Highlighting joint research results from The State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun, China, and 3Bs Research Group-Biomaterials, Biodegradables and Biomimetics, Department of Polymer Engineering, University of Minho, AvePark, Zona Industrial da Gandra, S. Cláudio do Barco, 4806-909 Caldas das Taipas – Guimarães, Portugal.

Title: Interactions between cells or proteins and surfaces exhibiting extreme wettabilities

This review gives an overview of recent studies on proteins or cells action on the superhydrophilic and superhydrophobic surfaces. The potential biomedical applications of such information will also be explored, aiming to give inspiration on the development of new diagnostic systems, implants, cell-based biosensors and devices for tissue engineering.

REVIEW

Interactions between cells or proteins and surfaces exhibiting extreme wettabilities

Wenlong Song[*a] and João F. Mano[*bc]

Regulation of protein adsorption and cell adhesion on surfaces is a key aspect in the field of biomedicine and tissue engineering. Beside the general studies on hydrophilic/hydrophobic surfaces, there are both fundamental and practical interests to extend the investigation of the interaction between proteins or cells and surfaces to the two extreme wettability ranges, namely superhydrophilicity and superhydrophobicity. This review gave an overview of recent studies on proteins or cells action on these two special wettability ranges. The first part will focus on the interaction between proteins and superhydrophilic/superhydrophobic surfaces. The second part will focus on cells adhesion on these extreme wettable surfaces. Surfaces can be patterned to control in space the wettability within extreme values. As an application of such substrates, flat chips for high-throughput screening are also addressed to offer new insight on the design of a new type of bioanalysis supports.

1   Introduction

In the field of biomedicine and tissue engineering, one important topic is the investigation of the interaction between cells and substrate because it is crucial to estimate the biological reaction of implantable biomaterials in both theoretical and practical significance.[1–11] From the point of view of cell biology, most known mammalian cells exhibit the instinct to adhere onto a surface in order to carry out normal metabolism, proliferation and differentiation.[12–15] Generally before the adhesion occurs, the implanted materials are firstly coated with proteins from blood and interstitial fluids. Upon anchoring

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onto a surface, cells will first sense the extra-environment through ion channels and receptors presenting at their membranes, then integrate the chemical and physical signals from the extra-environment and give the response that some transmembrane receptors form clusters known as the integrins, and associate intracellularly with groups of proteins which link them to the cytoskeleton. Subsequently focal adhesions take place through the binding between the cluster integrin receptor and the ligand of the extracellular matrix (ECM)[16–24] as shown in Fig. 1. Therefore the cells adhesion will be strongly affected by the physiological activity of cells, such as the cell metabolic state, the hydrophobicity of the cell, the charge on the cell surface and the contact time of the cells and materials.[25–29] In the case that the initial protein adsorption on the substrate is absent, cell adhesion can also occur through non-receptor chemical binding, such as electrostatic, ionic–polar interactions, hydrogen binding to surface functional groups.[30–32] Comparing to integrin-mediated cell adhesion, this kind of cell adhesion cannot ensure the transference of enough signals from extracellular environments into cells. If the cells are not able to synthesize and deposit their own ECM molecules in a relatively short time, they will undergo apoptosis.[33–36] From the materials side, it is well known that cell adhesion and protein adsorption onto a substrate are highly affected by distinct surface properties such as surface energy, roughness, and chemical composition.[37–49] All these properties are an integrated embodiment of surface wettability. Usually most studies have mainly focused on the surfaces ranging from hydrophilic to hydrophobic, as smooth surfaces are typically used, such as silicon wafer, glass slide.[28,50,51] Also many works on protein adsorption have been reported on such kinds of surfaces. Some research showed increased protein adsorption onto hydrophilic substrates[52] whereas the majority has found that proteins tended to absorb more extensively onto hydrophobic surfaces.[53] Regarding the effect of wettability on cell response, it can be said that it is quite dependent both on the cell type and the surface properties.

In order to increase the understanding on the interaction between cells or proteins and surface, there are both fundamental and practical interests in extending such studies towards the two extreme wettable surfaces: superhydrophilic and superhydrophobic surfaces.[54–57] New insights may be obtained on the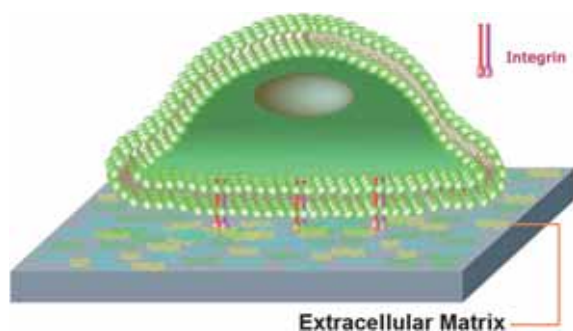 influence of such extreme environments on the physiological response of cells, including their contractile characteristics and signalling activity that may influence adhesion, morphology/anisotropy, migration, proliferation and differentiation. Superhydrophobic surfaces exhibit extreme water repellence with a water contact angle (WCA) higher than 150°.[58–61] A wide variety of materials, such as natural/synthesized polymers,[62–66] inorganic or hybridized organic–inorganic materials,[67–70] metals[71,72] and semiconductive materials,[73–76] are involved to fabricate superhydrophobic surfaces by different methods including chemical lithography,[77] layer-by-layer self-assembly,[78] electrospinning,[79] template synthesis,[80] electrochemical deposition,[81] or phase separation.[114,115] On the contrary, the superhydrophilic surface is defined as WCA $\sim$ 0° on which the water can spread on the surface completely.[82–84] Beside surface chemical composition, rough structures in micro- and/or nano-scale are necessary to obtain superhydrophilic or superhydrophobic surfaces.[84,85]

In this review recent studies on the interactions between cells or proteins and substrates with extreme wettable ranges will be presented. Tables 1 and 2 exhibit a brief summary on the interactions between cells/proteins and superhydrophobic/superhydrophilic surfaces. The potential biomedical applications of such information will also be explored, aiming to give inspirations on the development of new diagnostic systems, implants, cell-based biosensors and devices for tissue engineering. In particular, the possibility of using superhydrophobic surfaces for developing high-throughput chips will be discussed.

2 Protein adsorption on the extreme wettable surfaces

Protein adsorption onto a surface is the key factor to determine the fate of adherent cells.[86–89] When biomaterials first come into contact with the culture medium, interstitial fluids or blood, protein adsorption onto the material surface will first happen, and subsequently cell adhesion occurs. This adsorption is a complex and dynamic process involving non-covalent interactions, including hydrophobic interactions, electrostatic forces, hydrogen bonding, and van der Waals forces.[90–95] Protein parameters including primary structure, size, and structural stability as well as surface properties such as surface energy, roughness, and chemistry have been identified as key factors influencing the adsorption process.[96–98]

2.1 Protein adsorption on superhydrophobic surfaces

The surface structures and chemistry of the superhydrophobic surface can be foreseen to be strongly related to the proteins adsorption. Shirtcliffe et al.[99] reported hydrocarbon or fluorocarbon terminated superhydrophobic surfaces with the surface structures in different scales (micro-, submicro- and nanolevel) as shown in Fig. 2B–D. The Cassie–Baxter wetting model shown in Fig. 2A was used to analyze the prepared superhydrophobic state in theory. The protein adsorption experiment was carried out in both static and dynamic conditions. The experimental results (Fig. 2E and F) in the static condition showed that the amount of protein adsorption was similar on

Fig. 1 Schematic representation of cell–extracellular matrix.

Table 1 Brief summary of examples of protein adsorption on the superhydrophobic/superhydrophilic surfaces

Superhydrophobic surfaces	Protein types	Adsorption property	Ref.
Poly(methyl methacrylate)	Goat anti-rabbit IgG antibody labeled with Alexa Fluor® 488	Almost no adsorption	101
Hydrocarbon or fluorocarbon terminated methyltriethoxysilane sol–gel foams	Bovine serum albumin	There is protein adsorption on both hydrocarbon and fluorocarbon terminated substrates with 4 μm and 800 nm particle size in static condition; less protein adsorption in dynamic condition	99
Hydrocarbon or fluorocarbon terminated CuO nano-pillars	Bovine serum albumin	There is lower protein adsorption on both hydrocarbon and fluorocarbon terminated substrates with 10 nm pillar size in both static and dynamic conditions	99
Polystyrene	Fibronectin	Lower adsorption on the superhydrophobic PS surface than the smooth one and glass slide	100
Commercial expanded polytetrafluororethylene	Human serum albumin	Proteins adsorption on the superhydrophobic surface at ambient conditions; significant HSA adsorption was measured on the superhydrophobic surface with elimination of air from ePTFE adsorbent and degassing of solutions	54
Porous silica coatings with thermal treatment at 200 °C	Bovine serum albumin	Albumin adsorption was lower on superhydrophobic surface than on flat hydrophobic surfaces	102
	Bovine fibrinogen	Fibrinogen adsorption was lower on superhydrophobic surface than on flat hydrophobic surfaces	102

Superhydrophilic surfaces	Protein types	Adsorption property	Ref.
Alkali-treated micro-rough titanium substrates	Fibrinogen	Almost no adsorption	110
Porous silica coatings with thermal treatment at 450 °C	Bovine serum albumin	Albumin adsorption was lower on the superhydrophilic surface compared to hydrophilic flat surfaces	102
	Bovine fibrinogen	Fibrinogen adsorption was lower on the superhydrophilic surface compared to hydrophilic flat surfaces	102
UV-treated commercially pure titanium substrate	Bovine serum albumin	There is more protein adsorption on superhydrophilic Ti substrate than on the untreated substrate	52,112
Silicone nano-filaments coating terminated with amino or carboxyl groups	β-Lactoglobulin, α-chymotrypsin, and lysozyme	Excellent protein retention properties	113
UV/Ozone treated rough polystyrene	Human serum albumin and human plasma fibronectin	Almost no adsorption compared to the flat and other rough PS surfaces with wetting range from superhydrophobicity to hydrophilicity	104
Zwitterionic poly(sulfobetaine methacrylate) (PSBMA) prepared by electrospinning	Human plasma fibrinogen and bovine serum albumin	Resists to protein adsorption	127

smooth and nano-scale hydrocarbon terminated superhydrophobic surfaces, but a greater amount of adsorption occurred on micro-scale superhydrophobic surfaces compared to the nano-scale ones no matter if they were terminated with hydrocarbon or fluorocarbon. Furthermore there was a considerably larger proportion of adsorbed protein that desorbed from the superhydrophobic surfaces than from the smooth ones in buffer flow conditions; in fact, almost all the proteins could be removed from some nano-scale surfaces. This type of surface may be useful in microfluidic devices where protein sticking is a problem and fluid flow is present. Possible mechanisms for such findings could

include the decreasing contact area between proteins and surface, and greater shear stresses due to interfacial slipping between the superhydrophobic surface and the liquid. This suggests that it will not be possible to prevent protein adhesion entirely, but it may be possible to reduce the binding strength, facilitating protein removal by flow shear or other methods. Ballester-Beltrán et al.[100] also reported human plasma fibronectin (HFN) adsorption on superhydrophobic polystyrene (PS) surface. HFN adsorbed on superhydrophobic PS surfaces in lower density and altered conformation as compared with the corresponding standard smooth PS.

Table 2 Brief summary of examples of cell interactions on the superhydrophobic/superhydrophilic surfaces

Superhydrophobic surfaces	Cell types	Adhesion property	Ref.
Poly(L-lactic acid) (PLLA) substrates	Fibroblasts L929 cell line	Almost no adhesion on the superhydrophobic one compared to the smooth one after 24 h culture	114
	Rat bone marrow stromal cells	Superhydrophobic PLLA surfaces were found to prevent adhesion and proliferation of rat bone marrow stromal cells compared to the corresponding smooth ones	115
Polystyrene	SaOs2	SaOs2 cells can adhere on the superhydrophobic surface, but showed a certain inhibition in cell adhesion when the nano-scale roughness is particularly small and cannot proliferate on it	121, 122
	ATDC5	Cell adhesion takes place but proliferation is prevented	122
	Fibroblasts L929 cell line	Cells can adhere and proliferate on the superhydrophobic PS surface	122
	Mouse osteoblastic cells (MC3T3-E1)	Cells can adhere much less and proliferated slower onto such superhydrophobic PS substrates when compared to the standard smooth PS	100
	Adipose-derived stem cells	Compared to the flat substrate, the superhydrophobic one induced higher cell attachment but didn't change significantly cell proliferation rate	123
TiO_2 nanotubes with self-assembled monolayers of octadecylphosphonic acid	Mesenchymal stem cells	Cell attachment was considerably enhanced	116
Polytetrafluoroethylene (PTFE)	Osteoblasts	Promoted osteoblast adhesion and proliferation compared to the original PTFE substrates	124
Poly(carbonate urethane)s with fluorinated alkyl side chains coating on the carbon nanotubes film	Blood platelets	Almost no platelets adhered and there was much less platelet activation onto these materials compared with the smooth carbon nanotubes	132
Polydimethylsiloxane (PDMS) surfaces through CO_2-pulsed laser treatment	Blood platelets	Reduced platelet adhesion	133
TiO_2 nanotube layers on a titanium substrate	Platelets	Resisting to the adhesion and activation of blood platelets	117

Superhydrophilic surfaces	Cell types	Adhesion property	Ref.
PS surfaces with wettability controlled by UV-Ozone irradiation	SaOs2	Good adhesion and proliferation	122
Rough silicon oxide substrate treated by vacuum UV irradiation	Mouse 3T3 fibroblast cells	Cells adhesion was much higher on the superhydrophilic surface than the superhydrophobic one	125
Silicon nanowire	Mammalian cells (Chinese Hamster Ovary K1)	Cell cytoplasmic projections entered the silicon nanowire layer and coated the nanowires of the superhydrophilic substrate, which resulted in a strong adhesion between the cells and the superhydrophilic areas	57
Zwitterionic poly(sulfobetaine methacrylate) (PSBMA) prepared by electrospinning	Endothelial cell BAECs	PSBMA exhibited a resistant ability of cell adhesion	127
CO_2-pulsed laser treated polydimethylsiloxane surfaces by further grafting of hydroxyethylmethacrylate phosphatidylcholine	Blood platelets	Reduced platelet adhesion	133
TiO_2 nanotube layers by UV irradiation	Blood platelets	Large quantities of platelets adhered and spread	117

Furthermore the analytic protocol also affects the measurement of protein adsorption on the superhydrophobic surface. Leibner et al.[54] reported the adsorption of human serum albumin (HSA) onto a commercial superhydrophobic polytetrafluoroethylene (PTFE) in room and vacuum conditions by using either radiometry or electrophoresis as methods of protein quantification. Surface concentration of unlabeled HSA measured by the electrophoresis method was nearly 4-fold higher than using radiometry (^{125}I-labeled HSA) or electrophoresis. This may be attributed to the alteration of the properties

of protein adsorption induced by ^{125}I-radiolabeling: the subsequent measurement of bound protein significantly underestimates the total protein actually adsorbed from solution. On the other hand, the same authors investigated the effect of the trapped air in the rough structures of the superhydrophobic surface on the protein adsorption. They found that when adsorption was performed with degassed protein solutions under vacuum, the adsorption results were similar using the two methods. These results evidenced that the air trapped within the interstices of the superhydrophobic surface prevents
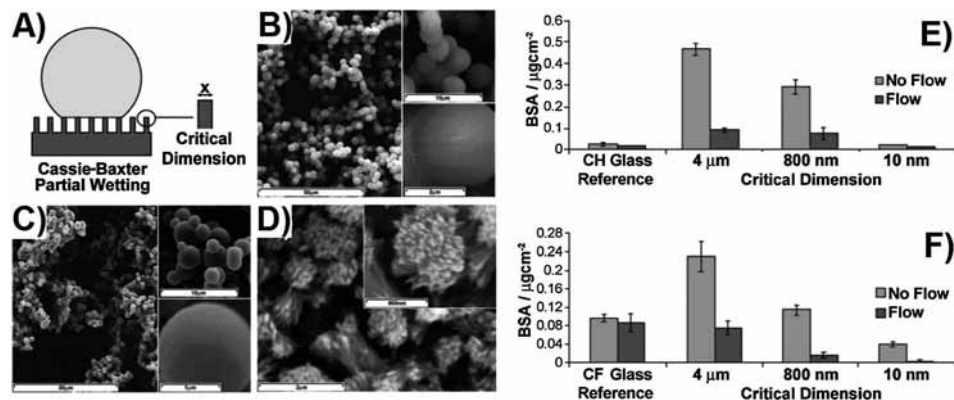
Fig. 2 (A) Scheme showing the Cassie–Baxter scenario for superhydrophobicity and the critical dimension used to define surfaces here. SEM images of (B) the larger scale sol–gel material, (C) the smaller scale sol–gel and (D) the copper oxide nano-pillars. Albumin adsorption onto micro-scale and nano-scale surfaces: (E) hydrocarbon terminated and (F) fluorocarbon terminated under static conditions and after subsequent flow of buffer. Reproduced with permission from ref. 99. Copyright 2008 The Royal Society of Chemistry.

intimate contact of the surface with the protein solution and may strongly inhibit protein adsorption.

All the works referred to in this section evidenced that protein adsorption onto superhydrophobic surfaces may occur, although it might be dependent on the rough feature sizes of the surface and the used protein. Two opposite effects are present regarding this situation. On one hand, one could expect an increase in protein adsorption on rough surfaces due to the increase in surface area. On the other hand, the topographic features of the surfaces have particular consequences in terms of wettability: the presence of air pockets in superhydrophobic surfaces, according to the Cassie-Baxter model, resist the contact of the culture medium or blood which could have an opposite effect of the increase of surface area in the rough surfaces, inhibiting protein adsorption.[101,102] Fig. 3 shows that almost no protein adsorption was observed on a superhydrophobic poly(methyl methacrylate) stripe fabricated by depositing a less than 15 nm Teflon-like film on the superhydrophilic stripe prepared by a deep anisotropic O$_2$ plasma etching technique;[101] on the contrary, significantly enhanced adsorption was seen on rough hydrophilic stripes. The reason can be attributed to partial contact protein solution with the

surface (Cassie–Baxter regime), being only a small fraction of the solid surface (column tops) available for adsorbing proteins, leading to significant reduction in the amount of protein immobilized on the superhydrophobic surface.

2.2 Protein adsorption on superhydrophilic surfaces

Superhydrophilic surfaces could lead to lower protein adsorption due to the large repulsive forces existing between proteins and the thin layer of water tightly linked to them. As reported by Jiang *et al.*[103] force–distance curves showed that the repulsive forces acting on the protein decrease in the following order: oligo (ethylene glycol)-terminated > OH-terminated > CH$_3$-terminated self-assembled monolayers (SAMs). Their results indicated that the tightly bound water layer adjacent to the SAMs is mainly responsible for the large repulsive force, and large number of hydrogen bonds with water molecules on SAMs exhibited a larger repulsive force on the protein.

HSA and HFN adsorption on PS surfaces with wettability controlled by UVO irradiation, ranging from superhydrophobic to superhydrophilic, was analysed by our previous work.[104] These proteins were chosen because HSA is present in high
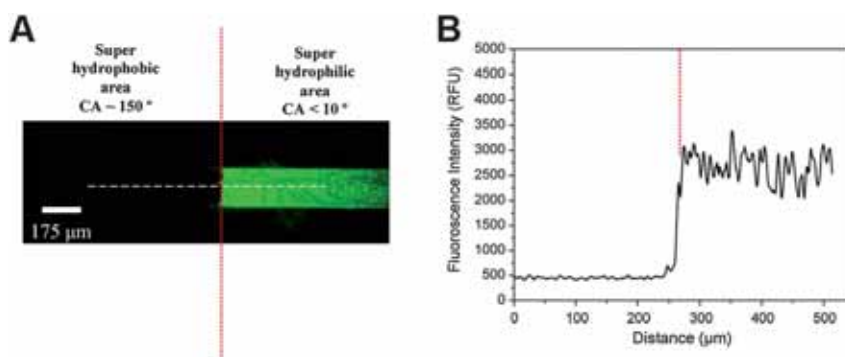

Fig. 3 (A) Fluorescence image of a microchannel with patterned wettability, superhydrophobic area in the left zone, and superhydrophilic area in the right zone and adsorption of AF488 labeled goat anti-rabbit IgG antibody. The graphic in (B) represents the fluorescence intensity across the white dotted lines in the image. Notice the abrupt increase in fluorescence from left to right for the channel and the large intensity in the superhydrophilic rough areas. Reproduced with permission from ref. 101. Copyright 2012 Elsevier.

quantities in human blood plasma and HFN is known to improve cell attachment onto biomaterial surfaces. A strong decrease in protein adsorption was observed for the case of the PS superhydrophilic surface compared to the other prepared and smooth PS surfaces, the two proteins essentially do not adsorb. Molecular dynamics studies showed that highly wettable surfaces produce large repulsive forces on the proteins, leading to lower protein adsorption, in agreement with the results of ref. 103. Moreover, it was found that the adsorbed density was almost the same for the superhydrophobic sample and the control smooth sample, and tended to gradually decrease for both proteins from the superhydrophobic situation to the hydrophilic state, this decrease being more evident for HSA.

Commercially available pure titanium (Ti) is widely used in osseointegrated implants. However the surface of Ti implantable materials can be easily contaminated. Many techniques were proposed to avoid or reduce hydrocarbon contamination.[105–109] Such methods are typically technically demanding, time consuming or use harsh chemical reaction conditions, which might affect the final clinical implant result. Tugulu et al.[110] developed a new strategy to prepare superhydrophilic micro-rough titanium implant surfaces, based on a rapid treatment with diluted aqueous sodium hydroxide solutions. The superhydrophilicity of alkali-treated micro-rough titanium substrates was mainly attributed to deprotonation and ion exchange processes in combination with a strong roughness enhancement of the substrates. The adsorption of a fibrinogen–Alexa Fluor conjugate on superhydrophilic Ti substrates decreased compared with the untreated Ti surface, which was consistent with the general tendency of better fibrinogen adsorption on hydrophobic substrates than on hydrophilic ones. At the same time fibrinogen has been reported to show a higher tendency to denature on hydrophobic surfaces compared to hydrophilic ones.[88,111] Superhydrophilic alkali treated sandblasting and thermal acid etching (SBA) Ti implants may led to enhanced osseointegration, and offer a clinically applicable route in in situ implantation without the need to modify both production and storage routines of the implants.

In order to quantify protein adsorption on surfaces with extreme wettability, Perry et al. used Nano Orange, a fluorometric assay, to assess the adsorption of bovine fibrinogen and albumin onto model superhydrophilic and superhydrophobic porous silica surfaces.[102] Enhanced binding affinities were found for proteins adsorbing onto porous materials as compared to flat surfaces, but protein density was significantly lower on the superhydrophilic surface than would have been expected, which might be attributed to the protein transport through the porous material. Compared to the superhydrophobic surface, albumin showed a higher affinity toward the superhydrophilic surface as shown in Fig. 4. However, fibrinogen showed a weaker affinity toward the superhydrophilic surface, but values were 70% lower than those on flat hydrophilic and hydrophobic surfaces, respectively.

It is interesting to notice that high protein adsorption can also be found on some superhydrophilic surfaces. Nearly complete bone–implant contact was reported on ultraviolet (UV)-photofunctionalized Ti substrates, which exhibited superhydrophilic characteristics. Ogawa et al.[52,112] found that albumin adsorption on such surfaces at pH 7.0 was considerably greater (6-fold after 3 h of incubation and 2.5-fold after 24
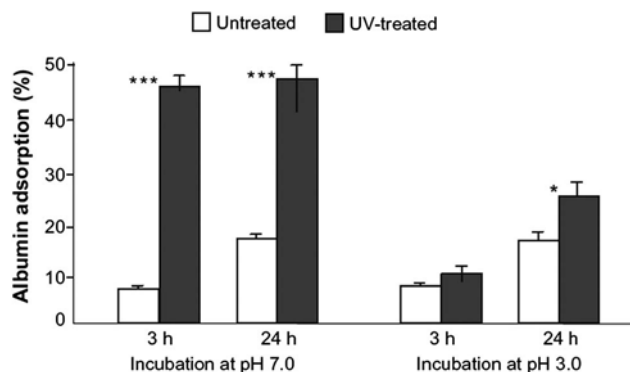
Fig. 5 Albumin adsorption on UV-treated and untreated titanium surfaces during 3 and 24 h of incubation at pH 7.0 and 3.0. Statistically significant differences are marked between untreated and UV-treated titanium surfaces. Reproduced with permission from ref. 52. Copyright 2010 Elsevier.
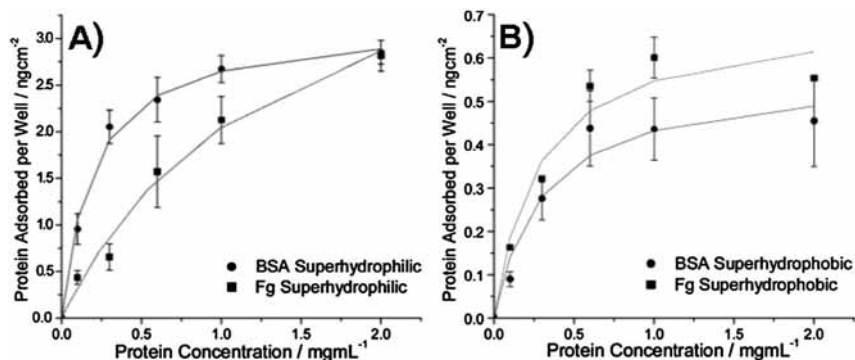
Fig. 4 Adsorption curves for fibrinogen and albumin onto (A) superhydrophilic and (B) superhydrophobic surfaces obtained using the Nano Orange assay. Reproduced with permission from ref. 102. Copyright 2006 American Chemical Society.

h) than on UV-untreated surfaces – see Fig. 5. When the pH was decreased to 3.0 or Ti surfaces were treated with anions, UV-enhanced albumin adsorption was abrogated while keeping superhydrophilicity. They also found that protein adsorption on superhydrophilic Ti surface increased after being treated with divalent cations but not with monovalent cations. The possible reason can be concluded by the bridge function of the divalent cations, such as Ca^{2+}, between the UV-treated Ti surface and those anionic proteins. It is this distinct UV-induced electrostatic property that predominantly regulates the protein adsorption capability of Ti, superseding the effect of the hydrophilic status, and converts titanium surfaces from bioinert to bioactive. Zimmermann et al.[113] also observed the protein retention on superhydrophilic silicone nano-filaments coating terminated with amino- or carboxyl groups. In their case adsorption of β-lactoglobulin, α-chymotrypsin, and lysozyme were investigated on the prepared surface in different pH values (3 and 6) for tuning the surface charge. The combination of the electrostatic condition of the proteins and the presence of the amino and carboxyl groups with the high contact area resulting from the nano-roughness allowed the obtaining of surfaces with excellent protein retention properties of high specificity toward these charged proteins.

3 Cell adhesion on extreme wettable surfaces

3.1 Cell adhesion on superhydrophobic surfaces

Cell behaviour on superhydrophobic surface is very complex, being affected by many factors as mentioned above. There is still no general understanding on how cell adhesion and proliferation proceeds on these substrates and contradictory trends have been reported. For examples, some works showed that cells poorly adhere on a superhydrophobic surface;[114–118] others showed that cells can not only adhere but also proliferate on superhydrophobic substrates.[119,120] Some representative examples of studies of cell adhesion on superhydrophobic surfaces will be now presented.

To investigate the interactions of superhydrophobic substrates with mammalian cells, superhydrophobic poly(ʟ-lactic acid) (PLLA) substrates were prepared by a phase inversion method, and the influence of their exposition time to Ar plasma
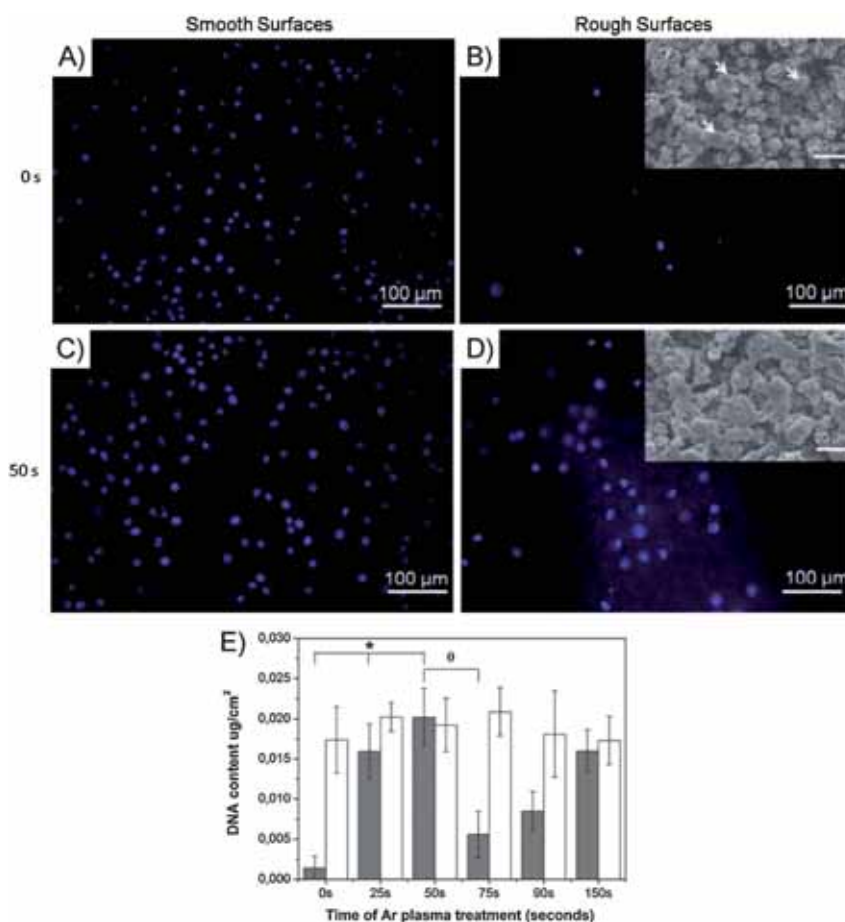

Fig. 6 Fluorescent microscopy images of smooth and rough poly(ʟ-lactic acid) surfaces treated with argon plasma or untreated (blue DAPI staining for the nuclei of cells). (A) Smooth PLLA, untreated. (B) Rough PLLA, untreated. (C) Smooth surface treated with argon plasma for 50 s. (D) Rough surface treated with argon plasma for 50 s. Insets: SEM images of cells attached to the rough surfaces. Inset scale bar: 20 μm. (E) DNA content in the untreated and treated samples after 24 h of culture. White and gray bars correspond to the smooth and rough surfaces, respectively. Reproduced with permission from ref. 114. Copyright 2009, Wiley-VCH Verlag GmbH & Co. KGaA.

on cell adhesion was analysed.[114] Fig. 6 shows fluorescent microscopy and SEM images with cells onto the surfaces (A–D); the corresponding quantitative analysis for cell attachment was carried out using a DNA assay (E). On the smooth surfaces, no significant difference on cell attachment was observed for different times of treatment. In the absence of plasma treatment, few cells adhered on the superhydrophobic surfaces (Fig. 6B), compared with the number of cells adhered onto the smooth ones (Fig. 6A). The water repellence of superhydrophobic surfaces may prevent the culture medium and cells from being in contact with the entire surface, as predicted by the Cassie–Baxter model. Therefore, cells mainly adhered to some points of the asperities at the surface. The cells adopted a round shape (Fig. 6B-inset) due to such unfavourable anchorage situations. For the rough surfaces, the Ar plasma treatment enhanced cell attachment and cell number reached the highest value after 50 s of treatment. These results suggested that for the surfaces treated under these conditions, the combination of roughness, surface chemistry and wettability presented the best environment for the cells. In this case, the cells exhibited a more flattened and extended morphology (Fig. 6D-inset). Besides the related works with cell lines or primary cells, there are also a few works that analysed the behaviour of stem cells on surfaces exhibiting extreme wettability. Superhydrophobic PLLA surfaces were also found to prevent adhesion and proliferation of rat bone marrow stromal cells, when compared with the corresponding smooth ones.[115]

PS is a widely used material in biological applications and, hence, is an obvious choice for performing studies concerning cell interactions with surfaces with extreme wettability ranges. Mundo et al.[121] analyzed the response of SaOs2 cells on superhydrophobic PS surfaces that presented nano-scale dots produced by a tailored plasma-etching process. Their results indicated that SaOs2 cells respond to surfaces with different nanoscale roughness, and showed a certain inhibition in cell adhesion when the nano-scale roughness is particularly small – see Fig. 7. The in vitro performance of different cell lines was analysed on superhydrophobic PS surfaces produced by phase separation.[122] Compared to standard tissue culture PS, it was found that ATDC5 and SaOs2 cell lines were not able to proliferate on such surfaces and that cell morphology was affected. Ballester-Beltrán et al.[100] also reported that mouse osteoblastic cells (MC3T3-E1) adhered much less and proliferated slower onto such superhydrophobic PS substrates when compared with the standard smooth PS – see Fig. 8. They found that MC3T3-E1 cell adhesion occurred without formation of mature focal adhesion plaques, together with scarce phosphorylation of focal adhesion kinases. Under these circumstances, neither cell contraction nor reorganization of adsorbed HFN nor newly secreted HFN fibrils occurred on the superhydrophobic PS.

A set of other works showed that improved cell adhesion can be found on superhydrophobic surfaces. Cha et al. analysed the response of adipose-derived stem cells on PS surfaces fabricated with lotus surface structures by a hot-embossing process.[123] They found that, compared to the flat substrate, the superhydrophobic one induced higher cell attachment but didn't significantly change the cell proliferation rate (Fig. 9A and B); in such surfaces cells exhibited relatively narrower spreading morphology and a less organized cytoskeleton. Topological modification has been pointed out as a promising tool for controlling stem cell differentiation. They also analysed the effect of using the peculiar topography of a lotus-inspired surface on stem cell differentiation. They found that the superhydrophobic surfaces induced higher adipogenic differentiation of the cells and decreased both chondrogenic and osteogenic differentiation than the flat surfaces (Fig. 9C). So, such surfaces could be potentially used as culture dishes, for an efficient increase of adipogenic differentiation of stem cells, which is attractive in the cosmetic and aesthetic industry. Bauer et al.[116] reported the response of mesenchymal stem cells on superhydrophobic substrates prepared by organic modification of TiO_2 nanotubes with self-assembled monolayers of octadecylphosphonic acid. Cell attachment was considerably enhanced after 24 h on the superhydrophobic surfaces. However, this effect was temporary and cell adhesion was lost after 3 days.
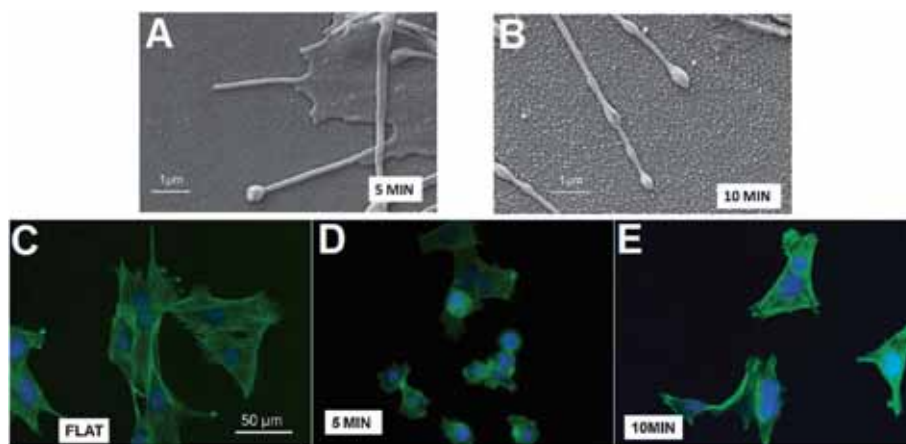

Fig. 7 (A) and (B) are the SEM images of SaOs2 osteoblast cells after culture for 24 h, on PS surfaces with plasma-etching for 5 min and 10 min showing in detail filopodia interacting with the surface nano-structures. (C–E) shows the effect of nanotopography on actin filaments assembly in SaOs2 cells grown for 48 h on the three fluorinated surfaces: green, actin; blue, nuclei. Reproduced with permission from ref. 121. Copyright 2011 American Chemical Society.
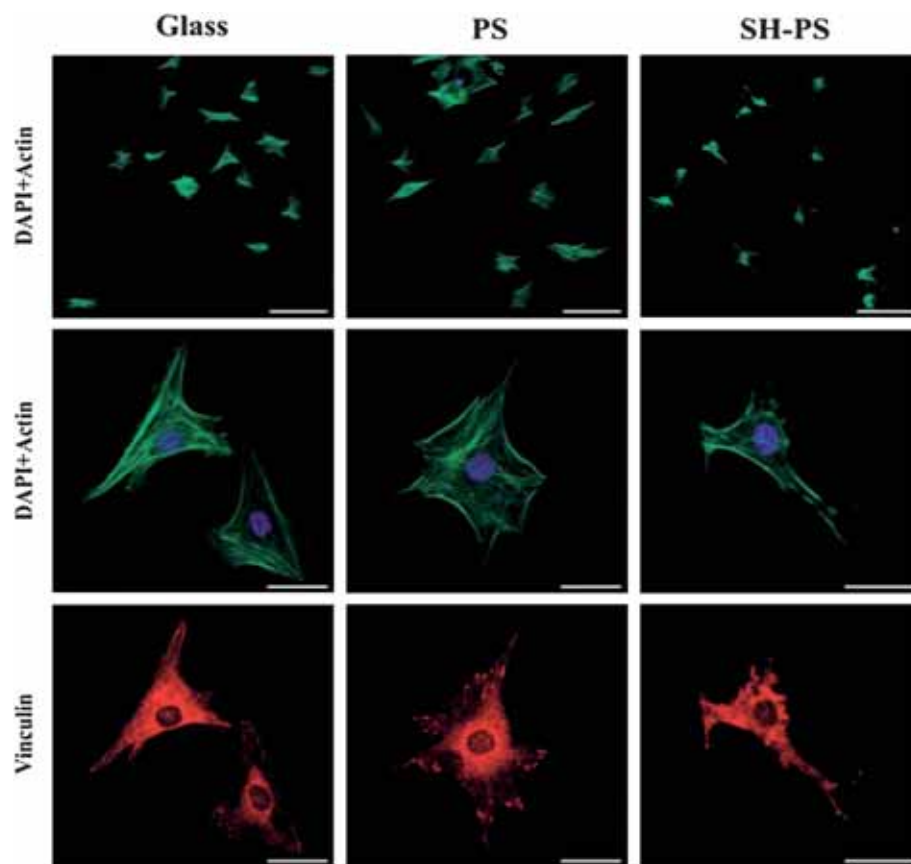
Fig. 8 Cytoskeleton organization and focal adhesion formation of MC3T3-E1 cells in different FN-coated substrates (glass, smooth PS and superhydrophobic PS) upon 3 hours of culture. First and second rows show F-actin cytoskeleton at different magnifications (scale bar 150 and 30 μm respectively). The third one shows the distribution of the focal adhesion protein vinculin (scale bar 30 μm). Nuclei were counterstained with DAPI. Reproduced with permission from ref. 100. Copyright 2011 The Royal Society of Chemistry.

Chu *et al.*[124] developed a superhydrophobic PTFE surface by plasma immersion ion implantation. Modified superhydrophobic surfaces promoted osteoblast adhesion and proliferation compared to the original PTFE substrates. Alkaline phosphatase, osteopontin and osteonectin expression of the seeded osteoblasts was also enhanced. The low cell adhesion on the original PTFE surface can be attributed to the low surface energy and lack of functional groups to interact with the cellular environment. The improvement of cells adhesion on the superhydrophobic PTFE can be explained by the presence of C–O and C=O groups.

3.2 Cells adhesion on superhydrophilic surfaces

Cells attachment on superhydrophilic surfaces is usually more efficient than on superhydrophobic ones. The attachment, morphology and proliferation of SaOs2 had been studied on rough and smooth PS surfaces with wettability controlled by UV-Ozone irradiation, ranging from superhydrophobic to superhydrophilic.[122] After 4 hours in culture, the attachment of SaOs2 was higher on the surfaces treated for 18 minutes, namely on rough superhydrophilic and highly hydrophilic smooth PS surfaces. It was also found that, for these PS surfaces, the proliferation after 6 days in culture was always higher in surfaces with water contact angles ranging from 13° to 30°, independently of

being rough or smooth. Superhydrophilic regions were also patterned on the superhydrophobic PS surfaces using hollowed masks to control the location of the UV-Ozone irradiation. It was found that the cells were preferably confined on such superhydrophilic spots, surrounded by the superhydrophobic regions – see Fig. 10.

Fibroblast cells adhesion behaviour was assessed on micropatterned superhydrophobic/superhydrophilic surfaces fabricated by CVD and VUV irradiation:[125] the cells attached to the superhydrophilic regions in a selective manner. The distance between superhydrophilic regions was also addressed. When the pattern distance was 200 μm, the cells adhered on the superhydrophilic regions partly extended to the neighboring cells. In contrast, when the pattern distance was greater than 400 μm, the cells did not exhibit this behavior. Similar results could also be found by using stem cells. Boukherroub *et al.*[57] analysed the behaviour of mammalian cells (Chinese Hamster Ovary K1) on patterned superhydrophilic/superhydrophobic silicon nanowire arrays and observed that the cells adhered preferentially to the superhydrophilic regions. Cell cytoplasmic projections entered the silicon nanowire layer and coated the nanowires of the superhydrophilic substrate, which resulted in a strong adhesion between the cells and the superhydrophilic areas. Compared to the superhydrophobic surface, the cytoplasmic projections
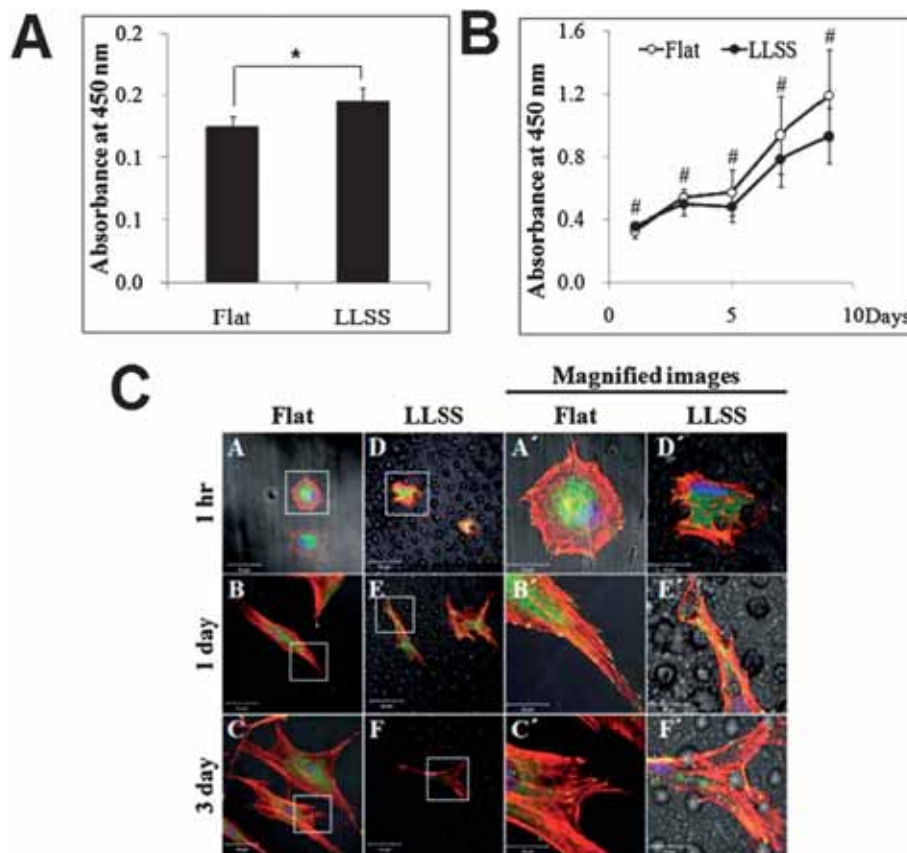
Fig. 9 (A) Cell attachment after 1 h of seeding and (B) proliferation during 9 days of adipose-derived stem cells (ASCs) on superhydrophobic and smooth substrates. (C) Immunocytochemical staining images of ASCs on the flat (A–C and A'–C') and superhydrophobic substrates (D–F and D'–F') was performed with anti-vinculin (green), phalloidin (red), and DAPI (blue) at 1 h, 1 day, and 3 days after seeding. On the superhydrophobic substrate, focal adhesion complexes were localized at the surrounding region of microbumps and action filaments of a relatively tiny size were formed (scale bar = 50 μm (A–F); 20 μm (A'–F')). Reproduced with permission from ref. 123. Copyright 2011 Wiley-VCH Verlag GmbH & Co. KGaA.
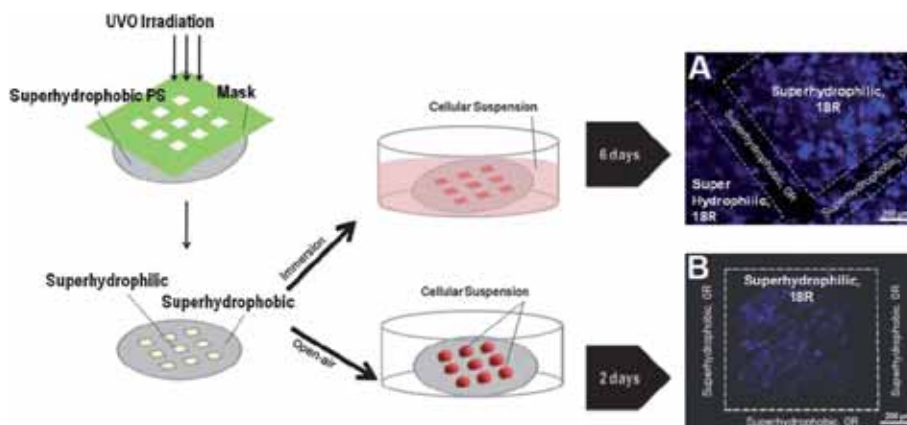

Fig. 10 Schematic representation of the patterning of superhydrophilic regions on superhydrophobic PS surfaces by UV-Ozone irradiation and using a hollowed mask; the two cell seedings were made by immersion and in open-air. Fluorescent staining of the SaOs2 cell nucleus with DAPI: (A) on samples where cells were seeded over the whole surface, after 6 days in culture; (B) and in open-air culture where 7 mL of the cellular suspension was dropped on the superhydrophilic region, after 2 days in culture. Reproduced with permission from ref. 122. Copyright 2011 The Royal Society of Chemistry.

remained on the top surface of the wires. This set of works evidenced that bioinspired platforms where cell attachment/proliferation could be controlled by patterning superhydrophilic spots on superhydrophobic substrates can be produced.

Beside the good cell adhesion property exhibited by most superhydrophilic surfaces, in some cases cell resistance can also be presented. Zwitterionic poly(sulfobetaine methacrylate) (PSBMA) is a well-known hydrophilic material, the hydrogel of

which is highly cell resistant both *in vitro* and *in vivo*.[126] Liu and Lalani[127] developed a superhydrophilic PSBMA surface using electrospinning. Compared to the electrospun polycaprolactone (PCL) surface and tissue culture polystyrene (TCPS), electrospun PSBMA exhibited a resistant ability of protein adsorption and cell adhesion. These unique characteristics are attributed to its strong hydration capacity, dictated by electrostatic attractions between charges on the polymer pendant groups and water molecules. The prepared electrospun PSBMA membrane can be foreseen to be used as a wound dressing.

4 Blood interactions with surfaces with extreme wettable values

The understanding of interactions between blood platelets and material surfaces is very important for constructing artificial heart valves, vascular stents and circulatory support devices.[117,128–132] In fact, blood coagulation and thrombosis are greatly affected by blood platelet adhesion and activation on the substrates used. The surface wettability is an important parameter in the adhesion and activation of platelets.

Sun *et al.*[132] analysed blood platelet adhesion on superhydrophobic carbon nanotubes and found that almost no platelets adhered and there was much less platelet activation on poly(carbonate urethane)s with fluorinated alkyl side chains coating the carbon nanotube film compared to the smooth ones. Khorasani *et al.*[133] prepared superhydrophobic polydimethylsiloxane (PDMS) surfaces through CO_2-pulsed laser treatment and superhydrophilic surfaces by further grafting of hydroxyethylmethacrylate phosphatidylcholine onto these superhydrophobic substrates. The *in vitro* results showed that both superhydrophobic and superhydrophilic surfaces reduced platelet adhesion and that these two extreme wettabilities exhibited better blood compatibility than the control smooth samples.

Recently Yang *et al.*[117] used a combination of electrochemical anodization and surface self-assembly technique to construct superhydrophobic TiO_2 nanotube layers on a titanium substrate. Superhydrophilic TiO_2 nanotube layers were also produced by exposing the superhydrophobic surface to UV irradiation. They found that the superhydrophobic surfaces presented remarkable blood compatibility and the ability of resisting to the adhesion and activation of blood platelets – see Fig. 11. On the other hand, large quantities of platelets adhered and spread either on the polished flat titanium substrate or on the superhydrophilic surfaces. So, the inhibition for blood platelet adhesion and blood compatibility generally observed in surfaces with extreme wettability could be advantageous in a variety of intracorporeal or extracorporeal medical devices in contact with blood, such as blood vessels or circulatory support devices.

5 Chips based on patterned surfaces with extreme wettable ranges for high-throughput screening

High-throughput screening permits the assessment of the relationships between many combinations of materials, surfaces and the corresponding biological responses, including cell adhesion, growth and differentiation or gene expression, in a single experiment. Different variables have been explored in such combinatorial analysis, including surfaces varying in roughness, surface chemistry/energy, mechanical properties or density of biochemical elements.[134,135] The substrates for high-throughput screening have been fabricated using distinct methods, including: robotic DNA spotters; microfabrication masking techniques, such as photolithography, soft-lithography, microfluidics, templating, imprint lithography, microelectronics and magnetic forces; and direct microfabrication techniques such as contact printing and non-contact printing, ink-jet printing, electron beam lithography and dip-pen
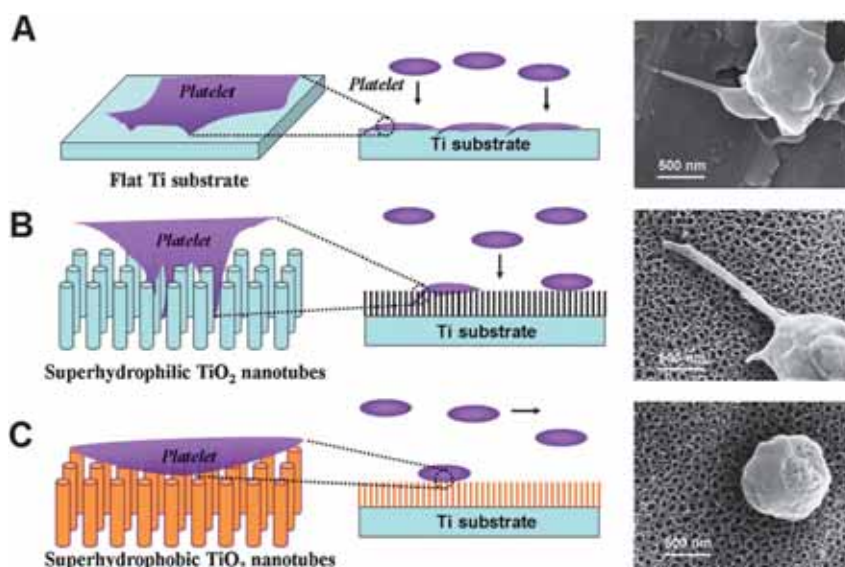

Fig. 11 Schematic illustration of platelet morphology and the corresponding interactions with different surfaces. (A) Plain Ti substrate; (B) superhydrophilic TiO_2 nanotubes; and (C) superhydrophobic TiO_2 nanotubes. Reproduced with permission from ref. 117. Copyright 2010 Elsevier.
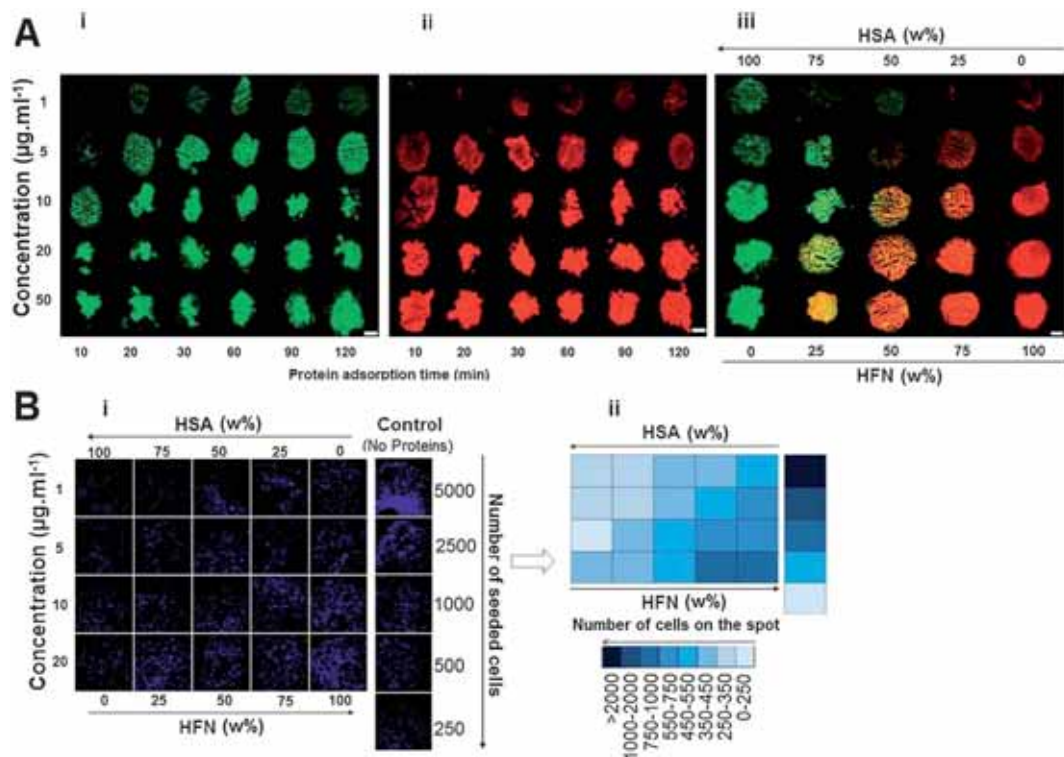
Fig. 12 (A) Fluorescence microscope images of substrates where spots were incubated with (Ai) HSA (green) and (Aii) HFN (red) with different concentrations (vertical axis) and during different adsorption times (horizontal axis). (Aiii) HSA and HFN fluorescent fingerprints in patterned surfaces after different relative amounts and protein concentrations were deposited in the hydrophilic spots. (Bi) Confocal microscope pictures of osteoblast-like cells cultured for 4 hours on the micropatterned array pre-adsorbed with different protein quantities (equivalent to the array in (Aiii)). The column on the right represents the number of seeded cells attached on hydrophilic spots without the protein pre-adsorbed. (Bii) The heat map for the cell number per spot corresponding to the same array tested with different combinations of proteins. Scale bars, 500 μm. Reproduced with permission from ref. 104. Copyright 2011 The Royal Society of Chemistry.

nanolithography.[136–141] The microarray format enables the rapid deposition of different materials and thereafter screening a large library of multiple biomaterials and microenvironments.

However, in most cases all the spots employed in the chip are usually tested in the same biological environment, which means the entire device is immersed in a unique culture medium. Advances in this field should offer a possibility to screen individually different combinations of biomaterials under different conditions in the same chip, including different cells, culture media or solutions with different proteins or other molecules. In this context, a new low-cost platform for high-throughput analysis that permits screening of the biological performance of independent combinations of biomaterials, cells and culture media was proposed.[104] Patterned super-hydrophobic PS substrates with controlled wettable spots were used to produce microarray chips for accelerated multiplexing evaluation. As volumes may be confined in the wettable regions due to strong contrasts in surface tension, this simple methodology enables the depositing of high control materials, cells and other substances. These patterned superhydrophobic PS substrates having pre-adsorbed combinations of proteins (HSA and HFN) were used for cell studies to demonstrate their applicability for high-throughput analysis – see Fig. 12. As a tendency, for the same total protein concentration, more cells are detected in the spots pre-adsorbed with higher relative HFN amounts. For the same HFN/HSA composition, the number of cells also tends to increase with increasing total protein concentration, also corresponding to an increase in the total amount of HFN. Such findings are consistent with the fact that albumin is a passivating protein and HFN has cell adhesive properties due to the existence of integrin binding domains in its structure. This inexpensive and simple bench-top method, or simple adaptations from it, could be integrated into tests involving larger libraries of substances that could be tested under distinct biological conditions, constituting a new tool accessible to virtually anyone to be used in the field of tissue engineering/regenerative medicine, cellular biology, diagnosis, drug discovery and drug delivery monitoring. In particular, this technology was transposed to assess the biological response of 3D-biomaterials, by encapsulating cells in arrays of miniaturized hydrogels dispensed onto patterned superhydrophobic substrates,[142] or even arrays of porous scaffolds.[143]

A protein microarray was also proposed by Shiu et al.[144] Superhydrophobic Teflon AF substrates were prepared by plasma treatment, and a switchable wetting character that could change from superhydrophobic to superhydrophilic was achieved using the electro-wetting technology. Micro-patterns were fabricated on these switchable superhydrophobic substrates. Each spot on this microarray can be addressed individually and different types of proteins or other biomolecules can be deposited on the microarray without losing their activity.

6 Conclusions

This contribution reviewed the interactions between cells or proteins and surfaces with extreme wettability, namely superhydrophobicity and superhydrophilicity. It can be said that although the behaviour is dependent on the cell type and used material, in general, few cells can adhere to superhydrophobic surfaces accordingly to the Cassie–Baxter model, and cells typically adopt a round morphology upon attachment. When both superhydrophobic and superhydrophilic regions are present in the surface, cells typically adhere selectively on the superhydrophilic regions. Regarding protein adsorption, superhydrophilic surfaces seem to be ideal for repellence of proteins and adsorption could occur on superhydrophobic surfaces depending on the chosen protein and mainly on the balance between the size of the rough features, the low wettability and the presence of air pockets at the surface. However, more studies are needed to get a general understanding on how roughness and extreme wettability affect protein conformation. In the case of blood interaction with surfaces with extreme wettability, studies performed until now showed that superhydrophobic surfaces generally inhibit blood platelet adhesion and exhibit blood compatibility, properties that could be obviously useful in a variety of intracorporeal or extracorporeal medical devices in contact with blood. The use of superhydrophobic/superhydrophilic surfaces to develop microarray chips for high-throughput screening, which could be applied on preparing highly efficient bio-analyzing devices, was also discussed. All these works will help us to search new insights on designing or improving devices for a variety of applications, including biomedicine, biology and in the environmental field.

Acknowledgements

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