

Supporting Information

The Amplified Effect of Surface Charge on Cell Adhesion by Nanostructures†

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KEYWORDS: nanostructure; surface charge; amplified effect; cell adhesion

1. Materials.

Sulfuric acid (98%, AR), hydrogen peroxide (30%, AR), acetone (> 99.5%, AR), alcohol (\geq 99.8%, GR) and dimethylsulfoxide (DMSO 99.5%, AR) were purchased from Beijing Chemical Works. Silicon tetrachloride (SiCl_4) and phosphate buffer solution (PBS) were purchased from Thermo Scientific. DMEM growth medium were purchased from Invitrogen. NIH/3T3 cell line was purchased from Beijing Xiehe Cell Resource Center. Triton X-100 was purchased from Aladdin Chemistry Co. Ltd. 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) was purchased from ROCHE. Fetal Bovine Serum (FBS) was purchased from Fisher Scientific. Six well cell culture plates were purchased from Corning Incorporated (Costar). 3-mercaptopropyl trimethoxysilane (MPTMS) was purchased from Sigma-Aldrich. 3-Maleimidopropionic acid (MPA) and N-(2-Aminoethyl)maleimide hydrochloride (AMH) were purchased from TCI (Tokyo Chemical Industry).

2. Preparation of silica nanostructured biointerfaces.

The silica nanostructured biointerfaces (nano-biointerfaces) with the size of 1 cm \times 1 cm were prepared according to our previous reports.^{1, 2} Firstly, the glass substrate was ultrasonicated in acetone, ethanol and deionized (DI) water for 10, 10 and 5 min at room temperature (R.T.), respectively. Then, the glass substrate was cleaned by boiling in a Piranha solution (a mixture of 98% H_2SO_4 and 30% H_2O_2 with 7:3 v/v; CAUTION! Piranha solution can react violently with organic materials, and should be handled carefully) for 30 min. Later, it was well rinsed with DI water and dried with nitrogen gas (N_2). Secondly, a layer of carbon-based template was successfully

deposited by placing the cleaned glass substrate on a carbon source (*i.e.*, burning candle). Thirdly, a layer of silica shell was coated on the carbon-based template. To absorb sufficient water, the substrate with surface carbon-based template and 3 mL DI water was separately placed in a well of six-well plate at 37 °C for ca. 1 h. Then, the substrate with carbon-based template was placed in an airproof petri dish containing 0.1 mL of silicon tetrachloride (SiCl₄) for ca. 1 h. Through the chemical deposition reaction between SiCl₄ and absorbed water, the carbon/silica nanocomposites can be readily fabricated by depositing silica shell on the surface of carbon-based template. After that, the carbon template can be easily removed by heating the as-prepared nanocomposites at 600 °C for ca. 2 h, leading to desired nano-biointerfaces. Therefore, by precisely controlling the deposition time, a series of nano-biointerfaces were successfully fabricated with the thicknesses of 3.9 ± 0.3 , 8.1 ± 0.4 and 13.4 ± 0.7 μm , respectively (Fig. S1).

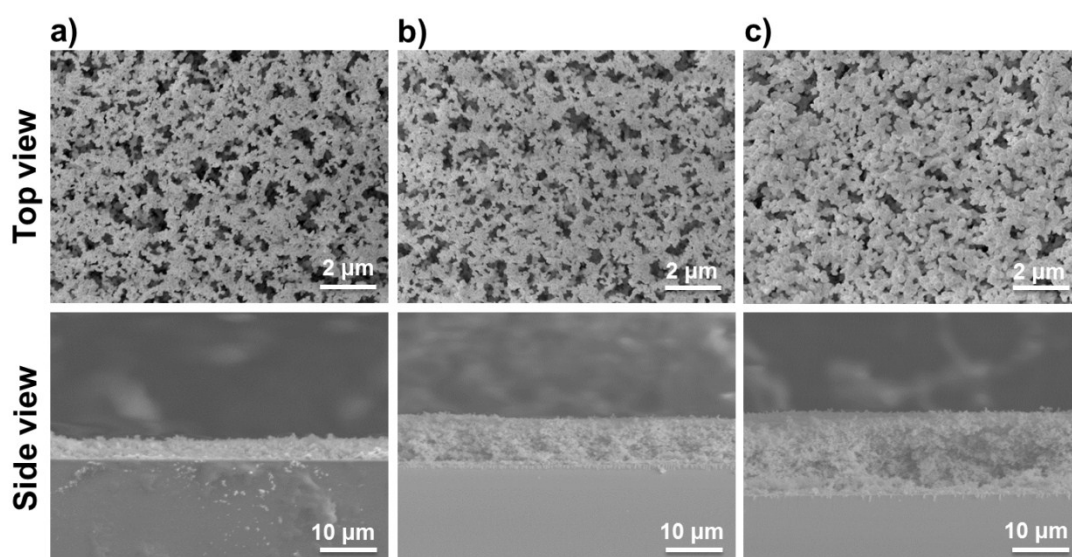


Fig. S1. Scanning emission microscopy (SEM) images of a series of nano-biointerfaces with the thicknesses of (a) 3.9 ± 0.3 , (b) 8.1 ± 0.4 and (c) 13.4 ± 0.7 μm ,

respectively. Error bars represent standard error of the mean ($n = 3$).

3. The modification of surface charge on silica biointerfaces.

Surface charges can be modified on silica biointerfaces by chemically grafting functional molecules including AMH with terminal amino ($-\text{NH}_2$) group for positive charges and MPA with carboxyl ($-\text{COOH}$) group for negative charges. As shown in Fig. S2, these biointerfaces were firstly treated with O_2 plasma for 300 s at 150 w. Then, 1% (v/v) MPTMS in ethanol is used to treat these substrates for 12 h (R.T.).³ After that, these substrates are sequentially washed with ethanol and DMSO. Later, charged substrates can be readily prepared by further grafting 2 mM functional molecules in DMSO for 6 h (R.T.) including AMH for positive charges and MPA for negative charges, respectively.³⁻⁵ Finally, these substrates are flushed with 1x PBS to remove excess functional molecules and stored in PBS solution ($\text{pH} \approx 7.2$) for 30 min to obtain charged surfaces. The pK_a value of AMH and MPA is 8.39 ± 0.10 and 4.18 ± 0.10 , respectively. During the process of cell capture and wash, the pH value of employed PBS and DMEM culture medium is ca. 7.2, which endow AMH and MPA-modified biointerfaces with different surface charges.

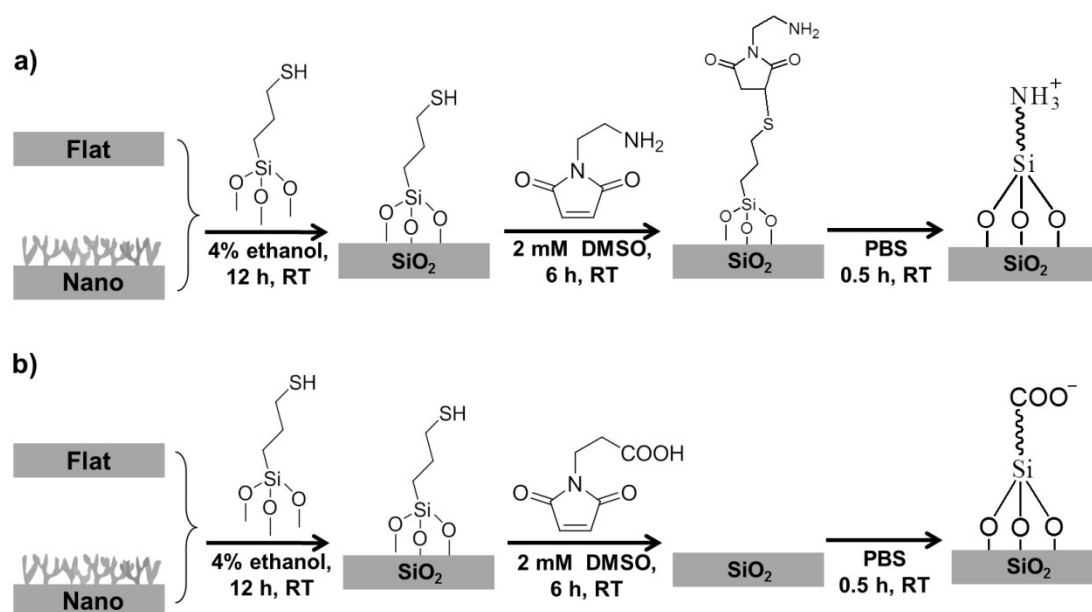


Fig. S2. The chemical modification process of silica biointerfaces with surface charges, providing (a) positively or (b) negatively charged surfaces by grafting functional molecules with terminal amino (-NH₂) or carboxyl (-COOH) group.

4. The characterizations of surface charge

The successful modification of surface charges on flat silica was performed by using a Kelvin probe force microscopy (KFM) (Bruker Inc.) in lift mode. Firstly, the samples with surface charge can be prepared by modifying MPTMS on flat silica. After immersing into DMSO solution of AMH or MPA, PDMS stamps are further employed to prepare strips on above MPTMS-modified silica. Finally, bright and dark strips with the potential difference of ca. 30 and 50 mV can be clearly observed in the KFM images of negatively and positively charged surface (Fig. S3a and S3b). Different from their KFM images, the corresponding atomic force microscope (AFM) images in tapping mode (Fig. S3c and S3d) exhibit negligible differences of topography, suggesting the successful modification of surface charges.

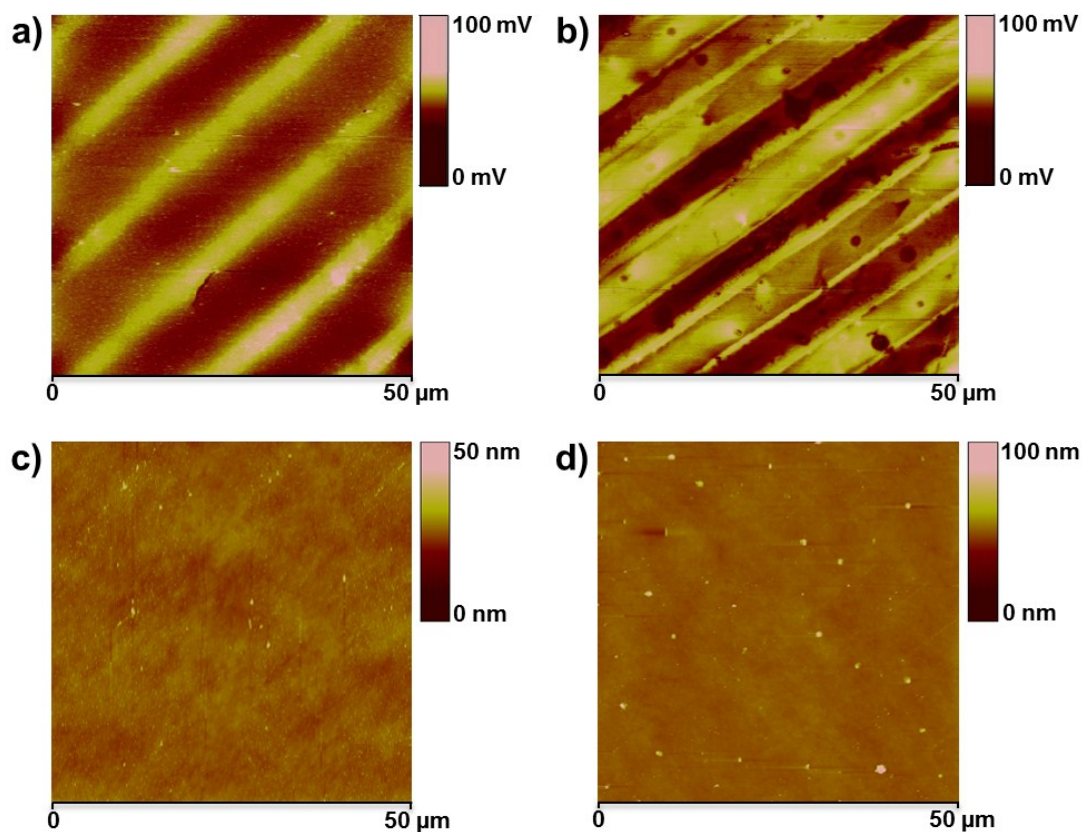


Fig. S3. The surface charge and roughness characterizations of functional group modified flat silica biointerfaces. The KFM images of (a) negatively and (b) positively charged surfaces by grafting terminal carboxyl (-COOH) and amino (-NH₂) group. The corresponding AFM topography images of (c) negatively and (d) positively charged biointerfaces. All the image sizes are 50 μm × 50 μm.

5. The procedure of cell capture.

After placing the charged biointerfaces into six-well cell culture plates, 3 mL NIH/3T3 cell suspensions with concentration of 1×10^5 cells/mL were carefully added into each well and incubated at 37 °C and 5% CO₂ in a cell incubator (Thermo Forma Series II, Thermo Scientific). To remove the unadhered cells, the substrates were gently rinsed with PBS for three times. To better observe the adhesive performance of these cells on charged substrates, the adhered cells were sequentially

treated in 2.5% (v/v) glutaraldehyde in PBS, 0.2% (v/v) TritonX-100 in PBS and 2 $\mu\text{g mL}^{-1}$ DAPI solution diluted by PBS for 20, 10 and 15 min, respectively. Finally, the fluorescent images of adhered cells can be observed by an inverted microscope (Nikon Ti-E).

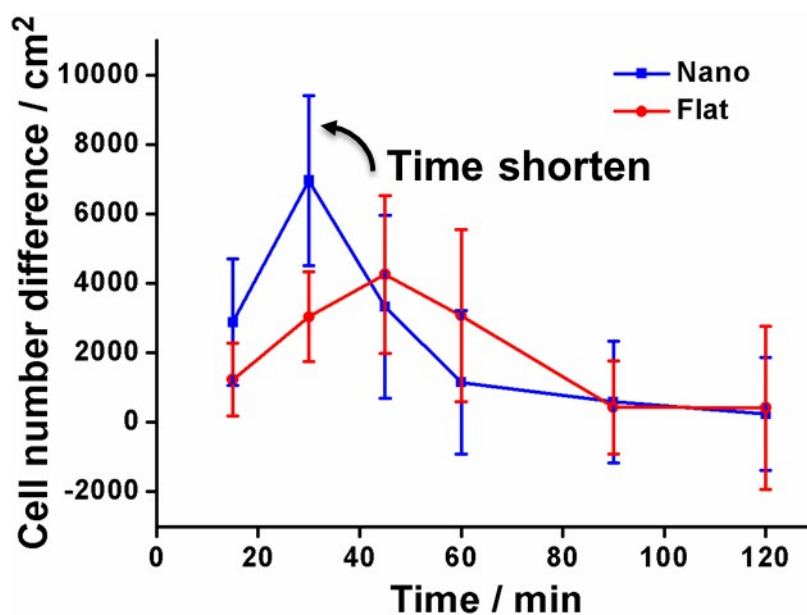


Fig. S4. The incubation time for the largest difference of cell adhesion between positive and negative biointerfaces is shortened from 45 min for flat to 30 min for nano, revealing that nanostructure is more benefit for the sufficient contact between cells and surface charge.

6. Environmental SEM (ESEM) observation.

The morphologies of adhered NIH/3T3 cells were characterized by an ESEM (FEI Quanta 200) in low vacuum mode. The NIH/3T3 cells were firstly incubated on charged biointerfaces (*i.e.*, flat and nano) for ca. 30 min. After gently rinsed with PBS for three times, the adhered cells were fixed with 2.5% (v/v) glutaraldehyde in PBS for ca. 12 h, followed by a PBS wash. Then, the cells were dehydrated using a series of gradient ethanol solution (30%, 50%, 70%, 85%, 95% and 100%) for ca. 15 min,

respectively. Finally, the samples were dried in critical CO₂ condition for ESEM observation.

Reference

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