Nano-scale Superhydrophobicity: Suppression of Protein Adsorption and Promotion of Flow-Induced Detachment

Yasemin Koc,[2] Andrew J. de Mello,[2] Glen McHale,[1] Michael I. Newton,[1] Paul Roach[1], Neil J. Shirtcliffe,[1]*

[1]School of Biomedical and Natural Sciences, Nottingham Trent University, Clifton Lane, Nottingham. NG11 8NS. UK

[2]Imperial College London, South Kensington, London SW7 2AZ, U.K

**Corresponding Author: Tel: +44(0)115-8483315 Fax: +44(0)115-8486616*

Email: Neil.shirtcliffe@ntu.ac.uk

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Abstract

Superhydrophobic surfaces are extremely water repellent[1],but proteins have been shown to adhere to them[2]. Here we show that although superhydrophobic surfaces can allow greater protein adsorption under static conditions, fluid flow over a nano-scale superhydrophobic surface can result in clean surfaces. Possible mechanisms that explain such behaviour include decreased contact between protein and surface and greater shear stress due to interfacial slip between the superhydrophobic surface and the liquid[3,4,5].

Keywords: superhydrophobic; Protein Adsorption; Slip; Anti-fouling, Topography

Text

Superhydrophobic surfaces have been explored for various applications including self-cleaning and anti-mist/fog surfaces, power-on-demand batteries and electrostatically controllable liquid optics. The basic correlations between surface roughness and water repellency were originally defined by Wenzel[6] and Cassie and Baxter[7]. In the simplest case, the Wenzel state, liquid conforms to the roughness; increasing its interfacial contact area. In contrast, the Cassie-Baxter state involves the liquid sitting on top of the roughness, giving potentially a lower solid-liquid contact area, (Figure 1a). Surfaces showing low interfacial areas allow water to slide or roll off very easily and are therefore of the most interest. A large number of techniques have been developed to produce different superhydrophobic surfaces for study, a small number have been developed into products.[8,9]

Anti-fouling surfaces that show low protein adsorption are important in many areas, especially for surfaces that cannot be cleaned for extended periods such as boat hulls and some biomedical devices. Biofouling of boat hulls and some pipes considerably increases energy consumption. Protein adsorption is the first stage in biological contamination of surfaces, with cells binding to a pre-adsorbed protein

layer before proliferating and spreading. Surfaces that hinder or obstruct this early adsorption process would reduce cell growth. Another area where protein adsorption is problematic is in enzyme catalysed reactions, where enzyme adsorption reduces the rate of reaction. This is particularly evident as the scale of a reaction environment is reduced and the surface area-to-volume ratio increases. Micro- and nano-fluidic devices, for example, are high-efficiency tools for chemical and biological processing typically consisting of wide, flat channels that maximise interfacial surface area and cross diffusion. Reducing protein adhesion has been approached in several ways in the past, including chemically coating the surfaces[10], filling the surface sites with other molecules[11] and attaching proteolytic enzymes to surfaces[12]. Surfaces that employ flow shear removal are used in a small number of applications, typically fast boats as the shear rate required is high and the material used can easily become damaged[13]. A recent publication highlights the possibilities of superhydrophobic coatings but also shows how little work has been undertaken in this area.[14]

It is understood that proteins which do bind to hydrophobic surfaces have a lower cell and platelet binding ability, possibly due to conformational changes.[15] It has been suggested that superhydrophobic surfaces could reduce the extent of protein adsorption due to the reduction in solid surface area at the liquid interface [16,17,18]. However, protein adsorption has been shown to occur over long time periods on superhydrophobic surfaces, which may indicate a dependence on the relaxation of the protein structure.[19,20] Furthermore, due to the surfactant nature of some proteins, adsorption onto pseudo-porous surfaces may be progressive, with an adsorbed protein layer driving the solvent front into the surface structure.[16, 21]

The current study demonstrates the effect of superhydrophobic surface dimensions and surface chemistry on static protein adsorption and efficacy of protein removal under flow. The hypothesis is that proteins may adhere to superhydrophobic surfaces, but several additional factors may contribute to their effective removal under flow, particularly if micro-metre scale roughness is replaced with nano-metre scale roughness. Interfacial slip between the liquid and solid would cause an increase in liquid flow rate near the surface; adsorbed molecules will experience greater shear forces and are therefore more likely

to be swept away. On nano-scale roughness the curvature of the surface approaches protein molecular dimensions, reducing the contact area unless the protein molecules deform.[22] The copper oxide nano-filaments used here have flat ends that are around 60 nm wide and 10 nm thick (Figure 1d) with possible roughness of a smaller scale[23]; so the thickness is similar to the dimensions of the protein used. In this case the interfacial surface area onto which a protein molecule could adsorb is smaller than the protein and so could greatly reduce its binding strength and resistance to flow shear. A reduction of protein adsorption due to flow shear has been reported on nano-wires[24] and polymer brushes[22,25]. Nano-scale superhydrophobic surfaces remain in the Cassie-Baxter bridging state under much higher hydrostatic pressure compared to micro-structured surfaces. Fluorocarbon terminated superhydrophobic surfaces are additionally relatively resistant to ingress of liquid into the structure under the action of surfactants (such as proteins).

Measurements were performed by adsorbing protein from solution for 1h, samples were then rinsed in buffer and then a portion of the samples were placed in a flow cell and buffer solution flowed over them. The amount of protein remaining adsorbed was then assessed by fluorometric assay after detachment from the surface. This technique has previously been demonstrated to quantify small amounts of protein; considerably less than a monolayer on a surface of a few cm^2.[16] Surfaces with micro- to nano-sized topographic structures were investigated. Rough sol-gel thin films of two different grain and pore sizes[26] and copper hydroxide needle[23] surfaces, having roughness with critical dimensions ca. 4 μm, 800 nm and 10 nm respectively, (Figure 2) were used as model surfaces. All surfaces were subsequently chemically modified to give hydrocarbon or fluorocarbon surface chemistry; giving water contact angles of 169^o for micron and 152^o for nano-rough fluorinated surfaces. Flat copper reference samples coated with either coating showed identical BSA adhesion properties to flat glass samples with the same coatings under both static conditions and after flow, indicating that the copper was not influencing the adsorption process. Details of surface preparation can be found in the electronic supplementary information.

Bovine serum albumin (BSA) was chosen as a model protein since it is known to adhere well to surfaces. Moreover, it is important in various biological applications such as PCR, found in high abundance in serum and is commonly used as a surface blocking agent due to its binding characteristics. This protein is of the order of 15 nm in size, but is known to deform when strongly adhered.[27]

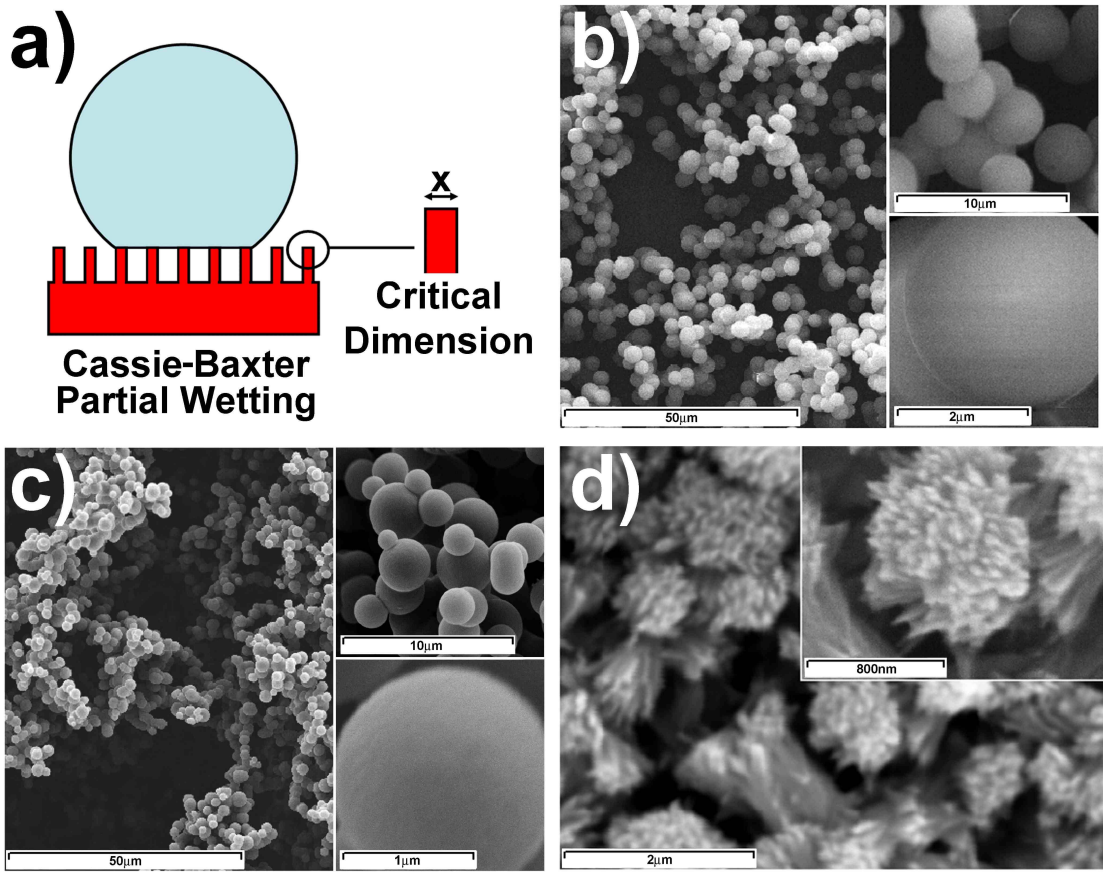

Figure 1: (a) diagram showing Cassie-Baxter superhydrophobicity and the critical dimension used to define surfaces here. Electron micrographs of (b) the larger scale sol-gel material used, (c) the smaller scale one and (d) the copper oxide nano-pillars.

Under static conditions similar amounts of albumin are observed to adsorb to flat glass and the nano-structured copper oxide surfaces with the sol-gel surfaces showing much higher adsorption (Figure 2). The small sized sol-gel surface (~800 nm particle size, ~4 μm pore size) had a lower degree of protein adsorption compared to the larger sized (~4 μm particle, ~20 μm pore) material. This indicates that the pressure in the system combined with the surfactant nature of the protein used, is sufficient to significantly penetrate the structure of the larger pored material, which presents a larger available surface area for adsorption.[16]

Greater adsorption was observed on fluorinated flat glass and copper oxide needle surfaces compared to the corresponding methylated surfaces. This may be due to the greater hydrophobicity of these surfaces[28]. It is possible that the fluorocarbon waterproofing agent generates some small scale roughness and thus increases the area available for adsorption, but results using silane coupling agents were similar (data not shown), which suggests that this result is due to the greater hydrophobicity of these surfaces than hydrocarbon surfaces, increasing the binding strength of hydrophobic interactions between BSA and the surface.

The structured fluorinated surfaces showed considerably lower protein adsorption than the equivalent hydrocarbon terminated surfaces. On fluorocarbon surfaces the increased hydrophobicity will result in a lower interfacial surface area available for protein adsorption. The nano-structured surfaces with both coatings were the most resistant to protein adhesion under static conditions ~12 ng cm^{-2}. It has previously been reported that proteins and peptides are affected by nano-structures of similar size to those used here[27,29,30].

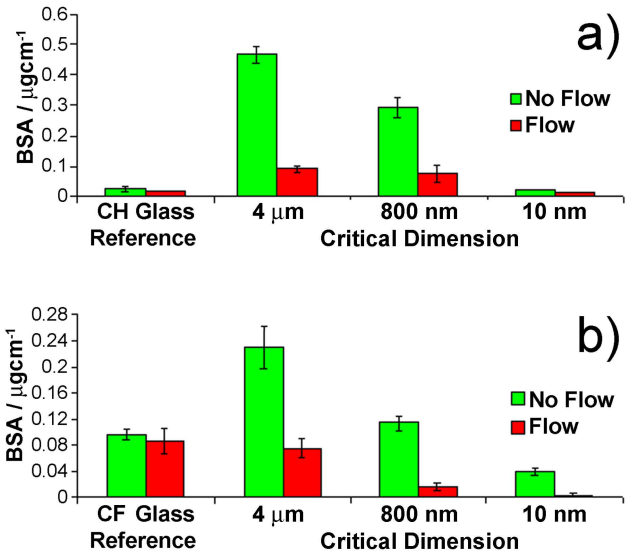

Figure 2: Albumin adsorption onto micro-scale and nano-scale surfaces: (a) hydrocarbon terminated and (b) fluorocarbon terminated under static conditions and after subsequent flow of buffer.

A proportion of the adsorbed protein was removed from all surfaces under flow conditions. Considerably greater amounts of protein, however, were lost from the superhydrophobic sol-gel surfaces than from flat surfaces, with the amount remaining being lower on successively smaller structured surfaces (Figure 2a). This suggests that micro-structures, despite being very large compared to the protein molecules, have a strong effect on protein retention under flow. Interfacial slip, if present, would create high shear-fields around the edges of contact areas, which would induce protein desorption. Our results demonstrate that on fluorocarbon terminated surfaces a higher degree of desorption was found on smaller structured surfaces, where higher shear fields would be expected (Figure 2b). This trend was also generally observed on the hydrocarbon surfaces, although a relatively large proportion of the protein was lost from the larger scale sol-gel surfaces.

The nano-structured copper oxide surfaces showed similar adsorption to flat surfaces under static conditions, but also showed greater losses after exposure to flow. The fluorinated surfaces show slightly lower levels of adsorption than flat surfaces under static conditions, but after flow were clear of protein

within the detection limits of the measurement ~3 ng cm^{-2}. This continues the trends observed on the sol-gel surfaces.

The amount of protein adsorbed onto superhydrophobic surfaces in the absence of flow was similar to or greater than that onto flat reference samples, except for fluorocarbon terminated nano-structured surfaces with critical dimension of ca. 10 nm. However, when buffer was flowed over the sample surfaces, more protein was removed from the superhydrophobic surfaces than flat ones. Fluorinated nano-structured surfaces became almost completely *clear* of protein where equivalent flat surfaces only lost around 10%-20% of their protein. It is not clear from these measurements if the enhanced effect at nano-structured surfaces is due to reduced distance of any point from an area of slipping fluid or to reduced contact area between protein molecule and surface due to the small size of the tips of the nano-pillars and their roughness. Reduced binding strength has previously been reported for BSA on high curvature surfaces[27].

The almost complete removal of protein films from some superhydrophobic surfaces under flow conditions is of significant interest in applications where flow is already present, such as in micro- and nano-fluidics or where flow cleaning is typically used, however, the use of surfactants may significantly reduce the effect observed. Other proteins may be affected to different extents, the size, shape and alignment of protein molecules on the structures will affect how much force the washing liquid can exert as it slips past on the surface. If intrusion into the structure occurs a large increase in adsorption would be expected.

Surfaces that hinder or prevent protein adsorption are sought after for use in many industries including biomedical, optical, electronics and engineering, where devices are prone to contamination. Here we have shown how nano-scale superhydrophobic surfaces can be used, firstly to obstruct adsorption taking place in the absence of fluid flow, but mainly to reduce the amount of adsorbed protein under flow

conditions. The effect demonstrated here is of particular use in micro- / nano-bioreactors, where the surface area to volume ratio favours enzyme loss from solution. We have also shown that larger scale superhydrophobic surfaces can have the opposite effect, causing increased adsorption; which goes some way to explaining the mixed results achieved by other studies.

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1 Furstner, R., Neinhuis, C., Barthlott, W. The lotus effect: Self-purification of microstructured surfaces., *Nachrichten aus der Chemie*, **48(1),** 24–28, (2000).

2 Zhang, H., Lamb, R., Lewis J., Engineering nanoscale roughness on hydrophobic surface - preliminary assessment of fouling behaviour. *Sci. Tech. Adv. Mat.*, **6 (3-4)**, 236–239, (2005).

3 Choi, C. and Kim, C., Large slip of aqueous liquid flow over a nanoengineered superhydrophobic surface., Phys. Rev. Lett, **96 (6),** Art. No. 066001, (2006) and comments on this paper.

4 Truesdell, R., Mammoli, A., Vorobieff, P., van Swol, F., Brinker, C. Drag reduction on a patterned superhydrophobic surface., *Phys. Rev. Lett.*, **97 (4)**, Art. No. 044504, (2006).

5 Ou, J., Rothstein, J.,Direct velocity measurements of the flow past drag-reducing ultrahydrophobic surfaces., *Phys. Fluids*, **17 (10)**: Art. No. 103606, (2005).

6 Wenzel,R., Resistance of solid surfaces to wetting by water. *Ind. Eng. Chem.*, **28**, 988–994 (1936).

7 Cassie, A., Baxter, S., Wettability of porous surfaces., *Trans. Faraday Soc.*, **40**, 546–551 (1944).

8 Ma, M. and Hill, R., Superhydrophobic surfaces. *Curr. Opinion Coll. Surf. Sci.*, **11 (4)**, 193-202, (2006).

9 Callies, M., Quere, D., On water repellency., *Soft Matter*, **1**, 55-61, (2005).

10 Bearinger, J., Terretaz, S., Michel, R., Tirelli, N., Vogel, H., Textor, M., Hubbell, J., Chemisorbed poly(propylene sulphide)-based copolymers resist biomolecular interactions., *Nature Materials*, **2**, 259-264, (2003).

11 Taylor, S., Smith, S., Windle, B., Guiseppi-Elie, A., Impact of surface chemistry and blocking strategies on DNA microarrays., *Nucleic Acids Res.*, **31 (16)**, Art. No. e87, (2003).

12 Asuri, P., Karajanagi, S., Kane, R., Dordick, J., Polymer–Nanotube–Enzyme Composites as Active Antifouling Films., *Small*, **3, 1**, 50–53, (2007).

13 Callow, M., Fletcher R., The influence of low surface energy materials on bioadhesion: a review., *International Biodeterioration and Biodegradation* **34**: 333–348, (1994).

14 Genzer, J., Efimenko, K., Recent developments in superhydrophobic surfaces and their relevance to marine fouling: a review., Biofouling, 22(5), 339–360, (2006).

15 Wu, Y., Simonovsky, F., Ratner, B., Horbett, T., *J. Biomed. Mat. Res. A*, **74A(4)**, 722–738, (2005).

16 Roach, P., Shirtcliffe, N., Farrar, D. Perry, C., Quantification of surface-bound proteins by fluorometric assay: Comparison with quartz crystal microbalance and amido black assay., *J. Phys Chem. B*, **110(41)**, 20572–20579, (2006).

17 Sun, T., Tan, H., Han, D., Fu, Q., Jiang, L., No platelet can adhere-largely improved blood compatibility on nanostructured superhydrophobic surfaces., *Small*, **1(10)**, 959–963, (2005).

18 Chen, P., Patterning proteins and cells using switchable superhydrophobic surfaces. *232nd ACS National Meeting*, **COLL-556**, (2006).

19 Toes G., van Muiswinkel K., van Oeveren W., Suurmeijer A., Timens W., Stokroos I., van den Dungen J., Superhydrophobic modification fails to improve the performance of small diameter expanded polytetrafluoroethylene vascular grafts., *Biomaterials*., **23 (1)**, 255–262, (2002).

20 Zhang H., Lamb R., Lewis J., Engineering nanoscale roughness on hydrophobic surface - preliminary assessment of fouling behaviour., *Sci. Technol. Adv. Mat.*, **6 (3-4)**, 236–239, (2005).

21 Vroman, L., Effect of adsorbed proteins on the wettability of hydrophilic and hydrophobic solids., *Nature*, **196**, 476–477, (1962).

22 de Vasconcelos, C., Bezerril, P., Dantas, T., Pereira, M., Fonseca, J., Adsorption of Bovine Serum Albumin on Template-Polymerized Chitosan/Poly(methacrylic acid) Complexes., *Langmuir*, (2007).

23 Wen, X, Zhang, W. Yang, S, Synthesis of Cu(OH)2 and CuO nanoribbon arrays on a copper surface., *Langmuir*, **19**, 5898–5903, (2003).

24 Ainslie K., Sharma G, Dyer M., Grimes C., Pishko M., Attenuation of protein adsorption on static and oscillating magnetostrictive nanowires., *Nano Lett.*, **5 (9)**, 1852–1856, (2005).

25 Anastassopoulos, D., Spiliopoulos, N., Vradis, A., Toprakcioglu, C., Baker, S., Menelle, A. Shear-Induced Desorption in Polymer Brushes, *Macromolecules*, **39**, 8901, (2006).

26 Shirtcliffe N., Mchale G, Newton M., Perry C., Roach P., Porous materials show superhydrophobic to superhydrophilic switching., *Chem. Comm.*, **25**, 3135–3137, (2005).

27 Roach P., Farrar D., Perry C., Surface tailoring for controlled protein adsorption: Effect of topography at the nanometer scale and chemistry., *J. Am. Chem. Soc.*, **128 (12)**, 3939–3945, (2006).

28 Prime, K., Whitesides, G., Self-assembled organic monolayers:model systems for studying adsorption of proteins at surfaces., *Science*, **252: 5009**, 1164–1167, (1991).

29 Mandal, H and Kraatz, H, Effect of the Surface Curvature on the Secondary Structure of Peptides Adsorbed on Nanoparticles., *J. Am. Chem. Soc.*, **129 (20)**, 6356–6357, (2007).

30 Pallandre, A., De Meersman, B., Blonbdeau, F., Nysten, B., Jonas, A., Tuning the orientation of an antigen by adsorption onto nanostriped templates., *J. Am. Chem Soc.,* **127**, 4320–4325, (2005).