# Menstrual blood donation for stem cell therapy

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# Abstract

Menstrual blood contains mesenchymal stem cells (MenSC), similar to those in bone marrow and umbilical cord blood. MenSC are highly proliferative and have the ability for multilineage differentiation, and considering the cyclicity and non-invasive nature of collection, are appealing candidates for regenerative medicine. Potential clinical applications include treating stroke, heart attack, spinal cord injury, sepsis, intrauterine adhesions, and COVID-19.

With these exciting clinical applications, very little research is focussed on the donor and the methods by which menstrual blood is collected. Associated with the menstrual taboo, people may be unwilling to donate MenSC. Without an acceptable donation method there are no MenSC. Therefore, this thesis has addressed the need to understand the attitudes to menstrual blood donation, optimise menstrual blood donation, and improve accessibility to MenSC donation and regenerative therapy.

In the literature the most popular method of MenSC isolation is via a menstrual cup during 'heaviest' flow. This work explored the possibility of MenSC isolation via sanitary pads and tampons, being more popular and accessible methods of menstrual hygiene management as well as being more stringently regulated. A comparison and categorisation of 14 menstrual cups was undertaken as a first step to improved regulation and safety surrounding menstrual cup use.

This work adopted a feminist approach to develop and propose optimum methods of MenSC isolation via sanitary pads and tampons, and was successful in isolating MenSC from sanitary pads and tampons as well as menstrual cups. When considering each product's MenSC isolation success rate and the participants' willingness to use, all three products have value, increasing MenSC donorship and accessibility to MenSC therapy. The product used to donate also had no impact on the MenSC in respect to processing time, number of MenSC, proliferation, and stemness, indicated by flow cytometry immunophenotyping and multilineage differentiation. It was found that MenSC donation was no more demanding than participants' normal monthly routine. Many participants celebrated MenSC donation, wishing to help others with this non-invasively collected 'waste' blood that can give menstruation a purpose beyond simply being a nuisance. It gives donors a reason to celebrate menstruation, and MenSC therapy will be instrumental in overcoming the menstrual taboo.

The ability to isolate MenSC from sanitary pads and tampons has valuable implications for all future MenSC research because it increases accessibility to a source of cell therapy, particularly where menstrual cups are not widely adopted and not regulated to the extent of sanitary pads and tampons. The research also positively contributes to broader reproductive health and diagnostics applications, for example the diagnosis of endometriosis and endometrial cancers. This work's success is evidence that this feminist, donor-led approach should be applied to all research for empowering outcomes that are appropriate to the needs and lifestyles of MenSC donors, future stem cell therapy recipients, and wider society.

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# Acronyms

| | |
|---|---|
| AHPMA | Absorbent Hygiene Product Manufacturers Association |
| BMI | Body Mass Index |
| CD | Cluster of differentiation molecules |
| DMEM | Dulbecco's Modified Eagle Medium |
| DMSO | Dimethylsulfoxide |
| EDANA | European Disposables and Nonwovens Association |
| EDTA | Ethylenediaminetetraacetic acid |
| EnMSC | Endometrium mesenchymal stem cell |
| FBS | Foetal bovine serum |
| FDA | Food and drug administration |
| ISO | The International Organisation for Standardisation |
| IUD | Intrauterine device |
| IUS | Intrauterine system |
| MenSC | Menstrual blood derived mesenchymal stem cell |
| MDR | Medical Device Regulation |
| MHRA | Medicines and Healthcare Products Regulatory Agency |
| MIR | Methodology for interdisciplinary research |
| MSC | Mesenchymal stem cell / Medicinal signalling cell |
| MSCGM | Mesenchymal stem cell growth medium |
| MTT | 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide |
| NASA | National Aeronautics and Space Administration |
| NASA-TLX | NASA Task Load Index |
| NICE | The National Institute for Health and Care Excellence |
| NIH | US National Institutes of Health |
| NWSP | Nonwoven Standard Procedures |
| P | Passage number |
| PBS | Phosphate-buffered saline |
| PPI | Patient and public involvement |
| SWAT | Subjective Workload Analysis |
| TPE | Thermoplastic elastomer |
| TSS | Toxic shock syndrome |

| | |
|---|---|
| WHO | World Health Organisation |

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Not all women menstruate and not all who menstruate are women. Gendered language regarding menstruation is harmful. Inclusive language surrounding menstrual health is important to minimise the alienation of communities surrounding an already taboo topic. This thesis uses inclusive language where possible.

# Chapter 1. Introduction

## 1.1 Context and research gap

Mesenchymal stem cells (MSC) are self-renewing cells able to differentiate into multiple cell types (Reya et al 2001; Weissman 2000) used in regenerative medicine, as found in bone marrow (Mamidi et al 2012; Prochazka et al 2009) and umbilical cord blood (Mo et al 2022; Ren et al 2018). Similar cells have also been found in menstrual blood (MenSC) (Akhavan-Tavakoli et al 2017; Azedi et al 2017; Chen et al 2019a; Meng et al 2007), and the potential clinical applications of these cells include heart attack (Xu et al 2017; Zhang et al 2013), spinal cord injury (He et al 2022a; Wu et al 2018), sepsis (Jin et al 2020; Martínez-Aguilar et al 2020), intrauterine adhesions (Ma et al 2020a; Tan et al 2016), COVID-19 (Fathi-Kazerooni et al 2022; Xu et al 2021) and acute respiratory distress syndrome (Chen et al 2020a).

With such exciting implications for regenerative medicine, and the taboo nature of menstruation (Delaney et al 1988; Johnston-Robeldo & Stubbs 2013; Kissling 2006; Laws 1993), this thesis investigates the delicate yet significant factors surrounding the donation of menstrual blood. Simplified in Figure 1.1, currently the literature focuses on MenSC isolation and potential clinical application, which is undoubtedly exciting and valuable. However, no research groups have explored the attitudes to donating menstrual blood to confirm whether people are willing to donate MenSC, and no research is focussed on the ease, comfort, and acceptability of menstrual blood donation methods.

Other research groups have collected menstrual blood with a variety of products, such as urine cup-tubing (Sun et al 2016), catheters (Sahraei et al 2022; Sheikholeslami et al 2021; Tan et al 2016), falcon tubes (Karadas et al 2014), a “proper collector pot” (Gonçalves et al 2020, p. 770), “sterile collection containers” (Arezoo et al 2021, p. 476) and a 20 mL injection syringe (Wang et al 2019; Zhang et al 2018), but the most common method of collection is via a menstrual cup (Alcayaga-Miranda et al 2015b; Azedi et al 2014; Nikoo et al 2012; Warren et al 2018). With menstrual cups being the preferred menstrual hygiene product for 8% of the UK (Mintel 2023), it is surprising and disappointing that other methods of menstrual blood collection have not been explored to make donation acceptable and easy for as many people as possible. Understanding the willingness to donate, and identifying collection methods that are more acceptable and suitable for as many people as possible, will increase MenSC donation rate, increasing MenSC availability for research, and later clinical application of MenSC, as outlined in Figure 1.1.

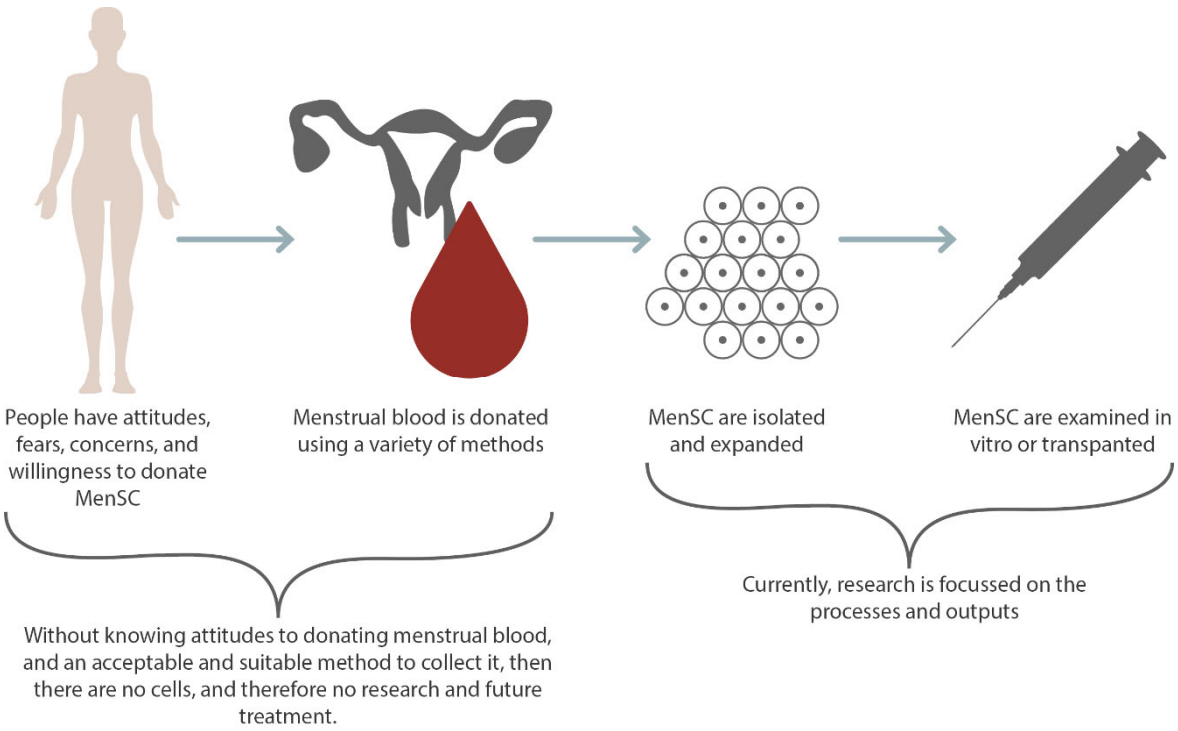



*Figure 1.1 Rationale for the current research*

The first gap in research to be approached in this thesis is a literature review to obtain and contextualise the existing knowledge relating to MenSC and their potential therapeutic uses, including menstrual blood donation: who is donating, how they are donating, and whether these methods are the best and appropriate for donors. This will open up the possibility of alternative, more suitable methods to be explored. **Research question 1: What are MenSC, and how are they currently donated?**

The next obvious research gap is the lack in research surrounding attitudes to MenSC donation. Without understanding the willingness of the public to donate menstrual blood, all other research is futile. **Research question 2: Do people even want to donate MenSC?**

When an understanding of current MenSC donation methods has been identified, and if people are willing to donate menstrual blood, the next research gap regards how this donation might be improved using menstrual cups or alternative methods; sanitary pads and tampons. People can not be expected to donate MenSC with these devices if they are not considered safe and fully understood by the researchers requiring the donation. This means identifying the function, material, and safety of sanitary pads, tampons, and menstrual cups. **Research question 3: What are sanitary pads, tampons, and menstrual cups, are they safe, and how are they being regulated?**

Once sanitary pads and tampons are identified as potential alternatives to menstrual cups for MenSC donation, the next research gap is the identification of methods for MenSC extraction in a laboratory setting. **Research question 4: How can MenSC be donated via sanitary pad or tampon?**

Finally, once appropriate methods for MenSC donation have been confirmed, the final research gap is to validate and compare menstrual blood donation methods via sanitary pads, tampons, and menstrual cups, in terms of MenSC isolation success, and donation experience. This will allow the proposal of optimum donation methods. **Research question 5: How do sanitary pads and tampons compare to menstrual cups in terms of MenSC isolation and donor experience?**

### 1.1.1 Aim and Objectives

Overall, this thesis aims to contribute novel MenSC donation and isolation methods using a feminist approach to understand the attitudes to menstrual blood donation, optimise menstrual blood donation, and improve accessibility to MenSC donation and regenerative therapy. Pursuing the five research questions outlined above will be achieved by the following objectives:

1. Understand the current position on MenSC in the literature, with an emphasis on donation methods and participant perspectives.
2. Understand the attitudes to donating menstrual blood.
3. Understand potential donation menstrual hygiene products regarding safety and regulation.
4. Identify suitable alternative MenSC donation methods (sanitary pads, tampons) and verify first with bone marrow MSCs, animal blood, and then menstrual blood.
5. Validate and compare MenSC donation methods in terms of participant attitudes and donation experience, as well as MenSC survival and isolation, compared to a menstrual cup. MenSC stemness to be confirmed via flow cytometry immunophenotyping and multilineage differentiation.

## 1.2 Overview of research methodology

This thesis adopts the approaches of interdisciplinary research, feminist research, and patient and public involvement (PPI), presented in Figure 1.2 and outlined in this section. The feminist approach is vital not only for the participants' benefit, but for the validity of the findings and the adoption of this approach in all future MenSC and reproductive health research. Utilising PPI ensures any outcome is appropriate to the needs and lifestyles of future MenSC donors.

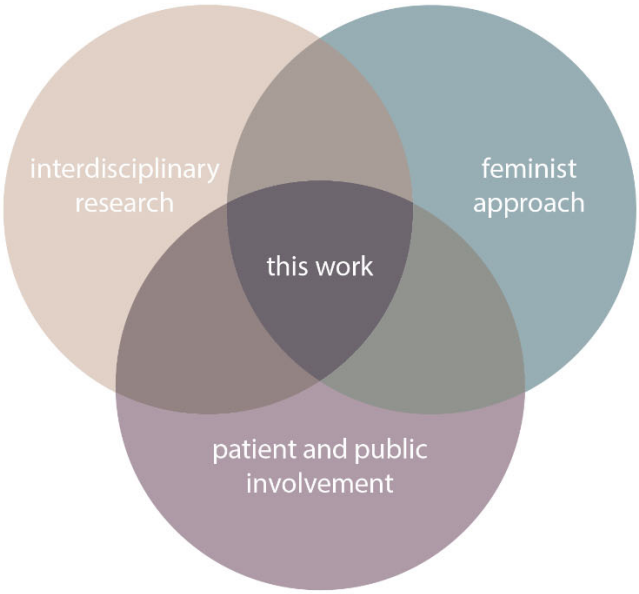



*Figure 1.2 Methodological approach to this research.*

### 1.2.1 Interdisciplinary research

This work straddles natural and social sciences, making this work interdisciplinary. Methodology for Interdisciplinary Research (MIR) framework has been outlined by Tobi and Kampen (2018), shown in Figure 1.3. This involves the conceptual design of the research, outlining the research objective and the operationalisation of the study. The technical design of the research, its execution, and finally the integration of the various branches of the research disciplines will result in the final recommendations for MenSC donation and isolation. Where many cases of MIR framework utilise an interdisciplinary team to collaborate on objectives, the author must identify methodologies from the natural and social sciences to successfully execute MIR. This methodology is systematic and practical but lacking complex constructs related to this research, such as sexism and misogyny in research, and the associated menstrual taboo. This requires a feminist approach.

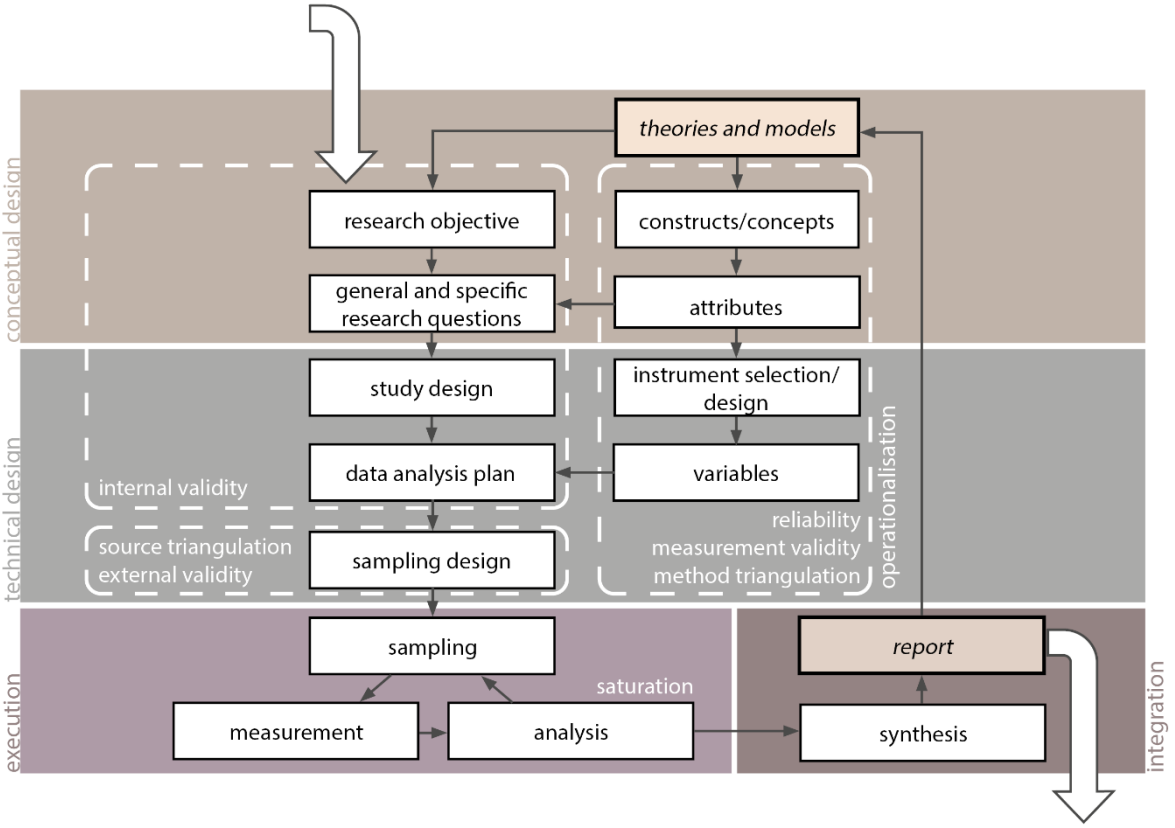



*Figure 1.3 Methodology for Interdisciplinary Research framework (Tobi & Kampen 2018)*

### 1.2.2 Feminist approach

Menstruation is a taboo topic. On top of women agreeing that menstruation is "inconvenient" (Blanchard 2003, p. 34; Farrington et al 2004, p. 1; Houppert 1999, p. 4; Kennet et al 2016, p. 553; Skultans 1988; Vostral 2008), women's experiences of menstruation are shaped and limited by this taboo (Delaney et al 1988; Johnston-Robledo & Chrisler 2020; Johnston-Robeldo & Stubbs 2013; Kissling 2006; Laws, 1993).

Globally the impact of the menstrual taboo varies. In certain societies, restrictions are imposed on menstruating women, such as food preparation, touching certain people or objects. In regions of Nepal, almost three quarters of women are restricted to a menstrual hut (Pandit et al 2021). Some Malay societies impose food restrictions taboos on menstruating women (Syed Abdullah 2022). Menstrual restrictions vary by religion, and women even report feeling pressure from other religions to adopt restrictions outside of their faith (Baumann et al 2021). Girls skip school when menstruating in Bangladesh (Hennegan & Sol 2020), Ethiopia (Azagew et al 2020), India (Vashisht et al 2018), Kenya (MacLean et al 2020), and this also occurs in the UK (BBC 2017). This being said, progress has been made: menstrual leave is recognised by law, in some cases permitting those menstruating state-paid leave from work in Indonesia (Labour Act No. 13 2003), Japan (Labour Standards Law 1967), South Korea (Labour Standards Law 2020), Spain (Proyecto de Ley Orgánica 2022), Taiwan (Act of Gender Equality in Employment 2014), and Zambia (called

Mother's Day) (Gender Equity and Equality Act 2015), which, when applied correctly, is instrumental in making people feel comfortable and empowered (Kanodia & Srivastava 2021; Levitt & Barnack-Tavlaris 2020).

Gottlieb argues that menstrual taboo is receiving greater resistance in the Western world (2020); Bodyform represented menstrual blood with red liquid for a TV-first (Bodyform 2017), and films surrounding menarche and menstrual hygiene Turning Red (2022) and Pad Man (2018) were released. In the UK, the tampon tax abolished the taxation of sanitary products (HM Treasury 2021), and the period product scheme provides free menstrual hygiene product in places of study (Department for Education 2023). More people reported feeling comfortable talking about menstruation in the UK (Glocalities 2020) which can be seen as a form of resistance[*] (McHugh 2020). Seeing positive or realistic representation of menstruation in the media and feeling supported by state schemes is instrumental in improving attitudes to menstruation.

Saying this, menstrual taboo is remains deeply ingrained (McHugh 2020), particularly for impoverished women (Boyers et al 2022). The menstrual taboo is dehumanising and is therefore a feminist issue. On top of this, menstrual blood donation could be a tool in overcoming the menstrual taboo.

It's widely acknowledged that women's health research is lacking (Kourany 2010). In terms of sharing research, women are less likely to speak up and when they do, they speak for less time (Salem et al 2021). High impact research conducted in women is deemed less publishable than research conducted in men, and research has a pro-male publication bias (Murrar et al 2021). This sexism and misogyny is also reflected in MenSC research. In 2021, Dr. Federica Helena Marinaro (a published MenSC researcher (2018; 2019)) revealed the Editor's rejection comments from a 9.26 impact factor Springer journal, sharing on Twitter. This quote has been reported in its entirety to highlight the misogyny and menstrual taboo in full context:

> In general, the topic idea is not that novel and could not be accepted as it is, since almost all articles in the literature reported the severe undesirable and toxic effects of menstrual blood and all its constituents (including MenSCs) on the human body. Even in all religions, it is well known that menstrual blood and its MenSCs are extremely very toxic and of very low quality. This blood contains the destructed metabolic constituents with very potent cytotoxic activities, thus in toxicological criminology, some women in some cultures, use very few drops of its potent toxic extract to secretly kill their husbands. In addition, why I have to spend that much money to enhance the immunomodulatory capacity of insecure toxic MenSCs when I can invest much less money in developing very safe synthetic and natural agents and of even more potency to satisfy the same purpose and more. This idea/matter also is not practically interesting/favorable since it

[*] Purely negative discourse surrounding menstruation feeds into the menstrual taboo (McHugh 2020).

> has significant privacy in females (do not forget that the menstrual blood contain their own ova, genomics, husbands' sperms/genetic material, etc.) along with the great possibility of increasing microbial blood infections transmission (Marinaro 2021a).

So commonplace was this response, that in the succeeding twitter thread, Marinaro described the need to use template responses to counter comments like this. Although shortly afterwards the Springer journal apologised and would ensure this would not happen again (Marino 2021b), this highlights the systemic sexism and continuation of problematic attitudes in the industry. MenSC research makes up only 0.25% of MSC research, and the resistance to MenSC research endures (Manica et al 2022). Most first authors are women and most last authors are men, highlighting the gender gap between last and corresponding authors: "women in benchwork, men as lab chiefs" (p. 957171). Menstrual scholar Owen recently found that menstrual researchers in general still experience challenges and need institutional support to publish (2022a). A feminist approach is essential to overcome this.

Research simply containing feminist content does not make it feminist work (Longino 1987); the research must change the surrounding social and political context, as "knowledge is shaped by the assumptions, values and interest of a culture" (ibid, p. 61). If the current research culture does not involve women in research, the research outcomes will be shaped by the menstrual taboo. Women have been excluded from meaningful participation in research, being treated as objects. Where objectification theory poses that women's bodies exist to gratify others, given value only for its use or consumption by others (Fredrickson & Roberts 1997), it is vital that menstrual blood donation does not simply become another commodity for consumption by others, and that research in this field does not objectify women further. As Maguire states, "traditional research processes are often alienating and dehumanizing" (1987, p. 37), and this is the case with MenSC. Chapter 2 highlights that very little research has been undertaken surrounding menstrual blood donation from the donor's perspective. Participatory research where participants have meaningful participation in the work has been described by Maguire as a "partnership";

> We both know some things; neither of us knows everything. Working together we will both know more, and we will both learn more about how to know (1987, p. 39).

This puts the researcher and participants on an even footing, both contributing, and therefore participatory research is a feminist approach. It is therefore an aim of this work for participants to be offered meaningful participation in the research, changing the social and political context surrounding it, resulting in this work being rooted in feminist practice.

### 1.2.3 Patient and public involvement

Within MenSC research, there continues to remain the lack of research focusing on the donor themselves: without donors, there is no MenSC treatment. Patient and public involvement (PPI)

has a positive impact during the initial stages of research, with users themselves identifying, prioritising, and developing research topics. This includes taking cultural issues into account, and improving the wording of sensitive issues (Brett et al 2014). PPI also contributes to outcomes that are "appropriate to the needs and lifestyles of the patient community it serves" (Bagley et al 2016, p. 6), although here the community served is a larger pool of healthy potential menstrual blood donors. PPI is not simply a matter of integrity or quality; it is a reflection of power, summarised by Arnstein, "citizen participation is a categorical term for citizen power" (1969, p. 216). Involving patients and the public, i.e. giving people meaningful power and participation in the research, is therefore fundamentally a feminist research technique.

Arnstein outlined the eight levels of PPI (ibid),[*] illustrated as a ladder, shown in Figure 1.4. Sometimes falsely celebrated as genuine participation, "manipulation" or "patient therapy" actually represent "nonparticipation" because they do not enable engagement with planning or operationalisation innovation. Therefore, simply educating the public about MenSC and menstrual blood donation is not enough. The next level of PPI, "degrees of tokenism", includes informing and consultation, which allows the public to be heard, but "they lack the power to ensure that their views will be *heeded* by the powerful" (ibid, p. 217). No change is affected. Placation allows for the public to advice in decision-making but those holding the power retain their decision-making. Therefore, this work strives for PPI permitting "degrees of citizen power", including partnership with public, as far as delegated power and citizen control. The author strives for MenSC donation in the future to be at least in part, if not fully, controlled by the public. Part of this research aimed for genuine partnership with the public to have been at the core of this work.

By including patients and participants in the story and giving them meaningful participation, this results in a "redistribution of power" (Arnstein 1969, p. 216), and is therefore intrinsically feminist. Figure 1.5 outlines the MIR utilised within this thesis with a feminist approach, including meaningful participation and adopting patient and public involvement.

[*] "Citizen participation" used synonymously with PPI

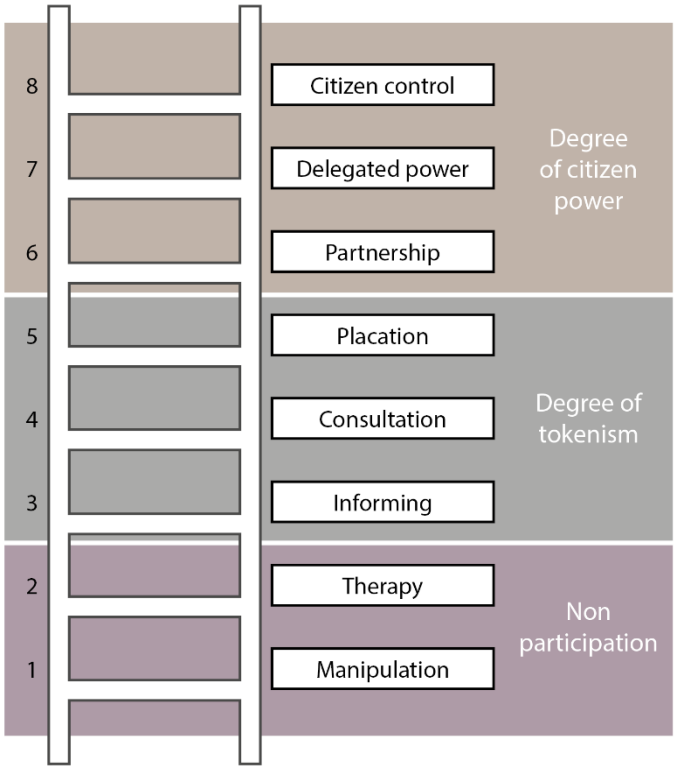



*Figure 1.4 Ladder of PPI, Arnstein 1969.*

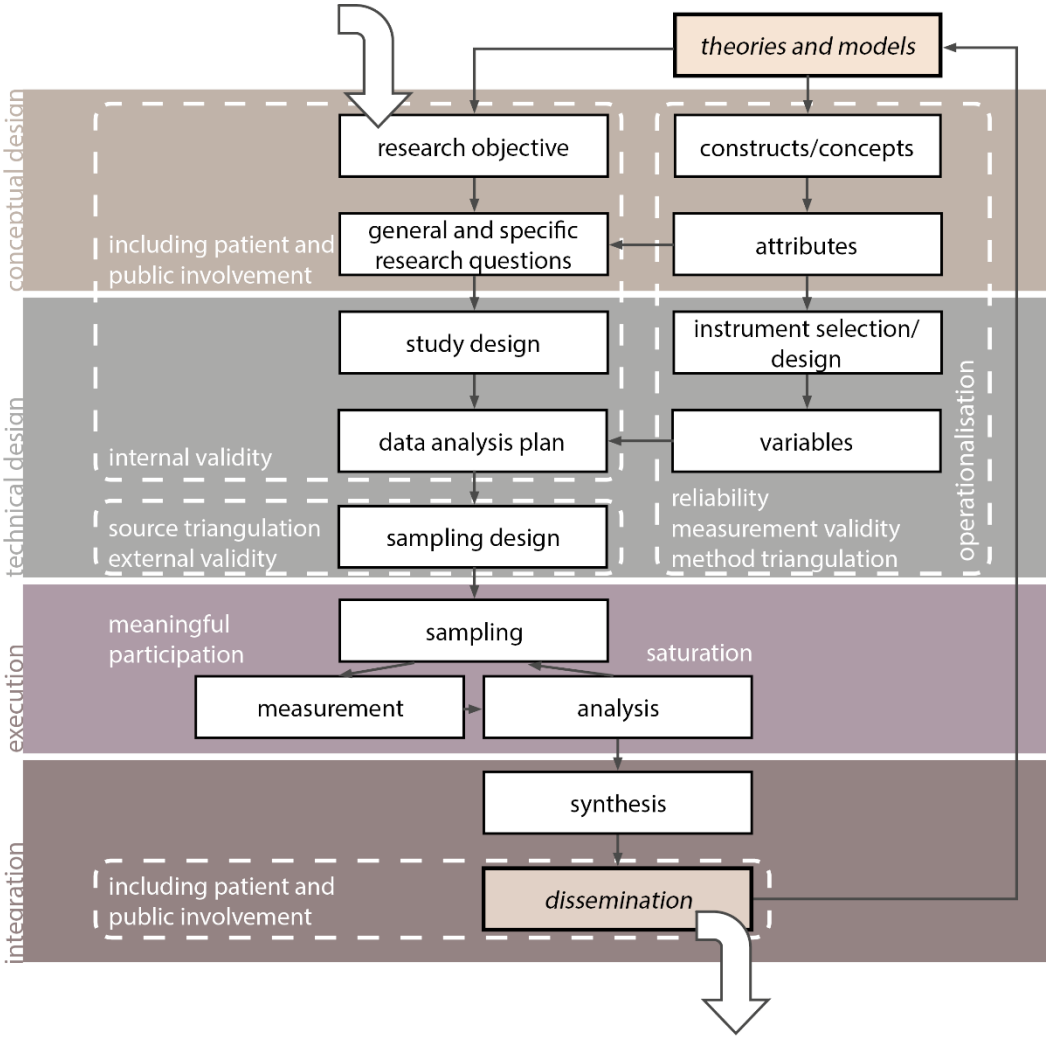



*Figure 1.5 Adapted methodology adopted in this thesis. Adapted from Tobi and Kampen (2018).*

*Note the addition of meaningful participation and adopting patient and public involvement for a feminist approach.*

## 1.3 Contribution to research

The key findings from this thesis include the understanding that there is an appetite for MenSC donation, with donors willing to help others and overcome the menstrual taboo, but also having concerns over the donation process.

This work had success isolating MenSC from sanitary pads and tampons as well as menstrual cups, and when MenSC success and willingness to use sanitary pads, tampons, and menstrual cups are taken into account, there is value in using all three products for future MenSC collection. It should be in the participants' hands which product is used as product had no effect on MenSC isolation success, growth, and stemness characteristics. Sanitary pads and tampons are also highly accessible and affordable.

These findings have implications in all future MenSC and broader MSC research and regenerative medicine. Broader implications include menstrual health and diagnostics and the design for reproductive health in relation to the design of a kit to allow menstrual blood donation. Finally, it

is a general aspiration for this thesis to show that a feminist approach to research produced findings that are valid scientifically as well as socially.

## 1.4 Structure of thesis

This research is interdisciplinary, and each research question follows the outcome of the research question before it. Many of the approaches, methods, and type of data vary between stages of the research. Therefore, it would not be useful to present this work by amalgamating all the methods, results, and discussion into separate chapters. As outlined by Phillips and Pugh when presenting theses as a series of projects or areas of research (2015), this thesis presents the research as divided into each research question and objective. See Figure 1.6.

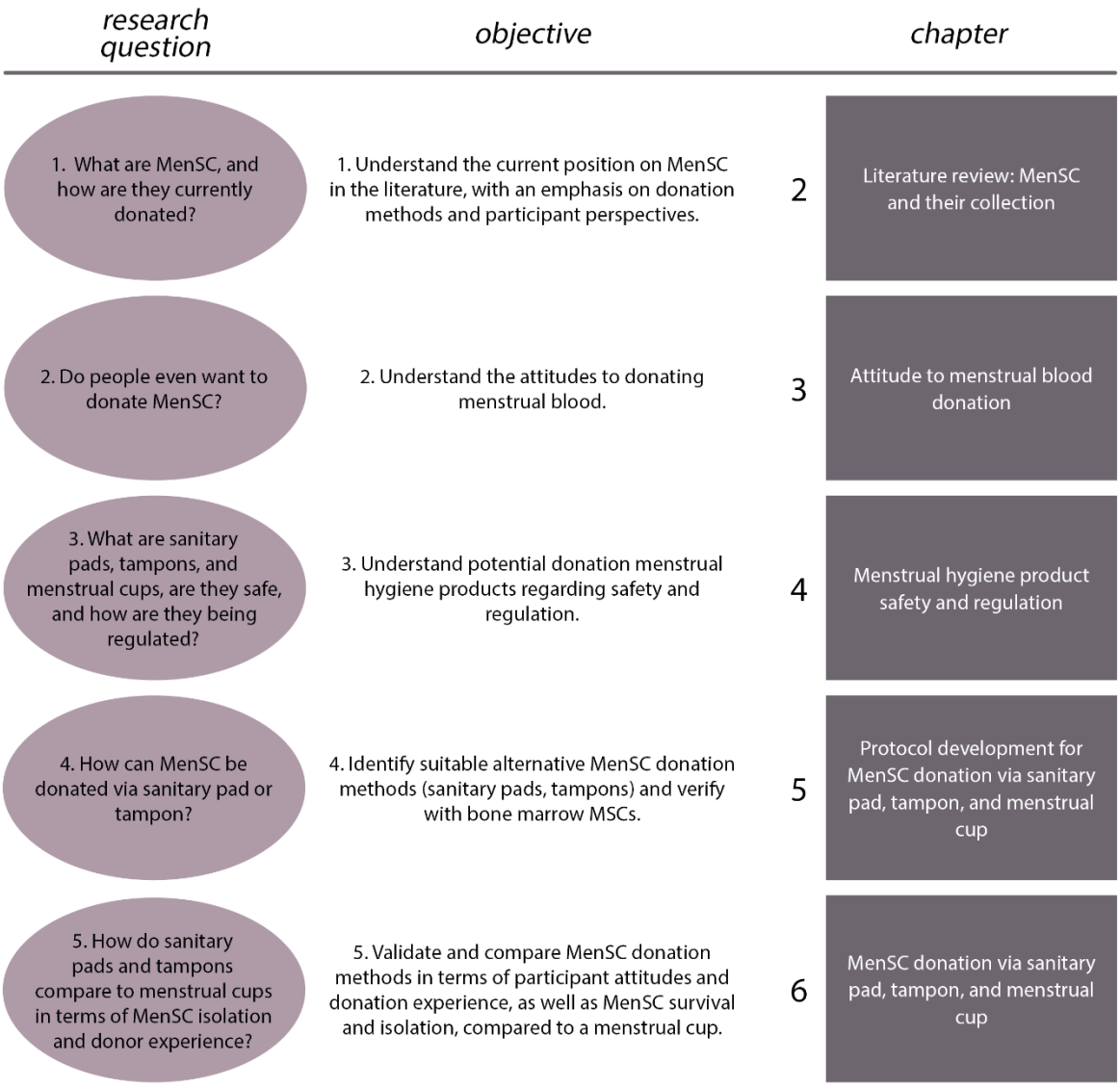



*Figure 1.6 The structure of this thesis, divided by the research questions posed in this thesis and their accompanying research objectives.*

The literature review in this thesis compiles the knowledge of MenSC characteristics within the context of other sources of MSCs and their clinical application. However, this literature review is unique in that beyond MenSC qualities and treatment, there is an emphasis on the menstrual

blood collection. While this is never the focus of other MenSC research, this literature review includes the donation device, participant engagement with the process, and logistical information such as menstrual blood flow, volume, and donation day.

0 reports the reflexive thematic analysis of the qualitative data collected in work previously published (Manley et al 2019). These findings include the motivation to help others, giving a 'waste' product a purpose, and potentially contributing to overcoming the menstrual taboo; donors are concerned with the donation, its convenience, and proposed menstrual cup use. It confirms that there is indeed an appetite for menstrual blood donation from a human factors' standpoint.

With the understanding that menstrual blood would be donated with sanitary pads, tampons, and menstrual cups, Chapter 4 outlines these products and their regulation. A case study and menstrual cup comparison highlights the lack of regulation of menstrual cups, leading to consumer confusion and potential safety implications.

0Chapter 5 outlines the protocol development for the isolation of MenSC from sanitary pads and tampons, utilising bone marrow MSC to validate the methods prior to menstrual blood collection. For the first time there is evidence that it is possible to isolate MenSC from sanitary pads and tampons.

All these chapters culminate in the main study, outlined in Chapter 6. Methods for isolating MenSC from both sanitary pads and tampons are described, as well as the general scientific methods adopted in this study including culture protocol, induced differentiation and flow cytometry to evidence the cells cultured are indeed MenSC and are not affected by the donation method. To analyse the participants' experience of donating menstrual blood, the National Aeronautics and Space Administration (NASA) Task Load Index is adopted, demonstrating that MenSC donation is no greater workload compared to undertaking one's normal menstrual hygiene routine from home. On top of this, qualitative data is collected and analysed using reflexive thematic analysis. This thesis reports comparable findings to those in Chapter 3 in terms of motivations for donating and celebrating menstruation, without many of the hypothetical donation concerns, but the addition of concerns such as leakage and the donation being fiddly.

Chapter 7 then synthesises all the findings from each chapter, with its holistic discussion considering the overarching themes, and the implications of this research for scientists, clinicians, and donors.

# Chapter 2. Literature review: MenSC and their collection

This literature review synthesises the current understanding of MenSC, their biological characteristics, and potential clinical application. Then, in response to **research question 1: What are MenSC, and how are they currently donated?**, the review centres upon the donors and the collection of menstrual blood, including the devices currently adopted, and logistics of donation, because if menstrual blood donation is not understood and optimised, donorship is limited. The focus has never been on the donor and these factors. The review reveals the sometimes unusual expectations of donors, and that utilising the most popular products (sanitary pads and tampons (Mintel 2023) has not been fully explored.

## 2.1 Menstruation

The female reproductive system involves the cyclical uterine preparation for pregnancy in four phases, the menstrual and proliferative phase, the ovulation phase, and the secretory phase. These coincide with the ovarian cycle, which includes the follicular phase, ovulation, and the luteal phase, as seen in Table 2.1. Key milestones are the development and release of an egg from an ovary, the thickening of the uterine lining in preparation for egg implantation, and, if there is no egg implantation, the shedding of the uterine lining through menstruation as the next cycle begins. This cycle varies and is considered to last around 28 days, with 65% of menstruators having a cycle length between 25-30 days (Bull et al 2019).

*Table 2.1 The menstrual cycle*

| | | *Day* | | | |
|---|---|---|---|---|---|
| | | *1-13* | | *14* | *15-28* |
| | | Pre-ovulation | | Ovulation | Post-ovulation |
| *Cycle* | *Ovarian* | Follicular phase | | | Luteal |
| | *Uterine* | Menstrual | Proliferative | | Secretory |

## 2.2 Menstrual blood

Menstrual blood is the combination of blood, mucous, and uterine tissues expelled during the menstrual phase of the menstrual cycle. Menstrual fluid is an average of 60.5% blood, although this ranges from 20.00%-99.80% (Reid 2006). Menstrual blood does not clot; it does not contain clotting agents such as fibrinogen (Beller 1971; Huggins et al 1943).[*]

[*] The endometrium releases fibrinolytic agents to initiate the enzymatic breakdown of the fibrin in any blood clots immediately prior to menstruation (Albrechtsen 1956; Salamonsen et al 1999). If menstrual bleeding is heavy, the fibrolytic enzymes do not have enough time to break down blood clots. Therefore, menstrual blood containing clots is an indicator that the loss is excessive (Salamonsen et al 1999).

Menstrual blood is more viscous than peripheral blood, with the highest viscosity on the first day of menstruation before reducing gradually over the following days (Reame 1983).

Menstrual blood samples' mean pH is 6.3, with a statistically significant range of mean values between participants (4.7-7.3), which was not influenced by collection time, duration of wear, of day of collection. Flow rate did significantly influence pH, with a faster flow rate having a higher pH (Reame 1983).

## 2.3 Stem cells

The cyclical deterioration, shedding, and regeneration of the uterine lining occurs with every menstrual cycle, particularly noting the complete lack of scarring with perfectly-functioning uterine tissue each month. This is non-existent in all other organs in the human body (Salamonsen 1998), suggests stem cell-like activity. Stem cells are cells with long-term self-renewing ability and with the capacity for differentiation into multiple lineages through differentiation (Reya et al 2001; Weissman 2000), including mature cells, progenitor cells, and stem cells of each tissue lineage (Wagers & Weissman 2004). These characteristics are therefore appealing in the field of regenerative medicine. A pluripotent stem cell has the ability to multiply indefinitely, as well as differentiate into cells from any of the three germ layers: endoderm, mesoderm, and ectoderm. A self-renewing and multiple mature cell lineage differentiation ability describes a cell that is multipotent. These cells self-renew, however may only differentiate into cell types from the same tissue. Unipotent stem cells continue to have the ability to multiply, however they can only differentiate into one type of cell (De Los Angeles et al 2015). Cells with a limited ability for self-renewal, often being unipotent, are described as progenitor cells (Seaberg & van der Kooy 2003).

Embryonic stem cells, derived from mammalian embryos during the blastocyst stage, are able to differentiate into all types of cell in the body, making them totipotent. With this source of human stem cell requiring the destruction of an embryo, it is associated with ethical implications, and therefore not seen by many as a suitable source of stem cells (Holm 2002; Janosky 2002). There are also the safety concerns of teratoma formation after transplantation of embryonic stem cells (Ben-David & Benvenisty 2011).[*]

Stem cells are also found within adult tissues. These cells maintain the tissue, generating and replacing cells within that specific tissue after damage or cell death (Slack 2000). Where it was thought adult stem cell plasticity is limited to its tissue of origin, adult stem cells have been shown

[*] Induced pluripotent stem cells are similar to embryonic stem cells due to their self-renewal capacity and ability to differentiate into multiple germlines. They are generated from adult cells through the recombineering of chromosomes (Okita et al 2007; Copeland et al 2001). However, being sourced from adult tissues, they often require a painful, invasive procedure for donation, and may also stand the risk of teratoma formation after transplantation (Deng et al 2018; Fong et al 2010).

to also replenish cells from other cell lineages, shifting between stem and progenitor cell (Blau et al 2001; Quesenberry et al 2002).

The term ‘mesenchymal stem cell’ was coined in 1991 by Caplan, referring to the middle mesodermal embryonic layer including bone and cartilage development, and the adult wound repair involving all skin, bone, and muscle damage. Mesenchymal stem cell and stromal cell have been used interchangeably, and there has been a demand for the discontinuation of the incorrect use of the word “stem”, with Seaberg and van der Kooy describing the trend with the “promiscuous use” of the term “stem cell’ without the precise nomenclature of the distinct cell (2003, p. 125). The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed minimal criteria for ‘human multipotent mesenchymal stromal’ cells being distinct from ‘stem’ cells (Horwitz et al 2005). However, this was disputed by Caplan, explaining that the use of the word ‘stromal’ is incorrect in the case of the cells not originating from connective tissues (2017). This nomenclature confusion must be rectified within the scientific community. The fact that there is no unique surface marker for mesenchymal (stromal) stem cell identification (Anisimov et al 2013) could perhaps mean that source-specific terminology must be adopted. However, it is not for this thesis to discuss. For the sake of this work, the continued use of the acronym ‘mesenchymal stem cell’ widely written as MSC will be used to describe these regenerative cells, and as Caplan suggests, to “Medicinal Signalling Cells” (2017, p. 1445) where the stemness is not verified.

MSCs have been sourced from adipose tissue (Hakki et al 2017; Li et al 2015a; Pendleton et al 2013), bone marrow (Conget et al 1999; Mamidi et al 2012; Prochazka et al 2009), dental tissues (Huang et al 2009; Seifrtova et al 2012; Thompson et al 2008), peripheral blood (Ab Kadir et al 2012; Li et al 2015b), and skin (Bartsch et al 2005; Riekstina et al 2008). Peripheral blood is the most widely-used adult source for regenerative medicine, treating patients with cancers of the blood, bone marrow and lymph nodes in nine out of ten cases in the UK (NHS England 2013), with bone marrow and umbilical cord blood also being available for treatment. However, collecting samples for the extraction of these sources of MSCs are either painful, invasive procedures, in the cases of bone marrow, adipose tissue, and peripheral blood; or they are only available in a limited supply, in the case of dental tissues. With adult MSCs having these disadvantages, there is demand for an alternative source of cells for regenerative medicine. One available source is through postnatal tissues, including placenta (Miao et al 2006; Raynaud et al 2012), amniotic fluid (Scherjon et al 2003; Tsai et al 2004) and membrane (Cai et al 2010; Wolbank et al 2010), sub amniotic umbilical cord lining membrane (Kita et al 2010), and Wharton’s jelly (Hou et al 2009; Wang et al 2004). However, the opportunity for this collection only occurs with the birth of a child, and the medical staff involved with the collection need to be specially trained. MSCs are

also isolated from umbilical cord blood (Mo et al 2022; Ren et al 2018). However, research has shown that a five-minute delay of cord clamping after birth significantly increased the baby's red blood cell-blood volume ratio, and the amount of haemoglobin for oxygen transportation (Mercer et al 2017). This delay also improved the iron levels and increased brain nerve protection after a four-month follow-up (Mercer et al 2018). With a cord clamping delay of minimum three minutes, at four years of age, significant improved scores in fine motor skills and social skills were seen, particularly in boys, compared to immediate cord clamping (Andersson et al 2015). The current World Health Organisation (WHO) protocol for cord clamping suggests clamping is undertaken "not earlier than one minute" (2014, p. 3), but "at least" a one-minute delay. Within the literature regarding the collection of umbilical cord blood MSCs, it is uncommon for the cord clamping timing to be mentioned at all, leaving the concern that newborn babies are not receiving the maximum blood volume available to them. The WHO recognises the need for further research, including clamping the cord "when the cord becomes flat" (2014, p. 10), which may not leave enough umbilical cord blood for MSC collection.[*] With more research in this area required, there is the possibility that umbilical cord blood donation may not be the most suitable source of MSCs after all. A source of MSCs that are ethical to harvest, with a cost-effective collection and painless, non-invasive procedure could revolutionise cell therapy research and treatment.

## 2.4 Menstrual blood-derived MSC

With this in mind, cells with stem cell characteristics have been found in the endometrium, first extracted via hysterectomy (Chan et al 2004). Due to the shedding of the endometrium during each menstrual cycle, it was then posited that menstrual blood also contains cells with stem cell activity. Meng et al (2007) were the first group to collect menstrual blood to source so-called "endometrial regenerative cells". Three cell types are candidates for contributing to the cyclical endometrial regeneration following each menstrual cycle: epithelial endometrial progenitor cells, mesenchymal stem-like cells, and endometrial endothelial progenitor cells (Cau et al 2019; Gargett & Masuda 2010).

### 2.4.1 Nomenclature

These stem cell-like cells are collected from endometrium tissue, via a hysterectomy or biopsy, and menstrual blood. Hysterectomy- or biopsy-sourced cells are generally named endometrium mesenchymal stem cells (Phermthai et al 2016), endometrial multipotent mesenchymal stromal cells (Schwab et al 2005; Rajaraman et al 2012), human endometrial mesenchymal stem cells (Kao et al 2011) and human endometrial stem cells (Navaei-Nigjeh et al 2014), and cells derived from

[*] Further issues arise if umbilical cord MSCs are banked for future autologous therapy, and stored for many years at high cost to the mother. If the umbilical cord MSCs are required for autologous treatment of a genetic condition, it is expected for the genetic mutation to present in these umbilical cord MSCs cells too.

menstrual blood are named menstrual blood stem cell (Akhavan-Tavakoli et al 2017; Fathi-Kazerooni et al 2017), menstrual blood-derived stem cell (Azedi et al 2017; Chen et al 2019a), or menstrual blood-derived mesenchymal stem cell (Guo et al 2019a; Chen et al 2020a; Wang et al 2017a), among other names. This is inconsistent in the literature. This work argues that MSCs harvested directly from the endometrium, via biopsy or after hysterectomy, should be titled endometrium-MSC (EnMSC), and menstrual blood-derived MSC (MenSC), to differentiate between the two.[*]

This research shall remain focused on MenSC due to the potential ease and non-invasive nature of collection, and the cyclical nature of menstrual bleeding.

Within the literature, clarification of nomenclature or confirmation of MenSC has sometimes proven difficult. This is in part due to the huge range of names for MenSC. Furthermore, researchers studying cells named "menstrual blood-derived stem cells" have been studying cells derived from the uterus of mice (Zhang et al 2022a, p.3826; Zhang et al 2022b), which is not an acceptable use of the nomenclature. It is assumed menstrual blood is human-derived; mice do not naturally menstruate (apart from the spiny mouse (Bellofiore et al 2018)). Therefore, to avoid confusion, MenSC nomenclature must only be used when referencing MSCs isolated from the menstrual blood of human donors.

## 2.5 Characteristics of MenSC

MenSCs that have been harvested from menstrual blood have been exciting the field, with research groups agreeing that these cells are ethical (Akhavan-Tavakoli et al 2017; Chen et al 2017a), easy to collect (Bozorgmehr et al 2014; Karadas et al 2014; Ren et al 2016), inexpensive (Du et al 2016; Khanmohammadi et al 2012), and available in abundance (Kazemnejad et al 2013a; Khanjani et al 2014). The following section discusses MenSC's stemness, profilerative capacity, and migration ability, which is useful in verifying their clinical potential.

[*] Where the MSC are described as "menstrual blood-derived", or where the methods within a study clearly report the extraction or collection of menstrual blood, it is easy to define them as MenSC. However, ambiguity arises when MSCs are described as "endometrial" (EnMSC). In some cases, these MSCs are collected via the menstrual blood, in other cases they are collected via a biopsy (Schüring et al 2011), and in other cases they are extracted post hysterectomy (Schwab et al 2005). Although Uzielienne et al (2018a) report MSCs from both of these sources to be the same, Shan et al compared the MSCs during various stages in the menstrual cycle. MSCs were reported to be more proliferative during the menstrual phase (2017, p. 184), which is positive regarding the understanding of MenSC, but also highlights that there is a difference between the MSCs secreted in menstrual blood and those taken from the endometrium throughout other phases of the menstrual cycle. Although Rodrigues et al report that MenSC are morphologically and functionally similar to EnMSCs extracted directly from the endometrium (2016), they are not identical, with stem cells being located in the stromal tissue of the basalis layer of the endometrium to prevent being shed during menstruation (Cho et al 2004). In this review, where MSCs have been sourced from a biopsy or hysterectomy, it can not be presumed that these cells are MenSCs. Therefore, they are not included in this review unless otherwise stated. The same applies for endometrial MSCs for which the collection or extraction methods are not described.

### 2.5.1 Stemness and potency

Firstly, the 'stemness' of MenSC must be validated. According to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, minimum criteria for human MSCs is to be plastic-adherent when in standard culture conditions. MSCs must express cluster of differentiation molecules (CD) CD105, CD73 and CD90, and not express CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Finally, MSCs must demonstrate a trilineage differential; being able to differentiate to osteoblasts, adipocytes and chondroblasts in vitro (Dominici et al 2006). 'Stem cell' terminology may only be accurately employed when stem cell activity has been clearly demonstrated (Horwitz et al 2005).

MenSC adhere strongly to plastic (Du et al 2016; Rossignoli et al 2013; Savilova et al 2016). MenSC have been reported to differentiate into osteoblasts, adipocytes, and chondroblasts (Li et al 2019a; Shokri et al 2019; Wang et al 2019; Yang et al 2022). As for the markers expressed by MenSC, they meet the criteria, see Table 2.2. Masuda et al were the first to report on the use of a novel single marker, W5C5, to identify MenSC (2012) and MenSC also exhibit the expression of the embryonic stem cell marker, OCT4 (Khanmohammadi et al 2014; Rahimi et al 2014a; Rajabi et al 2018), and SSEA-4 (Patel et al 2008; Sun et al 2019a) resulting in researchers arguing that MenSCs could be classified as a unique stem cell population (Darzi et al 2012; Khanmohammadi et al 2012). However, not all research groups reported an expression on SSEA-4 (Aleahmad et al 2018; Chen et al 2015a), meaning there is a need to clarify this. This disparity in marker expression has been described as potentially due to menstrual blood collection having differing collection days, collection techniques, and enrichment protocol (Bozorgmehr et al 2020), and it has been identified that further research is needed as MenSC have been loosely defined so far with disagreement regarding their cell markers (Sanchez-Mata & Gonzalez-Muñoz 2021).

*Table 2.2 MenSC marker expression*

| *Marker* | *Expression* | *References* |
|---|---|---|
| CD9 | + | Aleahmad et al 2018; Meng et al 2007; Nikoo et al 2012; Patel et al 2008; Verdi et al 2014 |
| | - | Sugawara et al 2014 |
| CD10 | + | Aleahmad et al 2018; Eyni et al 2017; Khanmohammadi et al 2014; Rajabi et al 2018; Sugawara et al 2014 |
| CD11a | - | Zemelko et al 2013 |
| CD11b | - | Alfano et al 2017; Xu et al 2023 |
| CD13 | + | Cui et al 2007; Verdi et al 2014; Wu et al 2014; Zemelko et al 2012; Zemelko et al 2013 |
| CD14 | - | Cuenca et al 2018; de Pedro et al 2021; Martínez-Aguila et al 2020; Uzieliene et al 2021; Wang et al 2019 |

| | | |
|---|---|---|
| CD19 | - | Dalirfardouei et al 2018; Domnina et al 2016; Li et al 2019a; Wang et al 2019; Zheng et al 2017 |
| CD20 | - | Álvarez et al 2018; De Pedro et al 2021 |
| CD29 | + | Chen et al 2015a; Dalirfardouei et al 2021; Du et al 2018; Guo et al 2019b; Liu et al 2019a |
| CD31 | - | Alcayaga-Miranda et al 2015a; Du et al 2016; Meng et al 2007; Verdi et al 2014; Wang et al 2021 |
| CD33 | - | Meng et al 2007 |
| CD34 | - | Alfano et al 2017; Bu et al 2016; Eyni et al 2017; Farzamfar et al 2017; Xiang et al 2017 |
| CD35 | - | Zheng et al 2017 |
| CD36 | - | Uzieliene et al 2021 |
| CD38 | - | Feng et al 2019; Martínez-Aguilar et al 2020; Nikoo et al 2014; Shokri et al 2019; Tan et al 2016 |
| CD40 | - | Liu et al 2019a; Moreno et al 2017 |
| CD41a | + | Meng et al 2007 |
| CD44 | + | De Pedro et al 2021; Kazemnejad et al 2012; Rahimi et al 2014a; Sun et al 2019b; Yan et al 2019 |
| CD45 | - | Akhavan-Tavakoli et al 2017; Alcayaga-Miranda et al 2015a; Alfano et al 2017; Chen et al 2017a; Wang et al 2017a |
| CD49d | + | Blázquez et al 2018 |
| CD49f | + | Allickson et al 2011; Blázquez et al 2018 |
| CD50 | - | Cui et al 2007 |
| CD54 | + | Cui et al 2007; Sugawara et al 2014; Uzieliene et al 2021 |
| CD56 | + | Blázquez et al 2018 |
| CD59 | + | Meng et al 2007 |
| CD63 | + | Lopez-Verrilli et al 2016; Uzieliene et al 2021 |
| CD73 | + | Chen et al 2017a; Du et al 2016; Eremichev et al 2018; Quintero-Espinosa et al 2021; Sheikholeslami et al 2021 |
| CD79a | + | Alfano et al 2017 |
| CD80 | + | van Phuc et al 2011 |
| | - | Álvarez et al 2018; de Pedro et al 2021; Liu et al 2019a |
| CD83 | + | Moreno et al 2017 |
| CD86 | + | van Phuc et al 2011 |
| | - | Liu et al 2019a; Moreno et al 2017 |
| CD90 | + | Eremichev et al 2018; Cen et al 2019; Chen et al 2020c; Jiang et al 2013; Ren et al 2016 |
| CD105 | + | Alcayaga-Miranda et al 2015a; Darzi et al 2012; He et al 2022a; Hida et al 2008; Rajabi et al 2018 |

| | | |
|---|---|---|
| CD106 | + | Arezoo et al 2021 |
| | - | Khanjani et al 2014 |
| CD117 | + | Allickson et al 2011; Álvarez et al 2018; Patel et al 2008; Verdi et al 2014 |
| | - | Dalirfardouei et al 2019; Kovina et al 2018; Wu et al 2019; Yang et al 2022; Zhao et al 2018a |
| CD130 | - | Zemelko et al 2012 |
| CD133 | + | Verdi et al 2014 |
| | - | Kazemnejad et al 2012; Martínez-Aguilar et al 2020; Rahimi et al 2014a; Sheikholeslami et al 2021; Shokri et al 2019 |
| CD144 | - | Arezoo et al 2021 |
| CD146 | + | Darzi et al 2012; Domnina et al 2016; Khanjani et al 2014; Rajabi et al 2018; Wu et al 2018 |
| | - | Hu et al 2019 |
| CD166 | + | Dalirfardouei et al 2018; Jiang et al 2013; Khanjani et al 2014; Verdi et al 2014; Wu et al 2014 |
| CD185 | + | Xiang et al 2017 |
| CD191 | + | Xiang et al 2017 |
| CD192 | + | Xiang et al 2017 |
| CD194 | + | Xiang et al 2017 |
| CD197 | + | Xiang et al 2017 |
| CD202b | + | Xiang et al 2017 |
| CD221 | + | Xiang et al 2017 |
| CD271 | - | Alcayaga-Miranda et al 2015a |
| c-kit | - | Cui et al 2007; Khanmohammadi et al 2014; Rajabi et al 2018 |
| c-MYC | + | Li et al 2012a; Liu et al 2018 |
| EPCAM | - | Alcayaga-Miranda et al 2015a |
| GFAP | - | Azedi et al 2017 |
| HLA ABC | + | Alcayaga-Miranda et al 2016; Cuenca et al 2018; Cui et al 2007; Liu et al 2018; Sugawara et al 2014 |
| HLA-1 | + | Lopez-Verrilli et al 2016 |
| HLA-DR | - | Álvarez et al 2018; Cen et al 2019; Chen et al 2015a; Chen et al 2020c; Martínez-Aguilar et al 2020 |
| HLA-G | - | Kovina et al 2018 |
| hTERT | + | Meng et al 2007; Patel et al 2008 |
| hTRA-1-60 | - | Alcayaga-Miranda et al 2015a |
| LIN | - | Patel et al 2008 |
| MAP 2 | + | Borlongan et al 2010 |
| MCH II | - | Patel et al 2008 |

| | | |
|---|---|---|
| Nanog | + | Allickson et al 2011; Borlongan et al 2010; Liu et al 2018; Sun et al 2019b |
| | - | Khanmohammedi et al 2012; Khanmohammadi et al 2014; Meng et al 2007; Patel et al 2008 |
| Nestin | + | Borlongan et al 2010; Domnina et al 2016; Zemelko et al 2012 |
| Oct-4 | + | Aleahmad et al 2018; Khanmohammadi et al 2012; Mirzadegan et al 2022; Nikoo et al 2014; Shokri et al 2019 |
| | - | Zemelko et al 2012 |
| SOX-2 | + | Liu et al 2018; Patel et al 2008; Sun et al 2019a |
| | - | Li et al 2012a |
| SSEA-1 | - | Meng et al 2007 |
| SSEA-3 | - | Alcayaga-Miranda et al 2015a |
| SSEA-4 | + | Allickson et al 2011; Borlongan et al 2010; Li et al 2012a; Liu et al 2018; Patel et al 2008 |
| | - | Aleahmad et al 2018; Chen et al 2015a; Khanmohammadi et al 2012; Khanmohammadi et al 2014; Tan et al 2016 |
| STRO-1 | + | Sheikholeslami et al 2021 |
| | - | Darzi et al 2012; Kazemnejad et al 2012; Khanmohammadi et al 2012; Nikoo et al 2014; Zheng et al 2018 |

The pluripotent stem cell markers exhibited by MenSC understandably led to some research groups describing MenSC as pluripotent (Li et al 2012a; Meng et al 2007; Peñailillo et al 2022; Rodrigues et al 2012; Sanberg et al 2011; Xiang et al 2017). As stated by Lin et al in 2011, ambiguity begins when the term 'pluripotent' is used to describe a cell when it may not exhibit all the characteristics of pluripotency. The US National Institutes of Health (NIH) lists the following criteria to establishing a pluripotent cell: the cell line must be proven to self-renew indefinitely, and the cell line must be evidenced to form a teratoma (tumour-like growth) containing cells from all three embryonic germ layers (endoderm, mesoderm, and ectoderm). However, alternative evidence may be provided to NIH for pluripotency to be considered (Smith et al 2009). The NIH website declares the in vitro studies to show differentiation into the three germ layers (Department of Health and Human Services 2016). De Los Angeles et al (2015) defines pluripotency with the combination of select core transcription factors: OCT4 (also known as POU5F1), SOX2 and NANOG (collectively called OSN). OCT4 is strongly exhibited in MenSC as discussed. NANOG has been reported as both positively exhibited (Allickson et al 2011; Liu et al 2019a; Sun et al 2019a) and negatively exhibited (Khanmohammadi et al 2014; Meng et al 2007; Patel et al 2008), and SOX2 also both positively exhibited (Liu et al 2018; Sun et al 2019a) and negatively exhibited (Li et al 2012a; Patel et al 2008). This ambiguity should be addressed, identifying whether the methods for SOX2 and NANOG detection in MenSC needs to be

addressed. This ambiguity is perhaps why MenSC are more heavily described as multipotent (de Carvalho Rodrigues et al 2012; Ren et al 2016; Zemelko et al 2012; Zheng et al 2018).

MenSC can have pluripotent status induced via nuclear reprogramming, with resulting cells showing the same characteristics as human embryonic stem cells in terms of their morphology, markers expressed, and genes expressed (Li et al 2012a). Another research group also reported success with inducing MenSC to pluripotent status with a much higher efficiency when compared to fibroblasts, 2-5% success compared to 0.01-0.1% (de Carvalho Rodrigues et al 2012), and Lopez-Caraballo et al found that MenSCs have unique expression and epigenetic and potency profiles, showing higher reprogramming efficiency than MSCs sourced from bone marrow and fibroblasts sourced from skin (2020). Reprogrammed MenSC have been used to treat autoimmune hepatitis in mice (Wang et al 2021).

### 2.5.2 Low tumorigenicity

Criteria to establish pluripotent cell lines by the NIH is the evidencing of a teratoma with cells from all three germ layers (Smith et al 2009). However, this criteria also identifies safety issues with MSC transplantations. Embryonic stem cells directly implanted in mouse heart formed teratomas and were ultimately rejected (Nussbaum et al 2007), Hentze et al found embryonic stem cells formed teratomas in multiple sites, independent of injection site (2009), and teratomas destroyed the knee joint after embryonic stem cell injection to the knee (Wakitani et al 2003). With this understanding that embryonic stem cells transplantations carry the risk of teratoma formation, it is encouraging to learn that MenSC have little or no tumorigenicity (Khanjani et al 2014; Kovina et al 2018; Liu et al 2018; Mou et al 2013). MenSC transplantation is lower risk than embryonic stem cell transplantation.

### 2.5.3 Proliferation and longevity

In vitro studies have been undertaken to understand the longevity of MenSC. Research groups reported MenSC ability to undergo as many as 45 population doublings before senescence (Domnina et al 2016; Zemelko et al 2012). MenSC have been subcultured into as many as 28 generations without affecting specific cardiomyogenic transdifferentiation ability (Hida et al 2008) and subcultured 47 times before complete senescence and death (Allickson et al 2011).

In vivo studies assess MenSC longevity. After the transplantation of $1 \times 10^6$ MenSCs treatment for cutaneous wounds in mice, MenSC were still present at the site of injury after at least 14 days (Cuenca et al 2018). Similarly, MenSC could survive for at least 14 days in vivo after the transplantation of $1 \times 10^4$ cells into mouse ovary (Liu et al 2014). MenSC survival as long as 21 days in vivo has been reported after an injection of $5 \times 10^6$ MenSC, with a small number of cells surviving 21 days in mouse tumour (Liu et al 2019a).

Compared to bone marrow MSCs, MenSCs were found to have 90% survival compared to 70% survival (Azedi et al 2014), and in another case MenSC numbers only decreased 2.5-fold compared to eightfold in bone marrow MSC numbers (Luz-Crawford et al 2016).

This level of survival is accompanied with high proliferative ability. MenSC was found to have comparable proliferative capacity to bone marrow (Azedi et al 2014), and on multiple occasions being found to have a greater proliferative capacity (Kazemnejad et al 2012; Rahimi et al 2014a; Uzieliene 2018b; Wu et al 2014). On one occasion MenSC were reported to have as high as threefold the proliferative capacity as bone marrow MSCs (Jiang et al 2013), and another study showed MenSC to have twice the proliferative capacity as bone marrow and adipose tissue MSCs (Kozhukharova et al 2017). However, MenSC have been reported as not being as proliferative as umbilical cord blood MSCs (Chen et al 2015b; Ren et al 2016). The maintenance of this proliferative capacity is to be fully understood due to conflicting reports, with it being reported that later passages, passage ten compared to passage five, showed proliferation was greatly reduced (Chen et al 2015a), but it also being reported that there was no difference in proliferation between passage three to passage ten (Du et al 2016).

### *Doubling times*

This proliferation can be measured by a cell population's doubling time; sometimes reported as an approximation, a range, and with varying degrees of accuracy. However, as seen in Table 2.3, the doubling time ranges from 19.4 hours (Meng et al 2007) to 72.4 hours (Mehrabani et al 2016). It is noted that several studies found MenSC to have faster doubling times than MSC from bone marrow (Darzi et al 2012; Khanmohammadi et al 2012; Moreno et al 2017) and adipose tissue (Chen et al 2015b; Kovina et al 2018).

*Table 2.3 MenSC doubling time*

| *Doubling Time (hours)* | *Author* |
|---|---|
| 19.4 | Meng et al 2007 |
| Approx. 20 | Wu et al 2014 |
| 20.5 | Darzi et al 2012; Khanmohammadi et al 2012 |
| 22-23 | Domnina et al 2016; Zemelko et al 2012 |
| 24 | Lai et al 2015 |
| Approx. 24 | Borlongan et al 2010; Mou et al 2013 |
| 24–36 | Cen et al 2019; Patel et al 2008 |
| "at first doubling every 26 h and then the proliferation rate decreased" | Kovina et al 2018, p. 367 |
| Approx. 26 | Chen et al 2015a |

| | |
|---|---|
| 27.6 | Rossignoli et al 2013 |
| 39.7 ±2.3 | Moreno et al 2017 |
| 39 ± 4 | Eremichev et al 2018 |
| 48 | Liu et al 2019a |
| 48-72 | Álvarez et al 2018 |
| 55.5 and 62 hours for 30-40 year olds, 70.4 and 72.4 hours for 40-50 year olds | Mehrabani et al 2016 |
| Approx. 80 | Skliutė et al 2021 |

### 2.5.4 Migration ability

MenSC have strong migratory capability, vital for cells to locate areas in need of growth or healing. The strong migration ability of MenSC allows for the straightforward treatment of an intravenous transplantation. MenSC have been injected directly to the sites of injury or have been injected intravenously. After intravenous injection, MenSCs reached ovarian tumour nodules within 48 hours in mice (Alfano et al 2017), and as fast as six to twelve hours in mice with premature ovarian failure (Lai et al 2015). One study in rats receiving MenSC transplants for tumour treatment either with intravenous or direct to tumour injection found similar reductions in tumour size, 49% compared to 46% respectively (Han et al 2009). MenSC were found to have significantly greater migration compared to umbilical cord blood MSCs under basal and pro-inflammatory conditions during wound treatment (Cuenca et al 2018). In one study, it was found that MenSC treated with plasma rich platelets increased the cells' migration rate (Wang et al 2019), and another claiming Bushen Tiaochong enhances the migration of MenSC (Guo et al 2019a). It is this migration ability that allows for the use of intravenous treatment of MenSC clinical application, whether direct to site (Tan et al 2016; Ma et al 2020a), or injected intravenously (Zhong et al 2009). However, after MenSC transplantation via tail vein injection in mice, MenSC successfully migrated to the lungs, liver, and spleen, but not the heart or kidneys (Yang et al 2022). This may affect future application of MenSC.

## 2.6 MenSC treatment

With MenSC holding these exciting characteristics of migration, longevity, proliferation, and stemness, the potential for MenSC to be used in regenerative medicine has been explored. Martínez-Aguilar et al highlight MenSCs' dynamic role in orchestrating the inflammatory response post-transplantation, with MenSCs preventing tissue damage and promoting homeostasis (2020). Research groups have begun to understand the potential for treatment in a range of diseases and injuries in animal and human models, including treating liver failure (Cen et al 2019; Chen et al 2017a; Fathi-Kazerooni et al 2017; Fathi-Kazerooni & Tavoosidana 2019; Lu et al 2016), heart

attack (Hida et al 2008; Jiang & Wang 2012; Jiang et al 2013; Zhaocai & Jianan 2012) Alzheimer's disease (Zhao et al 2018a), stroke (Borlongan et al 2010) and chemotherapy-induced premature ovarian failure (Guo et al 2019b; Liu et al 2014; Lai et al 2015; Manshadi et al 2019) in animal models, and Asherman's syndrome (Ma et al 2020a; Tan et al 2016), multiple sclerosis (Zhong et al 2009), and acute respiratory distress syndrome (Chen et al 2020a) in humans. See Table 2.4. MenSC are commonly transplanted via injection (Chen et al 2020a; Ma et al 2020a; Tan et al 2016; Zafardoust et al 2020), but have potential to be transferred via other methods, including MenSC seeded onto surgical mesh (Marinaro et al 2021; Paul et al 2019), hydrogel (Hao et al 2022), or scaffold (Fan et al 2022; He et al 2022b), or combination of these (hydrogel-scaffold) (He et al 2022a). Previously, it was believed transplantation was effective because cells were differentiating into the injured cells of interest. However, it is understood that MenSC exert paracrine activity on injured tissues (cell signalling).

*Table 2.4 MenSC treatment studies*

| *Model* | *Study treating* | *Author* |
|---|---|---|
| *Animal* | Ischemia | Murphy et al 2008; Vu et al 2015 |
| | Duchenne muscular dystrophy | Cui et al 2007 |
| | Wound healing | Cuenca et al 2018 |
| | Osteochondral injury | Khanmohammadi et al 2019 |
| | Type 1 diabetic hyperglycaemia | Wu et al 2014 |
| | Cardiac function after heart attack | Hida et al 2008; Jiang & Wang 2012; Jiang et al 2013; Zhaocai & Jianan 2012 |
| | Myocardial infarction | Lan et al 2017; Xu et al 2017; Zhang et al 2013 |
| | Cardiac allograft | Hu et al 2021 |
| | Transplant vasculopathy | Ye et al 2018 |
| | Lung injury | Ren et al 2018; Xiang et al 2017 |
| | Idiopathic pulmonary fibrosis | Sun et al 2019b; Zhao et al 2018b |
| | Liver failure | Alcayaga-Miranda et al 2015a; Cen et al 2019; Chen et al 2021a; Chen et al 2017a; Fathi-Kazerooni et al 2017; Fathi-Kazerooni & Gholamreza 2019; Fathi-Kazerooni et al 2019; Lu et al 2016 |
| | 2/3 liver removal | Mou et al 2013 |
| | Liver fibrosis | Chen et al 2017b |

| | |
|---|---|
| Liver injury | Yang et al 2022 |
| Colitis | Li et al 2019b; Lv et al 2014; Sun et al 2022b; Xu et al 2018 |
| Spinal cord injury | He et al 2022a; Shi et al 2023; Wu et al 2018 |
| Sciatic nerve injury | Farzamfar et al 2017 |
| Alzheimer's disease-like pathology | Zhao et al 2018a |
| Neurological and histological impairments after stroke | Borlongan et al 2010 |
| Endometrial injury repair | Hu et al 2019; Zhang et al 2016 |
| Intrauterine adhesion (Asherman's syndrome) | Domnina et al 2018; Chen et al 2020b; Hao et al 2022; Hu et al 2022; Zhang et al 2019; Zheng et al 2018 |
| Cervical cancer | Liu et al 2019a |
| Ovarian cancer | Bu et al 2016 |
| Chemotherapy-induced premature ovarian failure | Guo et al 2019b; Lai et al 2015; Liu et al 2014; Manshadi et al 2019 |
| Premature ovarian failure | Feng et al 2019; Wang et al 2017b; Wang et al 2017d; Yamchi et al 2021 |
| IVF outcome in age-related infertility | Marinaro et al 2018 |
| Embryo development in assisted reproduction | Gonçalves et al 2020 |
| Liver cancer | Wu et al 2019 |
| Brain tumour | Han et al 2009; Wang et al 2017a |
| Cancer | Moreno et al 2019 |
| Pulmonary fibrosis | Chen et al 2020c |
| Peritonitis | Martínez-Aguilar et al 2020 |
| Sepsis | Jin et al 2020; Martínez-Aguilar et al 2020 |
| Premature ovarian insufficiency | Zhang et al 2021a |
| Wound healing with diabetes | Fan et al 2022; Mirzadegan et al 2022 |
| Autoimmune encephalomyelitis | Li et al 2022 |
| Type 1 Diabetes | Sun et al 2022a |
| Hepatitis | Zhang et al 2022c; Zhang et al 2023 |
| Neuroinflammation due to infection | Xu et al 2023 |
| Acute respiratory distress sydrome | Alcayaga-Miranda et al 2023 |
| Non-alcoholic fatty liver disease | Du et al 2023 |

| | | |
|---|---|---|
| *Human* | Intrauterine adhesions (Asherman's syndrome) | Ma et al 2020a; Tan et al 2016 |
| | Multiple Sclerosis | Zhong et al 2009 |
| | Acute respiratory distress syndrome | Chen et al 2020a |
| | Poor Ovarian Responders | Zafardoust et al 2020 |
| | COVID-19 | Fathi-Kazerooni et al 2022;** Lu et al 2021;* Xu et al 2021 |
| | Premature ovarian failure | Zafardoust et al 2023 |

* In this case report it is unclear whether MenSC or other MSC were used in the first case of COVID-19 in Hangzhou, MenSC treatment was "proposed" (Lu et al 2021, p. 1707) without describing actual transplantation.
** Treated via MenSC secretome collected during MenSC culture.

### 2.6.1 MenSC Extracellular vesicles/secretome

Most cells excrete nano-sized extracellular vesicles allowing intercellular communication and promote regeneration (Tkach & Théry 2016). MenSC can be cultured and MenSC extracellular vesicles extracted from the medium (Mahdipour 2022) and transplanted, treating type 1 diabetes (Mahdipour et al 2019), intrauterine adhesions (Zhang et al 2021b), acute burns (Rohani Ivar & Mahdipour 2021), and in vitro improvement of fertilization outcomes and embryo quality during assisted reproduction (Blázquez et al 2018), although the exact mechanisms for this therapeutic benefit remains a mystery (Chen et al 2021b).

The MSC secretome,* is a complex mixture of protein products including growth factors, cytokines, microvesicles, and exosomes (Wangler et al 2021; Ahangar et al 2020). The secretome is released into the culture medium of MSC (Fathi-Kazerooni et al 2022), conditioning the media. Transfusion of the secretome repairs tissue damage, reduces inflammation, and increases tissue repair capacity (Kim et al 2013; Mancuso et al 2019). MenSC-conditioned media promoted the regeneration of injured tissues in mice (Shang et al 2023), eased the symptoms of mice with ulcerative colitis (Sun et al 2022b), improves embryo yield and quality in mice (Marinaro et al 2019) and cows (Amini et al 2022), has therapeutic affect for myocardial infarction in pigs (de Pedro et al 2022).

MenSC is no miracle cure, particularly in the treatment of various cancers. For example, MenSCs alone neither promoted nor inhibited primary liver cancer (Zhou et al 2023), and pancreatic cancer cells were not affected by MenSC, although it was observed that MenSC did inhibit further cancer growth (Alcayaga-Miranda et al 2016). MenSC treatment alone had no influence on tumour size after transplantation in mice, however using MenSC and their migratory abilities

* Also called 'bioactive materials' (Amini et al 2022)

meant that MenSC were successful in delivering oncolytic adenovirus to target cancer cells (Guo et al 2019b). This was also found by Barlabé et al treating mice with lung cancer with MenSC as delivery for oncolytic adenovirus (2020). MenSC have the ability to transport a number of types of adenoviruses and can home to the tumour after injection to the general area, allowing for oncolytic adenovirus replication in vivo, for these reasons being described as a realistic alternative to bone marrow MSC (Moreno et al 2017).

With the understanding of the potential of MenSC treatment for a range of injuries and diseases, the next step is to identify who the potential recipients of this treatment could be, and the limitations to collecting, isolating, and transplanting MenSC.

### 2.6.2 Autologous or allogeneic transplantation

An autologous transplant describes a recipient receiving a transplantation of their own cells. An allogeneic transplant describes the recipient receiving a transplantation from a donor. An allogeneic transplantation has the advantage that treatment can be accessed in an off-the-shelf manner, with patients receiving almost immediate care. This is particularly important in cases where patients are too ill to provide a sample themselves, or when there is no time to spend weeks preparing the cells for transplantation in labs, which must be expanded before delivery, limiting autologous cell transplants (Rodrigues et al 2012). However, allogeneic transplants carry the risk of graft versus host disease, where the recipient's immune system responds to the foreign transplant by attacking the tissue or cells. Therefore, allogeneic transplant donors and recipients are carefully matched to reduce this risk of immune response and fast recovery, which in itself is time-consuming, and a matching donor is not always possible to find.

Many research groups agree that MenSC treatment would be administered in an autologous capacity. This includes approaches in neurotransplantation (Azedi et al 2014), treating premature ovarian failure (Lai et al 2015), pelvic organ prolapse (Chen et al 2016) tendon repair (Zheng et al 2017), improve embryo development during assisted reproduction (Gonçalves et al 2020), as well as reprogramming MenSC into induced pluripotent stem cells (Hojjat et al 2023; Park et al 2011). Clinical trials treating patients with Asherman's syndrome successfully utilised autologous MenSC transplants to improve pregnancy rates (Ma et al 2020a; Tan et al 2016), and autologous transplantation to the ovary improved fertility and pregnancy rates in poor ovarian responder (POR) patients (Zafardoust et al 2020).

However, MenSC have been reported to have low immunogenicity (Lan et al 2017; Shilina et al 2018; Xu et al 2017) and describing MenSC as "immunosuppressive agents" (Hu et al 2021, p. 474). In studies specifically reporting non-immunocompromised animals with an observed therapeutic effect, it has been suggested that MenSC could be a source for allogeneic

transplantation (Domnina et al 2018; Li et al 2022; Murphy et al 2008), treating colitis (Lv et al 2014), damaged heart tissue (Jiang et al 2013), ischemia (Vu et al 2015). This is particularly pertinent after patients with multiple sclerosis received allogeneic transplants of menstrual blood. These patients were reported to be fully immunocompetent and did not suffer any immunological reactions after MenSC treatment (Zhong et al 2009). Patients with acute respiratory distress syndrome also received allogeneic MenSC transplantation, with a small number of patients returning for a 5-year follow-up and experiencing no negative or harmful effects (Chen et al 2020a). Finally, 26 patients suffering from severe and critical forms of COVID-19 received allogeneic MenSC transplantation with no immediately-transparent adverse effect as a result of the transfusions (Xu et al 2021). This builds the strong case that the future of MenSC treatment is allogeneic, with the option for people that menstruate to receive autologous treatment if required. Research groups such as Verdi et al (2014) stating MenSC treatment is limited to menstruating women and Yan et al suggesting "men population and menopause women seem to be deprived from this blessing" (2022, p. 495) are therefore mistaken.

### 2.6.3 Quantity of MenSC

With the understanding that MenSC have the potential to be used in various treatments as seen in Table 2.4, it is important to identify the quantities required in treatment to establish the protocol for the future. This will identify whether samples of MenSC must be pooled to have adequate quantities of MenSC, or how much these cells must be expanded in vitro before being administered to patients.

Volume of menstrual blood collected for each study is not always reported (Du et al 2018; Wang et al 2017c; Yan et al 2019; Yang et al 2022). Menstrual blood volume collected ranged from 0.5 mL (Karadas et al 2014) to 20 mL (Kovina et al 2018), as shown in Table 2.5. The majority of research groups collect 5 mL and successfully isolate MenSC.

*Table 2.5 Volume of menstrual blood collected*

| *Volume collected (mL)* | *Author* |
|---|---|
| 0.5 | Karadas et al 2014 |
| 1-2 | Sheikholeslami et al 2021; Zemelko et al 2012; Zemelko et al 2013 |
| 1-5 | Moreno et al 2017 |
| 1-17.5 | Fiorelli-Arazawa et al 2019 |
| 1-20 | van der Molen et al 2013 |
| 2 | Alfano et al 2017; Domnina et al 2016 |
| 2-5 | Azedi et al 2014 |

| | |
|---|---|
| 3-5 | Dalirfardouei et al 2018; Dalirfardouei et al 2019; Luz-Crawford et al 2016; Nikoo et al 2012; Rajabi et al 2018 |
| 4 | Arezoo et al 2021 |
| 5 | Akhavan-Tavakoli et al 2017; Arasteh et al 2018; Azedi et al 2017; Cen et al 2019; Chen et al 2016; Darzi et al 2012; Kazemnejad et al 2012; Kazemnejad et al 2013a; Kazemnejad et al 2013b; Khanmohammadi et al 2012; Khanmohammadi et al 2014; Li et al 2019a; Li et al 2019b; Mehrabani et al 2016; Meng et al 2007; Ren et al 2016; Sun et al 2016; Sun et al 2019b; Wang et al 2019; Xu et al 2018; Zhang et al 2018; Zheng et al 2018; Zhong et al 2009 |
| 5-10 | Uzieliene et al 2021 |
| 8 | Liu et al 2019a |
| 8-10 | Borlongan et al 2010 |
| Mean 9.8 (SD 5.0) | Wyatt et al 2021 |
| 10 | Hida et al 2008; Lv et al 2014; Lan et al 2017; Manshadi et al 2019; Wang et al 2017d |
| 10-15 | Quintero-Espinosa et al 2021 |
| 10-20 | Eremichev et al 2018 |
| 15-20 | Kovina et al 2018 |

Fiorelli-Arazawa et al very clearly define the number of cells isolatable from each menstrual blood sample; ranging from 0.81 to 14.0 × $10^6$ (median 5.2 × $10^6$) (2019), although it is not specified if these are all cells or MenSC. If the number of MenSCs isolatable from menstrual blood was reported more consistently, the average cells/mL menstrual blood provided would easily be comparable. However, as shown in Table 2.6, the number of cells isolatable were generally reported per sample. This value ranged from 2 × $10^4$ cells (Luz-Crawford et al 2016) to 3 × $10^7$ cells (Borlongan et al 2010). However, reporting the number of MenSC per sample is not overly useful when sample volumes range from 3-5 mL (Luz-Crawford et al 2016) to 10 mL (Lan et al 2017), and then are sometimes not stated at all.* Where possible, the value for MenSC per mL of menstrual blood collected were calculated. However, in cases where the data was not available and volume of blood was not specified, 5 mL was used as the estimated volume. This is regularly cited as the most popular volume collected as seen in Table 2.5, and in one case, the study methods cited

* "About 1–3 × $10^6$ nucleated cells with more than 90% viability could be procured from each milliliter of menstrual blood. When Ficoll separation step is omitted, about 3 × $10^6$ nucleated cells per each milliliter of menstrual blood are obtained". (Kazemnejad et al 2013a, p.164) Of these cells, how many are MenSC?

another research group that had collected 5 mL in their work, so it can be assumed 5 mL was collected (Khanmohammadi et al 2019). These findings show a great range in the number of MenSC isolatable per mL of menstrual blood, ranging from $6 \times 10^2$ MenSC (Alcayaga-Miranda et al 2015b) to an estimated $6 \times 10^6$ MenSC (See Table 2.6). This is currently difficult to explain. It could be due to the variation in collection and isolation methods, which will be discussed in greater depth in Section 2.8, or that only very few participants have been recruited in each study (see Table 2.7), with the majority of studies recruiting 1-5 participants. Future research must recruit a sample size from which significant findings can be extracted.

*Table 2.6 Number of MenSC isolated from menstrual blood*

| *Volume / mL* | *MenSC /sample* | *MenSC /mL* | *Reference* |
|---|---|---|---|
| - | $3 \times 10^3$* | $6 \times 10^2$ | Alcayaga-Miranda et al 2015b |
| - | $5 \times 10^4$ | $1 \times 10^4$* | Patel et al 2008 |
| - | $1 \times 10^5$ | $2 \times 10^4$* | Moreno et al 2019 |
| 10 | $1 \times 10^7$ | $1 \times 10^6$† | Lan et al 2017 |
| 3-5 | 3-7.5 $\times 10^6$‡ | 1–1.5 $\times 10^6$ | Nikoo et al 2012 |
| 5 | $1 \times 10^6$ | $2 \times 10^5$† | Cen et al 2019 |
| 1-2 | 4–9 $\times 10^6$ | 2-9 $\times 10^6$† | Zemelko et al 2012 |
| - | $3 \times 10^7$ | $6 \times 10^6$* | Borlongan et al 2010 |
| 10-15 | $6.7 \times 10^6 \pm 3 \times 10^6$, n = 3 | 2.5-9.7 $\times 10^5$† | Quintero-Espinosa et al 2021 |
| - | $1 \times 10^8$ | $2 \times 10^7$* | Wang et al 2021 |

* calculations made based on an estimated 5 mL sample of menstrual blood

† calculations made based on reported sample volume and cells/sample

‡ calculations made based on reported sample volume and cells/mL

*Table 2.7 Number of study recruits for menstrual blood donation*

| *Number of study recruits* | *Reference* |
|---|---|
| 1 | Borlongan et al 2010; Bu et al 2016; Chen et al 2020a; Domnina et al 2018; Lai et al 2015; Lai et al 2016; Shilina et al 2018; van Phuc et al 2011 |
| 2 | Kovina et al 2018 |
| 3 | Domnina et al 2016; Du et al 2016; Eremichev et al 2018; Guo et al 2019a; Jiang et al 2013; Moreno et al 2019; Quintero-Espinosa et al 2021; Ren et al 2018; Rossignoli et al 2013; Yamchi et al 2021; Yan et al 2019; Zhang et al 2019; Zhang et al 2021b |
| 3-6 | Rahimi et al 2014a |
| 4 | Alcayaga-Miranda et al 2015a; Alcayaga-Miranda et al 2016; Álvarez et al 2018; Blázquez et al 2018; Chen et al 2017b; Farzamfar et al 2017; Farzamfar et al 2018 |
| 5 | Alcayaga-Miranda et al 2015b; Azedi et al 2014; Fathi-Kazerooni et al 2017; Kazemnejad et al 2012; Kazemnejad et al 2014; Khanmohammadi et al 2019; Liu et al 2014; Manshadi et al 2019; Nikoo et al 2012; Rajabi et al 2018; Sun et al 2019a; Uzieliene et al 2018b; van der Molen et al 2013; Zheng et al 2018; |
| 6 | Arezoo et al 2021; Feng et al 2019; Hida et al 2008; Uzieliene et al 2021; Wang et al 2017d; Wang et al 2019; Xu et al 2017; Zhang et al 2018 |
| 7 | Luz-Crawford et al 2016; Moreno et al 2017 |
| 8 | Gonçalves et al 2020; Lv et al 2014 |
| 8 (5 with recurrent implantation failure (RIF), and 3 non-RIF) | Esmaeilzadeh et al 2020; |
| 9 | Wu et al 2014 |
| 9 (3 healthy, 3 with polycystic ovary syndrome, 3 with endometriosis) | Sheikholeslami et al 2021 |
| 10 | Aleahmad et al 2018; Chen et al 2019b; He et al 2022a; Hu et al 2019; Mehrabani et al 2016 |
| 11 | Shan et al 2017; Wyatt et al 2021 |
| 12 | Ma et al 2020a; Wu et al 2018 |

| | |
|---|---|
| 15 | Martínez-Aguilar et al 2020; Zafardoust et al 2020; Zafardoust et al 2023 |
| 17 | Chen et al 2015b |
| 18 | Chen et al 2015a; Liu et al 2018; Shokri et al 2019 |
| 12 healthy donors and 6 chronically HIV-infected donors | Sabbaj et al 2011 |
| 20 (10 healthy, 10 endometriosis patients) | Cressoni et al 2023 |
| 21 | Cui et al 2007; Sugawara et al 2014 |
| 29 (10 nulliparous, 10 multiparous, 9 preeclampsia) | Peñailillo et al 2022 |
| 31 | Li et al 2017 |

Perhaps it is easier to determine how many cells are extracted after time in culture. After 2 weeks in culture, Jin et al estimated $1 \times 10^7$ MenSC adhered (2020), presumably at passage 0 (P0). Meng et al reported a one to two thousand-fold increase in MenSC number after 3-4 passages, from $1 \times 10^5$ to $1\text{-}2 \times 10^8$ (2007). Patel et al reported an almost thousand-fold increase from initial cell count to cell expansion after 26 days, from $5.0 \times 10^4$ to $4.8 \times 10^7$ (2008). Zhong et al reported a higher increase, of ten to twenty thousand-fold increase after 3-4 passages, having started with around $1 \times 10^4$ MenSC cells and expanding to $1\text{-}2 \times 10^8$ (2009). Kovina et al reported they could yield up to $4 \times 10^6$ MenSC/mL of blood (2018), so $2 \times 10^7$ for a 5 mL sample. These quantities of MenSC are obviously exciting, but it is important that researchers remain realistic and participants confirmed to be healthy.[*] With an idea of how many MenSC are available in each sample, it is next important to identify how many MenSC are required for treatment, to establish clinical donation requirements.

As seen in Appendix A, the median and mode number of MenSC transplanted into various rodents and small animals for a number of diseases and injuries is $1 \times 10^6$ (Bu et al 2016; Feng et al 2019; Li et al 2019b; Murphy et al 2008), having inflammatory and immunosuppressive effects on colitis (Lv et al 2014), alleviating the severity of pathological changes after cardiac allograft (Lan et al 2017), and rescuing spatial learning and memory in mice with Alzheimer's disease (Zhao et al

[*] Kovina et al then go on to state, "up to 50 mL of blood per day or 200 mL per cycle can be collected" (p. 364), suggesting $1 \times 10^9$ MenSC could be donated in one cycle. A study measuring the volume of menstrual blood lost over 96 cycles found that menstrual blood volumes of more than 169 mL should be considered abnormal in multiparous women, and 162 mL in nonparous women (Donoso et al 2019), with The National Institute for Health and Care Excellence (NICE) defining menorrhagia (abnormally heavy or prolonged bleeding) as more than 80 mL per cycle (2018). Therefore, it should not be expected, or considered healthy or safe, for women to be donating more than 80 mL in 5-10 mL samples, and certainly not 200 mL in one cycle.

2018a) among others. Translating this to use in human transplantation does not always reflect in larger doses of MenSC. Chen et al do not specify how many samples of menstrual blood are collected, the size of the samples, and the expansion rates of MenSC. However, one donor is enough to treat 17 patients allogeneically. Chen et al define the MenSC transplantation dose as $1 \times 10^6$ per kg body weight (2020a). Without reporting the weights of the participants, it can be estimated an average European body of 70.8kg (Walpole et al 2012) requires a MenSC dose of approximately $7.1 \times 10^7$. Other clinical trials have transplanted fewer MenSC. As seen in Appendix B, autologous transplantation for treating Asherman's syndrome was still successful in single doses of $1 \times 10^6$ MenSC, although on two occasions transplantation was repeated once per patient during a different menstrual cycle (Tan et al 2016). In treating multiple sclerosis, four patients received slightly larger doses of MenSC of generally around $6 \times 10^6$ cells, but having up to five doses over up to ten days, transplanting a total of either 1.6 or $3 \times 10^7$ MenSC (Zhong et al 2009). Although the number of clinical trials administering transplantations of MenSC remains low, it is pertinent to establish that the quantities required in these instances are obtainable from a single sample of menstrual blood from healthy donor, which looks plausible judging by the available figures. One research group goes so far as to claim one donor can generate a potential 20,000 patient doses (Bockeria et al 2013).

## 2.7 Future for MenSC

With expansion figures looking positive but with limited data available, it could also be important to establish protocol for situations where single samples are not enough for treatment.

### 2.7.1 Pooling

Dalirfardouei et al collected menstrual blood in a urine cup, and reported successfully pooling the samples from three donors (2018). Where they successfully cultured MenSC without needing special growth factors, the pooled MenSC were not studied in vivo. Tan et al report the age of each donor, the volume of menstrual blood donated, the frequencies of donations and if they were pooled, treatment, intrauterine adhesion severity, and infertility history. In two out of seven patients, menorrhea (menstrual bleeding lasting for less than two days) meant that samples were insufficient for treatment. In these cases, another sample was taken, with the cells then pooled for transplantation (2016).[*] van der Molen et al were able to collect samples of menstrual blood from donors every 12 hours (2013). Van Phuc et al were able to collect 2-3 samples from donors, without reporting the volumes of the samples (2011). Kovina et al also report donors submitting

[*] This could be considered a setback for future collection of MenSC. However, Asherman's syndrome, for which these patients were being treated, is identifiable by amenorrhea (lack of menstruation) (Asherman 1948). Therefore, these patients would always be less likely to be able to donate full samples of menstrual blood. It highlights the need for healthy donors for allogeneic transplantations, particularly for those who are unable to donate their own menstrual blood due to pathogenic, hormonal, or other reasons.

4-6 samples of menstrual blood in one day (2018). MenSC from different donors were pooled to treat infected mice (Xu et al 2023). These studies not only suggest the future potential for MenSC collections to made repeatedly and pooled, but also that donors are open to the idea of multiple donations per cycle. Saying this, many research groups express that MenSC from a menstrual blood sample can be expandable to quantities of clinical application (Bozorgmehr et al 2014; Eyni et al 2017; Khanjani et al 2014; Wang et al 2019), potentially negating the need for pooling multiple samples.

### 2.7.2 Cryopreservation

Cryopreservation of cells is the gradual cooling to very low temperatures, typically −80 °C or −196 °C, which effectively stops any process causing damage or ageing to the cell. Bone marrow MSCs have been successfully cryopreserved for decades (Areman et al 1990; Rowley 1992). This has been successfully translated to MenSC, with high levels of viable MenSC being recovered (Allickson et al 2011), proliferation and clonogenicity being preserved (Du et al 2016), and stem cell phenotype being maintained (Liu et al 2018). Kovina et al even go to say that MenSC are the most suitable form of MSC for cryopreservation (2018). Some research groups acquired MenSC from banks for their studies without stating the percentage of viable cells from the samples (Domnina et al 2018; Eyni et al 2017). Xu et al thawed MenSC with >90% viability for transplantation (2021). Ma et al reported that MenSC were successfully cryopreserved before autologous transplantation, finding cryopreserved MenSC had similar therapeutic affect compared to fresh MenSC, and "Importantly, the patients have more time to prepare for the transplantation. Utilizing cryopreserved cells make it more convenient for clinical practice and can shorten the waiting time" (2020a, p. 2354). Disadvantages to cryopreserving MenSC are reported by Rodrigues et al, including its high cost and the affiliated potential bioethical dilemmas limited by high cryopreservation cost (2016). As with all medicine, the cost to benefit must be weighed to establish whether the cryopreservation of MenSC is suitable, and if it is not available within public healthcare parameters for these reasons, MenSC could rely on private banking. Finally, Moll et al discuss the potential issue of cryo-stunned MSC affecting responsiveness of MSC after cryopreservation. Saying this, recovering MSC in culture "should be just as good as fresh cells" (2016, p. 98). Research in this area is lacking; for MSC in general and certainly for MenSC. Future research should identify which approach is most appropriate.

### 2.7.3 Banking

It has been argued that the future of MenSC could be that of private banking (Paul et al 2019). Hida et al argue the case for public MenSC banks could be the future of healthcare, specifically for cardiac treatment (2008). MenSC have been repeatedly described as a potential 'off-the-shelf' solution for a number of diseases or conditions (Alcayaga-Miranda et al 2015a; Jiang et al 2013;

Murphy et al 2008; Verdi et al 2014; Zhong et al 2009), implying that MenSC could be processed, cryopreserved and thawed for allogeneic use for transplantation in patients with disease or injury.

Cryo-Cell founded C'elle in 2007, allowed people to cryopreserve samples of menstrual blood for potential future autologous use (private banking) (Cryo-Cell 2020), but this is no longer available on their website in 2023. The service has since been signed over to Hangzhou S-Evans Biosciences (2020), where it also appears to removed from the website in 2023. MenSC banking is still available through other companies such as ReeLabs private banking (2020) where the author was quoted 882 US$ for lifetime MenSC storage costs in October 2020 (Nimeesh 2020, personal communication, 23 October); and is reportedly available from Avicenna Infertility Centre in Iran (IFP 2020) with research groups reporting this centre as the source of MenSC (Eyni et al 2017; Farzamfar et al 2017), but this is not advertised on their website (Avicenna Infertility Group 2020).

An issue with private banking could be understood that the cryopreservation of menstrual blood relies too much on the "capitalisation of hope", as described in regard to other private MSC banking (Martin et al 2008, p. 128), and the fact that MenSC treatment is still in the very early exploratory stages of treatment options could be building into the idea of "early promise/later disappointment" (ibid, p. 133) as privately banked samples are not yet ready for treatment (and certainly weren't in 2007). Cryocell received public criticism in the press in 2007, charging £238 for collection and the first year's cryopreservation, and "capitalising on people's fear" (Roberts).

Private banks obviously rely on people wanting to pay for a service to store cells for the potential treatment of themselves or close family member. However, surveying 100 people in the UK, of those currently menstruating, the majority agreed they would donate for the treatment of anyone if possible (allogeneic), and at no financial cost to them (Manley et al 2019). This motivation for MenSC donation suggests that the future of MenSC banking will be for public banking.

Ethnic-representation public banking strategies should be implemented, as even though this does not allow the greatest percentage of a population to be matched for treatment, it is the strategy that best provides equality to members of minority ancestral/ethnic groups (Bok et al 2004). However, the need for MenSC banking may be less in demand than that for umbilical cord blood and other MSCs; it is so readily available. Could it be argued that most people have a close relative that menstruates? With 1.9 billion menstruators globally (Babbar et al 2022), potential donors are only ever a month away from MenSC donation, and recipients then a further few weeks away from transplantation.

### 2.7.4 Concerns

MenSC certainly looked to be a promising potential form of cell therapy, having discussed their high proliferation, strong potency, capacity for migration, and success in animal and human studies. As a source of MSC, menstrual blood is beneficial due to its ease of collection, monthly occurrence, and collection requiring minimal invasion. However, researchers have expressed their concerns; Li et al (2012a) and Moreno et al (2017) voiced the concern that only people of reproductive age are able to donate. To overcome this, uptake in donation must be adequate to meet the demand required for transplantation. This would be maximised by making the donors the centre of the process, designing the process and equipment required to be intuitive, easy, painless, quick, and preferably an overall positive experience.

#### *Contamination*

Another concern is that of contamination during the collection process (Ren et al 2018; Rodrigues et al 2016). As a defence mechanism against invasion and the proliferation of microbial pathogens in the genital tract, the vaginal microflora is vital for reproductive health (Linhares et al 2010). However, this microflora can contaminate the menstrual blood sample. Even though these research groups identify this concern in their work, the frequencies of contamination of menstrual blood samples rarely been reported. Van Phuc et al (2011), Vu et al (2015), Liu et al (2018) among others report that the menstrual blood samples collected in their study are tested for bacterial and fungal contamination and discarded if positive, but do not report whether this happened, or the frequency of this issue. Ren et al (2018) commented that MenSC were prone to microbial contamination during the collection process, but did not publish any relating data. Ma et al did report that a quarter of the samples collected had microbial contamination, and so these participants were asked to donate again (2020a). The literature rarely contains any indication of the number of successful samples over unsuccessful, whether that be due to contamination, or otherwise degraded samples or samples absent of MenSCs; Fiorelli-Arazawa et al report approximately 70% of the samples are not contaminated (2019). Future studies should report the occurrence of sample contamination in order to identify the prevalence of this contamination in reality, as it could be a major obstacle for MenSC therapy.

Antimicrobials are used upon sample collection and throughout MenSC culture to reduce contamination (Ma et al 2020a; van Phuc et al 2022; Zemelko et al 2012; Zucherato et al 2021). However, the addition of antibiotics adversely affected MSC physiology (Pountos et al 2014), growth within 24 hours (Skubis et al 2017; Turani et al 2015), increasing population doubling time by almost half (Cohen et al 2006), although this effect may be mitigated by type or combination of antibiotic and potentially source of MSC (Pountos et al 2014). Antifungals have been found to also negatively impact cell viability after 24-72 hours, but a less toxic form (AmB-$Cu^{2+}$) resulted in a

higher viability after 48 and 72 hours (Skubis et al 2017). Classic MSC marker expression was slightly higher after antibiotic treatment (ibid), and antibiotics may inhibit stem cell differentiation (zur Nieden et al 2004). Antimicrobials are necessary for MenSC collection and culture, and future research should identify optimum timeframes for exposure to antimicrobials, minimising contamination and adverse effect on MenSC.

#### *Endometriosis*

Endometriosis is a condition where endometrial tissue is found outside of the endometrium, continuing endometrium functions, leading to the tissue thickening, breaking down, and bleeding with each menstrual cycle. This has been reported around the body including in the vulvar (Yordanov et al 2020), sciatic nerve (Saar et al 2018), bowel (Parag et al 2021), umbilical cord (Zebbakh et al 2023), and rectum (Shi et al 2021). This causes a range in symptoms including pain and fertility complications (NICE 2017). The cause for endometriosis, a disease mainly affected by menstruators of reproductive age, has been debated. Possible causes include bacterial contamination (Khan et al 2018), changes in mesodermal-lined cavities (NICE 2017), or retrograded endometrial fragments containing stem/progenitor cells implanting in the pelvic cavity (Cousins & Gargett 2018; Ma et al 2020b; NICE 2017; Szukiewicz et al 2021). Endometriosis has also been present or connected to scarring: including surgery for ectopic pregnancy (Kotdawala et al 2021); by surgery for anal fissure during menstruation (Koca & Yildirim 2021), and caesarean surgery (Masereka et al 2022).

Some researchers voice their concern of MenSC transplantation causing endometriosis in the recipient (Figueira et al 2011; Verdi et al 2014). None of the studies transplanting MenSC report this, and with the exact cause of endometriosis being unknown, further research and improved follow-up reports must be done to quell this concern.

### 2.7.5 Donors

Understandably, research on MenSC and the potential future therapies derived from this source of cells has maintained an emphasis on the cell characteristics, processing methods, potential treatment, and general outputs of MenSC. Very little attention has been paid to those who would be donating MenSCs. Without an understanding of the limitations set out by the donor, researching anything else is futile, as there would be no menstrual blood to derive MenSC from. This review will focus on the available information on the donors of MenSC. The effects of age, health, and other donor characteristics on MenSC will be reviewed.

#### *Age*

Research groups have sometimes focussed their efforts on recruiting ‘young’ female donors for menstrual blood samples, which is defined as between 25-35 years old (Li et al 2012a), 20-35 years old (Martínez-Aguilar et al 2020), 20-45 (Guo et al 2019b), or sometimes not defined at all

(de Carvalho Rodrigues et al 2012). This methodology is not well justified, as there is limited research in this area, particularly before 2015. Zemelko et al state that they believe MenSC population doublings depends on donor age without evidencing this (2012). Studies identified samples from younger donors as more proliferative than those from older donors, such as the findings from Mehrabani et al (2016); the study comparing the doubling times of MenSC from 30-40 year olds and 40-50 year olds reported that with an increase in age comes an increase in doubling time. However, only five people were recruited in each group, so this is not an adequate sample size to glean effects of age on proliferation. Liu et al recruited more participants, with three age groups containing six donors (2018). They reported that the proliferation between each age group was only slightly different, with older donors having a slightly lower proliferative capacity, but this was not tested for statistical significance. In contrast, Chen et al reported MenSC population doubling trends to be basically comparable across the three age groups, again comparing samples from 18 participants across three age groups. Interestingly, they found the colony-forming rates of MenSC to be higher than MSC derived from bone marrow, regardless of donor age (2015a).

Further research must assess age with proliferative capacity of MenSC, using sample sizes suitable for testing significance. However, perhaps the reasoning for utilising younger participants should not come from attaining samples with high proliferation, but rather from the attitudes to donating menstrual blood generally. Younger people (aged 19-30) may have greater feelings of positivity regarding menstruation compared with 31-40 year olds, or 41-55 year olds, with 50% expressing extremely positive feelings compared to 30% and 20% respectively (Lee 2002). Feeling more positive about the subject, this age group may be more willing to donate menstrual blood. This topic is discussed in greater depth in Chapter 3.

### *Health*

In almost all cases, only those reported as 'healthy' were recruited for menstrual blood donation for MenSC research. In most cases the description is left at just that (Chen et al 2020a; Chen et al 2020b; Gonçalves et al 2020; Sun et al 2019a; Vu et al 2015). However, due to the intrinsic links with reproductive health, sometime research groups reported a more rigorous definition of 'healthy' to include qualities such as having regular menstrual cycles (Arasteh et al 2018; Hu et al 2019; Nikoo et al 2014; van der Molen et al 2013; Yan et al 2019), specifying the cycle length (Hu et al 2019), having a history of pregnancy and term delivery (Rajabi et al 2018; Zheng et al 2018) or having no history of pregnancy (van der Molen et al 2013),[*] having a normal menstrual blood loss volume (Zheng et al 2018), not receiving hormone therapy (Blázquez et al 2018; Nikoo et al

[*] Wyatt et al (2021) received total 39 menstrual blood samples from 11 women; six were described as fertile with parity =>1, five had unknown fertility with parity = 0, three experiencing heavy menstrual bleeding, either diagnosed or self-reported, and one with adenomyosis. MenSC were collected from all.

2014; van der Molen et al 2013; Wyatt et al 2021), showing no sign of vaginal infection or discharge (Aleahmad et al 2018; Kazemnejad et al 2012; Kazemnejad et al 2014; Khanmohammadi et al 2012; Ren et al 2016), not having a hormone-dependent or reproductive or gynaecological disease of any kind (Mehrabani et al 2016; Rajabi et al 2018; Wang et al 2019; Zhang et al 2018; Zhang et al 2019), and specifically not having endometriosis (Aleahmad et al 2018; Shokri et al 2019; Zheng et al 2018). Interestingly, van der Molen et al also reported non-pregnancy in the participants (2013). This is pertinent as people can still present bleeds during pregnancy.

In most cases, preventing the donation from those with health conditions avoids pathologies being transferred to the recipients of the transplantation, and also reduces the risk for those processing the menstrual blood samples. In the case of menstrual cycle regularity and 'normality', this not only suggests general reproductive health, but will also help improving the predictability of bleeds, so that donors and researchers can best plan the donation timing. Endometriosis was found to affect MenSC (de Oliveira et al 2022; Gargett et al 2015) with differing gene expression compared to healthy MenSC (Cressoni et al 2023), so screening against this could improve the quality of MenSC collected. This is reflected by Zemelko et al, suggesting that donor health, specifically endometrium state, could have an impact on MenSC population doublings. However, this is not evidenced (2012). Claims like these must be explored and proved in future studies.

Looking at the effects of respiratory health on MenSC, Musina et al reported that in some cases they failed to isolate MenSC from women with respiratory disease (2008). This highlights the need to identify all of the influences on MenSC quality, and that healthy donors must be recruited for MenSC collection when those with underlying disease or injury an unable to donate themselves. Zheng et al state that patients with severe intrauterine adhesions were less potent than women with a normal uterine cavity (2018), which links to the autologous transplantations by Tan et al, requiring the pooling of some patients' menstrual blood as they did not produce enough MenSC in one sample (2016), as previously discussed. Esmaeilzadeh et al collected menstrual blood from five patients experiencing recurrent implantation failure and three patients not experiencing recurrent implantation failure, not to explore treatment options in animal or human model, but to understand how receptivity markers differ among the two groups (2020). This study had one of the most rigorous and specific health criteria seen involving the study of MenSC.[*]

[*] Patients from both groups had a normal body mass index (BMI), were younger than 40 years old, and had regular menstrual cycles. Patients with recurrent implantation failure had at least three miscarriages after they received infertility treatment. Patients not experiencing recurrent implantation failure experienced fewer than three miscarriages and at least one live birth after receiving infertility treatment. Exclusion criteria for both groups included irregular menstrual cycles, and any endometrial or uterine abnormality such as endometriosis, endometrial polyps and hyperplasia, fibroids, endometriosis, and adenomyosis. Patients were also excluded for smoking and alcohol consumption, or any finding or health issue that may explain the cause of recurrent miscarriage.

Although many research groups assume donors with good reproductive health make the ideal donor, Sheikholeslami et al found that those with polycystic ovary syndrome and endometriosis were equally able to donate MenSC as healthy donors, with the MenSC differentiation potential similar to that of MenSCs taken from healthy donors, and even more promising in terms of the expression of some genes (2021). However, MenSC collected from healthy donors and patients experiencing infertility with unknown cause exhibited slightly differently pronounced surface markers and protein levels during decidualisation (Skliutė et al 2021), and therefore should be exclusion criteria for future clinical application.

Nikoo et al reported collecting menstrual blood from donors who had experienced pregnancy and live birth as well as those who had not, but unfortunately did not report whether this parameter had any impact on MenSC quality (2012). However, more recent research compared MenSC between nulliparous, multiparous, and preeclamptic donors. Migratory behaviour and proliferation rate were similar across these groups (Peñailillo et al 2022). Again, experiencing pregnancy and successful live births suggests good reproductive health, but this can be explored further to see if one of these demographics can be targeted if resulting in preferential characteristics.

In 2018, 29.2% of 15-49 year old menstruators in the UK were receiving a prescription for any contraceptive. 6.5% were using long-acting reversible contraception (such as intrauterine systems (IUS), intrauterine devices (IUD), injection, implant), 10.8% used the progesterone-only pill, and 14.3% used combined hormonal contraceptives (Pasvol et al 2022). Continuous use of combined hormonal contraceptives and the cessation of the natural menstrual cycle due to contraception results in an 'inactive' or noncycling endometrium (Hee et al 2013). There is limited research in this area, but it might be theorised that an inactive endometrium therefore does not contain MenSC, as it does not need to undergo natural building and shedding of the endometrium. However, Schwab et al collected endometrial tissue from 26 patients undergoing a hysterectomy for non-endometrial pathologies. Four of these patients were on a form of contraceptive pill (2 on combined, and 2 on progesterone-only contraceptive pill), and they were found to have similar levels of clonogenic stromal cells between the proliferative and secretory stages of the cycling endometrium. Schwab et al suggested the MenSC population maintains its proliferative potential in inactive endometrium (2005). Although this study involved hysterectomy, rather than the collection of menstrual blood, future studies can verify that even during the 'bleeds' that people undergo whilst on a contraceptive pill, while they are not true menstrual cycles, still contain MenSC. MenSC were successfully isolated from donors with and without hormonal contraceptive (Fiorelli-Arazawa et al 2019). This is promising for MenSC therapy, and future studies should verify whether MenSC stemness is maintained in noncycling endometrium.

Where taking contraceptive may or may not have an impact on the quality of MenSC in the endometrium and released during menstruation, it could be the case that those on the combined oral contraceptive pill bleed smaller volumes of menstrual blood. Comparing those taking this contraceptive pill long-term (longer than ten years) and short term (shorter than ten years), long-term users were significantly more likely to have a thinner endometrium (<7mm). (Talukdar et al 2012). Where this had no impact on birth rates, it could have an impact on the volume of menstrual blood expelled, and therefore the ease at which a donor on the combined contraceptive pill could donate an adequate quantity of menstrual blood for MenSC treatment

Tan et al have been the most rigorous in their description of menstrual blood donors, highlighting the age of donor and amount of menstrual blood collected in each instance (2016). However, this is due to the autologous nature of the transplantations, and therefore donor information was also recipient information. Fiorelli-Arazawa et al reported participant birth history, BMI, and contraceptive type. These were not found to affect MenSC (2019). Future studies should report basic information on donors, with the onus on researchers then being able to identify potential limitations to menstrual blood donation, such as sample contamination rates, volume of blood donated, number of donors willing to donate multiple samples, and general menstrual blood donation experience.

## 2.8 Donation process

As Liu et al state, there is a lack of standard protocols for MenSC collection and production (2019b). This makes it difficult to evaluate effectiveness and isolation success. The devices and procedures used to collect menstrual blood for MenSC extraction must be compared. However, difficulty arises when research groups do not report the methods of collecting menstrual blood (Fan et al 2022; Hu et al 2022; Jiang & Wang 2012; Luz-Crawford et al 2016; Sun et al 2019b), describe the methods as briefly as "from menstrual blood of healthy volunteers at the peak of flow" within the supplemental material (Lopez-Caraballo et al 2020), or purchase or receive MenSC lines externally and therefore do not describe the donation protocol (Chen et al 2015a; Fu et al 2022; Hao et al 2022; Hu et al 2014; Mo et al 2022;[*] Ren et al 2018; Sun et al 2022a; Wang et al 2017c; Wang et al 2020; Zhang et al 2022c). On a similar note, some research groups cite the methods of another group that also do not fully disclose their menstrual blood collection methods (Barlabé et al 2020; Fard et al 2017), or cite two research groups with differing methods (Blázquez et al 2018) making it impossible to distinguish the protocol at times. This is an example of the donors volunteering their time and not being the centre of research. Understanding the current menstrual blood donation procedures will help identify where improvements can be made and the donation experience enhanced. This will increase recruitment rates, reduce withdrawal rates,

[*] Mo et al 2022 translated from Chinese on Google

and therefore positively impact MenSC research and treatment. Because many research groups do not identify valuable information on menstrual blood collection, including the devices involved, donation time frames, blood storage time and temperature, etc., only studies that have disclosed two or more components to the donation process has been included in analysis. Menstrual blood collected during a hysterectomy or any other form of surgery has not been included. Biopsies have also not been included as it is invasive and requires trained personnel to undertake the procedure. All other non-surgical, less technical methods have been included in analysis.

### 2.8.1 Donation device

The most common device utilised for menstrual blood collection was a menstrual cup (Aleahmad et al 2018; Borlongan et al 2010; Chen et al 2015b). This is a silicon, thermoplastic elastomer (TPE), or rubber cup-shaped device that sits in the vagina, much like a tampon. It collects blood, rather than absorbs it, as it forms a seal against the vaginal wall. Menstrual cups can be removed and emptied up to every 12 hours.

The brands of menstrual cups are not always reported, but when they are specified, they included Mialuna cup (Alcayaga-Miranda et al 2015b; Cuenca et al 2018), Maggacup (Alfano et al 2017), Mooncup (Moreno et al 2017), Lady cup (Yamchi et al 2021), Lunette (Wyatt et al 2021), with the most common being Diva cup (Chen et al 2020c; Patel et al 2008; Rahimi et al 2018; Sun et al 2019a). A tampon has been reported to collect MenSC with no description of the extraction process and no further evidence of this, suggesting it is possible to extract MenSC from more popular menstrual hygiene products (Martínez-Aguilar et al 2020). Other less conventional donation devices include a urine cup (Chen et al 2016; Dalirfardouei et al 2018; Dalirfardouei et al 2019; Mahdipour 2022; Meng at al 2007), urine cup-tubing (Sun et al 2016), a catheter (Sahraei et al 2022; Sheikholeslami et al 2021; Tan et al 2016), falcon tubes (Karadas et al 2014), a "proper collector pot" (Gonçalves et al 2020, p. 770), "sterile collection containers" (Arezoo et al 2021, p. 476) and a 20 mL injection syringe (Wang et al 2019; Zhang et al 2018).[*] It is assumed that these unconventional[†] collection methods, particularly catheters, falcon tubes, and syringes, were undertaken by trained practitioners, and that the donors using urine cups were able to undertake the straightforward donation in the privacy of a bathroom, as this is not reported. Menstrual cups are conventional products that many people currently use, and do not require trained

[*] There are some concerns with the confusing use of the terminology "urine cup". In one case, a "urine cup" is listed in the collection methods, with a menstrual cup cited (Bozorgmehr et al 2014). In other cases, the use of a urine cup is reported (Meng at al 2007), and research groups citing this method then use a menstrual cup (Chen et al 2017b; Zhao et al 2018b). This highlights either the incorrect terminology, inaccurate translation of menstrual cups, or false citations. Future studies must define the methods fully to prevent future confusion. Similarly, what is a proper collector pot? This is perhaps an issue with translation.

[†] Unconventional being what people are not likely to be familiar using (compared to e.g. sanitary pads and tampons)

professionals to insert or remove, although it has been the case that a clinician inserts and removes a menstrual cup for the collection of MenSC (Ma et al 2020a; Zafardoust et al 2020). The same has been undertaken for private menstrual blood banking, (Cryo-Cell 2020), although this is no longer available. This clinician control can be beneficial for first time users, as it can be ensured that the menstrual cup is placed correctly. The donation process can be undertaken in a controlled, aseptic condition as possible. However, it does mean that control is taken away from donors, it is not private, and for those already able to use a menstrual cup, may be seen as an unnecessary, invasive step. It also requires specialist training, and the necessary clinic. It is sometimes reported that the donors themselves insert and remove the menstrual cup (Kazemnejad et al 2012; Kazemnejad et al 2013b; Nikoo et al 2014; Zhong et al 2009), or that donors inserted the menstrual cup (Vu et al 2015). Because the donor is never at the centre of these studies, it is unreported whether donors prefer being in control of the donation process, whether it is easy for the research group to manage, and whether relevant hygiene levels are maintained for samples to remain uncontaminated. As discussed in 2.7.4, unsuccessful donation procedures relating to methods have rarely been reported.

### 2.8.2 Time between donation and processing

The time from sample donation to processing varies by research group. In some cases, a menstrual blood sample is “immediately” processed (Bozorgmehr et al 2014; Nikoo et al 2014; Sheikholeslami et al 2021) or “quickly” transfer the sample to the laboratory for processing (van Phuc et al 2011; Vu et al 2015). However, the time menstrual blood is held in storage before processing varies from within two hours (Wyatt et al 2021), between 2-4 hours (Zemelko et al 2013), within 10-12 hours (Dalirfardouei et al 2018; Dalirfardouei et al 2019), within 24 hours (Allickson et al 2011; Du et al 2016; Ren et al 2016; Shan et al 2017), 24-48 hours (Patel et al 2008; Rossignoli et al 2013; Sun et al 2019a), within 48 hours (Chen et al 2020b), and up to 72 hours (Du et al 2018). The temperature of this storage varies as well. Often, a quick transfer is simply done on ice (Dalirfardouei et al 2019; van Phuc et al 2011; Vu et al 2015), kept in a cold chain (Shokri et al 2019), or kept at room temperature (van der Molen et al 2013). The samples have been successfully stored at a temperature ranging 1-10 °C (Allickson et al 2011), but the majority of research groups store the menstrual blood samples at 4 °C (Borlongan et al 2010; Chen et al 2020b; Kovina et al 2018; Liu et al 2018; Zemelko et al 2012).

With the disparity in sample transfer and storage methods, little research has focussed on optimum or most accessible procedure. Liu et al split menstrual samples into four, and stored these samples at 4 °C for 6, 24, 48, and 72 hours after donation. They reported finding no significant differences among the MenSCs isolated after these different storage timings (2018). Therefore, menstrual blood can be kept at 4 °C for at least 3 days before being processed further,

meaning processing need not be rushed, and also that donors can donate at a time suitable for them without negatively affecting the quality of MenSC.

### 2.8.3 Day of donation, and menstruation flow

Where no other information is available, the "heaviest" flow is reported to be the ideal for menstrual blood donation (Allickson et al 2011). However, "heaviest" flow is referred as the first three days of menstrual flow (Arasteh et al 2018; Kazemnejad et al 2013a). Other research groups identify menstrual blood collection days to be the first three days of menstrual flow (Borlongan et al 2010; Moreno et al 2017; van der Molen et al 2013) or simply "the first few days" of menstruation (Azedi et al 2014; Chen et al 2016; Cuenca et al 2018; Patel et al 2008; Rossignoli et al 2013). Wyatt et al asked donors to donate on the self-reported heaviest day of menstrual bleeding, which resulted in one sample on day 1, 36 samples on day 2, and two samples on day 3 (2021). It is good to give participants the freedom and responsibility to know when donation would work most suitably for them and ultimately results in the greatest volume of menstrual blood.

Although the volume of menstrual blood collected was not reported, Zhong et al were able to isolate enough MenSC from menstrual blood samples after a menstrual cup wear time between 30-60 minutes (2009). In other studies, menstrual cups were worn for 2-3 hours (van Phuc et al 2011; Vu et al 2015), less than 4 hours (Allickson et al 2011; Borlongan et al 2010), 4-6 hours (Wyatt et al 2021) or even as high as 10 (Warren et al 2018) or 12 hours (van der Molen et al 2013) or up to overnight (Arasteh et al 2018; Kazemnejad et al 2013a) in order to collect enough menstrual blood. Importantly, Kovina et al found there to be no decrease in MenSC yield in menstrual blood sample collected every day from the first to fifth day of menstruation (2018). This shows that so long as the volume of menstrual blood is adequate to extract MenSC, donors should be able to donate when is suitable for them, not being limited by menstrual cup wear time, or rate of flow.

As Chapter 2 shows, there is limited consistency in reported menstrual blood collection and storage methods, displayed by the quantity of empty sections of Appendix C. Wyatt et al were the only group to report information across each column (2021). This is not to criticise the research groups undertaking this research. It highlights that the emphasis is currently not on the donors of menstrual blood, but the outcomes of the MenSC collection: potential treatment, isolation methods, and MenSC characteristics.

On top of participant information and collection methods being reported inconsistently, factors including total menstrual fluid blood volume, pH, flow rate, and viscosity have never been reported, so there is no understanding of how these factors impact MenSC yield or viability.

Menstrual blood pH varies greatly, and a participant's flow rate also affects pH (Reame 1983), which presumably affects MenSC.

Within MenSC research, menstrual blood sample pH has never been reported, and the effect of menstrual blood pH or culture pH on MenSC have never been studied. MSC viability decreases with increased acidity; a pH of 6.8 was a threshold under which human adipose-derived MSC apoptosis and necrosis increased significantly (Li et al 2012b); seeing as menstrual blood pH can be as low as 4.7 (Reame 1983), this likely greatly impacts MenSC survival and viability. Murine embryonic stem cells were cultured in media with pH ranging from 6.7 to 7.9 and growth rate declined significantly beyond pH 7.75 and below 6.70. Promisingly, these cells were exposed to the altered pH media for 24 or 48 hours, after which remaining cells were returned to pH 7.3 medium and the growth rate (and differentiation potential) were restored (Chaudhry et al 2009).

On a similar note, menstrual blood contains blood, mucous, and uterine lining, with blood content varying between participants (Reid 2006), which would presumably impact MenSC yield. Menstrual blood samples have been occasionally strained of lining or mucous (Aleahmad et al 2018) without reporting whether this has impacted MenSC yield or viability. Blood, mucous, and uterine lining content would also affect the viscosity of each menstrual blood sample, which could affect the ease of handling the sample for untrained donors and laboratory staff, as well as lining, mucous, and cell aggregates impacting mass transfer (Antoni et al 2015). Again, these factors have never been reported or studied to understand the impact of menstrual blood content or viscosity on MenSC yield or viability.

In summary, factors such as participant age, menstrual cycle length, reproductive health, menstrual blood properties including day or flow of donation, sample pH and viscosity, and donation device and process must be a) reported in full and b) measured to understand how these factors affect MenSC. Furthermore, participants are rarely asked of their experiences and attitudes towards menstrual blood donation, and certainly in-depth, qualitative data has not been collected in this regard. This thesis argues for the need for donors to become the centre of attention. In Chapter 2, the characteristics and clinical potential of MenSC has been identified, but without willing donors and an understanding of the attitudes towards menstrual blood donation, and without an acceptable donation method, there will not be adequate numbers of donors and therefore MenSC. An in-depth understanding of potential donor's attitudes, concerns, and requirements will confirm that there is a potential for MenSC donation from the donor's point of view, as well as outline their needs and expectations for future application.

# Chapter 3. Attitude to menstrual blood donation

Early work by the author identified that when asked in a survey, 78% those that menstruate would be willing to donate MenSC (Manley et al 2019). There is currently little understanding of the potential uptake of menstrual blood donation for MenSC treatment, particularly qualitatively. While quantitative data on this topic has been published (ibid), the qualitative data collected concurrently was not analysed and reported in the detail is deserves and requires. This chapter outlines the known attitudes to menstruation in general and the potential for MenSC donation, in response to **research question 2: Do people even want to donate MenSC?** Responses revealed themes such as altruism, giving a 'waste' product a purpose, and menstrual blood donation being less invasive than other donations. Respondents are concerned about the practical factors for donation including ease, hygiene, convenience, and concerns with their unknown menstrual cup use. This work confirms reasons to neutralise and even celebrate menstruation, and provides strong evidence for public support of MenSC donation.

### 3.1.1 Attitude to menstruation

Attitudes to menstruation have often been reported as across a broad spectrum (Lee 2002), from beneficial to someone's sense of overall well-being at best (Skultans 1988), to a "disgusting mess" at worst (Martin 2001, p. 103). At times it has been found to be slightly more positive than negative (Lee 2002), with people stating menstruation indicates body normality, and evidence of not being pregnant (Fahs 2020). This being said, those taking contraception that reduced the volume of menstrual blood loss saw this side-effect as positive rather than negative (Schmidt 1966).

Larki et al found that people who experienced pain and cramping during menstruation (dysmenorrhea) were more likely to rate menstruation as more debilitating, bothersome, ad predictable, and higher perceived menstrual blood loss volume related to rating menstruation as natural, bothersome, and anticipatory (2022). Those with disabilities experience even greater disadvantages associated with menstruation (Phillips-Howard 2022). This could be due to a group already marginalised feeling the effects of a taboo, sexist issue as heightened.

Interestingly, adolescents with chronic conditions had significantly better attitudes towards menstruation than peers without chronic conditions (Serret-Montaya et al 2020). Perhaps menstruation is a signifier of health and reproductive health, and is therefore appreciated by those with chronic conditions.

However, literature generally agrees that menstruation is "our "dirty little secret"" (Kissling 2006, p. 9), associating menstruation with fear, embarrassment, and pain (Fahs 2020). This could be due

to menstrual blood itself being seen an intrinsically disgusting or harmful, however the consensus is that these negative experiences are worsened by the menstrual taboo.

### 3.1.2 Menstrual taboo

There maintains the general consensus that the experience of menstruation is shaped and limited by the menstrual taboo (Delaney et al 1988; Johnston-Robeldo and Stubbs 2013; Kissling 2006; Laws 1993). People admit to changing their clothing and behaviour to hide menstruating (Kolić et al 2022), those in sport are affected in terms of performance as well as abuse after a bad result when menstruating (McColgan 2022).

Many studies have identified the impact of education on menstrual perception, experience, and safety, among other factors. In China, it was found that a series of educational workshops improved adolescents' knowledge, confidence, and behaviours surrounding menstruation (Su & Lindell 2016). Of 1824 surveyed people, mostly from Hawaii, those with a higher level of education were more likely to have a positive perception of menstruation (Morrison et al 2010). It was identified that public ignorance regarding vaginal health fed into the surrounding taboos, which in turn contributed to individual ignorance (Nappi et al 2006), and this suggests that improved knowledge surrounding menstruation, and improved education, will result in a reduction in the menstrual taboo, potentially improving attitudes towards menstruation in general. Researchers tend to agree that an improvement in education will help combat the menstrual taboo (Arney 2017; Bhartiya 2013; Kapoor & Khari 2016; Lamborn 2017). Negative discourse surrounding menstruation is still very much part of day-to-day life, including with the internet and social media. Approximately 70% instagram meme posts analysed perpetuated menstrual taboo, often through humour. This included continuing to conceal menstruation, being emotionally unstable during menstruation, and pitting men against women. Only 3% menstrual memes surrounded solidarity during menstruation (Tomlinson 2021).

Feminists have also been challenging the menstrual taboo because the control of fertility and reproductive capacities is "essential to women's liberation" (Jackson 1993, p. 365). Houppert et al noted the benefits arising from the feminist movement, allowing women to think differently about their bodies (1999). However, some feminists go a step further. Describing themselves as third wave feminism menstrual activists, Kissling discusses "Celebrate-Your-Cycle feminists" (2006, p. 214) who embrace their relationship with nature. They see the similarities between the cyclicality of menstruation and that of nature, such as the seasons, tides, and lunar activity. This celebration is marked with returning menstrual blood to the earth, "or at least [watering] our houseplants with it" (ibid). Menstrual activists begin to overturn the menstrual taboo by boycotting disposable menstrual products, buying or making reusable products, and free bleeding (Fahs 2016; Hunter 2016; Vostral 2008). Interestingly, those with a negative attitude to

menstruation have been reported to feel more ambivalent about feminism (Lee 2002). Perhaps realising the sexist nature of the menstrual taboo, and its effects on menstrual experience, will draw people to feminism and overcoming the menstrual taboo together. Or perhaps some find menstrual blood intrinsically disgusting, and therefore any flamboyant display of menstrual activism is a step too far.

Some researchers have discussed a potential technique to overcome the self-objectification that has limited people's experience of menstruation (focussing on relation to reusable menstrual hygiene products) by concealing and hiding menstruation more effectively (Lamont et al 2019). However, because the language and concealment of menstruation is shaped by, and shapes, the menstrual taboo (Newton 2016), the decisions made, from the language chosen to deciding to hide menstruation completely, impacts this taboo. Therefore, it should be spoken of openly if not positively.

### 3.1.3 Attitude to menstrual blood donation

At present, very little is known regarding the attitudes to potentially donating MenSC or receiving MenSC treatment. Quantitative responses in this area have shown that up to 78% menstruating respondents are willing to donate their MenSC, and 91% of respondents would accept MenSC-derived treatment (Manley et al 2019).

Other literature has explored the perception and understanding of MenSC banking, although banking is merely an element of tissue or cell donation and transplantation. Private and public tissue banks are slightly dissimilar. Private banking is the processing and storage of tissues or cells for future regenerative therapies for oneself or a family member, with upfront and annual storage fees. Public banking is the processing and storage of tissues or cells that are donated for therapeutic purposes for anyone, or for research, with no cost to the donor. There is a more positive attitude toward public umbilical cord banks compared to private umbilical banks in pregnant people in France, Germany, Italy, Spain, and the UK; 76% choosing to donate to a public bank compared to 12% choosing a private bank (Katz et al 2011). In Switzerland, nearly 70% of umbilical cord blood donors opted for public banking due to altruism and the high costs of private banking (Manegold et al 2011). This perception that public banking is more charitable than private banking may influence general attitudes toward banking, including banking MenSC.

In a study by Hans and Kaur among nursing students in India, knowledge of MenSC banking was assessed and knowledge was shown to improve after four days' structured teaching on the subject, but the survey did not explore the perception of the banking, MenSC donation, or treatment (2016). Among health care professionals, another survey in India explored the

knowledge and perception of MB banking. 66% had an unfavourable attitude toward MenSC banking (Jomon et al 2019).

The attitudes to menstrual cups have never been explored in connection with donating MenSC, and MenSC may have implications for menstrual cup use or vice versa. Exploring attitudes to menstrual cups in the context of donating MenSC is also valuable as people may elect to only use a menstrual cup to donate MenSC, rather than in their normal monthly routine.

This early research shows that it is important to stimulate conversation and explore these attitudes. Again, qualitative exploration of these attitudes would add depth to these findings, as it is unclear whether participants were opposed to MenSC banking due to the menstrual taboo, the opposition of the use of private banks, or for any other reason. Qualitative research into attitudes and opinions would highlight this, as well as other trepidation, concern, and celebration surrounding MenSC.

### 3.1.4 Questionnaire: exploring attitudes to menstrual blood donation

Qualitative research will enrich the limited quantitative research on the topics of MenSC and MenSC donation using a menstrual cup (Manley et al 2019) by providing insight into attitudes to donation, including any concerns or worries that may need to be addressed. As such, this work aims to understand attitudes to MenSC, donating MenSC and using a menstrual cup to do so, and potentially receiving MenSC therapy. This will give voice to the ideas, misgivings, incentives, and needs, informing the future of MenSC donation and treatment.

### 3.1.5 Methods

A questionnaire was distributed to participants, recruited from a variety of locations within a multicultural city in the Midlands, UK, to fill in a questionnaire without incentive. The researcher visited local business offices, universities, libraries, and leisure centres with physical questionnaires, asking whether people would be willing to participate in an investigation about donating MenSC. Willing participants could either fill in a questionnaire immediately, or take the questionnaire and return it in an envelope to the researcher by hand, allowing for the completion in the comfort and security of their own home if desired.

Qualitative questions were phrased as a follow-up to quantitative questions, for example: (1) Had you heard before today that menstrual blood contains stem cells? What thoughts come to mind? (2) Would you donate your menstrual blood? Please explain your answer. (3) Would you donate your menstrual blood with a menstrual cup, or try using a menstrual cup for the first time in order to donate your menstrual blood? Please explain your answer. (4) Would you receive cell therapy from a menstrual blood sample if you needed the treatment? Please explain your answer.

Phase 1 of the study involved the statistical analysis of quantitative data derived from the questionnaire (Manley et al 2019). Phase 2 is reported here, presenting an analysis of the qualitative data from the open questions of the same questionnaire. This study was approved by the Nottingham Trent University Ethics Clearance Sub-Committee (21017-496205).

### *Analysis*

Data was anonymized before analysis. For qualitative data, realist reflexive thematic analysis was used (Braun & Clarke 2006; Braun & Clarke 2019a; Terry et al 2017). It is recognized that theory and knowledge is historically and culturally situated, i.e. in a culture limited by the menstrual taboo, so this approach is driven by perspectives and experiences. This allows researchers to tell the people's stories, data being 'interpreted' rather than 'discovered'. Reflexive thematic analysis has been used in other health research settings (Braun & Clarke 2014), and thematically-analysed questionnaires have been used for studying other intimate, feminist topics including pubic hair (Braun et al 2013), and clothing and body image (Frith & Gleeson 2008).

The data was coded inductively with QSR Nvivo 12.2 Pro, allowing themes and patterns to be extracted. Researchers explored and familiarized themselves with the data, then identified codes together. Codes revealed general themes and subthemes, which were mapped, revised, and refined. Two researchers met on three occasions: first to initially code some responses together and map the initial themes before working independently; second at the midway point to re-establish and revise the possible themes before continuing to work independently; and finally to go through each theme and subtheme to refine and define each theme together. Researchers agreed on all codes and themes, although nomenclature had to be agreed upon.

Inductive thematic analysis was undertaken as it was the first study to understand this topic qualitatively, so content, rather than existing ideas, drove the analysis (Braun & Clarke 2006; Gavin 2008). When coding, researchers attempted to mitigate personal experiences from influencing the analysis. This is described as researchers 'owning their perspectives' (Braun & Clarke 2019b). To briefly contextualize this: Researcher A had researched MenSC previously, whereas Researcher B had not heard of MenSC before engaging with this research. Both Researchers had heard of menstrual cups before. Researcher A had mixed experiences with a menstrual cup (mostly positive before a bad experience discontinued use), and three friends with mostly positive experiences. Researcher B had multiple friends and close family with positive experiences of using menstrual cups but never used one personally. Researcher A had donated blood before, neither had donated stem cells, and both had a positive attitude of and would be willing to donate MenSC. By voicing these opinions and 'owning their perspectives', the Researchers maintained this critical discussion of their personal and social standpoint and positioning during analysis, allowing the voices of the participants to remain in focus.

### 3.1.6 Analysis and Discussion

Several themes and subthemes were drawn out through the analysis. Helping others, increasing research, menstrual blood being a waste blood, and menstrual blood becoming useful were motivations to donate. The topic was described as interesting. Some people also agreed that menstrual blood has untapped potential, would be less invasive than donating blood, and thought group encouragement and support would be ideal. Reactions were also of disgust. Many respondents directly or indirectly spoke of overcoming the menstrual taboo; neutralizing and stating the source of treatment does not matter, that it is 'natural', or to celebrate menstruation to overcome the taboo. When discussing the donation, respondents would donate on the condition of it helping others, it being easy, and hygienic. Some found using a menstrual cup to be easy, already used it, or willing to try again. Others thought the menstrual cup to be painful or less comfortable, unhygienic, or had concerns with confidence. The themes and subthemes are discussed below. These are not presented per question because responses often spread across multiple themes and were occasionally mirrored or repeated. There are some noteworthy findings here that can inform the future of MenSC collection practices.

#### *Benefits of MenSC*

Respondents found MenSC to be beneficial for several reasons, including helping those in need using a non-invasive procedure that can make use of a 'waste' blood.

#### *Helping others*

A strong theme regarding MenSC research and treatment was the notion of being able to help someone through MenSC donation; "Helping others", as well as comments such as: "I love it. Fantastic to think the blood I have can be used to save people's lives". Increasing the rate of research and advancement in this therapy was also seen as a way to help: "Any medical progress is positive and I'd be happy to be involved".

Altruism was always in relation to the betterment of members of public in need. When asked broadly about MenSC and whether they would donate, no participant expressed a selfish or personal requirement for treatment, or mentioning a friend or family member that would benefit from this. The benefits of MenSC donation were always in the context of broader society, with this reflected in other areas.

#### *Group encouragement*

Respondents made comments of encouragement to take part: "I would encourage women to get involved"; "If it helps someone in need or to make medical advances then it should be encouraged". This could perhaps provide a feeling of togetherness or making progress together. It was also found that people might find it easier to partake in MenSC donation in groups, needing an element of support from a friend or acquaintance: "I guess I would have to see or know

someone that has done it first". A small study comparing levels of support in first-time menstrual cups used found that increased support and advice could improve users' experience (Manley 2018). Jackson and Falmagne (2013) found that women found safe spaces to discuss menstruation comfortably and openly, and it would benefit both women and the cause to have a MenSC donation platform that provides a level of group encouragement and support.

### *Untapped potential*

Another benefit of donating MenSC included the increased accessibility and therefore impact of this source of MSC due to the high numbers of menstruating respondents, generalized as 'untapped potential': "Thousands of women every month could be donating stem cells - the impact could be huge". Another response emphasized that almost half the population was affected by menstruation, so research in this field would be very accessible. This was highlighted again by one respondent who tried to donate MSCs from umbilical cord blood:

> It's handy to know that stem cells can be donated via menstruation. When pregnant with my second child I asked about donating stem cells from the umbilical cord and was "shut down" and told that it wasn't done in our area. It's a shame.

### *Menstrual blood waste can be useful*

Respondents identified that menstrual blood was a waste blood: "Fantastic as I view it as a 'waste' it should be used for something useful"; "'Waste not want not' - I'm going be producing it anyway, so it may as well be used for something helpful!". By donating MenSC, donors would be losing nothing, and giving an otherwise-discarded by-product a function. The element of making 'periods useful' was stated, exclaiming they could make their periods positive, rather than a nuisance: "Being able to donate would definitely make my periods feel like they have more of a purpose than just being annoying!".

### *Menstrual blood collection is less invasive*

People commented that the proposed donation would be less painful or invasive than blood donation: "The only reason I haven't donated blood or anything before is because I am scared of the pain and the process but I don't feel that this would be a problem with menstrual blood donation"; "Due to a heart condition I am unable to donate blood and would be interested in noninvasive donation I could participate in".

Compared to donating blood; menstrual blood donation does not involve needles and is affiliated with reduced pain, or it is considered more convenient than donating blood, which are two key factors influencing blood donation (McVittie et al 2006; Schreiber et al 2006), suggesting perhaps more people are willing to donate MenSC, further highlighting the need for painless and convenient donation to maximize potential donors.

All of these reasons to celebrate MenSC (helping others, increasing research, menstrual blood being useful) should be reasons to celebrate menstruation, opening conversation surrounding a menstruator's general mental, relational, and sexual health, and this will be instrumental in overcoming the menstrual taboo.

### *Disgust*

Several people responded with some form of disgust. Some people responded with disgust knowing that the menstrual taboo has influenced their perception of menstruation: "A little disgust – [we're] sometimes taught menstrual blood is unclean";

> I feel slightly more grossed out by the idea of the original substance being menstrual blood, but I think that might be something we have all learned from society - i.e. that women's bodily functions are disgusting and should be hidden!

However, feelings of disgust are also expressed without this metacognition; one participant mentioned general disgust at the MenSC coming from the vagina, and another simply stated: "Ew… gross… messy". It is therefore difficult to assess whether this level of disgust is due to society's framing of the subject, or whether menstrual blood is intrinsically disgusting for some.

### *Overcoming the menstrual taboo*

Some people had concern for society's reaction, as some expressed concern for the negative reaction from society, including finding it "icky", and this might affect uptake: "I would think this might not be popular".

For those that recognized the influence of the menstrual taboo, donation of MenSC was seen as an excellent way to neutralize it, and that menstrual blood is just blood.

### *It's just blood*

Participants stated that menstrual blood in this context should be seen as neutral: "If it helps you to get better, then there is no reason to be picky of where the blood comes from"; "It could be treated the same as any other blood sample"; "Of course - it's without question - it's just blood". Many respondents stated that the source of treatment should not or does not matter: "It does not matter how they are obtained"; "If I needed the treatment, I wouldn't care where it came from I would just care that it worked!". Similarly, some people made comments surrounding the naturalness of menstrual blood: "It's a naturally-occurring phenomenon"; "Periods are a natural process!", implying the stigma should be removed from it.

### *It's more than just blood*

However, others found this a reason to celebrate menstrual blood, and to go beyond neutralizing the subject. One participant stated:

> I think my experiences of my period would improve if I donated my blood as it would be almost rewarding in a way that you would be contributing to research and potentially helping someone, improving their health. Obviously, the potential benefit for stem cell research but also surrounding the stigma around periods in general. By donating menstrual blood, women may become more open in discussing their periods, something which I think is really important, rather than it being viewed as a dirty process or something to be embarrassed by.

"Menstruation is still very much a taboo subject. If menstrual blood was seen as something useful it may dispel some of the taboos around it [and] make young women more comfortable with the whole idea". Other comments in this respect included the menstrual taboo being "ridiculous", and MenSC donation being "instrumental" in removing stigma. One respondent made an interesting point regarding the symbolism of menstrual blood for those trying to conceive: "Helping life, with the blood that represents the time when life hasn't begun". The donation of MenSC for therapy gives a reason to be proud to menstruate, reducing the negative connotations of menstruation for the individual. There is also the opportunity for conversation, improving knowledge on the menstrual cycle, and reducing the taboo in society.

Blood donation does not appear to be affected by taboo, with Mathew et al (2007) finding that major factors preventing donation were personal fears of the donation, and the inconvenience, rather than taboo or stigma. Biobanking was also not associated with taboo, exploring American community members' knowledge, attitudes, and beliefs toward cancer-related biobanking (Luque et al 2012). Could it be that something offering therapeutic benefit reduces taboo? As sex toys have become increasingly associated with sexual health and general wellness, there has been a reduction in the taboo surrounding these items (Eaglin & Bardzell 2011). Sex toys are even considered "more acceptable" by members of the public when used for health reasons (Piha et al 2018, pp. 1091-1092). With the understanding that MenSC can be used for therapeutic purposes, donating and discussing the topic should become commonplace without rejection due to societal norms. Feminist scholar Kissling asked, "But can negative associations [with menstruation] be brushed aside and replaced with positive ones?" (1996, p. 497). Perhaps MenSC is the ultimate reason for people to celebrate and honour their menstrual cycle. If MenSC donation can also be strategic in overcoming the menstrual taboo, everyone will benefit from MenSC whether they donate or not.

### *Concern with donation MenSC*

Interestingly, there was more concern with the practicality of MenSC donation, such as ease, hygiene, and particularly using menstrual cups, rather than a discussion of the ethical implications and moral concerns of MenSC donation. Other concerns could not be themed easily: "My own personal views on blood transfusion... I am not refusing under religious reasons. I have the same

view on transplants"; "I have no problem with the technique, just the personal implications of donation"; "I feel guilty, but in all honesty I probably wouldn't be motivated enough to get round to donating even if I was eligible"; "I think it would be difficult to use since giving birth tampons don't fit as well". Where possible, these concerns should be addressed, particularly if they are having a detrimental effect on menstrual and life experiences. However, everyone's concerns are also entirely valid, and people should therefore not feel further shaped and limited by the menstrual taboo for having these concerns surrounding MenSC donation.

### *Conditions of donation*

Many people made tentative conditions of donating, rather than stating outright concerns; they would donate on condition of the MenSC donation being easy, others requiring donation being hygienic, and also mentioning they would donate if proven to help someone in need: "If it was easy to do, I would definitely donate"; "I've wanted to use [a menstrual cup] for a while - not sure how this would work though - just if easy and hygienic - yes!"; "If it's proven to work and be hygienic then yes".

### *Convenience*

One participant commented that a "monthly donation" would be "inconvenient". Another made a comment how to make the donation convenient:

> If the packaging came as a subscription so that every month it would be sent to your home I feel more people will use it, as it is in the home and if [menstrual blood] was postable, such as chlamydia home kits

It is clear that convenience and ease mean different things for each donor, and all donation strategies should be considered. The future MenSC donation should be flexible, offering the option for regular MenSC donations as well as providing kits for single-time donors to contribute without obligation.

### *Concerns with menstrual cup use*

Those with no prior experience of using a menstrual cup sometimes had concerns with the practicality of donating using one, but did not appear to be influenced by taboo. Some thought the menstrual cup would be painful or less comfortable, others feared it would be unhygienic: "More likely to get toxic shock syndrome". Some had other concerns, such as "more hassle" and "less convenient". Because these people had no experience of menstrual cups, these comments were based on assumptions rather than experience. This initial reaction to menstrual cups is seen elsewhere, including a focus group by Peberdy et al (2019) where one participant who had never heard of a menstrual cup before described it as "disgusting" initially. After discussing the subject openly and having questions answered, the participant went on to buy one herself. A similar experience is reported here: One respondent stated that she "was freaked out to begin with", but

after discussing it with a friend, found it to be “not as gross as I thought”, showing that initial reaction might change after open discussion. This is also where the theme of group encouragement might also ease some concerns. Where it is important to take all fears and concerns into account, assumptions should also be addressed to ensure misinformation is not spread. On a different note, someone may be willing to donate MenSC and should not need persuading to use a device they are not comfortable with. Ideally, further research would explore the feasibility of other popular menstrual hygiene products, such as tampons or sanitary pads, to be used for MenSC donation. This could further increase the pool of potential MenSC donors.

### *Concern with confidence*

Issues with confidence were highlighted, either in general, or about using a menstrual cup: “I’ve been curious about [menstrual cups] for a few years but have not been brave enough to try”; “I’ve never used a cup and I’m nervous about doing so”; “I would prefer to be at home all day to build trust in a product”. Confidence could be improved with education on the donation method, and a chance to build confidence in the product in the donor’s own time, as studies have found after 3 cycles, over 90% women using a menstrual cup for the first time would continue to do so (Donoso et al 2019; Howard et al 2011). MenSC donation would be most successful if potential donors new to menstrual cups are educated, supported, and are given the opportunity to use one for the days or months required to become comfortable with it. This also links with the comments made on group encouragement; learning to use a new product together might be less intimidating.

### *Happy to use a menstrual cup*

Multiple people stated they were already happy and comfortable using a menstrual cup, so donating MenSC would not be a difficult decision: “I use one anyway most of the time and wouldn't have a problem using one to donate”; “I use a menstrual cup so this would involve nothing different for me”. Generally, using a menstrual cup was described as an easy way to donate MenSC, as well as generally positive; “I wasn't sure what a menstrual cup was or how it worked but after looking it up on the internet it appears that it would be no more difficult to use than a tampon”; “I've heard people comment positively on this method generally. Why not kill two birds with one stone?”. Those who had never used menstrual cups remarked on their cost-effectiveness, eco-friendliness, and convenience, as found in other studies on menstrual cup attitudes (North & Oldham 2011; Stewart et al 2010). One participant stated they would buy one the following weekend after learning about MenSC and menstrual cups.

### *Giving the menstrual cup another go*

For two respondents, they had tried a menstrual cup before but not found it ideal. They would be willing to use it again for the purposes of MenSC donation: “For me menstrual cups weren't a very good form of sanitary protection. However, I think the difference it could make is so great that I

would be happy to use one to collect blood for stem cells"; "I would persist with it if I knew it was going to a good cause (I have tried a couple of times)".

### *Intrigue/interest*

Initial reactions were often that of intrigue or interest; one stating the topic was "fascinating", and several people agreeing that the subject was "interesting". One participant was intrigued to do further reading on the topic, a second stated they did their own research before completing the questionnaire, and a third mentioned they asked a friend for more information on the topic. 84% menstruating respondents had not heard of MenSC previously, meaning this questionnaire captured initial reactions to the topic. Alongside a general preparedness to use a menstrual cup to donate MenSC, this theme of overall interest is positive and indicates that as the population becomes aware of MenSC donation, a growing number of people will be intrigued and potentially willing to donate themselves. This overall positivity could also instigate discussion over general, menstrual, and sexual health, and with open discussion comes the reduction in taboo surrounding the subject (Kissling 1996; Peberdy et al 2019).

### *Limitations and concerns*

Being a convenience sample, this brings the question of whether the results of this questionnaire from 100 people sampled from various environments within a city in the Midlands, UK, can represent the UK. As menstruation is a taboo topic, participants may not have spoken openly about their thoughts, or not responded to the questionnaire at all. This would result in an inadvertent but unavoidable bias in the data. It is the hope that this study captured the attitudes from a range of people, but this will be difficult to ascertain, as with all research involved in taboo topics. However, this work as a minimum is an important first step in exploring these topics, and should remain in focus as MenSC therapy research continues.

Even though a questionnaire was chosen to enable people to share thoughts on these sensitive topics anonymously and freely, having an online platform could broaden the reach (Sue & Ritter 2012). The convenience of online questionnaires may encourage participation, although is restrictive in its own way: Online surveys require access to internet, computer or smartphone devices, and assume computer literacy. These are issues of social inequality, and relying on online surveys lead to coverage error (Couper 2000). Future studies could include online questionnaires.

Importantly, these findings must be contextualised within the cultural locatedness of the study. Attitudes to MenSC differ greatly from the UK to India; 78% menstruating respondents would be willing to donate their MenSC in the UK (Manley et al 2019) whereas 66% in India people hold an unfavourable attitude toward MenSC banking (Jomon et al 2019). Between India and the US, women scored differently in their attitudes towards menstruation, with Indian women having more positive attitudes than American women, but with American women felt better

preparedness for menstruation (Hoerster et al 2003). Attitudes differ from culture to culture, therefore future study of attitudes to MenSC donation should be undertaken cross-culturally and around the world.

This questionnaire focused on the opinions of potential donors: those having experienced at least one menstrual cycle. Gender identity was not assessed in this study. Future work should include the voices of trans men, non-binary and gender-expansive people: anyone who may be involved with MenSC donation and treatment. Additionally, the attitudes of post-menarcheal girls and teenagers would be worth exploring in the future if they are to be valuable potential MenSC donors, but these were currently rejected for ethical reasons. Information regarding ethnicity, social class, and sexual orientation were not collected for this study. With a larger sample size, any association with ethnicity, social class, and sexual orientation and the cultural influences that impact attitudes to menstruation and MenSC could be explored. Age, history of birth, history of whole blood and stem cell donation, menstruation status and experience rating, and preferred menstrual hygiene products were chosen to initially explore the attitudes towards MenSC and MenSC donation, but as race/ethnicity, social class, and sexual orientation could have important implications for attitudes to MenSC and MenSC donation, it is important that future work includes these factors.

It was evident here that there are a wide range of attitudes to MenSC, donating MenSC, and using menstrual cups. Given that this is a new and multi-faceted area of research, influenced by taboo, it's no surprise that the views and perceptions of the public vary greatly. Considering the lack of research in this space, this study has attempted to address the whole narrative. Future work should further explore and understand the prevalence of these identified themes.

This work is feminism-driven, with an ongoing goal to remove the sociocultural barriers that diminish the wellbeing and life experiences of people in relation to menstruation. MenSC could help remove the stigma around menstruation by celebrating the altruistic clinical potential of MenSC donation. However, there is the concern over the commodification of women's bodies, whether that is through surrogacy where women's bodies become "under the control of others" (Phillips 2013, p. 64), softened by referencing the "gift of life" (ibid, p. 62), or in embryonic stem cell research (Dodds 2004). It is vital that MenSC do not simply become another commodity for consumption by others. MenSC donation should be part of a strategy to empowerment.

### *Implications*

It is known that involving the public (in this case, potential MenSC donors) has a positive impact during initial stages of research, with users identifying, prioritizing, and developing research topics, improving wording of sensitive issues, and taking cultural issues into account (Brett et al 2014). It also contributes to outcomes that are "appropriate to the needs and lifestyles of the

patient community it serves" (Bagley et al 2016, p. 6). It is ideal to involve the public now, so that MenSC donation can be optimised before it is mainstream, rather than an afterthought. If MenSC donation methods are not appropriate for donors' needs and lifestyle, no one will donate. This work contributes to the scholarly research surrounding the menstrual taboo that has been described to remain lacking (Bobel 2010; Lamborn 2017; Laws 1991; Risling Baldy 2017), and is an important contribution to scholarly research surrounding attitudes to MenSC, using a menstrual cup to donate MenSC, and MenSC in relation to the menstrual taboo.

### *Conclusion*

This chapter presents a new impression and understanding of the attitudes, preferences, and concerns regarding MenSC donation. With MenSC being described as expandable for clinical applications, suitable for mass banking, and potentially donated up to every month in pre-menopausal menstruators, it would be foolish to continue research in MenSC without being informed by potential donors' opinions. Yet, this is the first qualitative study on the subject of MenSC donation.

Being able to help people, advance therapy, and overcome the menstrual taboo were key motivations for donation. Personal concerns were focused on the practicality of donation, rather than ethical trepidation, with the donation needing to be easy and hygienic before committing. MenSC donation should be flexible, and if kits can be sent to donor's homes on request with time to gain confidence in the product, uptake could be very high.

Currently, the most popular MenSC collection method is using a menstrual cup. It could be that those already comfortable using a menstrual cup are ideal candidates for the initial pool of MenSC donors. This study revealed that the new knowledge that menstrual cups could be used to donate MenSC was a) enough to give someone motivation to try the menstrual cup again, if it had not been the ideal product for them, or b) to try one for the first time, with one participant even stating they would buy one the following weekend. However, it is undeniable that sanitary pads and tampons are more popular (Mintel 2023) and accessible forms of menstrual hygiene products. These have never been compared or tested for MenSC donation potential.

With the objective that sanitary pads, tampons, and menstrual cups are to be explored as methods for menstrual blood donation, their function, materials, and regulation must first be understood to confirm that they are suitable and safe options to explore.

# Chapter 4. Menstrual hygiene product safety and regulation

It is an aim of this thesis to identify the most appropriate, accessible, acceptable methods of menstrual blood donation. While the use of menstrual cups is the most popular methods to collect MenSC in the literature, and their use is gaining traction and popularity, methods such as sanitary pads and tampons should also be assessed. These methods are globally more popular, are highly accessible, inexpensive, and regulated to a stricter degree than menstrual cups. This chapter looks to review sanitary pads, tampons, and menstrual cups[*] in terms of usability and safety in response to **research question 3: What are sanitary pads, tampons, and menstrual cups, are they safe, and how are they being regulated?** Sanitary pads and tampons, while not considered medical devices in the UK, are regulated in terms of absorbency, which has safety implications for tampon users by preventing toxic shock syndrome, and has usability implications for consumers knowing that across differing brands, they can have confidence in a product's absorbance. However, menstrual cups are not regulated at all. Available in a huge variety of size, shapes, and materials, a case study presents a consumer's difficulty in finding and using a suitable menstrual cup. Therefore, there is a need to compare menstrual cups in terms of size, shape, material, and firmness, which is a first step to categorisation and improvement for safety. This comparison reveals there is no correlation between a menstrual cup's size, shape, material, and firmness. This means consumers can not choose a suitable size visually before buying, and the firmness of a product could hinder use or even increase risk to injury or health.

## 4.1 Sanitary pads

Sanitary pads are worn within the wearer's underwear, absorbing menstrual blood as it flows from the vagina, composed of layers of a liquid-permeable upper layer, an absorbent core, and a non-permeable bottom layer. This absorbent core is made of a variety of materials, often marketed by companies as a trademarked material, rather than disclosing the actual contents, including Infinicel (Procter & Gamble 2020), Infinity Flex Foam (Always 2020), and Airlaid (GDM 2020). Table 4.1 shows the 'ingredients' disclosed in some of the top-selling sanitary pad brands.[†] Companies are not required by law to disclose this information, unlike the toy and cosmetic

[*] Other menstrual products include absorbent underwear such as Thinx (2020), washable pads, and natural absorbents. Washable pads, fitted to normal underwear with poppers or Velcro, can be homemade or purchased, an example being Charlie Banana (2020). Some people choose to use natural sea sponges similarly to tampons to absorb menstrual blood (Kanungo & Omar 2008; Stockburger et al 2013). The safety of the latter is questioned, as they have been found to contain contaminants (Richards 1983) and cause toxic shock syndrome (Svastisalee 1980; Tofte & Wiliams 2016). There are other products on the market, like the Tampliner (Callaly 2020), that combines a tampon with a panty liner attached by a cylindrical plastic film, designed to protect underwear and allow for mess-free tampon removal. In cases like these, the products fit within either the tampon or the sanitary pad category. However, because they are not prevalent in the menstrual hygiene market, they have not been included in analysis. For the sake of understanding the products most used by people, this chapter looks to sanitary pads, tampons, and menstrual cups.

[†] Some brands offer an organic version of the product, which is otherwise identical to the standard product, except for a 100% organic cotton top layer, and the omittance of perfumes (Always 2020).

industries (Desmedt et al 2020), but the superabsorbent polymer in the absorbent layer is typically made from sodium polyacrylate granules (Campbell et al 1987; Woeller & Hochwalt 2015). The materials and size of the pad determines its absorbency, ranging from panty liners for managing spotting or for protecting underwear worn in conjunction with other menstrual hygiene products, to super or 'maxi' for managing heavy flow or overnight use.

*Table 4.1 Sanitary pad 'ingredients'*

| *Brand* | *Top layer* | *Absorbent layer* | *Bottom layer* | *Reference* |
|---|---|---|---|---|
| Always | Polyolefins | Absorbent foam, or absorbent wood cellulose with absorbent gel, rayon, or polyester | Polyolefins | Always 2020 |
| Bodyform | Polypropene/ polyethylene polyester/ viscose fibre | Paper pulp and super absorbents | Polymers and synthetic resins | Bodyform 2020 |
| U by Kotex | Polypropylene and polyethylene fibres | Wood fluff pulp, polypropylene and polyethylene fibres, cellulose tissue, superabsorbent (sodium polyacrylate) | Polyethylene film | Kimberly-Clark 2020 |

These products are worn continuously for hours and days at a time. With this element of ambiguity surrounding products' composition, how are these products regulated to ensure they are safe to wear?

### 4.1.1 Regulation

Sanitary pads are not regulated as medical devices under the Medical Devices Regulation (MDR). (MDR 2023). Within the EU, sanitary pads sit under the absorbent hygiene products category by the European Disposables and Nonwovens Association (EDANA), for minimum safety and regularity information for product on the EU market (EDANA 2018), which are less stringent than the US Food and Drug Administration (FDA) (Bae et al 2018). However, even the FDA is not legally enforceable, being regarded as guidance or manufacturing control recommendations.

The International Organisation for Standardisation (ISO) has identified key regulations to maintain safety and transparency in products that applies to sanitary pads, shown in Table 4.2. As shown, regulations are built upon general medical device safety and product absorbency, and not specifically to absorbing menstrual blood. ISO have produced standards for comparing absorbance capacity of urine-absorbing aids (pads) for heavily incontinent adults (1996), stating the unknown transferability of the application of these standards to other users (e.g. babies) or other products (e.g. reusable, or not body-worn), and certainly not to other bodily fluids such as menstrual blood. Additional sets of standards regulate the specifications and testing methods for super absorbent polymers used in sanitary pads and other blood-absorbing products (ISO 2017a; ISO 2017b), highlighting formulation for making simulated blood. Again, these regulations are not specific for the absorption of menstrual blood, which differs to whole blood in its contents and consistency[*] (see Section 2.2), but are the closest matching standards. The superabsorbent polymer regulations therefore also do not transfer the regulations to sanitary pads containing other substances, such as cellulose tissue and various pulps as listed in Table 4.1.

*Table 4.2 ISO regulations applicable to sanitary pads*

| *Standard* | *Description* | *Reference* |
|---|---|---|
| ISO 9073-12:2002* | Evaluation of the absorbency of fabrics when one side is in contact with a liquid and the fabric is under mechanical pressure allowing for comparison. | ISO 2002a |
| ISO 10993-17:2002 | Establishment of allowable limits for leachable substances in medical devices. | ISO 2002b |
| ISO 9073-11:2002* | Assessment for comparing quantity of simulated urine run-off when poured down a nonwoven material. | ISO 2002c |
| ISO 9073-6:2003* | Evaluation of absorbency in nonwoven materials including liquid absorbency time, absorptive capacity and wicking rate. | ISO 2003 |
| ISO 9073-13:2006* | Repeated liquid strike through-time for nonwovens for assessing penetration time for simulated urine. | ISO 2006 |
| ISO 10993-10:2010 | The assessment of medical devices and their potential to produce irritation and skin sensitivities. | ISO 2010 |
| ISO 19699-1:2017 | Testing methods for superabsorbent polymer used in physical hygiene and medical products for | ISO 2017a |

[*] The FDA provides the formula for recreating menstrual blood, named “syngyna fluid”.

| | | |
|---|---|---|
| | absorbing blood, providing formula for simulated blood. | |
| ISO 19699-2:2017 | The requirements for properties, marking and packaging of superabsorbent polymer for absorbing blood. | ISO 2017b |
| ISO 20158:2018* | Test methods for determining water absorption time and capacity of all textile fabrics designed to absorb water | ISO 2018a |
| ISO 10993-18:2020 | The identification of biological hazards and the estimation and control of biological risks. | ISO 2020 |

*not specific to medical devices

EDANA and Association of the Nonwoven Fabric Industry have drawn up Nonwoven Standard Procedures (NWSP) document, a set of standardised experiments in order to test, compare, and analyse nonwoven products in Europe. A section is dedicated to Absorbent Hygiene Products, see Table 4.3. The document touches upon elements of sanitary pad design that goes beyond that of ISO, and meets users' needs in terms of usability, comfort, and success; specifically not all safety concerns (NWSP 2019), however, many of them are specific to incontinence products or tampons, and could be transferred to sanitary pads.

*Table 4.3 EDANA Nonwovens Standard Procedure, Absorbent Hygiene Products section*

| *EDANA test* | *Method Number* | *Description* |
|---|---|---|
| Menstrual Tampons Absorbency – Syngina Method | NWSP 350.1.R1 (15) | Methods for creating Syngina (derived from synthetic vagina) and testing the absorbency of tampons in a vagina-like environment. Does not include the testing of sanitary pads. |
| Determination of Ethanol- Extractable Organotin Species in Absorbent Hygiene Products and Materials, Absorbent Hygiene Materials-Organotin I | NWSP 351.0.R0 (15) | Method for identifying and quantifying organotin cation species (RnSnCl(4-n)) which has a high toxicity in liver and reproductive systems, from absorbent hygiene products and their raw materials. |
| Determination of Organotin Species | NWSP 352.0.R0 (15) | Method for identifying and quantifying organotin cation species (RnSnCl(4-n)) which |

| | | |
|---|---|---|
| Extracted From Absorbent Hygiene Products and Materials With Synthetic Urine, Absorbent Hygiene Products – Organotin ll | | has a high toxicity in liver and reproductive systems, from absorbent hygiene products and their raw materials by synthetic urine. Used with synthetic urine, not menstrual blood. |
| Determination of Aceton Extractable Finish on Nonwoven | NWSP 353.R0 (15) | Method to determine the amount of finish extracted from the product with acetone, which affects performance and processing properties but does not simulate in-use conditions of finished products. |
| Absorption Before Leakage Using an Adult Mannequin<br><br>NWSP 2019 | NWSP 354.0.R1 (15) | Method to evaluate the performance of incontinence products in non-ambulatory adults absorbing 300g to 1100g test liquid in residential and nursing home settings. This test method is for incontinence products, not menstrual hygiene products. |

The Bureau of Indian Standards introduced standards for sanitary pads in 1980, from which several sections should be relevant to today's standards. These regulations provided standards regardless of the materials within the absorbent layer, and recommending standardised dimensions for regular, large, and extra-large sanitary pads. A recommended thickness across all pad dimensions is provided at 15±2mm, although this is potentially outdated due to the improvement in superabsorbent polymers. Although the safety regulations are not as rigorous as those seen in current International Standards, it highlights procedure for maintaining a safe pH level, disposability of the product, and lack of leakage should all be transferred to contemporary standards, as well as considerations for user comfort.

Companies are not required by law to disclose sanitary pad composition (Desmedt et al 2020). For transparency, these should be disclosed for safety and comfort purposes, and to allow consumers to make informed choices. ISO regulations are often not applicable to menstrual blood absorption and do not consider user comfort. European NWSP do meet users' needs in terms of usability and comfort, although are often base upon incontinence products rather than menstrual hygiene

products. Regulation should emphasise comfort as well as safety, and propose dimension recommendation similar to the Bureau of Indian Standards, or regulation for categorisation of product size in regard to contraceptive diaphragms (ISO 2014). This call for improved regulation is mirrored for tampon manufacturers.

## 4.2 Tampons

Tampons are worn vaginally, where they expand and absorb menstrual blood. Digital tampons are inserted by hand, and applicator tampons provide the user with a cardboard or plastic tube to insert. Over the years, tampon design has been enhanced to produce a more streamlined, petal-like applicator shape (Suga 2002), and for improved manufacture and assembly (Crockford 1949).

As with sanitary pads, tampon composition varies by brand and product, see Table 4.4.

*Table 4.4 Tampon composition*

| *Brand and product* | *Absorbent core* | *Outer layer* | *String* | *Thread* | *References* |
|---|---|---|---|---|---|
| Lil-lets (main range) | Viscose | Polyester/ polyethylene | Polyester/cotton | - | Lil-lets 2020a |
| Lil-lets organic cotton | Organic cotton | - | Organic cotton | - | Lil-lets 2020a |
| o.b. ORIGINAL | Two types of rayon | Polyester/ polyethylene | Polyester | - | o.b. 2020a |
| o.b. PRO COMFORT | Two types of rayon | Polyethylene/ polypropylene | Polyester | - | o.b. 2020a |
| o.b. 100% ORGANIC COTTON | 100% organic cotton | 100% organic cotton | 100% organic cotton | - | o.b. 2020b |
| Tampax Pure | 100% organic cotton | 100% organic cotton | Cotton with polypropylene | Polyester | Tampax 2020 |
| Tampax Pearl | Cotton and/or rayon | Polyethylene and polypropylene | Cotton with polypropylene | Polyester | Tampax 2020 |
| Tampax Cardboard | Purified cotton and/or rayon | Polypropylene | Cotton-wrapped polyester | Polyester | Tampax 2020 |

Tampons come in a variety of absorbencies, ranging from light to ultra (see Table 4.5), allowing people to choose the right product for them depending on their flow. Smaller tampons are also available for children or teenagers shortly after menarche (Lil-lets 2020b).

### 4.2.1 Safety

Toxic shock syndrome (TSS) is caused by the vaginal colonisation of *Staphylococcus aureus*, and tampon use is associated with TSS risk because inserting a tampon introduces oxygen, and the naturally-occurring higher pH of the vagina during menstruation (Eschenbach et al 2000). This creates ideal conditions for *S. aureus* growth, leading to the production of the toxin causing TSS (Schlievert et al 1981). Menstrual TSS cases peaked between late 1970s to 1990s (Hajjeh et al 1999), because increased absorbency in new tampon designs meant people wore tampons for longer, and regardless of tampon brand and the chemical composition of the tampon, increasing the absorbency level of a tampon increased the odds ratio of illness with TSS (Berkley et al 1987; Osterholm et al 1982).

The Tampon Taskforce was established in 1982, made up of manufacturers, consumer groups, and feminist health advocates (Vostral 2017). This group pressured the FDA for uniform standards for tampon absorption capabilities and labelling, aiming to improve tampon absorbency transparency and safety. The syngyna (synthetic vagina) was taken on by the FDA to assess the absorbency accuracy across tampon brands, and is still in use today and allows for accurate absorbency levels to be comparable across tampon brands, ranging from light to ultra (see Table 4.5).

TSS is not the only danger with tampon use. Other dangers include chemical residue in perfumed tampons, strings breaking during use, lacerations caused by plastic applicators, and general material safety (Vostral 2017). Tampons are equally vague to sanitary pads in how the ingredients are disclosed. Some tampon brands also contained a carcinogen (WVE 2020) and volatile organic compounds (Lin et al 2020). Tampons may contribute to higher exposure to toxic compounds than sanitary pads (Ding et al 2020; Ding et al 2022).

### 4.2.2 Regulation

How are the dangers of tampons being mitigated, and the safety ensured, particularly in the UK? Tampons *should* sit within class IIa medical device by the European Legislation MDR EU 2017/745 (MDR 2023), as they would fit within the remit of an invasive device, which “in whole or in part, penetrates inside the body, either through a body orifice or through the surface of the body” (p. 10). As tampons are worn for a few hours at a time, they sit within Class IIa, as they are intended for short-term use (MRD 2023 Annex VIII 1.2). However, the MDR do not recognise this, as “Female sanitary products such as pads, tampons or menstrual cups are not intended to treat a

disease, an injury or a disability" (Kyriakides 2023). The Medicines and Healthcare Products Regulatory Agency (MHRA) state tampons are "not normally considered to be medical devices" (MHRA 2016, p. 4), even though incontinence products are. MHRA emphasise that their guidance is based on their own interpretation of the MDR. Even so, regulation and standardisation authorities should be unified in their classification of tampons as invasive medical devices, promoting the highest quality surveillance and regulation of these products. ISO define a medical product to include one with the purpose of "investigation, replacement, modification, or support of the anatomy or of a physiological process" (ISO 2018b, 3.27), and menstrual hygiene products should sit within that remit. Where it would be expected for tampons to have their own standards, much like the standards for intrauterine contraceptive devices (ibid), and female condoms (that are also worn vaginally) (ISO 2017c), this is not the case, particularly as tampons are worn for greater length of time than female condoms. The ISO standards seen in Table 4.2 applying to medical devices also apply to tampons, except for the standards referring specifically to products made from superabsorbent polymers.

In the UK, menstrual hygiene products are regulated and produced according to the General Product Safety Directive, with the UK government departments and retailers recognising the UK Voluntary Code of Practice for Tampon Manufacturers and Distributors. The General Product Safety Directive 2001/95/EC describes the necessary levels of health and safety protection of a product, including the raw materials as well as the finished tampon, and the traceability of each product. The regulations also cover the adjoining labelling, warnings and instructions for the product's use, and its disposal. The directive is acknowledged to be broad. In an attempt to meet this need, the UK Voluntary Code of Practice for Tampon Manufacturers and Distributors was introduced by the Absorbent Hygiene Product Manufacturers Association (AHPMA). This code of practise uses NWSP as described in Table 4.3. Similarly to the FDA in the US, the absorbency of tampons is standardised in order for tampons across UK brands to be comparable, and consumers can make informed decisions about the product they should purchase. This is shown in Table 4.5. There are similarities between the labelling regulations, as descriptors from light (or lite) to Super Plus generally matching. Tampon descriptors should be universal and internationally acknowledged.

*Table 4.5 Tampon absorbency terminology, UK vs. US*

| *AHPMA 2019 (UK)* | | | | *FDA 2019a (US)* | |
|---|---|---|---|---|---|
| *Absorbency* | *Droplet symbol* | *Primary descriptor* | *Secondary descriptor* | *Absorbency* | *Primary descriptor* |
| <6g |  | Lite(s) / Light(s) | "very light to light flow" | <6g | Light |
| 6-9g |  | Regular / Slender / Mini / Normal | "light to medium flow" | 6-9g | Regular |
| 9-12g |  | Super | "medium to heavy flow" | 9-12g | Super |
| 12-15g |  | Super Plus | "heavy flow" | 12-15g | Super Plus |
| 15-18g |  | Super Plus Extra | "very heavy flow" | 15-18g | Ultra |
| 18-21g |  | Ultra | "extremely heavy flow" | Above 18g | No term |

The EDANA lists tests for member tampon manufacturers and laboratories to undertake to meet their requirements, seen in Table 4.6 (EDANA 2018). Unfortunately, the tests are not as thorough as those for sanitary pads, and does not include tests for the abrasiveness of the materials to reduce laceration or irritation of the vaginal tissue, either of the tampon itself, or the applicator. Even EDANA states in its documents, "there is no guideline for the laboratories how to declare a product as safe!" (ibid, p. 8).

*Table 4.6 EDANA tampon tests*

| *EDANA test* | *Description* |
|---|---|
| Syngyna | Measuring the absorption capacity of the tampon in a simulated vagina environment. Determines the category of absorption. |
| Strength of the string | Ensuring the tampon string will withstand the pressure when being removed from the vagina. |
| Linting /fibre fluff-off | Measuring the quantity of the retention of loose fibres in the vagina after use. |

In summary, manufacturers are again not required to disclose product composition, and tampons have been found to potentially contribute more to higher exposure to toxic compounds than sanitary pads (Ding et al 2020; Ding et al 2022). While absorbency is regulated to reduce risk of TSS (AHPMA 2019; FDA 2019a), there is global inconsistency regarding whether or not they are considered medical devices (FDA 2019; MDR 2023; MHRA 2016), and even EDANA states in its documents, “there is no guideline for the laboratories how to declare a product as safe!” (2018, p. 8). This thesis calls for improved regulation of tampons, regardless of whether the tampons are used to donate MenSC.

## 4.3 Menstrual cups

This section will have a particular focus on menstrual cups, being the most popular product for MenSC collection, as discussed in Section 2.8.1. Menstrual cups are cup-shaped vessels made from silicon, TPE, or natural rubber, that are worn in the vagina to collect blood, rather than absorb it. Use shown in Figure 4.1, and it is inserted into the vaginal opening whilst folded, the firm rim opens within the vaginal walls, creating a seal preventing leaks. Sometimes trimmed for comfort, the stem (or based of the cup) is gently pulled down, with small air holes under the rim that release the suction of the cup, for removal after up to 12 hours of use. The menstrual cup can simply be washed between uses, and boiled in water for sterility between cycles. It has been proven that cleaning cups with soap and water before placing in boiling water is the most successful method for washing menstrual cups between cycles (Wunsch et al 2022). With menstrual cups lasting up to ten years (van Eijk et al 2019), people report choosing a menstrual cups for economic (Cheng et al 1995; Pena 1962; Stewart et al 2010), comfort (Beksinska et al 2015; Mason et al 2015), and environmental reasons (Milne & Barnack-Tavlaris 2019; Peberdy et al 2019; Stewart et al 2010).

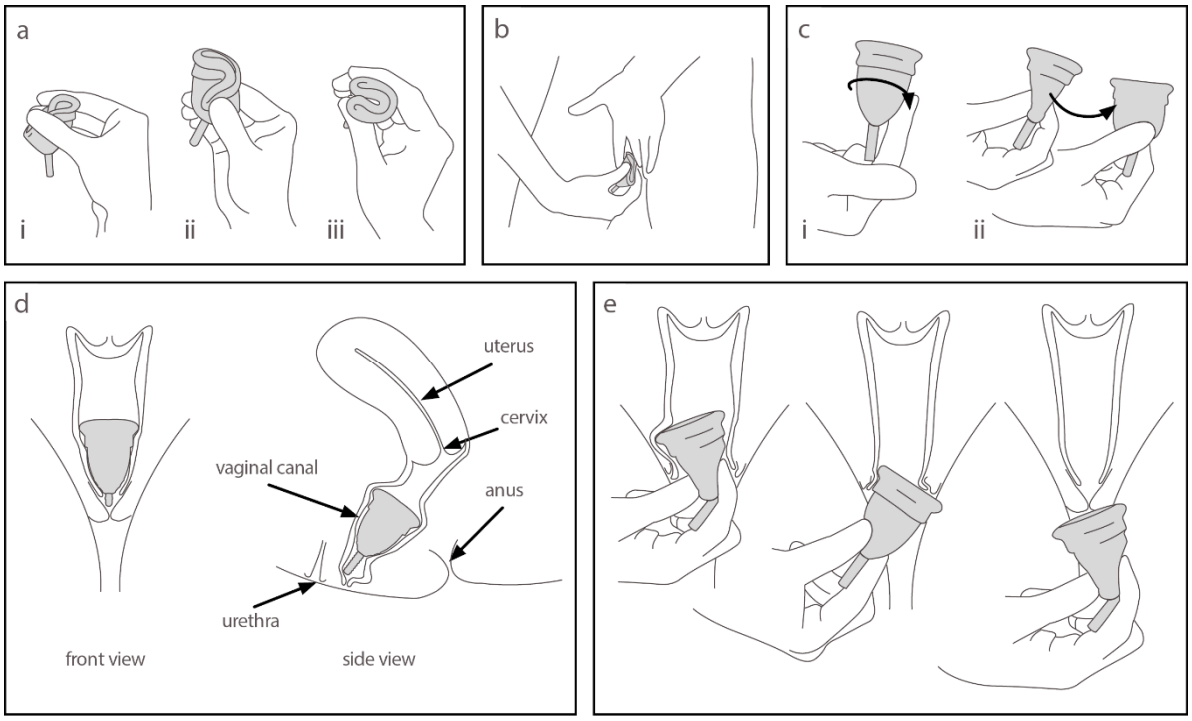



*Figure 4.1 Menstrual Cup Use.*

*a) Users find a fold suitable for them; (i) punchdown-fold, (ii) 7-fold, and (iii) c-fold illustrated. b) The folded menstrual cup is inserted and allowed to spring open. c) To ensure the menstrual cup is fully open and therefore creating a seal against the vaginal wall, users can (i) run a finger around the vessel to feel for bumps as a sign it is not open, or (ii) users can gently pinch and twist the menstrual cup. d) The menstrual cup sits lower than a tampon. The stem may need to be trimmed to avoid discomfort. The length of the vaginal canal will affect where the menstrual cup sits in relation to the cervix. e) To remove, users must ensure the seal is broken by running a finger cup the side of the menstrual cup, or pinching the base of the vessel. The cup is then gently removed, and it can help to shift the menstrual cup from side to side.*

Menstrual cups also come in a variety of shapes. According to Menstrual Cup Reviews, there are four basic menstrual cup shapes, Bell-shaped, V-shaped, Round-shaped, and Ergonomic-shaped (2020). This thesis recommends the alteration of a fourth category of menstrual cup shape to “Asymmetrical”, as Ergonomic implies the other shapes are not designed to be worn vaginally with ergonomics in mind. As shown in Figure 4.2, menstrual cup shape and size varies greatly, which is explored more in Section 4.3.5. Menstrual cup brands are often offered in multiple sizes. Generally, one smaller size is offered to users under the age of 30 who have not given birth vaginally, and then a larger one for those over the age of 30, or who have given birth. Some brands offer a shorter menstrual cup for people with shorter vaginal canals or a cervix that sits lower in the body (Me Luna Shorty), and some brands offer yet smaller cups marketed at teenagers or those with a particularly small build (DivaCup model 0). Finally, some menstrual cup brands make recommendations regarding the firmness of the product, offering softer menstrual cups for people with a weaker pelvic floor muscle, or history of prolapse, or firmer menstrual cups for those with firmer pelvic floors, examples given being gymnasts or dancers (Me Luna Classic, Sporty, and Soft).

### 4.3.1 Attitudes to menstrual cups

With menstrual cups seeming to be a safe option for handling menstruation (van Eijk et al 2019), are they perceived to be acceptable, easy, pain-free to use?

Meta-analysis over three studies by van Eijk et al found only 11-33% on interviewed women were already aware of menstrual cups (2019). In a focus group, one person had heard of menstrual cups for the first time. Her initial reaction was disgust, but after talking openly about the product and asking questions about it, her disgust became intrigue, and finally the desire to purchase one (Peberdy et al 2019). This theme of first-time attitudes to menstrual cups changing over time is not only seen in general perception of the product, but how people experience using them.

Several studies have examined attitudes to menstrual cups, often during first time use. This has often been done by simply comparing the experiences directly to other menstrual hygiene products. Generally, people preferred the menstrual cup to their current method of menstrual hygiene management (North & Oldham 2011). In one study, 110 participants used to tampons were split into two groups, with one group using menstrual cups for the first time. Their overall satisfaction was higher for menstrual cups (Howard et al 2011). In clinical testing, 37% participants rated menstrual cups better than, and 34% rated them equal to, tampons or sanitary pads after three cycles of use (North & Oldham 2011). This three-month or three-cycle mark has been used in many studies (Madziyire et al 2018), as it is clear the product requires a learning curve, and there is often an improvement in experiences once used to their menstrual cup (Donoso et al 2019; Howard et al 2011; Pena 1962; Stewart et al 2010).

Minimal leakage is important. In one study, leakage was found in 1% of participants which lead to discontinuation in the study (North & Oldham 2011). Therefore, minimising leakage leads to positive user experiences. 65% of people experienced staining or leaking with previous use of tampons or sanitary pads, which dropped to 0% of staining or leaking with menstrual cups (Cheng et al 1995). However, this is not the case when the menstrual cup isn't emptied enough (Pena 1962).

Positive attitudes stem from the menstrual cup being easy to use (Cheng et al 1995; Pena 1962), economical (Cheng et al 1995; Pena 1962; Stewart 2010), environmentally-friendly (Peberdy et al 2019; Stewart et al 2009; Stewart et al 2010), requiring carrying only a few supplies (Cheng et al 1995; Stewart et al 2009), comfortable (Howard et al 2011; North & Oldham 2011), convenient or practical (Cheng et al 1995; Howard et al 2011; Pena 1962), easy to maintain (Madziyire et al 2018), dry/minimal leaks (Howard et al 2011; North & Oldham 2011; Stewart et al 2010), hygienic (Cheng et al 1995; Pena 1962), and requiring few product changes (Stewart et al 2009; Stewart et al 2010).

In terms of contact with blood, Owen found that some new menstrual cups users found the different and direct contact with their own menstrual blood “beautiful” (2022b, p. 1106), “powerful”, “exciting”, and “uncomfortable in a completely different way to other blood” (ibid p. 1107).

Menstrual cup removal was negative compared to tampon removal (Howard et al 2011), rated negatively for their disposal and convenience (North & Oldham 2011), too long, being difficult to insert or position correctly, uncomfortable, messy, and difficult to use during light flow (Cheng et al 1995). One person’s husband did not agree with her use of a menstrual cup! (Madziyire et al 2018) People are also unhappy to clean their menstrual cup in kitchen (Stewart et al 2010), or empty or clean their cup in public toilets (Kim et al 2022; Stewart et al 2010).

A friend’s input when using a menstrual cup for the first time significantly improved usage success. The number of failed attempts was reduced, the number of total attempts increased, and initial successful usage increased (Oster & Thornton 2012). This links to early work by the author, exploring how people learned to use a menstrual cup for the first time. Face-to-face description, instruction, and support from a close friend or family member showed a better initial menstrual cup experience: a faster learning curve, fewer failed attempts, fewer leaks, and improved general comfort (Manley 2018).

It appears that after trying a menstrual cup for the first time, even with negative first impressions, after a few months, the user experience may well be a positive one. If people are supporting eachother, particularly friends and family, this experience can be optimised. This is important to know if menstrual cups are the future for MenSC donation. Disappointingly, donors’ attitudes towards menstrual cup use in MenSC donation has been researched minimally in this field. However, van der Molen et al, recruiting five people for menstrual blood donation, asked them to report their experience. All of them described the use of the menstrual cup as acceptable (2013). This is positive, and all future studies requiring a people to use a new menstrual hygiene product must take her opinion and experience into account.

In the UK, 8% of menstruators use a menstrual cup (Mintel 2023). There is now a huge range of menstrual cups available, and people enjoy, appreciate, or at least tolerate the use of these products. This leads to the question of how these products remain safe, and how they are being regulated.

### 4.3.2 Safety

In June 2020, a search on the FDA’s Manufacturer and User Facility Device Experience database for adverse effects as a result of using medical products searching for “cup, menstrual” finds 85 instances from 2001 to 2020, with the earliest record from 2010 (FDA 2020). See Table 4.7. Of

these instances, 22 of these events are categorised as “injuries”. Mooncup and Divacup saw higher levels of user injury. This is potentially because they have been around for longer, being available in 2002 and 2003 respectively (Divacup 2023; Mooncup 2023). However, Saalt, bringing their products to the market in 2018 (Saalt 2020a), is fairly new to the market, and it is worrying that 46 out of 85 adverse effects since 2010 are a result of using Saalt menstrual cups (see Table 4.7), and five out of 22 injuries (see

Table 4.8). There has been no potential explanation for this, and with the FDA Manufacturer and User Facility Device Experience reports being brief, it is difficult to extract further explanation.

As seen in Table 4.9, in four reports, these “injuries” were people unable to remove their menstrual cup, and requiring medical assistance for removal. It could be said that this should not be classed as an injury, as other adverse effects classed as “malfunctions” are cases where people could not remove their menstrual cup. However, it could be that these users had unspecified injuries as a result of requiring medical assistance to remove their menstrual cup, and certainly one of these occasions resulted in an infection as the menstrual cup was worn for 19 hours until medical assistance could be received. The next most frequent injuries caused by menstrual cups were unspecified or undiagnosed pain in four cases, and three cases of TSS. On two occasions, the cup was suctioned onto the cervix. IUD expulsion has not been reported much, but Schnyer et al found that users of both IUD and menstrual cups are significantly more likely to experience UID expulsion with concurrent menstrual cup use (2019).

*Table 4.7 FDA's list of reported adverse effects caused by menstrual cups*

| *Menstrual cup* | *No. reports of adverse effects* |
|---|---|
| Softcup | 5 |
| Femmycycle | 1 |
| Divacup | 4 |
| Mooncup | 7 |
| Lena | 19 |
| Smartcup | 1 |
| Saalt | 46 |
| Lilycup | 1 |
| Divacup OR Softcup | 1 |

*Table 4.8 FDA's list of reported injuries caused by menstrual brands*

| *Menstrual cup use resulting in injury* | *Frequency* |
|---|---|
| Mooncup | 6 |
| Lilycup | 1 |
| Saalt | 5 |
| Divacup | 5 |
| Softcup | 4 |
| Femmcycle | 1 |

*Table 4.9 FDA's list of reported injuries by injury and brand of menstrual cup*

| *Injury* | *Frequency* | *Brand* | *Frequency* |
|---|---|---|---|
| Unknown | 3 | Mooncup | 1 |
| | | Saalt | 2 |
| Suction onto cervix | 2 | Divacup | 1 |
| | | Lilycup | 1 |
| Inability to remove | 4 | Saalt | 2 |
| | | Softcup | 2 |
| Inability to remove resulting in infection | 1 | Saalt | 1 |
| Prolapse | 1 | Mooncup | 1 |
| Pain without specified diagnosis | 4 | Divacup | 1 |
| | | Femmycycle | 1 |
| | | Mooncup | 1 |
| | | Softcup | 1 |
| TSS | 3 | Divacup | 2 |
| | | Mooncup | 1 |
| Silicone allergy | 1 | Mooncup | 1 |
| Expulsion of IUD | 1 | Mooncup | 1 |
| Uurinary tract infection | 1 | Softcup | 1 |
| Vaginal wound | 1 | Divacup or Softcup | 1 |

In the UK, menstrual cups not being classed as a medical device, the MHRA's Yellow Card scheme does not cover adverse effects from menstrual cups.

Published papers reporting adverse effects as a result of menstrual cup use are described in better detail. There is a small number of peer-reviewed, published illness or injury as a result of

menstrual cup use. For example, a case reported in a 17 year old as probable TSS caused by menstrual cup (Stanke et al 2020). Another patient was using the menstrual cup for the first time, and reported causing a "small abrasion" on one of her first insertions (Mitchell et al 2015, p. 218). Another otherwise healthy 20 year old used a menstrual cup safely for a year, but one month ran out of soap so did not wash her menstrual cup between emptying and developed TSS (El Soufi et al 2021).

Testing menstrual cups in vitro for their influence on *Staphylococcus aureus* growth showed that menstrual cups did not inhibit growth, meaning that TSS risk is not reduced by using menstrual cups over tampons. The level of *S. aureus* growth was significantly lower with TPE menstrual cups made from compared to silicone menstrual cups, which could be explained by the oxygen permeability of silicone. This being said, it was found that aeration influences toxin production more than the composition of the menstrual cup, and aeration was inevitable in this study where *S. aureus* was incubated in menstrual cups sitting in plastic bags, from which it was difficult to remove all the air. This study therefore highlighted the importance of the advice given to users on emptying their menstrual cups early and frequently enough, before the volume of menstrual fluid exceeds that of the menstrual cup, preventing menstrual blood contact with the vaginal mucosa which would cause toxin transfer into the blood and the development of TSS (Nonfoux et al 2018).

This shows the importance of education surrounding TSS: it is well known that tampons are a cause of TSS, but it needs to be understood that it is not simply the introduction of the tampon that causes the *S. aureus* toxin to develop. If everyone understood that *S. aureus* can cause infection and be introduced to the bloodstream through any cutaneous and soft tissue abrasion (Miller & Cho 2011), an abrasion to the vaginal wall would be a clear red flag to remove any product from the vagina, including menstrual cups.

Several cases of renal colic have been reported, with confirmation of the menstrual cup blocking the urinary tract and causing pain for a number of hours. In two of these cases it was confirmed that the menstrual cup was inserted incorrectly (Athiel et al 2019; Stolz et al 2019), and in the third case the menstrual cup was "introduced oriented to the right side" (Nunes-Carneiro et al 2018, p. 29). Similarly, a case of renal swelling was caused by "deeply inserted" menstrual cup suctioning on the fornix and entrapping the ureter (Wilhite & Rogers 2020, p. 6). Another case, this time of renal swelling, was caused by menstrual cup use, with no comment on incorrect use, causing abdomen pain on two separate menstrual cycles (Umaramanan et al 2019). In this case, a patient experienced abdominal pain when wearing a menstrual cup for two periods, and did not immediately remove the menstrual cup. In all cases, removal of the menstrual cup resulted in almost immediate alleviation of symptoms. In response to these cases, Wilhite and Rogers diplomatically state "Removal of a menstrual cup is simple, and subsequent resolution of

symptoms may obviate the need for further expensive work-up or exposure to ionizing radiation" (2020, p. 6). People must use common sense when using vaginally-worn devices, and make their removal the first port of call when experiencing any abdominal pain or discomfort. This also highlights the need for good instruction in correct use of menstrual cups and any vaginally-worn device.

In 2020, BBC's Victoria Derbyshire programme brought further issues to light regarding menstrual cup use. Interviewing Physiotherapist Katie Lough representing the Chartered Society of Physiotherapy, concerns have been raised over the instructions for removing menstrual cups, which direct users to pull down on the stem or base of the cup (Adams 2020). Even with the addition of air holes, due to the suction effect of the rim (needed to prevent leaks when worn), pulling the cup down creates negative pressure in the vaginal canal, which is the suspected cause for an unknown number of prolapses in the UK. There is no warning of this on the product's safety label. The instruction to also 'bear down' to push the menstrual cup lower in the vagina during removal is also described as conflicting with the Chartered Society of Physiotherapists' advice on exercises to reduce prolapse (ibid), providing a potential two-pronged increase in prolapse risk when using a menstrual cup.

### 4.3.3 Regulation

This thesis calls for the regulation of menstrual cups, as currently there is none, even though they *should* fit within class IIa invasive medical device by the MDR, as they are worn inside the body (MDR 2023, p. 10) for several hours at a time (ibid Annex VIII 1.2). The EDANA Guidelines for the Testing of Feminine Hygiene Products are clear, stating "Products like incontinence products and menstrual cups are outside the scope of these guidelines" (EDANA 2018, pg. 3). The FDA (2019a) recognizes menstrual cups as Class II medical devices (Title 21 FDA Chapter I Subchapter H Medical Devices). However, unlike tampons which are labelled and categorized according to their absorbency (21 CFR 801.430), menstrual cups are not categorized in any way. British standards do not mention menstrual cups within medical device nomenclature (BSI 2005). Due to this lack of regulation, there is a huge array of menstrual cups on the market, with an Amazon search for menstrual cups producing over 2,000 results as of April 2020 (amazon.co.uk).

A systematic review and meta-analysis across 43 studies found menstrual cups to be a safe option for handling menstruation. It also found the use of the menstrual cup showed no adverse effects on the vaginal flora, across four studies involving 507 people (van Eijk et al 2019). However, there are concerns for the safe use of menstrual cups. There are fears menstrual cups could increase the likelihood of developing endometriosis or adenomyosis (Cousins & Gargett 2018; Ma et al 2020b; NICE 2017; Spechler et al 2003). This emphasises on the need for menstrual cups to be regarded as medical devices, with their effects on the body fully understood and regulated.

### 4.3.4 Case study: problems using a menstrual cup

After discussing this work with the public, one person recounted their personal experiences and difficulties choosing and trying a menstrual cup for the first time. This person wished for their story to be documented and agreed to the case study being included in this thesis.

Approval for reporting this case study was granted by the Nottingham Trent University School of Science and Technology Non-Invasive Human Research Ethics Committee, (ID 1605748).

A nonparous 24 year old (JS) wanted to try a menstrual cup for economical and ecological reasons. With a healthy BMI, undertaking physical exercise 6 times a week, and a light to moderate menstrual flow, it was thought a firmer menstrual cup for those under the age of 30 with no history of childbirth would be appropriate. Being most popular in the UK, JS chose a Mooncup in size B, with one firmness option available. JS required the stem to be trimmed for comfort. The menstrual cup took a few attempts for initial insertion, but then opened within the vagina fairly easily. It seemed slightly too large, not for its volume, rather having difficulty unfolding the product after insertion, and particularly product removal was sometimes painful. Leaks sometimes occurred when the menstrual cup was not opened after insertion, but leaks and spillages were minimal; the main concern was the discomfort of potentially having too large a menstrual cup.

After a particularly painful experience, JS searched for a slightly smaller menstrual cup, still choosing a V-shape product. Me Luna offered Classic size small menstrual cup, so JS purchased one with a stem similar to that on the Mooncup. The size of this product was much more suitable, as insertion and removal were more comfortable. However, the product was much softer, being made of TPE, not silicon, and JS could not get the menstrual cup to unfold at all. The meant the menstrual cup was not usable.

Finally, JS searched for the same size and shape, and purchased the Me Luna Sport version, described as a “slightly harder menstrual cup… the cup unfolds perfectly and repositioning is not usually necessary” (Me Luna 2021). Being identical in size and shape as the previous choice, this menstrual cup was expected to open easily while remaining a suitable size for painless removal. This menstrual cup, although the firmest Me Luna product, was still softer than the Mooncup, and would remain unfolded in the vagina after the recommended twisting, pushing, and squeezing of the product. Menstrual cups summarised in Table 4.10.

*Table 4.10 Case study menstrual cup choices and reasons for failure. Scale bars 10mm.*

| *Attempt 1* | *Attempt 2* | *Attempt 3* |
|---|---|---|
| MoonCup | Me Luna | Me Luna |
| - | Classic | Sport |
| Size B | Size Small | Size Small |
| Silicon | TPE | TPE |
|  |  |  |
| Too large | Too soft | Too soft |

In total, JS spent 4 months attempting to find a suitable menstrual cup, paying total £62.79 on non-returnable items, and was not able to identify an ideal product. Where it was easy as a consumer to identify a menstrual cup shape and size that might be more suitable when JS had some personal experience, the ideal material and firmness was impossible to identify with the information available. It is clear that menstrual cups must be compared and categorised objectively to aid consumers in purchasing the correct menstrual cup for them.

### 4.3.5 Menstrual cup comparison

Peer-reviewed menstrual cup comparisons have been undertaken. One comparison by Shihata and Brody asks 834 participants to compare the Femmycycle menstrual cup against the menstrual cup that people are already comfortable using (2019). The Femmycycle is categorised as "bulbous" by Menstrual Cup Reviews (2020), and is different to the traditional "V" shape the participants were used to. Furthermore, it is designed with a folding lip that catches menstrual blood from overflowing or leaking when upturned. However, the methods of the study are not clear, with no statistical analysis. Were the participants gifted the Femmycycle to try, or did they purchase it themselves? Did each participant wear the product, or were reviews based on seeing or reading about the product? Literature has shown there to be a learning curve with menstrual cups: if used, for how many cycles were the products worn? The article makes a comparison to traditionally-shaped menstrual cups, without comparing the lengths, diameters, volumes, or firmness metrically, giving little context to the qualitative reviews. Finally, the first author is the

designer of the Femmycycle, so there may have been a conflict of interests in reporting this. Future menstrual cup comparisons must be objective, scientific, and provide consumers with the information required to make educated decisions on choosing a suitable internally-worn menstrual hygiene product.

In non-peer reviewed, non-scientific articles, menstrual cups have been qualitatively compared, including in terms of firmness. Examples of soft cups have been reported as the Bella Cup, Lily Cup, Si-Bell Cup, and the Super Jennie Cup, and examples of firm cups are the Lena Original Cup, Lunette Cup, MeLuna Sport Cup, and the Yuuki Classic Cup (Goldberg 2019). It is not clear how these menstrual cups were compared, with methods not being reported. In online video menstrual cup comparisons, firmness comparisons are done by simply squeezing the cups against eachother and seeing which compresses more (Farmer 2018). In online menstrual cup comparison charts, firmness is rated out of five (Hearn 2020; Menstrual Cup Reviews 2020). There is a lack in quantified comparisons of menstrual cups.

There are companies that offer menstrual cups in varying firmnesses, including Lena offering Original and Sensitive menstrual cups; MeLuna offering Soft, Classic, or Sport menstrual cups; Saalt offering Original or Soft menstrual cups; and Yuuki offering Soft, or Classic menstrual cups. Information on the cup types available explain the differing sizes available, but not the different firmnesses available (Me Luna 2020; Saalt 2020b; Yuuki 2020) and certainly don't provide metric information on the menstrual cups' firmnesses, providing minimal support for people choosing their menstrual cup and preventing an easy comparison of one brand's menstrual cup firmness to another.

Menstrual cups also come in a variety of shapes. According to Menstrual Cup Reviews, there are four basic menstrual cup shapes, Bell-shaped, V-shaped, Round-shaped, and Ergonomic-shaped (2020). Bell-shaped cups can have a flared rim and include the Lena Cup, LoulouCup, Sckoon Cup, and Si-Bell Cup. Bell-shaped cups without a flared rim include the LaliCup, Ruby Cup, Saalt Cup, and Super Jennie Cup. V-Shaped cups include the Casco Cup, Diva Cup, Mermaid Cup, and Monthly Cup. Round-shaped cups include the FemmyCycle Cup, Formoonsa Cup, Merula Cup, and Tieut Cup. Only one Ergonomic-shaped menstrual cup is listed, being the Fun Cup (Goldberg 2020). However, on the original comparison chart, the shapes are still listed as, "V" shape, "bell" shape, "spherical/bulbous", or "other" (2020). This thesis recommends that menstrual cup shapes are generalised into: V-shaped, Bell-shaped, Round-shape, and Asymmetrical-shape, as this minimises emotive descriptors such as "bulbous" or "ergonomic" and best describes the menstrual cups.

This work proposes that consumers must be able to identify which menstrual cup would work for them, because it's size, shape, and firmness have direct implications for comfort, usability, and safety, as summarised in Table 4.11.

*Table 4.11 Menstrual cup improper fit matrix*

| *Fit* | *Potential minor issues* | *Potential major issues* |
|---|---|---|
| Too small | Menstrual cup will not form a seal against the vaginal wall, causing leaks. | Menstrual cup rim could suction around the cervix, causing pain or prolapse if pulled during removal. |
| Too soft | Menstrual cup will not open, causing leaks. | - |
| Too large | Discomfort experienced during insertion and removal. | Obstruction of urine flow, causing renal colic. Difficulty removing menstrual cup could cause prolapse. |
| Too firm | Discomfort experienced during insertion and removal. | Obstruction of urine flow, causing renal colic. |

It is concerning that menstrual cups are not classed as medical devices, with little regulation to their design and manufacture in comparison to female condoms, vaginal rings, and vaginal suppositories, even though all of these products are worn vaginally for hours or days at a time. In the next part of this thesis, the aim is to compare a number of menstrual cups to establish a baseline understanding of the products available. The outcome of this comparison is to answer the following questions:

- How do menstrual cups across different brands compare in terms of general size, shape, material, volume, and firmness?
- With a consumer approximately knowing the rate of their menstrual flow, can they estimate the volume of a menstrual cup by a its general size?
- Is menstrual cup volume consistent across menstrual cup shape types, and can a consumer estimate the volume based on a menstrual cup's shape?
- Is a menstrual cup's compressive strength (firmness) consistent across menstrual cup material types, and can a consumer estimate a cup's compressive strength based on a menstrual cup's material?
- Is a menstrual cup's compressive strength (firmness) consistent across menstrual cup shape types, and can a consumer estimate a cup's compressive strength based on a menstrual cup's shape?

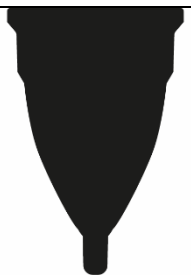
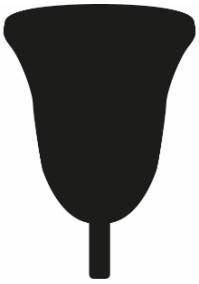
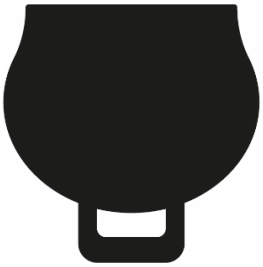
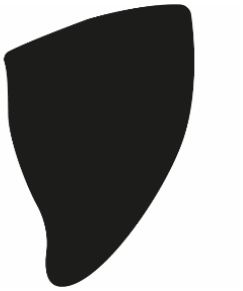
Menstrual Cup Reviews, the largest collection of objective menstrual cup ratings to be found on the internet, was used as a reference to identify which menstrual cups to study. The following cups were chosen because of their high ratings and popularity, true of April 2020. A range of menstrual cups were chosen to span across the general shapes: V-shaped, Bell-shaped, Round-shape, and Asymmetrical-shape, and across the three materials: Silicon, TPE, and Natural Rubber. See Figure 4.2 for the menstrual cups chosen for this study, Table 4.12 for the menstrual cup category matrix chosen for this study, and Table 4.13 for shape category definitions. A minimum of two menstrual cups in each category were chosen. Being the most prevalent material on the market, it was possible to study silicon menstrual cups from all four shape categories. Being the most prevalent shape on the market, it was possible to study V-shape menstrual cups in the three available materials.


*Figure 4.2 Menstrual cups compared in this study. Scale bars 10 mm.*

*Table 4.12 Menstrual cup categories*

| | | *Shape* | | | |
|---|---|---|---|---|---|
| | | *V-shape* | *Bell-shape* | *Round-shape* | *Asymmetrical-shape* |
| *Material* | *Silicon* | Lunette | Sckoon Cup | Merula | Lily Cup |
| | | DivaCup | Lena Cup | Femmycycle | Fun Cup |
| | | Mooncup | | | |
| | | Organicup | | | |
| | *TPE* | Me Luna | | | |
| | | Hello Cup | | | |
| | *Rubber* | Fair Squared | | | |
| | | The Keeper | | | |

*Table 4.13 Menstrual cup shape category definitions*

| *Shape category* | *Example/Visual* | *Definition* |
|---|---|---|
| V-shape |  | Menstrual cup vessel tapers in gradually from the rim to the stem, with the rim as the widest part of the menstrual cup. The vessel is longer than it is wide. |
| Bell-shape |  | A rounder vessel that may flare up at the rim, with bell-shaped curves. The vessel is longer than it is wide. |
| Round-shape |  | A more spherical-shaped vessel, the vessel is wider than it is long, with the widest point of the vessel being below the rim. |
| Asymmetrical-shape |  | Any asymmetrical menstrual cup. These are designed to sit at a particular rotation and angle under the cervix, with the rim not necessarily being perpendicular to the axis of the vessel. The vessel is longer than it is wide. |

Several cup manufacturers offer a variety of size options, which often includes two options; one smaller option for people under the age of 30, with no history of pregnancy and childbirth, and a larger option for people over the age of 30, or with history of pregnancy and childbirth.[*] However, some companies offer yet a smaller menstrual cup for teenagers or beginners (Me Luna, Organicup, Hello Cup), and some larger still (Me Luna). It was not possible to purchase every menstrual cup available. Therefore, one menstrual cup size was chosen from each manufacturer, advertised to those under 30 years old who have not given birth. This category was consistent across most menstrual cup manufacturers, even if the size category names were not consistent (i.e. size Small, size B), as seen in Table 4.14. There were some exceptions: Hello Cup and Me Luna categories differed. From Hello Cup, "one size fits most" size menstrual cup for "under 35 years old and/or super sporty" was chosen. From Me Luna, "Size M mainly used by women of all ages with normal muscles and medium flow" was chosen.

*Table 4.14 Menstrual Cup Properties*

| *Name* | *Size* | *Material* | *Shape* | *Additional feature* |
|---|---|---|---|---|
| DivaCup | Model 1 | Silicone | V-shape | - |
| Fair Squared | Size M | Rubber | V-shape | - |
| Femmycycle | Regular | Silicone | Round-spherical-shape | Anti-spill |
| Fun Cup | Size A | Silicone | Asymmetrical-shape | - |
| Hello Cup | Size S/M | TPE | V-shape | - |
| Lena Cup | Small | Silicone | Bell-shape | - |
| Lily Cup | Size A | Silicone | Asymmetrical-shape | - |
| Lunette | Model 1 | Silicone | V-shape | - |
| Me Luna | Classic size M | TPE | V-shape | - |
| Merula | One size available | Silicone | Round-spherical-shape | - |
| Mooncup | Size B | Silicone | V-shape | - |
| Organicup | Size A | Silicone | V-shape | - |
| Sckoon Cup | Size 1 | Silicone | Bell-shape | - |
| The Keeper | Size B | Rubber | V-shape | - |

[*] There is limited literature on which to base this variety in menstrual cup size. With the vaginal canal being muscular, it is thought that after birth this would return to its original size. However, little research has been done on this. One explanation of the increase in size of menstrual cup post-birth could be due to the increase in menstrual blood volume loss measured using menstrual cups, with nulliparous women having a mean menstrual blood loss volume of 45.9 mL, and multiparous having volume of 99. mL (Donoso et al 2019). This needs to be clarified.

*Dimensions*

Digital callipers (RS Components) were used for all length measurements: total length, vessel length, stem length, rim width, and width at widest part. Care was taken to not compress or distort the menstrual cups when measuring. It was also necessary to examine a menstrual cup's general size, because consumers are unlikely to know the actual volume of menstrual blood loss each month, and where sanitary pads and tampons have absorbency ratings to aid in correct product selection, menstrual cups do not. Consumers will therefore judge menstrual cup size visually. General size was quantified by a menstrual cup's vessel length × vessel maximum diameter.

*Volume*

To calculate menstrual cup volume, each menstrual cup was filled with water to the desired volume: volume to holes, and total volume. Volume was calculated, with volume = mass/density, and 1 $cm^3$ water weighing 1g, measured using Kern PCB 1000 g × 0.01 g laboratory balance (Kern).

*Compressive strength/ firmness*

Menstrual cup firmness could not simply be undertaken by comparing each product's material's shore hardness following testing standards in isolation to its overall design, because these findings would not be translatable to real menstrual cup use. Product thickness, material, and overall shape heavily influence its firmness.

Compression testing was undertaken using the Instron Universal Testing System 3367 (Instron) in compression mode, fitted with a 500 N load cell and using Bluehill 2 software control. The menstrual cup was placed between the centre of the plates and compressed just enough to hold in place at its widest point. The product was compressed at a constant rate of 5mm/min to a compression of 50 ± 0.5% of the menstrual cup's maximum diameter. Each menstrual cup was compressed five times, rotating the menstrual cup approximately 30 ° after each test.

*Data Analysis*

A Shapiro-Wilk test examined whether continuous variables were normally distributed*, and a means analysis was used to test the data's homoscedasticity, to examine whether or not data violated the assumptions required for ANOVA and Pearson's correlation test. Assumptions for Pearson's correlation test were violated, so a Spearman's rank-order correlation was undertaken. As sample sizes between the dependent variable groups were not equal, a non-parametric

* A Shapiro-Wilk test was undertaken for menstrual cup size, volume, and compressive strength variables. The test showed the menstrual cup size and compressive strength data did not show a significant departure from normality, $W(14) = 0.927$, $p = 0.279$ and $W(14) = 0.939$, $p = 0.403$ respectively, but that volume data did show a significant departure form normality $W(14) = 0.856$, $p = 0.027$.

Levene's test was used to test homogeneity of variances (Nordstokke & Zumbo 2010)[*]. A Kruskal-Wallis test was used when data was not normally distributed but displayed homogeneity of variances shown via a non-parametric Levene's test, or when data was not homoscedastic, being a violation of ANOVA assumptions. Descriptive statistics were used when appropriate. Quantitative data analysis was undertaken using IBM SPSS Statistics 26.0 (IBM Corp.).

## *Results*

### *Menstrual cup size, shape, material, volume, and compressive strength*

Menstrual cup properties varied greatly across the differing brands, as shown in Table 4.15. The range in materials available allows a consumer with allergy concerns to purchase a menstrual cup safely. Menstrual cup shape could certainly be a factor in choosing a menstrual cup, and the variety in shape available is broad, with V-shape being the most prevalent on the market.

The dimensions of each menstrual cup were sometimes difficult to measure or slightly inaccurate, although arguably to a negligible degree.[†] Menstrual cup total length ranged from 50.00 mm (Fun Cup) to 78.60 mm (The Keeper) (*M* = 67.98, *SD* = 7.26), vessel length ranged from 40.00 mm (Merula) to 68.00 mm (Lily Cup) (*M* = 49.21, *SD* = 6.69), and maximum diameter ranged from 39.00 mm (Lily Cup) to 48.70 mm (Femmycycle) (*M* = 42.16, *SD* = 2.49). General vessel size varied from Hello Cup being visually the smallest, and Lily Cup being the largest, even though Lily Cup was the narrowest and Femmycle was the widest menstrual cup at its widest point, and despite all the menstrual cups being marketed to people under the age of 30 having never given birth. This shows that menstrual cup size across the brands could be confusing or misleading, and there should be some guidance or structure for comparing menstrual cup size.

Volume to holes varied from 13.03 mL (The Keeper) to 22.16 mL (DivaCup) (*M* = 18.37, *SD* = 2.65). Although volume to holes is a more meaningful value to rate a menstrual cup's volume as it shows a true usable volume, three menstrual cups did not have air holes. Total volume is the only volume comparable across the full range of menstrual cups, and ranges from 18.88 mL (Sckoon Cup) to 38.14 mL (Merula) (*M* = 26.03, *SD* = 4.21), meaning a user will be required to empty the Sckoon Cup around twice as often as the Merula with the same rate of menstrual blood loss.

Compressive strength, or firmness, measured by compressive load at 50% diameter compressive extension varied from 3.385 N (Fair Squared) to 13.915 N (Hello Cup) (*M* = 8.83, *SD* = 3.25), meaning one menstrual cup marketed to a nonparous 25 year old will be too soft and not open

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* A non-parametric Levene's test showed the variances for compressive strength among both the shape groups and the material groups were equal; $F(3,10) = 1.65$, $p = 0.24$, and $F(2,11) = 0.28$, $p = 0.76$ respectively. The non-parametric Levene's test showed the variances for volume among shape groups were also equal; $F(3,10) = 0.41$, $p = 0.75$.

† Imperfections and warping in the stem (Sckooncup and Organicup), and the rim (Femmycycle, Organicup and Hello Cup) due to the casting process made them difficult to measure. Organic shapes were particularly difficult to measure, notably as its widest part was not transverse to the length of the vessel (Fun Cup).

inside the vagina, causing leaks, and another marketed to the same person would be too firm and may feel uncomfortable, or could even cause prolapse in a serious case. Therefore, this thesis proposes that menstrual cup firmness is categorised by the following table to empower consumers to find a suitable menstrual cup (Table 4.16). As more menstrual cups are tested, they can be included in the table, with the categorisation brackets perhaps evolving with new information.

| Name | Size | Material | Shape | Total length / mm | Vessel length / mm | Stem length / mm | Rim width / mm | Maximum diameter / mm | General Size / $mm^2$ | Volume to holes / mL | Total volume / mL | Compressive load to compress menstrual cup 50% (±0.5%) maximum diameter / N | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Compression cycle | | | | | |
| | | | | | | | | | | | | 1 | 2 | 3 | 4 | 5 | Mean (SD) |
| DivaCup | Model 1 | Silicone | V-shape | 66.00 | 56.00 | 10.00 | 42.50 | 42.50 | 2380.00 | 22.16 | 27.81 | 6.12 | 5.58 | 5.68 | 5.06 | 7.67 | 6.02 (*0.89*) |
| Fair Squared | Size M | Rubber | V-shape | 65.20 | 48.20 | 17.00 | 40.80 | 40.80 | 1966.56 | 14.26 | 21.13 | 3.38 | 3.35 | 3.49 | 3.34 | 3.37 | 3.39 (*0.05*) |
| Femmycycle | Regular | Silicone | Round-shape | 64.00 | 42.00 | 22.00 | 39.00 | 48.70 | 2045.40 | …[a] | 27.59[b] | 12.01 | 11.83 | 11.84 | 11.60 | 11.77 | 11.81 (*0.13*) |
| Fun Cup | Size A | Silicone | Asymmetrical-shape | 50.00 | 50.00 | …[c] | 37.90 | 41.00 | 2050.00 | 19.62 | 26.24 | 15.66 | 14.49 | 11.18 | 10.70 | 13.20 | 13.05 (*1.89*) |
| Hello Cup | Size S/M | TPE | V-shape | 60.00 | 41.70 | 18.30 | 41.00 | 41.00 | 1709.70 | 21.00 | 25.44 | 13.28 | 14.38 | 14.06 | 13.12 | 14.74 | 13.92 (*0.62*) |
| Lena Cup | Small | Silicone | Bell-shape | 71.00 | 47.50 | 23.50 | 41.00 | 41.00 | 1947.50 | 20.46 | 25.03 | 10.22 | 10.67 | 10.29 | 10.54 | 10.64 | 10.47 (*0.18*) |
| Lily Cup | Size A | Silicone | Asymmetrical- shape | 78.00 | 68.00 | 10.00 | …[d] | 39.00 | 2652.00 | …[a] | 28.15 | 5.89 | 5.92 | 5.37 | 5.16 | 5.13 | 5.49 (*0.35*) |
| Lunette | Model 1 | Silicone | V-shape | 72.40 | 49.00 | 23.40 | 41.00 | 41.00 | 2009.00 | 18.57 | 25.06 | 5.75 | 5.73 | 6.32 | 7.16 | 6.54 | 6.30 (*0.53*) |
| Me Luna | Classic M[e] | TPE | V-shape | 63.00 | 50.00 | 13.00 | 41.00 | 41.00 | 2050.00 | 18.01 | 23.92 | 9.03 | 8.07 | 8.53 | 12.72 | 7.83 | 9.24 (*1.79*) |
| Merula | …[f] | Silicone | Round-shape | 73.00 | 40.00 | 33.00 | 40.00 | 46.00 | 1840.00 | …[a] | 38.14 | 11.60 | 10.87 | 14.17 | 13.98 | 18.27 | 13.78 (*2.59*) |
| Mooncup | Size B | Silicone | V-shape | 73.00 | 50.30 | 22.60 | 43.30 | 43.30 | 2177.99 | 17.18 | 24.71 | 6.26 | 6.39 | 5.92 | 5.46 | 5.82 | 5.97 (*0.33*) |
| Organicup | Size A | Silicone | V-shape | 67.00 | 48.60 | 18.40 | 41.00 | 41.00 | 1992.60 | 20.02 | 28.06 | 7.34 | 6.10 | 6.59 | 6.56 | 7.25 | 6.77 (*0.46*) |
| Sckoon Cup | Size 1 | Silicone | Bell-shape | 70.50 | 45.20 | 25.30 | 40.00 | 40.00 | 1808.00 | 17.81 | 18.88 | 8.60 | 8.55 | 8.83 | 8.69 | 8.49 | 8.63 (*0.12*) |
| The Keeper | Size B | Rubber | V-shape | 78.60 | 52.50 | 26.10 | 44.00 | 44.00 | 2310.00 | 13.03 | 24.22 | 8.20 | 9.18 | 9.04 | 8.94 | 8.83 | 8.84 (*0.34*) |

a = No air holes, b = Measured to flipped anti-spill lip, c = No stem, d = No rim, e = With stem, f = No specific name

*Table 4.16 Proposed menstrual cup firmness categories*

| *Category Name* | *Symbol* | *Compressive load at 50% diameter compression / N* | *Brand Examples* |
|---|---|---|---|
| Very soft | ● | x ≤ 5.2 | Fair Squared |
| Soft | ●● | 5.2 < x ≤ 7.4 | DivaCup, Lily Cup, Lunette, Mooncup, Organicup |
| Medium | ●●● | 7.4 < x ≤ 9.6 | Me Luna, Sckooncup, The Keeper |
| Firm | ●●●● | 9.6 < x ≤ 11.8 | Lena Cup |
| Very firm | ●●●●● | 11.8 < x ≤ 14.0 | Femmycycle, Fun Cup, Hello Cup, Merula |

*General size and volume*

A Spearman's correlation test showed there was no statistically-significant correlation between menstrual cup's general size and its volume, $r_s(14) = 0.183$, $p = 0.532$. This means a consumer cannot visually judge a menstrual cup's size (the vessel's height × width) and estimate the volume of menstrual blood it can contain. A difficulty arises in rating menstrual cups on either their size or volume. It is the aim for this work to propose a table, as suggested with Table 4.16, to allow consumers to easily differentiate between the menstrual cup brands. However, as these characteristics are independent of each other, which is most suitable to compare? Would it be more suitable to categorise menstrual cups by volume, allowing consumers to judge by their menstrual blood loss? This might result in a suitable volume not being a suitable shape for the consumer: too wide, or too long. Conversely, categorising menstrual cups by size, allowing consumers to choose based on their vaginal length or cervix height for example, will not reflect its volume. Similarly, consumers choosing the 'largest' menstrual cup thinking that it will meet their heavy menstrual flow may be disappointed. This remains an unanswered question in this thesis. Fortunately, menstrual cup brands are generally very clear on the volume of menstrual blood collected in each menstrual cup: it is consumers who perhaps do not know their menstrual blood volume.

*Shape and volume*

A Kruskal-Wallis test showed that distribution of volume was not significantly different across menstrual cup shape categories, $\chi2(3) = 6.000$, $p = 0.112$. This means a consumer can not predict the volume of a menstrual cup based on its shape, and may lead to a consumer selecting an inappropriate menstrual cup.

### *Material and compressive strength*

A Kruskal-Wallis test showed that distribution of compressive strength was not significantly different across menstrual cup material categories, $\chi2(2) = 2.880$, $p = 0.237$. This means a consumer cannot estimate a menstrual cup's firmness by its material. A menstrual cup with the incorrect firmness may be purchased, being unusable for the consumer, being uncomfortable or increasing the risk of injury.

### *Shape and compressive strength*

A Kruskal-Wallis test showed that distribution of compressive strength was not significantly different across menstrual cup shape categories, $\chi2(3) = 3.171$, $p = 0.366$. This means a consumer can not estimate a menstrual cup's compressive strength based on its shape, and may buy a menstrual cup too firm or too soft for them, making the product unusable or increasing the risk of discomfort or injury.

### *Discussion and conclusion*

Choosing the 14 most popular and highly-rated menstrual cups according to Menstrual Cup Reviews (2020), this study was a first step to compare menstrual cups across different brands in terms of design, material, mechanical, and physical properties.

In this comparison of 14 menstrual cups, dimensions varied greatly, as did total volume which ranged from 18.88 mL (Sckoon Cup) to 38.14 mL (Merula), meaning users will be required to empty the Sckoon Cup around twice as often as the Merula with the same rate of menstrual blood loss. All the menstrual cups tested here were marketed to be suitable for people under the age of 30 having never given birth, and as the range in shape and size in Figure 4.2 shows, variation in menstrual cups could be confusing. General menstrual cup size is not related to its volume, so judging by size can be misleading. There should be some guidance or structure for comparing menstrual cup size, which was an aim for this thesis. However, as there is no correlation between general size and volume, it is unclear whether it would be more suitable to categorize menstrual cups by volume or size. Judging by visual size, allowing consumers to judge by their menstrual blood loss, might result in a suitable volume not being a suitable shape for the consumer: too wide, or too long. Conversely, categorising menstrual cups by size and allowing consumers to choose based on their vaginal length or cervix height for example, will not reflect the menstrual cup's volume. Similarly, consumers choosing the visually 'largest' menstrual cup thinking that it will meet their heavy menstrual flow may be disappointed. International Standards for contraceptive diaphragms (ISO 2014) contraceptive regulate dimensions of vaginally-worn contraceptive devices, specifying that size must sit within certain limits, from 55 mm to 100 mm, but this is not applicable to menstrual cups. If menstrual cup size was categorised in a similar way, and this categorisation was standardised across brands, it would provide transparency for

consumers to make informed decisions. Size and volume categorisation remain an unanswered question in this thesis.

Firmness measured by the compressive load required to compress the menstrual cup to 50% (±0.5%) maximum diameter varied from 3.39 N (Fair Squared) to 13.92 N (Hello Cup). This means one menstrual cup marketed to a nonparous user under 30 years old will be too soft and not open inside the vagina, causing leaks, and another would be too firm, may feel uncomfortable, and could potentially cause injury such as renal colic. A menstrual cup's material or shape was not related to its firmness, meaning people would not be able to estimate which would be most suitable based on these. On the product packaging, Sckoon Cup advertises its product as "the softest and most advanced menstrual cup". However, this menstrual cup was found to sit within the 'medium' firmness category in this study. This work recommends manufacturers clearly label products with firmness categories, proposed in Table 4.16. A firmness comparison chart and clarity on packaging would prevent any misconceptions. This work proposes firmness categories ranging from, 'very soft', 'soft', 'medium', 'firm', and 'very firm'. In the same way that tampons are categorised in terms of absorbency for ease of use and comfort, as well as reducing risk of TSS (FDA 2019a), these categories can improve consumers' comfort and safety. If more responsibility lies with manufacturers to display the firmness of the menstrual cups, and practice shifts to educate healthcare practitioners to be able to support people in finding a suitable menstrual cup, consumers will find greater success and improved safety in using one. There will also be better understanding of the prevalence of injuries such as prolapse as a result of using a menstrual cup, as well as more accurate reportings of adverse incidents if healthcare professionals are more engaged in the process.

Finally, the menstrual taboo has forced silence surrounding menstrual hygiene practice, and by extension consumer inattention to the menstrual hygiene market (Soni & Ram 2020). By openly reporting and discussing the topic surrounding menstruation and menstrual hygiene practice, experience and safety can be improved.

### *Research since*

Since this work was published (Manley et al 2021), Sica et al completed a Procter & Gamble Company-funded comparison assessing safety and usability of their Tampax Cup (2022). They found the product to be safe to use, including in terms of microflora assay, microbiota safety, and the growth of *Staphylococcus aureus* and risk for toxic shock syndrome. They tested firmness but not as robustly as the methods presented here; the rim is only deformed by 10mm, but Sica et al did test more areas of the menstrual cup than the current study. They also report an interesting figure showing the Tampax Cup within a vaginal canal. Unfortunately the menstrual cup that the

Tampax Cup is compared against is not revealed, and there are no images of the product, meaning it is difficult to contextualise the results with the findings of the current study.

In terms of usability, 9.5% subjects reported impacts on bowel movements and 12.7% subjects reported impacts on urination using the Tampax Cup, and statistically significantly more subjects reported bowel movement changes for the Tampax Cup when compared to the other brand. Users of Tampax Cups perceived statistically significantly more discomfort when inserting compared to the other brand. There was greater discomfort during insertion and removal for both cups on the first day of the menstrual cycle and there was more insertion discomfort for the 25–34 year olds, wearing discomfort for shorter wear times and lower flow, and removal discomfort by subjects at lower body weights (ibid). The level of usability included in this study is excellent, unlike any other menstrual cup evaluation seen before, and should be industry standard.

Another menstrual cup analysis studied the effects of the Violeta Cup® and vaginal resting pressure, maximum voluntary contraction, and pelvic floor muscle tone. 10 participants used the cup for three months, with those not adapting to the product being excluded from the study. Seven participants reported discomfort during the first cycle, and three reporting discomfort throughout the study. It was found that the use of the vaginal cup for a period of three menstrual cycles changes the vaginal resting pressure, maximum voluntary contraction, and pelvic floor muscle tone, as well as improves the repetitions of pelvic floor muscle assessed by digital palpation (Schevchenco et al 2023). It is a shame that participants were not asked why they did not get on or adapt to the menstrual cup as this is valuable data. This is the first instance of quantitative data revealing the changes in pelvic floor function as a result of menstrual cup use, and could be an explanation for injury reported elsewhere (see Section 4.3.2).

*Limitations and future study*

Exploring inter-brand menstrual cup variations, particularly varying firmness categories, is missing from this first comparison. By only picking the most popular and highly-rated menstrual cup brands, this study is missing the lesser-known or newer brands, and importantly the very inexpensive menstrual cups that have been criticized in popular media for being potentially dangerous (Hearn 2018).

Size and volume categorisation remains an unanswered question and must be explored in future studies to identify how menstrual cups can be regulated properly. It is recommended for future studies to categorise all menstrual cups in terms of shape, material, size, total volume, firmness, etc. in a single table for easier decision-making by consumers. This understanding of menstrual cup physical and mechanical properties is a first step to later identifying whether certain menstrual cups hold inherently more risk of injury than others.

Compression testing was limited. Testing was undertaken at five points at room temperature, rotating the menstrual cup approximately 30 ° for each test. It does not represent the dynamic, three-dimensional compression of the vaginal canal and surrounding tissues. Where this study is an important initial comparison, future analysis of the mechanical properties of menstrual cups could be undertaken in more a realistic, dynamic setting. The FDA 'Syngyna testing' method could be utilised (FDA 2019a) which can be undertaken at 37 °C.

Van Eijk et al identified that menstrual cups ranged in price from 0.72 US$ to 46.72 US$ (2019). Menstrual cup cost was not included in this analysis. Particularly as Karnani found that targeting 'bottom of the period consumers' was not viable as a business model, worsening equality and access to safe menstrual products (2022), product cost will ultimately have an influence on a consumer's decision. Future analysis should measure whether product cost is associated with either initial menstrual cup uptake, or factors such as material quality, product longevity, or comfort (including chafing or abrasion due to the product being manufactured via slightly cheaper methods).

Future research of a user's age, history of childbirth, BMI, and even level of fitness could indicate which firmness category would be most suitable, removing the element of trial and error, and improving menstrual cup user experience. It was found that increased support and education in reproductive anatomy might improve menstrual cup use willingness and success (Beksinska et al 2021; Manley 2018). Future study is needed to show how an improved knowledge on reproductive anatomy could also help people choose which menstrual cup might be more suitable, for example after learning of having a "low" cervix. It would then be important to examine which factors are most significant in terms of safety and acceptability. Future study is needed to explore what consumers themselves are looking for when choosing a menstrual cup and how they would prefer to be supported in this experience.

### *Conclusion*

This is the first objective comparison of 14 menstrual cups, comparing DivaCup, Fair Squared, Femmycycle, Fun Cup, Hello Cup, Lena Cup, Lily Cup, Lunette, Me Luna, Merula, Mooncup, Organicup, Sckoon Cup, and The Keeper, which vary greatly. There is no correlation between a menstrual cup's size, shape, and volume, or a menstrual cup's material, shape, and firmness. Consumers guess which shape or material might suit them, and if incorrect may experience discomfort, leaks, and increase the risk of injury such as prolapse and renal colic. More research is clearly needed to further empower people to choose the correct menstrual cup, and improve their regulation from the FDA, the MHRA, and similar regulatory authorities worldwide. Consumers need more support and guidance from healthcare providers when choosing a

menstrual cup to make better decisions in their reproductive life. With this information, other people like JS (4.3.4) can save time, money, hassle, and reduce discomfort and pain.

It is not within the scope of this thesis to propose alternate safety and regulation advice beyond the suggestion of firmness categories for menstrual cups because it was not successful in proposing volume, size, or shape recommendations.

In general, people that use sanitary pads, tampons, and menstrual cups are being let down by regulators and product manufacturers. Product materials are not fully disclosed to customers, absorbency is not tested with menstrual blood, and even EDANA declares there are no guidelines for laboratories to declare a product as safe (2018, p. 8). While regulations are in place for sanitary pads and tampons to be labelled clearly with absorbency values to reduce risk of TSS, this should be the same for menstrual cups. Regulation should require all menstrual hygiene manufacturers to fully declare product composition, and absorbency/ capacity must be tested with a Syngyna where applicable, and using blood rather than saline for realistic values as undertaken by DeLoughery et al in their ground-breaking use of red blood cells to test product absorbency (2023). Consumers must currently still guess as to their ideal menstrual cup. Future research could identify whether an adapted Syngyna might reflect the variation in reproductive anatomy; for example a shorter, stouter Syngyna suited for “Round-shape” menstrual cups, or a longer, narrower Syngyna to test a “V-shape” menstrual cup. If menstrual hygiene products were considered medical devices as this thesis proposes, regulation should include microflora assay, microbiota safety, and the growth of *Staphylococcus aureus* to assess and mitigate risk for toxic shock syndrome. Firmness should be reported and categorised by manufacturers to mitigate risk of prolapse, and impact of menstrual cup use on bowel movement, urination, and general insertion and removal comfort. Finally, improved regulation should encourage a greater involvement with medical practitioners who would then report injuries and side effects to the MHRA to get a holistic and realistic understanding of the risks of menstrual hygiene products, feeding into a culture of recognition, accountability, and improvement. This will inevitably improve adoption and product satisfaction as progress trickles to the consumer.

So long as the sanitary pads, tampons, and menstrual cups adopted in MenSC donation comply to the existing regulation, there is nothing preventing the exploration of all three methods in MenSC isolation. Therefore, the next step is to explore potential protocol for MenSC extraction from sanitary pads and tampons to increase accessibility to MenSC donation and research.

# Chapter 5. Protocol development for MenSC donation via sanitary pad, tampon, and menstrual cup

It is possible to collect menstrual blood from a variety of products. Most popularly, menstrual cups are used. However, other products such as urine cups, catheters, and test tubes have also been used. Within the literature, it is common for one method to be adopted, however both tampons and menstrual cups have been used to collect MenSC without reporting how these methods compare, and without outlining the methods by which tampons have been used to extract MenSC (Martínez-Aguilar et al 2020). In response to **research question 4: How can MenSC be donated via sanitary pad or tampon?**, this chapter outlines the exploration and optimisation of proposed MenSC extraction from sanitary pads and tampons. This will increase accessibility to MenSC donation and research and clinical application of MenSC in general.

Protocol has been reported for collecting MenSC from menstrual cups and other devices, but never sanitary pads or tampons. This chapter describes the optimisation of suitable MenSC isolation techniques for both sanitary pads and tampons, initially simulating menstrual blood with bone marrow MSCs, then isolating MSCs from horse blood, before finally exploring MenSC isolation from menstrual blood. It is presented largely chronologically, with further information on each experiment in Appendix D. See Figure 5.1 for protocol optimisation strategy.

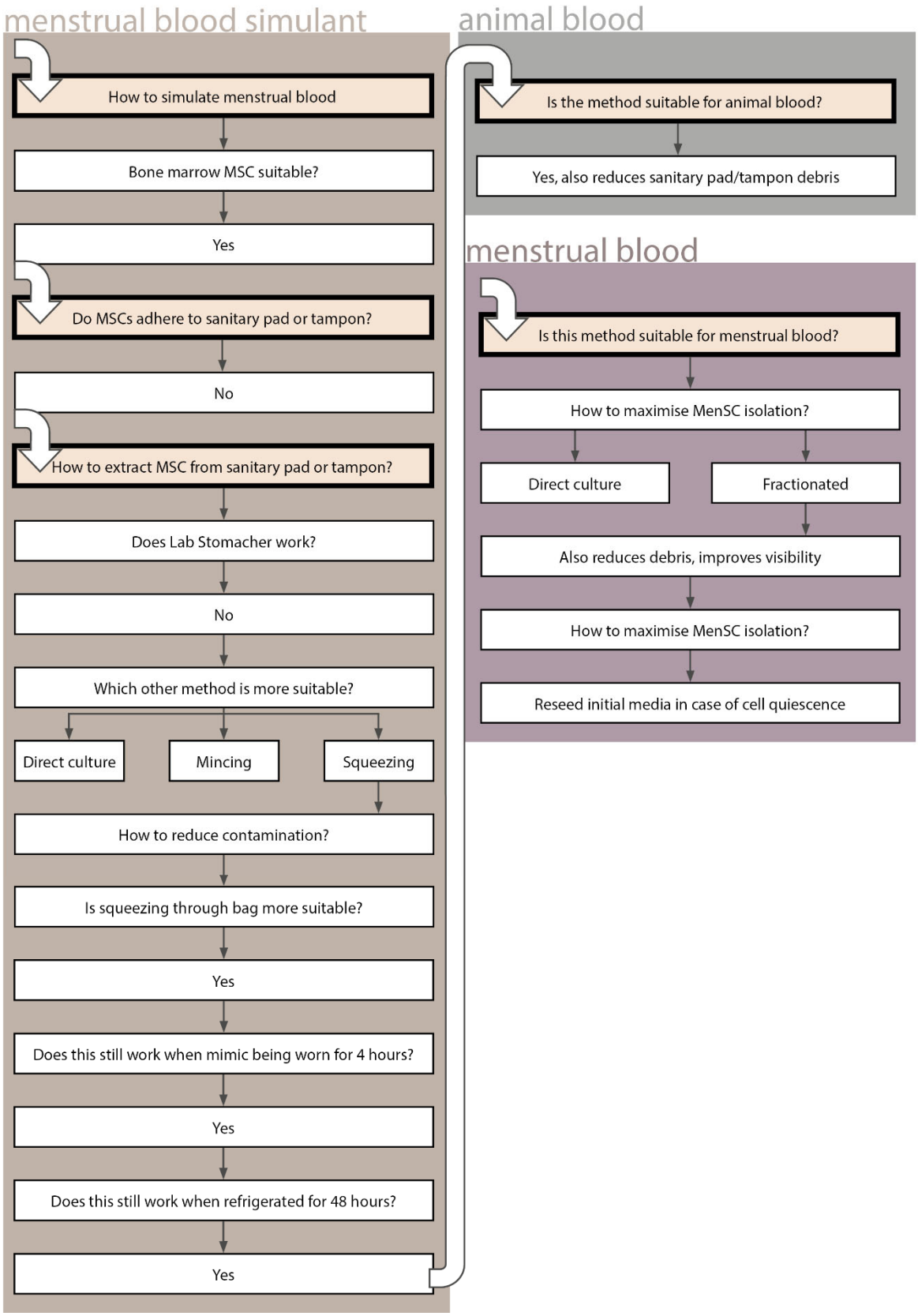


*Figure 5.1 Workflow for MenSC extraction protocol optimisation*

## 5.1 Menstrual blood simulant

Before menstrual blood was collected, the protocol was optimised using a substitute cell type. Bone marrow MSC and MenSC have been studied in tandem. Both cell lines were cultured using

the same methods (Wang et al 2017c; Rahimi et al 2018), or the same methods apart from antibiotic/antimycotic supplements (Hida et al 2008). Bone marrow MSC and MenSC are similar (see Figure 5.2), although not identical (Alcayaga-Miranda et al 2015b; Khanmohammadi et al 2012; Wang et al 2012), so bone marrow MSC were used in lieu of MenSC. This is particularly useful where MenSC have been described to be frequently contaminated due to the environment they are collected in (Ren et al 2018), so using commercially-available bone marrow MSC eliminates this risk in early protocol development.

The number of MenSC recovered from menstrual blood has been reported between 600 cells/mL of menstrual blood, which would be approximately 3 x $10^3$ MenSC per sample, (Alcayaga-Miranda et al 2015b), and as high as 3 x $10^7$ per sample (Borlongan et al 2010). Therefore, 1 x $10^6$ bone marrow MSC suspended in 5 mL medium were used as the substitute for MenSC, named menstrual blood simulant.

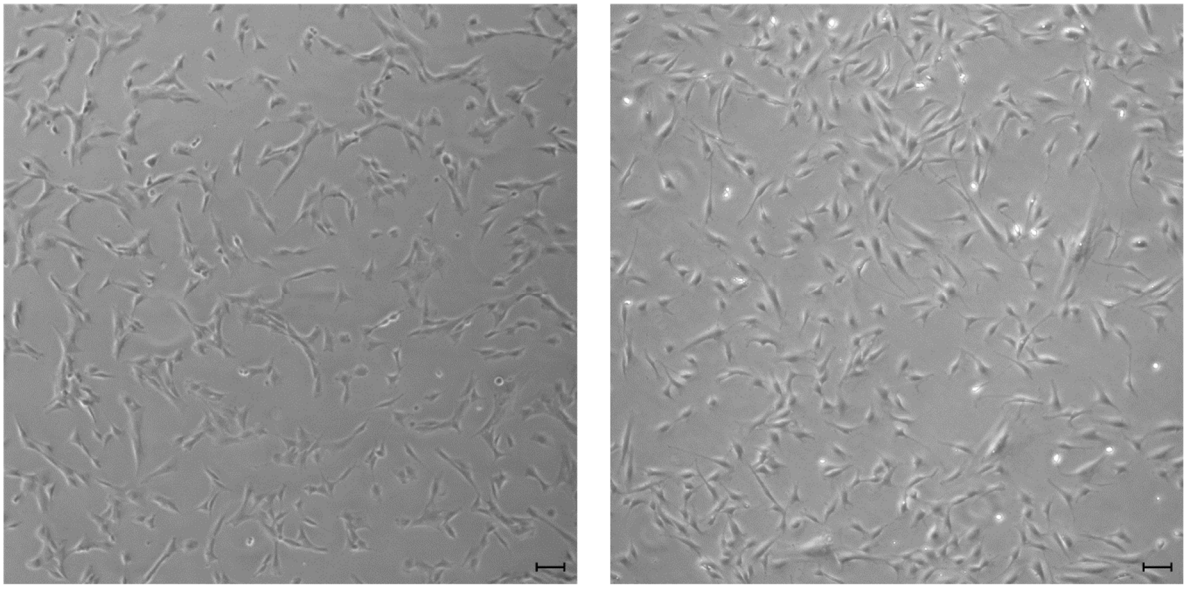

*Figure 5.2 Bone marrow MSC (left) and MenSC (right) in culture.*

*The cells adopt the same spindle-shaped morphology. Scale bars 100 μm.*

## 5.2 MSC adherence

The majority or MenSC isolation was undertaken with a menstrual cup, and there was no evidence of MenSC adhering to menstrual cups, so there was no need for a cell detachment step. MenSC adherence onto rayon, cotton, or superabsorbent polymer has never been reported in the literature, particularly in relation to tampon and sanitary pads. It was therefore unknown whether it would be necessary to have a detachment procedure.

Placing an entire menstrual blood simulant-absorbed product into a T75 flask or dish and viewing under a microscope for 14 days determined MSCs did not adhere. Visibility of any cells was challenging, as depicted in Figure 5.3. However, when cells were visible on the surface of the

flask, cells were adhering to the flask, rather than the sanitary pad or tampon, as seen in Figure 5.4. This demonstrated that a further detachment step would not be necessary in MenSC isolation.

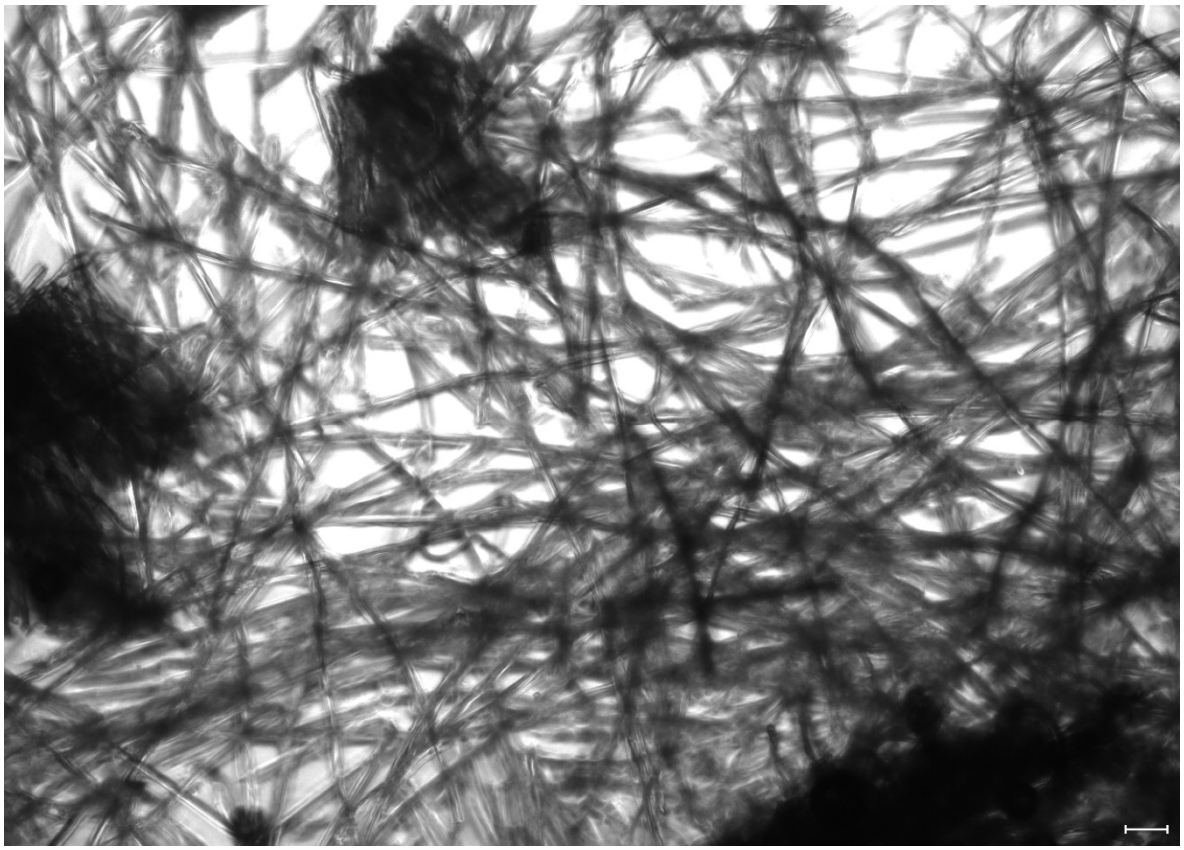

*Figure 5.3 Direct culture of the entire sanitary pad or tampon made it very difficult to observe cells, obstructed by tangle of fibres. Scale bar 100 µm.*

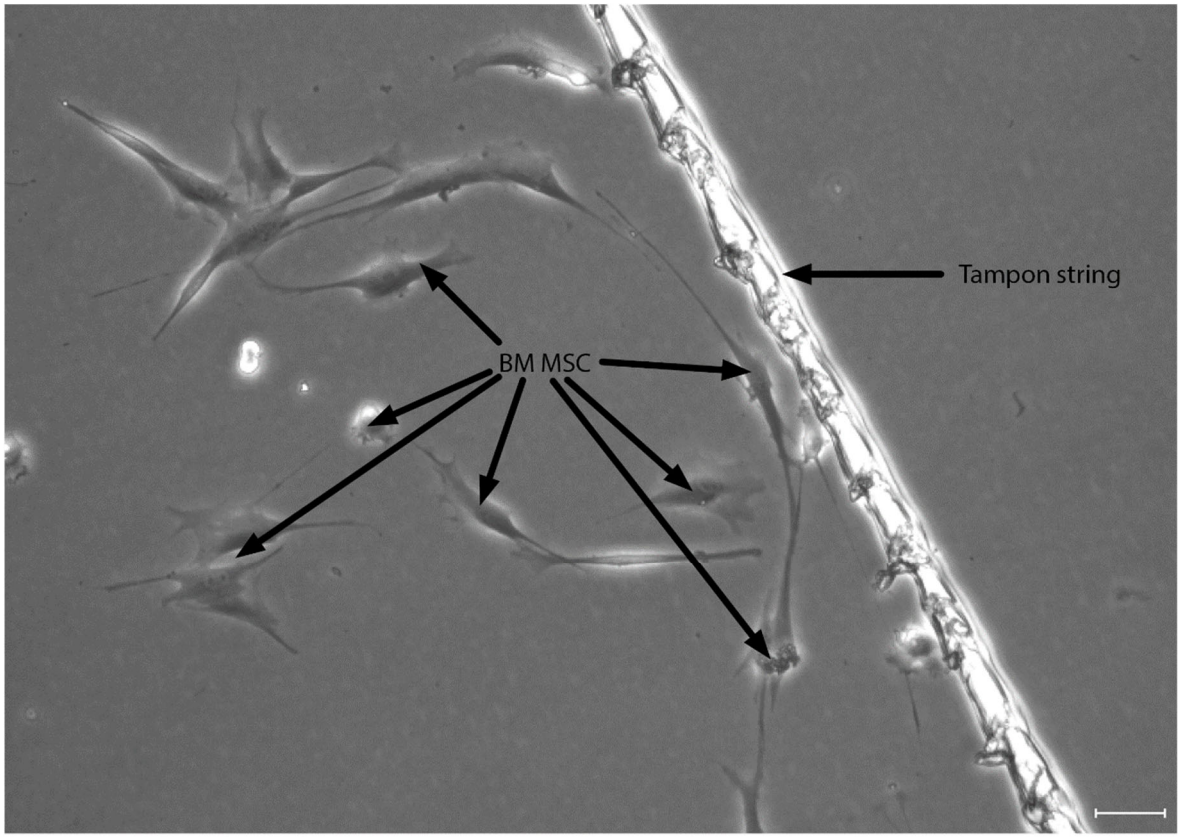


*Figure 5.4 Bone marrow MSCs adhering to the surface of the flask, rather than the tampon string. Scale bar 50 µL.*

## 5.3 Lab Stomacher

As an initial step to explore how menstrual blood may be extracted from sanitary pads and tampons, methods for measuring menstrual blood loss were explored. One method of measuring this is utilising the alkaline hematin method, converting haemoglobin to alkaline haematin which determines volume of blood in a sample via colorimetric analysis. Initially the used products were soaked in a solution for 48 hours before measuring blood loss (Haynes et al 1977). However, it was found that homogenising the sample improved accuracy, and removed the need for a 48 hour-long soak. Using a Smallboy benchtop washing machine (Tefal) for 10 minutes homogenised the sample (Gannon et al 1996, p. 2030), and a Lab Stomacher has also been used to pummel the samples (Magnay et al 2011; Newton et al 1977). Therefore, Lab Stomacher was initially identified as potential equipment to extract the MenSC from menstrual blood.

Bone marrow MSC were cultured in T75 flask containing mesenchymal stem cell growth medium (MSCGM) (Lonza) supplemented with 10% foetal bovine serum (FBS), 100 unit/mL of penicillin, 100 mg/mL of streptomycin and 0.25 µg/mL of Amphotericin B. Cells were incubated at 37 °C in a fully humidified environment with 5% CO2. The cell culture medium was changed every 2-3 days. When the cells reached 80% confluence, they were detached using 0.25% trypsin–ethylenediaminetetraacetic acid (EDTA) and passaged at a ratio of 1:3. bone marrow MSC at P5-11 were used for menstrual blood simulant.

5 mL menstrual blood simulant was pipetted either onto the tip of a tampon, or the centre of a sanitary pad in aseptic conditions. The entire tampon or sanitary pad was then placed and sealed in a Lab Stomacher bag (Seward), and run on a variety of settings, see Appendix D.

The homogenised supernatant was then pipetted into 50 mL centrifuge tubes and centrifuged at 300 × g for 5 minutes. The pellets were then combined to a single 15 mL centrifuge tube, centrifuged at 300 × g for 5 minutes, and the pellet resuspended in 1 mL MSCGM before viewing under the microscope and counting with a c-chip haemocytometer. Bone marrow MSC extraction rate was calculated total live cells post-extraction / total live cells pre-extraction × 100.

### *Result and discussion*

None of the experiments returned MenSC. After supernatant extraction and centrifugation, there appeared to be a visible cell pellet. However, after assessment in the haemocytometer there were no visible cells, rather there was a visible pellet of fibrous debris as well as a cloud of debris remaining in the medium after centrifugation (see Figure 5.5). This was confirmed when there were no cells in culture after seeding the pellet. It was concluded that the Lab Stomacher, even on its lowest setting, was too aggressive and the MenSC underwent lysis.

## 5.4 Mincing

It was then decided that treating the tampon or sanitary pad like a human tissue might be more suitable, for example finely mincing uterine tissue and clots (Wyatt et al 2021), by mincing with dissecting scissors.

After pipetting the menstrual blood simulant onto the tip of the tampon or the centre of the sanitary pad, the product was then immediately finely minced using dissecting scissors and tweezers, with pieces dropping into a 100mm petri dish containing growth medium (see Figure 5.6). The contents were then gently rocked from side to side to distribute the fibres evenly. The dish was then incubated in a cell culture incubator at 37 °C humidified environment and 5% CO2. After 24 hours, the largest fibres were lifted from the plate and transferred into fresh plates containing 12 mL of media to see if MSC had remained within the fibres. The original plate was washed three times with phosphate buffered saline (PBS). Media was changed every 2-3 days. Cell adherence and growth was visually determined on a daily basis using phase contrast microscopy for the next 14 days.

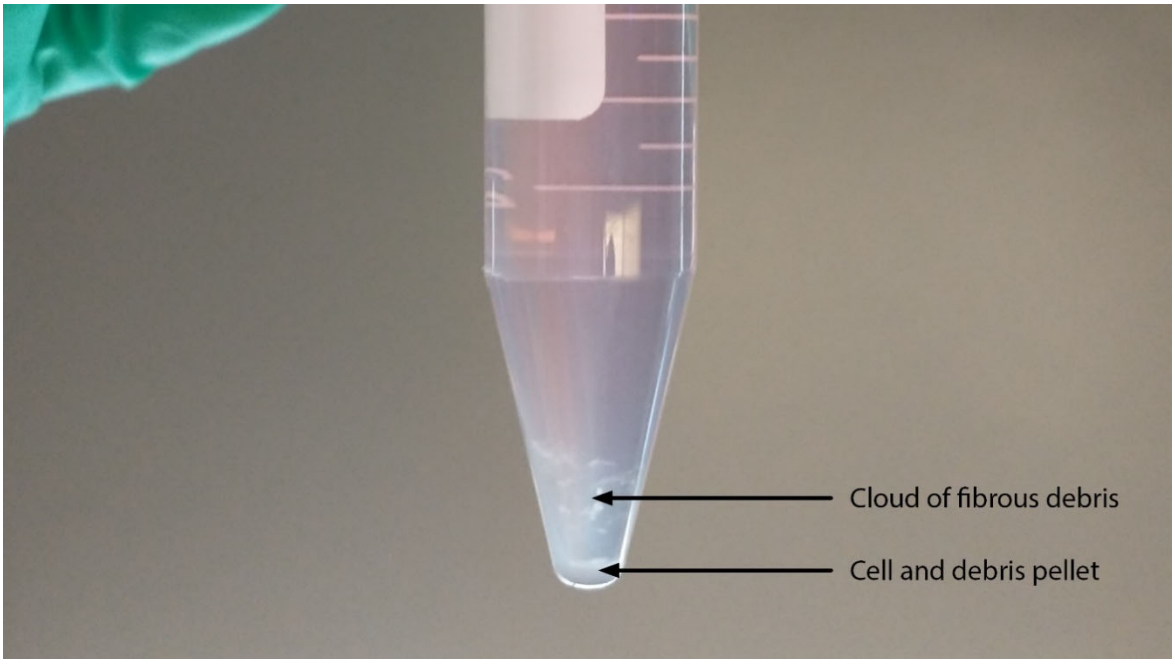


*Figure 5.5 Visible debris in and above the BM MSC pellet after centrifugation following extraction via mincing tampon.*

*Figure 5.6 Mincing a tampon in which menstrual blood simulant has been absorbed with dissecting scissors.*

### *Results and discussion*

Mincing the tampon or sanitary pad could take up to 30 minutes. This was identified as a factor determining the method's success; if other methods were more successful in extracting MSC then time taken to isolate the cells would be prioritised.

It was noted that the mechanical process or mincing the products squeezed the products, forcing menstrual blood simulant to drip down into the dish. If this proved the cells did not adhere to the fibres, it was thought that simply squeezing the liquid out of the product may be enough to extract the cells, provided this did not damage the MSCs in the process.

The minced fibres and segments of sanitary pad and tampon were left in the dish for one day. After this, the largest clumps of product were gently lifted from the dish without disturbing the cell monolayer and placed into a fresh dish with media. After removing the larger clumps from the dish after one day in culture, it was easier to see the cells adhere and grow in the dish under the microscope. See Figure 5.7. Very few cells were also recovered after the initial culture step, meaning that cells did not remain within the clumps and fibres of the sanitary pad or tampon once they had been minced and floated in the culture medium.

This proved that cells would not remain entangled within or adhered to the fibres and materials of sanitary pads or tampons, and indicated that the action of squeezing the product might be enough to release MSC from the product, potentially removing the need to mince.

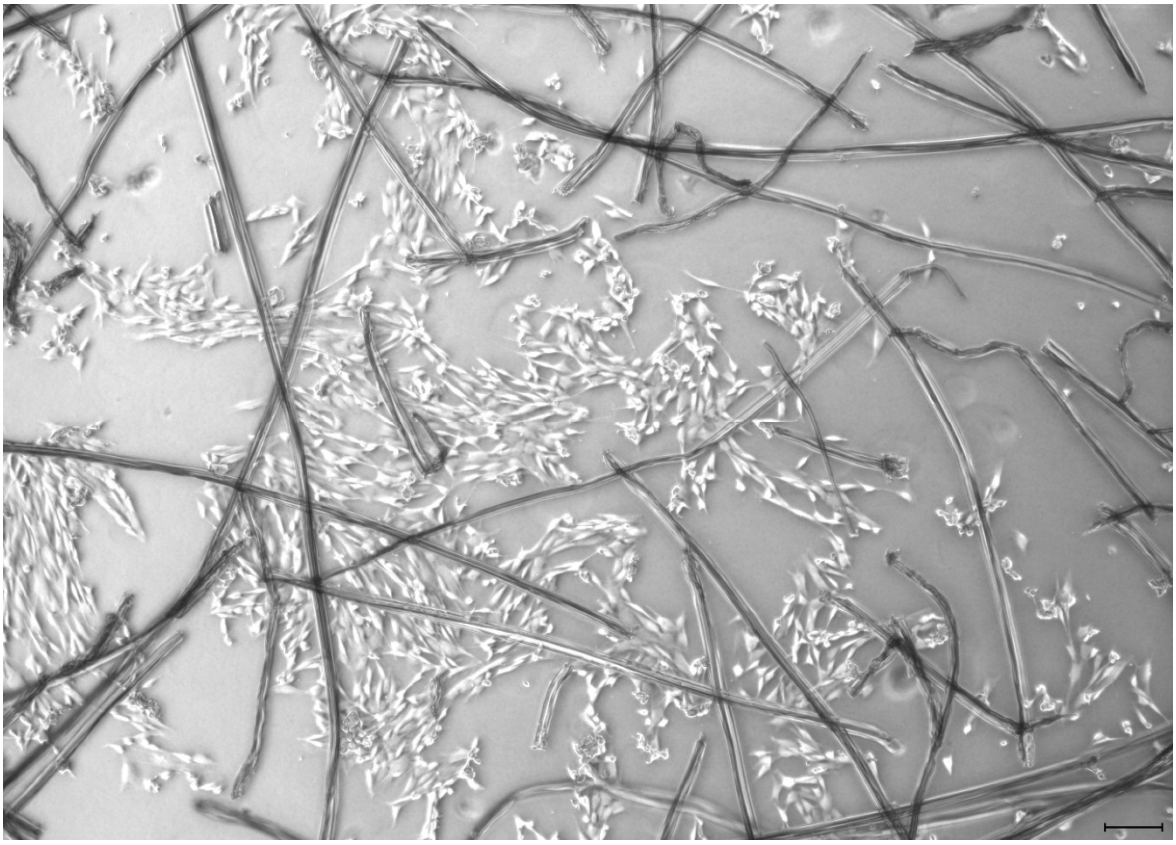

*Figure 5.7 Minced tampon fibres visible during MenSC culture.*

*These fibres remain in the dish after larger clumps of debris have been removed, before second wash. The cells are clearly adhered to the surface of the dish, rather than adhering to the fibres themselves. With this level of debris floating in the media, there was the concern that this would damage the cells. Scale bar 100 μm.*

## 5.5 Squeezing

Initially the tampon or sanitary pad, once impregnated with menstrual blood simulant, was squeezed by double-gloved hand directly into the culture dish. This resulted in the highest recovery of MSCs. It was decided that the sanitary pad or tampon would be squeezed through a sealable bag snipped at one corner. The benefits of this method include it being tidier, not directly handling the menstrual blood, being able to control the sample flow rate, and the fact that the

samples could come in the sealable bag directly from each participant reduced the number of handling steps. As samples collected by menstrual cup were added to a tube already containing 10 mL saline-antibiotics cocktail (collection mixture), the sealable bag would already contain the collection mixture, and this could be massaged to increase the number of MenSC recovered.

The next phase of protocol development explored whether the process identified so far would translate into success isolating mononuclear cells derived from animal blood, before moving onto human menstrual blood.

## 5.6 Animal Blood

Menstrual blood simulant containing bone marrow MSC was useful for identifying whether MSC could survive the proposed protocol for extraction from sanitary pad or tampon. The next level of complexity was to include the element of extracting the mononuclear cell fraction from blood (the fraction of cells of which MSC are part). The red blood cells and plasma is removed from the sample using density gradient fractionation. Horse blood was used as it is analogous to human menstrual blood, particularly defibrinated blood (removing the clotting agents of blood) without the need to use human samples.

Horse blood was chosen for its accessibility and affordability. Using whole horse blood made the protocol more realistic and relevant and did not affect the extraction of the mononuclear cells. The next step to protocol optimisation was to translate the procedure to collecting mononuclear cells from human menstrual blood.

## 5.7 Menstrual blood

The MenSC isolation protocol developed and tested with bone marrow MSC and horse blood was applied to optimise the MenSC isolation protocol, the final steps included identifying the required steps for use with human menstrual blood. Within the literature, it was common for the menstrual blood to be fractionated, with the mononuclear cells seeded in tissue culture flasks (Li et al 2022; Liu et al 2018; Skliutė et al 2021). However, researchers also reported having greater success by removing the fractionation step and culturing directly (Álvarez et al 2018; Dalirfardouei et al 2021; de Pedro et al 2021). The MenSC would naturally sediment to the bottom of the flask, adhere to the surface, and populate the dish. It was therefore important to identify which method would be most suitable to isolate maximum MenSC.

### 5.7.1 Direct culture vs. fractionated

Directly culturing the menstrual blood sample involved adding to the culture flask with the medium and is quick and straightforward. Fractionating the sample prior to seeding required additional equipment and time. Although it is not complicated, layering the menstrual blood over

the Ficoll-Paque must be done very slowly and carefully, and the centrifugation and washing steps alone took approximately 60 minutes.

Red blood cells create a barrier due to their high density, affecting mass transfer for oxygen and nutrient supply to the cells. Red blood cell death also affected media composition. As shown by Figure 5.8, the red blood cells from the menstrual blood samples greatly reduced the visibility of any MenSC adhered to the flask. Contamination was difficult to identify. On two occasions samples were contaminated, once by bacteria and once by candida yeast, and it was only detected after several days because of poor visibility. This means that although Table 5.1 might reveal that direct culture appears the more suitable protocol by having fewer steps and is faster, the necessity to control the media and see the MenSC necessitate the need for a fractionation step to purify the mononuclear cells prior to culture.

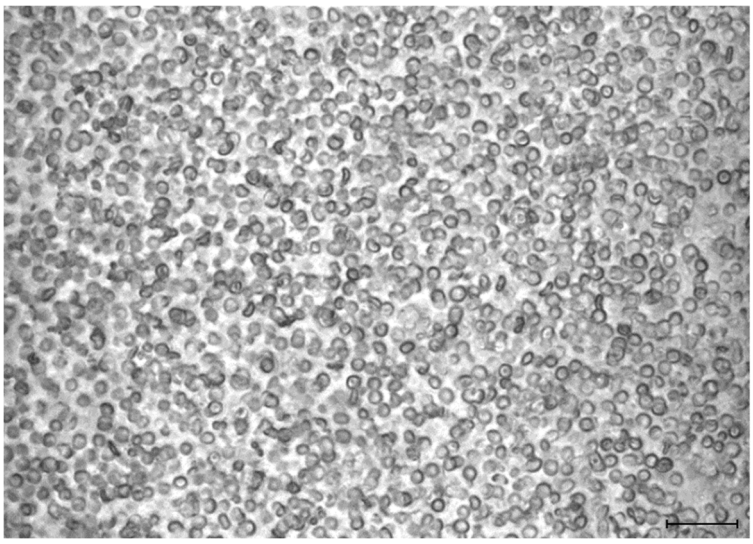

*Figure 5.8 Sample after direct culture. Red blood cells prevent visibility of any adherent MenSC. Scale bar 50 µm.*

*Table 5.1 Direct menstrual blood culture versus fractionating menstrual blood prior to seeding mononuclear cells.*

| | *Direct Culture* | *Fractionated* |
|---|---|---|
| *Advantages* | Quicker (~20 mins).<br>Improved cell signalling for improved growth.<br>Mimics environment. | Can clearly see MenSC adhering and growing.<br>More easily recognise contamination. |
| *Disadvantages* | Impossible to see when MenSC adhere.<br>More difficult to recognise contamination.<br>Platelet aggregation and other clotting mechanisms.*<br>Heavy cells could disrupt/damage MenSC adherence. | Slower isolation process (~120 mins).<br>Requires more material.<br>MenSC undergo more disruption/damage during handling.<br>Reduced cell signalling for growth in culture. |

### 5.7.2 Cell quiescence and washing time

Having identified that fractionating the menstrual blood sample prior to seeding allows for the removal of uncontrolled death and release of factors from contaminating cells, improved visibility of cell adherence and growth, the next steps were to identify when to wash the non-adherent mononuclear cells, cellular debris, and other debris away. Within the literature, MenSC are seeded and commonly washed and fed after one day (Álvarez et al 2018; Mirzadegan et al 2022). Sometimes the samples are left in culture up to five (Esmaeilzadeh et al 2020) or seven (Patel et al 2008) days before removing and replacing the old medium. This timing is variable and a selected timing rarely justified in the literature.

With each precious sample, it was of the highest importance to ensure that as many MenSC as possible were isolated from every sample. Washing cells is important for continued growth, viability and visibility, as shown in Figure 5.9. It remained a concern that MenSC could be quiescent; not adhering to the flask surface but also still alive. If this were the case, these quiescent MenSC would be discarded with the old medium. For this reason, rather than discarding old medium, the sample would be retained and reseeded into a fresh flask or well, meaning two parallel populations were taken forwards: the adherent MenSC, and the suspended potentially quiescent cells. Cells in suspension in media changes on days 1, 2 and 3 were found to contain MenSC that did eventually grow to form colonies and could in some cases reach confluence. Day 3 reseeded MenSC shown in Figure 5.10.

* Menstrual blood does not contain fibrinogen, meaning the blood does not clot with this factor.

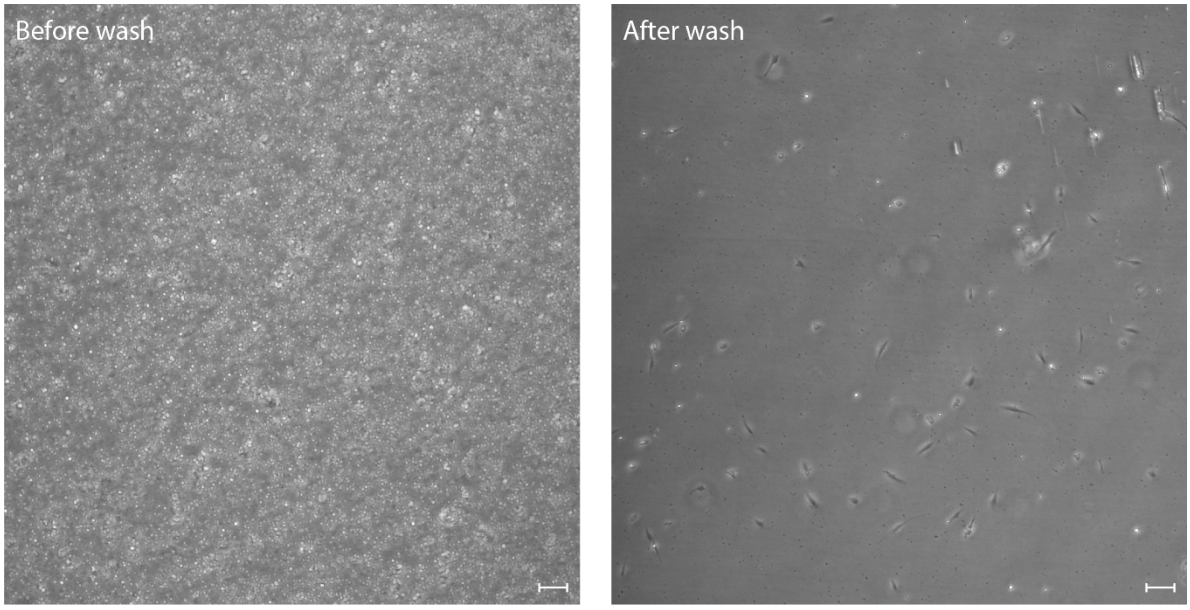


*Figure 5.9 MenSC isolated from a tampon after two days in culture, before and after washing non-adherent cells away from 2D culture.*

*Prior to washing the MenSC are very difficult to observe, and particularly any contamination is very difficult to identify. Scale bars 100 µm.*

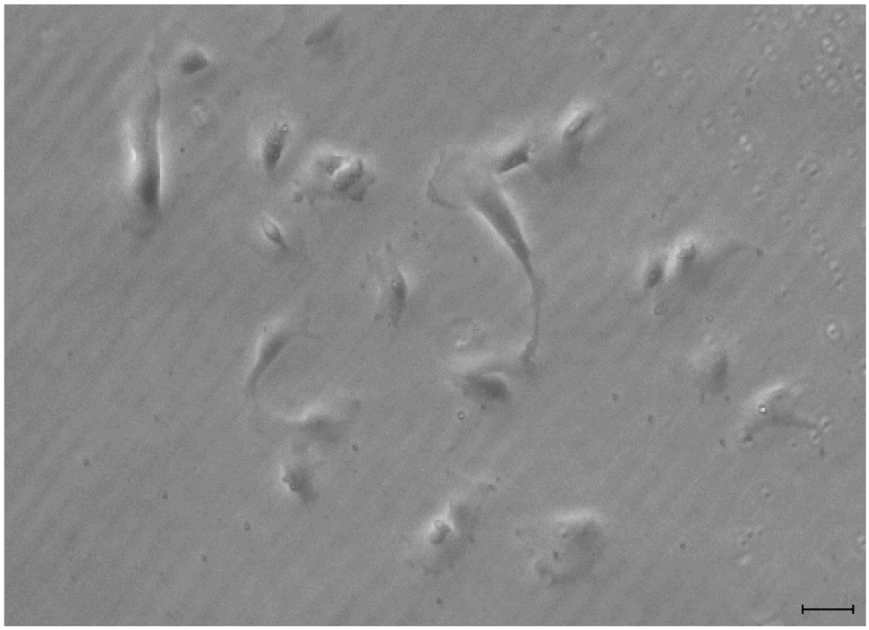

*Figure 5.10 Day 3 reseeded MenSC after a further 15 days in culture. These MenSC collected via tampon, delayed processing. Scale bar 50 µm.*

### 5.7.3 Sample volume, early culture, and MenSC expansion

As seen in Figure 5.11, adhered MenSC cell numbers can look very low at early culture time points, but without infection these cultures can be allowed time to expand, only discarding samples after four weeks of no change or growth. When samples contain many MenSC, they can reach 80% confluence within a week, and in these cases the MenSC can be seen clearly, well-dispersed across the flask. However, in other cases, some samples contain far fewer MenSC. In

these cases, the cells should be monitored daily, as seen in Figure 5.12; nothing was originally seen under the microscope at day 5, and after 2-3 weeks small colonies are seen. Therefore, some samples will not reach 80% confluence, but the MenSC should be passaged as soon as the smaller colonies reach high confluence, or daily monitoring reveals cell growth is slowing. The growth of small colonies shows that MenSC can multiply from a single cell.

Menstrual cup collection allows for precise sample volume measurement, however this is more difficult with sanitary pads and tampons. Here it was simply the sample colour that indicated sample volume; the darker the colour the higher the volume. This is seen in Table 5.2.

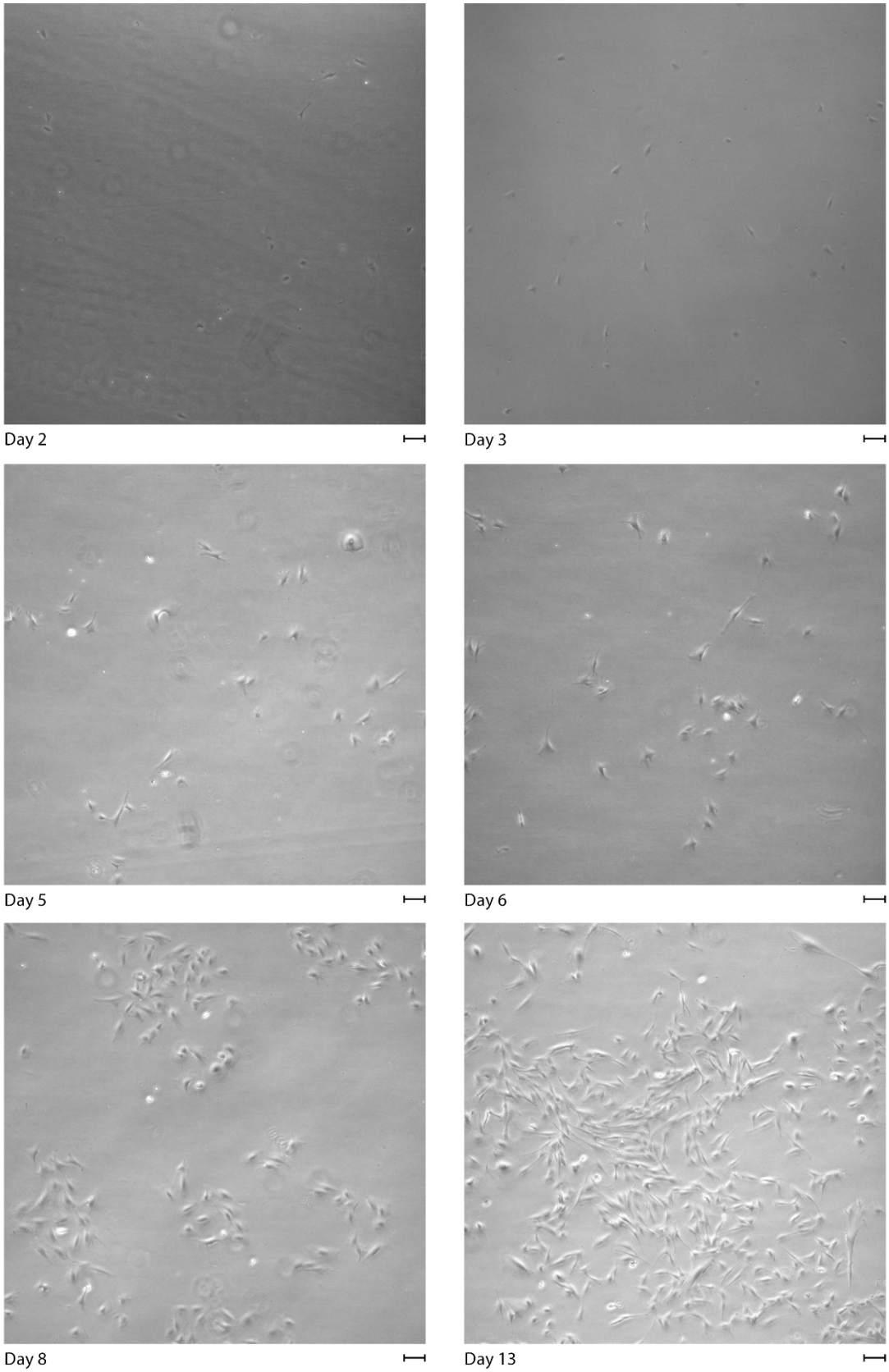


*Figure 5.11 P0 MenSC growth from days 2 to 13. These were isolated from a tampon, processed immediately. Scale bars 100 μm.*

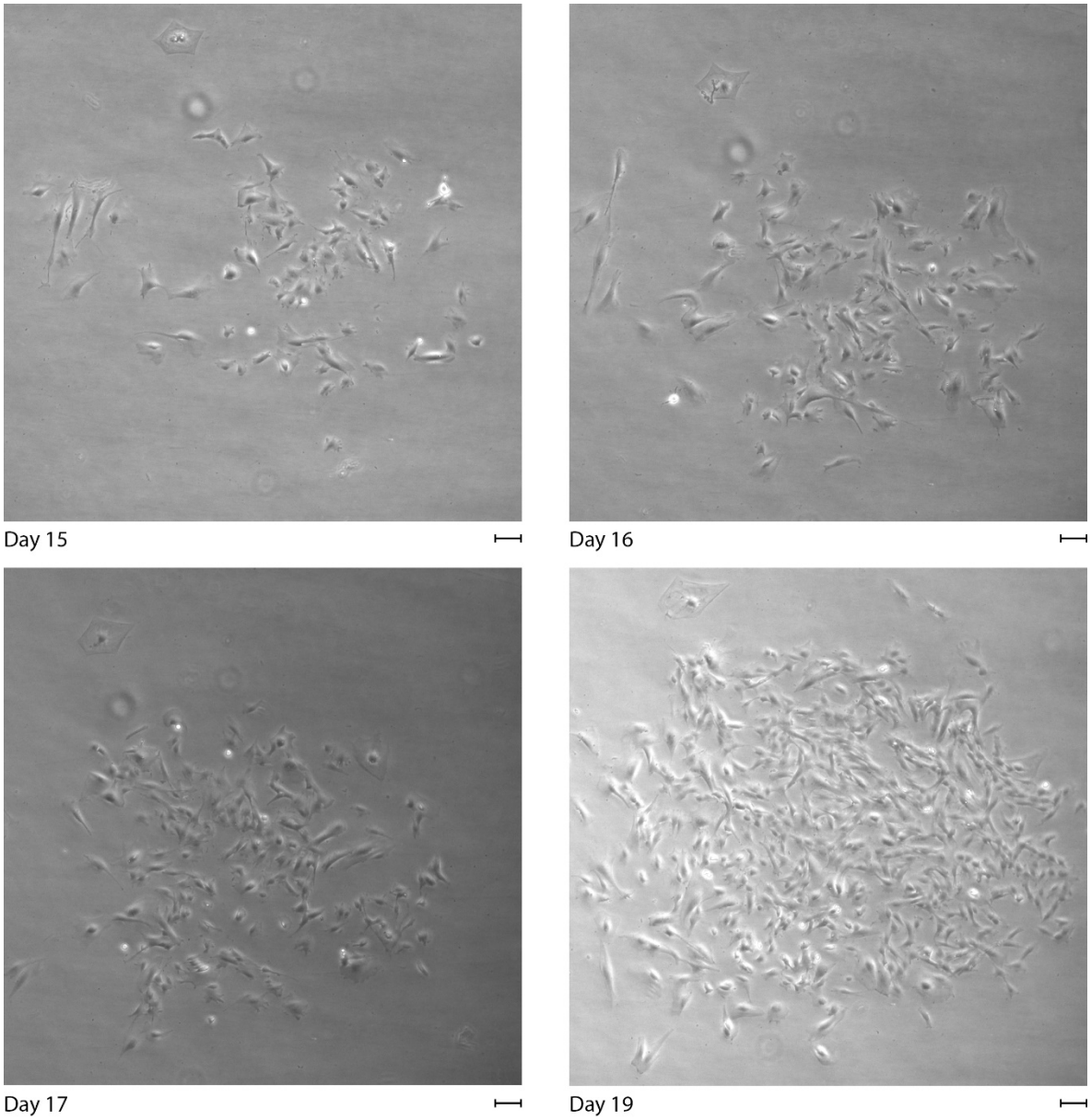


*Figure 5.12 Tracking the growth of a single colony of P0 MenSC from days 15-19, isolated from a sanitary pad, processed after 48 hours.*

*Prior to this colony being noticed, lab notes tracking the growth of cells from this sample read, "day 0 – seems a very small volume", "day 5 – not a sausage, leave", "day 8 – visible cells! Not looking good", "day 9 – few cells", "day 11 – three small colonies", "day 17 – definitely growing!", "day 19 – Great! Count and split". From this sample, 33,000 MenSC were isolated. Scale bars 100 µm.*

*Table 5.2 Two menstrual blood samples.*

*One sample collected via sanitary pad had a visibly lower menstrual blood volume, which was reflected in cell count and number of MenSC isolated after initial culture.*

| *449.2* | *838.1* |
|---|---|
|  |  |
| Sanitary pad | Tampon |
| Delayed processing | Immediate processing |
| Visibly more diluted sample (lower volume) | Visibly darker sample (higher volume) |
| Original cell count: 55,000 (22.7% viability) | Original cell count: 1,830,000 (55.6% viability) |
| Isolated MenSC: 33,000 after 19 days culture | Isolated MenSC: 647,500 after 13 days culture |

## 5.8 MenSC isolation from tampons and sanitary pads

Having optimised the protocol, this section clearly outlines the protocol for isolating and culturing MenSC from sanitary pads and tampons.

### 5.8.1 Materials

Key resources in Table 5.3.

1) Menstrual blood collection media: 100 mL of calcium/magnesium-free PBS, 100 U/mL penicillin, 100 mg/mL streptomycin, and 0.5 mM EDTA. Prepare this fresh before use. Transfer to sealable collection bag for donor use. Store at 4 °C until donation.
2) MenSC culture media: Dulbecco's Modified Eagle Medium (DMEM): Nutrient Mixture F12 (DMEM/F12), supplemented with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, 2.5 µg/mL amphotericin B. Pre-warm media to 37 °C prior to use.
3) Density media: Ficoll-Paque PLUS. Bring to 20 °C prior to use.
4) MenSC culture: incubated at 37 °C, 5% $CO_2$, 85% humidity.
5) MenSC passaging: Pre-warm Trypsin/EDTA solution 0.25% and PBS to 37 °C prior to use.
6) MenSC freezing media: FBS with 10% Dimethylsulfoxide (DMSO). Prepare fresh before use, keep at 4 °C.

*Table 5.3 Key resources*

| *Reagent or resource* | *Source* | *Supplier Catalogue Identifier* |
|---|---|---|
| | *Chemicals, peptides, and recombinant proteins* | |
| Amphotericin-B | Thermo Fisher | 15290018 |
| DMEM/F12 | Thermo Fisher | 11320074 |
| DMSO | Merck | D2650-100 mL |
| EDTA | Thermo Fisher | AM9260G |
| Ficoll-Paque PLUS | Merck | Ge17-1440-02 |
| Foetal Bovine Serum | Merck | F4135-500 mL |
| PBS calcium/magnesium-free | Fisher Scientific | 18912014 |
| Penicillin-Streptomycin | Thermo Fisher | 15140148 |
| Trypan Blue | Fisher Scientific | 15250061 |
| Trypsin/EDTA 0.25% | Merck | T4049 |
| | *Other* | |
| Always Ultra Sanitary pad size 1 normal with wings | Boots | 4994183 |
| Bag | FermionX | BA6041 |
| Bag clips | Fisher Scientific | 1152288 |
| Tampax Compak regular tampon | Boots | 8139393 |
| 70% IPA wipes | Vernacare Azowipe | 81103 |

### 5.8.2 Method

*Collection of menstrual blood via sanitary pad or tampon*

The samples are collected with the informed consent of the participant as approved by Local Ethics Review Board. Participants donate menstrual blood on their heaviest flow day, collecting the sample themselves in a designated bathroom, and use a menstrual hygiene product they are comfortable with.

1) Participants track their menstrual cycle to ease donation planning. Participants provide informed written consent prior to donation. Researchers provide participants with sanitary pad or tampon.
2) Disinfect the surfaces and touch points of the designated bathroom (equipped with table) prior to participant visit.
3) Participants arrive having worn sanitary pad or tampon for 4–6 hours prior to donation.

4) Participants are instructed to wash hands, remove menstrual hygiene product, and place in sealable bag containing collection mixture. The collection bag is gently massaged for 10 seconds, before being labelled, sanitised with 70% IPA wipes, and placed in transportation box.
5) Transport the sample to the laboratory immediately at room temperature, or store the sample at 4 °C for up to 48 hours.

*Isolation of MenSC*

See Figure 5.13 for MenSC isolation protocol.

1) Prepare 50 mL tube with 8 mL Ficoll-Paque PLUS (D=1.077 g/mL) and bring to 20 °C.
2) Note: if the menstrual blood sample is particularly high volume, prepare 2 × 50 mL tubes with 8 mL Ficoll-Paque PLUS (D=1.077 g/mL).
3) Massage collection bag prior to extraction, particularly after 48 hours storage.
4) Using sterile scissors, cut 1 mm from the corner of the sealable collection bag and pour menstrual blood sample into empty 50 mL tubes, squeezing the bag and contents to maximise extraction.
5) Centrifuge at 250 × g at 20 °C for 10 min with gradual acceleration and deceleration.
6) Collect the precipitate (approximately 3-4 mL from each tube), discarding supernatant.
7) Dilute the precipitate in 1:1 ratio with PBS.
8) Gently layer the menstrual blood sample over the Ficoll-Paque PLUS. If the sample contains unmanageable clots or mucous, see Figure 5.14. It is critical to layer the menstrual blood sample on top of the Ficoll drop by drop down the side wall of the tube slowly to prevent turbulence and mixing.
9) Centrifuge at 400 × g at 20 °C for 30 min with gradual acceleration and deceleration to pellet red blood cells.
10) Collect the narrow band that appears white and cloudy containing mononuclear cells (also known as the buffy coat).
11) Place this extracted layer into a fresh tube and add 10 mL PBS, then wash the cells: Centrifuge at 250 × g at 20 °C for 10 min, discarding supernatant. Flick the outside of the tube resuspend the cells. Add fresh 10 mL PBS and centrifuge at 250 × g at 20 °C for 10 min.
12) Discard the supernatant and resuspend the pellet in 1 mL MenSC culture medium by gentle trituration.
13) Transfer cell suspension to T25 flask with 3 mL MenSC culture medium.
14) Place in a 5% $CO_2$, 37 °C incubator, changing the medium every 2-3 days.

Note: to ensure maximum MenSC are isolated, during first media change, transfer used media in fresh T25 flask rather than discarding. Quiescent cells may take up to three days to adhere to the flask.

### *Passaging MenSC*

1) Observe MenSC growth daily. At P0, it is typical for colonies to form rather than an entire monolayer.
2) When MenSC reach 70-80% confluence (this can take 3-28 days), discard media and wash gently with PBS. Dissociate by adding 2 mL Trypsin/EDTA to T25 flask and incubating at 37 °C for 5 min. Observe cells to confirm MenSC are dispersed.
3) Inactivate Trypsin/EDTA with 2 mL MenSC culture medium. Triturate cells and transfer to 15 mL tube.
4) Add 2 mL MenSC culture medium to flask, triturate any remaining MenSC and transfer to 15 mL tube.
5) Centrifuge at 270 × g at 20 °C for 5 min to pellet MenSC.
6) Discard supernatant, resuspend MenSC in 1 mL MenSC culture medium.
7) Count cells by Trypan Blue exclusion. Passage MenSC when they reach 70-80% confluence.

It is possible to resuspend MenSC in MenSC freezing media and transfer to store MenSC at -80 °C for short-term storage or -150 °C for long-term storage.

### *Verification of MenSC*

Minimal criteria for MSC confirmation include adherence to plastic, multilineage differentiation and expression of markers by flow cytometry (Dominici et al 2006). MenSC undergo adipogenic, chondrogenic, and osteogenic differentiation (Li et al 2019a; Shokri et al 2019; Wang et al 2019), and positively express CD73, CD90, and CD105, and are negative for CD34 and CD45 (Ma et al 2020a; Skliutė et al 2021; Hao et al 2022).

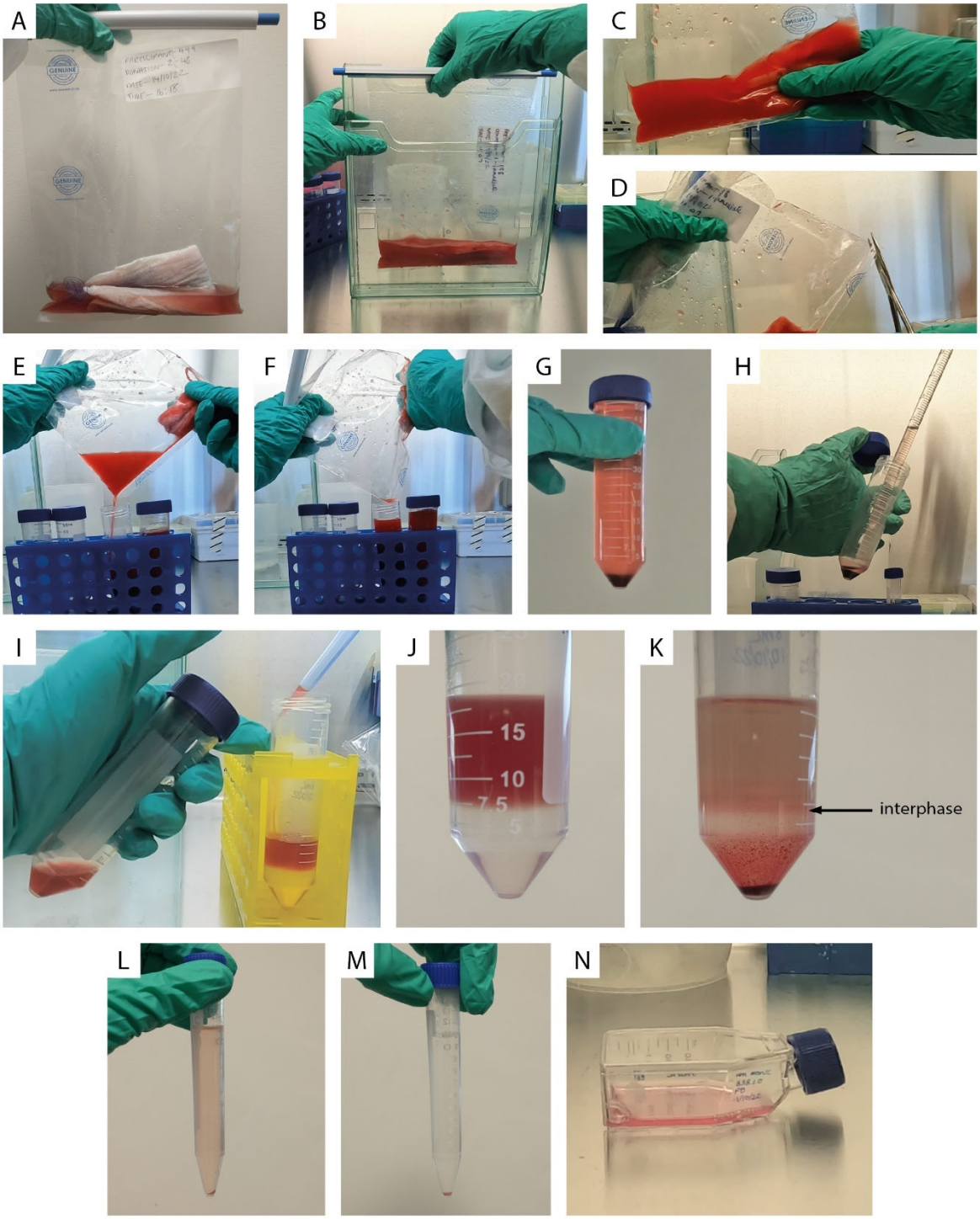


*Figure 5.13 MenSC isolation protocol.*

*A) Menstrual blood collected via sanitary pad and B) Tampon. C) Massage the contents of the bag gently. D) Cut the corner of the bag. E) Pour the menstrual blood-PBS mixture into 50 mL tubes. F) Squeeze the bag to collect maximum volume of menstrual blood. G) Centrifuge at 250 × g at 20 °C for 10 min with gradual acceleration and deceleration. H) Discard supernatant and dilute precipitate 1:1 with PBS. I) Layer gently over the Ficoll-Paque PLUS. It is important to layer this drop by drop down the tube wall to minimise disturbing the Ficoll-Paque PLUS layer. J) Menstrual blood-PBS mixture layered over the Ficoll-Paque PLUS. Centrifuge at 400 × g at 20 °C for 30 min with gradual acceleration and deceleration. K) The mononuclear cells are at the interphase between the Ficoll-Paque PLUS and the plasma (indicated by the arrow). These are visible as a white-coloured cloud. Discard the plasma and collect the mononuclear cells. L-M) Wash the cells. N) Seed MenSC in T25 flask.*

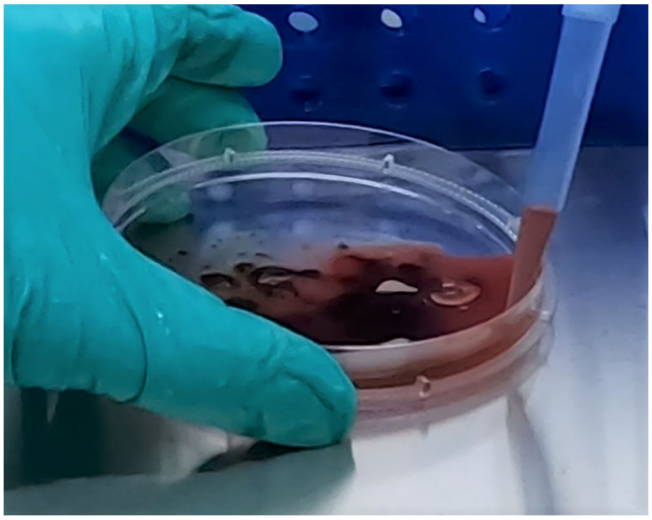

*Figure 5.14 Transfer the menstrual blood sample to a petri dish if the number of clots of mucous make it difficult to handle. Pipette from there.*

A strainer has been occasionally used to remove clots and uterine lining, reducing the toxic effects of dying cells on MenSC (Aleahmad et al 2018). Future collection should identify the value of straining samples, or using a deoxyribonuclease enzyme to break down the toxic extracellular DNA excreted by apoptotic or necrotic cells, as used for bone marrow MSC (Dray et al 2021) and adipose MSC (Boquest & Collas 2012).

During haemostasis, platelets form a plug either via collagen-bound von Willebrand factor or via thrombin generation, activating platelets to bind to collagen, forming aggregations by converting fibrinogen to fibrin (Davies & Kadir 2012). Calcium ions are responsible for platelet activation and several coagulation factors (Singh et al 2019). EDTA combines with calcium, preventing blood clotting after collection (Banfi et al 2007), therefore EDTA is used for MenSC isolation (Bozorgmehr et al 2014; Chen et al 2020b; Kazemnejad et al 2013; Zafardoust et al 2023). However, EDTA does not break down pre-existing clots. Therefore, an enzymatic approach could improve MenSC yield. Menstrual blood was treated with collagenase II by Skliutė et al (2021). A masters thesis comparing enzymatic (collagenase I) and non-enzymatic isolation found enzymatic MenSC isolation did not affect cell morphology or surface marker expression, but cell viability was increased in non-enzymatic isolated MenSC (Şener 2023). Future research should identify whether MenSC yield or handling is optimised with enzymatic isolation.

# Chapter 6. MenSC donation via sanitary pad, tampon, and menstrual cup

This chapter outlines the collection and isolation of MenSC via sanitary pads, tampons, and menstrual cups in response to **research question 5: How do sanitary pads and tampons compare to menstrual cups in terms of MenSC isolation and donor experience?** It details the research design and methods, results, and discussion of MenSC isolation, culture, phenotype analysis and participants' experience and task load analysis.

The primary research objective for this thesis was executed in two phases. Phase 1 was the eligibility and recruitment phase, where participants' eligibility was approved, contact details were collected, and participants began to track their menstrual cycle in order to establish appropriate donation scheduling. Phase 2 was the menstrual blood collection, MenSC isolation and analysis, participant donation experience, data collection and analysis. Appendix E provides a graphical overview of the research.

## 6.1 Ethical Approval

All samples and procedures in this study were approved by the Nottingham Trent University Ethical Committee for Human Biological Investigation in conjunction with NHS Health Research Authority approval. NTU Phase 1 approval number: 646. NTU Phase 2 approval number: 721. East Midlands Nottingham 2 Research Ethics Committee REC reference: 21/EM/0266.

## 6.2 Participants

Menstruating participants over the age of 18, determined as healthy, not pregnant or breastfeeding, not experiencing unusual vaginal discharge, and being comfortable using either a sanitary pad, tampon, or menstrual cup to donate menstrual blood were recruited in this study. All participants provided informed, written consent. Health of participants was determined with the NTU Health Screening with some adjustments (Appendix F). Health and blood collection preferences were self-reported, including history of HIV and hepatitis.

## 6.3 Study Design

### 6.3.1 Recruitment

Participants were recruited via a physical and digital poster that was displayed around Nottingham Trent University Clifton site and on University-approved intranet platforms such as Yammer and Microsoft Teams (Appendix G). It is possible that participants were also recruited via word of mouth.

### 6.3.2 Eligibility meeting

After potential participants had expressed an interest they were sent the participant information sheet (Appendix H) and they were invited for an eligibility meeting to discuss the research in more detail, their eligibility, and the chance to have any questions answered. This meeting was undertaken over the phone, Microsoft Teams, or in person.

### 6.3.3 Introductory meeting

After a minimum of 24 hours after the Eligibility meeting, participants were invited for an Introductory meeting. This in-person meeting was a second chance for questions to be answered. Participants signed consent forms (Appendix I), and were given the first NASA Task Load Index (NASA-TLX), (see Section 6.3.11, see Appendix J) to undertake at home. Part of the consent form included selecting the menstrual hygiene products the participants were already comfortable using. If participants were comfortable using more than one product, this was randomised via Microsoft Excel. Participants were provided the products to be used for the study.

The menstrual hygiene products used in this study were Always Ultra Sanitary pad size 1 normal with wings, Tampax Compak regular tampon, and Original DIVA™ Cup. Participants chose between Model 1 and Model 2 DIVA™ Cup, as determined by their personal experience and product instructions. Model 1 and Model 2 DIVA™ Cups were kindly donated by Diva International Inc. pro bono. Diva International Inc. had no input in the research design or analysis.

Participants were requested to track their cycle prior to donation if they did not already do so. This allowed the two donation dates to be planned with as much accuracy as possible.

### 6.3.4 Preparation for sample collection

An accessible bathroom was identified as study-use only, and equipped with a small table/storage, instructions, and disinfecting materials. The designated bathroom surfaces and touchpoints were disinfected before use. The small table/storage was equipped with spare sanitary pads and tampons, any paperwork, and the polystyrene donation box was placed on top of this. Instructions were fixed to the wall (Appendix K).

A collection mixture was prepared, containing PBS without Ca2+, Mg2+, containing 100 µg/mL penicillin, 100 µg/mL streptomycin, 2.5 µg/mL amphotericin B, 0.5 mM (EDTA), as Kazemnejad et al (2012) and Zafardoust et al (2020) have reported, although here this collection mixture was either added to a 50 mL collection tube (10 mL), or to sealable bag (100 mL). This collection tube or bag was placed into the donation box.

### 6.3.5 Sample collection

Participants were invited to donate menstrual blood on the heaviest day of flow, wearing the menstrual hygiene product for an appropriate and safe length of time to collect approximately 5 mL of menstrual blood.

Participants were invited to use the designated bathroom for donation. Participants used and removed the menstrual hygiene product as normal, and placed the product into the collection bag, or tipped the sample into the collection tube. The collection bag or tube was sealed. The collection bag was massaged by hand for ten seconds, with instructions to "try to change the colour of the liquid", or the collection tube was gently tipped from side to side for ten seconds, to mix the menstrual blood and collection mixture. The container was then labelled and disinfected by the participants with 70% IPA wipes before being placed in the box ready for collection by the researcher.

### 6.3.6 MenSC isolation and culture

Sanzhez-Mata et al published the first protocol to extract (and reprogramme) MenSC from sample of menstrual blood in 2020. The methods were adapted and optimised, as outlined in Chapter 5 and described here.

Menstrual blood mononuclear cells were separated using Ficoll-Paque PLUS (D = 1.077 g/mL)) (400 × g for 30 minutes) and washed with PBS by centrifugation (250 × g for 10 min) at 20 °C. For separation from menstrual blood samples collected with a menstrual cup, the menstrual blood and collection mixture was simply layered over the Ficoll-Paque as described by Sanzhez-Mata et al (2021). In the case of tampon and sanitary pad collection, the corner of the collection bag was cut away allowing the menstrual blood collection mixture to drain from the bag into 50 mL centrifuge tubes. The sealable bag could be squeezed by hand to maximise the extraction from the sanitary pad or tampon. The tubes were then centrifuged at 250 × g for 10 min with gradual acceleration and deceleration, collecting the precipitate (approximately 3-4 mL from each tube), which was then diluted 1:1 ratio with PBS, layered over the Ficoll-Paque and separated as above. The cell pellet was seeded in a T25 culture flask and cultured at 37 °C in 5% humidified CO2. After 2-3 days, rather than discarding the media before replacing, this is seeded in a fresh T25 flask to determine the presence of quiescent MenSC. Media was subsequently changed every 2-3 days before reaching 70–80% confluence, when cells were detached by 0.25% trypsin/1 mM EDTA and subcultured to new flasks at approximately $4 \times 10^3$ MenSC/$cm^2$.

### 6.3.7 Proliferation

MenSC proliferation was determined by MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide). This has been undertaken in other capacities when studying

MenSC (Khanmohammadi et al 2012; Wang et al 2017d, Mahdipour 2022). In this assay, formazan crystals are formed as the tetrazolium salt is reduced in the mitochondria of metabolically active cells to yield purple formazan crystals. When these are dissolved, the darker the purple solution, the greater the cell metabolism, which is quantified on a plate reader. Undertaking MTT assay first with known cell counts to establish a standard curve permits the use of MTT to assess cell number change in culture based on metabolic activity.

P3 cells were previously utilised for MTT assay (Li et al 2019a; Liu et al 2019a; Moreno et al 2019; Sun et al 2019a). All assays are undertaken with 5 experimental replicates, minimum 3 biological replicates. P3 MenSC were seeded at $5.0 \times 10^2$ cells/well in 96-well plates, cultured in maintenance conditions.

On days 1, 4, 7, and 10, MTT was added to the cell medium at the same time point, and the cells were then incubated at 37 °C for an additional 90 mins. A 100 µL volume of DMSO was then added to each well to terminate the reaction and dissolve the crystals. Absorbance values were determined at 570 nm using a Thermo Scientific Varioskan Lux plate reader. The assay was performed in quintuplicate for each sample. This permitted the identification of factors such as product donation methods affecting MenSC.

To supplement MTT assay, MenSC under maintenance conditions were subcultured up to P5. At each subcultivation, the population doubling was calculated as follows:

$$Doubling\ time = \frac{Duration \cdot \ln(2)}{\ln\left(\frac{Final\ concentration}{Initial\ concentration}\right)}$$

### 6.3.8 Media comparison

MenSC culture has been achieved with high glucose DMEM (Alcayaga-Miranda et al 2015b; Liu et al 2018; Meng et al 2007), low glucose DMEM (Quintero-Espinosa et al 2021; Uzieliene et al 2021; Yamchi et al 2021; Zhong et al 2009), and other media include Chang medium (Chen et al 2017b; Wu et al 2014), Chang Medium composed of α-MEM, Chang B, and Chang C medium (Patel et al 2008), and α-MEM (Moreno et al 2017). Numerous research groups report culturing MenSC in DMEM-F12 medium (Arezoo et al 2021; Darzi et al 2012; Farzamfar et al 2017; Khanmohammadi et al 2012; Li et al 2012a; Mehrabani et al 2016; Tan et al 2016; Wyatt et al 2021). Media choice is not justified within the literature. No media comparison has been undertaken for MenSC culture.

Determination of the most suitable media for MenSC culture was assessed using the MTT assay. P3 MenSC were seeded at $5.0 \times 10^2$ cells/well in 96-well plates, cultured in varying media (DMEM high glucose (4.50 g/L), DMEM low glucose (1.00 g/L), DMEM-F12 (3.15 gm/L)), supplemented with 10% FBS, 100 unit/mL of penicillin, 100 mg/mL of streptomycin and 0.25 µg/mL of Amphotericin B. These media were chosen to compare because these were the most commonly

used in the literature, and were three among five compared by Pal et al when comparing optimum culture conditions of bone marrow MSCs (2009).

MTT assay was undertaken as described above on days 1, 4, 7, and 10. All assays are undertaken with 5 experimental replicates, minimum 3 biological replicates.

### 6.3.9 Phenotype analysis

Flow cytometry for MenSC phenotype analysis has been undertaken at P3-P5 flow cytometry (He et al 2022a), P4 (Ma et al 2020a), P2-P6 (Martínez-Aguilar et al 2020), and P4-P6 (Wu et al 2014). Li et al found that MSC surface markers stabilised by P3-P4 (2022a).

MenSC phenotype was evaluated at P5. Cryopreserved MenSC thawed and were stained with fluorescent antibodies (dye) seen in Table 6.1, and analysed using a 3 laser Cytek® Aurora and SpectroFlo® Flow Cytometry Software. Fixable Viability Stain 510 (BD, UK) was used to exclude dead cells. The following markers were chosen as they were used by Tan et al when identifying MenSC to be used in the clinical trial (2016) which at the time of protocol identification and optimisation was the highest quality study:

*Table 6.1 Surface markers chosen to assess MenSC phenotype, as reported by Tan et al (2016) and the corresponding fluorochromes used in this study.*

| *Specificity* | *Marker* | *Expecting to see?* | *Fluorochrome* |
|---|---|---|---|
| CD34 | Hematopoietic stem cell marker | Negative | Mouse anti-human CD34 BV605 |
| CD38 | Hematopoietic stem cell marker | Negative | Mouse anti-human CD38 APC-H7 |
| CD44 | Lymphohematopoietic, MSC marker | Positive | Mouse anti-human CD44 Alexa Fluor 700 |
| CD45 | Hematopoietic stem cell marker | Negative | Mouse anti-human CD45 PE-CF594 |
| CD73 | MSC marker | Positive | Mouse anti-human CD73 BB700 |
| CD90 | MSC marker | Positive | Mouse anti-human CD90 BV650 |
| CD105 | MSC marker | Positive | Mouse anti-human CD105 BV786 |
| SSEA-4 | Pluripotent embryonic stem cell marker | Mixed in literature | Mouse anti-human SSEA-4 Alexa Fluor 647 |
| | Viability | | Fixable Viability Stain 510 |

*Flow cytometry panel design*

As the Cytek Aurora simultaneously emits all lasers and detects via all detector channels for each cell (spectral flow cytometry), it must be setup to compensate for spectral emission overlaps. Cytek's Complexity™ Index is an overall measure of uniqueness of the antibodies in a full spectrum cytometry panel. The lower the value, the easier it will be to analyse the antibodies in

the panel as the emission overlaps in the panel will be low. The higher the Complexity™ Index, the more challenging it will be to work with the panel. As the panel used in this study uses eight fluorochromes, a well-designed panel of this size will have a Complexity Index around 2 to 3. As seen in Table 6.2, this Complexity™ Index of 2.88 indicates a well designed panel.

*Table 6.2 Similarity™ Indices from Cytek, showing the panel to be well designed.*

| | PE-CF594 | BB700 | BV650 | BV786 | BV605 | Alexa Fluor 647 | Alexa Fluor 700 | APC-H7 |
|---|---|---|---|---|---|---|---|---|
| PE-CF594 | 1.00 | 0.38 | 0.17 | 0.02 | 0.33 | 0.00 | 0.01 | 0.00 |
| BB700 | 0.38 | 1.00 | 0.36 | 0.22 | 0.16 | 0.33 | 0.37 | 0.14 |
| BV650 | 0.17 | 0.36 | 1.00 | 0.19 | 0.53 | 0.16 | 0.14 | 0.04 |
| BV786 | 0.02 | 0.22 | 0.19 | 1.00 | 0.09 | 0.01 | 0.10 | 0.21 |
| BV605 | 0.33 | 0.16 | 0.53 | 0.09 | 1.00 | 0.00 | 0.02 | 0.01 |
| Alexa Fluor 647 | 0.00 | 0.33 | 0.16 | 0.01 | 0.00 | 1.00 | 0.53 | 0.15 |
| Alexa Fluor 700 | 0.01 | 0.37 | 0.14 | 0.10 | 0.02 | 0.53 | 1.00 | 0.32 |
| APC-H7 | 0.00 | 0.14 | 0.04 | 0.21 | 0.01 | 0.15 | 0.32 | 1.00 |

Configuration: 3L 16V-14B-8R
Complexity™ Index: 2.88

Geometric mean of intensity was produced by transferring the unmixed data to Kaluza Analysis 2.2 (Beckman Coulter).

### 6.3.10 Induced differentiation

To confirm multilineage differentiation capacity of MenSC and whether collection method affects this, MenSC were induced to differentiate down adipogenic, chondrogenic, and osteogenic pathways.

P3 immediately processed MenSC isolated via sanitary pad, tampon, and menstrual cup were thawed and prepared for differentiation. Thawed samples with > 90% viability were considered for experimentation. Cells were seeded in 96 well plates at $2 \times 10^3$ MenSC/well and cultured following the procedure outlined in Section 6.3.6*. StemPro™ differentiation media were prepared following the manufacturer's instructions, supplementing with 100 U/mL penicillin, 100

* Originally, chondrogenic differentiation was undertaken in 3D, the optimal condition for chondrogenesis. This entailed adding $1 \times 10^6$ MenSC to 2 mL tubes, centrifuging at 270 × g for 5 minutes at room temperature, and continuing culture within the tubes, where MenSC produce spheroids. However, not only did this require a large quantity of MenSC, while 3D culture is optimum condition for chondrocytes, 3D culture is not optimal condition for MenSC, so prior to differentiation there was a high cell death and failure rate. When MenSC were seeded in 2D and cultured in chondrogenic differentiation medium, the MenSC spontaneously formed spheroids, negating the need for high cell quantities and more complicated culture conditions. Therefore 2D culture was adopted for all remaining sample differentiation.

µg/mL streptomycin, and 2.5 µg/mL Amphotericin B. When MenSC reached 70-80% confluence, MenSC were washed and differentiation media subsequently added. Media was changed every 2-4 days. At days 1, 14, and 21, MenSC were fixed with formalin before histological staining, as outlined in Figure 6.1. Adipogenic differentiation was assessed with Oil Red O, which stains the lipid vacuoles as an indicator of adipogenesis. Chondrogenic differentiation was assessed with Alcian Blue, which stains sulfated glycosaminoglycans and glycoproteins found in cartilage. Osteogenic differentiation with Alizarin Red, which stains cationic metals such as calcium, localised nodules of which are an indicator of bone production. Cells were stained for 30 minutes, washed, and imaged on Leica Thunder Cell Imager, with differentiation validated qualitatively.

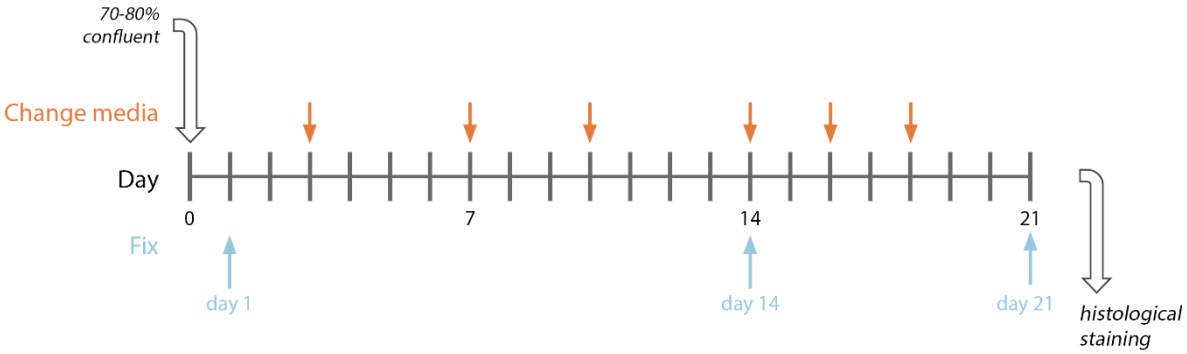


*Figure 6.1 Induced differentiation experimental design.*

### 6.3.11 Participant experience and donation success

#### *NASA Task Load Index*

NASA has produced the NASA-TLX, a set of six scales to assess workload based on three dimensions related to the demands imposed on a participant (mental, physical, and temporal), and three dimensions related to the interaction of the participant with the task (effort, frustration, and performance). The sensitivity of the scale is then enhanced by participants rating the NASA-TLX factors by importance, and task load is rated and analysed per participant and task via pairwise comparison (NASA Ames Research Center 2020). NASA-TLX has been used to determine task load in healthcare settings, including in intensive care settings (Levin et al 2006), safe drug administration (Lin et al 2001), and surgeon workload analysis (Law et al 2020).

Another similar method is the Subjective Workload Analysis (SWAT), a measure of time load, mental effort, and physiological stress, each on a three-point scale, without the participants' ratings of each category. In healthcare settings, both NASA-TLX and SWAT have been used (Carswell et al 2005; Jacobson et al 2011). When comparing NASA-TLX and SWAT in the measurement of cancer clinic nurse workload, NASA-TLX could predict the result of SWAT, but not the reverse, and SWAT failed to include the dimensions of performance and frustration (Huggins & Claudio 2018). The NASA-TLX is therefore a more useful tool of human factors analysis,

particularly in healthcare settings, and will be adopted in comparing menstrual blood donation methods in terms of donor usability and task success.

Where NASA-TLX is a useful method to quantitively understanding task load (here used as understanding an element of 'user experience'), the results must be assessed tentatively. The understanding and experience of an activity is experienced and given meaning in context of this activity and the greater context (Bornstein 1995). In the case of this study, experience of menstrual blood donation is influenced by participants' prior menstrual experiences, the menstrual taboo, and the context of the study itself, as well as time passing. This context will evolve, for example with the gained knowledge of MenSC, the donation of menstrual blood for research purposes, and simply as time passes. This is outlined as beta change by Golembiewski et al (1976). Although the distinction of this change is described as potentially "splitting hairs" (p. 136), it is important to note. Particularly studying such a taboo topic, this can never be eliminated from the study, but should be considered even if "matters get more complicated if the possibility of beta change is acknowledged" (ibid, p. 137).

Bustamante and Spain (2008) identify that the first step of the NASA-TLX protocol, completing the six scales to assess workload, is more suitable than completing the protocol in full, for convenience and to avoid the compounding of error from the scale assessment and pairwise comparisons. It was also indicated that NASA-TLX lacked scalar invariance (ibid). In a study testing mean differences across groups, this invariance would suggest one item intercept differs across the groups, and invariance and group difference testing should be discontinued (Putnick & Bornstein 2016). However, the use of the NASA-TLX in this study is a means to an end; the NASA-TLX is not the primary study, and data will not be used to compare results between groups. Rather, results will be analysed comparing at-home experience to donation number one, and donation number one to donation number two; two paired tests. This would assess whether there is a great difference in user experience (task load) from normal routine to donating menstrual blood, and then donating menstrual blood for the first time to the second time, to see if experience is affected by familiarity with the process. Any general results from the NASA-TLX will not be used to compare groups, with the aim they will provide an overview of donor's experience of menstrual blood donation.

In this study, the NASA-TLX guidance protocol was followed. The only alteration made is each participant was asked to indicate the marker on each scale with an arrow, rather than an 'x' as directed in the guidance (NASA Ames Research Center 2020). This is to encourage participants to indicate a specific point on the scale, rather than highlight a general area (described as "between two ticks"), negating the need for the researcher to "round up" (ibid, p. 4) on behalf of the participant. See Appendix J the worksheets used in this study.

To compare menstrual blood donation experience, participants were asked to complete the NASA-TLX on three occasions. First, the participants completed the six-part scale from home as the baseline control. They received a physical NASA-TLX questionnaire with the instruction to complete it after carrying out their normal menstrual hygiene routine from the comfort of their home. The participants were then asked to complete the same six-part scale immediately after donating their menstrual blood on the two occasions for the study. The second part of the protocol, pairwise comparison of the six scales, were completed by participants once as described in the protocol (ibid).

## 6.4 Qualitative analysis

The methods adopted here do not deviate much from those described in Section 3.1.5. At the end of the NASA-TLX form, an open-ended section of the form permitted participants to write further detail that was not captured within the conventional TLX form, including general experience taking part in the study, motivations for taking part, and further thoughts. This qualitative data was digitised and analysed through reflexive thematic analysis.

The data was coded inductively with QSR Nvivo 12.2 Pro. This involved reading and rereading the data, familiarising oneself with the data. The content drove the analysis (Braun & Clarke 2006; Gavin 2008), meaning no prior themes and subthemes were drawn upon. This allowed true themes and patterns to be interpreted, which were organised into general themes and subthemes (codes), which were mapped, revised, and refined. While the codes were produced digitally, it was more intuitive and flexible to print and map the themes physically before refining and reporting.

## 6.5 Statistical analysis

Statistical analysis was undertaken via SPSS (version 28.0.1.1; IBM), including descriptive statistics. See Table 6.3 for the statistical tests used in this analysis. 5% significance level was an indication of statistical significance. All descriptive statistics are reported as mean and standard deviation.

*Table 6.3 Statistical analysis undertaken for this study.*

| *Dependent variable* | *Independent variable* | *Test* |
|---|---|---|
| MenSC isolation success | Product type | Fisher's exact |
| Processing time | Product type | Kruskall-Wallis |
| P0 MenSC count | Product type | Kruskall-Wallis |
| P0 MenSC viability | Product type | Kruskall-Wallis |
| P0 time to reach confluence | Product type | ANOVA, Kruskall-Wallis |

| | | |
|---|---|---|
| Doubling time | Passage no., product type, processing time | 3-way ANOVA |
| Marker expression | MenSC population | Student's t (dependent) |
| Marker expression | Hormonal contraception use | Student's t (independent) |
| Marker expression | Product type | Kruskall-Wallis |
| Marker expression | Processing time | Mann-Whitney U |
| MenSC growth | Product type | ANOVA |
| MenSC growth | Processing time | Student's t (dependent) |
| MenSC growth | Media type | ANOVA |
| TLX | Home vs. donation 1, home vs. donation 2, donation 1 vs. donation 2. | Wilcoxon signed-ranks |

## 6.6 Results and discussion

Healthy participants (*n* = 21) aged between 20-41, mean age 26.86 years (*SD* = 6.25), had a menstrual length of 29.55 days (*SD* = 4.41) and menstrual phase of 5.15 days (*SD* = 1.29). 95% participants were nulliparous . 33% Participants were taking a hormonal contraceptive. 81% were willing to use a sanitary pad, 57% a tampon, and 38% a menstrual cup. See Appendix L for participant information.

Participants donated menstrual blood samples (*n* = 41) using a sanitary pad (*n* = 17), tampon (*n* = 13), or menstrual cup (*n* = 11), having worn the product for between 1.6 – 9.75 hours, mean time 4.92 hours (*SD* = 1.86), on their heaviest flow day (day 2.33 (*SD* = 0.65)). When donated with a menstrual cup and volume was easily measured, mean sample volume was 5.27 mL (*SD* = 2.51). Samples were processed immediately (1.78 hours (*SD* = 0.25)) or after 48 hours' storage at 4 °C (48.22 hours (*SD* = 1.59)) to isolate MenSC.

### 6.6.1 Success with sanitary pads, tampons, and menstrual cups

Table 6.4 presents the results of MenSC isolation comparing sanitary pads, tampons, and menstrual cups, after immediate and delayed processing. Success Rate was assessed simply by whether MenSC were isolated and successfully cultured after collection and processing.[*] The lowest Success Rates were via sanitary pad; immediate (44%) and delayed processing (50%). The highest were via tampons; immediate (100%) and delayed processing (86%).

[*] Isolation was considered a failure when the sample was contaminated. There was one case of bacterial contamination (tampon), and one candida (yeast) (menstrual cup). These are slightly better than the contamination rates reported by other research groups, at 25% (Ma et al 2020a) and 30% (Fiorelli-Arazawa et al 2019).

However, success rate is not the only important factor, with willingness to use the product arguable equally as important to factor into product choice validation. Willingness Rate was assessed by whether a participant was comfortable using the product already, not the hypothetical adoption of a new product. When Willingness Rate (sanitary pad = 81%, tampon = 57%, menstrual cup = 38%) and mean average from immediate and delayed Product Rating is taken into account, sanitary pad = 0.64, tampon = 0.75, and menstrual cup = 0.56.

As UK menstrual cup adoption is 8% (Mintel 2023), a higher rate of 38% reported here is surprisingly high. Because this study does not collect specific data on justification for this willingness and participation, it can only be speculated. Due to the recruitment poster containing clear imagery of a menstrual cup, and the fact that most menstrual cup users recommend menstrual cup use (Howard et al 2011), perhaps their enthusiasm for the product meant that the poster grabbed their attention.

To explore whether MenSC isolation success was affected by the product used to donate menstrual blood, a Fisher's exact test was executed. The results do not indicate a significant association between product used to collect menstrual blood and MenSC isolation success, for immediate processing ($p$ = 0.058), and delayed processing ($p$ = 0.397). This means that for future MenSC research, researchers should encourage each participant to use a product they are comfortable using, to offer participants agency in their donation process, and encouraging as high participant retention rates as possible. Sanitary pads and tampons are also more accessible and inexpensive compared to menstrual cups which is desirable for research groups. There is value in using all three products to donate menstrual blood for MenSC isolation.

*Table 6.4 Comparing sanitary pads, tampons, and menstrual cups in terms of MenSC isolation 'success rate' (whether MenSC were isolated), as well as Participants' willingness to use the product.*

*'Product Rating' is calculated using the equation $R = (0.5 \times S) + (0.5 \times W)$ where S is success rate of isolating MenSC from each product and W is the willingness rate of the participants using each product. Overall product rating is the mean average from both immediate and delayed rates.*

| *Immediate processing* | | | | *Delayed processing* | | | | |
|---|---|---|---|---|---|---|---|---|
| Product | Success Rate | Willingness Rate | Product Rating | Product | Success Rate | Willingness Rate | Product Rating | ***Overall Product Rating*** |
| Pad ($n$ = 9) | 44% | 81% | 0.63 | Pad ($n$ = 8) | 50% | 81% | 0.66 | **0.64** |
| Tampon ($n$ = 6) | 100% | 57% | 0.79 | Tampon ($n$ = 7) | 86% | 57% | 0.72 | **0.75** |
| Cup ($n$ = 6) | 67% | 38% | 0.53 | Cup ($n$ = 5) | 80% | 38% | 0.59 | **0.56** |

### 6.6.2 MenSC processing

Menstrual blood samples were processed by Ficoll-Paque isolation and cultured. Immediate processing took 1.78 hours (*SD* = 0.25) and delayed processing 48.22 hours (*SD* = 1.59).

Statistical analysis was undertaken to explore whether product affected processing time. A Kruskall-Wallis test showed that product used to donate menstrual blood did not affect processing time; $\chi^2(2) = 3.312$, $p = 0.191$ (immediate processing); $\chi^2(2) = 3.663$, $p = 0.160$ (delayed processing). There is no justification for a research group to prefer one product over another, regarding MenSC isolation or the overheads incurred with time spent in lab, permitting the participants to choose which product they prefer to use.

In this study, sample storage for 48 hours prior to processing was not found to affect MenSC. Allickson et al transported menstrual blood samples on chilling bricks in styrofoam shipping containers at 1-10 °C within 24 hours (2011), with success in isolating MenSC. Liu et al found no significant differences in MenSC isolation between 6, 24, 48, and 72 hours' storage at 4 °C after donation (2018). Chen et al found MenSC were stored best at 4 °C, partly due to the release of autophagy-produced energy (2022). Therefore, future research can establish the potential for donors to undertake sample collection at home or work and posting or couriering the sample to the laboratory.

### 6.6.3 MenSC isolation and culture

MenSC displayed a spindle-shaped morphology which did not change up to passage 5, as shown in Figure 6.2. MenSC were passaged after reaching 70-80% confluence, or after 28 days in culture, whichever occurred first. At P0 for both immediate and delayed processing this took approximately 2 weeks, with this decreasing with each passage to 3-4 days by P3. A one-way ANOVA undertaken to explore whether product affected P0 MenSC confluence time did not indicate a significant difference in MenSC viability between product used for immediate processed samples ($F(2,11) = 2.455$, $p = 0.131$) or delayed ($F(2,12) = 2.891$, $p = 0.094$). This is a further indication that MenSC should be collected via the product that participants prefer.

MenSC were seeded at approximately $4 \times 10^3$ MenSC/cm$^2$. With low cell counts, particularly at P1, MenSC still expanded at lower cell densities. At approximately $2 \times 10^3$ MenSC/cm$^2$, cells took approximately up to 9 days to reach 70-80% confluence. At around $1 \times 10^2$ MenSC/cm$^2$, this increased to 11 days. It is promising that even lower MenSC densities permit recovery and healthy growth, showing the possibility to expand MenSC for clinical application.

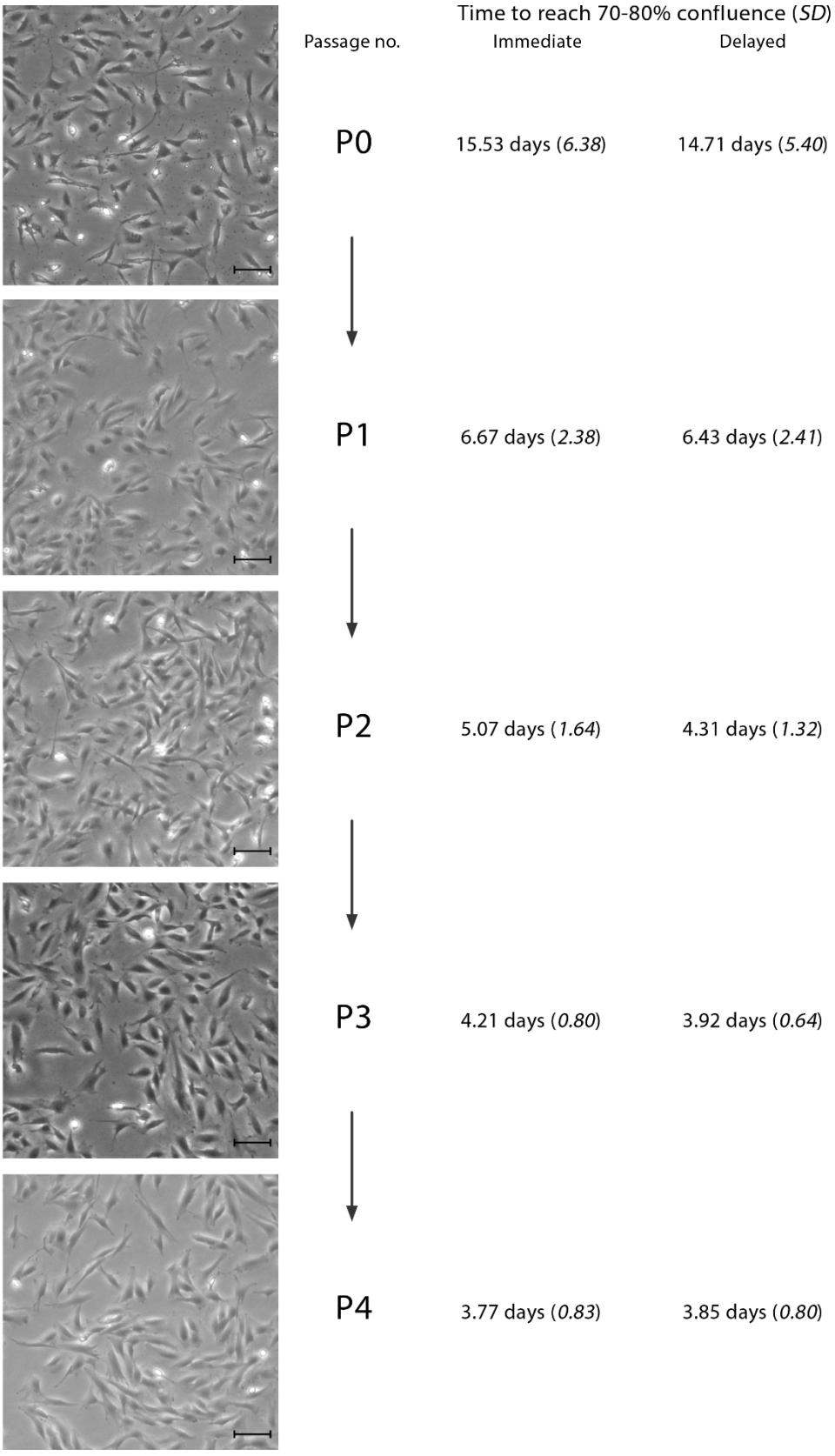


*Figure 6.2 MenSC cultured up to P5 showing no change in morphology.*

*Time taken to reach 70-80% confluence decreases from around 14 days at P0, to 3-4 days by P3. Scale bars 100 µm.*

At each passage, cells were counted via Trypan Blue exclusion using a c-chip haemocytometer. Figure 6.3 presents an example of the cells at P0; platelets make up the majority of the sample,

small, round cells 4 µm in diameter. The sample also contains leukocytes, including monocytes (pictured), recognised by their larger diameter (15-30 µm) and kidney-shaped nucleus. MenSC are approximately 8-10 µm in diameter, with slightly irregular shape. The introduction of Trypan Blue stain inside the cell walls is indicative of membrane rupture and therefore cell death, shown by the darkened cell.

To examine whether product affected MenSC viability, a Kruskall-Wallis test was executed. This showed that there was not a significant difference in MenSC viability from menstrual blood depending on which product was used to donate, for immediate processing ($\chi^2(2) = 1.404$, $p = 0.495$), or delayed processing ($\chi^2(2) = 5.252$, $p = 0.072$). This is another indicator that participants can donate with their preferred product without impacting MenSC viability.

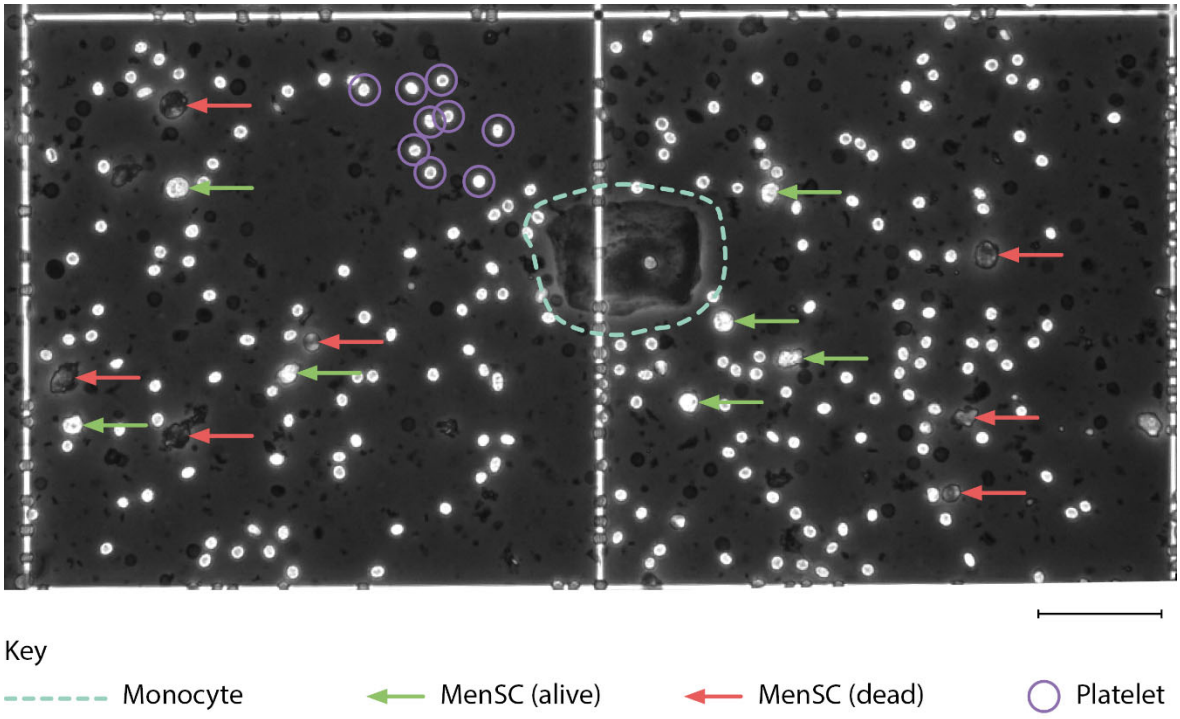


*Figure 6.3 The processed menstrual blood sample in the haemocytometer after Trypan Blue staining. The mononuclear cells are visible, including live and dead MenSC. Scale bar 50 µm.*

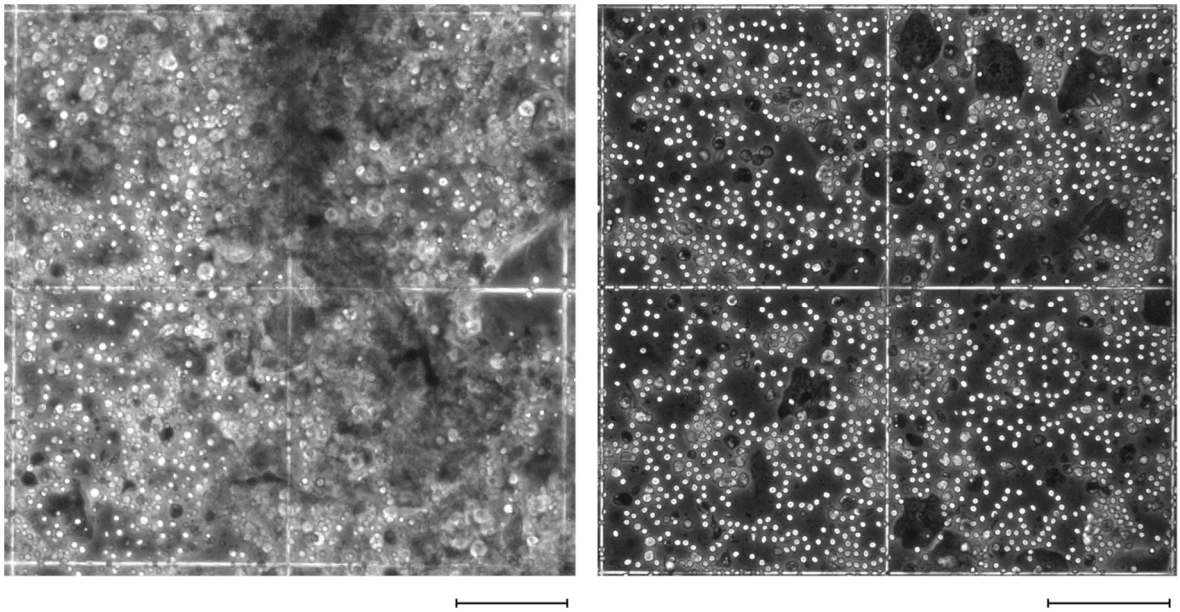

*Figure 6.4 Two menstrual blood samples in the haemocytometer after Trypan Blue staining. Note the difference in visibility of the samples due to mucous. Scale bar 100 µm.*

Figure 6.3 shows successful imaging and count via Trypan Blue exclusion. However, due to the nature of the sample containing e.g. mucous as shown in Figure 6.4, imaging the cells was often difficult. Even when cells were visible, there is also the element of subjectivity in differentiating between live and dead or dying cells. Many samples were simply not possible to image in this way. Therefore, a second method was used to estimate initial cell count.

By counting cell concentration at each passage and measuring the time taken between passage, doubling time is calculated using the following equation (Żuławińska 2023):

$$Doubling\ time = \frac{Duration \cdot \ln(2)}{\ln\left(\frac{Final\ concentration}{Initial\ concentration}\right)}$$

Duration and final concentration is known at each passage. However, initial cell concentration is not known at P0, therefore doubling time can not be calculated from P1. To estimate P0 cell count, the trend of the superseding passages could be used to extrapolate the doubling time for P0. Here, linear regression has been calculated mirroring the cell growth kinetics of bone marrowMSC that were found to have a linear popular doubling potential until P10 (before becoming logarithmic non-linear expression of growth kinetics as MSCs lost proliferation capacity) (Bruder et al 1997). This allows for P1 doubling time to be extrapolated, allowing for initial concentration to be calculated using the following equation:

$$Initial\ concentration = \frac{Final\ Concentration}{e^{\left(\frac{Duration \cdot \ln(2)}{Doubling\ time}\right)}}$$

Mean MenSC P0 count per sample were therefore estimated $7.3 \times 10^3$ cells ($SD = 1.5 \times 10^4$) for immediately processed samples, and $1.2 \times 10^4$ cells ($SD = 2.3 \times 10^4$) for delayed processed samples.

To assess whether initial MenSC count was affected by product used to collect menstrual blood, a Kruskall-Wallis test was executed. This showed that there was not a significant difference in number of MenSC isolated from menstrual blood depending on which product was used to donate, for immediate processing ($\chi^2(2) = 3.495$, $p = 0.174$), or delayed processing ($\chi^2(2) = 2.124$, $p = 0.346$). This is another indicator that the product used to donate menstrual blood has not been demonstrated to affect MenSC isolation.

P0 estimated doubling time was 49.38 hours ($SD = 14.26$) (immediate processing) and 53.04 hours ($SD = 22.31$) (delayed processing). This decreased to 36.08 hours ($SD = 6.37$) (immediate processing) and 36.52 hours ($SD = 8.95$) (delayed processing), as shown in Figure 6.5. A 3-way ANOVA was executed to explore whether MenSC doubling time was affected by passage number, product used to donate, and processing time (immediate vs. delayed). There were no statistically significant three-way interaction between these variables ($F(6,84) = 0.645$, $p = 0.694$), meaning that passage number, product used to donate, and processing time were not proven to affect MenSC doubling time up to P5. This is promising for future MenSC research as it is one indicator that menstrual blood can be temporarily stored or transported before processing without impacting MenSC growth.

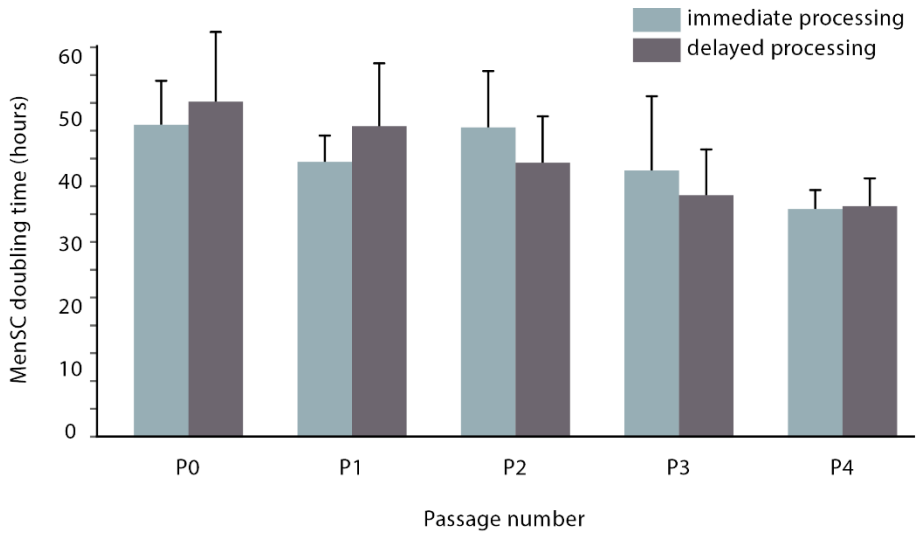


*Figure 6.5 MenSC doubling time from P0 to P4 for both immediately (n = 14) and delayed processed samples (n = 13). A 3-way ANOVA found no statistically significant three-way interaction between passage number, product used to donate, and processing time.*

### 6.6.4 Flow cytometry

Phenotypic analysis via flow cytometry was undertaken to confirm MSC status of MenSC and explore whether factors such as collection product and processing time affected cell phenotype.

Thawed P5 MenSC were gated for singlet, live, CD45- cells, as shown in Figure 6.6 (to explore CD45 expression, MenSC were gated for singlet, live cells). MenSC were positive for CD38, CD44, CD73, CD90, CD105. MenSC were positive for SSEA-4 to differing degrees (see Page 140). MenSC were negative for CD34 and CD45.

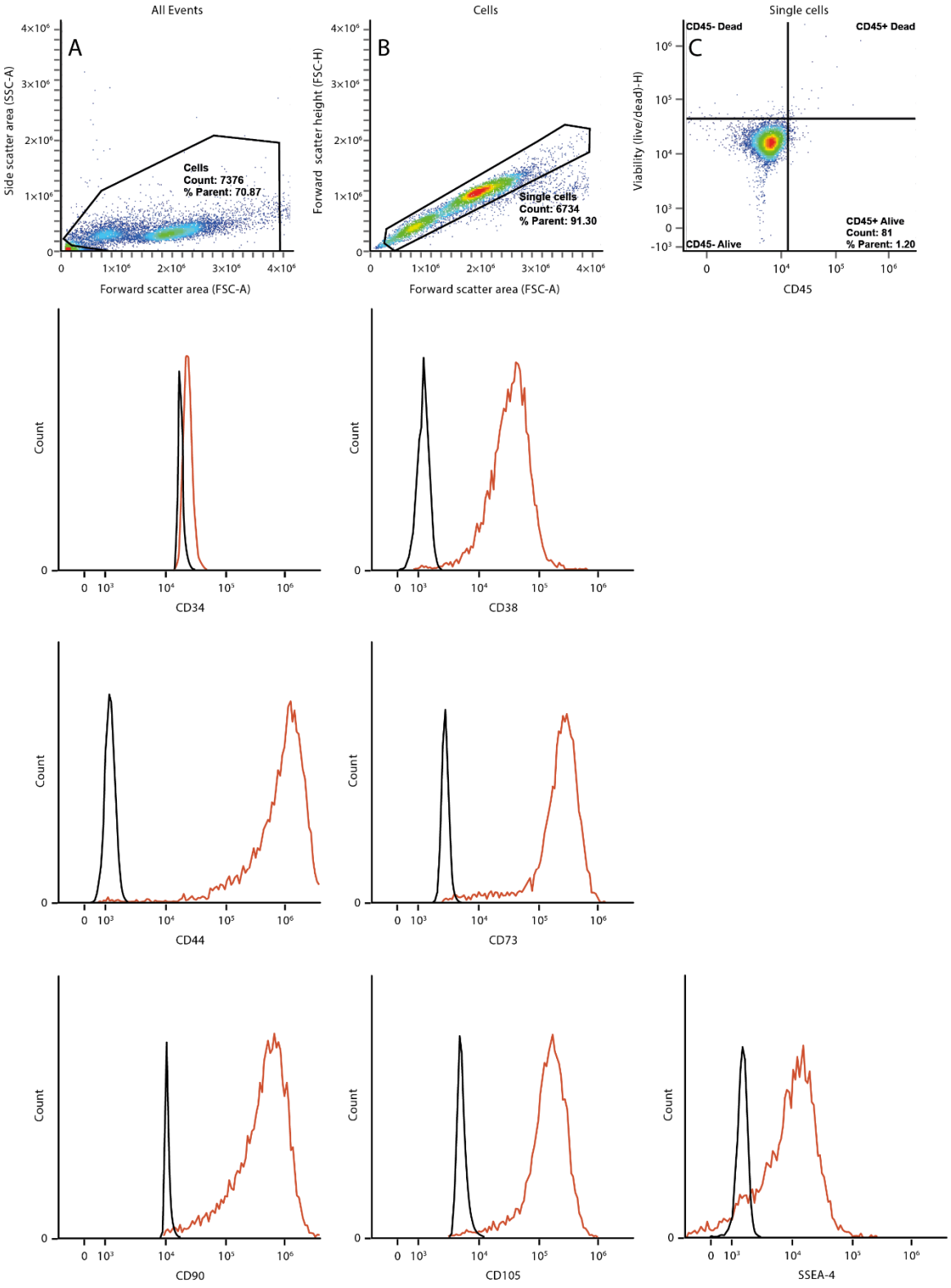


*Figure 6.6 P5 MenSC were gated for singlet, live, CD45- cells.*

*This example was collected via sanitary pad, immediately processed. A: Gating for cells. B: Gating for single cells. C: Gating for live, CD45- cells. Flow cytometry data showing control (black) and MenSC experimental data (red). For gating and raw data, see Appendix M.*

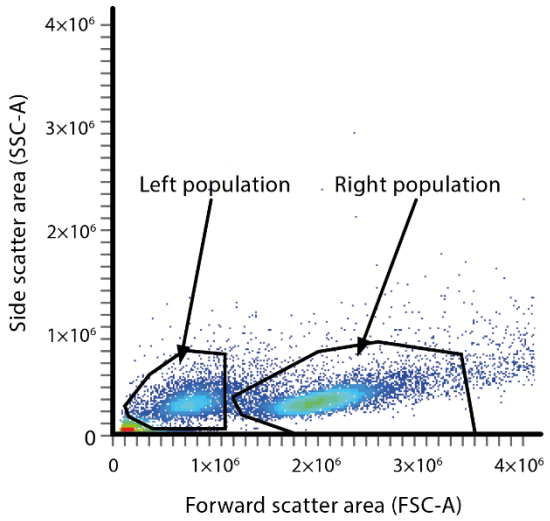


*Figure 6.7 Thawed P5 MenSC flow cytometry revealed two distinct populations.*

*Note: the population close to the origin contains particles of debris so is not included in analysis.*

Figure 6.7 shows the MenSC contained two cell populations of differing size and granularity (left population and right population). It has never been reported that MenSC contain two cell populations, so it was first necessary to confirm the cells were indeed MenSC. Statistical analysis was first undertaken to explore whether these two population differed in terms of marker expression. A paired t-test was undertaken for the following groups ($n$ = 3): sanitary pad, immediate processing; menstrual cup, immediate processing; sanitary pad, delayed processing; tampon, delayed processing; menstrual cup, delayed processing. For both immediate and delayed processing of menstrual cup samples, there were no statistically significant differences between the left and right populations. There were statistically significant differences in the other groups, which appeared inconsistently, as shown in Table 6.5.

*Table 6.5 MenSC marker expression via flow cytometry, exploring the difference between the two cell populations (left and right) visible when gating cells.*

*Paired t-tests found some statistically significant differences in marker expression between the populations, presented by thick outlines.*

| | *Marker expression / mean % (SD)* | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *CD34* | | *CD38* | | *CD44* | | *CD45* | | *CD73* | | *CD90* | | *CD105* | | *SSEA-4* | |
| | *Left* | *Right* | *Left* | *Right* | *Left* | *Right* | *Left* | *Right* | *Left* | *Right* | *Left* | *Right* | *Left* | *Right* | *Left* | *Right* |
| a | 0.12 *(0.11)* | 0.21 *(0.18)* | 87.41 *(19.32)* | 82.32 *(30.26)* | 99.07 *(0.15)* | 99.64 *(0.41)* | 2.26 *(1.97)* | 3.31 *(3.39)* | 93.70 *(1.41)* | 99.20 *(0.72)* | 86.97 *(13.64)* | 89.60 *(15.70)* | 95.92 *(2.41)* | 99.28 *(0.87)* | 75.57 *(7.60)* | 91.21 *(6.11)* |
| c | 0.07 *(0.18)* | 0.21 *(1.00)* | 96.35 *(4.76)* | 99.94 *(0.07)* | 97.88 *(2.00)* | 98.33 *(2.02)* | 1.43 *(0.76)* | 2.35 *(1.84)* | 95.86 *(2.91)* | 99.53 *(0.37)* | 92.50 *(7.11)* | 99.08 *(0.73)* | 95.66 *(2.54)* | 99.64 *(0.15)* | 52.24 *(7.05)* | 68.18 *(15.17)* |
| d | 0.18 *(0.31)* | 0.12 *(0.21)* | 82.82 *(18.11)* | 76.48 *(25.10)* | 97.28 *(1.24)* | 98.62 *(1.54)* | 0.51 *(0.94)* | 0.73 *(1.27)* | 91.30 *(2.30)* | 97.66 *(1.89)* | 90.19 *(3.05)* | 97.25 *(3.28)* | 93.29 *(2.18)* | 98.53 *(2.49)* | 64.57 *(21.32)* | 78.16 *(20.30)* |
| e | 0.13 *(0.07)* | 0.25 *(0.35)* | 98.73 *(0.94)* | 99.98 *(0.03)* | 97.52 *(2.28)* | 93.69 *(8.38)* | 3.33 *(1.12)* | 3.59 *(1.17)* | 95.54 *(2.15)* | 99.43 *(0.79)* | 95.83 *(0.65)* | 99.30 *(0.08)* | 95.24 *(1.91)* | 99.64 *(0.51)* | 45.92 *(36.26)* | 51.81 *(37.54)* |
| f | 0.23 *(0.40)* | 0.09 *(0.16)* | 95.48 *(5.71)* | 99.62 *(0.39)* | 96.03 *(5.75)* | 99.33 *(0.16)* | 3.09 *(2.48)* | 3.48 *(4.37)* | 91.29 *(5.83)* | 98.93 *(1.04)* | 93.81 *(5.95)* | 99.37 *(0.23)* | 92.95 *(6.40)* | 99.53 *(0.27)* | 72.40 *(9.32)* | 84.82 *(18.07)* |

Statistically different left and right population marker expression

Low expression (≤5%)

Slight expression (5.01-74.99%)

High expression (75-89.99%)

Very high expression (≥90%)

a = sanitary pad, immediate processing , (*n* = 3)

not enough data for tampon, immediate processing; not shown (*n* = 2)

c = menstrual cup, immediate processing, (*n* = 3)

d = sanitary pad, delayed processing, (*n* = 3)

e = tampon, delayed processing, (*n* = 3)

f = menstrual cup, delayed processing, (*n* = 3)

It is worth noting that some of these statistically significant differences should be regarded as inconsequential. For example, CD34 expression between the left and right population for delayed processing tampon was 0.13% (*SD* = 0.07) and 0.25% (*SD* = 0.35) respectively, so while statistically significant, these still represent undoubtedly low CD34 expression. With this in mind, MSC markers CD73, CD90, and CD105 have at times statistically slightly higher expression in the right population than the left population, and in general the right population has a slightly higher expression than the left. Pluripotent marker SSEA-4 vary greatly across participants, discussed in more detail later in this section, and appear to be contained in greater concentrations within the right population. Both left and right population, while of distinctly differing size and granularity, display phenotypically indistinguishable cells. As both populations were confirmed to be MenSC, all cells were included in subsequent further analyses.

To test whether marker expression is affected by hormonal contraceptive use, a nonparametric Mann-Whitney U Test was undertaken. There was no significant different in marker expression between participants not taking hormonal contraceptive and participants who were taking hormonal contraceptive, as shown in Table 6.6. This is in line with the finding that the MenSC population maintains its proliferative potential during hormonal contraceptive use (Schwab et al

2005), and MenSC that have been isolated from participants with and without hormonal contraceptive express the same markers (Fiorelli-Arazawa et al 2019). This promising result broadens MenSC donorship, and allows participants to continue with their preferred contraceptive status without compromising MenSC quality. However, no other contraception or hormone therapy has been studied in respect to MenSC quantity or quality, or ease of collection. Future research should identify whether IUS use, IUD use, perimenopause, and hormone replacement therapy affects MenSC.

*Table 6.6 MenSC marker expression via flow cytometry, exploring the difference between MenSC from participants taking hormonal contraceptive and not.*

*Paired t-tests showing no statistically significant difference between these groups.*

*Marker expression / mean % (SD)*

| | *CD34* | *CD38* | *CD44* | *CD45* | *CD73* | *CD90* | *CD105* | *SSEA-4* |
|---|---|---|---|---|---|---|---|---|
| a | 0.23 *(0.03)* | 99.27 *(0.52)* | 94.95 *(5.79)* | 3.51 *(1.27)* | 97.79 *(1.30)* | 97.78 *(0.57)* | 97.50 *(0.78)* | 49.17 *(36.61)* |
| b | 0.25 *(0.39)* | 96.00 *(5.38)* | 95.11 *(3.83)* | 6.50 *(5.46)* | 92.15 *(10.30)* | 94.20 *(7.51)* | 94.11 *(7.71)* | 71.95 *(10.00)* |

Statistically different marker expression

Low expression (≤5%)

Slight expression (5.01-74.99%)

High expression (75-89.99%)

Very high expression (≥90%)

a = tampon, delayed processing, no hormonal contraceptive, (*n* = 3)

b = tampon, delayed processing, taking hormonal contraceptive, (*n* = 3)

To explore whether marker expression was affected by product used to donate menstrual blood, a Kruskall-Wallis H test was undertaken to compare mean rank. These generally found no statistically significant difference between cell marker expression when comparing between product used to donate. There were statistically significant differences comparing the expression of CD44 for immediate processed samples ($\chi^2(2) = 6.676$, $p = 0.036$), and the expression of CD45 for delayed processed samples ($\chi^2(2) = 8.081$, $p = 0.018$), shown in Table 6.7. Because marker expression remains very high for CD44 and low for CD45 when collected via sanitary pad, tampon, and menstrual cup, it is argued here that collection method does not impact MenSC phenotype. This finding is promising for future MenSC collection and confirms that MenSC should be donated with a product participants feel comfortable using.

*Table 6.7 MenSC marker expression via flow cytometry, exploring the difference between MenSC collected via sanitary pad, tampon, and menstrual cup for immediate and delayed processing samples.*

*Paired t-tests showing some statistically significant difference between these groups, presented by thick outlines.*

| | *Marker expression / mean % (SD)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *CD34* | *CD38* | *CD44* | *CD45* | *CD73* | *CD90* | *CD105* | *SSEA-4* |
| a | 0.19 *(0.18)* | 88.69 *(20.98)* | 99.20 *(0.28)* | 2.58 *(2.37)* | 97.00 *(1.29)* | 91.00 *(13.07)* | 97.98 *(1.08)* | 82.09 *(6.51)* |
| b | 0.12 *(0.11)* | 84.13 *(19.79)* | 96.62 *(1.69)* | 4.56 *(4.91)* | 93.82 *(4.19)* | 94.12 *(5.06)* | 95.13 *(4.72)* | 55.42 *(21.62)* |
| c | 0.08 *(0.10)* | 96.56 *(4.95)* | 97.46 *(1.44)* | 1.78 *(1.29)* | 96.84 *(3.15)* | 93.96 *(6.44)* | 96.70 *(2.89)* | 55.55 *(8.21)* |
| d | 0.09 *(0.17)* | 85.58 *(19.50)* | 98.10 *(1.41)* | 0.95 *(0.82)* | 94.02 *(5.04)* | 93.19 *(4.90)* | 95.43 *(3.86)* | 68.41 *(19.44)* |
| e | 0.42 *(0.25)* | 97.64 *(3.86)* | 95.03 *(4.39)* | 5.00 *(3.91)* | 94.97 *(7.26)* | 95.99 *(5.15)* | 95.81 *(5.24)* | 60.56 *(27.05)* |
| f | 0.21 *(0.05)* | 88.30 *(18.35)* | 97.78 *(3.07)* | 3.04 *(2.68)* | 98.85 *(3.17)* | 96.81 *(2.92)* | 96.52 *(3.42)* | 81.03 *(11.05)* |

Statistically different marker expression

Low expression (≤5%)

Slight expression (5.01-74.99%)

High expression (75-89.99%)

Very high expression (≥90%)

a = sanitary pad, immediate processing, (*n* = 4)

b = tampon, immediate processing, (*n* = 5)

c = menstrual cup, immediate processing, (*n* = 3)

d = sanitary pad, delayed processing, (*n* = 4)

e = tampon, delayed processing, (*n* = 6)

f = menstrual cup, delayed processing, (*n* = 4)

To explore whether marker expression was affected by processing time, a Mann-Whitney U test was undertaken. For samples collected by sanitary pad, tampon, and menstrual cup, processing time (immediate vs. delayed) was not associated with a difference in marker expression across all markers.

The markers chosen in this study were chosen because they were reported by Tan et al as criteria for their MenSC transplantation for Asherman's syndrome (2016). The MenSC in this study expressed the markers in line with the literature, except for CD38 and SSEA-4, seen in Table 6.8. Geometric mean of intensity is presented in Table 6.9. CD38 was reported as negative and SSEA-4 is reported with mixed results in the literature. These discrepancies will be discussed.

Hao et al (2022), Sheikholeslami et al (2021), Martínez-Aguilar et al (2020), Feng et al (2019) report MenSC as negative for CD38 expression, described as haematopoietic marker (Aleahmad et al 2018; Feng et al 2019). The presence of CD38 is involved with interaction with the endothelium in peripheral blood, although the functional presence of CD38 has not been determined in many tissues including menstrual blood, and "the unspoken hunch circulating in the CD38 community is that the molecule may be ubiquitously present, albeit it at different levels of expression" (Deaglio et al 2001, p. 3). The International Society for Cellular Therapy does not include CD38 within the minimal criteria for MSC (Dominici et al 2006), so it is evidenced that CD38 is not the most useful

marker for understanding MenSC further. Importantly, all MenSC in this study were positive for CD73, CD90, and CD105, necessary markers for MSC definition (ibid).

MenSC SSEA-4 expression has been reported as positive (Allickson et al 2011; Borlongan et al 2010; Li et al 2012a; Liu et al 2018; Patel et al 2008) and negative in the literature (Aleahmad et al 2018; Chen et al 2015a; Khanmohammadi et al 2012; Khanmohammadi et al 2014; Tan et al 2016). This study found variation in SSEA-4 expression, approximately 65%, which was also reported by Li et al (2012a). SSEA-4 is also not included in the International Society for Cellular Therapy minimal criteria for MSC (Dominici et al 2006). It is an embryonic stem cell marker (Carpenter et al 2003). SSEA-4 has been reported to have large variation in expression between patients and between tissue sources while classical MSC marker expression (CD73, CD90, and CD105) remains consistently high (Qadan et al 2018). SSEA-4 may not be a useful identifier of multipotency (Schrobback et al 2011), and this variation in SSEA-4 expression does not close a gap in the understanding of the variation in phenotype and biological potential of MSCs from differing sources and donors (Qadan et al 2018). Further research should identify why MenSC have such a variation in SSEA-4 expression, and whether this is important for future clinical application, although it appears that may be unimportant.

*Table 6.8 Marker expression for MenSC (n = 26)*

| *Specificity* | *Marker* | *Expecting to see?* | *Result (% (SD))* |
|---|---|---|---|
| CD34 | Hematopoietic stem cell marker | Negative | 0.16 (*0.16*) |
| CD38 | Hematopoietic stem cell marker | Negative | 90.25 (*15.37*) |
| CD44 | Lymphohematopoietic, MSC marker | Positive | 97.15 (*2.81*) |
| CD45 | Hematopoietic stem cell marker | Negative | 3.25 (*3.30*) |
| CD73 | MSC marker | Positive | 95.11 (*4.48*) |
| CD90 | MSC marker | Positive | 94.32 (*6.39*) |
| CD105 | MSC marker | Positive | 96.17 (*3.74*) |
| SSEA-4 | Pluripotent embryonic stem cell marker | Mixed in literature | 66.66 (*20.30*) |

*Table 6.9 Geometric mean of intensity of marker expression for MenSC*

| *Marker* | *Expecting to see?* | *Geometric mean of intensity* | |
|---|---|---|---|
| | | *Control* | *Experimental data* |
| CD34 | Negative | 17,063.52 | 46,568.99 |
| CD38 | Negative | 767.82 | 28,995.18 |
| CD44 | Positive | 1,099.25 | 764,033.06 |
| CD45 | Negative | 5,446.89 | 5,661.57 |
| CD73 | Positive | 3,014.98 | 202,528.00 |
| CD90 | Positive | 10,879.99 | 391,798.69 |
| CD105 | Positive | 5,221.20 | 132,281.13 |
| SSEA-4 | Mixed in literature | 1,169.66 | 11,975.73 |

### 6.6.5 MTT

MTT assay was undertaken to assess MenSC metabolism, results in Figure 6.8. Producing a standard curve with known cell count permits the use of MTT to assess cell number change over 10 days assuming the metabolic rate of the cells in a population remain linear. Normalising these data to each experimental sample's day 1 cell count permits the use of MTT to infer and compare normalised cell count across different groups.

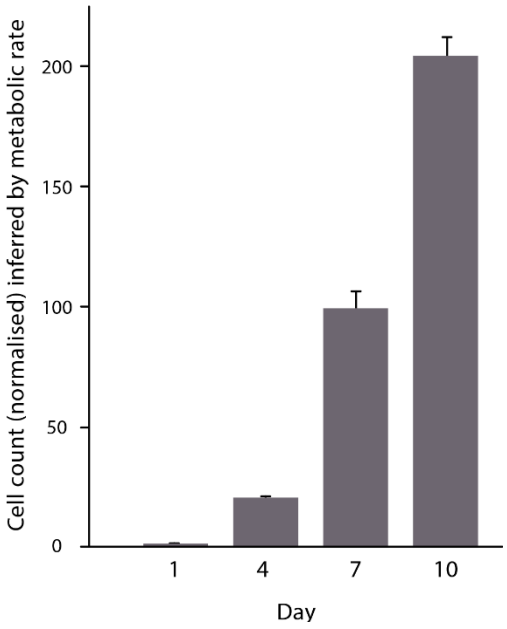


*Figure 6.8 10 day MTT assay showing an increase in absorbance as MenSC are cultured (n = 1). An increase in absorbance is demonstrative of MenSC metabolic rate and therefore MenSC count can be inferred.*

Analysis was undertaken to explore whether product used to donate menstrual blood effected MenSC growth over ten days. See Figure 6.9. A one-way ANOVA for assessing whether product

affected MenSC growth did not indicate a significant difference in MenSC growth between product used for immediate processed samples ($F(2,7) = 2.012$, $p = 0.204$) or delayed processing ($F(2,7) = 1.619$, $p = 0.264$).

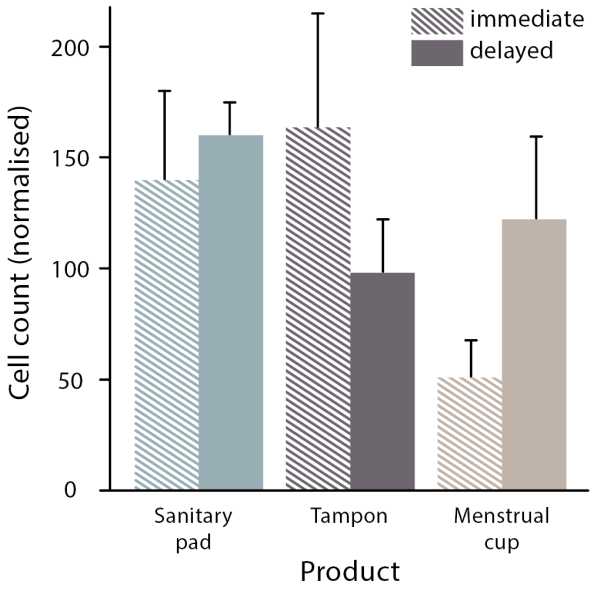


*Figure 6.9 Normalised cell count inferred by metabolic activity after ten days, comparing collection products via immediate and delayed processing.*

*Statistical analysis did not show a difference in cell growth across the three products, sanitary pad (n = 3), tampon (n = 4), and menstrual cup (n = 3).*

A paired t-test was undertaken to explore whether processing time (immediate versus delayed) affected MenSC growth. There was only enough data for a paired t-test when using the MTT results and calculated growth factor of MenSC collected by tampon ($n = 3$), seen in Figure 6.10. The results of a Shapiro-Wilk test showed data was normally distributed; $W(3) = 0.875$, $p = 0.309$ (immediate processing); $W(3) = 0.794$, $p = 0.100$ (delayed processing). The paired t-test did not prove there was a significant change in MenSC growth between immediate and delayed processing, $t(2) = 0.583$, $p = 0.619$.

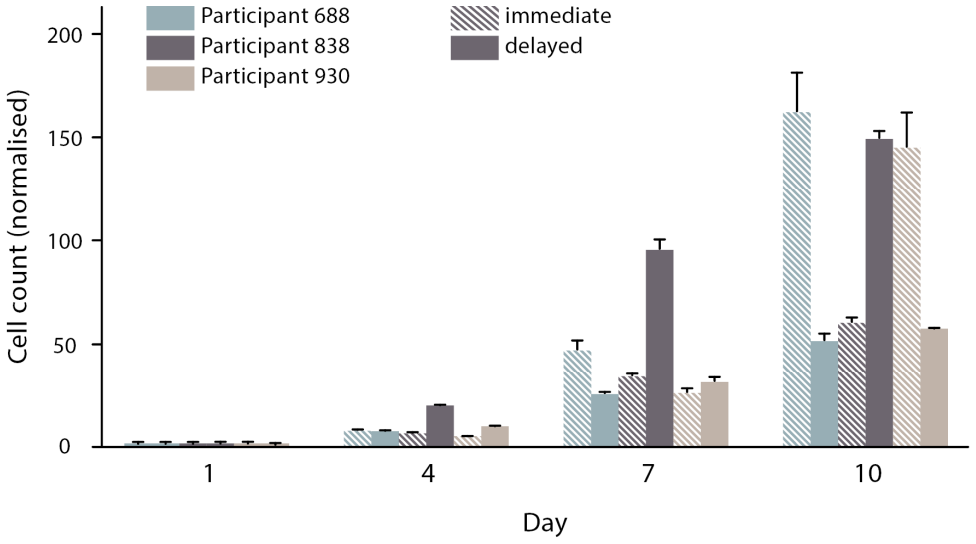


*Figure 6.10 Normalised cell count inferred by metabolic activity over ten days for MenSC collected via tampons, comparing immediate and delayed processing.*

*Statistical analysis did not show a difference in cell growth between immediate and delayed processing.*

### *Media comparison*

Another MTT was undertaken in the same manner to assess MenSC metabolism when cultured in differing media; DMEM high glucose (4.50 g/L), DMEM low glucose (1.00 g/L), and DMEM-F12 (3.15 gm/L)). See Figure 6.11. A one-way ANOVA was undertaken to explore whether media type affected MenSC growth over a ten day culture period ($n$ = 18). On days 4, 7, and 10, media type had statistically-significant effect on MenSC growth; $F(2,53) = 3.211$, $p = 0.048$, $\eta^2 = 0.108$ (day 4), $F(2,53) = 7.030$, $p = 0.002$, $\eta^2 = 0.210$ (day 7), $F(2,53) = 3.419$, $p = 0.040$, $\eta^2 = 0.114$ (day 10)[*]. A post-hoc Tukey's test showed that on day 4, F12 media had resulted in significantly greater MenSC growth than high glucose media ($p = 0.041$), enduring to day 7 ($p = 0.001$), and day 10 ($p = 0.031$). There was no statistically-significant difference between high and low glucose media, or F12 and low glucose media. These findings are shown in Figure 6.12.

Similar findings are reported by Pal et al for bone marrow MSC culture (2009). Low glucose and F12 behaved similarly, growing rapidly, although Pal et al found the high glucose led to an initial spike in growth before failing to support growth beyond passage 5, which is not seen in the study here as cells have not been expanded beyond P5. After passaging, low glucose then underwent morphology change and also displayed fewer MSC-characteristic surface markers, although Pal et al do not discuss why these media affect MSCs (ibid). High glucose levels have detrimental effects on MSCs (D'Esposito et al 2020). High glucose media has previously been reported to have a negative effect on rat MSC colony formation and differentiation (Stolzing et al 2012), reduced proliferative capacity in humans both with and without diabetes (Cramer et al 2010), and in

[*] This displays a very large effect size for days 4, 7 and 10.

relation to diabetes, has limited the therapeutic effects of MSC (Li et al 2021).* There are many factors involving high glucose-induced damage (ibid).

Although not statistically significant here, Pal et al suggested that the additional amino acids, vitamins, inorganic salts and other components in F12 may improve cell proliferation beyond the levels of low glucose media (2009). The research in this thesis supports the use of DMEM F12 supplemented with 10% FBS as the optimum culture conditions for MenSC isolation and expansion from the medias compared.

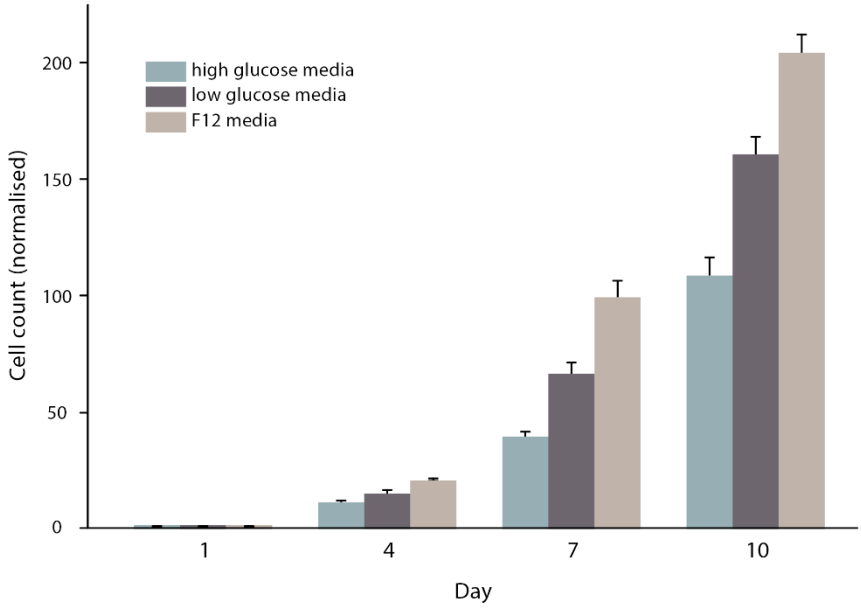


*Figure 6.11 Normalised cell number inferred by metabolic activity over ten days for sample (n = 1) of MenSC collected via sanitary pad with immediate processing (415.1).*

* In this case, high glucose is defined as 30mM glucose or approximately 5.4 g/L which is higher than the high glucose used in this study.

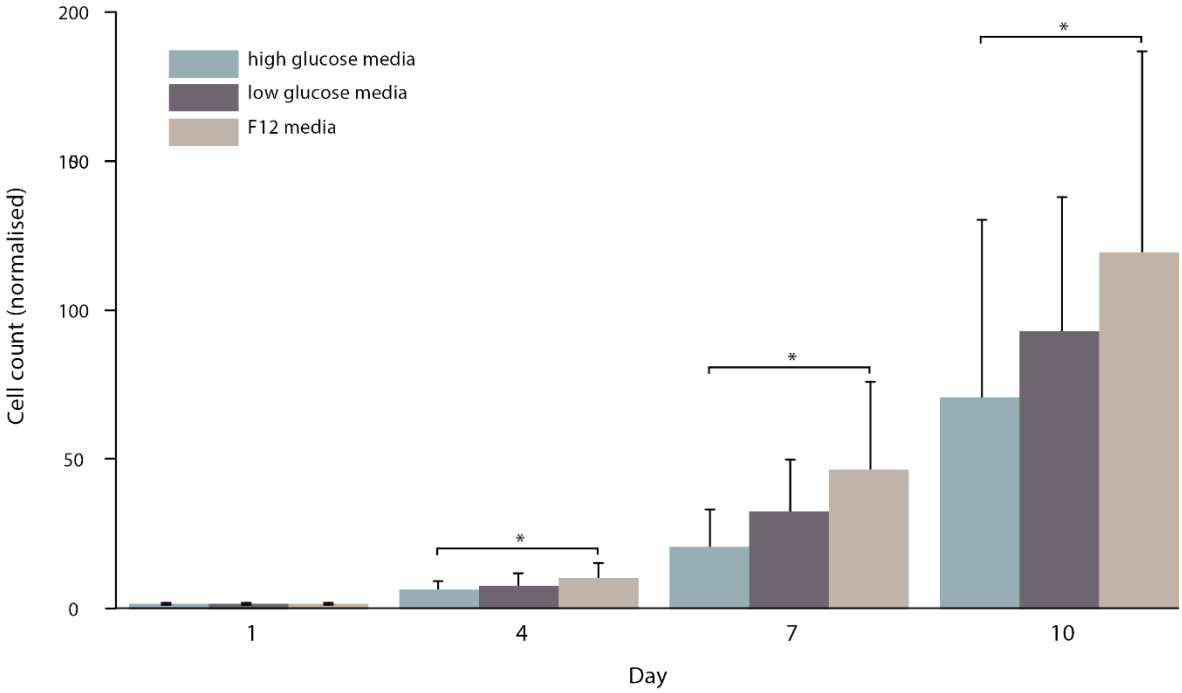


*Figure 6.12 Normalised cell number inferred by metabolic activity over ten days for MenSC (n = 18) comparing media types.*

*A one-way ANOVA found statistically significant increase in cell count (as inferred by metabolic rate) between high glucose media and F12 media on days 4, 7, and 10, indicated by an asterisk.*

### 6.6.6 Induced differentiation

The MenSC ability to differentiate was not affected by collection method as MenSC isolated from sanitary pad, tampon, and menstrual cup all responded consistently to induced differentiation. The MenSC formed spontaneous spheroids as a result of chondrogenic differentiation when seeded in 2D. As seen in Figure 6.13, by day 14 the cells migrate to form a nodule, which forms a spheroid by day 21. For MenSC undergoing osteogenic differentiation, by day 21, Alizarin Red stained the cationic metals such as calcium, an indicator of bone production. These results are seen across the three groups; MenSC isolated by sanitary pad, tampon, and menstrual cup, shown in Figure 6.14.

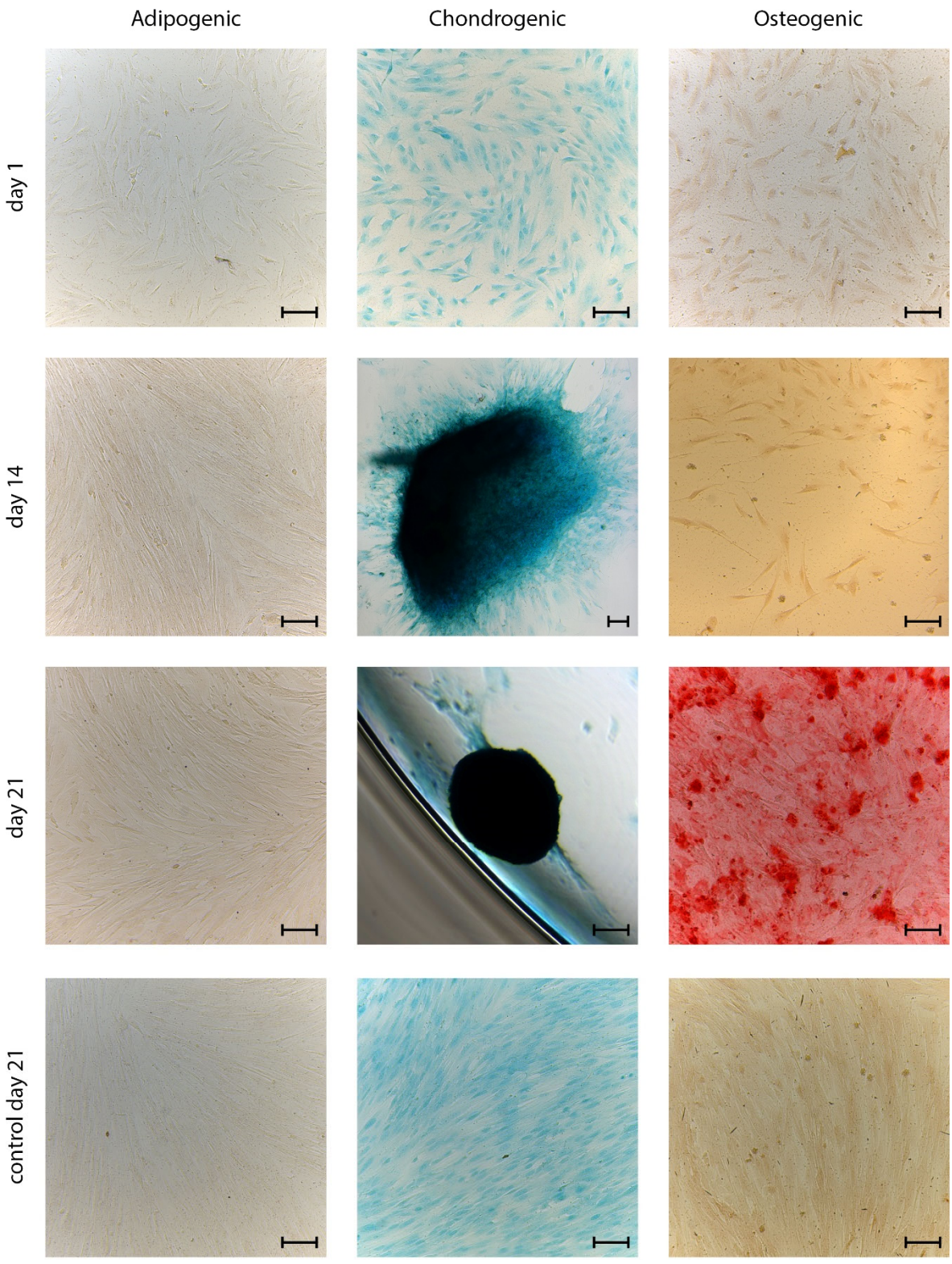


*Figure 6.13 Induced differentiation of MenSC collected from a sanitary pad over 21 days. Scale bar 100 µm.*

*Cells stained with Oil Red O for adipogenic differentiation, Alcian Blue for chondrogenic differentiation, and Alizarin Red for osteogenic differentiation.*

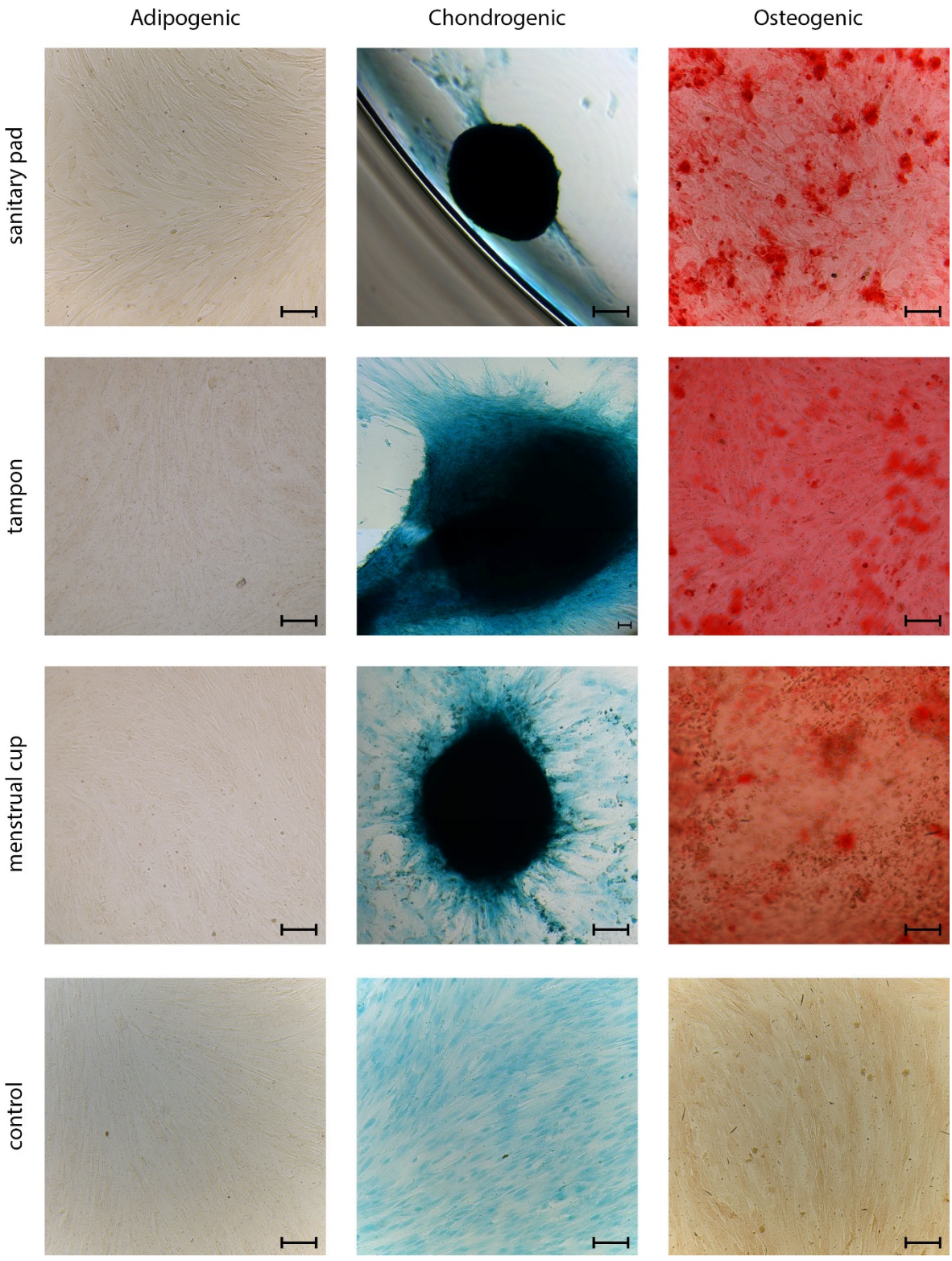


*Figure 6.14 Induced differentiation of MenSC at day 21 collected from a sanitary pad, tampon, and menstrual cup. Scale bar 100 µm.*

MenSC did not appear to differentiate into adipocytes using Oil Red O staining to indicate the differentiation. There do appear to be morphological changes that are indicative of more mature white, beige, or brown adipocytes (Ussar et al 2014), as shown in Figure 6.15, and appear similar to those reported by Sun et al 2019a. However, positive staining is absent.

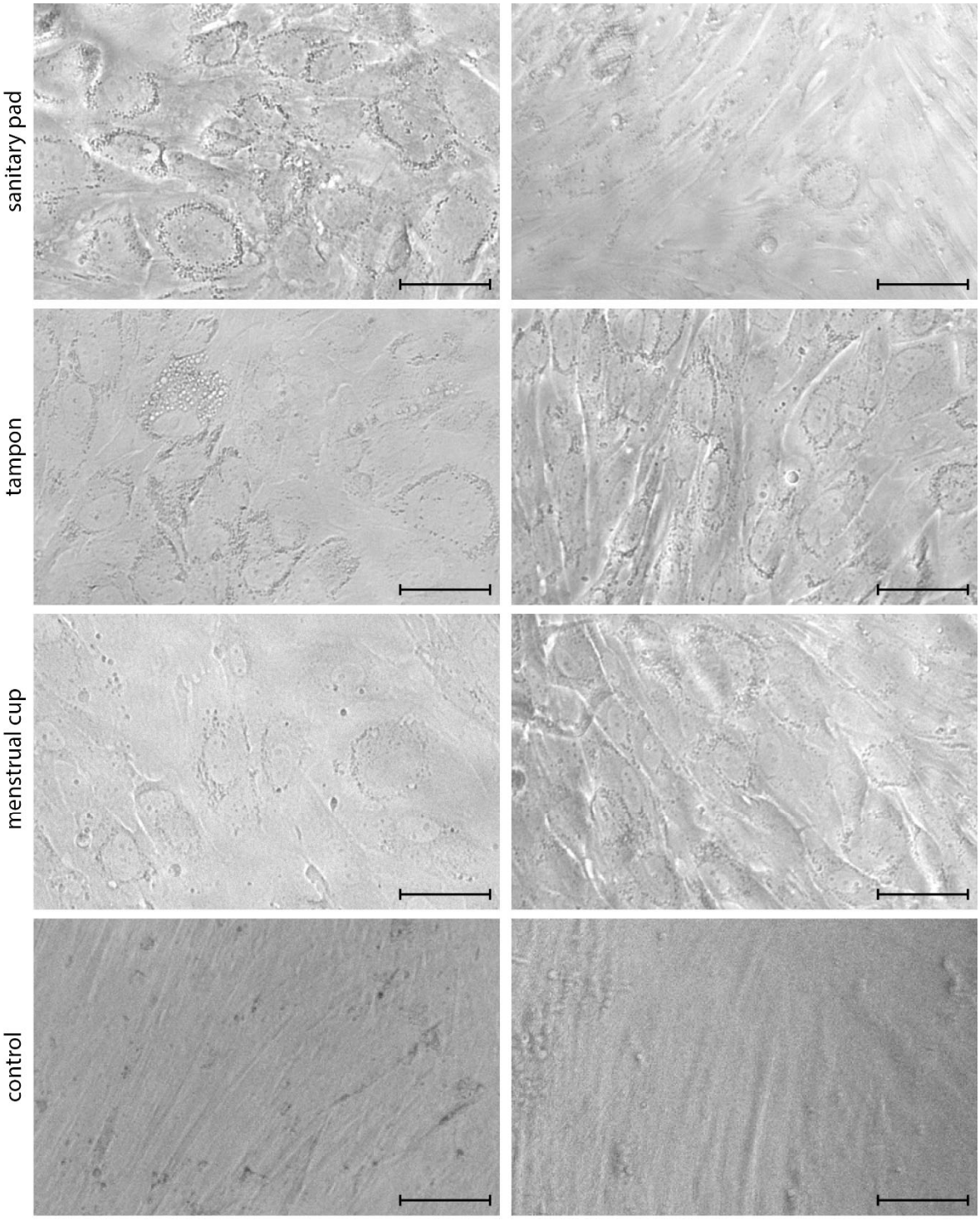


*Figure 6.15 Induced adipogenesis at day 21.*

*While the cells did not stain with Oil Red O, the rounded morphology with 'bubbles' is suggestive of mature white, beige, or brown fat cells, compared to the highly overpopulated, elongated control MenSC. Scale bars 50µm.*

Several research groups have reported success in trilineage differentiation, particularly adipogenic differentiation (Khanmohammadi et al 2014; Patel et al 2008; Wu et al 2014) which was negative in this study. Khanjani et al reported MenSC negative oil red O staining of differentiated adipocytes, (2015). The low adipogenesis of MenSC could be attributed to a number of factors. It

could be due to media volume (100 µL) being too high: Sheng et al investigated the impact of media volume on adipogenic differentiation (2014) and found that a lower media volume above the cell layer supported adipogenesis. This is likely due oxygen exchange being a physiological regulator of adipogenesis, with hypoxia inhibiting adipogenic differentiation (Yun et al 2002). Sheng et al recommended ideal volume for adipogenic differentiation (2014), around half the volume used in the current study.

MSC tissue of origin impacts its multilineage differentiation capacity, and each tissue requires a unique protocol (Ng et al 2020), so it may be that further optimisation is required to achieve adipogenesis. Wu et al (2014) and Khanmohammadi et al (2014) reported intensive protocols for achieving adipogenesis which involved cycles alternating between induction and maintenance media. Other research groups achieved adipogenesis after four weeks' induction (Liu et al 2019a), and even five weeks (Shilina et al 2018). Musina et al reported fewer than 30% of cells had differentiated by day 35 and concluded that a low capacity for adipogenic differentiation is a specific feature of MenSCs (2008). Availability of reagent was very limited in this study. In the future, a positive control as well as negative control would identify whether failure for adipogenesis was due to reagent failure or lack of MenSC ability for adipocyte differentiation. The outcome of the study here is due to possible faulty batch of reagent, incomplete optimisation for which further study can identify, or low adipogenic potential of these MenSC. Further optimisation is required for future MenSC trilineage differentiation, and running qRT-PCR to study changes in gene expression to assess markers for particular cell types such as white or brown adipocytes would be valuable. However, these promising results of high capacity for osteogenic and chondrogenic differentiation are a functional verification of multilineage potential of MenSC. Furthermore, MenSC via sanitary pads, tampons, and menstrual cups differentiating in a similar manner is indicative that the product used to donate MenSC does not impact their differentiation potential.

### *Limitations and future characterisation*

MenSC characterisation in this study was limited due to time and financial constraints. Without these constraints, further characterisation could include;

RT-qPCR is performed to detect gene expression. Western blot qualitatively or quantitatively assesses protein expression, and immunofluorescence qualitatively or semi quantitatively assesses protein expression. Selecting transcription factors such as Nanog, Oct-4, SOX2 would evidence stemness of MenSC via RT-qPCR (Kaupinis & Valius 2021; Rodda et al 2005), western blot (Li et al 2012a), or immunofluorescence (Borlongan et al 2010, Sun et al 2019a).

MenSC ability for proliferation could be performed via colony forming unit assay (Chen et al 2015b) or cellular mitochondrial dehydrogenase measurement (Uzieliene et al 2021), which could

be compared between participants, to other sources of MSC, MenSC at increasing passage numbers.

A scratch assay would evidence MenSC ability to migrate in vitro (Hao et al 2022), or alternatively in vivo via fluorescent dye and in vivo (Yang et al 2022) or vitro imaging after MenSC transplantation (Liu et al 2018).

Chromosome analysis would confirm MenSC karyotypic stability (Zafardoust et al 2020).

MenSC ability to differentiate into more lineages, such as cardiomyocytes, myocytes, hepatocytes, and neural cells should be explored. Differentiation would be quantified via RT-qPCR, for example using peroxysome proliferator-activated receptor for adipogenesis (Alcayaga-Miranda et al 2015b), osteocalcin for osteogenesis (ibid), Collagen 2A1 for chondrogenesis (Khanmohammadi et al 2012), rather than qualitatively as undertaken here, and furthermore connexin-43 for cardiomyocytes (Rahimi et al 2014a), nestin for neural cells (Azedi et al 2017), and albumin for hepatocytes (Khanjani et al 2015).

MenSC paracrine effect could be studied via immunofluorescence (Borlongan et al 2010; Liu et al 2018) or qPCR detecting growth factors in the MenSC secretome (de Pedro et al 2023).

MenSC reparative effect can be studied in vivo via organ or tissue damage (Borlongan et al 2010; Tan et al 2016), or drug-induced injury in animal models (Fathi-Kazerooni et al 2019; Zhang et al 2023).

Proteomics can be performed to study global protein expression of MenSC or RNA sequencing can be performed for global transcriptome analysis to report all genes which can be compared to other sources of MSC (Li et al 2012a; Skliutė et al 2021; Valatkaitė et al 2021).

### 6.6.7 Participant Task Load

Participants completed the NASA-TLX forms, indicating Task Load on scales from 0-100, rating Mental Demand, Physical Demand, Temporal Demand, Performance, Effort, and Frustration, with 0 being the lowest load and 100 being the highest. Participants completed the TLX forms after undergoing normal menstrual hygiene routine (home) ($n$ = 18), after donation 1 ($n$ = 21), and after donation 2 ($n$ = 18). These were combined with participants' individual pairwise analyses to produce an Adjusted score (see Figure 6.16), and the Work Load categories were combined into a total Weighted Rating for each Task, following the NASA-TLX methodological approach. The home TLX Weighted Rating was 14.48 ($SD$ = 11.77). Donation 1 was 17.21 ($SD$ = 11.53). Donation 2 was 20.00 ($SD$ = 14.49), see Figure 6.17.

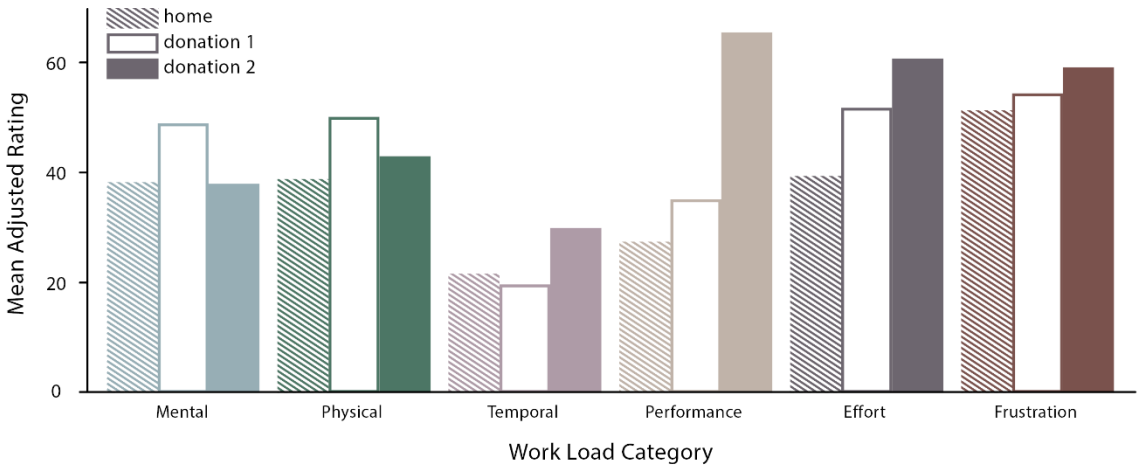


*Figure 6.16 Mean Adjusted TLX Rating for each Work Load category, including home, donation 1, and donation 2.*

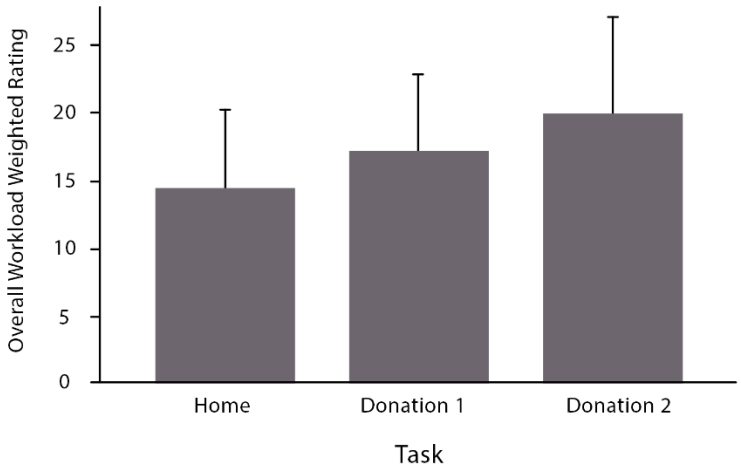


*Figure 6.17 Overall Workload Weighted Rating for each task, from home, donation 1, and donation 2. There were no statistically significant differences between the TLX results of the three tasks.*

Statistical analysis was undertaken to explore whether there was a difference between the TLX when undergoing normal menstrual hygiene routine (home) versus donation 1 and donation 2. The results of a Wilcoxon Signed-Ranks test did not find a statistically-significant difference in TLX from home compared to donation 1 ($Z$ = -0.224, $p$ = 0.221) or donation 2 ($Z$ = -1.528, $p$ = 0.124), and did not find a statistically-significant difference between donation 1 and donation 2 ($Z$ = -0.314, $p$ = 0.753). These encouraging results quantitively show that donating menstrual blood is acceptable for participants and is not considered a mental, physical, temporal, or emotional burden compared to their normal monthly routine. This should be supplemented by qualitative data for a richer understanding of donation experience and general attitudes to MenSC donation.

### 6.6.8 Qualitative data

Very little is known about donor experience of MenSC donation, beyond being “simple and painless, and the protocol was easy to follow” (Fiorelli-Arazawa et al 2019, p. 640). In the present study, 16 participants were willing to complete the optional open-ended hand-written form to

share their experience of donating menstrual blood. The participants used a product they were comfortable using (sanitary pad, tampon, or menstrual cup), so the discourse surrounded the experience of menstrual blood donation, rather than e.g., using a menstrual cup for the first time. All participants had donated menstrual blood twice, so in some cases discussion included how these donations differed.

### *Complimenting the research*

Participants generally celebrated the research, describing as "marvellous"; being "fascinated" by the study; finding it "interesting and innovative". One participant stated;

> For me personally, I think the reward of taking part in the study, and potentially contributing to the development of regenerative medicine, far outweighs any demands of the donation process. I am registered with Anthony Nolan charity as a potential stem cell donor, which is why I was interested in this study. The possibility of an additional way for me to donate stem cells in something that appeals to me… It has been a pleasure to take part in this study, and I think that using menstrual blood donations for the development of regenerative medicine is incredibly positive and progressive.

Other comments include the satisfaction of taking part in the research, and the study being "worthwhile and meaningful". Menstruation being "natural" was also mentioned by one participant, and another person highlighted that this method of MSC collection would be "renewable, sustainable".

### *Non-invasive*

Two participants highlighted that MenSC collection is non-invasive;

> This is a non-invasive and natural method to isolate stem cells that can help to overcome the problems related to obtaining this type of cells from sources more difficult or more painful to access.

### *Accessible, many can donate*

Some participants discussed the accessibility of the research, and that menstrual blood donation would be available to many people, stating "I think it's a very easy process that lots of women can do", "every woman can easily donate", and;

> Utilising menstrual blood for stem cell extraction could make regenerative medicine a far more feasible treatment option for more diseases, making it accessible to more people.

### *Wanting to help*

Participants stated they took part in the research generally because they wanted to help people, whether that was to progress research in the field, or support by donating menstrual blood for

clinical application; “I would be open to donating for treatment on the NHS, as I know that it’ll be going towards benefiting others”;

> I see this as a way of giving back almost, having family members that have needed stem cell donations also pushed me to help with developing different ways.

And this includes being able to help when a participant is unable to donate blood conventionally; “I really wanted to be involved in this research project as have always wanted to give blood but haven't been able to”. One participant didn’t mind which specific application the MenSC were being used, so long as it helped, either donating for example for treatment on the NHS, or a charity like The Anthony Nolan Trust; “Regardless of the end result of my donation, the donation process would still be the same in my eyes.”

*Payment*

This participant also went on to say that they would not expect monetary payment for donating MenSC:

> I guess it’s the same as donating blood to a blood bank- people don’t get paid for that! The whole point of a donation is that you volunteer to donate something. It’s no effort at all, so definitely wouldn’t expect payment.

One participant explained that they would want payment if the sample was used for treatment, but more participants stated they would not expect payment for the donation of MenSC. This might be specific to UK (most countries ban the compensation for blood donation, but some countries do compensate (Jaworski 2020)).

This links perhaps to the general theme of altruism; giving time and energy to help others, a key motivation for taking part. This was often a vague sense of helping, and sometimes more specific, either by increasing research, or hypothetically helping someone in need of cell therapy. As well as this, there was the attitude that MenSC donation would also have wider benefit to society as the menstrual taboo was condemned. The menstrual taboo was also discussed in terms of making sure the topic and MenSC donation was approached with sensitively, knowing that for many people it would be considered unacceptable, with the overall theme being that the menstrual taboo is to be eradicated.

This research confirmed many themes anticipated by early research exploring attitudes to menstrual blood donation. Many participants complimented the research, excited by the topic, and certainly inspired to take part. This is evidence that the public would welcome a future MenSC donation and treatment programme similar to that of bone marrow MSC. In addition to being interesting and exciting, key benefits of MenSC include non-invasive nature of donating and broad donorship, making MenSC donation and potential future treatment very accessible.

### *Taboo in society*

Multiple participants mentioned or implied the menstrual taboo;

> However, I can understand why the process could be demanding for some people. Menstruation can be a sensitive subject, some people might be embarrassed by the donation process, and find it mentally demanding.

Another expected theme to arise, the menstrual taboo was discussed by participants either in anticipation for limited uptake of future MenSC donation, or negative connotations for donors. However, one participant hinting that MenSC donation could be instrumental in educating people around the world;

> Menstruation is considered as a taboo in most of the countries even now. So a study like this will be an eyeopener for those who still believe that.

This strengthens the argument that MenSC donation and therapy will be instrumental in overcoming the menstrual taboo, or at least improving education on the topic as a first step.

### *Fiddly, difficult donation*

Participants discussed the fiddlier aspects of the donation, including closing the bag, the "the logistics of placing tampon in bag without spilling whilst also thinking about cleaning up/putting in another tampon", and;

> It was a little difficult when you're trying to take the pad off in a normal way in the toilet and your pants/trousers are still down and the biohazard box is far away. Other than that the procedure is easy.

In addition to finding elements of donation fiddly or tricky, one participant highlighted they found the process of writing in the toilet the most unpleasant part of the donation experience. This includes writing on the sample label, and completing the NASA Task Load Index.

Another participant mentioned the general stress of being on their period at the time of donation, including the stress of the heavy flow, bleeding onto the bedsheets, and being exasperated, which was not necessarily linked to the donation; "Frustration was high but I was also mainly just exasperated. As I mentioned - something like this will happen every cycle so I'm used to it".

Another participant made a reference to the difficulty of timing the donation; "you have to time the insertion of the tampon with collection time so that you know you'll have enough blood to donate".

### *Donation was easy, routine*

Saying this, almost all participants mentioned the ease or routine nature of the MenSC donation; "The overall experience was very easy, easy instructions and wasn't distressing or uncomfortable

at any point"; "The task was very straightforward and not demanding because it's something that's already part of my daily routine when I'm on my period";

> The overall process of removing the pad and putting it in the container was very straightforward, easy to massage as a lot of liquid. Did not affect the way I usually remove pads, requires the use of two hands, however, this is usually the case normally.

In addition to this, one participant mentioned that they were not worried about getting blood on them, and that donation did not increase exposure to blood compared to normal routine.

In terms of implications for menstrual blood donation, this research contributes a number of points to take forward. When designing this research effort was made to ensure the menstrual blood donation process was as dignified, straight-forward, and quick as possible. This included as much autonomy for the participants as feasible, clear instructions, and practical donation method. Participants were in an accessible bathroom with more space than a conventional cubicle, and were provided with a writing surface/drawer storage on wheels so that they could access spare pads and tampons, spare paperwork etc., and they could move the surface to convenient. Most participants discussed the easy, routine nature of the donation. However, an increased working surface might help participants lay the equipment out, and as one participant mentioned, the ability to wheel the storage to where convenient needs to be made clearer to participants. More writing space would make the labelling process easier. As there was concern for spilling the collection mixture, a suitable device to hold the collection rather than simply being placed in a box would make it less fiddly and more accessible in general. It is an aim for future donation to be possible one-handed. Many of these concerns involve the logistics and practicality of donation, so future product development should engage with PPI and rigorous product testing when designing the donation kit.

### *Repeat donation*

Two participants stated that the second donation felt no different, or no more easy or difficult the second time round, and a few participants mentioned that they found it easier the second donation. Most participants did not explain this in further depth, although one did say "The second time I donated, I was more relaxed as I knew what to do… it was a much more easier donation."

### *Donate from home, or give the option*

Several participants stated that they would be comfortable or happy donating MenSC from home, one stating they would be happy to donate "with a send home pack to do in more comfortable surroundings", and another;

> It's quick and easy to do- it's even more discrete at home, so even if someone was nervous about doing it in a laboratory setting, this solves that issue.

While not talking about personal opinion, one person did say, "for some people, it could be inconvenient and physically demanding to travel to make a donation." Although the preference of donating from home is not universal, as one participant stated they would not prefer to donate from home;

> I would prefer to donate in-person, as I would be less likely to make a mistake and it would lower chances of error such as, transporting of sample from home to research site.

The inconvenience of travelling to donate and the improved discretion and comfort of donating at home were mentioned by several participants, so future research should explore the feasibility of donating from home, particularly as this study suggests that MenSC still remain viable after 48 hours' storage at 4 °C.

This provides evidence that there is appetite for repeat MenSC donation, repeat donation is either no more difficult or easier second time round. However, to be made more accessible and convenient for donors, future research should consider providing multiple options for donation; from home or work, or from the lab, to cater for the needs of individuals. This is similar to the increase in at-home swab kits for STI diagnosis, or that peripheral blood donation has become more flexible to encourage donation (NHS Blood and Transplant 2023).

### *The product makes a difference*

The products provided for MenSC donation were sometimes a brand donors were familiar with, and sometimes were a different brand or style to their preferred product. The ease of donation was sometimes attributed to the fact that the brand of product used to collect menstrual blood was the same as their usual brand. On the contrary, two participants (both using sanitary pads) stated that they were asked to donate MenSC using an unfamiliar brand or size. One person seemed neutral on the topic; "The pads I used in the study were not my regulars - but I usually choose what's on offer!", but the other felt uncomfortable; "The collection pad for me was the wrong size and felt like I was going to leak through it during wear".

Two participants who donated with a menstrual cup generally complimented the menstrual cup, commenting, "Using the cup once getting used to it, is easy and straightforward",

> Although I now have lean more towards reusable pads, the cup is a good alternative for zero waste periods and makes the donation process easy and straightforward.

The most popular and readily-available menstrual hygiene products were chosen for this study. However, it is unacceptable that some participants were scared of or experienced leaks during the

study, so it is imperative that future research explores a wider variety of menstrual hygiene brands and products, to cater for as many participants as possible. This includes difference size/absorbency products, as well as reusable pads or period underwear.

### *Added pressure of donation, period stress, and trickiness of donation*

Participants stated they felt pressure or insecure about donating:

> The added 'pressure' of ensuring I didn't throw the pad in the sanitary towel bin or fasten the bag incorrectly made me slightly anxious as I didn't want to spoil my sample.

Furthermore, it needs to be understood that participants are donating at an already potentially stressful time; their period. While most participants did not mention this, the stress of the donation or period in general was discussed, particularly timing the donation to ensure there was 'enough' sample. Especially as menstrual cycles can be a very unpredictable time for some people that menstruate, the menstrual blood donation process including the planning needs to be sensitive to the participants' mood and situation. In some weeks, 70% of scheduled donations were cancelled last minute, mostly due to the flow being unpredictable, starting early or late, and falling at an inconvenient time for participants. Future menstrual blood collection research should put more power in participants' hands: they should be able to schedule entirely themselves, perhaps following an approach similar to conventional whole blood donation where online portals allow donors to select a time slot independently (NHS Blood and Transplant 2023). The lab will then receive an alert for when to prepare for the collection and isolation, hopefully saving time and reagents, as well as giving participants more autonomy. This relates to one of the foundational aspects to this research, the degree of citizen power, outlined in the Ladder of PPI by Arnstein (1969).

Improved scheduling might encourage repeat donation, which would feed into even better donation experiences as participants familiarise themselves with the process, as even during the second donation participants discussed finding it easier.

One participant described they "Felt a little insecure about donating but Hannah was very reassuring". This links to the idea that the researcher has an impact on how the donation is experienced as a participant.

### *Researcher has positive impact*

Multiple participants discussed the researcher directly, complimenting the conduct or discussing the trust developed between researcher and participant; "Hannah explained clearly all the steps of the blood donation and MSCs isolation, making me feel confident and enthusiastic to donate"; "I trust the individual conducting the research and so, was more willing and comfortable with

taking part in the study"; "Hannah was very much friendly in explaining and answering all the queries. She also made an effort to follow up".

Another participant commented that they felt comfortable in their surroundings as they donated, so not only does the researcher have a positive impact on donation experience, but the environment itself also has an impact on donation experience.

This shows the importance of conducting research in a friendly, approachable, patient manner. This is a foundation of feminist research; understanding that the researcher has an influence on how participants experience a study, and that they should understand the power dynamic between researcher and participant to avoid exploiting that power. Particularly as this work revolves around taboo and sensitive topics, and that participants were bearing personal and medical information including reproductive and sexual health, this dynamic had to be approached delicately. As this study was undergoing ethical approval, a Clinician overlooking the application noted the levels of consideration that was made for the participants. This shows the success of the current study, and future MenSC research should be a continuation and improvement on this.

### *Use for waste product/monthly burden*

Several participants discussed that their menstrual blood is going to waste anyway, or considered a "burden", questioning what their menstrual blood "would achieve"; "Using menstrual blood seems the most effortless and advantageous as it is essentially a waste product anyway"; "It's only going to get chucked in the bin anyway, so why not!".

This links to the findings in Chapter 3. Participants appreciate giving what might be considered a nuisance, burden, or waste a purpose. Instead of throwing something away, it can be put to good use. This is further evidence highlighting the appetite for MenSC donation, giving people a reason to celebrate menstruation, and is a key motivation for donorship.

### *Hypothetical vs. actual MenSC donation attitudes*

Because Chapter 3 analysed the data collected surrounding MenSC donation on a hypothetical basis, it is possible to note where attitudes and opinions overlap with the attitudes and experiences of participants in this study undertaking actual menstrual blood donation, and where actual MenSC donation has produced differing opinions. These attitudes are shown in Figure 6.18.

Unsurprisingly, between the members of the public that have not donated MenSC (public) and the participants in this study who have donated MenSC (participants), there are themes that are independent of each other. These include the public having fears or concerns over the practicality of donating MenSC, and fears or concerns of using a menstrual cup to donate, and the idea of encouraging each other to donate. This is down to any uncertainty surrounding actual donation being clarified for donors, and donors only donating with a product they are comfortable with,

rather than the public who were asked about donating with a menstrual cup, the most popular method in the literature. The public also commented about the factor of disgust when donating menstrual blood. No participants mentioned this in their response, and this could be because participants who did donate might have thought the process would be disgusting and were surprised that it was not, or more likely that only those who would not be disgusted or concerned about menstrual blood would be willing to take part in the study in the first place, removing this factor from discussion. Intriguingly, the comment that menstrual blood is "just blood" was only mentioned by the public, and this might be due to participants elevating menstrual blood status beyond "just blood" and into the realm of being helpful, empowering, or impressive. It certainly removed the idea of menstrual blood being a 'waste' blood.

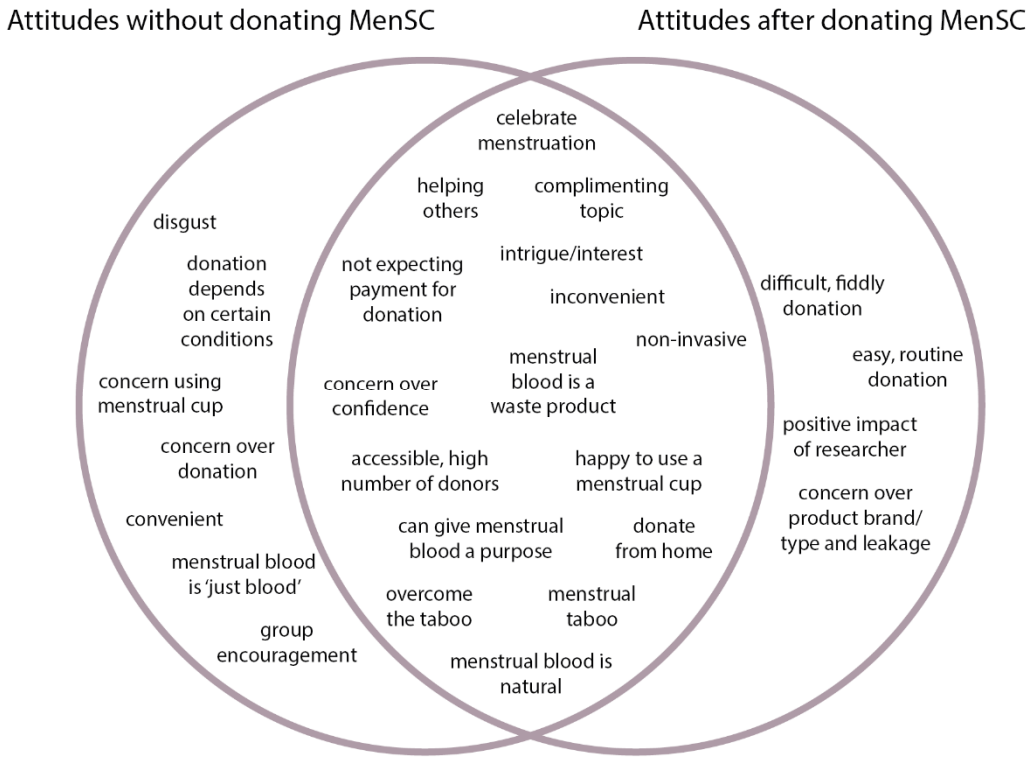


*Figure 6.18 Venn diagram showing the public's attitudes to MenSC donation on a hypothetical basis (had not donated MenSC), and the attitudes of participants in this study (people who had donated MenSC).*

All the comments made by participants were specific to donating MenSC, including being fiddly, ease, and concern over product leakage which is not surprising. What is interesting however, is the high quantity of overlapping themes that have appeared between the public and participants. Many celebrate MenSC donation, wishing to help others without pay with this non-invasive, interesting research. Menstrual blood is considered a 'waste' blood, that can give menstruation a purpose beyond simply being a nuisance. Although menstruation is a natural process, it should be celebrated, and it is impacted by menstrual taboo, which could be overcome by MenSC donation and therapy.

It was assumed that hypothetical donation attitudes by the public would be too far removed from reality and would therefore be greatly deviated from the participants' thoughts which is not the case. What can be taken forward with this information is that approaching and supporting members of the public as well as repeat donors can largely utilise the same approach. If concerns and questions about product use and logistics can be appeased, then most people agree on complimenting the donation of MenSC for research or treatment purposes, and are empowered and intrigued to donate, wanting to help others. It is considered more accessible than e.g. blood, bone marrow, or umbilical cord blood donation, gives the 'waste' blood a purpose, and by extension being a tool to see menstruation as something to celebrate and eventually overcome the menstrual taboo. Further research should be done to make the donation even more convenient, potentially possible from home to reduce travel time and offer a more comfortable and confident experience. Later development will identify whether MenSC adopts the current blood donation strategy or whether donors receive monetary compensation for samples.

#### *Limitations with qualitative findings*

It is important to note that while the qualitative data from respondents who had not donated menstrual blood in Chapter 3 reached data saturation, only 16 participants were willing to share their opinions after donating menstrual blood for the MenSC study. After thematic analysis, this study has not yet reached data saturation. The current themes revealed by the 16 participants remain a valuable initial exploration of this topic. This study collected handwritten qualitative data, meaning that participants could privately share their thoughts and concerns, but it meant that certain ideas could not be probed or deepened by researcher enquiry as it might with an interview or focus group.

### 6.6.9 Participant retention

The study had a high drop-out rate (46%). This is due in part to the mutable nature of reproductive health status such as pregnancy or menopause, the complicated and sometimes-unpredictable nature of the menstrual cycle and organising the timing of sample donation, perhaps the intrinsic link to taboo and maybe disgust of donating menstrual blood, as well as being based in an academic setting and participants moving in and out of the area between term times. Recruitment for future research involving MenSC or general menstrual blood donation should bear this in mind.

### 6.6.10 Planning collection

It was difficult planning donation times due to donation being limited to one day per month per participant, and participant availability and fluctuations in menstrual cycle. Many scheduled donation appointments were cancelled or rearranged last minute making this a limiting factor to menstrual blood donation.

Unpredictability or changes in menstrual cycle could be due to a number of factors including stress (Yamamoto et al 2009), receiving a COVID-19 vaccine (Edelman et al 2022; Laganà et al 2022) and even stressors due to the COVID-19 pandemic (Medina-Perucha et al 2022). These factors should be taken into account when planning and undertaking future MenSC research.

Related to this, it was expected participants would be engaged in the research for 3 months. They were engaged on average for 5.78 months (*SD* = 3.84), in part due to this cycle unpredictability and planning difficulty. For future research involving menstrual blood collection, the demand on participant's time as well as the logistics of organising donation timings should be taken into account and communicated clearly for participants to make informed decisions. This long engagement likely also contributed to the low participant retention rate. If participants are better informed about realistic expectations, they will have an improved experience, and this will save researchers time and equipment. A consequence of this would perhaps be decreasing recruitment rates but improving participant retention.

### 6.6.11 Donation comfort

It was important that participants donated menstrual blood with a product they were already comfortable with to make the process as straightforward and stress-free as possible. The sanitary pad (Always ULTRA size 1 normal with wings) and tampon (Tampax Compak regular) were chosen as they were accessible and popular in the UK. However, 2 participants mentioned the sanitary pad brand and style was not the style they would usually choose, and in one participant the absorbency levels were not high enough for the four-hour recommended wear time and she experienced slight leaking during the first sample donation. This is not acceptable for participants to experience leakage during donation. Future research should explore different brand and styles in MenSC collection, with the aim that all donors can be comfortable with a brand they prefer.

#### *Self collection*

Qualitative research regarding self swabs for sexually transmitted diseases revealed that women feel more responsible for their sexual health when confident in collecting their own samples (Verhoeven et al 2002), and that the 'do it yourself' aspect of self collection increased privacy (Rohner et al 2020). A strength of this work if that participants undertook the donations themselves, creating the sense of responsibility, privacy, and autonomy in this study.

### 6.6.12 Low volume samples

Although not measured, some samples via sanitary pads appeared to have a very low volume prior to processing. See Table 5.2 for examples. It was possible to isolate MenSC from most participants at least once, however for 2 participants and both via sanitary pads, both sample volumes appeared very low, and no MenSC were isolated. In these cases, it was a sad that the

participants expressed shame or guilt during donation due to the low volume and difficulty timing it with the heaviest flow. Future researchers should ensure to never shame donors, and thank them for their time, stating “this is what research is all about!”. It was considered for participants to estimate their menstrual blood loss here using a pictorial assessment tool to be completed by participants before placing into the donation bag. However, pictorial methods in various forms have not been found to be a valid method to estimate actual menstrual blood loss (Larsen et al 2013; Reid et al 2006; Reid et al 2000). It was deemed not valuable enough to add another step to the donation for participants. In reality, during future MenSC research, when samples are delivered with a visibly nil volume, it would simply be discarded.

Tampon samples appeared to have higher volumes than the samples from sanitary pads. Tampons and sanitary pads hold similar volumes of blood (DeLoughery et al 2023). It is posited that participants with a higher menstrual blood volume are perhaps more likely to use a tampon, and therefore it has higher MenSC isolation success rate, however there are no data to support this contemplation. Further research should identify whether people have tendencies towards certain products depending on their menstrual blood flow.

### 6.6.13 Clots

It was expected that samples collected via menstrual cup would be more straightforward than sanitary pads or tampons due to requiring fewer processing steps. However, samples collected via sanitary pad and tampon had far fewer clots and mucous and were easier to work with. It is posited that the product fibres dispersed or filtered any clots or mucous. Although only demonstrated by anecdotal evidence, this is an interesting finding that indicate that sanitary pads and tampons could be preferred methods of collection to save researchers time. Further research can confirm this.

### 6.6.14 Menstrual cup willingness

In this study, there was a higher menstrual cup willingness rate than the general public (38% vs. 4%). It is posited that participants already using a menstrual cup were more likely to engage with this research because the recruitment poster included an image of a menstrual cup, and users of this more unconventional product were more passionate or interested in seeing this product represented in research. Future MenSC research should celebrate the passion of menstrual cup users and other unconventional forms of menstrual hygiene management, which could be instrumental in future recruitment methods.

# Chapter 7. Discussion and conclusion

This chapter seeks to critically review the overarching themes of this thesis, synthesising the results of each chapter in response to each research question and discussing the implications of this research for MSC scientists, clinicians, and future menstrual blood donors.

In pursuit of answering **research question 1: What are MenSC, and how are they currently donated?**, this thesis identifies the exciting characteristics of MenSC, their suitability for regenerative medicine, and the clinical applications MenSC offer. MenSC are multipotent MSCs, with high proliferative ability and low tumorigenicity, with research groups exploring both autologous and allogeneic transplantation. Clinical application of MenSC include intrauterine adhesions, multiple sclerosis, acute respiratory distress syndrome, poor ovarian responders, and COVID-19. In addition to identifying the exciting clinical applications of MenSC, this thesis emphasises that the focus of research has never been on the donors; no single donation method has been identified as the most appropriate, either in terms of MenSC survival, or donor's preferences. While remaining underreported, it was possible to compare and analyse the logistics of menstrual blood donation for cell therapy or MenSC research; i.e. the device used to collect, the wear time, and time between donation and processing. The most popular method was via a menstrual cup, during 'heaviest' flow, often processed immediately or within 24 hours. This is helpful to understand what is required from the scientists' perspectives, but what is lacking is the research exploring whether people are even willing to donate menstrual blood, and their experiences donating MenSC. There has never been a comparison of differing methods to explore which would be more appropriate for donors, and therefore improve their experience as well as increase MenSC donation rates.

This led to posing **research question 2: Do people even want to donate MenSC?** Without an understanding of the public's attitudes to donating a taboo blood, their concerns, and needs, there runs the risk there are no willing menstrual blood donors, and therefore no MenSC. With this knowledge, MenSC donation can be adapted and citizens can be empowered with contribution or control of the menstrual blood donation process. By placing donors at the centre of this research, the outcomes of a feminist approach become "appropriate to the needs and lifestyles of the patient community it serves" (Bagley et al 2016, p. 6). Generally, the public are willing to donate menstrual blood, and concerns relay to logistics, convenience, and confidence, as well as concern surrounding the menstrual taboo. It was indicated that MenSC donation and treatment might be influential in overcoming the menstrual taboo, which has positive connotations for all; the experience of menstruation is shaped and limited by the menstrual taboo (Delaney et al 1988; Johnston-Robeldo and Stubbs 2013; Kissling 2006; Laws 1993). Regarding donation logistics, while many are willing to donate with a menstrual cup, why limit menstrual

blood donation to menstrual cups alone? This enquiry introduced the opportunity to explore more accessible forms of menstrual blood donation to cater for more people: now that it is understood many are willing to donate menstrual blood, as a minimum this would be exploring sanitary pads and tampons as potential donation methods.

This provoked the pursuit of **research question 3: What are sanitary pads, tampons, and menstrual cups, are they safe, and how are they being regulated?** In the context of menstrual blood donation, with the proposition of requiring participants to use existing products in this novel manner, it is vital that these products are understood. Researchers can only advocate for the utilisation of methods in the possession of a thorough understanding of their functionality, usability, and safety. The investigation of sanitary pads, tampons, and menstrual cups revealed the FDA considers sanitary pads and tampons as medical devices (MDR 2023), and absorbency is regulated to reduce risk of TSS (AHPMA 2019; FDA 2019a). However, the MHRA state tampons are "not normally considered to be medical devices" (2016, p. 4), and companies are not required to disclose menstrual hygiene product composition (Desmedt et al 2020). Seeing as toxic compounds have been found in both, and tampons hold higher risk than sanitary pads (Ding et al 2020; Ding et al 2022), regulation is still lacking for these products. There is a greater problem for menstrual cups. Although they are considered generally safe to use (van Eijk et al 2019), adverse effects and injuries are reported by the FDA (2020), and published works detail TSS (El Soufi et al 2021; Mitchell et al 2015; Stanke et al 2020), and renal colic as a result of menstrual cup use (Athiel et al 2019; Nunes-Carneiro et al 2018; Stolz et al 2019; Umaramanan et al 2019). The large range of menstrual cup styles (shape, size, material, and firmness) that remain unregulated contribute to consumers purchasing the wrong products (see Section 4.3.4) and have implications for comfort, usability, and safety (Table 4.11). As a first step to this regulation, this thesis compared 14 popular menstrual cups. There is no correlation between a menstrual cup's size, shape, and volume, or a menstrual cup's material, shape, and firmness, and proposing suitable categories for size or shape remains unanswered in this work. However, the categorisation of menstrual cup firmness, ranging from very firm to very soft, is a first step to supporting people purchasing a menstrual cup suitable for them, and aiming to reduce risk of injury. This work has not included the study of alternative menstrual hygiene products such as menstrual discs, reusable sanitary pads, and washable period underwear. More research and regulation must be in place to make all menstrual hygiene products safe and transparent for those who menstruate to make informed decisions regarding the products they choose.

Chapter 5 reports the protocol optimisation of MenSC isolation via sanitary pads and tampons in response to **research question 4: How can MenSC be donated via sanitary pad or tampon?** Protocol for MenSC isolation via menstrual cup has been previously reported (Sanzhez-Mata et al

2021), but never from sanitary pads or tampons. By optimising isolation methods first using bone marrow MSCs, then animal blood, before confirming with menstrual blood, this chapter confirms that MenSC isolation is a low-cost, straightforward process that is suitable from the researcher's perspective. This directly fed into the methods of the main study of this thesis, to compare MenSC donation methods in terms of MenSC isolation success, and suitability and acceptability for donors.

The findings from Chapters 2 to 5 accumulated into **research question 5: How do sanitary pads and tampons compare to menstrual cups in terms of MenSC isolation and donor experience?** Most commonly donated via menstrual cup, this thesis found success isolating MenSC from sanitary pads and tampons as well as menstrual cups. When considering each product's MenSC isolation success rate and the participants' willingness to use sanitary pads, tampons, and menstrual cups, all three products have value, increasing MenSC donorship and accessibility to MenSC application.

MenSC isolation success and willingness are a snapshot of the many factors contributing to identifying the optimum MenSC donation methods. Should MenSC donation move to clinical or commercial contexts, further steps to identify and optimise sample collection and MenSC isolation costs would be necessary: van Eijk report mean menstrual cup mean cost at $23.30 (2019) and in this study the sanitary pads and tampons cost £3.40 and £2.79 per pack respectively (Boots). After including all other equipment and reagents, overheads, and storage costs, it would be fascinating to calculate overall cost and compare to other sources of MSC. This cost value could incorporate success rate; Zhang et al achieved a 90% umbilical cord MSC isolation success rate (2011) but Kern et al achieved only 63% success rate for umbilical cord and 100% for bone marrow and adipose tissue MSC (2006). A formal cost analysis would firstly identify whether sanitary pads, tampons, or menstrual cups are more cost-effective as methods of MenSC isolation, and then to contextualise these costs and compare to MSC isolation from adipose tissue, bone marrow, dental pulp, and umbilical cord blood. As a first comparison, isolation success and donor willingness were reported as the two key topics of this thesis, and in reality cost will have huge implications for shifting MenSC into clinical and commercial settings.

Comparing sanitary pads, tampons, and menstrual cups, the product used to donate had no impact on the MenSC in respect to processing time, number of MenSC, time to reach confluence, proliferation, and stemness. These factors were not affected by immediate vs. delayed processing, showing that in the future, MenSC could be donated from home or off-site before being transferred and processed within 48 hours, which aligns with work by Patel et al (2008), Rossignoli et al (2013) and Sun et al (2019a).

MenSC display excellent proliferative capacity, even when seeded at low densities of $1 \times 10^2$ MenSC/$cm^2$; at this density MenSC took 11 days to reach confluence. This is promising for future MenSC application, as even with low cell counts, MenSC can be easily expanded to quantities required for cell therapy.

Sun et al (2019a) found that MenSC isolation was increased by directly culturing the otherwise-discarded red blood cell pellet after red blood cell lysis. However, this method has not been widely adopted, and further publications from this research group do not confirm using this method (Chen et al 2022; Li et al 2023) which brings the question as to why this is not explored. Perhaps cell count was very low, or the time taken or additional equipment required was not deemed worthwhile. This was not explored in this thesis, however future research should identify the value in utilising this method to maximise every menstrual blood sample in the future.

A strength of this thesis is the transparency with which the menstrual blood donation and MenSC isolation process is reported. Currently, very few researchers (Wyatt et al 2021) report all the logistics of the MenSC donation process, and the donation process from the participants' point of view has never been described and analysed. This work clearly reports these processes. Reporting this and descriptive statistics such as menstrual cycle length, product wear time, and processing time improves reproducibility of the protocol and allows researchers to compare these factors in future studies.

There are factors beyond these which should be explored in the future: information on menstrual blood pH and viscosity is firstly outdated (Reame 1983), and has never been studied in regard to MenSC. These should be reported and studied alongside factors such as menstrual blood volume and flow rate, blood content, as well as factors such as participant race and ethnicity, both as biological factors affecting MenSC and cultural expression and implications for willingness to donate menstrual blood. The factors affecting MenSC donation, yield, and viability is summarised in Figure 7.1.

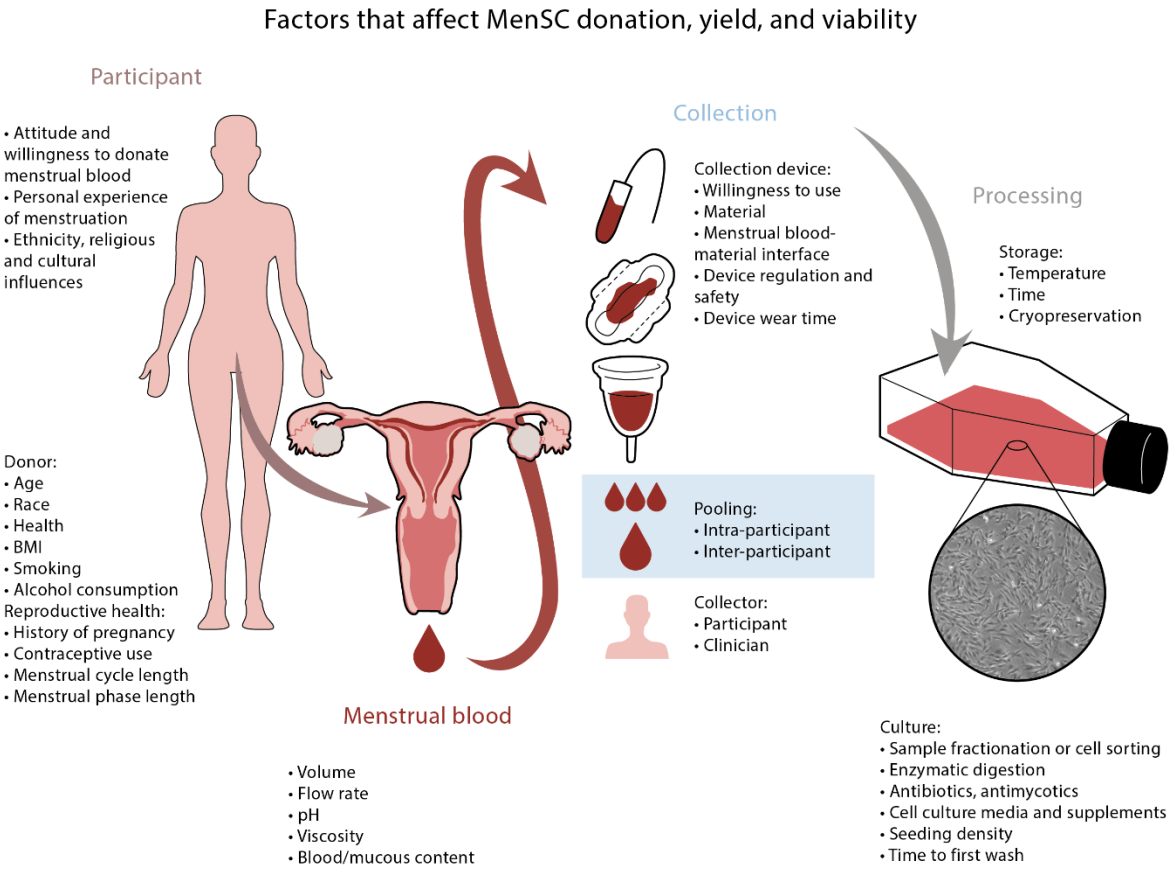


*Figure 7.1 Factors affecting willingness to donate menstrual blood, MenSC yield, and viability.*

Menstrual hygiene products should be considered medical devices. One of the benefits of this would include improved safety and accurate reportings of injury or side effects due to their use. This would bring sanitary pads, tampons, and menstrual cups (among other product such as discs) up to the standards benefitted by products like condoms, vaginal rings, incontinence products, resulting in better products. These can also be tested in regard to their biocompatibility with MenSC.

The thesis reported the first media comparison for MenSC culture, and recommends the use of DMEM-F12 (3.15 gm/L), supplemented with 10% FBS, 100 unit/mL of penicillin, 100 mg/mL of streptomycin and 0.25 µg/mL of Amphotericin B for optimum results.

This work has adopted a feminist approach, meaning the participants are at the centre of this research and given meaningful participation. To this end, it was imperative that MenSC donation was also analysed in terms of acceptability, taking donation experience into account. Participants compliment the research, praising MenSC donation for its non-invasive, accessible nature and general ease of donation, and while there are improvements to be made to reduce the fiddly nature of donation and to allow a broader range of brand and product for collection, MenSC donation and clinical application is worth celebrating and considered instrumental in overcoming the menstrual taboo. Menstrual blood donation is not considered more challenging or demanding

when compared to participants' normal menstrual hygiene routine at home, but interestingly there was also no statistically significant difference between donation 1 and 2, meaning participants do not feel more comfortable or challenged when they are repeating the donation process. It is the hope that future MenSC donation will encourage repeated donation, so future work should focus on improving and simplifying the donation process.

Adopting this feminist approach firstly resulted in discourse surrounding MenSC and their collection from a perspective previously never reported. It is argued here that the lack of feminist approach in the MSC research community is the reason that an appropriate MenSC donation method had not previously been explored. By being donor-focused, exciting donation opportunities have been identified, improving accessibility to MenSC donation and therapy. Beyond this, the qualitative data collected here support the argument that a feminist approach resulted in participants having a more positive experience, with multiple participants singling out the researcher as a reason for feeling more confident, trusting, and comfortable. This feminist approach has avoided the alienating and dehumanising processes traditional research often falls into (Maguire 1987). As MenSC research progresses, it should go beyond a degree of tokenism and employ citizen partnership and control, outlined by Arnstein (1969). All future MenSC research should adopt a feminist approach because this uncovers exciting results and empowers the public.

While this work celebrates the success of utilising sanitary pads and tampons to improve MenSC donation, improving this experience has implications for other avenues of research involving menstrual blood collection. Menstrual blood can be analysed to diagnose endometriosis (Cressoni et al 2023; Nayyar et al 2020; Nikoo et al 2014; Madjid & Hernowo 2019; Warren et al 2018) a great improvement on the current 4-11 year delay (Agarwal et al 2019). Future menstrual blood collection could be used for monitoring diabetes mellitus (Naseri et al 2022a), detecting endometrial cancer (Quynh & Radisch 2008), HPV diagnosis (Naseri et al 2022b), and diagnosing reproductive failure by analysing lymphocyte subpopulations (Marron & Harrity 2022). Therefore, the collection of menstrual blood via sanitary pads and tampons may have application in diagnostics of several conditions as well as MenSC isolation. Furthermore, educating people on MenSC and donating MenSC could open conversation surrounding general mental, relational, and sexual health. MenSC donation could provide society with a tool to help overcome the menstrual taboo and remove the sociocultural barriers that diminish menstruators' wellbeing and life experiences.

In summary, menstrual blood contains MSCs that are not only clinically relevant for treating musculoskeletal disorders aligning to uses for MSCs, human and animal clinical studies have proven their potential to deliver therapies for conditions such as stroke, liver failure, sepsis,

premature ovarian failure, intrauterine adhesions, and COVID-19. It has a huge potential donorship, and menstrual blood is considered a 'waste' product. The ability to isolate MenSC from sanitary pads and tampons has implications for all future MenSC and broader MSC research and the ability to deliver cellular therapies at scale, as it increases accessibility to a source of MSCs even further, particularly where menstrual cups are not widely adopted and not regulated to the extent of sanitary pads and tampons. This work also has wider implications for reproductive health and diagnostics, for example the diagnosis of endometriosis and endometrial cancers, and this feminist approach should be applied to all research for outcomes that are appropriate to the needs and lifestyles to all involved.

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# Appendix A MenSC treatment in animal models

| *Experimental method* | | | | | | *Disease* | *Outcome* | *Author* |
|---|---|---|---|---|---|---|---|---|
| *Model* | *Administration method* | *Transplantation* | | | | | | |
| | | *MenSC / transplant* | *no. transplant* | *treatment timing* | *total no. MenSC transplant* | | | |
| Mouse | Uterine cavity injection | $2 \times 10^5$ | 1 | - | - | Endometrial injury | Mice treated with MenSC exhibited increased endometrial thickness and resulted in increased pregnancy rates | Hu et al 2019 |
| Mouse | Intramuscular injection | $2 \times 10^7$ | 1 | - | - | Duchenne muscular dystrophy | Improved efficiency of muscle regeneration and dystrophin delivery to dystrophic muscle in mice, differentiation into myogenic cells | Cui et al 2007 |
| Mouse | Embryo co-culture with MenSC | - | - | - | - | Assisted reproduction | Coculture with MenSC increased blastocyst rates during in vitro fertilisation treatment | Gonçalves et al 2020 |
| Rat | Injection to centre and around myocardial border zone, two sites | $2\text{-}4 \times 10^5$ | 1 | - | - | Myocardial infarction | MenSC transplantation decreased the fibrosis area and restored the left ventricular systolic function, achieving cardiomyogenesis | Hida et al 2008 |
| Mouse | Intramuscular injection | $1 \times 10^6$ | 3 | 4 | $3 \times 10^6$ | Ischemia | By day 14 the leg tissue of all control mice had died, where MenSC treated mice had intact limbs, with 2/8 displaying signs of impeded walking | Murphy et al 2008 |

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| Rat | Intravenous injection | $3 \times 10^6$ | 1 | - | - | Brain tumour | MenSC had inhibitory effect on brain tumours, with 49% tumour volume reduction after intravenous injection, and 46% tumour volume reduction after intratumoral administration | Han et al 2009 |
| | Intratumoral injection | $1 \times 10^6$ | 1 | - | - | | | |
| Rat | Intracerebral injection | $4 \times 10^5$ | 1 | - | - | Stroke | Transplantation of MenSC, either intracerebrally or intravenously and without immunosuppressio n, significantly reduced behavioural and histological impairments of stroke | Borlongan et al 2010 |
| | Intravenous injection via jugular vein | $4 \times 10^6$ | 1 | - | - | | | |
| Rat | Intramyocardia l injection | $2 \times 10^6$ | 1 | - | - | Myocardial infarction | MenSC can attenuate cardiac fibrosis after myocardial infarction; MenSC contribute to improved cardiac remodelling after myocardial infarction | Zhaocai & Jianan 2012 |
| Rat | Injection around myocardial border zone, five sites | $1.5 \times 10^6$ | 1 | - | - | Myocardial infarction | Echocardiography showed improved cardiac function seven days after MensC transplantation, with a small amount of myocardial regeneration by day 28 | Jiang et al 2013 |
| Mouse | Intravenous injection via splenic pulp | $1.5 \times 10^6$ | 1 | - | - | Partial liver removal (2/3) | MenSC were induced into hepatogenic differentiation (liver cells) | Mou et al 2013 |
| Rat | Intramyocardia l injection | $2 \times 10^6$ | 1 | - | - | Myocardial infarction | MenSC increased left ventricle thickness, and decreased infarct size 7 days post transplantation. There was a | Zhang et al 2013 |

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| | | | | | | | significant reduction in cardiac collagen deposition, although MenSC did not differentiate into cardiac cells apart from a small number of endothelial cells | |
| Mouse | Intraperitoneal injection | $1 \times 10^4$ | 1 | - | - | premature ovarian failure | Increased number of mature follicles, differentiation into ovarian granulosa cells | Liu et al 2014 |
| Mouse | Intravenous injection | $1 \times 10^6$ | 3 | over 7 days | $3 \times 10^6$ | Colitis | MenSC had anti-inflammatory and immunosuppressive effects; mice showed less body weight loss, firmer stool, and increased food and water consumption | Lv et al 2014 |
| Mouse | Intravenous injection via tail vein | $3 \times 10^5$ | 1 | - | - | Type 1 diabetes | MenSC improved hyperglycaemia, with a survival rate of 95% compared to 30% nontreated mice, had a more stable weight, and reduced blood glucose levels | Wu et al 2014 |
| Mouse | Intraperitonal injection | $7.5 \times 10^5$ | 1 | - | - | Sepsis | MenSCs and antibiotics resulted in a higher survival rate in mice with sepsis. Liver function was markedly improved in mice receiving MenSC or MenSC with antibiotics | Alcayaga-Miranda et al 2015a |
| | Intravenous injection | $7.5 \times 10^5$ | 1 | - | - | | | |
| Mouse | Intravenous injection via tail vein | $2 \times 10^6$ | 1 | - | - | Premature ovarian failure | MenSC transplantation increased animal weight and restored (although irregular) cyclicity in sterile mice. Litter size in pregnant mice was improved | Lai et al 2015 |

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| Mouse | Injection to ischemic region | $5 \times 10^6$ | 1 | - | - | Ischemia | MenSC injection and infusion both improved degree of ischemia, however, the best result was with mice that were injected and infused with doses of Mensc | Vu et al 2015 |
| | Intravenous injection via tail vein | $5 \times 10^6$ | 1 | - | - | | | |
| | Injection to ischemic region AND intravenous injection via tail vein | $5 \times 10^6$ | 1 | - | - | | | |
| Mouse | Subcutaneous injection | $1 \times 10^6$ | 1 | - | - | Epithelial ovarian cancer | Tumour volume was significantly reduced after MenSC transplantation by inducing cell cycle arrest, promoting apoptosis, disturbing mitochondria membrane potential and decreasing pro-angiogenic ability | Bu et al 2016 |
| Rat | Intrauterine injection | $1 \times 10^5$ | 1 | - | - | Infertility | MenSC resulted in intensive development of all the elements of decidual tissue, resulting in increased decidua size | Domnina et al 2016 |
| Mouse | Intravenous injection | $1 \times 10^6$ | 1 | - | - | Acute liver injury | Liver function improved by MenSC, reducing inflammation, discolouration, and increased liver volume caused by damage | Lu et al 2016 |
| Mouse | Intraperitoneal injection | $1 \times 10^6$ | 1 | - | - | Graft vs. host disease | MenSCs exert a beneficial effect in the experimental graft vs. host disease model, increasing the survival rate in mice, not associated with their capacity to suppress inflammation | Luz-Crawford et al 2016 |

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| Mouse | Intravenous injection via tail vein | $1 \times 10^5$ | 1 | - | - | Injured endometrium | MenSC resulted in significantly accelerated restoration, with increased endometrial thickness and microvessel density | Zhang et al 2016 |
| Mouse | Intravenous injection via tail vein | $5 \times 10^5$ | 1 | - | - | Liver fibrosis | After two weeks, liver fibrosis was reduced | Chen et al 2017b |
| Rat | Injection around sciatic nerve damage | $3 \times 10^4$ | 1 | - | - | Sciatica | MenSC improved leg function after nerve damage and significantly prevented muscle weight-loss | Farzamfar et al 2017 |
| Mouse | Intravenous injection via tail vein | $8 \times 10^5$ | 1 | - | - | Acute liver failure | 70–80% survival rate in MenSC-treated group opposed to 0% survival rate in untreated group after prolonged period, with an improvement in liver function after 30 days | Fathi-Kazerooni et al 2017 |
| Mouse | Intravenous injection | $1 \times 10^6$ | 1 | - | - | Cardiac allograft | MenSC alleviated the severity of pathological changes in the cardiac grafts | Lan et al 2017 |
| Mouse | Intraperitoneal injection | $5 \times 10^6$ | 1 | - | - | Lung cancer tumour | MenSC were cell carriers for regional delivery of an oncolytic adenovirus (cancer treatment) in the tumour, and could eliminate 50% of cancer cells in 5 days. MenSC were reported in tumours, regardless of administration method | Moreno et al 2017 |
| | Intratumoral injection | $5 \times 10^6$ | 1 | - | - | | | |
| Rat | Injection around myocardial border zone, five sites | $1 \times 10^6$ | 1 | - | - | Myocardial infarction | Paracrine effects of MenSCs on myocardial infarction, with a superior therapeutic potential | Wang et al 2017c |

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| | | | | | | | compared to bone marrow and adipose MSC | |
| Mouse | Intravenous injection | $2 \times 10^6$ | 2 | over 3 days | $4 \times 10^6$ | Premature ovarian failure | Mouse bodyweight and ovary weight was significantly higher in the MenSC-treated group, showing health improvement. MenSC treatment lead to significantly more primordial, primary, secondary, early antral, and pre-ovulatory follicles after one week. MenSCs reduced injury to the ovary by inhibiting apoptosis | Wang et al 2017d |
| Mouse | Intravenous injection via tail vein | $1 \times 10^6$ | 1 | - | - | Acute lung injury | MenSC reduced inflammation, thinning the thickened texture and improving the dry/wet ratio of the lung | Xiang et al 2017 |
| Mouse | Intravenous injection | $1 \times 10^6$ | 1 | - | - | Cardiac allograft | MenSC treatment doubled the mean survival time of cardiac allografts | Xu et al 2017 |
| Mouse | Intradermal injection, four sites | $1 \times 10^6$ | 1 | - | - | Cutaneous wound | MenSCs treatment increase the rate of wound closure, promoting proper tissue regeneration | Cuenca et al 2018 |
| Rat | Intravenous injection | $1 \times 10^7$ | 1 | - | - | Injured endometrium | Pregnancy rates were greater with intrauterine rather than intravenous administration, but number of pups was not different between the administration | Domnina et al 2018 |
| | Intrauterine injection | $1 \times 10^6$ | 1 | - | - | | | |
| Mouse | Intravenous injection via tail vein | $1 \times 10^6$ | 1 | - | - | Acute lung injury | MenSC reduced inflammatory infiltrates, interalveolar septal thickening, and other | Ren et al 2018 |

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| | | | | | | | structural destruction after acute lung injury. MSCs from umbilical cord blood resulted in a more significant reduction in lung injury compared with MenSC | |
| Rat | Epidural injection | $1 \times 10^5$ | 1 | - | - | Injured spinal cord | MenSCs transplantation markedly reduced cavity formation in the lesion site and improved the movement of spinal cord injured rat | Wu et al 2018 |
| Mouse | Intravenous injection | $1 \times 10^6$ | 3 | over 7 days | $3 \times 10^6$ | Colitis | MenSC decreased the damaged epithelium and crypt structure, glandular disorders, and inflammatory cell infiltration in the mucosa and submucosa. MenSC significantly reduced the elevated level of proinflammatory cytokines | Xu et al 2018 |
| Mouse | Intravenous injection via tail vein | $1 \times 10^6$ | 1 | - | - | Transplant Vasculopathy | MenSC can reduce the severity of transplant vasculopathy. MenSC increase the population of regulatory immune cells | Ye et al 2018 |
| Mouse | Intracerebral injection | $1 \times 10^5$ | 1 | - | - | Alzheimer's disease | Spatial learning and memory were improved by treatment with MenSC | Zhao et al 2018a |
| Mouse | Intravenous injection via tail vein | $1 \times 10^6$ | 1 | - | - | Idiopathic pulmonary fibrosis | MenSC maintained lung structure and attenuated inflammation levels by reducing pulmonary oedema. There was a small degree | Zhao et al 2018b |

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| | | | | | | | of interstitial hyperplasia, but the alveolar structure remained complete after MenSC treatment | |
| Mouse | Subcutaneous injection | $1 \times 10^6$ | 1 | - | - | Severe intrauterine adhesions (Asherman's syndrome) | MenSCs could rebuild endometrial tissue in mice after administering oestrogen and progesterone | Zheng et al 2018 |
| Pig | Intravenous injection via portal vein | $2.5 \times 10^6$ /kg | 1 | - | - | Acute liver failure | Prolonged survival time and improved liver function after MenSC transplantation | Cen et al 2019 |
| Mouse | Intravenous injection via tail vein | $1 \times 10^6$ | 1 | - | - | Premature Ovarian Insufficiency | MenSC transplantation resulted in increased ovary weight. MenSCs transplantation could restore the follicle injury, and were capable of regulating follicle-stimulating hormone levels to normal conditions. MenSCs could dramatically reduce the occurrence of apoptosis. | Feng et al 2019 |
| Mouse | Intravenous injection via tail vein | $1 \times 10^6$ | 1 | - | - | Premature ovarian failure | MenSC repaired chemotherapy-induced damage by inhibiting a gene that stops cell growth | Guo et al 2019a |
| Rabbit | Orthopotic scaffold implantation | $7 \times 10^5$ | 1 | - | - | Osteochondral defect | MenSC encapsulated in fibrin glue resulted in the defects being completely filled with firm, smooth, opaque hyaline cartilage-like tissue in most rabbits, including significant increase in blood vessel repair | Khanmohammadi et al 2019 |

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| Mouse | Intravenous injection | $1 \times 10^6$ | 3 | over 7 days | $3 \times 10^6$ | Colitis | MenSC pretreated with SDF-1 (protein) induced an immunoregulatory or tolerogenic response which could support damaged organ repair. In addition, SDF-1 effectively markedly improved the therapeutic effect of MenSC in alleviating colitis | Li et al 2019b |
| Mouse | Subcutaneous injection | $5 \times 10^6$ | 1 | - | - | Cervical cancer | Decreased volume and weight of tumour | Liu et al 2019a |
| Rat | Intravenous injection | $5 \times 10^6$ | 1 | - | - | Premature ovarian failure | MenSC transplantation had a significant effect on follicle formation and ovulation in the treatment group | Manshadi et al 2019 |
| Mouse | Intravenous injection via tail vein | $5 \times 10^6$ | 3 | over 6 days | $1.5 \times 10^7$ | Liver cancer | MenSC have an anticancer effect by suppressing oncogenic pathways | Wu et al 2019 |
| Rat | MenSC scaffold, and MenSC injection via tail vein | $5 \times 10^7$ FGF2-MenSCs on scaffold | 1 | | | Intrauterine adhesions (Asherman's sundrome) | Improved endometrial regeneration and successful pregnancy. FGF2-transfected* MenSC had an enhanced regenerative effect. | Chen et al 2020b |
| Mouse | Intravenous injection via tail vein | $5 \times 10^5$ | 2 | On days 2 and 7 | $1 \times 10^6$ | Pulmonary fibrosis | Transplantation of MenSC significantly improved pulmonary fibrosis mouse through evaluations of pathological lesions, collagen deposition, and inflammation. | Chen et al 2020c |
| Mouse | Injection via the peritoneum | $2 \times 10^6$ | 1 | At 4 or 24h | | Peritonitis | MenSC injected at 4 or 24 h after peritonitis administration | Martínez-Aguilar et al 2020 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | decreased the number and percentage of macrophages recruited to the peritoneum. | |
| | | | 1 | At 4h | | Sepsis | MenSC reduced the number of phagocytes—macrophages and neutrophils—recruited to the peritoneum. | |
| Mouse | Injection via the peritoneum | $3 \times 10^6$ | 1 | - | - | Acute liver failure | MenSC significantly reversed acute liver injury and improved survival rates. | Chen et al 2021a |
| Mouse | Injection via uterus | $5 \times 10^5$ (or the paracrine secretions from extracellular vesicles from $5 \times 10^5$ MenSC) | 1 | - | - | Intrauterine adhesions | Both significantly promoted endometrial regeneration and increased gland numbers. MenSCs exhibited a continuous repair effect in the damaged endometrium. | Zhang et al 2021b |
| Rat | Injection via ovarian tissue | ~$2 \times 10^5$ MenSCs per ovary (simultaneously) | 2 | - | ~$4 \times 10^5$ | Premature ovarian failure | CD146+ MenSC transplantation increased the number of normal follicles and reduced follicular degeneration, improving pregnancy rates. | Yamchi et al 2021 |
| Mouse | Injection via tail vein | $5 \times 10^5$ | 2 | At week 2 and 4 | $1 \times 10^6$ | Cholestatic liver injury | MenSC treatment improved size and colour of liver and gallbladders, restored liver damage and damage-induced jaundice, promoting repair function by upregulating the expression of specific protein.** | Yang et al 2022 |
| Rat | Injection via uterus | $1 \times 10^5$ | 1 | immediate | | Intrauterine adhesions | MenSC-loaded scaffold resulted in | Hu et al 2022 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MenSC-loaded scaffold transplant | unknown | | | | | significant increase in the number of endometrial glands, significant reduction in the number of collagen decreased fibrosis, and no adverse affects after 90 days. | |
| Rat | MenSC-loaded wound dressing | $3 \times 10^4$ per scaffold | 1 | | | Diabetic wound | MenSC-loaded dressing demonstrated a significantly higher rate of wound closure than other Dressings, with highest rate of collagen deposition. | Fan et al 2022 |
| Mouse | Intravenously or intraperitoneally | $1 \times 10^6$ | 1 | | | Experimental autoimmune encephalomyelitis (EAE) | MenSC suppressed development of disease before EAE-induction meaning it can be a preventative measure. MenSC displayed a comparable therapeutic efficacy to UC-MSCs. Peritoneal injection and intravenous injection had similar effect. | Li et al 2022 |
| Mouse | Injection via tail vein | $1 \times 10^6$ | 3 | On days 0, 7, and 14 | $3 \times 10^6$ | Type 1 diabetes | MenSC transplantation significantly improved the blood glucose and serum insulin levels, equal to umbilical cord MSC treatment. | Sun et al 2022a |
| Rat | MenSC-loaded hydrogel transplant | $5 \times 10^5$ | 1 | | | Intrauterine adhesions | Amniotic membrane extract-enriched hydrogel transplant promotes the proliferation and secretion of MenSC, improves | Hao et al 2022 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | the retention and viability of MenSC in vivo, and improves MenSC's therapeutic effect. | |
| Rat | MenSC transplant or MenSC-loaded scaffold | $1 \times 10^6$ | 1 | | Spinal cord injury | MenSC-loaded gel composite scaffold improved motor function, reduced the inflammatory response, and promoted neuronal differentiation after transplantation. | He et al 2022a |
| Mouse | MenSC or pretreated MenSC*** | $1 \times 10^6$ | 1 | | Sepsis | MenSC transplant alleviated symptoms, reducing tissue damage, regulating inflammatory response, and reducing oxidative stress, with further therapeutic affect when MenSC were pretreated.*** | Jin et al 2020 |
| Mouse | Intravenous injection via tail vein | $5 \times 10^5$ | 1 | | Autoimmune hepatitis | MenSC significantly alleviated hepatitis, decreasing the percentage of macrophages that promote inflammation. | Zhang et al 2022c |
| Rat | MenSC within hydrogels | $5 \times 10^5$ | 1 | | Intrauterine adhesions | Co-transplantation of MenSCs with hydrogel and amniotic membrene extract-enriched hydrogel restored uterus morphology and endometrial receptivity | Hao et al 2022 |
| Mouse | Injection via tail vein | $10^7$ cells/kg | 1 | | Neuroinflammation due to infection | MenSCs suppressed the neuroinflammatory reactions in the brain after infection. | Xu et al 2023 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mouse | Intravenous injection | Various concentrations | 1 | | | Acute respiratory distress syndrome | Optimal dose was of $4 \times 10^6$ cells/kg improved mortality. | Alcayaga-Miranda et al 2023 |
| Mouse | Intrathecal injection | $2 \times 10^5$ | | | | Spinal cord injury | MenSCs significantly improved locomotive function after spinal cord injury; transplantation significantly reduces macrophages. | Shi et al 2023 |
| Mouse | Injection via tail vein | $5 \times 10^5$ cells | 3 | 3 weeks apart | $1.5 \times 10^6$ | Non- alcoholic fatty liver disease | MenSC transplantation can inhibit lipid accumulation as it suppressed the expression of genes involved in fatty acid synthesis. | Du et al 2023 |
| Mouse | Injection via tail vein | $5 \times 10^5$ | 1 | | | Autoimmune hepatitis | MenSC significantly reduced mortality and reduced hepatitis-induced apoptosis. | Zhang et al 2023 |

* The FGF2 gene can promote mitosis and the proliferation of vascular endothelial cells, smooth muscle cells, and other cells, promoting new blood vessel formation.
** β-catenin.
*** Pretreated with CXCR4 antagonist AMD3100 or Stromal cell-derived factor-1 by co-culture with

# Appendix B MenSC treatment in human models

| *Experimental method* | | | | | | *Disease* | *Outcome* | *Author* |
|---|---|---|---|---|---|---|---|---|
| *Participants* | *Administration method* | *Transplantation* | | | | | | |
| | | *MenSC / transplant* | *no. transplant* | *treatment timing* | *total no. MenSC transplant* | | | |
| Participant 1 | Intravenous injection | $3 \times 10^6$ | 3 | over 4 days, on day 1, 3, and 4 | $1.6 \times 10^7$ | Multiple sclerosis | MenSC were safely administered allogeneically in four patients with multiple sclerosis, reporting on the safety of the cells and no disease progression occurring in the patients after one year | Zhong et al 2009 |
| | Intrathecal injection | $6 \times 10^6$ | 1 | day 2 | | | | |
| Participant 2 | Intrathecal injection | $6 \times 10^6$ | 5 | over 10 days | $3 \times 10^7$ | | | |
| Participant 3 | | | | over 9 days | | | | |
| Participant 4 | | | | | | | | |
| Participant 1 | Intrauterine injection | $1 \times 10^6$ | 1 | - | - | Intrauterine adhesions (Asherman's syndrome) | Autologous MenSC transplants lead to a significant increase in endometrial thickness in all the patients, with recovery of endometrial morphology to a normal status. One patient had a spontaneous pregnancy, and embryo transfers in four patients resulted in pregnancy in two. No complications or immune rejection was found in any of the patients | Tan et al 2016 |
| Participant 2 | | $1 \times 10^6$ | 1 | - | - | | | |
| Participant 3 | | $1 \times 10^6$ | 2 | over multiple cycles | | | | |
| Participant 4 | | $1 \times 10^6$ | 2 | over multiple cycles | | | | |
| Participant 5 | | $1 \times 10^6$ | 1 | - | - | | | |
| Participant 6 | | $1 \times 10^6$ | 1 | - | - | | | |
| Participant 7 | | $1 \times 10^6$ | 1 | - | - | | | |
| 3 participants (at early infection stage) | Intravenous injection | $1 \times 10^6$/kg body weight | 3 | - | $3 \times 10^6$/kg body weight | Acute respiratory distress syndrome | MenSC transplantation significantly lower the mortality. No harmful effects exerted after five-year follow up | Chen et al 2020a |
| 6 participants (at late infection stage) | | $1 \times 10^6$/kg body weight | 3 | - | $3 \times 10^6$/kg body weight | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8 participants (at late infection stage) | | 1 × $10^6$/kg body weight | 4 | - | 4 × $10^6$/kg body weight | | | |
| 15 participants | Intraovarian injection | 2 × $10^7$ | 1 | - | - | Poor ovarian responder | MenSC transplantation increased fertility and pregnancy rates. | Zafardoust et al 2020 |
| 12 participants | Intrauterine injection | | 3 sites | Second day of menstrual cycle | 1 × $10^7$ | Intrauterine adhesions (Asherman's syndrome) | MenSC transplantation led to improvements in endometrial thickness, menstrual duration, and pregnancy rates. | Ma et al 2020a |
| 15 participants (9 men, 15 women) with severe pneumonia due to COVID-19 | Intravenous injection | 5 mL of MenSC-derived secretome diluted in 100 mL, 60 mins over 5 consecutive days | | | | COVID-19 | MenSC secretome improved survival rate in patients, improve of intubation rate, patient oxygenation, preventing progression of the disease. | Fathi-Kazerooni et al 2022 |
| 26 patients (17 men, 9 women) with severe or critically ill with COVID-19 | Intravenous injection | 3 × $10^7$ | 3 | Over five says, on day 1, 3, 5 | 9 × $10^7$ | COVID-19 | MenSC transplantation immediately improved coughing symptoms and breathing significantly improved at day 1 compared to control group (although long-term improvements were probably result of other medications patients received), with the survival rate and 'time to improve' significantly improved, although one patient experienced severe liver function abnormality. | Xu et al 2021 |
| 15 | Intravaginal injection | 6 × $10^6$ | 1 | - | - | Premature ovarian failure | Menstruation returned in 4 of the 15 patients for average 2 cycles. | Zafardoust et al 2023 |

# Appendix C Menstrual blood collection methods

| *Device* | *Day* | *Flow* | *Wear time* | *Storage time/ time until processing* | *Storage temp* | *Volume / mL* | *No. Samples / donor* | *Collected by donor?* | *Cited by* | *Reference* |
|---|---|---|---|---|---|---|---|---|---|---|
| Mooncup | | | | Immed-iately | Ice cold | | | | | Esmaeilzadeh et al 2020 |
| Urine cup | | | | | | 5 | | | Zhao et al 2018a; Han et al 2009; Chen et al 2017a; Mou et al 2013; Wang et al 2017a; Xiang et al 2017; Lu et al 2016; Chen et al 2017b | Meng at al 2007 |
| | 1 | | | | | 10 | | | | Hida et al 2008 |
| Diva cup | first few days of menstr-uation | | | within 24–48 h | 4 °C | | | | Chen et al 2019b; Wu et al 2018 | Patel et al 2008 |
| Divacup | | | 30-60 minutes | | | 5 | | Yes | | Zhong et al 2009 |
| Menstrual cup | 1, 2, 3 | | <4 hours | | 4 °C | 8–10 | | | | Borlongan et al 2010 |
| Menstrual cup | | Heaviest | <4 hours | within 24 hours | 1-10 °C | | | | | Allickson et al 2011 |
| Menstrual cup | | | 2-3 hours | quickly moved to the lab. | on ice | 2-3 | | | | van Phuc et al 2011 |
| Diva cup | 2 | | | | | 5 | | | Khanjani et al 2015 | Darzi et al 2012 |
| Divacup | first few days of menstrual cycle | | | | | 5 | 2 or 3 | Yes | Khanjani et al 2015 | Kazemnejad et al 2012 |
| Diva cup | | | | | | 5 | | | | Khanmohammadi et al 2012 |
| Diva cup | 2 | | | | | 3-5 | | | | Nikoo et al 2012 |
| Diva cup | 1, 2, 3 | Heaviest | <3 hours - | same day of sample | | 5 | | | | Kazemnejad et al 2013a |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | overnight | collection if possible | | | | | | |
| Divacup | 2 | | | | 5 | | Yes | | Kazemnejad et al 2013b |
| Diva cup | first few days of menstruation | | within 24–48 h | 4 °C | | | | | Rossignoli et al 2013 |
| Menstrual cup | 1, 2, 3 | 12 h | | room temp. | 1- 20 | 6 | | | van der Molen et al 2013 |
| aspirator Ipas MVA plus | 2 | | 2-4 hours | 4 °C | 1-2 | | | Blázquez et al 2018 | Zemelko et al 2013 |
| DivaCup | first few days of menstrual cycle | | | | 2–5 | | | | Azedi et al 2014 |
| Urine cup* | 2 | | immediately | | | | | | Bozorgmehr et al 2014 |
| Falcon tubes | 1 | | | | 0.5 | | | | Karadas et al 2014 |
| Diva cup | 2 | | | | | | | | Khanjani et al 2014 |
| Diva cup | 2 | | | | 5 | | | Fard et al 2017 | Khanmohammadi et al 2014 |
| | 1 | | | | 10 | | | | Lv et al 2014 |
| Diva cup | 2 | | immediately | | | | Yes | | Nikoo et al 2014 |
| Diva cup | 2 | | | | | | | | Rahimi et al 2014a |
| Diva cup | 2 | | | | | | | | Rahimi et al 2014b |
| Mialuna cup | earliest days of the menstrual cycle | | | | | | | Lopez-Verrilli et al 2016; Alcayaga-Miranda et al 2016; Alcayaga-Miranda et al 2015a; Alcayaga-Miranda et al 2023 | Alcayaga-Miranda et al 2015b |
| Menstrual cup | 2 | | | | | | | | Chen et al 2015b |
| Diva cup | 1 | | | | | | | Lai et al 2016 | Lai et al 2015 |
| Menstrual cup | | 2-3 hours | Quickly | kept on ice | | 2 or 3 | | | Vu et al 2015 |
| Urine cup | first few days of | | | | 5 mL | | | | Chen et al 2016 |

* Urine cup but sourced from DivaCup so potentially a mistranslation

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | menstruation | | | | | | | | | |
| | 2 | | | | | 2 mL | | | | Domnina et al 2016 |
| Menstrual cup | 1, 2, 3 | | 2-4 hours | within 24 h | 4 °C | | | | Guo et al 2019a | Du et al 2016 |
| Diva cup | 3 | | | | | 5 mL | | | | Mehrabani et al 2016 |
| Diva cup | 2 | | | within 24 h | 4 °C | 5 mL | | | | Ren et al 2016 |
| Urine cup-tubing | | | | | | 5 mL | | | | Sun et al 2016 |
| Catheter | 2 | | | | | 1.5 - 3.5 mL | 1 or 2 | | Feng et al 2019 | Tan et al 2016 |
| Diva cup | 2 | | | | | 5 mL | | | | Akhavan-Tavakoli et al 2017 |
| Maggacup | 2 | | | | | 2mL | | | | Alfano et al 2017 |
| Diva cup | 2 | | | | | 5 mL | | | | Azedi et al 2017 |
| Diva cup | | | | | | | | | Liu et al 2019a | Chen et al 2017b |
| Diva cup | 1, 2 | | | | | | | | | Fathi-Kazerooni et al 2017 |
| Menstrual cup | 1 | | | | | 10 mL | | | Sun et al 2022b | Lan et al 2017 |
| Mooncup | 1, 2, 3 | | | | | 1-5 mL | | | Blázquez et al 2018 | Moreno et al 2017 |
| | 2, 3 | | | within 24 h | | | | | | Shan et al 2017 |
| Menstrual cup | 1 | | | | | 10 | | | | Wang et al 2017d |
| Menstrual cup | 1 | | | | | | | | | Xu et al 2017 |
| Menstrual cup | 2 | | | | | | | | | Aleahmad et al 2018 |
| Diva cup | 1, 2, 3 | Heaviest | 3 h – over-night | same day | | 5 mL | | Yes | | Arasteh et al 2018 |
| Menstrual cup | 2, 3 | | | | | | | | Marinaro et al 2018; Fard et al 2017; Marinaro et al 2019 | Blázquez et al 2018 |
| Mialuna cup | earliest days of a menstrual cycle. | | | | | | | | | Cuenca et al 2018 |
| Urine cup | 2, 3 | | | within 10–12 hours | on ice | 3-5 mL | 1 or 2 | | Dalirfardouei et al 2021 | Dalirfardouei et al 2018 |
| | 2 | | | | | 10–20 mL | | | | Eremichev et al 2018 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Menstrual cup | 1, 2, 3, 4, 5 | | 3-5 hours | within 12 h | 4 °C | 15-20 mL | 4-6 times a day | | Kovina et al 2018 |
| Menstrual cup | first few days of menstrual cycle | | | within 6, 24, 48 and 72 hrs | 4 °C | | | | Liu et al 2018 |
| Menstrual cup (single-use) | | | | | | 10 mL | | | Qin et al 2018 |
| Divacup | 2 | | | | | | | | Rahimi et al 2018 |
| | 2 | | | | | 3-5 mL | | | Rajabi et al 2018 |
| DivaCup | 1, 2, 3 | | 6–10 hour | approx. 24 h | | | | | Warren et al 2018 |
| Divacup | 1 | | | | | 5mL | | | Xu et al 2018 |
| Menstrual cup | 1 | | | | | | | | Ye et al 2018 |
| 20-mL injection syringe | 2 | | | | | 5 mL | | Zhang et al 2019; Zhang et al 2021b | Zhang et al 2018 |
| Menstrual cup | 1 | | | | | | | | Zhao et al 2018b |
| Menstrual cup | 2 | | | | | 5 mL | | | Zheng et al 2018 |
| Urine collection cup | 2, 3 | | | within 10–12 hours. | on ice | 3–5 mL | | | Dalirfardouei et al 2019 |
| Divacup | 1 | | | | | | | | Khanmohammadi et al 2019 |
| DivaCup | first few days of menstrual cycle | | | | | 5 mL | | | Li et al 2019a |
| Menstrual cup | | | | | | 5 mL | | | Li et al 2019b |
| Menstrual cup | | | | | | | | Barlabé et al 2020 | Moreno et al 2019 |
| Diva cup | 2 | | | | Trans-ported in cold chain | | | | Shokri et al 2019 |
| DivaCup | 1, 2, 3 | | | within 48 h | | | | | Sun et al 2019a |
| 20-mL injection syringe | 2 | | | | | 5 mL | | | Wang et al 2019 |
| Silicone collector | 2, 3 | Most intense flow | 3 hours | Within 24 hours | 1 – 24 °C | 1 – 17.5 mL | 2 | | Fiorelli-Arazawa et al 2019 |
| Diva cup | 2 | | | | | | 1 | No | Zafardoust et al 2020 |
| Diva Cup | 2, 3 | | | Within 48 hours | 4 °C | | | | Chen et al 2020b |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Menstrual cup or tampon | 2 | | | | | | | | | Martínez-Aguilar et al 2020 |
| Pipelle catheter | 2 or 3 | | | | | 1-2 mL | | | | Sheikholeslami et al 2021 |
| Menstrual cup | 2, 3, or 4 | | 3 hours | 4 hours | 4 °C | | | | | Zucherato et al 2021 |
| Cup | 1, 2, or 3 | | | Within 24 hours | | 10-15 | | | | Quintero-Espinosa et al 2021 |
| iCare menstrual cup | 2 | | | | | 5-10 | | | | Uzieliene et al 2021 |
| Lunette | Self-reported heaviest flow; 1 sample on Day 1, 36 samples on Day 2, 2 samples on Day 3 of menses | Heaviest | 4-6 | Within 2 hours | 4 °C | Mean 9.8 (SD =5.0) | 1-5 | Yes | | Wyatt et al 2021 |
| Divacup | 2 | | 2-4 (mean 3) | | | 4 | | | | Arezoo et al 2021 |
| Lady cup | 2 | | | | | | | | | Yamchi et al 2021 |
| Menstrual cup | 1 | | | | | | | | | Wang et al 2021 |
| Menstrual cup | 2 to 3 | | Several hours | | | | | | De Pedro et al 2021; Marinaro et al 2021 | Álvarez et al 2018 |
| Menstrual cup | 2 | | | | 4 °C | | 1, 2 if first sample contaminated | No | | Ma et al 2020a |
| MoonCup | 1-2 | "as soon as flow became heavy" | at least 4h or overnight | Processed immed-iately or next day | 4 °C | Min. ~ 7 mL blood | 1-2 | | | Cindrova-Davies et al 2021* |
| Lunette menstrual cup | 2 | | 4-6h | received within 2 h of collection. Processed immed-iately or within 24 h of collection | 4 °C | | 2 | Trans-ferred by partici-pant | | Filby et al 2021* |
| Inciclo menstrual cup | 2-4 | | 3h | | | | | | | de Oliveira et al 2022 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Divacup | 2 | | | | | He et al 2022a |
| Menstrual cup | 1 | | | | | Jin et al 2020 |
| Menstrual cup | First 48 hours | overnight | | | Self collected | Peñailillo et al 2022 |
| DivaCup | 2 | | Immed-iately | | | Amini et al 2022 |
| DivaCup | 2 | | | | | |
| Menstrual cup | 2, 3, 4 | 3 hours | Up to 4 hours | 4 °C | | Cressoni et al 2023 |

*organoids isolated from menstrual blood rather than MenSC

# Appendix D Protocol development

*Menstrual blood simulant*

Taking the figures reported in Table 2.6, the mean number of MenSC per sample is approximately 6.5 x $10^6$ MenSC; the median is 7.6 x $10^6$ MenSC per sample. Because 6 or 7 million BM MSC would require multiple T75 flasks in culture, the development of this protocol will aim for minimum 1 million cells per menstrual blood simulant sample.*

*Lab stomacher*

The Lab Stomacher was tested using the following variations:

*Lab stomacher protocol development settings*

| Volume PBS (mL) | Lab stomacher setting | Time (minutes) |
|---|---|---|
| 50* | Medium | 5 |
| 50* | Medium | 15 |
| 100 | Low | 5 |
| 100 | Low | 15 |
| 100 | Medium | 5 |
| 100 | Medium | 15 |
| 100 | High | 15 |
| 100 | High | 30 |
| 200† | Low | 5 |
| *50 mL was too low volume for the tampon or sanitary pad to be adequately pummelled<br>† 200 mL required too great a volume to centrifuge the supernatant, greatly increasing the number of steps required | | |

*Direct culture*

*Direct in flask*

*Methods*

The BM MSC were cultured as described above, and BM MSC suspended in MSCGM as before being pipetted a tampon. The entire tampon was then placed in a T75 flask, with 10 mL MSCGM,

* Mean 64,653,000 / 10 = 34,653,000 = approx. 6.5 x 106 MenSC
Median = between 3-7.5 × 106 and 1 × 107
= between 5.25 x 106 and 1 × 107 = mean of those = 7.6 x 106
So could aim for 6.5-7.5 x 106 MenSC?
Or just straight up 1 x 106 MenSC

BM MSC will be used as a reasonable quantity of cells per menstrual blood sample, as is the median cell count
5697545 (5.7 × 106) is the mean cell count

and the flask incubated at 37 °C in a fully humidified environment with 5% CO2, and left overnight.

*Result and discussion*

No MenSC adhered to the T75 flask. The tampon completely absorbed all the MSCGM, perhaps MenSC were deeply submerged within the fibres of the tampon. At this point trypsinisation failed because the tampon was impregnated with MSCGM.

*Mincing*

*Experiment 2.1*

(Tampon; whole, cut, dropped into media)

*Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 4, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 2.9 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. One 100mm diameter culture plate was prepared with 12 mL DMEM. 5mL menstrual blood simulant was slowly pipetted onto the tip of a tampon. Using dissecting scissors and tweezers, the tampon was finely cut and fibres dropped into the plate. 20 mL additional DMEM was pipetted into the plate to ensure the plate surface and fibres were submerged and was incubated. After approximately 24 hours, the largest fibres were lifted from the plate and transferred into fresh plates containing 12 mL. The original plate was washed three times with PBS before 12 mL DMEM added for culture.

*Result and discussion*

Very few cells adhered to the plate after washing. Perhaps the density of the fibres is too great, due to a whole tampon being placed in one plate. At Day 7 the BM MSC were seeded onto a T25 flask, where the surface was populated more suitably for cells growth. At Day 12 (6/8/21) approximately 1 million BM MSC were retrieved from the original 2.9 million BM MSC.

*Experiment 2.2*

(Pad; middle third, cut, dropped into media)

*Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 4, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 3 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. One 100mm diameter culture plate was

prepared with 12 mL DMEM. 5 mL menstrual blood simulant was slowly pipetted onto the centre of a sanitary pad. Using dissecting scissors and tweezers, the middle section of the sanitary pad was finely cut and fibres dropped into the plate. 2 0mL additional DMEM was pipetted into the plate to ensure the plate surface and fibres were submerged and was incubated. After approximately 24 hours, the largest fibres were lifted from the plate and transferred into fresh plates containing 12 mL. The original plate was washed three times with PBS before 12 mL DMEM added for culture.

*Result and discussion*

The cells were contaminated by Day 1. It became clear that where tampons are sealed within secondary packaging, the secondary packaging surrounding sanitary pads are not fully sealed and therefore were contaminated before undertaking the experiment. These cells were discarded. The contamination then affected the plate below described in

*Experiment 3*

(Tampon; top and mid cut and dropped into dry plate, bottom squeezed into media)

*Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 4, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 2.7 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. 5 mL menstrual blood simulant was slowly pipetted onto the tip of a tampon. Using dissecting scissors and tweezers, the top third of the tampon was finely cut and fibres dropped into the first plate, the middle third of the tampon was finely cut and fibres were dropped into the second plate, and the final third of the tampon was finely cut and fibres were dropped into the third plate. 22 mL DMEM was then pipetted into the plates, so the 'dry' fibres were submerged. The plates were incubated. After approximately 24 hours, the largest fibres were lifted from each plate and transferred into fresh plates containing 12 mL DMEM to examine whether BM MSC adhered to the fibres or could be transferred to a new surface to adhere to. The fibres were transferred with tweezers, and care was taken to avoid touching the surface of the plate, disturbing the BM MSC as little as possible. The original plates were washed three times with PBS before 12 mL DMEM added to plate for culture. The BM MSC were cultured, with cell growth analysed under the microscope each day.

*Result and discussion*

Adding the fibres 'dry' to the plates were not as adherent to the plates. It would appear the fibres falling into the media would loosen the BM MSC from the fibres, allowing them to fall to the surface of the place and adhere. Cells were still able to adhere, but not as successfully as when

the plate is already prepared with DMEM. At Day 7, BM MSC from all three dishes were seeded onto a T25 flask where the surface was populated more suitably for cells growth. By Day 18 BM MSC were confluent, with 1 million retrieved from the original 2.7 million BM MSC pipetted onto the tampon. More cells were collected in the tip of the tampon, with the middle and lower sections with no or very few MSC surviving or adhering to the dish.

### *Experiment 4*

(Tampon, cut, 3 dishes, 22 mL media)

#### *Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 5, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 2.5 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. 5 mL menstrual blood simulant was slowly pipetted onto the tip of a tampon. Three 100mm diameter culture dishes were prepared with 12 mL DMEM Using dissecting scissors and tweezers, the top third of the tampon was finely cut and fibres dropped into the first plate, the middle third of the tampon was finely cut and fibres were dropped into the second plate, and the final third of the tampon was finely cut and fibres were dropped into the third plate. A further 10 mL DMEM was then pipetted into the plates, so the fibres were submerged. The plates were incubated. After four days, the largest fibres were lifted from each plate. The fibres were transferred with tweezers, and care was taken to avoid touching the surface of the plate, disturbing the BM MSC as little as possible. The plates were washed three times with PBS before a further 12 mL DMEM added to plate for culture. The BM MSC were cultured, with cell growth analysed under the microscope each day.

#### *Result and discussion*

Dropping the fibres into already-prepared culture dishes seemed to dislodge more cells for adherence. At Day 6 the BM MSC were transferred to a T25 flask where the surface was populated more suitably for cells growth. At Day x x BM MSC were retrieved from the original 2.5 million BM MSC.

### *Experiment 5.1*

Sanitary pad, poly, cut, mid third, 150 mm dish, 50 mL media

#### *Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 5, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue

exclusion confirmed cell count and >95% viability. Approximately 1.4 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. A 150mm culture dish was prepared with 30 mL DMEM. 5 mL menstrual blood simulant was slowly pipetted onto the centre of an original (polymer) sanitary pad. Using dissecting scissors and tweezers, the centre third of the sanitary pad that visibly contained media was removed and finely cut, with fibres dropped into the dish. A further 20 mL DMEM was then pipetted into the plates, so all fibres were submerged. The plate was incubated. After approximately 24 hours, the largest fibres were lifted from the plate. The fibres were transferred with tweezers, and care was taken to avoid touching the surface of the plate, disturbing the BM MSC as little as possible. The plate was washed three times with PBS before 30 mL DMEM added to plate for culture. The BM MSC were cultured, with cell growth analysed under the microscope each day.

### *Result and discussion*

Sanitary pad texture differed from the hair-like fibres from the tampon. Where some hair-like fibres were present, there was also silicon-like crystals of super-absorbent polymer fragments. This super-absorbent polymer much more difficult to wash from plate as it was more dense, it blocked the pipette tips when washing, and its high friction was harder to wash away compared to fibres that float on the surface of PBS and DMEM. In future experiments and in clinical applications it may be necessary to strain the fragments out.

By Day 8 the T25 flask was confluent. Approximately 800,000 BM MSC were collected with 94% viability.

### *Experiment 5.2*

Sanitary pad, cotton, cut, mid third, 150mm dish, 50 mL media

### *Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 5, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 1.4 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. A 150mm culture dish was prepared with 30 mL DMEM. 5 mL menstrual blood simulant was slowly pipetted onto the centre of an organic (cotton) sanitary pad. Using dissecting scissors and tweezers, the centre third of the sanitary pad that visibly contained media was removed and finely cut, with fibres dropped into the dish. A further 2 0mL DMEM was then pipetted into the plates, so all fibres were submerged. The plate was incubated. After approximately 24 hours, the largest fibres were lifted from the plate. The fibres were transferred with tweezers, and care was taken to avoid touching the surface of the plate, disturbing the BM MSC as little as possible. The plate was washed three times with PBS

before 30 mL DMEM added to plate for culture. The BM MSC were cultured, with cell growth analysed under the microscope each day.

*Result and discussion*

Same as above. There was no noticeable difference between the polymer and cotton sanitary pads. Cells do not appear to adhere to cotton, rayon, or superabsorbent polymer centre of sanitary pads.

By Day 8 the T25 flask was confluent. Approximately 850,000 BM MSC were collected with 99% viability.

*Experiment 6.1*

Tampon, whole, cut, 150 mm dish, 50 mL media

*Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 5, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 1.8 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. A 150 mm culture dish was prepared with 30 mL DMEM. 5 mL menstrual blood simulant was slowly pipetted onto the tip of a tampon. Using dissecting scissors and tweezers, the tampon that visibly contained media was finely cut, with fibres dropping into the dish. A further 20 mL DMEM was then pipetted into the plate, so all fibres were submerged. The plate was incubated. After approximately 24 hours, the largest fibres were lifted from the plate. The fibres were transferred with tweezers, and care was taken to avoid touching the surface of the plate, disturbing the BM MSC as little as possible. The plate was washed three times with PBS before 30 mL DMEM added to plate for culture. The BM MSC were cultured, with cell growth analysed under the microscope each day.

*Result and discussion*

Contamination occurred on Day 3 of this experiment, potentially caused by the contamination of Experiment 6.2 outlined below.

*Squeezing*

*Experiment 1*

(Tampon; top and mid cut and dropped into media, bottom squeezed into media)

*Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 4, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue

exclusion confirmed cell count and >95% viability. Approximately 1.2 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. Three 100mm diameter culture plates were prepared with 12 mL DMEM. 5 mL menstrual blood simulant was slowly pipetted onto the tip of a tampon. Using dissecting scissors and tweezers, the top third of the tampon was finely cut and fibres dropped into the first plate, the middle third of the tampon was finely cut and fibres were dropped into the second plate, and the final third of the tampon was squeezed with excess menstrual blood simulant dripping into the third plate. 20 mL additional DMEM was pipetted into the plates containing fibres to ensure the plate surface and fibres were submerged. The plates were incubated. After approximately 24 hours, the largest fibres were lifted from each plate and transferred into fresh plates containing 12 mL DMEM to examine whether BM MSC adhered to the fibres or could be transferred to a new surface to adhere to. The fibres were transferred with tweezers, and care was taken to avoid touching the surface of the plate, disturbing the BM MSC as little as possible. The original plates were washed three times with PBS before 12 mL DMEM added to plate for culture. The BM MSC were cultured, with cell growth analysed under the microscope each day.

*Result and discussion*

BM MSC do not appear to adhere to the fibres of a tampon. As the menstrual blood simulant was pipetted from the top of the tampon to mimic absorbing blood released from the cervix, more cells were absorbed into the top section of the tampon compared to the middle or bottom. Transferring the fibres to fresh plates with DMEM resulted in few BM MSC being transferred. Gently lifting the fibres off from the original plates and washing fibres away allowed BM MSC to grow. The floating fibres may be abrasive to the adhered BM MSC, and the movement and scraping appears to damage BM MSC as some lay smeared on the surface of the flask.

At Day 8, the BM MSC cells from the dishes were reseeded in T25 flask where the surface was populated more suitably for cells growth. At Day 12, the cells were lifted and counted, retrieving 1 million BM MSC from original 1.2 million.

*Experiment 6.2*

Tampon, whole, squeeze, 150mm dish, 50 mL media

*Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 5, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 1.8 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. A 150mm culture dish was prepared with 30 mL DMEM. 5 mL menstrual blood simulant was slowly pipetted onto the tip of a tampon. The

tampon was squeezed and wrung with gloved hands to remove as much menstrual simulant as possible. The plate was incubated. After approximately 24 hours, the largest fibres were lifted from the plate. The fibres were transferred with tweezers, and care was taken to avoid touching the surface of the plate, disturbing the BM MSC as little as possible. The plate was washed three times with PBS before 30 mL DMEM added to plate for culture. The BM MSC were cultured, with cell growth analysed under the microscope each day.

### *Result and discussion*

This method is not ideal – contamination occurred on Day 2. This is potentially due to gloves being in direct contact with the tampon. Next steps will be to squeeze the product through Seward sealable bag by snipping the end like a piping bag.

### *Experiment 7*

Tampon, whole, squeeze from bag, T25, 4 mL media

### *Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 5, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 1 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. A T25 flask was prepared with 4 mL DMEM, and a Seward closure bag was prepared with 75 mL of PBS at 4 °C. 5 mL menstrual blood simulant was slowly pipetted onto the tip of a tampon. The tampon was placed in the Seward bag and gently massaged in the PBS. Approximately 1mm was snipped form the corner of the bag, with PBS poured in to two 50 mL tubes. The tampon was squeezed through the bag to remove as much menstrual simulant as possible. The tubes were centrifuged and the BM MSC were seeded onto the T25 flask. The BM MSC were cultured, with cell growth analysed under the microscope each day.

### *Result and discussion*

The tampon was visibly ‘cleaned’ in the PBS, with all visible DMEM dispersing in the PBS. Squeezing the tampon in this way retrieved all but approximately 6 mL of the BM MSC/DMEM/PBS mix. Although no visible pellet could be seen in the 50 mL tubes after centrifugation, BM MSC were seen in the T25 flask and were adhered by Day 1. By Day 9, 1 million cells of 99% viability were extracted from the T25 flask.

### *Experiment 8.1*

Polymer sanitary pad, whole, squeeze from bag, T25, 4 mL media

*Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 5, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 1.7 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood. A T25 flask was prepared with 4 mL DMEM, and a Seward closure bag was prepared with 150 mL of PBS at 4 °C. 5mL menstrual blood simulant was slowly pipetted onto the centre of the sanitary pad. The sanitary pad was placed in the Seward bag and gently massaged in the PBS. Approximately 1mm was snipped form the corner of the bag, with PBS poured into four 50 mL tubes. The sanitary pad was squeezed through the bag to remove as much menstrual simulant as possible. The tubes were centrifuged and the BM MSC were seeded onto the T25 flask. The BM MSC were cultured, with cell growth analysed under the microscope each day.

*Result and discussion*

The sanitary pad was not as visibly 'cleaned' in the PBS as the tampon was, but there was still a reduction in the DMEM visible in the sanitary pad. Although no visible pellet could be seen in the 50 mL tubes after centrifugation, BM MSC were seen floating in the T25 flask before incubation. However, by Day 1 the sample was contaminated. There is a step in the protocol where contamination is occurring. There are many occasions this could occur. It could be that the sealable seward bag in which the sanitary pads are kept has been compromised, or the sealable seward bag in which the PBS is poured and sanitary pad is inserted in compromised. The seward bad is held in a rack to prevent the bag from tipping over, and this could also be a source of contamination. Although the hood surface is sprayed with IMS before each step, placing the sanitary pad on this could be a source of contamination. The next step will be to repeat the experiment without allowing the sanitary pad to touch the hood surface, or if this is not possible when pipetting the menstrual blood simulant onto the centre of the sanitary pad, laying the sanitary pad on its own packaging.

*Experiment 8.2*

Polymer sanitary pad, whole, squeeze from bag, T25, 4 mL media repeat

*Methods*

Repeated experiment 8.1 with cells at passage 6. Approximately 2 million BM MSC with 98% viability were used for this experiment.

*Result and discussion*

By Day 1 cells were adhered with no sign of contamination. By Day 3 1.9 million BM MSC of 98% viability were extracted, remaining contamination free.

### *Experiment 9.1*

Tampon, whole, incubator 4 hours, fridge 48, squeeze from bag, T25, 4 mL media

#### *Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 5, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 1 million BM MSC were resuspended in 5 mL DMEM to mimic menstrual blood, which was slowly pipetted onto the tip of the tampon. The tampon was then placed in a sealable Seward bag, and stored in the incubator for four hours to mimic being worn vaginally. After four hours, 75 mL PBS was added to the bag, and the tampon was gently massaged in the PBS. This was then stored at 4 °C for 48 hours. Approximately 1mm was snipped from the corner of the bag, with PBS poured into four 50 mL tubes. The tampon was squeezed through the bag to remove as much menstrual simulant as possible. The tubes were centrifuged and the BM MSC were seeded onto the T25 flask. The BM MSC were cultured, with cell growth analysed under the microscope each day.

#### *Result and discussion*

By Day 1 cells were adhered with no sign of contamination. By Day 8 approximately 1.1 million BM MSC of 98% viability were counted from this experiment.

### *Experiment 9.2*

Sanitary pad, whole, incubator 4 hours, fridge 48, squeeze from bag, T25, 4 mL media

#### *Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 5, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 1 million BM MSC with 95% viability were resuspended in 5 mL DMEM to mimic menstrual blood, which was slowly pipetted onto the centre of the sanitary pad. The sanitary pad was then placed in a sealable Seward bag, and stored in the incubator for four hours to mimic being worn close to the body. After four hours, 150 mL PBS was added to the bag, and the sanitary pad was gently massaged in the PBS. This was then stored at 4 °C for 48 hours. Approximately 1mm was snipped from the corner of the bag, with PBS poured into four 50 mL tubes. The sanitary pad was squeezed through the bag to remove as much menstrual simulant as possible. The tubes were centrifuged and the BM MSC were seeded onto the T25 flask. The BM MSC were cultured, with cell growth analysed under the microscope each day.

*Result and discussion*

By Day 1 cells were adhered with no sign of contamination. By Day 8 approximately half a million BM MSC of 98% viability were counted from this experiment.

*Experiment 10.1*

Tampon, whole, incubator 4 hours, squeeze from bag, T25, 4 mL media

*Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 6, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 1 million BM MSC with 95% viability were resuspended in 5 mL DMEM to mimic menstrual blood, which was slowly pipetted onto the tip of the tampon. The tampon was then placed in a sealable Seward bag, and stored in the incubator for four hours to mimic being worn vaginally. After four hours, 75 mL PBS was added to the bag, and the tampon was gently massaged in the PBS. Approximately 1mm was snipped from the corner of the bag, with PBS poured into four 50 mL tubes. The tampon was squeezed through the bag to remove as much menstrual simulant as possible. The tubes were centrifuged and the BM MSC were seeded onto the T25 flask. The BM MSC were cultured, with cell growth analysed under the microscope each day.

*Result and discussion*

From 75 mL PBS added to the menstrual blood simulant in the sealable bag, approximately 70 mL was drawn from the bag. By Day 1 cells were adhered with no sign of contamination. By Day 5 approximately 1.4 million BM MSC of 99% viability were counted from this experiment.

*Experiment 10.2*

Sanitary pad, whole, incubator 4 hours, squeeze from bag, T25, 4 mL media

*Methods*

BM MSC were cultured in T75 flasks in DMEM high glucose supplemented with 10% FBS, incubated in a humidified atmosphere with 5% CO2 at 37 °C. At passage 6, the BM MSC were dissociated with Trypsin-EDTA and cell count with c-chip haemocytometer and Trypan Blue exclusion confirmed cell count and >95% viability. Approximately 1 million BM MSC with 95% viability were resuspended in 5 mL DMEM to mimic menstrual blood, which was slowly pipetted onto the centre of the sanitary pad. The sanitary pad was then placed in a sealable Seward bag, and stored in the incubator for four hours to mimic being worn close to the body. After four hours, 150 mL PBS was added to the bag, and the sanitary pad was gently massaged in the PBS. Approximately 1mm was snipped from the corner of the bag, with PBS poured into four 50 mL

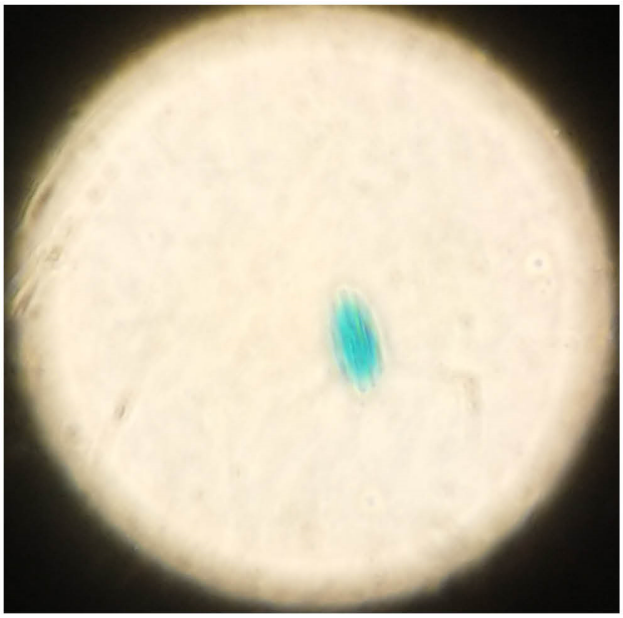
tubes. The sanitary pad was squeezed through the bag to remove as much menstrual simulant as possible. The tubes were centrifuged and the BM MSC were seeded onto the T25 flask. The BM MSC were cultured, with cell growth analysed under the microscope each day.

### *Result and discussion*

From 150 mL PBS added to the menstrual blood simulant in the sealable bag, approximately 125 mL was drawn from the bag. Where there was still substantial amounts of superabsorbent polymer fragments in the flask, these were considerably smaller than the fragments created when cutting the sanitary pad up. These smaller fragments appear to interfere with the BM MSC less once they've adhered, as there are no visibly damaged cells caused potentially by the non-buoyant superabsorbent polymer fragments dragging across the adhered cells. By Day 1 cells were adhered with no sign of contamination. By Day 5 approximately 600,000 BM MSC of 98% viability were counted from this experiment. Refrigeration clearly slows cell metabolism and takes slightly longer to continue to proliferate but temporary sample storage certainly seems viable for MenSC donation at this point and might make donation easier and more accessible for women.

### *Mystery of the blue pellet*

Centrifuging supernatant taken from experiments occasionally resulted in a pellet with a slight but noticeable blue tint to it. This only sometimes occurred with samples from sanitary pad extraction, never tampon extraction. When the cells were resuspended into media, the blue tint was no longer visible. It turns out that there were very few fragments of fibres that were dyed blue and are clearly blue under the microscope, but as micrography was undertaken in black and white this was not identified in early experiments. The fibres were spread out on the flask so often not visible from monolayer, but when concentrated gave a blue tint to the cell pellet. This seems to be inert and not affect the BM MSC, however for clinical application it may be that future MenSC are run through a Cell Sorter so that fibres of a similar size to MenSC are safely discarded.



*Fragment of sanitary pad in BM MSC culture after extraction*

### *Animal Blood*

The next step to mimicking menstrual blood was to mimic needing to separate mononuclear cells from red blood cells and plasma from a menstrual blood sample. BM MSC were useful for learning that MSC would not adhere to tampons or sanitary pads, but it was unclear how red blood cells and plasma might interfere in the extraction process. To do this, whole defibrinated animal blood (horse) was used.

### *Methods*

A sealable collection bag was prepared with 120 mL PBS. 5 mL aseptically-collected defibrinated horse blood is slowly pipetted onto the tampon or centre of the sanitary pad. The tampon is placed in the sealable collection bag, or the wings of the sanitary pad are folded to the back, and the whole sanitary pad is folded in half blood side outwards before being placed in the sealable collection bag. The tampon or sanitary pad is massaged for ten seconds, allowing the PBS to change colour and the product to be as visibly 'washed' as possible. The tip of the sealable bag is snipped away and the contents is poured into four 50 mL centrifuge tubes. The tampon or sanitary pad was squeezed through the sealable bag to release as much mixture into the tubes. The tubes are then centrifuged at 1000RPM for 5 minutes at 25 °C, leaving a pellet of red blood cells and mononuclear cells. These pellets are resuspended in PBS, with final volume of 10 mL. The blood PBS mixture is gently layered over 3 mL Ficoll paque, pipetting one drop at a time down the wall of the tube and disturbing the ficoll-paque layer as little as possible. This is centrifuged at 400G for 30 minutes at 25 °C. The buffy coat is collected as described by Sanzhez-Mata et al 2020, and resuspended in 10 mL PBS. This is washed by centrifugation at 250G for 10 minutes at 25 °C. The washing step is repeated a further two times. The pellet is resuspended in 1 mL PBS and

counted using trypan blue method and haemocytometer. Each experiment was undertaken in triplicate.

### *Results and discussion*

From 5 mL blood, collection using a tampon, 2,690,000 (*SD* = 512,000) mononuclear cells with 82.9% (*SD* = 6.50%) viability were extracted. Collecting using a sanitary pad, 2,900,000 (*SD* = 1,470,000) mononuclear cells with 76.8% (*SD* = 3.23%) viability were extracted.

Debris, fibres, and bits of superabsorbent polymers from the tampon and sanitary pad generally had a larger density than mononuclear cells so could be seen within the ficoll-paque and was therefore not extracted, although smaller bits of debris was still collected as part of the buffy coat. Some red blood cells were also sometimes visible in the buffy coat and washed pellet, and it is suspected these are lysed cells that have lost cell membrane integrity, affecting the cells density, and therefore being extracted with the mononuclear cells. After seeding these cells would be simply washed away. Small bubbles and smaller bits of debris from the tampon and sanitary pad made it sometimes difficult to distinguish between dead cells and debris as debris would take on the Trypan blue. This debris was often more irregularly-shaped than cells so were distinguishable, although this still may have affected reliability of the results.

# Protocol


Recruitment poster (physical and digital)

Participants declare interest via email

## Eligibility meeting

Phone

Video call

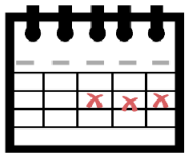
Face to face

**Inclusion criteria:**
Healthy, aged 18+, menstruate regularly, comfortable using tampon, sanitary pad, or menstrual cup

**Exclusion criteria:**
Abnormal vaginal discharge, pregnant or breastfeeding, history of Hepatitis B and C and HIV-1 and HIV-2

## Introductory visit

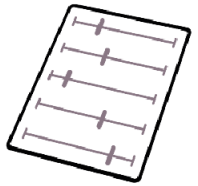

Participants provide informed written consent



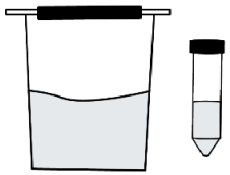
Participants track menstrual cycle

Participants indicate which products they are comfortable using

Of these, the product is chosen at random. The participants are provided with the product for the study



Participants take NASA-TLX 1 to complete at home



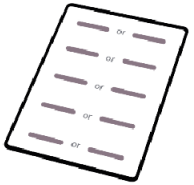
Prepare collection mixture: 100mL in sealable bag for sanitary pad and tampon collection, or 10mL in 50mL tube for menstrual cup collection.

Designated bathroom is prepared with table, necessary equipment, and sanitised before visit.

## Donation visit 1

Participants donate during their heaviest flow

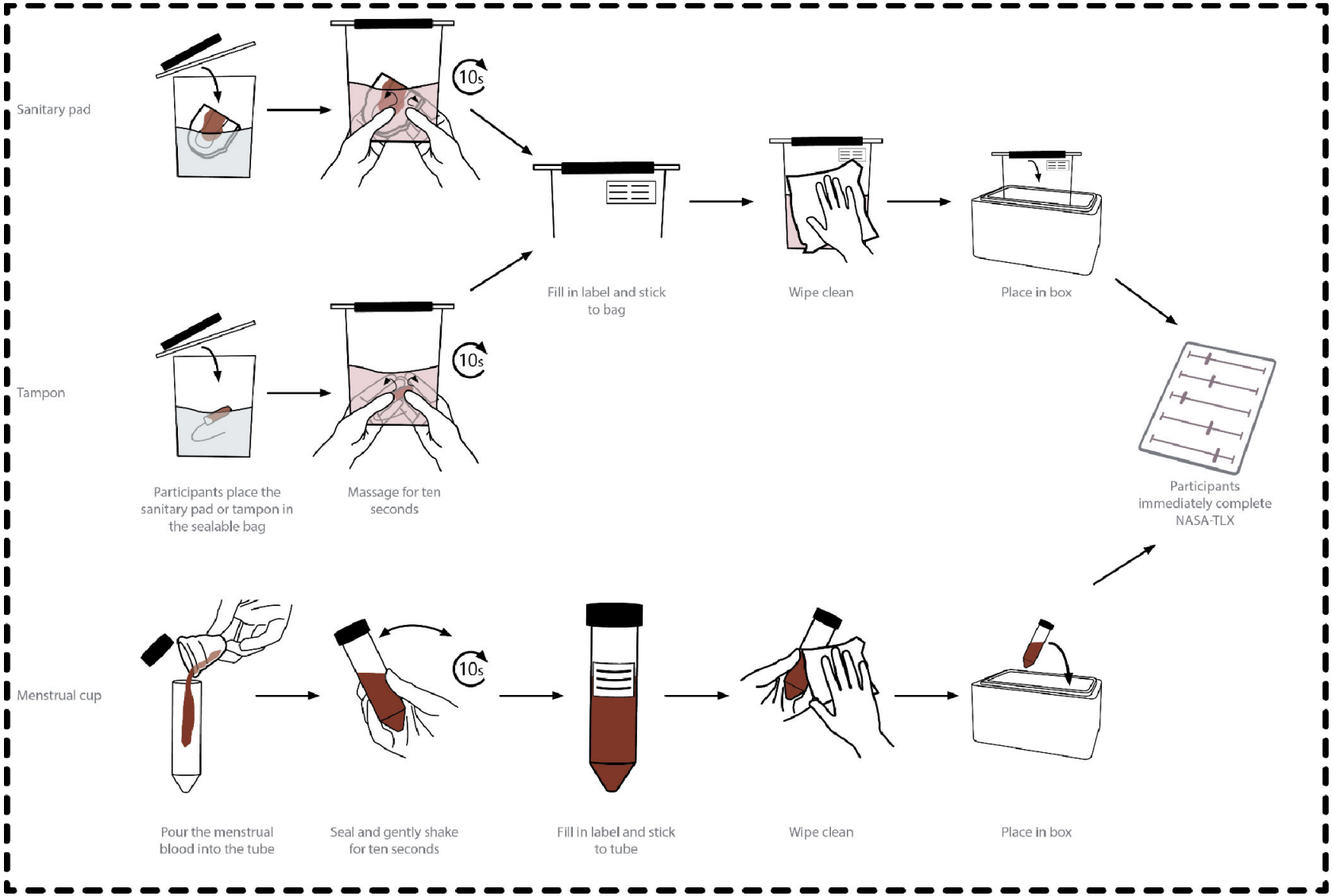

Participants arrive having worn the product for approximately four hours



Participants complete NASA-TLX comparison

Participants are led to designated bathroom for donation

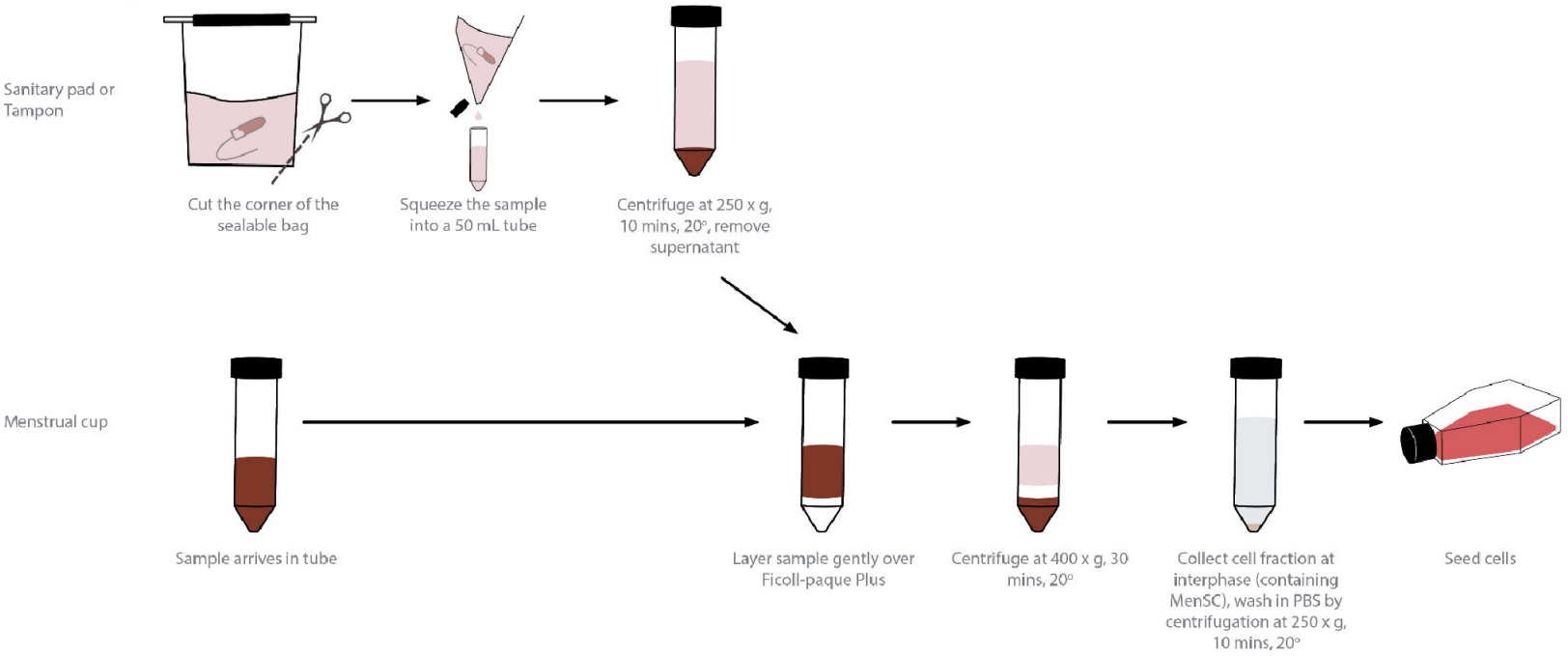
## Donation visit 1



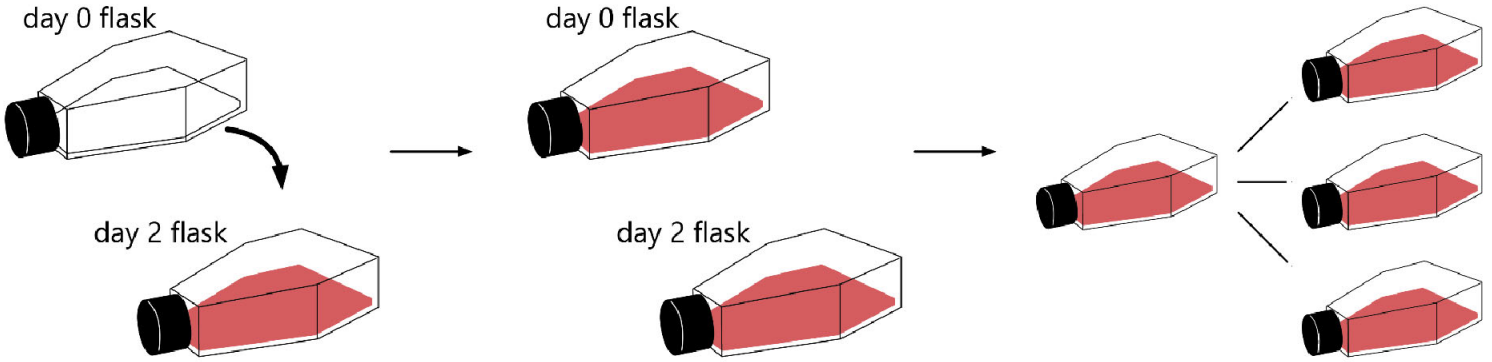
## Donation visit 2

The donation process is repeated. One sample is processed immediately, and the other sample is stored at 4 °C and processed after 48 hours

## Processing



## Expanding

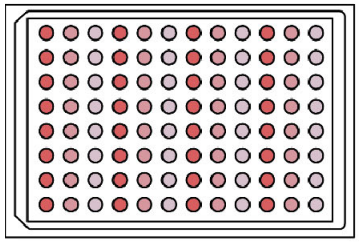



On day 1-3, transfer media to fresh flask to preserve any quiescient cells

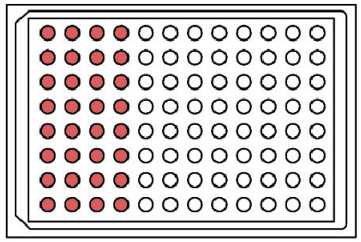
Rinse day 0 non-adherent cells with PBS, feeding cells every 2-3 days. Observe day 2 flask over 28 days for MenSC growth

Passage cells when 70-80% confluent, seed at $4 \times 10^3$ MenSC/cm$^2$

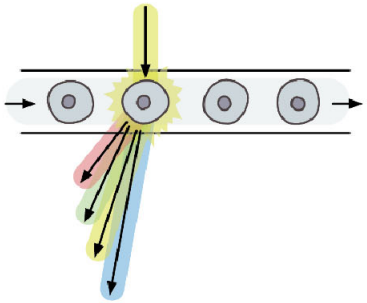
## Experiments



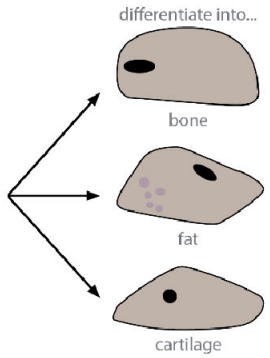
10-day media comparison
MTT assay at P3



10-day MenSC proliferation
MTT assay at P3



Flow cytometry
undertaken at P5




P3 trilineage
differentiation

# Appendix F Health Screen

## HEALTH SCREEN

**Health form** for the study titled: *"Comparing sanitary pads, tampons, and menstrual cups for menstrual blood collection and stem cell extraction"*

| | |
|---|---|
| IRAS ID | 301566 |
| Participant number | ..... |
| Date of birth | ..... |
| Date | ..... |
| Version (date) | 1 (18.10.21) |

Please complete this brief questionnaire to confirm fitness to participate:

| | | |
|---|---|---|
| 1. | At present, do you have any health problem for which you are: | |
| (a) | on medication, prescribed or otherwise | ☐ Yes ☐ No |
| (b) | attending your general practitioner | ☐ Yes ☐ No |
| (c) | on a hospital waiting list | ☐ Yes ☐ No |
| 2. | In the past two years, have you had any illness which require you to: | |
| (a) | consult your GP | ☐ Yes ☐ No |
| (b) | attend a hospital outpatient department | ☐ Yes ☐ No |
| (c) | be admitted to hospital | ☐ Yes ☐ No |
| 3. | Have you ever had any of the following? | |
| (a) | Convulsions/epilepsy | ☐ Yes ☐ No |
| (b) | Asthma | ☐ Yes ☐ No |
| (c) | Eczema | ☐ Yes ☐ No |
| (d) | Diabetes | ☐ Yes ☐ No |
| (e) | A blood disorder | ☐ Yes ☐ No |
| (f) | Head injury | ☐ Yes ☐ No |
| (g) | Digestive problems | ☐ Yes ☐ No |
| (h) | Heart problems | ☐ Yes ☐ No |
| (i) | Problems with bones or joints | ☐ Yes ☐ No |
| (j) | Disturbance of balance / coordination | ☐ Yes ☐ No |
| (k) | Numbness in hands or feet | ☐ Yes ☐ No |
| (l) | Disturbance of vision | ☐ Yes ☐ No |
| (m) | Ear / hearing problems | ☐ Yes ☐ No |
| (n) | Thyroid problems | ☐ Yes ☐ No |
| (o) | Kidney or liver problems | ☐ Yes ☐ No |

| | | |
|---|---|---|
| (p) | Allergy to nuts, alcohol etc. | ☐ Yes ☐ No |
| (q) | Any problems affecting your nose e.g. recurrent nose bleeds | |
| (r) | Any nasal fracture or deviated nasal septum | ☐ Yes ☐ No |
| 4. | Has any, otherwise healthy, member of your family under the age of 50 died suddenly during or soon after exercise? | ☐ Yes ☐ No |
| 5. | Are there any reasons why blood sampling may be difficult? | ☐ Yes ☐ No |
| 6. | Have you had a blood sample taken previously? | ☐ Yes ☐ No |
| 7. | Have you had a cold, flu or any flu like symptoms in the last month? | ☐ Yes ☐ No |
| 8. | Have you ever tested positive for covid-19? | ☐ Yes ☐ No |
| 9. | Are you a smoker? | ☐ Yes ☐ No |
| 10. | Are you pregnant, trying to become pregnant, or breastfeeding? | ☐ Yes ☐ No |
| 11. | Have you ever given birth? | ☐ Yes ☐ No |
| 12. | Do you have a history of | |
| (a) | Hepatitis B | ☐ Yes ☐ No |
| (b) | Hepatitis C | ☐ Yes ☐ No |
| (c) | HIV-1 | ☐ Yes ☐ No |
| (d) | HIV-2 | ☐ Yes ☐ No |
| 13. | Are you currently experiencing unusual vaginal discharge? | ☐ Yes ☐ No |
| 14. | Are you currently taking a contraceptive? | ☐ Yes ☐ No |

If YES to any question, please describe briefly if you wish (e.g. to confirm problem was/is short-lived, insignificant or well controlled.)

.....

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.....

# COVID-19 SYMPTOM QUESTIONNAIRE

**Covid-19 form** for the study titled: *"Comparing sanitary pads, tampons, and menstrual cups for menstrual blood collection and stem cell extraction"*

| | |
|---|---|
| IRAS ID | 301566 |
| Participant number | ..... |
| Date | ..... |
| Version (date) | 1 (18.10.21) |

1. Do you have:

| | | |
|---|---|---|
| A high temperature / fever | Yes ☐ | No ☐ |
| A sore throat | Yes ☐ | No ☐ |
| A new continuous cough* | Yes ☐ | No ☐ |
| Loss of, or change in, taste or smell | Yes ☐ | No ☐ |

* A new, continuous cough means coughing for longer than an hour, or three or more coughing episodes in 24 hours.

2. Have you, or anyone you share a house with, been in close contact with anyone with a suspected or confirmed case of COVID-19 in the last two weeks? Yes ☐ No ☐

3. Please confirm that ALL of the questions 4-6 have been answered "NO" and that there are no reasons why you should not participate in the research study:

Yes – I can confirm that all of my responses to questions 1 & 2 above were "NO" ☐

No – I answered "Yes" to some or all of the questions 1 & 2 above. ☐

# Would you be willing to donate your menstrual blood?

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*If you are 18 or over, this study might be for you.*

## Menstrual blood stem cell donation

We're looking for healthy menstruators to donate menstrual blood using either tampons, sanitary pads, or a menstrual cup. We will be seeing whether this donation is successful in terms of ease of use, and stem cell survival, with the aim for this research to help develop regenerative medicine.

Participants will be asked to attend:

- 1 eligibility session
- 1 introductory session
- 2 donation sessions
- You will be asked to track your menstrual cycle to make donation easier

During eligibilty and introductory sessions, you will be asked to fill in forms regarding your health and menstrual experiences. During the donation sessions, you will be asked to give us your menstrual blood samples (rather than throwing them away), and to fill in forms regarding your menstrual experiences.

You will receive £20 voucher as thanks for your time.

**Where?**
Nottingham Trent University
Clifton campus

**Am I eligible?**
Healthy
18 or over
Willing to donate menstrual blood with sanitary pads, tampons, or a menstrual cup

**Contact**
Hannah Manley
Engineering Building 108, NTU
Clifton campus
hannah.manley@ntu.ac.uk
+44 (0) 1158 484790

IRAS ID 301566 | 18.10.21 | Version 1
Date approved 06.01.22 | Expires 30.09.2023

*To sign up or discuss eligibility, grab a ticket or get in contact with us. We look forward to hearing from you!*

Menstrual blood donation
hannah.manley@ntu.ac.uk
+44 (0) 1158 484790

Menstrual blood donation
hannah.manley@ntu.ac.uk
+44 (0) 1158 484790
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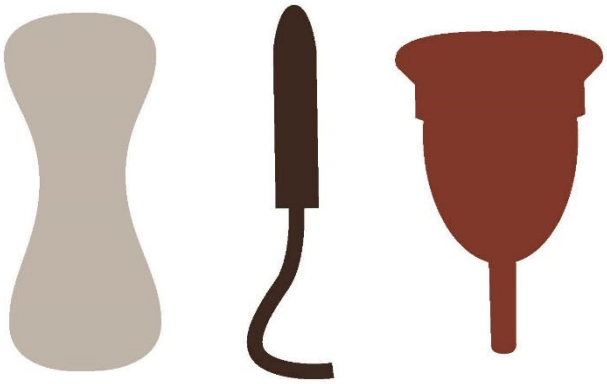
hannah.manley@ntu.ac.uk
+44 (0) 1158 484790

NOTTINGHAM TRENT UNIVERSITY



# Menstrual blood stem cell donation

*Comparing sanitary pads, tampons, and menstrual cups for menstrual blood collection and stem cell extraction*

## Participant information

IRAS ID 301566 | Date approved 06.01.22 | Expires 30.09.23 | Version 2 (08.12.21)

## Summary

Menstrual blood contains stem cells, and women just throw them away! We are asking healthy women with regular menstrual cycles to donate their menstrual blood on one day of two of their periods using a menstrual hygiene product they are comfortable with: a sanitary pad, tampon, or menstrual cup. We will then extract the stem cells from the menstrual blood to see which method is best, and ask you to rate your experience. This will be a next step in making menstrual blood donation for cell therapy achievable.

## Background to the research

Menstrual blood contains cells that have the potential to be used in therapy, much like the stem cells in bone marrow or umbilical cord blood. However, unlike donating bone marrow where a painful, invasive procedure is required, and unlike donating umbilical cord blood that can only be donated when a child is born, menstrual blood is a waste product that can be donated quickly, easily, and painlessly, and as often as every month. Clinical trials show there could be a great future for menstrual blood stem cells, with successful transplants in patients with multiple sclerosis, Asherman's syndrome (a reproductive syndrome), and acute respiratory distress syndrome. Animals have also been treated for liver failure, spinal cord injury, and stroke-like symptoms with these menstrual blood stem cells.

The exciting treatments made available by menstrual blood-derived stem cells are being researched. However, if the donation methods of menstrual blood are not acceptable to women, then they will not donate. So it's our job to make the donation as easy, quick, and appealing as possible! That is where we need your help.

Normally, menstrual blood is collected with a menstrual cup. Only 4% of women in the UK use a menstrual cup as part of their monthly menstrual hygiene routine, and the majority of women use a tampon or sanitary pad as their menstrual hygiene product of choice. However, there is no research on whether using these products collects menstrual blood successfully. There no comparison as to which method might be superior, either from the donors' perspective (you), or the perspective of cell survival and viability for cell therapy (the science).

This research will therefore be the first study of sanitary pads and tampons: are women happy to donate in this way, and will the cells survive? This work will contribute to the development of menstrual blood donation, with the intention to invent a device specifically for stem cell donation. This work will therefore also be relevant to easy-to-use, painless menstrual blood donation for other applications, such as the diagnosis of conditions such as endometriosis.

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# What's involved?

This is your first visit, with three more. Today you will fill in some paperwork and begin tracking your menstrual cycle if you do not already. Please make sure you ask any questions that you have. Once you understand your role, we will provide you with the menstrual hygiene product of choice which you will use for the other visits. You will also be given a questionnaire to fill in at home. We will also establish when you think you will be on your next period, so that we can plan your visits, and find a time that suits you best.

For visit three and four, we will ask you to use the menstrual hygiene products that we gave you ready for donation. This will mean wearing/ changing it four hours before your appointment here. You will come in, we will chat about your experience and make sure everything is ok, before taking you to a private bathroom where you can remove or change your menstrual hygiene product. The only difference this time, is you will not be throwing your blood away! Instead, it will be in a plastic bag, that you can place in a box. We will then take it off you for processing. Remember that questionnaire that you will have filled in at home? On both visits we will give you the same questionnaire to fill in again. This will help us to see if there are any differences. We will also ask you about your experiences, thoughts, ideas, worries, and feelings you have about the whole thing.

When you have finished both donations, we will provide you with a £20 voucher as a thank you and travel compensation.

You will be emailed all of this information as we know it is a lot to take in.

## What will happen with my menstrual blood?

We are collecting your menstrual blood to extract the stem cells it contains. We will then grow these cells to see whether using a sanitary pad, tampon, or menstrual cup makes a difference to the survival of these cells. We will also see whether it is possible to store the sample temporarily, so one sample will be processed by us within 24 hours, and the other sample will be refrigerated and processed after 48 hours. This is to see if temporary storage makes a difference to the survival of these cells. We may also see if a woman's age has any effect on the cells. The questionnaire you fill in after donating your menstrual blood will also give us an indication of your experience.

The data we collect will make up an important part of Hannah Manley's PhD. This means she will be writing about this study in her thesis and other papers and forms of research, such as conferences. Your sensitive data will never be shared. In Hannah's research outputs, your name, medical history, and any information that makes you identifiable will be removed. You will be completely anonymous. The only way people will ever know you took part is if you tell them yourself!

If at any point you decide to withdraw from the study, your data will be destroyed.

## What are the risks?

As with the use of all menstrual hygiene products, there is a risk of discomfort, chafing, and in very rare cases in the use of tampons or menstrual cups, toxic shock syndrome (there are 40 cases each year in the UK). The important thing is to read all the instructions carefully, and follow all the labelled advice. If something feels wrong, immediately remove the product and seek medical advice.

## How will we use information about you?

We will need to use information from you for this research project.

This information will include your
- name
- contact details
- health information including history of birth and use of contraception

People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

## What are your choices about how your information is used?

- You can stop being part of the study at any time, without giving a reason, and your data will be destroyed.
- We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

## Further research

The Human Tissue Authority is part of the government controlling the storage of human tissue for research. This study involves the use of your menstrual blood, which is "relevant material". At the end of this study, if the University is licensed by the Human Tissue Authority, the samples might be useful in other studies. Although we do not know at this stage what these might be, they will be in similar areas to this study. For example, in better understanding these cells, and potential treatment using these cells. Any other studies will be approved through the University's ethical approval process before they begin. The Human Tissue Authority allows this because it means that we don't have to keep taking new samples from people. If you agree to this, it means that you have given your enduring consent. Your tissues will not be used in particular studies if you would prefer them not to be, for example you might not want the stem cells to be used in animal testing, or used for genetics or DNA testing. There will be a section of the consent form surrounding this. All samples (classed as "relevant material" by the Human Tissue Authority) will be discarded if NTU does not have a HTA license in place.

## Supporting information

If something goes wrong, please tell us. We can replace sanitary items, or rearrange visit dates, but the important thing is to let us know.

If you don't want to take part anymore, you simply need to tell us. We will then delete all of your contact details, administrative information, and research data from the study. However, because we will be using the data for writing the PhD thesis and other papers, we ask that you tell us within six months of signing the consent form. This is to give us time to remove all the data before submitting work.

Everything you tell us will remain confidential. All data is saved securely on a password-protected computer, and the visits will all be in private settings.

This study is funded by the Nottingham Trent University Studentship Bursary, meaning Hannah is on a Scholarship to study here. No external companies are involved, meaning that this study is fair, and there will be no commercial exploitation of the findings. It also means we are organising the study ourselves, meaning we can put you first.

# Contact

Contact
We will need to stay in contact with you throughout the study. We might also see if you would like to take part in further studies. This will all be discussed with you, and will be clear on the consent form.

You can always get in contact with us if you have any questions:



Hannah Manley
Post Graduate Research student and Hourly Paid Lecturer in Product Design
Nottingham Trent University, Department of Engineering,
School of Science & Technology, Clifton Lane, Clifton, Nottingham, NG11 8NS
Mob: +44(0) 7999 395732 Work: +44 (0) 1158 484790
Office: Engineering Building EB108
Email: Hannah.manley@ntu.ac.uk

Here are some other support numbers from menstrual hygiene companies not affiliated with this study but can offer help and advice:

Sanitary pads
Always
UK
Phone: 0800 028 5884
Online chat (no email address) : https://www.always.co.uk/en-gb/contact-us

Tampons
Tampax
UK
Phone: 0800 378 135
Online chat (no email address) : https://tampax.co.uk/en-gb/contact-us

Menstrual cups
Mooncup Ltd
Vantage Point, New England Road
Brighton, BN1 4GW
UK
Phone: 44(0) 1273 673845
Email: info@mooncup.co.uk

# Where can you find out more about how your information is used?

You can find out more about how we use your information
- by asking Hannah Manley (above)
- by sending an email to Data Protection Officer tracy.landon@ntu.ac.uk, or
- by ringing Data Protection Officer on +44 (0)115 848 8754.

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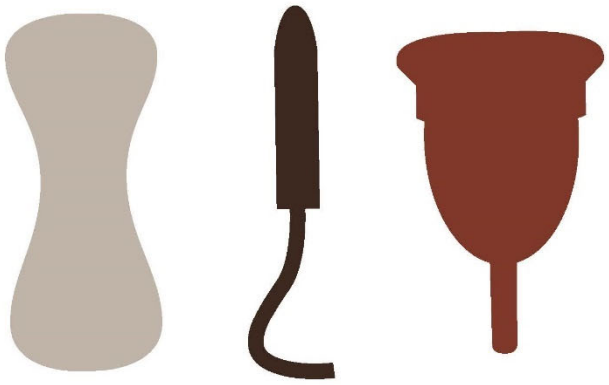
# Special covid-19 safety measures

To ensure covid-19 safety, you will be treated as asymptomatic positive for covid-19. You will wear masks for the duration, will sign in and out of the building, use the hand sanitising stations, and will only have access to rooms designated to the study. The meeting room and designated bathroom will be closed for study access only, and all surfaces will be sanitised before and after each visit. Researchers and participants will remain 2m apart where possible, remaining side by side, rather than face to face. The entire building is well ventilated.

Researchers will wear the necessary personal protective equipment, including mask, lab coat, and face shield while we are meeting, and latex gloves when handling the sealed sample.



Thank you for taking the time to read this document. We are here to answer all your questions, and we look forward to working with you!

# Appendix I Consent Forms

## CONSENT

**Donation** for the study titled: *"Comparing sanitary pads, tampons, and menstrual cups for menstrual blood collection and stem cell extraction"*

| | |
|---|---|
| IRAS ID | 301566 |
| Location | Nottingham Trent University |
| Study Number | 21/EM/0266 |
| Participant number | ..... |
| Researcher | Hannah Manley |
| Version (date) | 3 (06.01.22) |

Please initial box

| | | |
|---|---|---|
| 1) | I, ..... agree to partake as a participant in the above study. | |
| 2) | I understand from the participant information sheet (Dated 08.12.21 \| Version 2), which I have read in full, and from my discussion(s) with Hannah Manley that this will involve me donating my menstrual blood using a product I am comfortable with (a sanitary pad, tampon, or menstrual cup) on two occasions and filling in three questionnaires as well as other paperwork. | ☐ |
| 3) | It has also been explained to me by Hannah Manley that the risks and side effects that may result from my participation are as follows: discomfort, chafing, and in very rare cases in the use of tampons or menstrual cups, toxic shock syndrome. | ☐ |
| 4) | I confirm that I have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction. | ☐ |
| 5) | I undertake to abide by University regulations and the advice of researchers regarding safety. | ☐ |
| 6) | I am aware that I can withdraw my consent to participate in the procedure for any reason, without having to explain my withdrawal and that my personal data will be destroyed and that my medical care or legal rights will not be affected, within six months of signing this form. | ☐ |
| 7) | I understand that any personal information regarding me, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous. | ☐ |
| 8) | I confirm that I have had the University's policy relating to the storage and subsequent destruction of sensitive information explained to me. I understand that sensitive information I have provided through my participation in this study, in the form of administrative information, menstrual blood samples, and questionnaire results, will be handled in accordance with this policy. | ☐ |
| 9) | I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise, that would prevent me from partaking in this research. | ☐ |
| 10) | The Human Tissue Authority is part of the government controlling the storage of human tissue for research. As has been explained, this study | |

involves the use of your menstrual blood. At the end of this study, if the University is licensed by the Human Tissue Authority, the samples might be useful in other studies, although we do not know at this stage what these might be. However, they will be in similar areas to this study. For example, in better understanding these cells, and potential treatment using these cells. Any other studies will be approved through the University's ethical approval process before they begin. The Human Tissue Authority allows this because it means that we don't have to keep taking new samples from people. If you agree to this, it means that you have given your enduring consent.

I give my enduring consent for my cells to be used in future ethically approved studies, if NTU has a license from the Human Tissue Authority. Your tissues will not be used in particular studies if you would prefer them not to be. ☐

11) Please comment below if there are any studies you do not wish for your samples to be used in (e.g. genetics, animal testing etc.)

.....

.....

You can also withdraw your enduring consent and request destruction of your donated samples at any time by using the contact details in the information sheet provided. It is likely that the samples you provide will continue to be stored for a few months in a designated freezer on the University's Clifton Campus. Once the useful parts of the samples have been used up, the material that remains will be disposed of by incineration.

12) It has been explained to me that there may be additional risks arising from the current COVID pandemic. I have read the NTU recommendations for undertaking 'Research with human participants' and undertake to abide by the special measures which have been explained to me for this study together with such Government Guidelines that are at the time prevailing. ☐

13) Menstrual hygiene products I am comfortable using (tick all that apply)

- ☐ Sanitary pad
- ☐ Tampon
- ☐ Menstrual cup

Participant signature: Date:

Primary Researcher signature: Date:

Please provide your preferred method of communication for organising donation times. You can withdraw at any time in the timeframe mentioned above and this information will be deleted from our system.

.....

Thank you for your time.

*When completed: 1 for participant; 1 for researcher site file; 1 to be kept in medical notes (if appropriate).

# RESULTS

**Results form** for the study titled: *"Comparing sanitary pads, tampons, and menstrual cups for menstrual blood collection and stem cell extraction"*

| | |
|---|---|
| IRAS ID | 301566 |
| Participant number | ..... |
| Date | ..... |
| Version (date) | 1 (18.10.21) |

1. Would you like to receive a copy of the results when this study is complete?

☐ Yes ☐ No

If yes, please provide your preferred method of communication for receiving a copy of the results.

.....

.....

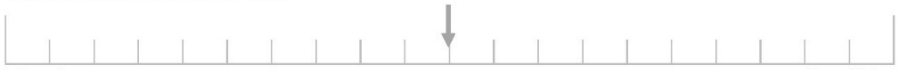
# Appendix J NASA-TLX

**TLX form** for the study titled: *"Comparing sanitary pads, tampons, and menstrual cups for menstrual blood collection and stem cell extraction"*

| | |
|---|---|
| IRAS ID | 301566 |
| Location | Nottingham Trent University |
| Study Number | 21/EM/0266 |
| Participant number | ..... |
| Researcher | Hannah Manley |
| Version (date) | 1 (18.10.21) |

*Hart and Staveland's NASA Task Load Index (TLX) method assesses work load on six scales. Increments of high, medium, and low estimates for each point result in 21 graduations on the scales.*

Example: add an arrow to the scale




**Mental Demand-** *How mentally demanding was the task?*


**Physical Demand-** *How physically demanding was the task?*


**Temporal Demand-** *How hurried or rushed was the pace of the task?*


**Performance-** *How successful were you in accomplishing on what you were asked to do?*


**Effort-** *How hard did you have to work to accomplish your level of performance?*


**Frustration-** *How insecure, discouraged, irritated, stressed, and annoyed were you?*

If you are happy to, please write down further thoughts and comments on the donation process; think about:

**Mental Demand-** *How mentally demanding was the task?*

**Physical Demand-** *How physically demanding was the task?*

**Temporal Demand-** *How hurried or rushed was the pace of the task?*

**Performance-** *How successful were you in accomplishing on what you were asked to do?*

**Effort-** *How hard did you have to work to accomplish your level of performance?*

**Frustration-** *How insecure, discouraged, irritated, stressed, and annoyed were you?*

**General feelings surrounding menstrual blood donation for potential regenerative medicine**

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| | |
|---|---|
| IRAS ID | 301566 |
| Location | Nottingham Trent University |
| Study Number | 21/EM/0266 |
| Participant number | ..... |
| Donation | ..... |
| Researcher | Hannah Manley |
| Version (date) | 1 (18.10.21) |

**Mental Demand-** *How mentally demanding was the task?*

**Physical Demand-** *How physically demanding was the task?*

**Temporal Demand-** *How hurried or rushed was the pace of the task?*

**Performance-** *How successful were you in accomplishing on what you were asked to do?*

**Effort-** *How hard did you have to work to accomplish your level of performance?*

**Frustration-** *How insecure, discouraged, irritated, stressed, and annoyed were you?*

*Please select the factor that is most important to you within each pair of factors (circle).*

| | | |
|---|---|---|
| **Effort** | or | **Performance** |
| **Temporal Demand** | or | **Frustration** |
| **Temporal Demand** | or | **Effort** |
| **Physical Demand** | or | **Frustration** |
| **Performance** | or | **Frustration** |
| **Physical Demand** | or | **Temporal Demand** |
| **Physical Demand** | or | **Performance** |
| **Temporal Demand** | or | **Mental Demand** |
| **Frustration** | or | **Effort** |
| **Performance** | or | **Mental Demand** |
| **Performance** | or | **Temporal Demand** |
| **Mental Demand** | or | **Effort** |
| **Mental Demand** | or | **Physical Demand** |
| **Effort** | or | **Physical Demand** |
| **Frustration** | or | **Mental Demand** |

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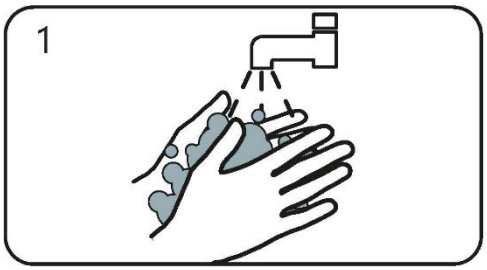
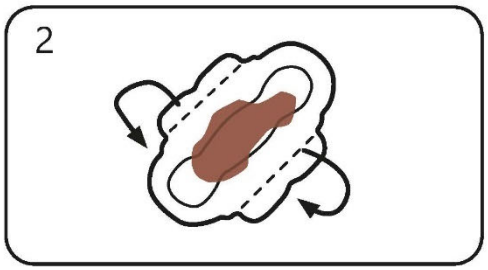
# Participant instructions

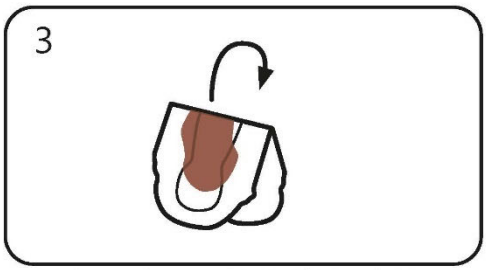
*Comparing sanitary pads, tampons, and menstrual cups for menstrual blood collection and stem cell extraction*



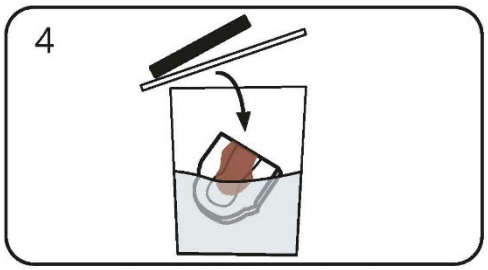

Wash your hands



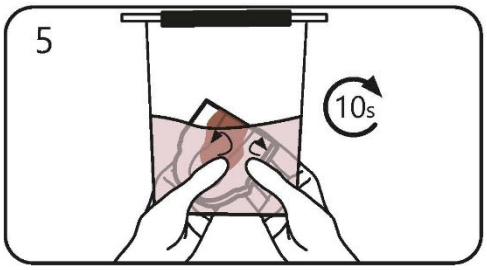

Remove the sanitary pad, and fold the wings to the back



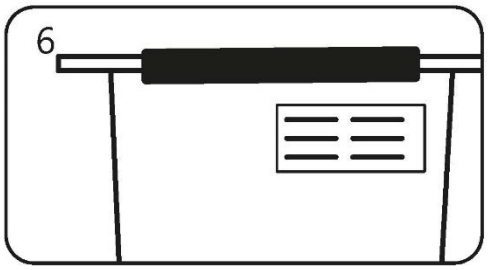

Fold the sanitary pad in half, blood-side out



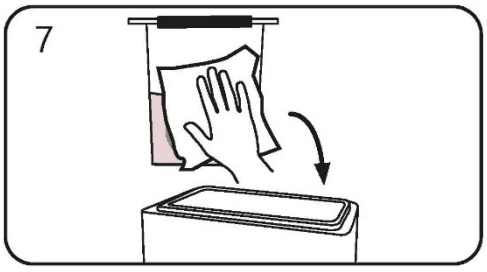

Place inside bag and seal




Massage for ten seconds. Try to change the colour of the liquid




Complete the information on the label and stick to the bag



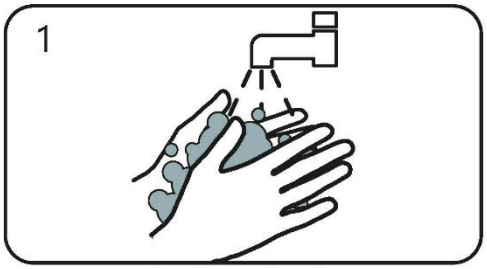

Wipe clean and place in box

*Thank you!*

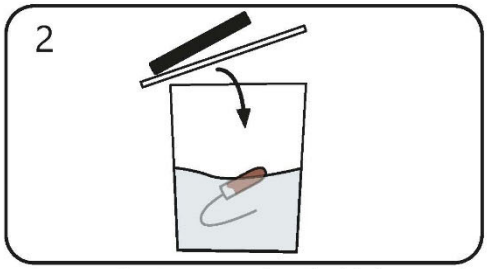
NOTTINGHAM TRENT UNIVERSITY

# Participant instructions

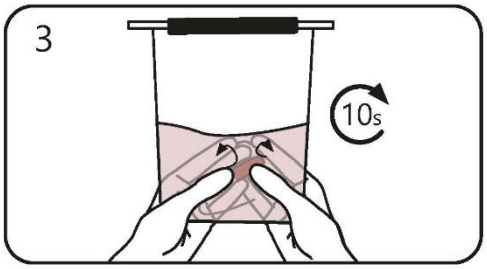
*Comparing sanitary pads, tampons, and menstrual cups for menstrual blood collection and stem cell extraction*



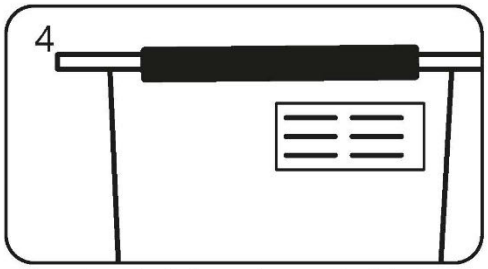

Wash your hands



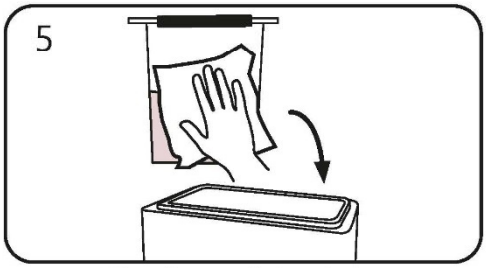

Remove the tampon, place inside bag and seal




Massage for ten seconds. Try to change the colour of the liquid




Complete the information on the label and stick to the bag



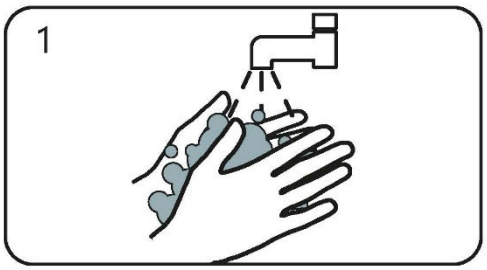

Wipe clean and place in box

*Thank you!*

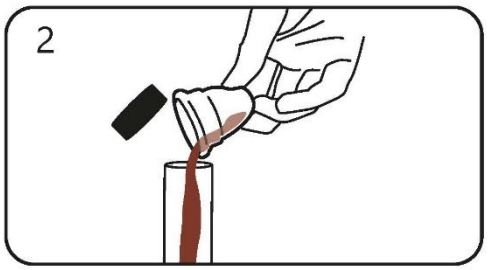
NOTTINGHAM TRENT UNIVERSITY

# Participant instructions

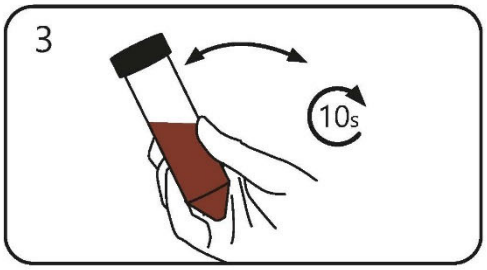
*Comparing sanitary pads, tampons, and menstrual cups for menstrual blood collection and stem cell extraction*



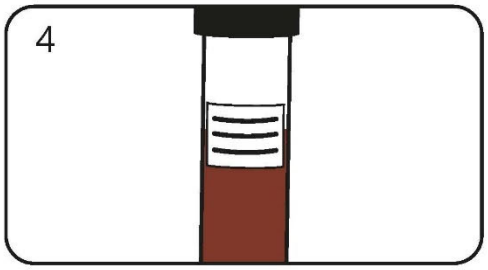

Wash your hands



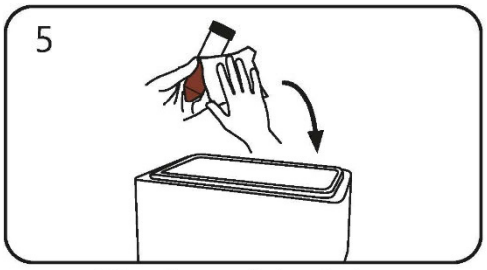

Remove the menstrual cup and tip blood into tube




Tip the tube from side to side for 10 seconds to mix




Complete the information on the label and stick to the tube



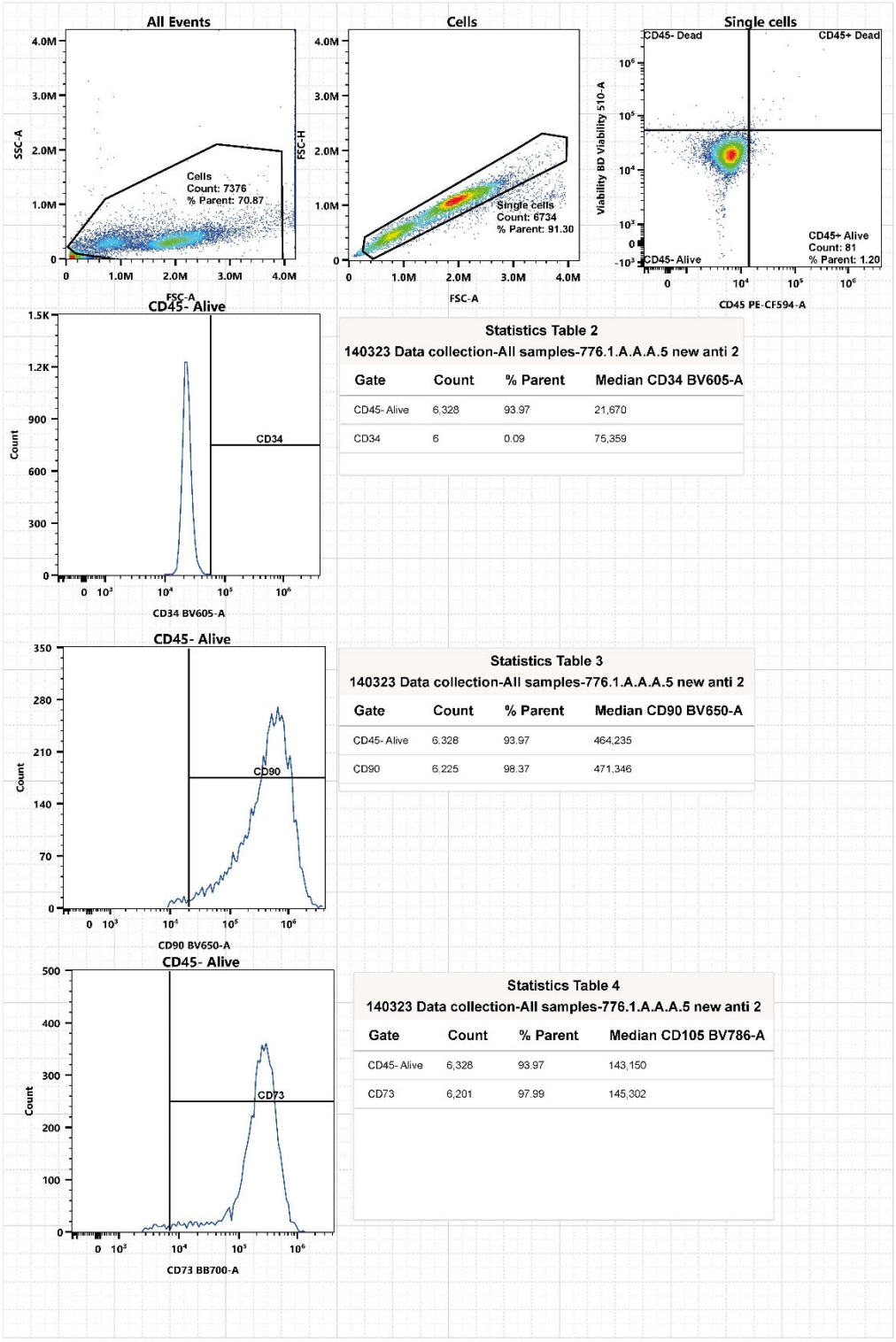

Wipe clean and place in box

*Thank you!*

| | | Willing to use | | | | | | | | Success? | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Participant* | *Age* | *Sanitary pad* | *Tampon* | *Menstrual* | *No. births* | *Hormonal contraceptive* | *Menstrual phase / days* | *Menstrual cycle / days* | *Product* | *Immediate* | *Delayed* |
| 775 | 41 | Yes | No | No | 0 | No | 6 | 29 | Pad | No | Yes |
| 776 | 21 | Yes | No | No | 0 | Yes* | 6 | 28 | Pad | Yes | No |
| 425 | 35 | Yes | No | No | 0 | No | 7.5 | 28 | Pad | No | Yes |
| 052 | 23 | Yes | Yes | Yes | 0 | No | 3.5 | 28 | Cup | No, Yes** | Yes |
| 536 | 31 | No | No | Yes | 0 | No | 4 | 26 | Cup | Yes | Yes |
| 276 | 36 | Yes | Yes | Yes | 2 | No | 6 | 38 | Tampon | Yes | No† |
| 836 | 22 | Yes | Yes | Yes | 0 | Yes* | 5 | 28 | Cup | No | Yes |
| 752 | 29 | No | Yes | No | 0 | No | 3.2 | 28 | Tampon | Yes | Yes |
| 428 | 27 | Yes | Yes | No | 0 | No | 5 | 28 | Pad | No | No |
| 415 | 36 | Yes | No | No | 0 | No | 4 | 25 | Pad | Yes | No |
| 614 | 31 | No | Yes | Yes | 0 | No | 5 | 28 | Cup | Yes | No‡ |
| 688 | 25 | Yes | Yes | No | 0 | Yes* | 5 | 28 | Tampon | Yes | Yes |
| 838 | 26 | Yes | Yes | No | 0 | Yes* | 4 | 28 | Tampon | Yes | Yes |
| 587 | 20 | Yes | Yes | Yes | 0 | No | 7 | …§ | Tampon | …‖ | Yes |
| 808 | 22 | Yes | No | No | 0 | No | 7 | 37 | Pad | Yes | Yes |
| 930 | 22 | No | Yes | No | 0 | No | 4 | 29 | Tampon | Yes | Yes |
| 557 | 22 | Yes | No | No | 0 | No | 5 | 43 | Pad | Yes | …‖ |
| 449 | 23 | Yes | Yes | No | 0 | Yes* | 4 | 28 | Pad | No | Yes |
| 637 | 31 | Yes | No | Yes | 0 | No | 4 | 29 | Pad | No | No |
| 265 | 20 | Yes | No | Yes | 0 | No | 6 | 28 | Cup | Yes | Yes |
| 470 | 21 | Yes | Yes | No | 0 | Yes* | 7 | 28 | Tampon | Yes | Yes |

*combined oral contraceptive pill, **donated twice, † sample contamination (bacteria), ‡ sample contamination (yeast), § missing data, ‖ participant did not donate

# Appendix M Flow cytometry gating and data example




**Statistics Table 2**

**140323 Data collection-All samples-776.1.A.A.A.5 new anti 2**

| Gate | Count | % Parent | Median CD34 BV605-A |
|---|---|---|---|
| CD45- Alive | 6,328 | 93.97 | 21,670 |
| CD34 | 6 | 0.09 | 75,359 |

**Statistics Table 3**

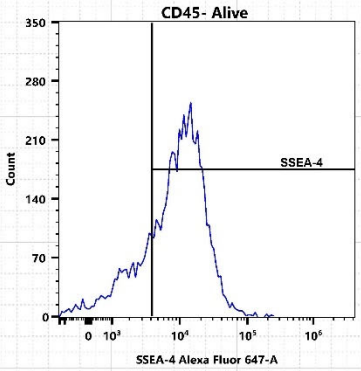
**140323 Data collection-All samples-776.1.A.A.A.5 new anti 2**

| Gate | Count | % Parent | Median CD90 BV650-A |
|---|---|---|---|
| CD45- Alive | 6,328 | 93.97 | 464,235 |
| CD90 | 6,225 | 98.37 | 471,346 |

**Statistics Table 4**

**140323 Data collection-All samples-776.1.A.A.A.5 new anti 2**

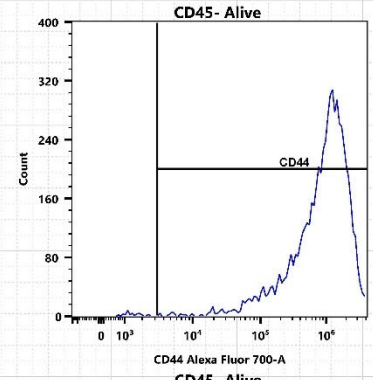
| Gate | Count | % Parent | Median CD105 BV786-A |
|---|---|---|---|
| CD45- Alive | 6,328 | 93.97 | 143,150 |
| CD73 | 6,201 | 97.99 | 145,302 |




**Statistics Table 5**

**140323 Data collection-All samples-776.1.A.A.A.5 new anti 2**

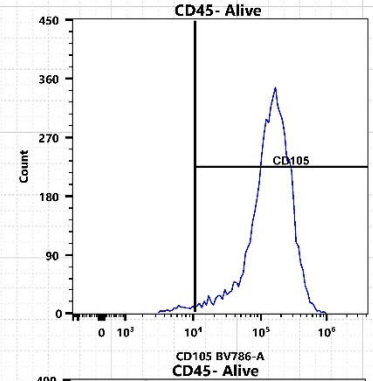
| Gate | Count | % Parent | Median CD73 BB700-A |
|---|---|---|---|
| CD45- Alive | 6,328 | 93.97 | 232,888 |
| SSEA-4 | 4,806 | 75.95 | 265,449 |




**Statistics Table 6**

**140323 Data collection-All samples-776.1.A.A.A.5 new anti 2**

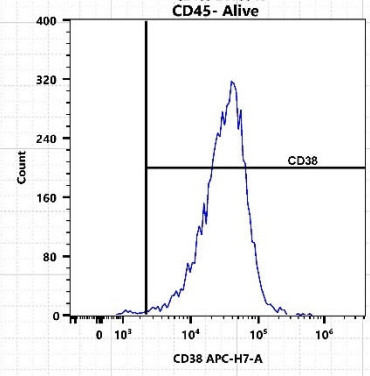
| Gate | Count | % Parent | Median SSEA-4 Alexa Fluor 647-A |
|---|---|---|---|
| CD45- Alive | 6,328 | 93.97 | 9,277 |
| CD44 | 6,272 | 99.12 | 9,313 |




**Statistics Table 7**

**140323 Data collection-All samples-776.1.A.A.A.5 new anti 2**

| Gate | Count | % Parent | Median CD44 Alexa Fluor 700-A |
|---|---|---|---|
| CD45- Alive | 6,328 | 93.97 | 982,317 |
| CD105 | 6,220 | 98.29 | 1,000,123 |



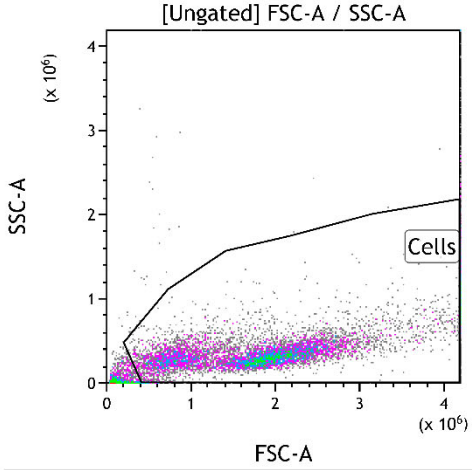

**Statistics Table 8**

**140323 Data collection-All samples-776.1.A.A.A.5 new anti 2**

| Gate | Count | % Parent | Median CD38 APC-H7-A |
|---|---|---|---|
| CD45- Alive | 6,328 | 93.97 | 31,722 |
| CD38 | 6,280 | 99.24 | 31,887 |

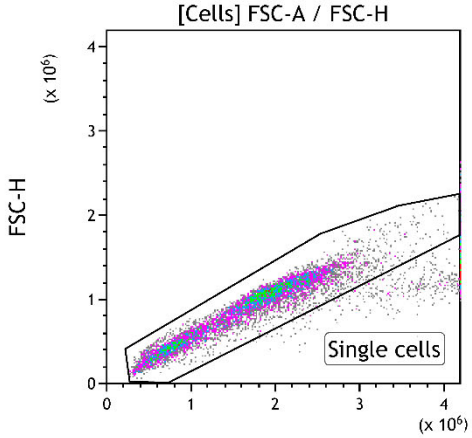
776.1.A.A.A.5 new anti 2_1
Data all cells blank - Plot Sheet 1

[Ungated] FSC-A / SSC-A



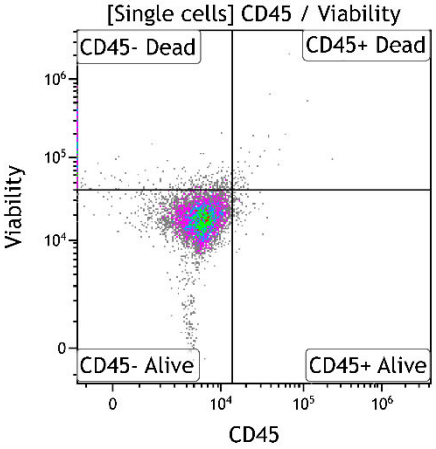

| Gate | Number | %Gated |
|---|---|---|
| All | 10,408 | 100.00 |
| Cells | 7,693 | 73.91 |

[Cells] FSC-A / FSC-H




| Gate | Number | %Gated |
|---|---|---|
| All | 7,693 | 100.00 |
| Single cells | 7,009 | 91.11 |

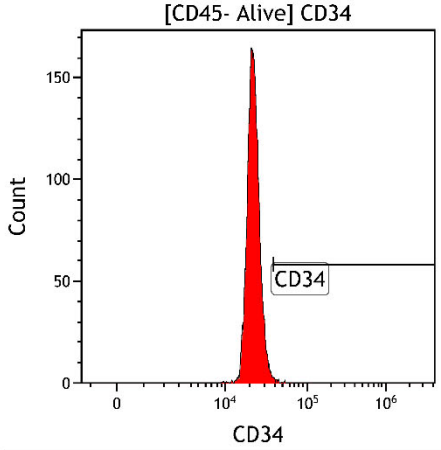
[Single cells] CD45 / Viability




| Gate | Number | %Gated | X-GMean |
|---|---|---|---|
| All | 7,009 | 100.00 | 4,963.79 |
| CD45- Alive | 6,299 | 89.87 | 5,661.57 |
| CD45- Dead | 568 | 8.10 | 464.23 |
| CD45+ Alive | 89 | 1.27 | 17,683.85 |
| CD45+ Dead | 53 | 0.76 | 22,630.46 |

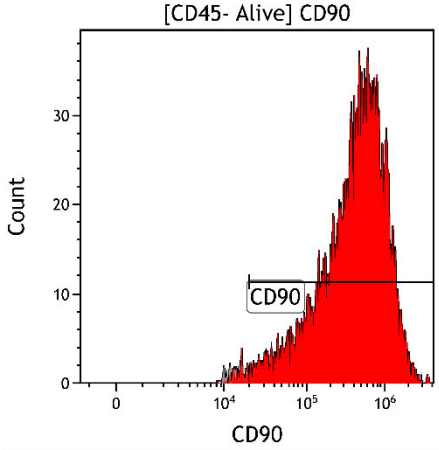
776.1.A.A.A.5 new anti 2_1
Data all cells blank - Plot Sheet 1

[CD45- Alive] CD34



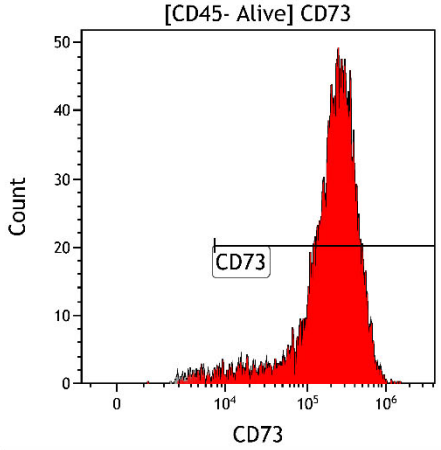

| Gate | Number | %Gated | X-GMean |
|---|---|---|---|
| All | 6,299 | 100.00 | 21,794.71 |
| CD34 | 35 | 0.56 | 46,568.99 |

[CD45- Alive] CD90



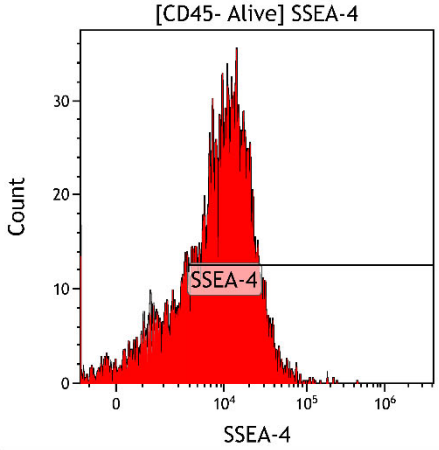

| Gate | Number | %Gated | X-GMean |
|---|---|---|---|
| All | 6,299 | 100.00 | 372,341.53 |
| CD90 | 6,205 | 98.51 | 391,798.69 |

[CD45- Alive] CD73




| Gate | Number | %Gated | X-GMean |
|---|---|---|---|
| All | 6,299 | 100.00 | 188,352.66 |
| CD73 | 6,184 | 98.17 | 202,528.00 |

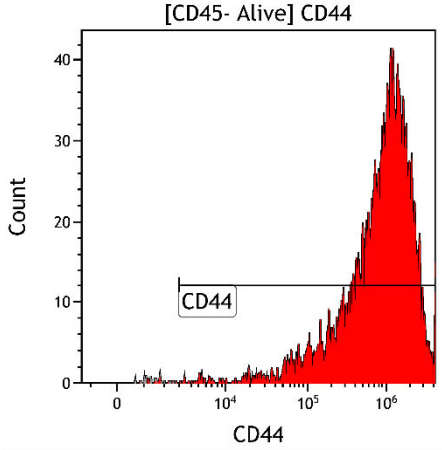
[CD45- Alive] SSEA-4




| Gate | Number | %Gated | X-GMean |
|---|---|---|---|
| All | 6,299 | 100.00 | 7,052.63 |
| SSEA-4 | 4,785 | 75.96 | 11,975.73 |

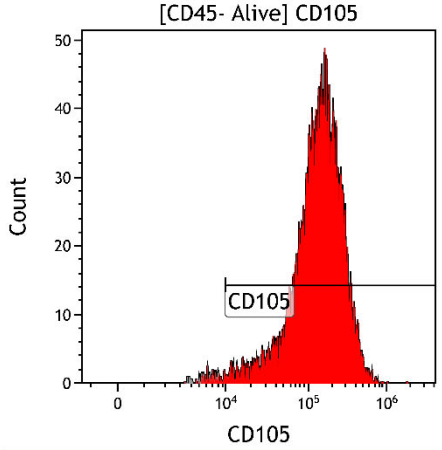
776.1.A.A.A.5 new anti 2_1
Data all cells blank - Plot Sheet 1

[CD45- Alive] CD44



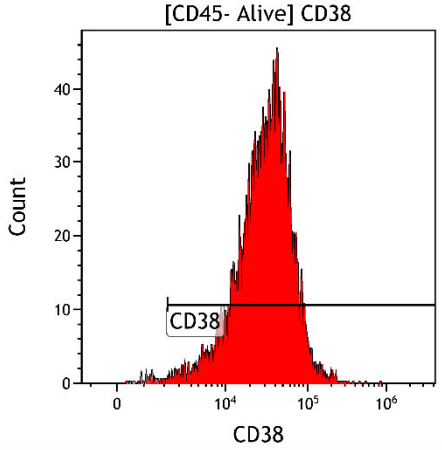

| Gate | Number | %Gated | X-GMean |
|---|---|---|---|
| All | 6,299 | 100.00 | 738,377.06 |
| CD44 | 6,266 | 99.48 | 764,033.06 |

[CD45- Alive] CD105




| Gate | Number | %Gated | X-GMean |
|---|---|---|---|
| All | 6,299 | 100.00 | 126,063.24 |
| CD105 | 6,202 | 98.46 | 132,281.13 |

[CD45- Alive] CD38




| Gate | Number | %Gated | X-GMean |
|---|---|---|---|
| All | 6,299 | 100.00 | 28,339.06 |
| CD38 | 6,254 | 99.29 | 28,995.18 |

Original Research Article

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# Comparison between menstrual cups: first step to categorization and improved safety

Hannah Manley[1], John A Hunt[2,3], Lívia Santos[4] and Philip Breedon[1,2]

**Abstract**

**Objectives:** Menstrual cups come in a range of shapes, sizes, and firmnesses, but unlike tampons are not categorized in any way. With these factors having an impact on product leaks and comfort, as well as being linked to illness and injury, women need the same level of transparency when purchasing a menstrual cup. The comparison of physical and mechanical properties of menstrual cups will be the first step to achieve this.

**Methods:** In October 2020, 14 popular and highly rated menstrual cups underwent quantitative comparison in laboratory settings (the United Kingdom), and they were compared in terms of their dimensions, volume, and compressive strength (firmness) using the Instron Universal Testing System. The overall designs were compared including shape, material, and features.

**Results:** Although all the products in this comparison were marketed to women below 30 years of age having never given birth, total volume varied from 18.88 mL to 38.14 mL, and compressive load to compress the menstrual cup 50% (±0.5%) maximum diameter varied from 3.39 N to 13.92 N.

**Conclusions:** Women are not sufficiently informed when choosing a menstrual cup. With no correlation between menstrual cup size, shape, and its volume, or material, shape, and its firmness, consumers cannot estimate which menstrual cup might be most suitable, and incorrect choice could cause injury. Transparency is needed across menstrual cup brands. With this and further regulation, women will make an informed decision to choose the correct menstrual cup and minimize injury. This work recommends firmness categories, ranging from 'very soft' to 'very firm' as a first step.

**Keywords**

comparison, medical device, menstrual cup, menstrual hygiene product, menstrual management, menstruation, women's health

Date received: 23 July 2021; revised: 15 October 2021; accepted: 21 October 2021

## Introduction

Menstrual cups are vessels made of silicone, thermoplastic elastomers (TPE), or natural rubber, designed to be worn vaginally to collect menstrual blood for up to 12 h before being emptied, cleaned, and reinserted. Market analysis shows 4% women in the United Kingdom (UK) use menstrual cups as their preferred menstrual hygiene product of choice.[1] They are available in a variety of shapes, with additional features available such as valves and anti-spill lips. A systematic review by Van Eijk et al.[2] identified that by 2019, there were 199 brands of menstrual cups on the market, available in 99 countries, with 145 of these brands ranging in price from US$0.72 to US$46.72. No scientific,

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quantitative, peer-reviewed menstrual cup comparison exists between more than two menstrual cups, and literature often focuses on women's attitudes to menstrual cups rather than their physical and mechanical properties.

With menstrual cups lasting up to 10 years,[2] women report choosing a menstrual cup for economic,[3–5] comfort,[6,7] and environmental reasons.[5,8,9] Generally, women prefer the menstrual cup to previously used methods of menstrual hygiene management (pads or tampons),[10] and menstrual cup satisfaction has been reported across multiple locations, (e.g. India,[11] Iran,[12] North America,[13] South Africa,[6,14] Thailand,[15] and the UK,[5] with many other studies not reporting location).[2] Studies have reported that menstrual cup users require familiarization to the product; one study reported that 23% of women had difficulties using the menstrual cup during first cycle use, citing difficult insertion and discomfort. However, once familiarized, more than 90% of participants found the menstrual cups easy to use by their third cycle of use and would recommend to others.[16]

User reviews have been published surrounding menstrual cup use. Shihata and Brody[17] asked 834 participants to compare the Femmycycle menstrual cup against the menstrual cup they were already comfortable using, but it is unclear whether participants used the product, or reviews were based on seeing it. Online user reviews have largely depended on visual inspection and 'squeeze tests' of one or two products.[18] In online menstrual cup comparison charts, firmness is rated out of five,[19,20] although categorization methods are not reported. Where user reviews have their value, standardized details on menstrual cup dimension, shape, material, and firmness would inform consumers, and provide context to the user reviews.

Here, it is argued that women are not sufficiently informed when using a menstrual cup for the first time, leading to women choosing a menstrual cup that is not suitable. With this having safety and comfort implications, this article provides a comparison and menstrual cup categorization to allow women to make educated decisions when choosing a menstrual cup.

## Function and safety

Menstrual cups are folded before insertion. The menstrual cup is then allowed to spring open, forming a seal against the vaginal wall. For removal, the base of the cup is pinched, and the product moved gently side to side while pulling down (see Figure 1).

Issues arise with incorrect sizing or firmness, see Table 1. If the menstrual cup is too small or the menstrual cup is too soft and the pressure from the vaginal wall prevents the menstrual cup from unfolding, it will leak. A rim with a smaller diameter may become suctioned around the cervix. If the menstrual cup is too large or firm, it is uncomfortable for the wearer and could cause injury.

The majority of evidence shows that menstrual cups are safe to use.[2] Rare cases of renal colic have been reported, caused by a menstrual cup blocking the urinary tract.[21–23] These could be attributed to menstrual cups being too large or firm and exerting pressure on the tissues surrounding the vagina. Similarly, a case of hydroureteronephrosis (blockage leading to kidney breakdown) was caused by 'deeply inserted' menstrual cup suctioning on the fornix (the recess from the protrusion of cervix).[24] This could be caused by a smaller rim diameter being able to suction around the cervix. These are little known issues, to the extent where patients experience hydronephrosis for multiple cycles before seeking medical attention.[25]

In 2020, a UK current affairs and debate programme named BBC's Victoria Derbyshire highlighted that even with the addition of air holes, due to the suction effect of the rim, pulling the cup down to remove is the suspected cause for an unknown number of prolapses in the UK and around the world.[26] There is no warning of this on the product's safety label. Difficulty removing a menstrual cup increases when it is too large.

## Variation

Menstrual cups vary in size, shape, material, and firmness. Many brands offer two sizes (e.g. Fun Cup, Merula, Mooncup/MCUK), where another has 269 variations allowing users to select size, firmness, stem, and colour (Me Luna,[27] December 2020). Menstrual cups are often categorized by their shape because it is one of the few ways women can judge a menstrual cup before it is purchased and used. This article recommends that menstrual cup shapes are generalized into v-shape, bell-shape, round-shape, and asymmetrical-shape. These categories are defined in Table 2.

Menstrual cups range in firmness, depending on their material and thickness. Some companies offer menstrual cups in varying firmness levels, including Lena (original and sensitive) and Me Luna (soft, classic, or sport). Manufacturers don't provide metric information on firmness.

## Current study

There is no level of regulation and standardization of menstrual cups in terms of size, shape, volume, or firmness. With menstrual cup size and firmness having a direct effect on safety, usability, comfort, and leaks, it is important to identify acceptable physical and mechanical properties of menstrual cups, with categorization similar to the absorbency levels of tampons or sanitary pads. Standardization across menstrual cup types and brands would empower women to make the choices necessary for a successful menstrual cup experience and improve safety overall.

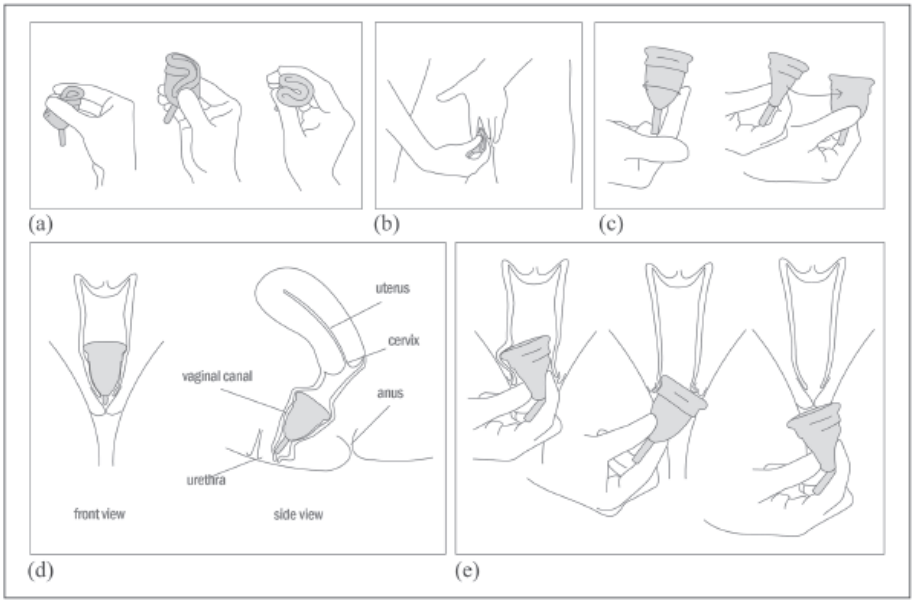


**Figure 1.** Menstrual cup use. (a) Users find a fold suitable for them; punchdown-fold, 7-fold, and c-fold illustrated. (b) The folded menstrual cup is inserted and allowed to spring open. (c) To ensure the menstrual cup is fully open and therefore creating a seal against the vaginal wall, users can run a finger around the vessel to feel for bumps as a sign it is not open, or users can gently pinch and twist the menstrual cup. (d) The menstrual cup sits lower than a tampon. The stem may need to be trimmed to avoid discomfort. The length of the vaginal canal will affect where the menstrual cup sits in relation to the cervix. (e) To remove, users must ensure the seal is broken by running a finger cup the side of the menstrual cup, or pinching the base of the vessel. The cup is then gently removed, and it can help shift the menstrual cup from side to side.

**Table 1.** Menstrual cup improper fit matrix.

| | Potential minor issues | Potential major issues |
|---|---|---|
| Too small | Menstrual cup will not form a seal against the vaginal wall, causing leaks. | Menstrual cup rim could suction around the cervix, causing pain or prolapse if pulled during removal. |
| Too soft | Menstrual cup will not open, causing leaks. | – |
| Too large | Discomfort experienced during insertion and removal. | Obstruction of urine flow, causing renal colic. Difficulty removing menstrual cup could cause prolapse. |
| Too firm | Discomfort experienced during insertion and removal. | Obstruction of urine flow, causing renal colic. |

The aims of this menstrual cup comparison are to understand how menstrual cups across different brands compare in terms of general size, shape, material, volume, and firmness; to understand whether a consumer can estimate the volume of a menstrual cup judging by its general size or shape; and whether consumers can estimate a cup's firmness based on a menstrual cup's material or shape. It will also attempt to identify potential size and firmness categorization to improve safety and transparency to ultimately help women choose the most appropriate menstrual cup from the outset. This is the first study reporting an objective, quantitative comparison of 14 menstrual cups.

## Method

Quantitative, ex-vivo comparison of 14 menstrual cups was undertaken in laboratory setting at Nottingham Trent University, Nottingham, UK, in October 2020.

### *Determination of menstrual cups to study*

With no peer-reviewed or scientific information available, and being accessible to the general public, website MenstrualCupReviews[19] was used as a reference to identify which menstrual cups to study. With almost 200 menstrual

**Table 2.** Menstrual cup shape category definitions.

| Shape category | Example/Visual | Definition |
|---|---|---|
| V-shape |  | Menstrual cup vessel tapers in gradually from the rim to the stem, with the rim as the widest part of the menstrual cup. The vessel is longer than it is wide. |
| Bell-shape |  | A rounder vessel that may flare up at the rim, with bell-shaped curves. The vessel is longer than it is wide. |
| Round-shape |  | A more spherical-shaped vessel, the vessel is wider than it is long, with the widest point of the vessel being below the rim. |
| Asymmetrical-shape |  | Any asymmetrical menstrual cup. These are designed to sit at a particular rotation and angle under the cervix, with the rim not necessarily being perpendicular to the axis of the vessel. The vessel is longer than it is wide. |

cups listed, it was the largest collection of objective menstrual cup information found online, found searching ('menstrual cup') AND rating OR review on Google. The following cups were chosen: DivaCup, Fair Squared, Femmycycle, Fun Cup, Hello Cup, Lena Cup, Lily Cup, Lunette, Me Luna, Merula, Mooncup/MCUK, Organicup, Sckoon Cup, and The Keeper (see Figure 2). A range of menstrual cups were chosen to span across the general shapes – v-shape, bell-shape, round-shape, and asymmetrical-shape – and across the three materials: silicone, TPE, and natural rubber. See Table 2 for shape category definitions, and Table 3 for the menstrual cup category matrix chosen for this study. A minimum of two menstrual cups in each category were chosen. Being the most prevalent material on the market, it was possible to study silicone menstrual cups from all four shape categories. Being the most prevalent shape on the market, it was possible to study v-shape menstrual cups in the three available materials. The highest-rated menstrual cups within each shape and material category were chosen, confirmed in April 2020. Therefore, within the round-shape category, for example, the menstrual cups are not as highly rated as other shapes.

Several cup manufacturers offer a variety of size options, which often includes two options: one smaller option for women less than the age of 30 years, with no history of pregnancy and childbirth, and a larger option for women above the age of 30 years, or with history of pregnancy and childbirth. However, some companies offer yet a smaller menstrual cup for teenagers or beginners (Me Luna, Organicup, Hello Cup), and some larger still (Me Luna). It was not possible to purchase every menstrual cup available. Therefore, one menstrual cup size was chosen from each manufacturer, advertised to women less than 30 years of age who have not given birth. This category was consistent across most menstrual cup manufacturers, even if the size category names were not consistent (i.e. size Small, size B), as seen in Table 4. There were two exceptions: from Hello Cup, 'one size fits most' size menstrual cup for women 'under 35 years old and/or super sporty' was chosen. From Me Luna, 'Size M mainly used by women of all ages with normal muscles and medium flow' was chosen.

### *Dimensions*

Digital vernier callipers (RS Components) were used for all length measurements: total length, vessel length, stem length, rim width, and maximum diameter. Care was taken to not compress or distort the menstrual cups when


**Figure 2.** Menstrual cups compared in this study.

measuring. It was also necessary to examine a menstrual cup's general size because consumers are unlikely to know the actual volume of menstrual blood loss each month, and where sanitary pads and tampons have absorbency ratings to aid in correct product selection, menstrual cups do not. Consumers may therefore judge menstrual cup's size visually. General size was quantified by a menstrual cup's vessel length × vessel maximum diameter.

### *Volume*

To calculate menstrual cup volume, each menstrual cup was filled with water to the desired volume: volume to holes and total volume. Volume was calculated, with volume = mass/density, and 1 $cm^3$ water weighing 1 g, measured using Kern PCB 1000 g × 0.01 g laboratory balance (Kern).

**Table 3.** Menstrual cup shape and material categories.

| Material | Shape | | | |
|---|---|---|---|---|
| | V-shape | Bell-shape | Round-shape | Asymmetrical-shape |
| Silicone | Lunette<br>DivaCup<br>Mooncup/MCUK<br>Organicup | Sckoon Cup<br>Lena Cup | Merula<br>Femmycycle | Lily Cup<br>Fun Cup |
| TPE | Me Luna<br>Hello Cup | | | |
| Rubber | Fair Squared<br>The Keeper | | | |

TPE: thermoplastic elastomers.

**Table 4.** Menstrual cup comparison: models, dimensions, and volume.

| Name | Size | Material | Shape | Total length (mm) | Vessel length (mm) | Stem length (mm) | Rim width (mm) | Maximum diameter (mm) | General size ($mm^2$) | Volume to holes (mL) | Total volume (mL) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DivaCup | Model 1 | Silicone | V-shape | 66.00 | 56.00 | 10.00 | 42.50 | 42.50 | 2380.00 | 22.16 | 27.81 |
| Fair Squared | Size M | Rubber | V-shape | 65.20 | 48.20 | 17.00 | 40.80 | 40.80 | 1966.56 | 14.26 | 21.13 |
| Femmycycle | Regular | Silicone | Round-shape | 64.00 | 42.00 | 22.00 | 39.00 | 48.70 | 2045.40 | . . .[a] | 27.59[b] |
| Fun Cup | Size A | Silicone | Asymmetrical-shape | 50.00 | 50.00 | . . .[c] | 37.90 | 41.00 | 2050.00 | 19.62 | 26.24 |
| Hello Cup | Size S/M | TPE | V-shape | 60.00 | 41.70 | 18.30 | 41.00 | 41.00 | 1709.70 | 21.00 | 25.44 |
| Lena Cup | Small | Silicone | Bell-shape | 71.00 | 47.50 | 23.50 | 41.00 | 41.00 | 1947.50 | 20.46 | 25.03 |
| Lily Cup | Size A | Silicone | Asymmetrical-shape | 78.00 | 68.00 | 10.00 | . . .[d] | 39.00 | 2652.00 | . . .[a] | 28.15 |
| Lunette | Model 1 | Silicone | V-shape | 72.40 | 49.00 | 23.40 | 41.00 | 41.00 | 2009.00 | 18.57 | 25.06 |
| Me Luna | Classic M[e] | TPE | V-shape | 63.00 | 50.00 | 13.00 | 41.00 | 41.00 | 2050.00 | 18.01 | 23.92 |
| Merula | . . .[f] | Silicone | Round-shape | 73.00 | 40.00 | 33.00 | 40.00 | 46.00 | 1840.00 | . . .[a] | 38.14 |
| Mooncup/MCUK | Size B | Silicone | V-shape | 73.00 | 50.30 | 22.60 | 43.30 | 43.30 | 2177.99 | 17.18 | 24.71 |
| OrganiCup | Size A | Silicone | V-shape | 67.00 | 48.60 | 18.40 | 41.00 | 41.00 | 1992.60 | 20.02 | 28.06 |
| SckoonCup | Size 1 | Silicone | Bell-shape | 70.50 | 45.20 | 25.30 | 40.00 | 40.00 | 1808.00 | 17.81 | 18.88 |
| The Keeper | Size B | Rubber | V-shape | 78.60 | 52.50 | 26.10 | 44.00 | 44.00 | 2310.00 | 13.03 | 24.22 |

*Note.* TPE: thermoplastic elastomers.
[a]no air holes; [b]measured to flipped anti-spill lip; [c]no stem; [d]no rim; [e]with stem; [f]no specific name.

### *Compressive strength*

International Standards for contraceptive diaphragms include methods for regulating general quality and freedom from defects, minimal tensile strength, and compression and twisting resistance.[28] Where these regulate the general safety, quality, and longevity of the product, none of the standards engage with comfort or usability. Menstrual cup mechanical properties could not simply be undertaken by comparing each product's material's shore hardness following testing standards in isolation to its overall design because these findings would not be translatable to real menstrual cup use. Product thickness, material, and overall shape heavily influence its firmness.

Compression testing was undertaken using an Instron Universal Testing System 3367 in compression mode, fitted with a 500 N load cell and using Bluehill 2 software control. The menstrual cup was placed between the centre of the plates and compressed just enough to hold in place at its maximum diameter. The product was compressed at a constant rate of 5 mm/min to 50% (±0.5%) of the menstrual cup's maximum diameter. Each menstrual cup was compressed 5 times, rotating the menstrual cup approximately 30° after each test.

### *Data analysis*

A Shapiro-Wilk test examined whether continuous variables were normally distributed, and a means analysis was used to test homoscedasticity. Spearman's rank-order correlation was undertaken. As sample sizes between the dependent variable groups were not equal, a nonparametric Levene's test was used to test homogeneity of variances.[29] A Kruskal–Wallis test was used when data were not normally distributed but displayed homogeneity of variances shown via a nonparametric Levene's test, or

**Table 5.** Menstrual cup comparison: compressive strength (firmness).

| Name | Compressive load to compress menstrual cup 50% (±0.5%) maximum diameter/N | | | | | |
|---|---|---|---|---|---|---|
| | Compression cycle | | | | | *Mean (SD)* |
| | 1 | 2 | 3 | 4 | 5 | |
| DivaCup | 6.12 | 5.58 | 5.68 | 5.06 | 7.67 | 6.02 (0.89) |
| Fair Squared | 3.38 | 3.35 | 3.49 | 3.34 | 3.37 | 3.39 (0.05) |
| Femmycycle | 12.01 | 11.83 | 11.84 | 11.60 | 11.77 | 11.81 (0.13) |
| Fun Cup | 15.66 | 14.49 | 11.18 | 10.70 | 13.20 | 13.05 (1.89) |
| Hello Cup | 13.28 | 14.38 | 14.06 | 13.12 | 14.74 | 13.92 (0.62) |
| Lena Cup | 10.22 | 10.67 | 10.29 | 10.54 | 10.64 | 10.47 (0.18) |
| Lily Cup | 5.89 | 5.92 | 5.37 | 5.16 | 5.13 | 5.49 (0.35) |
| Lunette | 5.75 | 5.73 | 6.32 | 7.16 | 6.54 | 6.30 (0.53) |
| Me Luna | 9.03 | 8.07 | 8.53 | 12.72 | 7.83 | 9.24 (1.79) |
| Merula | 11.60 | 10.87 | 14.17 | 13.98 | 18.27 | 13.78 (2.59) |
| Mooncup/MCUK | 6.26 | 6.39 | 5.92 | 5.46 | 5.82 | 5.97 (0.33) |
| Organicup | 7.34 | 6.10 | 6.59 | 6.56 | 7.25 | 6.77 (0.46) |
| Sckoon Cup | 8.60 | 8.55 | 8.83 | 8.69 | 8.49 | 8.63 (0.12) |
| The Keeper | 8.20 | 9.18 | 9.04 | 8.94 | 8.83 | 8.84 (0.34) |

Each menstrual cup underwent five separate compression cycles compressing each menstrual cup to 50% (±0.5%) maximum diameter, rotating the menstrual cup approximately 30° after each test; *SD*: standard deviation.

when data was not homoscedastic. Quantitative data analysis was undertaken using IBM SPSS Statistics 26.0 (IBM Corp.). Data collection was undertaken in October 2020, and data analysis was undertaken in December 2020.

Ethical approval was not required for this study as it did not involve human participants, human tissues or data, or animals according to Nottingham Trent University Code of Practice for Research.

## Results

### *Menstrual cup size, shape, material, volume, and compressive strength*

Menstrual cup properties varied greatly across the differing brands, as shown in Tables 4 and 5. Menstrual cup total length ranged from 40.00 mm (Merula) to 78.60 mm (The Keeper; $M=67.98$ mm, $SD=7.26$ mm), and maximum diameter ranged from 39.00 mm (Lily Cup) to 48.70 mm (Femmycycle; $M=42.16$ mm, $SD=2.49$ mm).

Volume to holes varied from 13.03 mL (The Keeper) to 22.16 mL (DivaCup; $M=18.37$ mL, $SD=2.65$ mL). Although volume to holes is a more meaningful value to rate a menstrual cup's volume as it shows a true usable volume, three menstrual cups did not have air holes. Total volume is the only volume comparable across the full range of menstrual cups, and it ranged from 18.88 mL (Sckoon Cup) to 38.14 mL (Merula; $M=26.03$ mL, $SD=4.21$ mL).

Menstrual cup firmness, measured by the compressive load required to compress the menstrual cup to 50% (±0.5%) maximum diameter measured in N (1 N = 1 kg m/s$^2$), varied from 3.39 N (Fair Squared) to 13.92 N (Hello Cup; $M=8.83$, $SD=3.25$). Where Table 5 provides the total compressive load, menstrual cups behave differently when compressed: some menstrual cups compressed smoothly and gradually (DivaCup, Fair Squared, MoonCup/MCUK), whereas others compressed in an irregular fashion as seen in Figure 3 (Fun Cup, Merula). This article proposes that menstrual cup firmness is categorized by the following table to empower consumers to find a suitable menstrual cup (Table 6).

### *General size and volume*

A Spearman's correlation test showed there was no statistically significant correlation between menstrual cup's general size and its volume, $rs(14)=.18$, $p=.53$.

### *Shape and volume*

A Kruskal–Wallis test showed that distribution of volume was not significantly different across menstrual cup shape categories, $\chi^2(3)=6.00$, $p=.11$.

### *Material and compressive strength*

A Kruskal–Wallis test showed that distribution of firmness was not significantly different across menstrual cup material categories, $\chi^2(2)=2.88$, $p=.24$.

### *Shape and compressive strength*

A Kruskal–Wallis test showed that distribution of firmness was not significantly different across menstrual cup shape categories, $\chi^2(3)=3.17$, $p=.37$.

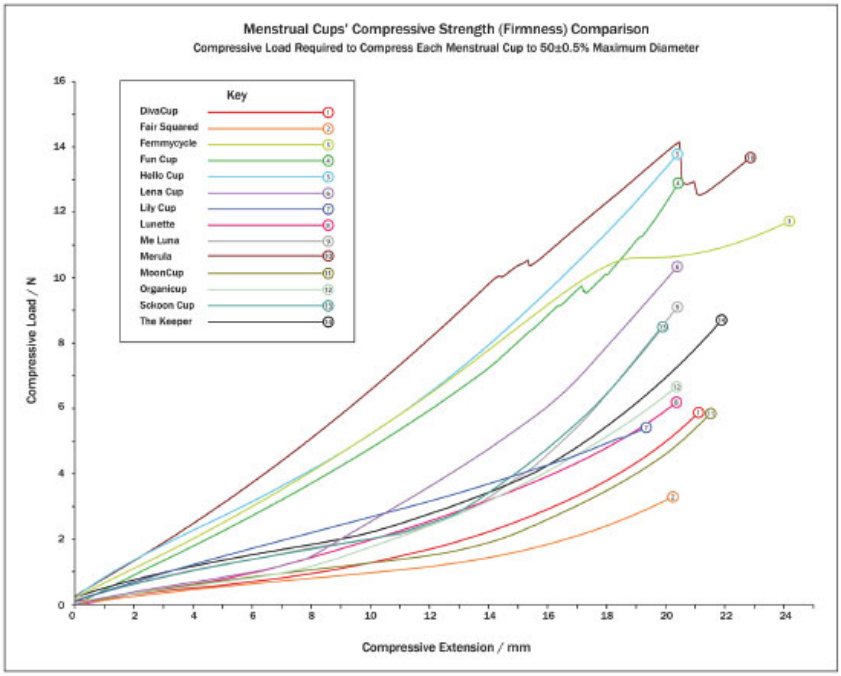


**Figure 3.** Menstrual cups' compressive strength (firmness) comparison.

**Table 6.** Proposed menstrual cup firmness categories.

| Category | Symbol | Compressive load required for 50% (±0.5%) diameter compression (N) | Brand examples |
|---|---|---|---|
| Very soft | ● | $x \leq 5.2$ | Fair Squared |
| Soft | ●● | $5.2 < x \leq 7.4$ | DivaCup, Lily Cup, Lunette, Mooncup/MCUK, Organicup |
| Medium | ●●● | $7.4 < x \leq 9.6$ | Me Luna, Sckoon Cup, The Keeper |
| Firm | ●●●● | $9.6 < x \leq 11.8$ | Lena Cup |
| Very firm | ●●●●● | $11.8 < x \leq 14.0$ | Femmycycle, Fun Cup, Hello Cup, Merula |

## Discussion

Choosing the 14 most popular and highly rated menstrual cups according to MenstrualCupReviews,[19] this study was a starting point to explore how menstrual cups across different brands compare in terms of design, material, and mechanical and physical properties.

In this comparison of 14 menstrual cups, dimensions varied greatly, with total length ranging from 40.0 mm (Merula) to 78.6 mm (The Keeper), and maximum diameter ranging from 39.0 mm (Lily Cup) to 48.7 mm (Femmycycle). Total volume ranged from 18.88 mL (Sckoon Cup) to 38.14 mL (Merula), meaning users will be required to empty the Sckoon Cup around twice as often as the Merula with the same rate of menstrual blood loss. All the menstrual cups tested here were marketed to women less than the age of 30 years having never given birth, and as the range in shape and size in Figure 1 shows, menstrual cup variations across the brands could be confusing. General menstrual cup size does not relate to its volume, so size can be misleading. There should be some guidance or structure for comparing menstrual cup size, which was an aim for this article. However, as there is no correlation between general size and volume, it is unclear whether it would be more suitable to categorize menstrual cups by volume, allowing consumers to judge by their

menstrual blood loss. This might result in a suitable volume not being a suitable shape for the consumer: too wide or too long. Conversely, categorizing menstrual cups by size, allowing consumers to choose based on their vaginal length or cervix height, for example, will not reflect its volume. Similarly, consumers choosing the visually 'largest' menstrual cup thinking that it will meet their heavy menstrual flow may be disappointed. International Standards for contraceptive diaphragms ISO 2014 contraceptive regulate dimensions of vaginally worn contraceptive devices, specifying that size must sit within certain limits, from 55 mm to 100 mm,[28] but this is not applicable to menstrual cups. If menstrual cup size was categorized in a similar way, and this categorization standardized across brands, it would provide transparency for women to make informed decisions. Size and volume categorization remain an unanswered question in this article.

Firmness measured by the compressive load required to compress the menstrual cup to 50% (±0.5%) maximum diameter varied from 3.39 N (Fair Squared) to 13.92 N (Hello Cup). This means one menstrual cup marketed to a nonparous 29-year-old will be too soft and not open inside the vagina, causing leaks, and another would be too firm, may feel uncomfortable, and could potentially cause injury. Material or shape of menstrual cup was not correlated to its firmness, meaning women would not be able to estimate which would be most suitable based on these. On the product packaging, Sckoon Cup advertises its product as 'the softest and most advanced menstrual cup'. However, Sckoon Cup was found to sit within the 'medium' firmness category in this study. This work recommends manufacturers clearly label products with firmness categories, proposed in Table 6. A firmness comparison chart and clarity on packaging would prevent any misconceptions. This work proposes the first recommended categorization of menstrual cups into firmness categories: ranging from, 'very soft', 'soft', 'medium', 'firm', and 'very firm'. In the same way that tampons are categorized in terms of absorbency for ease of use and comfort, as well as reducing risk of toxic shock syndrome,[30] these categories can improve consumers' comfort and safety. If more responsibility lies with manufacturers to display the firmness of the menstrual cups, and practice shifts to educate healthcare practitioners to be able to support women in finding a suitable menstrual cup, women will find greater success and improved safety in using one. There will also be better understanding of the prevalence of injuries such as prolapse as a result of using a menstrual cup, as well as accurate reportings of adverse incidents if healthcare professionals are more engaged in the process.

With more research, a user's age, history of childbirth, body mass index, and level of fitness could indicate which firmness category would be most suitable, removing the element of trial and error, and improving menstrual cup user experience.

### *Strengths*

This study was undertaken in a laboratory setting, offering a high level of control over the environment and variables measured. Along with being replicable, just as the Food and Drug Administration (FDA) compare tampons in a laboratory setting when establishing their absorbency levels,[30] these methods can be undertaken in comparable settings for other researchers and menstrual cup manufacturers to accurately assess menstrual cup properties. This study is the first step to demystifying menstrual cup size, shapes, material, and firmness, with the aims for manufacturers to clearly present this information. This will improve comfort and safety, as the knowledge will help women know which menstrual cup might be well suited. In particular, the quantification of menstrual cup firmness allows women to clearly compare menstrual cups to make informed decisions.

### *Limitations and future study*

Only 14 menstrual cup brands were chosen for this first comparison. Exploring inter-brand menstrual cup variations, particularly varying firmness categories, is missing from this study. By only picking the most popular and highly rated menstrual cup brands, this study is missing the lesser-known or newer brands, and importantly the very inexpensive menstrual cups that have been criticized for being potentially dangerous.[31]

Size and volume categorization remain an unanswered question in this study, and it must be explored in future studies to identify how menstrual cups can be regulated properly. It is recommended future studies categorize all brands in terms of shape, material, size, total volume, firmness, and so on in a single table for easier decision-making by consumers. This is the first objective, quantitative comparison of 14 menstrual cups. The understanding of menstrual cup's physical and mechanical properties is a first step to later identify whether certain menstrual cups hold inherently more risk of injury than others.

Compression testing was limited. Testing was undertaken at five points at room temperature, rotating the menstrual cup approximately 30° after each test. It does not represent the dynamic, three-dimensional compression of the vaginal canal. Where this study is an important initial comparison, future analysis of the mechanical properties of menstrual cups could be undertaken in more a realistic, dynamic setting. The FDA 'Syngyna testing' method could be utilized:[30] a three-dimensional vagina model currently used to hold tampons in place as 'syngyna' fluid is used to measure tampon absorbency. This can also be undertaken at 37 °C.

Van Eijk et al.[2] identified that menstrual cup brands ranged in price from US$0.72 to US$46.72. Menstrual cup cost was not included in this analysis. Product cost will ultimately have an influence on a consumer's decision.

Future analysis should measure whether product cost is associated with either menstrual cup uptake or factors such as material quality, product longevity, or comfort.

It was found that increased support and education in reproductive anatomy might improve menstrual cup use willingness and success.[14,32] This increased support and education could come from medical professionals, but future work should also explore how girls and women without access to medical professionals, including those living in refugee camps and low-income settings, can find a suitable menstrual cup. Future study is needed to show how an improved knowledge on reproductive anatomy could also help women choose which menstrual cup might be more suitable. It would then be important to examine which factors are most significant in terms of safety and acceptability. Future study is needed to explore what women themselves are looking for when choosing a menstrual cup and how they would prefer to be supported in this experience.

## Conclusion

This work is the first objective comparison of 14 of the most popular and highly rated menstrual cups of differing shapes and materials, comparing DivaCup, Fair Squared, Femmycycle, Fun Cup, Hello Cup, Lena Cup, Lily Cup, Lunette, Me Luna, Merula, Mooncup/MCUK, Organicup, Sckoon Cup, and The Keeper, which vary greatly. There is no correlation between a menstrual cup's size, shape, and volume, or a menstrual cup's material, shape, and firmness. Women guess which shape or material might suit them, and if incorrect may experience discomfort, leaks, and increase the risk of injury. More research is clearly needed to further empower women to choose the correct menstrual cup, and improve their regulation from the FDA, the Medicines and Healthcare products Regulatory Agency, and similar regulatory authorities worldwide. Women need more support and guidance from healthcare providers when choosing a menstrual cup to make better decisions in their reproductive life.

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### Contributorship

H.M. conceived and carried out the study, analysed the data, and wrote the article. J.A.H, L.S., and P.B. supervised the project. All authors discussed the results and commented on the article.

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# Menstrual Blood-Derived Mesenchymal Stem Cells: Women's Attitudes, Willingness, and Barriers to Donation of Menstrual Blood

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**Abstract**

***Background:*** Menstrual blood contains mesenchymal stem cells (MenSC), considered a potential ''off-the-shelf'' treatment for a range of diseases and medical conditions. Samples of menstrual blood can be collected painlessly, inexpensively, and as frequently as every month for cell therapy. While there has been considerable previous research into the clinical advantages of MenSC, there is currently little understanding of potential donors' attitudes regarding menstrual blood donation and MenSC.
***Methods:*** One hundred women 18 years of age or over were surveyed to understand attitudes and potential barriers to menstrual blood donation. The questionnaire assessed participant age and brief medical history (giving birth, donating blood, donating stem cells), menstrual experience (period rating, preferred menstrual hygiene products), and whether participants would donate MenSC or accept MenSC therapy.
***Results:*** MenSC was met with a generally positive response, with 78% of menstruating women willing to donate menstrual blood. No significant relationship was recognized between willingness to donate menstrual blood with age, history of childbirth or blood donation, menstruation perception, and preferred menstrual hygiene product. Women rated their period experience better after being made aware of the ability to donate menstrual blood, meaning MenSC therapy can be beneficial for donors as well as patients.
***Conclusions:*** Considering women's attitudes to MenSC and donation of menstrual blood, the future of MenSC therapy is positive; women are generally willing to donate menstrual blood, independent of age, perception of periods, and history of childbirth and blood donation.

**Keywords:** menstrual blood-derived mesenchymal stem cells, donation, human factors, menstrual blood, stem cells, menstruation

## Introduction

Stem cells are cells with the ability for long-term self-renewal and differentiation into multiple specialized cells.[1] Embryonic stem cells are pluripotent stem cells (differentiating into all adult cell types) that would therefore appear to have huge potential for treating a huge number of diseases and conditions. However, embryonic stem cells that come with the ethical dilemma of requiring the destruction of an embryo, are not widely available, and are susceptible to teratoma formation, which is a major health risk for the embryonic stem cell transplant recipients.[2-4] Adult stem cells are found across the body, and are ethically donated with consent from bone marrow, fat tissue, dental pulp, and peripheral blood. However, harvesting these cells require painful, invasive procedures, needing specialist staff and equipment, all at a high cost. Postnatal tissues, such as placental tissue and umbilical cord blood, do not require these painful invasive procedures, and can be donated with consent, but the opportunity to donate these cells does not occur regularly. Also, early clamping and draining of the umbilical cord blood has been found to significantly lower hemoglobin levels in new borns,[5] and also affects the child's neurodevelopment.[6] Subsequently the World Health Organization (WHO) recognizes the dangers of early cord clamping and recommend the cord should not be clamped ''earlier than necessary''.[7]

With our ever-growing, ever-aging population, the hunt for potential treatment of many more diseases and conditions continues, and the need for a safe, cheap, abundant source of stem cells grows.

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*Menstrual blood-derived mesenchymal stem cells*

From menarche to menopause, healthy women undergo a continuous reproductive cycle in constant preparation for conception and childbirth. Single eggs are matured and released, and the uterus prepares for possible conception. If this does not happen, the woman menstruates, and the cycle starts again. Remarkably, this cyclical deterioration, shedding, and regeneration of the uterine lining occurs without the formation of any scarring: each cycle bringing new, perfectly functioning uterine tissue, suggesting stem cell-like activity. This level of controlled tissue remodeling is not found in any other organ in the body,[8] and in 2004 stromal stem cells were first harvested through a hysterectomy.[9]

Even though 37,000 women undergo a hysterectomy each year on the NHS,[10] this invasive, nonreversible procedure has the same disadvantages to the other adult stem cell sources. Also, being required by women with conditions affecting the reproductive system means a transplantation could be detrimental for the recipient.

As the menstrual cycle involves the building and shedding of the endometrium, in 2007 it was correctly postulated that menstruated blood contains mesenchymal stromal cells.[11] Menstrual blood-derived mesenchymal stem cells (MenSC) are not limited in the way embryonic and other adult stem cells are: menstrual blood is a waste blood, shed from every healthy woman of reproductive age. It can be painlessly collected with every menstrual cycle, holding the potential for cell therapy that meets the growing demand on health care.

Researchers are beginning to understand the potential future applications of these cells. In animal models, MenSC have been used in the treatment of lung injury,[12] spinal cord injury,[13] soft tissue damage,[14] and critical limb ischemia,[15] as well as improve cardiac function,[16–18] and treatment of heart failure.[19,20] Peripheral nervous system disorders have been treated,[21] the outcomes of impairments after ischemic stroke have been improved with a transplantation of MenSC,[22] and Alzheimer's disease-like symptoms have also been bettered.[23] Premature ovarian failure,[24] ovary injury as a result of cancer treatment,[25] and a range of cancer types have been treated with MenSC,[26–29] including allogeneic MenSC treatment in mice.[30] Further success has been found in the treatment of liver failure,[31,32] liver injury,[33] including that from liver fibrosis,[34] and liver function has been improved after sepsis.[35] Finally, hyperglycemia was improved in mice with type 1 diabetes.[36] In human models, with further clinical trials underway for patients with type 1 diabetes, lung damage, and liver cirrhosis, women with Asherman's syndrome received successful treatment from autologous MenSC transplants,[37] and four patients with multiple sclerosis received allogeneic transplantations in four separate injections with no adverse effects and no disease progression after a 1-year follow-up,[38] suggesting that future treatment could be sourced from allogeneic MenSC transplants.

With these experiments showing MenSC having the potential to treat a range of diseases and conditions, being described as potential "off-the-shelf" treatments for a range of ailments,[3,20,36] it can certainly be argued that MenSC will be part of the future of medicine. There is an ever-growing understanding of the clinical outputs of MenSC, particularly in comparison to other sources of stem cells, such as bone marrow,[31,39–44] or isolation,[2,44,45] culture,[18,46,47] and differentiation protocols.[14,48] However, minimal work has been done to understand the attitude women (and men) have toward this therapy. Without the support of the public, and women willing to donate menstrual blood samples, there is little sense in continuing research. By speaking to the public, it will be understood whether there would be a positive reception of the potential therapy, and particularly whether the source of the cells will be morally accepted by patients. The collection method should also be discussed, as this will directly influence a woman's willingness to donate menstrual blood; without willing donors and an acceptable donation protocol, there will be insufficient quantities of MenSC for use in research and eventually therapy. With a positive reception from the public, pressure would be put on the science and medical communities to further the research in this field. These attitudes and understanding of the human factors related to the donation of menstrual blood, including the potential barriers of menstrual blood collection, were collected using a questionnaire.

Even though samples from younger donors have faster proliferation rates,[49,50] MenSC have still been successfully derived and used *in* and *ex vivo* from women up to 45 years of age.[48,51,52] Being such a cheap, easily accessible, and readily available source of cells for therapy, it must be argued that it is still worth collecting samples of menstrual blood from all premenopausal women when they can still be used for clinical application. Therefore, it is still important to include women from a broad age range in the survey.

## Materials and Methods

*Participants and recruitment*

Women over the age of 18 were recruited from local universities, offices, and leisure centers to fill in a questionnaire after reading an introduction to the topic with a description of general terms. These locations were chosen to recruit participants from as broad a demographic as possible. All participants were asked to provide informed consent and were provided the opportunity to ask questions. A total number of 170 questionnaires were handed out, with 102 being returned. Two questionnaires were excluded from the study for failing to follow the questionnaire format. No incentive was provided for questionnaire completion. This research was approved by the Nottingham Trent University Ethics Clearance Subcommittee.

*Survey*

The questionnaire consisted of 20 questions with extra space for additional comments. Questions stemmed from five key themes: age and medical history, menstruation perception and experience, knowledge of MenSC, donation of MenSC, and receiving MenSC therapy. The brief medical history included questions such as whether the participant had given birth or donated peripheral blood in the past, and whether she menstruated. In addition to general demographic questions we also gathered information on childbirth and blood donation history. The reason for doing this is to consider the hypotheses that women with history of childbirth and therefore having been in contact with a series of bodily fluids are more comfortable donating menstrual blood. Similarly, women already happy to donate peripheral blood are happy to donate a similar, waste blood. Women who did not menstruate were not asked if they would donate their menstrual blood and the corresponding

Table 1. Participants' Willingness to Donate Menstrual Blood Depending on Age Group, Previous Blood Donation, and Motherhood

| *Study* | *Percentage of participants surveyed* | *Percentage that would donate menstrual blood* | p |
|---|---|---|---|
| Age group | | | 0.125 |
| 18–24 years | 18 | 64 | |
| 25–32 years | 28 | 84 | |
| 33–40 years | 21 | 89 | |
| 41–48 years | 16 | 70 | |
| 49–55 years | 9 | n/a[a] | |
| 56+ years | 8 | n/a[a] | |
| Previous blood donation[b] | | | 0.816 |
| Has previously donated blood | 34 | 76 | |
| Has not previously donated | 66 | 79 | |
| Motherhood | | | 0.520 |
| Has given birth | 36 | 83 | |
| Has not given birth | 64 | 76 | |

[a]Figures not available as participants are no longer able to donate.
[b]Blood donations other than menstrual blood.

questions, and they were directed straight to the last section of the questionnaire.

### *Data analysis*

Data analysis was undertaken for quantitative components. Descriptive statistics were utilized where appropriate. Pearson chi-squared tests, and paired *t*-tests were used. *p*-Values <0.05 were considered significant. Open-ended, free text questions supplemented quantitative data, adding context to participants' quantitative responses.

## Results

### *Age and medical history*

A total of 100 participants completed questionnaires. Only adult women were considered (18+ years). Asking the participants for a brief medical history, 36% of participants had given birth before, 34% had donated blood before, and none of the participants had donated stem cells in the past, including donating umbilical cord blood after giving birth. Nine percent were on contraception that stops a monthly bleed, and 18% of participants did not menstruate due to being menopausal or for other health reasons, such as being severely underweight.

There was no significant relationship between age and willingness of menstruating women to donate, as shown by a Pearson chi-squared test ($X(4) = 7.207$, $p = 0.125$) (Table 1). However, in women 25–32 years of age and 33–40 years of age, 84% and 89%, respectively, were willing to donate their menstrual blood, showing that women of these ages are perhaps most open to the concept. There was also no significant relationship between women having given blood before and being willing to donate, as found with a Pearson chi-squared test ($X(1) = 0.054$, $p = 0.816$), 76% of women who had given blood would donate menstrual blood, compared with 79% of women who had not. The same was found of women who had previously given birth with their willingness to donate ($X(1) = 0.414$, $p = 0.520$), 83% of women who had given birth would donate menstrual blood, whereas 76% of women who had not given birth would donate.

### *Menstruation perception and experience*

Regarding the menstruating participants' perception of their periods, they were asked to rate their experiences of menstruation on a scale 1 to 10, from very negative to very positive, respectively. A Pearson chi-squared test showed there was no significant association between a woman's period rating and willingness to donate ($X(8) = 4.582$, $p = 0.801$). The mean (standard error) period experience score was 4.5 (±0.4), with

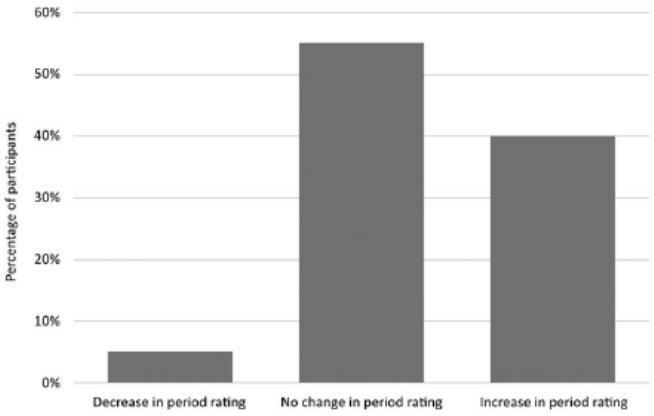


**FIG. 1.** Effect of knowledge of MenSC donation on participants' period experience rating. MenSC, mesenchymal stem cells.

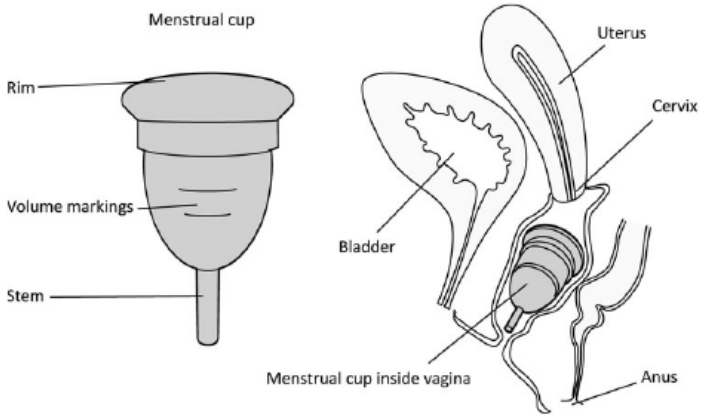


**FIG. 2.** Menstrual cup and its use.



26% of menstruating women rating their opinion on their period as positive (a score of >5), and the remaining 74% rating it as negative or neutral (a score of ≤5). The same question was asked again, but on the pretence that the participants were able to donate MenSC. The period rating increased in 40% of cases and decreased in 5%, with 55% of participants' period rating remaining unchanged (Fig. 1). A paired *t*-test shows a significant increase in participants' rating of their period ($t(69) = -4.524, p = 0.001$) after being made aware about MenSC donation (mean score = 5.6 ± 0.4).

Participants were also asked about their preferred menstrual hygiene products, because menstrual blood samples would be collected in a menstrual cup-style device, which is worn internally in the vaginal canal similarly to a tampon (Fig. 2).

When relating a menstruating woman's preferred menstrual hygiene products with their willingness to donate menstrual blood, a Pearson chi-squared test showed there was no significant association between the hygiene product groups ($X(6) = 2.919, p = 0.819$), with the percentage of menstruating women willing to donate ranging between 69% and 100% (Table 2). There was also no significant relationship between willingness to donate menstrual blood with preferred menstrual hygiene products worn internally, such as tampons or menstrual cups ($X(1) = 0.012, p = 0.914$). Overall, 78% of women who have used an internal menstrual hygiene product were willing to donate menstrual blood, compared with 77% of women who have not.

### *Knowledge of MenSC*

Menstruating participants were asked if they had heard of MenSC before, with 16% answering affirmatively, and of them 73% rated their opinion on MenSC as positive (a score of ≥6, where 1 is very negative and 10 is very positive). The mode score was 10, median 8, and only 3% of participants gave a score below 5 (Fig. 3).

To add context to these scores, open-ended questions of participant thoughts on MenSC discloses themes, including menstrual blood being waste blood, so it being good to put to use, a want to develop treatment, but conversely and negatively that the idea could be "gross," or "messy" (Table 3).

### *Donation of MenSC*

The majority of women were generally willing to donate MenSC; 78% of menstruating participants would donate a

TABLE 2. PARTICIPANTS' WILLINGNESS TO DONATE MENSTRUAL BLOOD DEPENDING ON PREFERRED MENSTRUAL HYGIENE PRODUCT (INTERNAL AND EXTERNAL)

| *Study* | *Percentage of participants surveyed* | *Percentage that would donate menstrual blood* | p |
|---|---|---|---|
| Preferred menstrual hygiene product | | | 0.819 |
| Sanitary towel | 36 | 77 | |
| Tampon | 30 | 75 | |
| Menstrual cup | 3 | 100 | |
| Combination[a]: Tampons and sanitary towels | 22 | 69 | |
| Combination: Menstrual cups and sanitary towels | 5 | 100 | |
| Combination: Tampons and menstrual cups | 4 | 100 | |
| Type of menstrual hygiene product used: | | | 0.914 |
| Internal (*i.e.*, tampon, menstrual cup) | 64 | 78 | |
| External (*i.e.*, sanitary towel) | 36 | 77 | |

[a]Participants that regularly use more than one type of menstrual hygiene product.

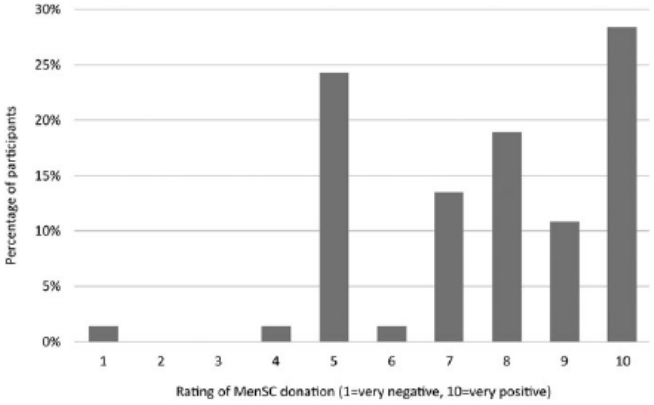


**FIG. 3.** Opinion of MenSC donation, for participants who have knowledge of it.



TABLE 3. ATTITUDES TOWARD MESENCHYMAL STEM CELLS AND MENSTRUAL BLOOD DONATION

| *Initial question and answer* | | *Follow-up question* | *Participant response examples* |
|---|---|---|---|
| Had you heard before today that menstrual blood contains stem cells? | Yes | What thoughts come to mind when you think that menstrual blood could be used in cell therapy? | "Menstruation affects almost 50% of the population, making it a very accessible means of cell research" |
| | | | "I love it. Fantastic to think the blood I have can be used to save people's lives" |
| | No | | "How? What happens to the blood between collection + administration as cell therapy? Also, ew/gross/messy!" |
| | | | "Seems like an excellent use of a waste product but some people might feel icky about it" |
| Would you donate your menstrual blood? | Yes | Explain your answer | "I don't see why you wouldn't when it's just a waste product from your body it might as well be put to good use" |
| | | | "If I felt I could help in any way to improve the lives of others in such a simple way it can only be a good thing" |
| | | | "It seems a very easy donation to make for an important reason" |
| | No | | "Because I would feel disgusted maybe hygiene reasons giving where it comes from, *i.e.*, your vagina" |
| | | | "wouldn't feel confident and necessary" |
| | | | "theoretically I would, but practically I probably wouldn't for health reasons, I can't donate blood, would the same apply here?" |
| Would you use a menstrual cup, or try using one for the first time, to donate your menstrual blood? | Yes | Explain your answer | "I have never used a cup before but I would be willing to give it a go to help someone with stem cells that would help someone else" |
| | | | "I currently use a menstrual cup, I found it empowering" |
| | | | "I've heard people comment positively on this method generally. Why not kill two birds with one stone?" |
| | No | | "have tried cups, but didn't get on with them, tried for environmental reasons" |
| | | | "I want my periods to be as unobtrusive as possible, with minimal fuss or potential for embarrassment. I imagine using a cup is less comfortable, more hassle + less convenient" |
| | | | "menstrual cups are not very hygienic more likely to get toxic shock syndrome" |

Table 4. Receiving Mesenchymal Stem Cells Therapy

| *Initial question and answer* | | *Follow-up question* | *Participant response examples* |
|---|---|---|---|
| Would you accept therapy from a menstrual blood sample if you needed the treatment? | Yes | Explain your answer | "I don't see why using menstrual blood is any different from other blood- it all comes from our bodies!"<br>"I have chronic kidney disease + high blood pressure, so if it would save my life, then yes"<br>"I imagine I would be willing to try anything" |
| | No | | "Undecided would rather not have a blood transfusion"<br>"Would like to know a bit more on how you donate before actually donating, depends if being on the pill affects it, depends if I'm having a bad period… if it's proven to work & be hygienic then yes [would donate]"<br>"Does not feel right. Would have to think about it in more detail" |



sample of menstrual blood for cell therapy. When asked to explain their answer, of the woman willing to donate, key themes included wanting to help people and the development of medicine, menstrual blood being easy to donate, and using a waste substance. Of participants not willing to donate, fears of health affecting whether donation is possible and requiring more information prevented willingness to donate. Confidence and feelings of the donation being unhygienic were also reasons for not donating MenSC (Table 3). A Pearson chi-squared test showed a significant relationship between women willing to donate menstrual blood and those willing to use a menstrual cup to do so ($X(1)=40.846$, $p=0.001$). Overall, 92% of women who would donate menstrual blood would use a cup, and while this is perhaps unsurprising for those who already do so regularly, it is worth noting that 73% would use a cup for the first time to donate. Reasons given for this include having already heard positive things about menstrual cups, and the fact it would be for a beneficial purpose (Table 3). For those that would not be willing to use a menstrual cup to donate, women were adverse due to having already tried it and not got on with it, having the pre-existing thoughts that they would be uncomfortable, and the cups not being very hygienic (Table 3).

### *Receiving MenSC therapy*

All women, whether they menstruated or not, were asked if they would accept cell therapy from MenSC. While 7% of participants did not answer the question, 91% of menstruating participants said they would receive MenSC. Open-ended responses explaining participants' answers include that the source of treatment did not matter, and if help was needed, then any treatment would be welcome (Table 4). As the dependant and independent variables were categorical, a Pearson chi-squared test was used. As expected, the test showed a significant relationship between women who would donate MenSC for therapy and those accepting therapy ($X(1)=7.883$, $p=0.005$), with 98% of women willing to donate MenSC also willing to accept MenSC therapy. Of the women who would not donate their MenSC, 77% would still accept MenSC cell therapy. This would often be due to women having a chronic illness, as women felt they were unfit for donation for health

Table 5. Further Comments

| *Further comments theme* | *Participant response examples* |
|---|---|
| Contraception | "I don't currently have periods (contraception) but would be happy to use a menstrual cup to donate when I do"<br>"If I was to menstruate every month and it could be donated, I would do this" |
| Blood donation | "The only reason I haven't donated blood or anything before is because I am scared of the pain & the process but I don't feel that this would be a problem with menstrual blood donation"<br>"And it's all good for people with a fear of needles" |
| Health | "My contraception is an IUD for treatment of endometriosis. I did not choose to not menstruate, it is a side effect of my treatment. If I could be involved in something like this I would, but would have doubts about whether my hormone imbalance or treatment would affect it."<br>"Would the blood be ok if I'm on levothyroxine?" |
| Taboo | "By donating menstrual blood, women may become more open in discussing their periods, something which I think is really important, rather than it being viewed as a dirty process or something to be embarrassed by"<br>"If menstrual blood was seen as something useful it may dispel some of the taboos around it + make young women more comfortable with the whole idea" |

IUD, intrauterine device.

reasons but would appreciate treatment. Others would feel uncomfortable donating but would still accept MenSC treatment if they were ill (Table 4). Of the women that would not accept MenSC therapy, women stated the treatment would make them feel uncomfortable, or they choose to not accept blood transfusions for personal reasons.

Further comments revealed several women wanted to reiterate their ill health and the wish to donate or receive MenSC if they were able to do it. Women who were currently on contraception that prevented menstruation stated they would donate when they stopped taking it. Donating MenSC was more appealing than donating blood or other stem cells as the donation does not require needles and would be relatively painless. Finally, the menstrual taboo was mentioned, the donation of MenSC being a potential dispelling agent of the stigma surrounding menstruation (Table 5).

### *Limitations*

With little prior research to the topic of menstrual blood donation and attitudes toward MenSC therapy, there has been little consideration of the barriers to acceptance. History of childbirth, whole blood donation, and stem cell donation were used as key variables among age and menstruation to minimize the number of demographic questions on the questionnaire. This was to increase the number of returned questionnaires. There is an unlimited number of potential socioeconomic, cultural, and medical factors that could affect women's attitudes and willingness to donate menstrual blood or MenSC therapy. Therefore, future work can overcome this limitation by exploring other factors.

Revolving around this taboo subject, another limitation of this questionnaire is that data would be taken from women willing to do the survey, which may be women less affected by the menstrual taboo.

Physical questionnaires were distributed from one geographical region. Where participant comfort and wellbeing is paramount, physical questionnaires were less intrusive for the participants and women could choose how much they engaged with the researcher, including asking questions. Where this could limit the range of demographics within the sample, there is no reason to believe the sample is nonrepresentative of the population.

## Discussion

MenSC undoubtedly is part of the future of cell therapy,[3,14,25,47,53] becoming more attractive than other stem cell banks, such as umbilical cord blood.[54] With this in mind, it is important that donor acceptance and barriers to uptake be fully considered. Without the acceptance and support from women, there would be no future for MenSC therapy.

As a subject, MenSC therapy and the existence of MenSC does not have a huge public awareness: 16% of women surveyed were aware of MenSC.

With a questionnaire return rate of 60%, this shows that many women are prepared to talk about menstruation, cell therapy, and other sensitive topics. With popular culture beginning to show realistic depictions of menstrual blood, including TV adverts[55] and film,[56] and parity in medicine improving with the increase in awareness and improvement of treatment for conditions such as endometriosis,[57] and urinary incontinence and pelvic organ prolapse,[58] it is possible that the menstrual taboo is beginning to dissipate. However, some women may have avoided participating in the survey due to this menstrual taboo.

This study highlights the overwhelmingly positive attitude the female public has to the donation of MenSC as well as using a menstrual cup to donate the menstrual blood sample, and expressing a positive response to receiving cell therapy from MenSC. Encouragingly, it appears that women of all ages are willing to donate menstrual blood, with no difference being made as to whether they had given birth. This means efforts to encourage women to donate their menstrual blood for MenSC therapy will not have to be directed to a particular demographic of women, and a large pool of women will be available for recruitment in preparation for MenSC therapy to become a reality. All ages of women, mothers and nonmothers, women with and without previous donation experience, can be supported and support each other in their MenSC donation.

Although not statistically significant, it could be argued that younger women are slightly less inclined to donate MenSC compared with women 25–40 years of age. This could be due to less knowledge on stem cell therapy, and being generally fitter and healthier: health care not being on their minds. Educating the younger audience could be all it takes to maximize the number of women willing to donate MenSC. Data tailed off for women 41 years of age onward because biologically they were less likely to still be menstruating.

Although whether or not a woman had donated blood previously had no significant impact on her willingness to donate MenSC, several women had a fear of needles, preventing them from donating blood. Therefore, donating MenSC would be more appealing to these women, so the lack of needles could be a persuading factor utilized when recruiting potential donors.

There is no significant relationship between how a woman chooses to deal with her period (her preferred menstrual hygiene products), and her willingness to donate menstrual blood. This is a useful finding, as adverts and campaigns do not need to be targeted to women who, for example, are extremely ecoconscious and only ever use reusable menstrual hygiene products. Saying this, women who already have experience using a menstrual cup are already happy to donate menstrual blood in this way. For these women, donating MenSC would require minimal change to their monthly routine; they would not need to learn how to use a new product, which can take a multiple cycles' practice, and would be available to donate virtually immediately. However, with such a large percentage of women so willing to donate their menstrual blood they would be prepared to use an entirely new menstrual hygiene product, there is extremely promising future for the donation of menstrual blood with a menstrual cup. With the knowledge that many women could be suddenly learning how to use a new product, it is important to understand how best to support this choice. A small preliminary study showed that for women learning to use a menstrual cup for the first time, the more support and guidance she received, the better her experience, and faster the learning curve.[59] Therefore, for women who have never used a menstrual cup before, a more positive experience and consequently possibly higher returning rate can be achieved by being given a menstrual cup and supported through first use. As several women surveyed also had incorrect preexisting

ideas surrounding menstrual cups, education on the subject, their safety, comfort, and ease of use must be highlighted for future donors.

No participants had previously donated stem cells, sourced from bone marrow, umbilical cord blood, or any other source. Therefore, no conclusion could be drawn from a woman's likeliness to donate had she previously donated stem cells.

Finally, no significant association was found between women's perception of menstruation, and their willingness to donate: women who thought their periods were "terrible'' as well as women who rated their period maximally were similarly willing to donate their MenSC. Therefore, with the aim of recruiting donors of MenSC, there is no need to find women, for example, who find their periods a joyous occasion, or those who never have period cramps. Interestingly, it was found that women's rating of their period improved in 40% of respondents after being able to donate MenSC: participant comments suggest it was the idea of helping someone in need, or using a waste blood that would otherwise be thrown away that increased this experience rating, and the feelings of shame associated with menstruation being reduced. If donating MenSC can be a benefit for society as well as help individual women feel more positive about a natural bodily process, then the donation of MenSC is something to be celebrated. This must be highlighted to women when recruiting donors.

### *Implications*

The results of this study show there is no need to urge women of specific demographics, including age, childbirth, and blood donation to donate menstrual blood, as these factors have no significant impact on women's willingness to donate. Other factors such as an increase in education, possibly in schools, could mean that more women are aware of donation and sample collection safety and hygiene. Support given to donating women could also lead to a positive donation experience and an improvement in their perception of menstruation, overcoming the menstrual taboo. Further investigation is needed regarding attitudes toward MenSC and donation requirements, to reveal potential barriers to MenSC donation and investigate how menstrual blood could be donated. Collection devices and donation systems can then be optimized for MenSC therapy needs and introduced into current health care systems.

## Conclusions

As the first study of human factors surrounding MenSC and the donation of menstrual blood, there is a generally positive perception of MenSC, with 78% of menstruating women willing to donate and 91% of all women willing to accept MenSC therapy. This support of MenSC is highly positive, as the support from the public and individual future donors of menstrual blood suggests there is potential for MenSC therapy from a human factors' angle. Age, history of childbirth and donating blood, perception of menstruation, and preferred menstrual hygiene product have no significant relationship with a woman's willingness to donate, meaning that all women can be encouraged to donate. The exact donation method should also now be analyzed in terms of women's acceptance and clinical suitability.

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# The Potential for Menstrually-Derived Stem Cell Banking in the UK

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## Abstract

In the UK, there is a growing demand for the NHS to provide cost-effective medical treatment for the ever-increasing, ever-aging population, suffering from chronic non-communicable diseases such as heart disease, diabetes, cancer, and chronic degenerative diseases, such as Alzheimer's disease, Huntington's disease, and multiple sclerosis.

Well-established stem cell treatment includes that for blood and immune system diseases and conditions, such as treating leukaemia with a bone marrow transplant. Skin grafts are grown from stem cells for severe burns cases; and cornea damage (surface of the eye) can be repaired with stem cells. However, stem cell treatment is currently limited by the painful, invasive, and expensive harvesting procedures required.

Stem cells have been found in menstrual blood. Harvesting menstrually-derived stem cells does not require an invasive procedure, can be donated monthly, and can be collected within the donor's home using a menstrual cup. This new source of stem cells could lead to greater accessibility to stem cell therapy and increase the rate of stem cell therapy research.

This paper explores the potential for a menstrually-derived banking system in the UK from a *scientific* and *human factor* standpoint. The scientific community views menstrually-derived stem cells as having potential for application in stem cell therapy. However, the potential for menstrually-derived banking in the UK is driven by the willingness for women to donate their menstrual blood: without the support from women, the entire system is void. The study looks to explore women's initial thoughts, concerns, and inclination to donate, in addition to their first experiences with a menstrual cup, and how guidance and support throughout the process affects this experience. With potential from a *scientific* and *human factor* perspective, the scientific and medical community can anticipate and prepare for the potential for banking menstrually-derived stem cells in the UK.

## Note

This paper discusses topics that may be offensive to some readers, frankly discussing the collection and use of menstrual blood, and vaginas. This paper does not set out to offend, but positively contribute to the field of science and medicine for the treatment of a number of diseases and conditions.

**Key Words**: Menstrually-derived stem cell; Stem cell banking; Stem cells

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## 1. Introduction

The aim of this paper is to determine the potential for menstrually-derived stem cell banking in the UK from a scientific and human factors standpoint.

Stem cells are cells capable of unlimited self- renewal with the capacity to differentiate into a range of cell types. These cells can therefore be harvested and transplanted into a patient that requires a specific cell type. For example, if a patient is suffering with leukaemia, a stem cell transplant from either the patient or a close tissue match could save the patient's life by replacing deficient white blood cells. Stem cells are typically sourced from human embryos, raising the ethical dilemma related to the destruction of an embryo, but they are also available in adult cells. They can be donated with consent. Whilst adult stem cells are ethically sourced, cost and practicalities are detrimental to their use as a sustainable source of cells. Extraction can cause pain to the patient, and the procedure may take days of recovery. Specialised staff and equipment may be necessary to undergo the harvesting procedure, all at a cost. It is these factors that limit the number of patients that can receive stem cell therapy. The cost of the procedure must be balanced with the benefits and chance of success. Stem cells have been found in menstrual blood. This source of stem cells is pain-free, easy, and cheap to procure, while still sharing the many beneficial characteristic of adult stem cells such as those from bone marrow. Although menstrually-derived stem cells are not effective in curing all conditions, such as failing to completely restore heart function, and not treating all cancer types, the potential for menstrually-derived stem cells to treat a number of diseases and injuries is ever growing. Lung injury, multiple sclerosis, and a number of cancers have been treated by menstrually-derived stem cells, with their medicinal properties transferable to countless other applications. From a scientific standpoint, there is huge potential for the banking of menstrually-derived stem cells in the UK.

Regardless of whether or not women have the ability to donate menstrual blood for stem cell therapy, the perception and experience of donating menstrual blood may have an impact on the true potential of menstrually-derived stem cell banking in the UK. The project aimed to explore women's perception of donating menstrual blood, their experience with the physical donation process using a menstrual cup, and the system to support women during their menstrual blood donation.

The study was undertaken in order to understand the potential for menstrual blood donation from the views of the women who would be donating themselves. General reactions, opinions, and fears of the prospect of donating menstrual blood for use in stem cell therapy were gathered. The use of a menstrual cup was utilised for safe, painless, quick menstrual blood collection. Many women already use a menstrual cup on a regular basis as part of their normal menstrual hygiene routine. In the case for these women, it is understood that the menstrual blood donation process would therefore not require the learning curve and new experience that using a menstrual cup entails. This study was carried out to acquire an understanding of women's experiences when using a menstrual cup for the first time. It also aimed to establish how a range of initial information, instruction, and advice would

affect the positivity and success of a woman's overall first-time experience of a menstrual cup (success being how informed one felt, affecting the confidence of the first time use, and ultimately how comfortable the first series of insertions and removals would therefore be).

An understanding of the potential for menstrually-derived stem cell banking in the UK will provide a clear, more realistic vision of the future of stem cell therapy. Possible problems can be anticipated and the scientific and medical community can prepare for the potential for banking menstrually-derived stem cells.

## Literature Review

The literature review looked to scientific and medical papers to explore the potential for banking menstrually-derived stem cells, especially to review whether human factors had been considered. It was discovered that there was minimal literature on this topic.

### Stem Cells

Stem cells describe undifferentiated cells with the capacity for unlimited self-renewal under the correct conditions, and the potential to differentiate into a range of specialised cell types; progenitors, precursor and fully committed cells. Although stem cells are typically thought to be isolated from embryos, adult stem cells are also present in most adult tissues (Allickson et al. 2011; Gargett and Masuda 2010; Ryan et al. 2005). These stem cells are from stromal (connective) tissue sources, including bone marrow, umbilical cord, adipose (fat) tissue, molar cells, amniotic fluid, and peripheral blood and are described as mesenchymal stem cells. Source type impacts stem cell potency (the range of cell types a stem cell can differentiate into). Embryonic stem cells are pluripotent, having the capacity to differentiate into all adult cell lineages in the human body. With the capacity to differentiate into several cell lineages, including cartilage, bone, muscle, tendon, ligament, and adipose tissue, mesenchymal stem cells are generally categorised as multipotent (Gargett and Masuda 2010; Kern et al. 2006; Mehrabani et al. 2016; Ryan et al. 2005). However, studies have found bone marrow stem cells can differentiate into neural cells, proving mesenchymal stem cells have the capability to differentiate into lineages "other than the tissue of origin", suggesting these stem cells have pluripotent characteristics (Jiang et al. 2002, p.41). Due to their self-renewal and flexible differentiation potential, stem cells are already used in stem cell therapy, with huge promise for further repair and regeneration of damaged tissue, both allogeneically (host and donor cells are of the same species) and autologously (host and donor are the same individual) (Meng et al. 2007; Zhong et al. 2009).

The development of stem cell therapy is underway, but with the planet's aging population bringing ever more degenerative disease, and the moral imperative to improve success rates for the stem cell therapy that is currently only offered to a small fraction of the population, there is a demand for stem cell therapy to be improved (Daley 2012; Daley and Scadden 2008; Holm 2002; McKay 2000). The author posits that the chosen source for therapeutic stem cells is the key for the development of stem cell therapy. Being pluripotent, embryonic stem cells have a superior potency to mesenchymal stem

cells, but are related to the ethical dilemma of the destruction of an embryo. They can also cause the formation of teretomas (tumours) (Erdö et al. 2003; Amariglio et al. 2009; Hentze et al. 2009). Mesenchymal stem cells are sourced from bone marrow, umbilical cord, adipose tissue, molar cells, amniotic fluid, and peripheral blood. They can be donated with consent, and allow autologous treatment for specific needs, so therefore are not banked. However, they all have retrieval disadvantages. Bone marrow, adipose tissue, molar cells, and peripheral blood-derived stem cells all require procedures with varying degrees of invasiveness and pain. Stem cells from umbilical cord blood and amniotic fluid are not painful to procure, but are only obtained when a woman gives birth, costing the NHS an estimated £70,000 per transplant. Mesenchymal stem cells have more recently been discovered in menstrual blood (Meng et al. 2007), without the disadvantages of pain and high cost, as mentioned with other stem cell sources.

### Menstrually-derived Stem Cells

Menstrually-derived stem cells exhibit many characteristics similarly to those of mesenchymal stem cells from other sources (Alcayaga-Miranda et al. 2015a; Gargett and Masuda 2010; Mehrabani et al. 2016). They have been proven to show a strong ability to travel to the injured area (Alcayaga-Miranda et al. 2015a; Lopez-Verrilli et al. 2016; Luz-Crawford et al. 2016; Xiang et al. 2017), with a higher expansion, proliferation and survival rate than bone marrow-derived stem cells (Alcayaga-Miranda et al. 2015a; Lopez-Verrilli et al. 2016; Luz-Crawford et al. 2016; Meng et al. 2007; Nikoo et al. 2012). -n addition they show a higher number of early progenitor colonies - capable of differentiating into white blood cells, red blood cells, and platelets (Alcayaga-Miranda et al. 2015a). Menstrually-derived stem cells have been found to have superior longevity. For example, Allickson et al (2011) discovered they were passaged up to 47 times before dying. Sharing characteristics with other mesenchymal stem cell sources, menstrually-derived stem cells are often described as *multipotent* (Alcayaga-Miranda et al. 2015b; Allickson et al. 2011; Wu et al. 2014). However, menstrually-derived stem cells have the ability to differentiate into all three germ lines (the endoderm (interior stomach lining, gastrointestinal tract, lungs), the mesoderm (muscle, blood, bone), and the ectoderm (skin and nervous system), including lung epithelial cells, and cardiomyocyte (heart muscle), without chromosome mutation (Hida et al. 2008; Meng et al. 2007; Xiang et al. 2017). This reflects the characteristics of *pluripotent* stem cells (Borlongan et al. 2010; Khoury et al. 2014; Meng et al. 2007; Xiang et al. 2017; Zhong et al. 2009). Menstrually-derived stem cells have been successful in treating a wide variety of conditions. These include: acute lung injury (Xiang et al. 2017): type diabetes mellitus (Wu et al. 2014); stroke (Borlongan et al. 2010); multiple sclerosis (Zhong et al. 2009;, some cancers (Alcayaga-Miranda et al. 2016); sepsis (Alcayaga-Miranda et al. 2015b); and critical limb ischemia (Murphy et al. 2008) As well as rebuilding heart tissue (Hida et al. 2008) and beneficially affecting neuronal outgrowth for treatment of neurological diseases (Lopez-Verrilli et al. 2016).

These cells do have their disadvantages: injecting menstrually-derived stem cells to the heart only had a limited effect when restoring function (Hida et al. 2008). In another experiment, the cells were

unsuccessful in reducing the progression of arthritis (Luz-Crawford et al. 2016). Although some cancers were treatable with menstrually-derived stem cells, not all cancers were suppressed by the treatment, including pancreatic cancer (Alcayaga-Miranda et al. 2016). In one experiment, menstrually-derived stem cells were incapable of differentiating into blood cells (Xiang et al. 2017). Specific to menstrually-derived stem cell collection, questions must be answered as to whether age, hormonal status (pre- or post-puberty or pre- or post-menopause), and contraceptive use affects the stem cells in menstrual blood (Khoury et al. 2014). And as with all stem cell therapy, multiple risks including adverse immune responses and tumour growth are associated with menstrually-derived stem cell therapy (Lopez-Verrilli et al. 2016).

### Collection of menstrually derived stem cells

Menstrual blood for menstrually-derived stem cell collection is almost exclusively collected using menstrual cups: small medical-grade silicon cups that sit in the vagina to collect, rather than absorb, menstrual blood (Fig. 1). The menstrual blood can then be added to buffering saline and antibiotics, and posted to an NHS laboratory alongside necessary consent forms and sample information, to be processed for culture (Fig. 2). Woman across the UK already use menstrual cups, and as with tampons, they are simple to use, requiring no aid from a medical practitioner to insert or remove. Because of this, the author posits that the menstrual blood donation process would require no trip to a medical practice or designated centre for women to insert or remove their menstrual cup, and therefore could be collected from home.

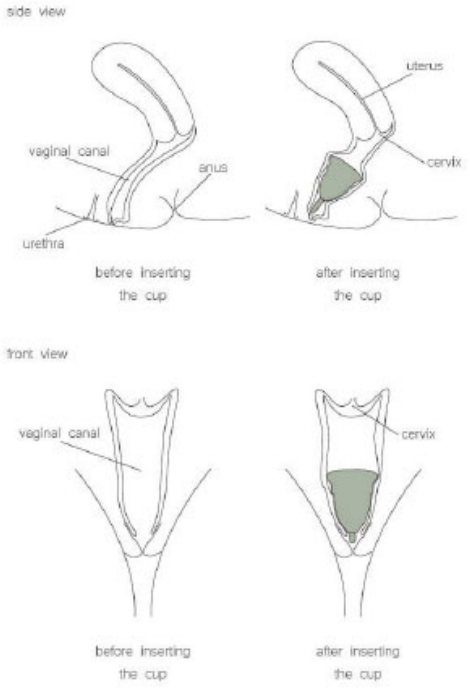


***Figure 1***: Menstrual blood collection with a menstrual cup

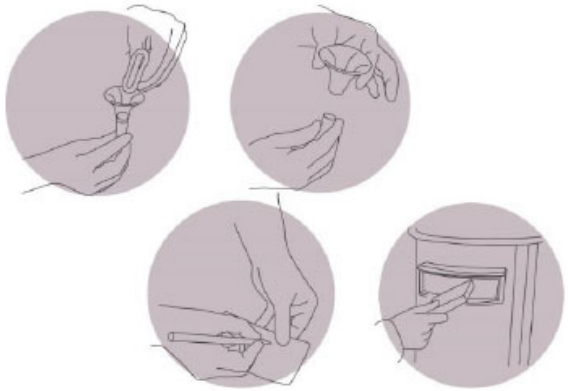

***Figure 2***: Menstrual blood donation process

## Research methodology

To ascertain women's views of using the menstrual cup for the first time, volunteers where sought to try out the cups. They were asked to record their experience in writing. Semi-structured interviews were also held to gather qualitative data regarding the experience and the potential of them donating menstrual blood. The findings from the study were supplemented with six focus groups to receive a better understanding of the perception of menstrual blood and the theme of donating menstrually-derived stem cells from a broader range of women. The study received ethical approval from the Nottingham Trent University School of Architecture, Design, and the Built Environment Ethical Clearance Sub-Committee.

### Research activity

After receiving their menstrual cups, the women were asked to provide feedback on the instructions regarding the text, images used, general content, and how easily they could be followed. This was achieved verbally through informal, semi- structured interviews and asking the participants to annotate the instructions provided with single words, phrases, or images. Having participants critique the instructions directly onto the paper would mean there would be absolutely no misinterpretation as to where the instructions worked well or otherwise, and also meant the participants could analyse the instructions in real time, and make notes as their thoughts and ideas were fresh, providing valid data. These instructions could also be easily passed on to the author and analysed with no misconception. Verbal feedback was provided regarding general experiences and emotions while using the cup for the first time in the form of informal, semi- structured interviews. These interviews would be relaxed in order for the participants to feel comfortable while discussing intimate topics, and participant-led. When conversations dropped, the author asked some questions in order to prompt participants (for example: "how did you find seeing the menstrual blood in the cup?", "did you spill any menstrual blood?", and "did you find you could open the menstrual cup easily or did you struggle to open the menstrual cup?"). The conversation was mostly participant-led, as the author understood each participant would have their own thoughts and emotions that the author could not anticipate. The participant would also feel the conversation was more natural-feeling and therefore share their thoughts more openly. Because using a menstrual cup for the first time would generally be a five to seven day experience, or even a two to three month experience, the participants were also encouraged to contact the author with further thoughts, ideas, and feedback in the days, weeks, and even months following the initial first menstrual cup use. This was achieved with personal messaging, as this was convenient, quick, informal, and comfortable method of communication that could be done on the go, or even as the participant inserted or removed the menstrual cup. This would again feel like natural communication, the participants would feel comfortable, and share their opinions and experiences openly, leading to more valid data.

To explore the effect of differing levels of support and instruction on how easy and comfortable the participants found first-time menstrual cup use, varying levels of support and instruction had to be designed (shown in Table 1). One group of participants simply had the standard instructions provided

with the menstrual cup. This was the group with *minimal* instruction and advice. Another group of participants had access to the standard menstrual cup instructions, and a menstrual cup diary: a first-hand, honest record of a woman's first two menstrual cycles using the menstrual cup, with photographs of the used menstrual cups, and anecdotal advice. This was the group with *medial* instruction and advice. The final group of participants had access to the standard menstrual cup instructions, the menstrual cup diary, face-to-face conversation and instruction on using a menstrual cup with another woman confident in using menstrual cups, and finally the opportunity to receive advice in real time during the menstrual cup use from the same woman (this woman is referred to as "the menstrual cup user"). This was the group with *maximal* instruction and advice. How the Participants were split into the support groups is shown in Table 1.

| Participant | Support Group | Support |
|---|---|---|
| Participant 1 | Medial | Menstrual cup instructions; menstrual cup diary |
| Participant 2 | Maximal | Menstrual cup instructions; menstrual cup diary; face-to-face support from menstrual cup user |
| Participant 3 | Maximal | Menstrual cup instructions; menstrual cup diary; face-to-face support from menstrual cup user |
| Participant 4 | Medial | Menstrual cup instructions; menstrual cup diary |
| Participant 5 | Minimal | Menstrual cup instructions |
| Participant 6 | Minimal | Menstrual cup instructions |
| Participant 7 | Medial | Menstrual cup instructions; menstrual cup diary |

***Table 1:*** Participants, Instructions and Support Groups

### Focus groups

The findings from the research activity were supplemented with six focus groups to receive a better understanding of the perception of menstrual blood and the theme of donating menstrually-derived stem cells from a broader range of women.

### Participants

The participants selected for the study were all friends and family of the author. This was due to the sensitive nature of the study. Having a close relationship to the participants broke down the barriers when talking about menstruation and vaginas on a very personal level. All the participants invited to the study accepted.

Seven female participants from well-educated backgrounds (students/working professionals) were invited to use a menstrual cup for the first time. They were aged between 18 and 23 years of age, and

had all never given birth. Informed consent was received by each participant, after fully understanding the nature of the study. The author kept the participants anonymous. The participants understood their feedback would be kept confidential, with the study abiding to the Data Protection Act (1998). The physical annotated instructions were collected for analysis by the author, and notes were made throughout any interviews with the consent of all the participants. The focus group participants were aged between 18 and 70 years old.

## Data Analysis

The data was analysed by the author, and the study findings were produced using abductive reasoning to form the hypothesis that the better informed and supported a woman was throughout the menstrual blood donation process, the better her experience and the more successful the process. Themes including understanding the instructions, discomfort, and mess were anticipated by the author. However, the informal, open quality of the interviews meant that there were themes that the author had not foreseen that were explored in this study, including: approaching other first time users and the education of female anatomy. The raw data from each participant was cross referenced to the others to find links between varying menstrual cup experiences. On all counts, the participants responded calmly, confidently, and naturally, giving the author no reason to assume the participants were not telling the truth.

## Results

All the participants had some form of initial understanding of menstrual cups and their use. They all understood the general principles around the menstrual blood collection, opposed to absorption, but not all the participants understood how actual insertion and removal took place. All the participants were happy to try the cup, with a couple of participants expressing hope: "*hoping* [it] *will feel better than using pads*" (Participant 5); "*hopeful it would work*" (Participant 6). All the participants had initial concerns, including the menstrual cup's "*comfort*" (Participant 5), and "*it would leak*" (Participant 6).

| Participant | Support Group | Attempts required to comfortably insert/remove menstrual cup | Comments on experience |
|---|---|---|---|
| **Participant 2** | **Maximal** | **1** | **"easy", "fully confident"** |
| Participant 3 | Maximal | 1 | "easy", "fine" |
| Participant 1 | Medial | 2 | "minimal discomfort", "quickly improved" |
| Participant 4 | Medial | 3 | "comfortable when fully sealed", "got easier" |
| Participant 7 | Medial | 2 | "okay", "uncomfortable at first" |
| Participant 5 | Minimal | 1 day of attempts | "scared", "painful" |
| Participant 6 | Minimal | 2 months of attempts | "discomfort", "leakages" |

***Table 2:*** Participants' menstrual cup experience and number of attempts required to comfortably insert/remove menstrual cup

The standard menstrual cup instructions had flaws: Almost all of the participants recognised improvements that could be made to the instructions. The single participant to think otherwise stated: "I read through all of the instructions on the pamphlet first, but I knew what to do anyway from speaking to [the menstrual cup user]" (Participant 2 – Maximal group). All of the participants agreed that the standard instructions, both text and images, were too small. Participant 2 mentioned, "*This* [image of menstrual cup inside vagina] *should be bigger! Cause less panic*". When trying to learn how to trim the menstrual cup to fit, Participant 4 (Medial Group) stated, "[the information was] *easy to understand but* [the image] *difficult to see how much to leave*". This statement highlights the importance of clear, large images, leaving nothing for women to guess: when it comes to trimming the menstrual cup stem, making an error can leave the cup difficult and in some cases painful to remove. The content of the instructions was complimented, however, by Participants 1, and 3 to 7.. Highlighting different sections of the leaflet, content was described as "*good*" (Participant 5 – Minimal group)), "*massively useful*" (Participant 4 – Medial group), "*friendly but not too colloquial*" (Participant 1 – Medial group), "*helpful*" (Participant 3 – Maximal group) and "*clear*" (Participant 6 – Minimal group).

When it came to fitting and trimming the menstrual cup, it was met with varying levels of understanding. Participant 1 cut the entire stem off after feeling it protrude out the vagina on first insertion, but feared she "*prematurely snipped the stem*". It was the correct fit, but Participant 1 is an example of overconfidence and was the only one rushing into the trimming. Participants 2-4, and 6-7

all trimmed their stems without risk of over-trimming, and in some instances "*just doing little bits at a time*" (Participant 7 – Medial group) ensured the participant did not over-trim. Gradual trimming helps women not to trim the stem so short as to make it difficult to remove from the vagina. This is an issue of comfort and confidence: the menstrual cup could still be removed were it to have no stem, but the woman would have to reach further inside her vagina with a thumb and finger to pinch the base of the menstrual cup for retrieval. Having a stem at the ideal length gives the woman confidence she can easily pull at the stem to lower the menstrual cup for retrieval, with the comfort of not having the stem protrude painfully from the vaginal opening:. Primarily participants wanted a comfortable experience, however, the author hypothesises that the cost of the menstrual cup also aids in women being wary of over-trimming. The menstrual cup being worth approximately £20 influenced the women to be more cautious to make the correct trim first time.

There was a range of user experiences, especially regarding comfort and confidence, for the participants when using the cup for the first time. Participant 5 (Minimal group) made these comments, regarding the general experience, "*not comfortable*"; "*scared to take out*"; "*leaked twice*". The discomfort felt and cup leaking suggests the cup was not inserted properly. Feeling discomfort signifies the cup was not sat above the pelvic bone in the vagina, where the vaginal walls cradle the cup. The leaking suggests the cup was not opened correctly inside the vagina; either it remained completely folded, or a crease in the silicon prevented a seal from forming. This is either down to the cup being too big, too flexible, or the participant not understanding how the menstrual cup works. If the latter, it is suggested the participant did not receive adequate instruction and advice before and during menstrual cup use. Following a similar theme, Participant 6 (Minimal group) stated, "*I still haven't fully got used to it, I've only been using it for 2 months* (so 2 weeks actual use) *and I've had a few instances of leakages and discomfort but hopefully that will get better with practice*". Participant 6 may also have not received adequate information and advice in order for her first time using a menstrual cup to go smoothly.

Participant 4 (Medial group) stated "*It took me about three attempts to get myself in the correct position and the correct alignment of the cup with enough force to push it all the way in… the more I practised, the easier it* [became]". To a similar effect, Participant 1 (Medial group) explained she had a "menstrual cup revelation" with the third use of the menstrual cup; "I'm consistently removing and placing the cup quickly with minimal discomfort". Participant 7 (Medial group) also inserted the menstrual cup correctly on the first few attempts: "*One time I got it in but I could feel it was folded, the second time I thought it was properly in*". These reports show a fairly quick learning time for all of these slightly more confident menstrual cup users. The faster learning time and better general menstrual cup experience suggests that these women had an adequate amount of information and advice available to them before and during their first menstrual cup use.

Participant 3 (Maximal group) had a very comfortable and problem-free first-time menstrual cup use. She explained, "[I] *just popped* [the menstrual cup] *in for the first time and it was absolutely fine, tried taking it out and putting it back in, equally easy! If anything really anticlimactic*". Participant 2 (Maximal

group) also had a very positive first menstrual cup experience. She stated, "*Well that was much easier than I thought it would be!... I knew what to do anyway from speaking to [*the menstrual cup user*]. I was expecting it to be A LOT harder to get in but it just slipped right in… I think my experience was made easier because I know what my vagina feels like and I am comfortable inserting fingers, tampons etc. inside".*

These positive accounts of first-time menstrual cup use suggest that these participants were knowledgeable, having received an abundance of instruction and advice before using a menstrual cup for the first time. Participant 2 also uncovered an unanticipated theme: that of education and understanding female anatomy. Understanding the vagina tilts backwards towards the base of the spine, rather than directly upwards as illustrated in many tampon instructions, is key to successfully inserting a menstrual cup.

Another example of unforeseen themes being highlighted by participants regarding first time menstrual cup use is that of approaching first-time users: *"Would some women be scared off by there being lots of [preparation] beforehand?... You might want to look at how to make* [it*] clear that it's also super easy and nothing to be scared of!... I had in mind that it would sit in the same place as a tampon, but knowing that you are easily able to touch it definitely made me think of it as less of a big deal!"* (Participant 3).

This indicates a balance is possibly required when approaching women with first time menstrual cup use. Where one amount of advice or information would create the perfect preparation for successful first time use, but too much, and the woman is potentially overwhelmed to the point of not going forward with it. Once having tried using a menstrual cup, all of the participants were extremely willing to donate their menstrual blood. It could be suggested that the use of a menstrual cup is the crux of a woman's decision to donate. The balance to inform but not repel future donors, and make the decision to use a menstrual cup for the first time will be further considered in the discussion.

All the participants bar one discussed how the menstrual cup is better for the environment as it produces no landfill waste, and the majority of participants mentioned the potential to save money on menstrual hygiene products, despite the initial cost of the menstrual cup.

The focus groups covered an age range of women between 18 and 70 years old,. The key themes from the focus groups were that the majority of the participants women react positively to menstrually-derived stem cell donation, and women firstly consider themselves when donating. 49/51 participants responded positively to the idea of menstrually-derived stem cell donation, stating they would consider donating their menstrual blood. The two participants who responded negatively to the theme, mentioning the "ick factor", were aged 18 and approximately 60 years old. Understanding that around 96% the focus group accepted the idea of menstrual blood donation, and those that rejected the idea of donation had an age range of 42 years, age and donation acceptance appear to have no correlation. Generally, the older the women, the longer it took for them to understand the idea of menstrually-derived stem cell donation. When it came to discussing donating menstrual blood, once the women generally understood the stem cell treatment process, they were only concerned with how

the process would directly affect them: how does it feel to use the menstrual cup. Approximately 75% of discussion time revolved around this theme. This mirrors the findings in the study and emphasised the importance of information in the menstrually-derived stem cell donation process.

## Discussion and recommendations

It can be argued that because the author knew the participants it may present bias in the findings. However, the participants not feeling ashamed or embarrassed allowed for honest responses, which in turn can be argued provided valid data.

The participants had a basic and generally positive preconception of menstrual cups, but none of the participants knew of the existence of stem cells in menstrual blood, or the potential for their use in medicine. This knowledge, with the understanding that menstrual cups would be the tool for menstrually-derived stem cell collection, could be extremely influential in the general acceptance of menstrual cups.

A campaign introducing women to the power of menstrually-derived stem cells and their collection and easy donation with the use of menstrual cups could improve the prospect of the UK having a menstrually-derived stem cell bank on the NHS. For those women who already use menstrual cups regularly, this campaign would encourage the donation of blood that they are already collecting. Women with no menstrual cup experience could be offered a menstrual cup for free, with the expectation that they donate their menstrual blood at least once. The free menstrual cup would give women incentive and liberty to give the menstrual cup a try, with no risk of losing money, at a small cost to the NHS in regard to the huge increase in stem cell donations it might receive.

The amount of information, instruction, advice, and support women receive before and during their first menstrual cup uses affected how assured, pain-free, comfortable, and generally positive the experience was. The minimal, medial, and maximal levels of advice and support offered to the first time menstrual cup users were echoed in their reflection of the experience. Those receiving *minimal* support and advice when using a menstrual cup for the first time experienced the most leaks and discomfort, and took up to two months for the users to get used to the cup with two (Participant 5) or "a few" (Participant 6) cases of leakage. These women relied purely on the written instructions provided by the menstrual cup manufacturer. Those receiving *medial* support and advice when using a menstrual cup for the first time took two to three attempts to correctly insert and remove the menstrual cup without discomfort and leakage. This is a vast improvement on the minimal support group. These women received the standard instructions, as well as a copy of a "menstrual cup diary": a personal, detailed, and honest account of a woman's first time menstrual cup use, offering insights and tips, as well as images, presenting the medial support and advice group with realistic expectations of menstrual cup use. Those receiving *maximal* support and advice when using a menstrual cup for the first time were provided with the standard menstrual cup instructions, the "menstrual cup diary", and finally face-to-face verbal advice and instructions, descriptions, and tips for first time menstrual cup use from a regular menstrual cup user. This group was also offered real-time

help with using the menstrual cups; the users could call or message the menstrual cup user, or have the menstrual cup user physically help with the menstrual cup should they have needed or wanted it. This elevated level of support was again reflected in how quickly and confidently the women inserted and removed the menstrual cup for the first time: the women were able to use the menstrual cup confidently and correctly from first use, with no problem with leakage or discomfort. The better informed, advised, and supported a woman was before and during her first time menstrual cup use, the faster, more successful, and more positive the experience was.

Written instruction given to women preparing to donate menstrual blood should be easy to read. All text and imagery must be large enough for women aged 18-55 to read clearly. Written instructions may not be the most appropriate method to learn how to use a menstrual cup. Participant 2 had one of the most positive and successful first time menstrual cup uses, and she was primarily given first-hand advice and instruction verbally: "*I knew what to do… from speaking to* [the menstrual cup user]". Participant 3 also had one of the most successful first time menstrual cups uses, having access to instructions, the menstrual cup diary and verbal guidance and instruction. She described the experience as "*easy*". These findings suggest that offering future menstrual blood donors listed instructions as only a secondary resource, and offering anecdotal diaries and verbal advice (in the form of conversations, audio messages and videos) as primary resources for using a menstrual cup for the first time would provide the best preparation for the donation experience.

One participant rushed into trimming the menstrual cup stem. Itr would be beneficial if the instruction and advice for using the menstrual cup insists the trimming is to be done slowly and gradually. This would prevent the menstrual cup stem being too short, and therefore slightly more uncomfortable to remove. It is proposed by the author that a choice from a range of menstrual cup sizes, determined following an algorithm for a woman's best fit, would be the most appropriate method for most women choosing to donate to have a comfortably-sized menstrual cup for years to come. All the menstrual cups would have the same length stem, to be trimmed down by each individual to their suiting.

The cost of the menstrual cup may influence how careful someone might be trimming the menstrual cup stem. The author proposes that the whole menstrual blood donation process could be designed for women to care for their menstrual cup, and as an extension, experience a positive and successful donation process. It is already advised that women do not share their menstrual cup with anyone for hygiene and health reasons. Women owning their own menstrual cup, having filled in a survey for their "perfect" menstrual cup fit, may increase the pride and excitement a woman will feel for her cup (and therefore the whole menstrual blood donation process). The menstrual cup packaging, storage items, and any online profile branding could be personalised for each donor, providing a sense of ownership, emotional attachment, and motivation to donate. This would in turn provide a higher readiness for donors and an increase in stem cells banked in the UK.

Participant 2 uncovered an unanticipated theme: that of education and understanding female anatomy. Understanding the vagina tilts backwards towards the base of the spine, rather than directly

upwards as illustrated in many tampon instructions, is key to successfully inserting a menstrual cup. The author therefore hypothesises that further research would support a positive correlation between great female anatomical understanding, and not only the correct placement, but comfortable placement of menstrual cups. The author hypothesises that improving a woman's knowledge of the female anatomy would result in a far superior first time menstrual cup experience.

Participant 3 highlighted that the more information provided to the women, the more likely it would be for them to become overwhelmed or repelled by the process. However, as the findings in this paper show, women who are better prepared for menstrual cup use, found the experience easier and more pleasant. A balance is required, between informing women and not repulsing them. The use of autonomy in this menstrual blood donation process, not only opens up the possibility for women to donate their menstrually-derived stem cells for the use in medicine, it also permits women to decide how they wish to proceed. Offering a wide selection of resources to women (videos on how to insert the menstrual cup; r  written accounts of menstrual cup use; or contact with a regular cup user via online messaging), would help make the menstrual blood donation process viable. It is through a range of information and support that the menstrual blood donation process will succeed, and therefore provide the potential for a menstrually-derived stem cell banking system in the UK.

The focus group outcomes indicates that menstrually-derived stem cell banking holds potential for not only younger audiences, but a broad range of women in the UK. Where older women may need more advice, or longer to consider the donation process, there was no evidence to suggest that any age group of women were more or less inclined to donate menstrual blood for stem cell therapy.

### Further Research

This small study identified the potential for menstrually-derived stem cell donation and the importance of adequate guidance and support for the women undergoing the donation process. It is clear that a study with a much larger sample size would provide more valid data, with defined correlations being drawn. Sampling women from a broader age range, including women who have given birth, would be useful to understand whether the menstrual cup donation experience is affected by age or life experience, and whether the donation process learning curve changes with age. It is important to recognise that the participants in this study were well-educated young women, either studying at University, or a young professional with a degree. Continuing this research sampling women from a range of backgrounds and education levels will establish whether this proposed donation system is a system that *any* healthy woman of menstruating age, regardless of background and education level, can access. Therefore exploring the true potential for menstrually-derived stem cell banking.

## Conclusion

This paper set out to discuss the potential for menstrually-derived stem cell banking in the UK. Science and medical papers point toward the characteristics of the pluripotent stem cells sourced from menstrual blood being suitable for treatment of an ever-growing list of conditions and diseases including diabetes, lung injury, stroke, sepsis, and some cancers. From a scientific standpoint, there

holds potential for menstrually-derived stem cells to be utilised in stem cell banking and therapy, employing the use of menstrual cups for the menstrual blood collection. From a human factors' perspective there is potential for menstrually-derived stem cell banking in the UK. The study found the participants to be happy to donate their menstrual blood for stem cell therapy. Regarding first-time menstrual cup use, the better informed, advised, and supported a woman felt before and during her first time menstrual cup use, the more successful and positive the experience was. Anticipating the problems misinformation and inadequate support causes, the scientific and medical community can prepare for the reality of menstrually-derived stem cell banking in the UK.

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