Supporting Information

In Situ Growth of a Polyphosphazene Nanoparticles Coating on a Honeycomb Surface: Facilely Forming of Hierarchical Structure for Bioapplication

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Supporting information for this article is given via a link at the end of the document.

1.1. Materials

Polystyrene (PS, Mw = 250 kDa) was purchased from Acros Reagents. Phosphonitrilic chloride trimer (HCCP, 99%) was obtained from Admas. 4,4-Dihydroxydiphenylsulfone (BPS, 99%), triethylamine (TEA, 99%), and the solvents toluene and ethanol were from Sinopharm Chemical Reagent Co., Ltd. HeLa cells were obtained from the Cell Resource Center of Life Sciences in Shanghai. Bovine serum albumin (66 kDa, >98% purity), fetal bovine serum (FBS), and Dulbecco's modified Eagle medium (DMEM) were obtained from Gibco BRL. Cell-staining dyes and all other cell culture reagents were purchased from the Beyotime Institute of Biotechnology. Water used in the experiments was purified with a Hitech system to a resistivity of above18.2 MΩ·cm.

1.2. Characterization of membranes

The morphologies of PSHCF and PNPs-pebbled PSHCF were examined by scanning electron microscopy (SEM) using an FEI Nova NanoSEM (FEI, USA). Contact angle were measured on an OCA 20 apparatus (Dataphysics, Germany). Water and diiodomethane contact angles were analyzed through the Owens–Wendt–Rabel–Kaelble (OWRK) method to calculate the surface energy. The contact angle of the PNPs powder was measured on an SCAT 11 unit (Dataphysics, Germany). To analyze the element distribution, energy-dispersive spectrometry was performed on a Sirion 200 field emission scanning electron microscope (operated at a voltage of 20 kV; FEI, USA) equipped with an INCA X-Act energy-dispersive spectrometer (Oxford, UK).

1.3. Fabrication and characterization of PSHCF

PS (1 g, 250 kDa molecular weight) was added to 100 mL of toluene, and the resulting mixture was stored overnight at room temperature, after which PS was completely dissolved. Subsequently, 40 μL of the solution was casted onto a 10 × 10 mm glass substrate in a chamber constructed in-house. Air (~60% RH, 4 L/min) flowed across the surface of the substrate.

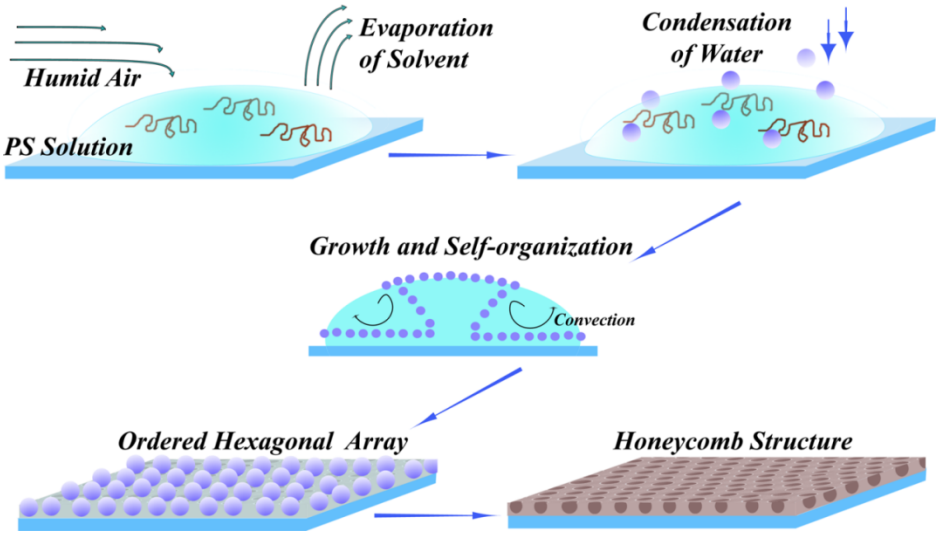

Figure S1. Scheme of preparation of the honeycomb-structured surface through the standard breath-figure method. The PS solution was casted onto the substrates, over which a stream of humid air flowed. Evaporation of the solvent cooled the solution surface, leading to condensation of water droplets from the humid air. The condensed water droplets self-assemble, resulting in convectional flow and capillary force. After the solvent and water dried off, well-organized hexagonally porous arrays comprising the honeycomb structure formed.

1.4. Decoration of PSHCF with PNPs

HCCP and BPS at a mole ratio of 1:3 were dissolved in ethanol ending with a concentration of HCCP at about 3 mg/mL, and 2 mL of the resulting solution was added to a Φ3.5 cm culture dish that contained two pieces of PSHCFs. Subsequently, 50 μL of TEA was added. Growth of PNPs onto the PSHCF was stirred for 2 h at ambient conditions. The resulting films were rinsed twice with deionized water and ethanol to remove byproducts and unattached PNPs, and then dried at 40 °C in a vacuum overnight.

The surface energy was calculated on the basis of the OWRK method. This method is widely used in calculating the surface free energy of a solid from its contact angle with several liquids. In the present study, water and diiodomethane were used to measure the contact angle.

To measure the surface energy of polyphosphazene nanoparticles (PNPs), PNPs were synthesized in solution. PNPs in the solvent was centrifuged, rinsed twice with water and ethanol, and then dried at 40 °C in a vacuum overnight. Apparent and intrinsic surface energies were

measured. As shown in Figure S2, the intrinsic surface energy was calculated after the nanoparticles were ground and pressed into a film; the apparent surface energy of polyphosphazene nanoparticles was obtained from measurement of the surface energy of the powder based on the capillary effect.

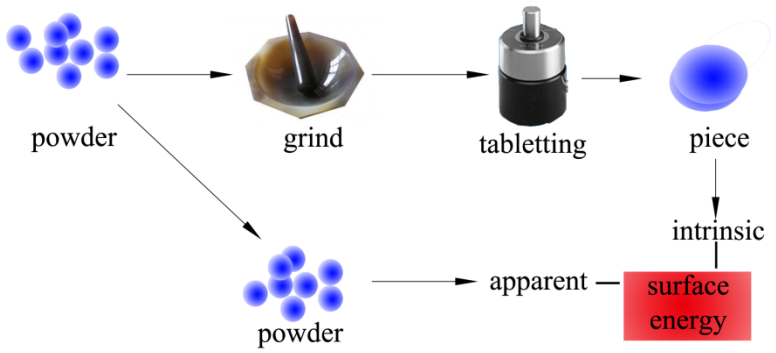

Figure S2. Measurement of the apparent and intrinsic surface energies of polyphosphazene nanoparticles.

1.5. Simulation

To understand the growth of PNPs on PSHCF, we established a model to investigate the approach of PNPs to substrates of untreated and plasma-treated PSHCF. In this model, PS repeat units approach the PNPs unit from six directions from 10 to 3 Å. The potential energy was calculated by using the Gaussian 09W package (Figure S3). Calculations used density functional theory at B3LYP level based on the 6-31G basis set. First, the geometry corresponding to the minimum potential energy of the monomers was optimized by solving a self-consistent field equation. The system energy of the system at 10 and 3 Å were then calculated at the amber force field. As shown in Table S1, the energy difference for the PNPs–PS system is 8.9199 kcal/mol, which indicates that it takes 8.9199 kcal/mol to support this approach. Meanwhile, we performed calculations for the approach of PNPs to plasma-treated PSHCF substrate. We found that approach to the plasma-treated substrate requires more than10 kcal/mol. Thus, we believe that attachment of PNPs onto a plasma-treated PS substrate is more difficult.

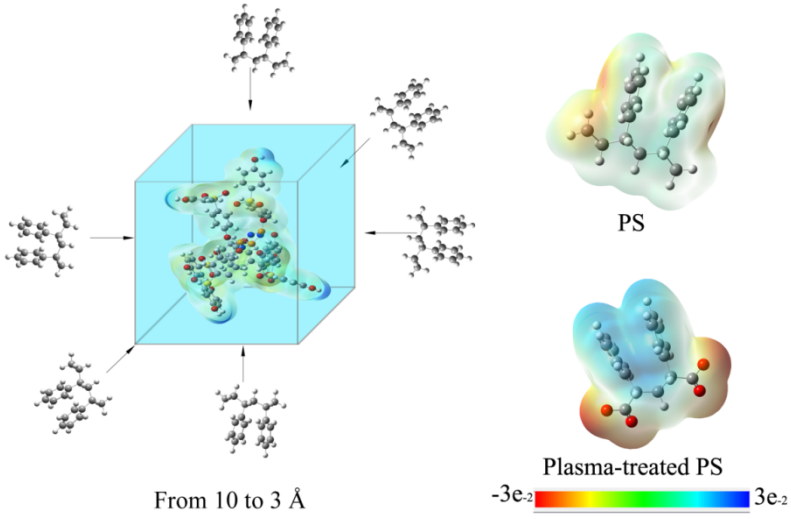

Figure S3. Model of the approach of polyphosphazene to substrates. Here, two repeat units of styrene were used to simulate the movement of the PS substrate from six directions, from 10 to 3 Å. This movement describes the attachment of polyphosphazene onto the substrate.

Table S1. Energies resulting from the approach of 10 to 3 Å

System	Energy (kcal/mol)	Δ*E* (kcal/mol)
PNPs-6PS-10	17,459.0059	8.9199
PNPs-6PS-3	17,450.0860	
PNPs-6PL-PS-10	17,465.3300	10.9290
PNPs-6PL-PS-3	17,454.4010	

Note: The PNPs-6PS system describes the approach of PNPs to untreated PS; the PNPs-6PL-PS system describes the approach of PNPs to plasma-treated PS.

1.6. The controllable size of PNPs

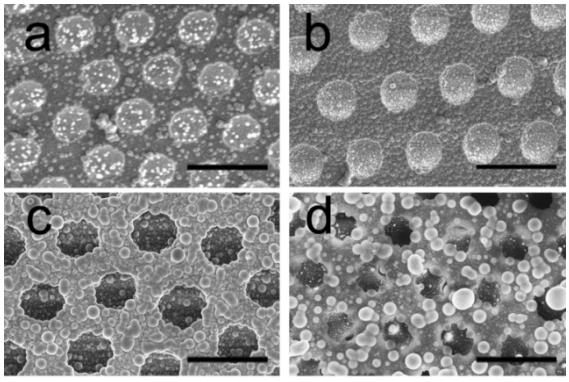

Figure S4. (a - d)SEM images of PSHCF decorated with PNPs. The HCCP/BPS ratio was fixed at 1:3. The HCCP monomer concentrations were a) 1 mg.mL^{-1}, b) 2 mg.mL^{-1}, c) 3 mg.mL^{-1}, and d) 4 mg.mL^{-1}. Scale bar = 10 μm.

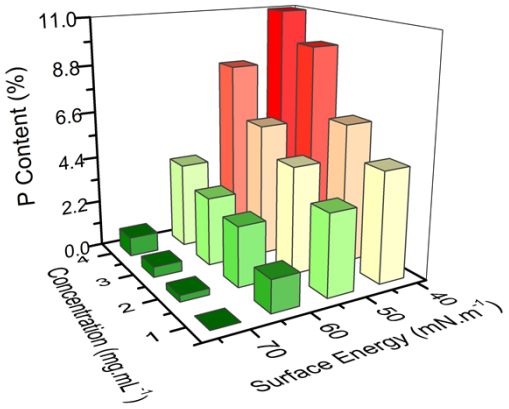

Figure S5. The relationship of polyphosphazene nanoparticles content (in weight, based on the EDS analysis) with concentration of monomers and the surface energy of polystyrene substrate in a 3-dimontional bar figure.

1.7. Plasma treatment

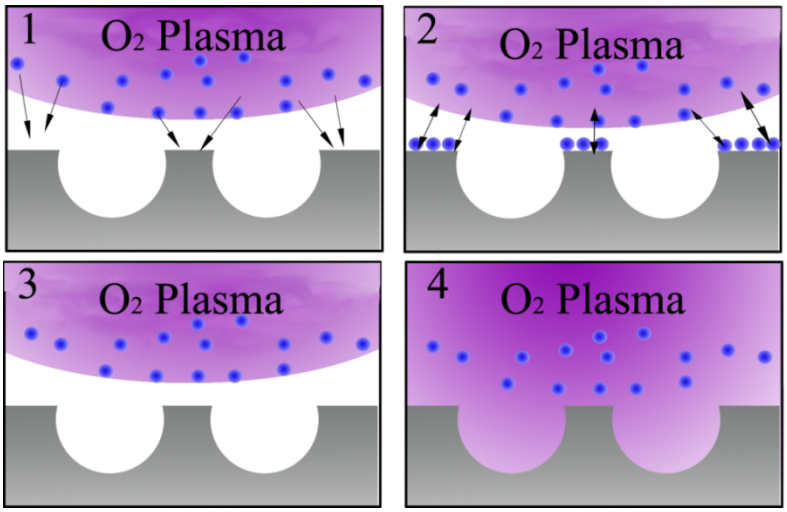

Figure S6. Schematic of the boundary electric field during plasma treatment. 1, the plasma sheath arriving to the surface of honeycomb; 2 the moment that the plasma sheath attaching to the surface of honeycomb endows the surface with the same charge as that of the plasma sheath; 3, surficial charge would be pumped away; 4, with the extended irradiation time, the inner surface would be modified as well.

We selectively modified the surface of PSHCF, which originally had hydrophobic honeycomb pores, to form a hydrophilic surface through plasma treatment with high-intensity O_2 plasma (350 W) for 15 s. A plausible mechanism of selective modification is shown in Figure S5. During brief irradiation, the surface of the honeycomb has the same charge as that of the plasma sheath. The resulting mutual repulsion due to an electric field restrains further modification of the honeycomb pores, thus retaining the hydrophobicity of the holes. With extended irradiation time, the electric field around the charged particles on the surface dissipates, and the positive charge in

the sheath affects the boundary electric field. Finally, the porous domain and the top surface undergo modification (Figure S5-4).

1.8. EDS line scanning of selectively patterned polyphosphazene

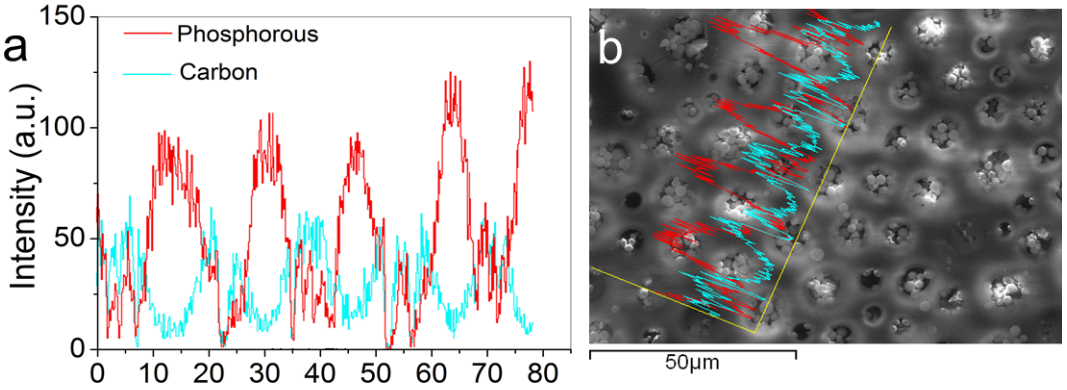

Figure S7. a) EDS spectrum and b) image of nanoparticles selectively modified in the hole of honeycomb structure.

1.9. Cell culture

The membranes were sterilized with medicinal alcohol, exposed to UV light for 24 h, placed in a 24-well plate, and then rinsed with PBS (2 × 1 mL). Cells were cultured in DMEM with 10% FBS and 1% penicillin–streptomycin solution, seeded at a density of 10^5 cells per well, and then incubated at 37 °C in a humid atmosphere with 5% CO_2 for 24 h, 48h and 72h. Membranes with adherent cells were then rinsed twice with PBS to remove unattached cells. Cell viability was assessed by dead/live (EB/AO) double staining and CCK-8 assay. An inverted fluorescence microscope (IX 71, Olympus) equipped with a CCD camera was used for cell imaging. SEM was also used for analysis of the cellular morphology. Cells were fixed with 2.5% glutaraldehyde solution for 20 min and then dehydrated with graded ethanol solutions. Samples were sputtered with Pt and then examined under an FEI Nova NanoSEM 450.

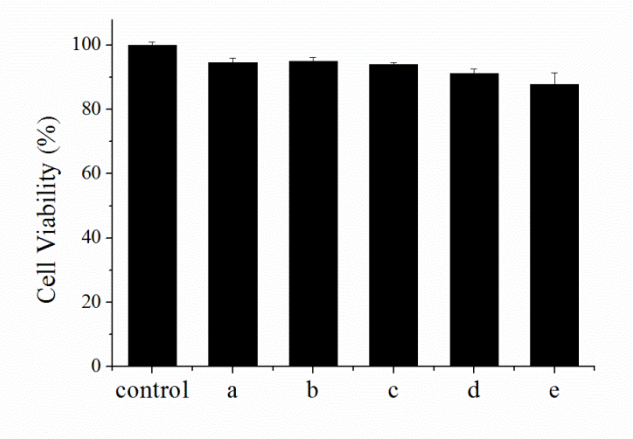

Figure S8. Cell viability on plates of PS (control) and PSHCF (a), hydrophilic PSHCF decorated with PNPs (water contact angles at 60°) (b), and PSHCF decorated with PNPs prepared at HCCP concentrations of 1 (c), 3 (d), and 4 mg/mL (e). Results of CCK-8 assay show that all the membranes were compatible with cells (Figure S6),

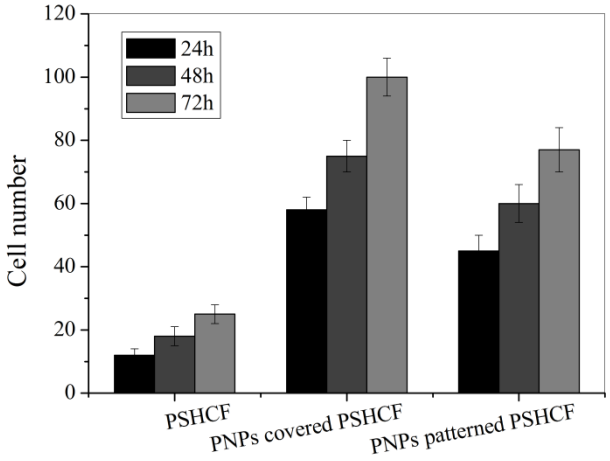

Figure S9. Statistical count of cell number on the membranes cultured for 24h, 48h and 72h. The data was analyzed and calculated based on the fluorescent images in ImageJ software. With the time going, the difference between original PSHCF and PNPs modified PSHCF become obviously larger.

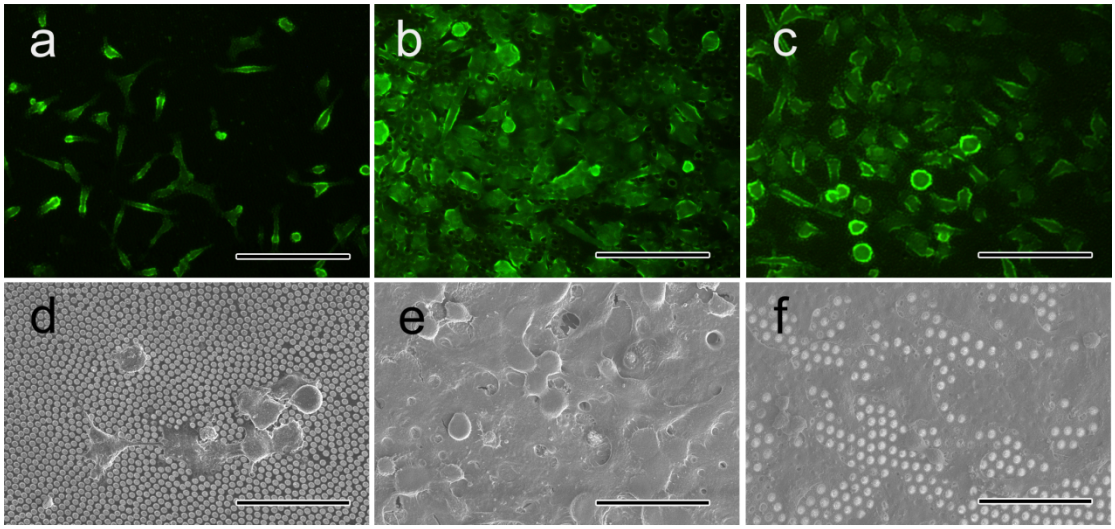

Figure S10. Fluorescence images (line 1, scale bar =200 μm) and SEM images (line 2, scale bar =50 μm) of Cells cultured for 72 h on PSHCF (column 1), PNPs covered PSHCF (column 2) and PNPs selectively patterned PSHCF (column 3). The difference between the original PSHCF and PNPs modified surfaces become obviously larger when cultured for longer time. Besides, cells on the PNPs modified surface exhibited more spreading morphologies.