Mesenchymal-like cells isolated from menstrual blood. Comparing sanitary pads, tampons, and menstrual cups for accessible donation

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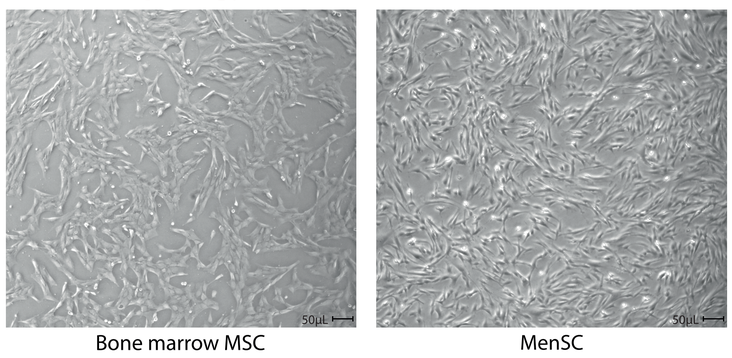
Sanitary pads, **tampons**, and **menstrual cups** can all be used to donate **mesenchymal-like cells** for future regenerative therapy

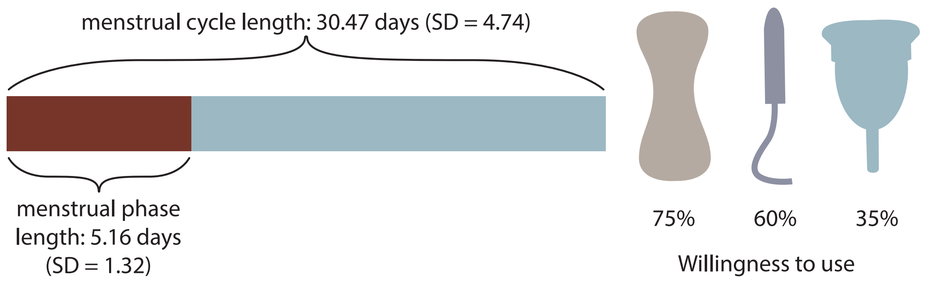
MenSC morphology



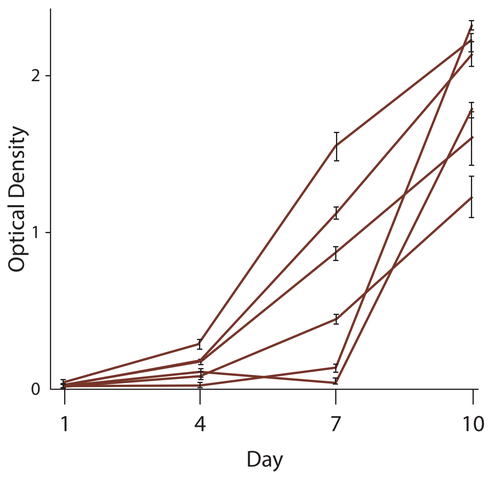
Bone marrow MSC MenSC

Bone marrow MSC and MenSC share similar morphology.

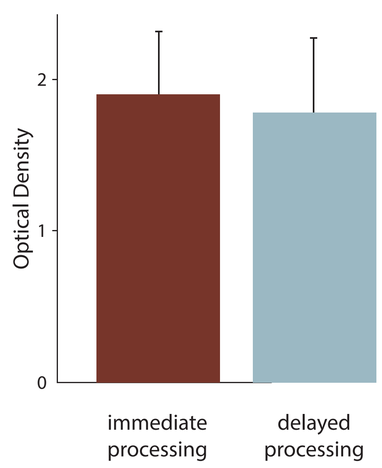
Participant information



MTT



P3 MenSC metabolic activity (n = 6) during 10 day culture.



Comparing metabolic activity (n = 6) of P3 MenSC after immediate vs. delayed processing.

Flow cytometry

	Marker	Expecting	Expression / Mean % (SD)
CD34	Haematopoietic stem cell marker	-	6.94 *(8.14)*
CD38	Haematopoietic stem cell marker	-	97.4 *(3.48)*
CD44	Lymphohaematopoietic, MSC marker	+	96.42 *(2.74)*
CD45	Haematopoietic stem cell marker	-	20.03 *(5.92)*
CD73	MSC marker	+	99.28 *(0.06)*
CD90	MSC marker	+	99.57 *(0.10)*
CD105	MSC marker	+	99.09 *(0.29)*
SSEA-4	Pluripotent embryonic stem cell marker	+/-	20.13 *(22.25)*

Flow cytometry data for P5 MenSC (n = 7). Gated for singlet, live CD45- cells.

Sanitary pad, tampon, and menstrual cup success

	Immediate processing			Delayed processing			Overall product rating
	Isolation success rate	Willingness rate	Combined rating	Isolation success rate	Willingness rate	Combined rating	
pad	40% *n = 10*	75%	0.58	57% *n = 7*	75%	0.66	0.62
tampon	100% *n = 7*	60%	0.80	85% *n = 7*	60%	0.73	0.77
cup	60% *n = 5*	35%	0.48	75% *n = 4*	35%	0.55	0.52

Immediate processing vs. delayed processing rating, calculated with the equation R = (0.5 × S) + (0.5 × 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.

Background
Menstrual blood contains mesenchymal-like cells (MenSC) which are highly proliferative, capable of multilineage differentiation, with strong migratory capacity and immunomodulatory abilities. The cyclicity and non-invasive nature of menstrual blood donation makes MenSC a promising candidate for regenerative medicine.

A common donation method is via a menstrual cup. However, only 4% of the UK currently use one, with most preferring tampons and sanitary pads. Therefore, this study aimed to identify MenSC isolation methods from sanitary pads and tampons to extend the accessibility of MenSC research and future clinical applications.

Methods
In this ongoing study, healthy women aged between 21-41 (n = 22) donated menstrual blood samples (n = 40) on their heaviest flow day, using a sanitary pad (n = 17), tampon (n = 14), or menstrual cup (n = 9).

Isolation success rate and metabolic activity is assessed via 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay, an indicator of cell metabolism and therefore viability. Stemness is confirmed using antibodies and their detection by flow cytometry; trilineage differentiation potential is confirmed using fat, bone and cartilage differentiation. Cell viability and immediate versus delayed (48 h) processing is evaluated using MTT.

Results
MenSC were isolated and cultured from all three products; sanitary pad, tampon, and menstrual cup for the first time, including immediate and delayed processing. Tampons and menstrual cups had a greater MenSC isolation success rate (immediate processing: sanitary pad = 40%, tampon = 60%, menstrual cups = 100% success rate; delayed processing: sanitary pad = 57%, tampon = 85%, menstrual cups = 75% success rate), but sanitary pad are the most popular menstrual blood donation method (SP= 75%, tampon = 60%, and menstrual cups = 35% willingness rate). MenSC displayed high proliferative capacity, a doubling time between 21-89 h.

Markers expressed by the MenSC were evaluated by flow cytometry, and were generally expressed as expected. However, interparticipant expression of SSEA-4 (embryonic stem cell marker) ranged from x - x% positive, and CD73 (haematopoietic stem cell marker) was expressed positively by x participants. Further data collection and analysis ongoing.

MenSC behaved similarly to bone marrow MSC, sharing similar morphology. Confirmation of stemness and trilineage potential are currently ongoing. Participants described the donation as easy and empowering.

Discussion & Conclusions
This research is the first comparison of menstrual blood donation techniques, comparing sanitary pads, tampons, and menstrual cups. Although tampons and menstrual cups had a greater MenSC isolation success rate, sanitary pads are the more popular method among participants and the population, therefore there is value in using all three methods. The ability to temporarily store menstrual blood samples at 4°C makes menstrual blood donation practical. MenSC offer unique advantages including non-invasive, cyclical collection, and are a promising source of cells for regenerative therapies in the future. The ability to donate menstrual blood with sanitary pad and tampons in addition to menstrual cup makes this approach to cell donation even more accessible.