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Supplementary Information

Anti-bioadhesion on Hierarchically Structured, Superhydrophobic Surfaces

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Table of Contents

1. Experimental details

Synthesis of spherical silica particles

Three types of silica particle with different diameters were synthesized according to the modified Stöber method as previously reported.[1]

Large silica particles (1200 nm) were prepared with a seed method.[2] Briefly, tetraethylorthosilicate (TEOS) (1 mL) was added to the mixture of 100 mL of ethanol and 20 ml of saturated ammonia solution (28-30%). The mixture was stirred at 600 rpm for 4 h at room temperature, leading to the seed suspension for the next step. Then a mixture of TEOS (10 mL) and ethanol (40 mL) was slowly added into the above seed suspension. Finally, particles were centrifuged and washed several times with ethanol, and dried in vacuum oven overnight.

Medium particles (160 nm) were prepared by one-step synthesis. TEOS (1.6 mL) was added into ammonia solution (3.2 mL) with ethanol (50 mL) as the solvent. The whole system was stirred at 600 rpm for 18 h at room temperature. The particles were collected and dried as mentioned above.

Small particles with the diameter of 40 nm were prepared by the reaction of the mixture (60 mL of ethanol, 40 mL of methanol, 7.5 mL of saturated ammonia solution) at 70° for 2 h. Small particles with the diameter of 10 nm, Ludox HS-40, were purchased from Aldrich, and diluted by 10 times with water before use.

Fabrication of single-scale (F-S), dual-scale (F-D) and triple-scale (T-S) structured surfaces

Glass slides (2×2 cm^2) used as substrates were cleaned by acetone, then thoroughly rinsed with ethanol and dried by air flow. Epon resin 1004 (1.78 g, 2 mmol epoxy groups) was dissolved in ethyl acetate (10 mL) at 70 °C, followed by the addition of Jeffamine D230 (0.117 g, 1 mmol amine groups). The mixture was further spin-coated on glass slides at 2000 rpm for 15 s, and the epoxy coated films were pre-cured at 100 °C for 30 min with about 50% conversion. The suspension of larger silica particles (10%) in ethanol was spin-coated on the pre-cured epoxy film surfaces at 300 rpm for 2 min. After that, these samples were completely cured at 100 °C for 24 h. Finally, these coatings were sonicated in an ethanol bath for 30 min to remove possibly loosely attached particles and dried by air flow. These samples with

single-scale roughness were labeled as Film-Single (**F-S** in short).

Fabrication of dual-scale and triple-scale roughened surface

The dual-scale roughened surfaces were prepared according to the following procedure: F-S samples were immersed in 3-aminopropyl-triethoxysiloxane (APS) toluene solution (1%) for 30 min, rinsed with dry toluene and ethanol, leading to amine-functionalized surface. Subsequently, these samples were immersed in HCl solution (0.1 mol/L) for 30 s to render the surface positively charged. Then the protonated surfaces were dipped into the suspension of medium silica particle to obtain dual-scale structured surface by electrostatic attraction between protonated surface and negatively charged silica particle. Afterwards, the samples were rinsed several times in ethanol, dried in oven and covalently cross-linked by $SiCl_4$/toluene solution (0.1 vol %). Finally, the raspberry-like particle surfaces with dual-scale roughness structure were obtained. All these samples were labeled as **F-D**.

The fabrication of triple-scale structured surfaces was similar to the preparation of F-D described above by depositing a layer of nanosilica (40 or 10 nm) onto the D-S surface, except that a 0.05 % $SiCl_4$ toluene solution was used to consolidate the structured surface. These resulted samples with different nanosilica particles (40 & 10 nm) were labeled as **F-T40** and **F-T10**, respectively.

Surface Fluorination

To render these structured samples superhydrophobic, the sample surface was chemically modified with *1H,1H,2H,2H*-perfluorodecyl trichlorosilane via the chemical vapor deposition at 100 °C for 20-40 min in sealed vials. At the end of reaction, the treated samples were rinsed with anhydrous toluene, sonicated for 5 min to remove residue, and then dried in an oven at 100 °C for 30 min.

Characterizations and measurements

Static water contact angles and the roll-off angles were measured with deionized water on a Ramé-Hart 290 contact angle goniometer at room temperature (~21 °C). Advancing and receding angles were not provided in this work because even slight vibration could cause the water droplets to roll off of the samples of F-T40-Rf and F-T10-Rf. Instead, static water contact angles on the sample surface were measured by dropping water droplets with different volume (2, 5 and 10 μL) on the film

surfaces. In addition, roll-off angles were collected for water droplets (2, 5 and 10 μL) by slowly tilting the sample stage. All the contact angles and roll-off angles were determined by averaging values measured at 3-4 different points on each sample surface. SEM images were recorded using a JEOL scanning electron microscope under high-vacuum mode operated at a 1 kV acceleration voltage; samples were coated with a thin layer of gold before observation.

Protein Adsorption Test by BCA assay

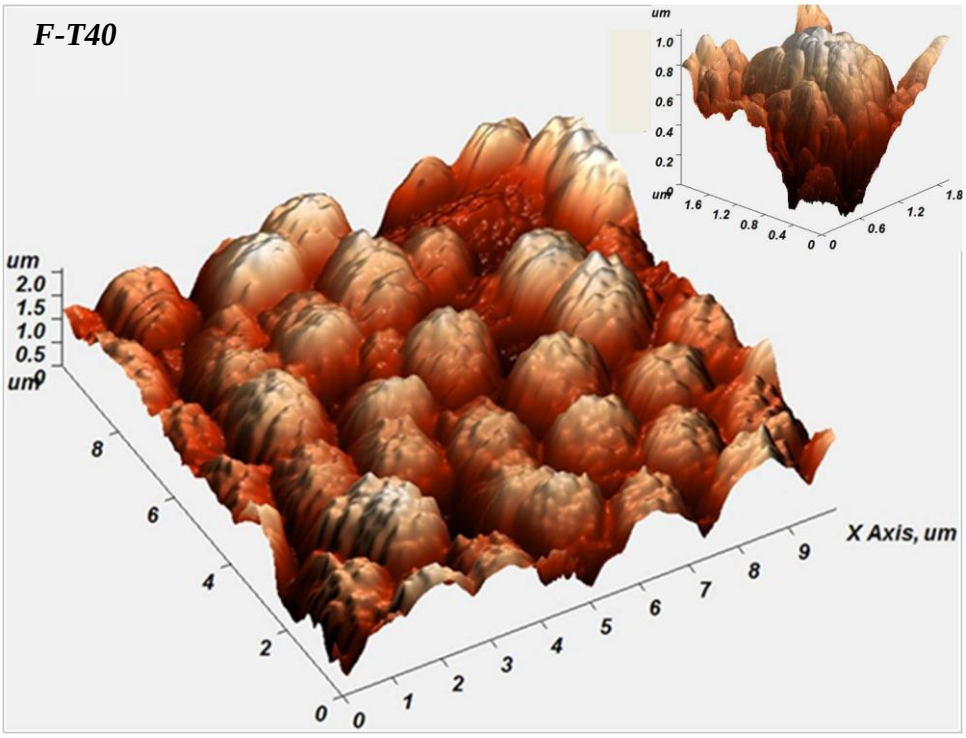
To evaluate nonspecific protein adsorption on samples, bovine serum albumin (BSA) was used as a test protein and the bicinchoninic acid (BCA) assay was adopted as the qualitative and quantitative method.[3,4] The samples (2×2 cm^2) were soaked in PBS solution at 37 °C for 1 h, and then incubated in PBS solution containing BSA (1 mg mL^{-1}) at 37 °C for 1 h. After being rinsed five times with fresh PBS solution, the samples were soaked in an aqueous solution of 1.0 wt% SDS, and oscillated at 37 °C for 1 h to remove the adsorbed proteins on the surfaces. Based on the BCA protein assay kit method, the amount of protein remaining in the SDS solution was determined by a microplate reader (Tecan Sunrise, Switzerland). The adsorbed BSA on the samples was thus calculated. The reported data were the mean values of triplicate specimens for each sample.

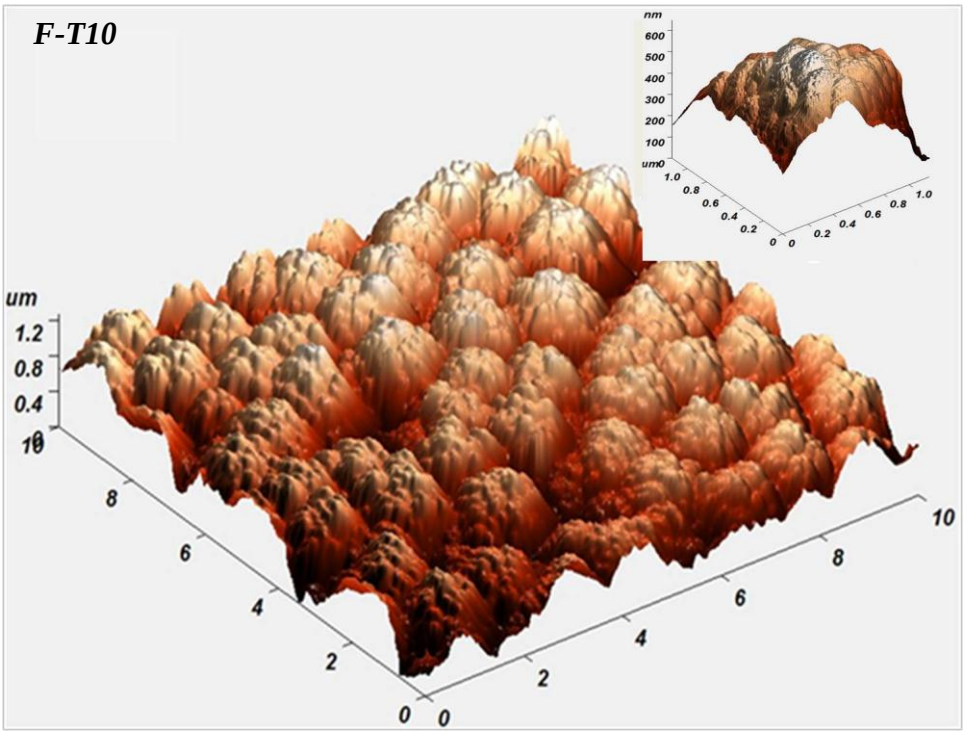
Platelet Adhesion

All samples were placed into a 6-well polystyrene plate, sterilized by ethanol for 2 h, dried under vacuum at room temperature for 12 h, and then equilibrated by PBS at 37 °C for 30 min. The samples were covered completely by 1 mL of fresh PRP (platelet-rich plasma obtained from the fresh rabbit blood by centrifuge at 800 rpm for 10 min), followed by incubation for 60 min at 37 °C. After being rinsed 3 times with PBS solution, the adhered platelet on the sample was fixated with 2.5 wt% glutaraldehyde at 4 °C for 8 h. Finally, the samples were cleaned several times with PBS solution, and sequentially dehydrated with a series of ethanol aqueous solution (10, 30, 50, 70, 90, 100 vol%). The samples were observed with a field emission scanning electron microscopy (FESEM, XL 30 ESEM FEG, FEI Company).[5]

2. AFM images of triple-scale structured surfaces

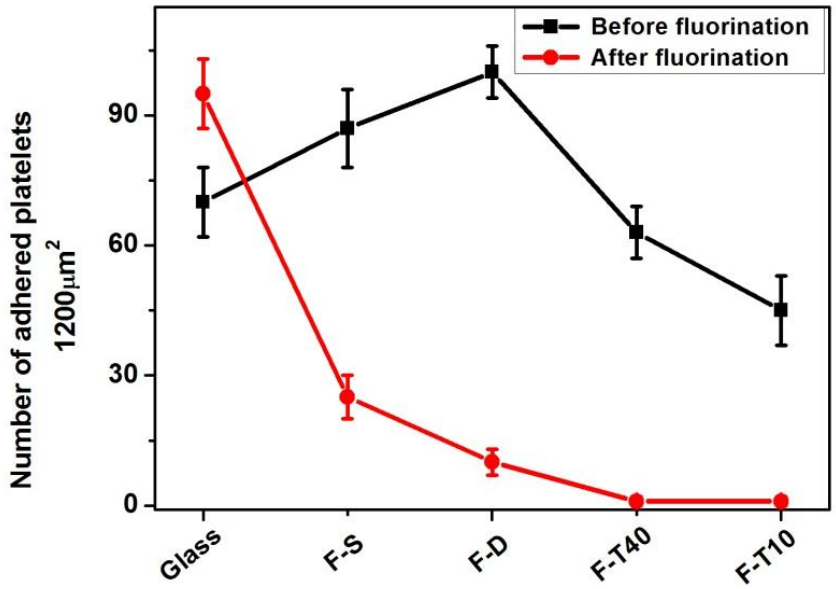
The triple-scale structured surfaces (both F-T40 and F-T10) were also examined by AFM, as shown below; the secondary- and tertiary-level particles are clearly shown, especially in the insets.





3. Quantitative analysis of reduced platelet adhesion on hierarchically structured surfaces

The figure below shows quantitative results on reduced platelet adhesion on hierarchically structured surfaces over the area of 1200 μm^2. Compared to the control glass (non-fluorinated), the platelet adhesion was reduced by ~15% and 35% on F-T40 and F-T10, respectively. For superhydrophobic surfaces, the reduction was much more significant: compared to the control glass (non-fluorinated), the platelet adhesion was reduced by ~65% and 85% on F-S-Rf and F-D-Rf, respectively; no platelet adhesion was observed on F-T40-Rf and F-T10-Rf at all.



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