Supplementary Material (ESI) for Soft Matter

Supporting Information

High-throughput evaluation of interactions between biomaterials, proteins and cells using patterned superhydrophobic substrates

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Microstructure of the superhydrophobic surfaces

The phase-separation based methodology employed in this work permitted to produce superhydrophobic polystyrene (PS) substrates exhibiting a hierarchical micro/nano-structured organization (Fig. S1 A). The structure of this superhydrophobic surface was induced when the polymer was dissolved in tetrahydrofuran and mixture with ethanol (nonsolvent) forcing polymer precipitation. The PS precipitation on the surface leads to formation of a rough surface due to the following mechanism: the mixture of a solvent and a nonsolvent of PS form both poor and rich PS phases. In the poor PS phase, polymer nuclei are formed by precipitation. The rich PS phase aggregates around these nuclei in order to decrease surface tension. During polymer precipitation within the rich PS phase, a continuous deposition of spheres on the surface takes place.[1]

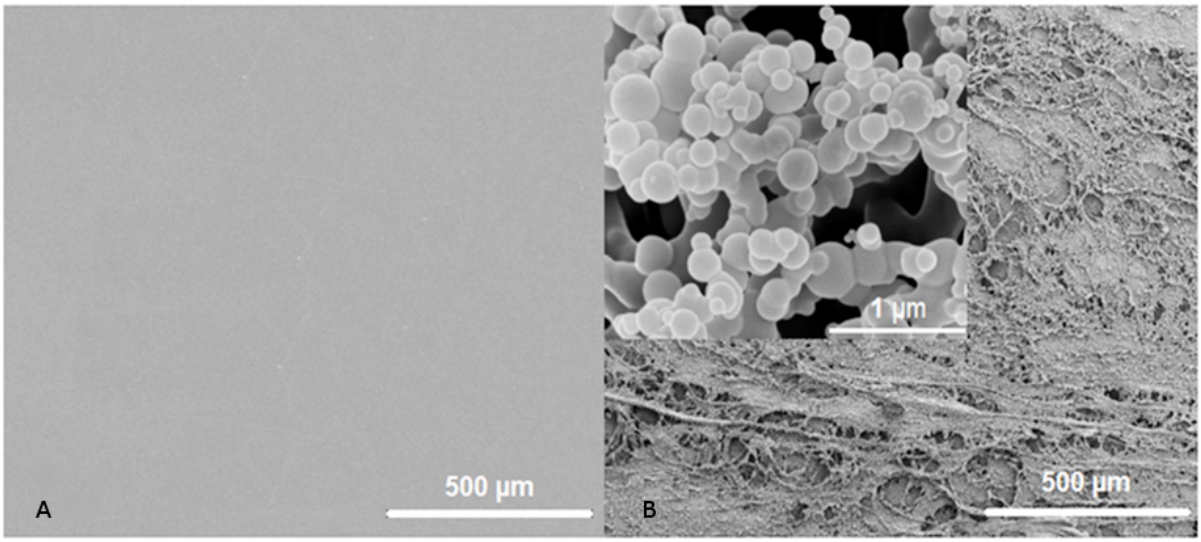

Fig. S1: Representative SEM image of smooth (A) and superhydrophobic rough PS surfaces (B)

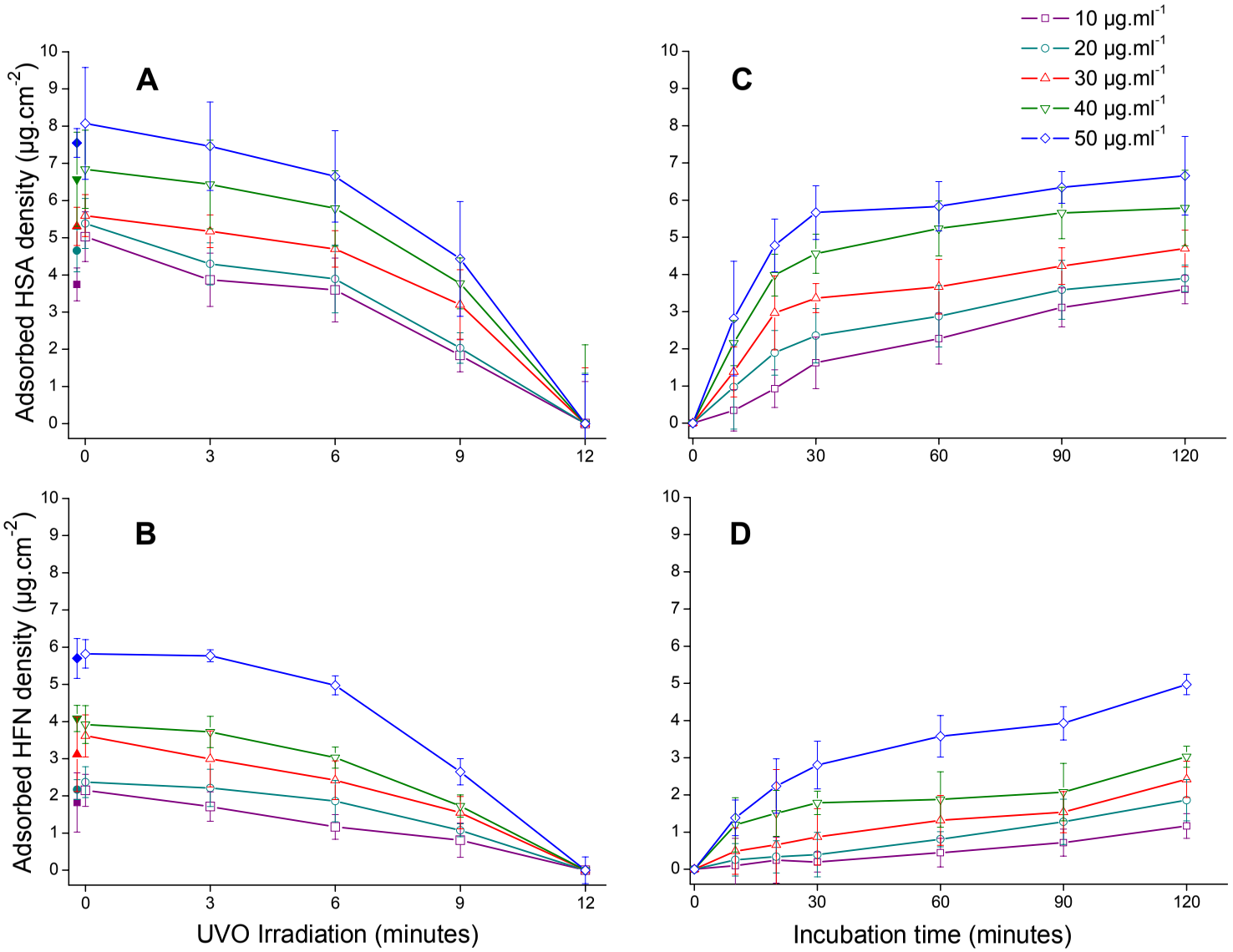

Fig. S2: HSA (A) and HFN (B) adsorption after 2 hours of incubation in solutions with different protein concentrations (different symbols as a function of UVO treatment time. The open symbols are for rough surface and the filled symbols are for the control smooth surface (no treatment). Kinetics adsorption at 37ºC of HSA (C) and HFN (D) onto rough surfaces after 6 min of UVO irradiation using solutions with different protein concentrations (different symbols).

Protein adsorption studies

For the proof-of-concept superhydrophobic PS substrates were patterned using UV/Ozone (UVO) irradiation and different combinations and amounts of human serum albumin (HSA) and human plasma fibronectin (HFN) were adsorbed onto the hydrophilic regions. We used such example as it is crucial to investigate the protein adsorption in the cell culture medium in order to correlate the effect of protein adsorption on cells behaviour.[2] 13mm discs of samples with different wetabilities were placed in 24-well plates and 1 ml of HSA and HFN with different concentrations was deposited on each well. Solution depletion method was used to measure the total amount of protein adsorbed to the disc immersed in a protein solution. The adsorption of proteins on solid substrates involves the transport of proteins from the solution to the interface, their binding to the surface usually via hydrophobic and electrostatic interactions, and their relaxation on the surface via conformational changes.[3] The adsorption of both HSA and HFN was studied on surfaces with different wetabilities (Fig. S2 A,B), showing the densities of adsorbed HSA and HFN after substrates with different wetabilities were put in contact for 2h in protein solutions with distinct concentrations. The amounts of HSA and HFN adsorbed increase with increasing solution concentrations. In general, for the same protein concentration used, the amount of adsorbed HSA was higher than adsorbed HFN. The effect of wettability on protein adsorption has been controversial in the literature. Some authors have reported increased protein adsorption onto hydrophilic substrates,[4] whereas the majority have found that proteins tended to absorb more extensively onto hydrophobic surfaces.[5] The adsorption of proteins onto treated area occurs by polar dispersion interaction between the substrates and the proteins and possibly with water molecules forming intermediate structure at the interface.[6] This has been explained by the strong hydrophobic interactions occurring at these surfaces, in contrast to the repulsive solvation forces arising from strongly bound water at the hydrophilic surface.[7] To our knowledge adsorption studies of proteins in substrates covering extreme ranges of wettability were never reported. Along the broad range of water contact angle (WCA) covered in this study the general behaviour observed is the following: in both cases, the adsorbed protein density tends to decrease slowly from the superhydrophobic situation (no irradiation) to the hydrophilic state (9 minutes of UVO irradiation); this decrease is more evident for the case of HSA and for lower concentrations of protein. A strong decrease in protein adsorption is observed for the case of the superhydrophilic surface: for all concentrations, the two proteins essentially do not adsorb. Apparently, such superhydrophilic surfaces act similarly as antifouling polymers, such as polyacrylates or poly(ethylene glycol), immobilized on surfaces. However, the mechanisms assigned to the resistance to protein adsorption in these two cases should be distinct.

The influence of surface topography on cell behaviour has been extensively studied but the literature on the influence of surface roughness on protein adsorption is sparse.[8] One could expected an increase in protein adsorption on rough surfaces due to the increase in surface area; one could also hypothesized that roughening may create surfaces with a highly heterogeneous pattern of surface free energy that might promote the adsorption of proteins.[9] However the topographic features of the surfaces investigated have particular consequences in terms of wettability: for superhydrophobic surfaces one can have the formation of air pockets that forces the liquid to be suspended over the micro/nano asperities of the surface (the so-called Cassie- Baxter model) having an opposite effect of the increase of surface area in the rough surfaces and complicating the description of the observed results. The wettability of the smooth samples is similar to the one of the rough surfaces after 3 minutes of UV/ozone (UVO) irradiation (Fig. S2 A,B). The protein adsorption levels in such two surfaces are similar, suggesting that the topography does not influence the protein adsorption provided that the wettability is the same. The substrates prepared for the high-throughput tests are based on superhydrophobic surfaces patterned with hydrophilic regions. The micro-patterned chips were produced by irradiating with UVO for 6 minutes through a hollowed square mask. Therefore, a study on the adsorption kinetics of both HSA and HFN were conducted in rough surfaces

subjected to this treatment. This could provide more evidences of the time-dependent process associate with the adhesion of proteins, which can involve relatively large energy scales in addition to dynamic conformational changes following contact with the surface.[10] The isothermal adsorption curves for both proteins was also shown (Fig. S2 C,D). For both HSA and HFN, as expected, adsorbed protein increases with increasing concentrations of protein in solution. Especially for HSA, most of the adsorption of the proteins takes place in the first 30 minutes, for all the concentrations tested. The HFN adsorption increases more slowly along the time-axis. The results for HSA suggest that the amount of adsorbed protein stabilizes up to 30 min incubation followed by a small increase until to 120 min of incubation time. Baujard-Lamotte et al.[11] showed that the adsorption kinetics for HFN involves two steps: the fast phase in which most of protein adsorption takes place in the first 5 minutes. After that, in a second phase the protein adsorption is much slower, showing an almost steady-state value at longer times. Cell attachment to biomaterials will depend strongly on the surface characteristics, including the nature and organization of pre-adsorbed proteins. To demonstrate the applicability of the developed chips for HTS, the substrates prepared with the same combinations and quantities of HSA and HFN used for the cells studies. The same threshold criteria were used to transform each image into black and white images enabling the quantification of black spots.

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