Electronic Supplementary Material (ESI) for Polymer Chemistry.

Self-defensive bilayer hydrogel coating with bacteria triggered switch from cell adhesion to antibacterial adhesion

Min He[a], Qian Wang[a], Weifeng Zhao[a] *, Jianshu Li and Changsheng Zhao[a]**

[a] College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu 610065, People's Republic of China

* Corresponding author. Tel: +86-28-85400453, Fax: +86-28-85405402, E-mail: zhaoscukth@163.com or weifeng@kth.se (W.F. Zhao); zhaochsh70@163.com or zhaochsh70@scu.edu.cn (C.S. Zhao)

1.1 Synthesis of ene-functionalized P(SBMA-co-AA-co-HEMA)

The free radical polymerization was initiated by KPS in water at 70 °C. For ene-functionalization, the condensation reaction was carried out in pure water in the presence of EDC/NHS, and the mole ratio of AA /allylamine/EDC/NHS was set at 1:2:2:2. Typically, the copolymers were completely dissolved in pure water; then, weighted EDC/NHS was added to the solution with a pH of 4.5. After stirring for 2 h, aqueous allylamine solution was added to the medium associated with the adjustment of the pH to 8.0 by 0.1 mol/L NaOH solution. After 48 h reaction, the solution was dialyzed in pure water to remove the unreacted EDC/NHS for 4 days. Finally, the functionalized polymers were achieved by freeze-drying.

1.2 Protein adsorption test

The wafers with the area of 1×1 cm^2 for each were incubated in physiological saline at 4 °C for 24 h and equilibrated at 37 °C for 1 h, and then immersed in the protein solution at 37 °C for 2 h. Afterwards, the wafers were gently rinsed with PBS and then incubated in 2 wt. % aqueous sodium dodecyl sulfate (SDS) solution at 37 °C for 1 h under agitation to remove the adsorbed protein. The protein concentrations in the solutions were quantified by Micro BCA™ protein assay reagent kits using an UV–vis spectrophotometer (UV-1750, Shimadzu, Japan) at a wavelength of 562 nm.

1.3 Cytocompatibility

The viabilities of L929 cells proliferated on the bilayer coating attached wafers were assessed by CCK-8 assay. The wafers were sterilized by washing with 75% (v/v) ethanol solution, subsequently rinsed three times with PBS solution, and then treated with UV light sterilization for 2 h. After sterilization, the wafers were fixed in a 24-well plate. L929 cells were seeded with a density of 2.0×10^4 cells/mL. The plate was incubated at 37 °C under 5% CO_2 atmosphere; and the medium was changed every two days. After incubating for 1, 3, 5, and 7 days, the wafers were washed with PBS for three times to remove the cells that did not attach onto the wafers, and then 100 μL of CCK-8 (FINAL DILUTION: 1:10) was added into each well to test the live cells. After incubating for another 4 h, 100 μL of the supernatant was transferred into a 96-well plate for optical density (O.D.) measurements using a microplate reader (Model 550, Bio-Rad) at 450 nm.

To observe the cell morphology, the cell-cultured Si wafers were washed with PBS for three times, then fixed with 3 vol.% glutaraldehyde at 4 °C for 2 h, soaked in 0.18 M sucrose solutions at 4 °C for 2 h, dehydrated through a series of graded ethanol solutions (15, 30, 45, 70, 90 and 100 vol.%), and lyophilized overnight. The morphologies of the cells on the membranes were observed by SEM.

2.2 The chemical compositions of the heparin-mimicking polymer chains.

To characterize the chemical composition of the heparin-mimicking polymer, 100 U CE were dissolved into 6 mL heavy water. 3 mL of the prepared CE solution were directly detected by ^{13}C-NMR. Another 3 mL CE solutions were used to detach the heparin-mimicking chains from five pieces of Si30 sample, then the solutions were detected by ^{13}C-NMR. As shown in Fig. S3, the peaks at 38, 57, and 46 were attributed to the segments of sodium allylsulfonate (SAS), while the peaks at 38, 49, and 170 were attributed to sodium acrylate (AANa). The mole ratio of SAS to AANa was calculated by the peak area ratio of peak 2 to peak 6. The results indicated that the proportion of AANa in the polymer chain was around 57 %.

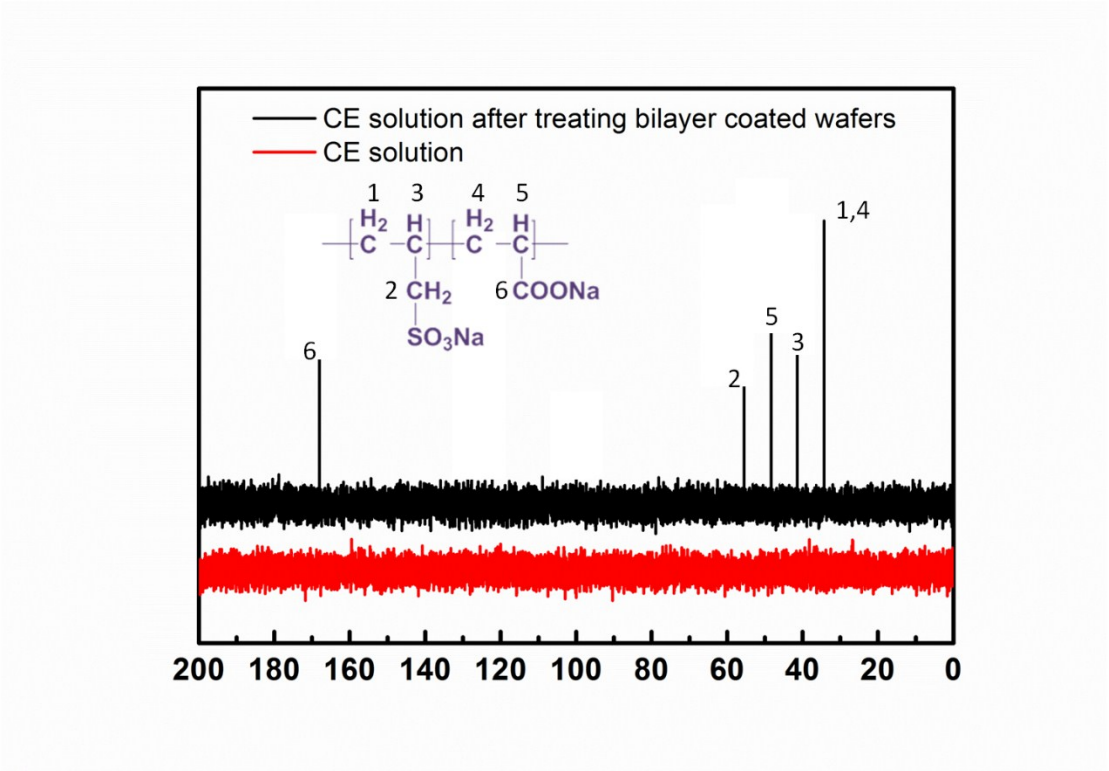

Fig. S1. ^{13}C-NMR spectrum of CE solution and CE solution after treating bilayer coated wafers.

2.1 The surface morphologies of the modified Si wafers

The surface morphologies of the bilayer coatings were observed by SEM, as shown in Fig. S1. Compared with pure Si wafer, dense cuspidal outshoots were observed on the bilayer coating surfaces, which might be formed via the shrinking of the hydrogel layer and the aggregation of the grafted polymer chains. The size of the cuspidal outshoots increased with the increase of the polymer brush density. To further investigate the surface morphology variations after each step of the modification, AFM was utilized to detect the morphology of pure Si wafer, the hydrogel layer attached Si wafer, and the bilayer coated Si wafer with a tapping mode at a scan rate of 0.8 Hz over an area of 5.0×5.0 μm^2 (Fig. S2). After attaching the hydrogel layer, the surface became rougher, with the root-mean-squared (Rq) value increased from 0.5 to 1.5 nm. After grafting the polymer brushes, the Rq further increased to 2.1 nm. The morphology of the contour pictures was greatly influenced by the size of scale bar. In this study, we setted a smaller scale bar of 10 nm, so the contour

pictures showed a rougher morphology. If we set a bigger scale bar, a smoother morphology could be observed, as shown in Fig. S4.

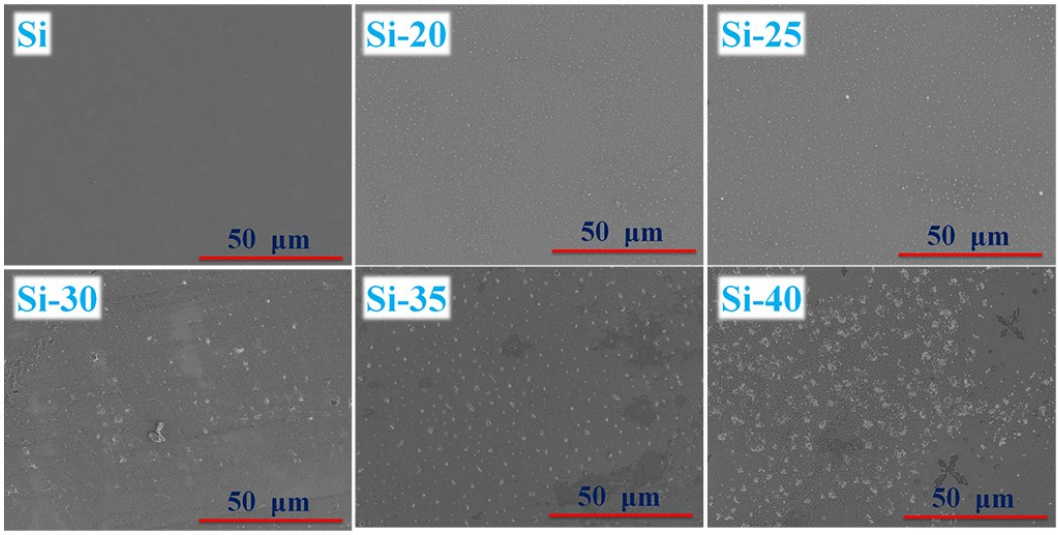

Fig. S2 Surface morphologies of the pure Si wafer and the modified Si wafers.

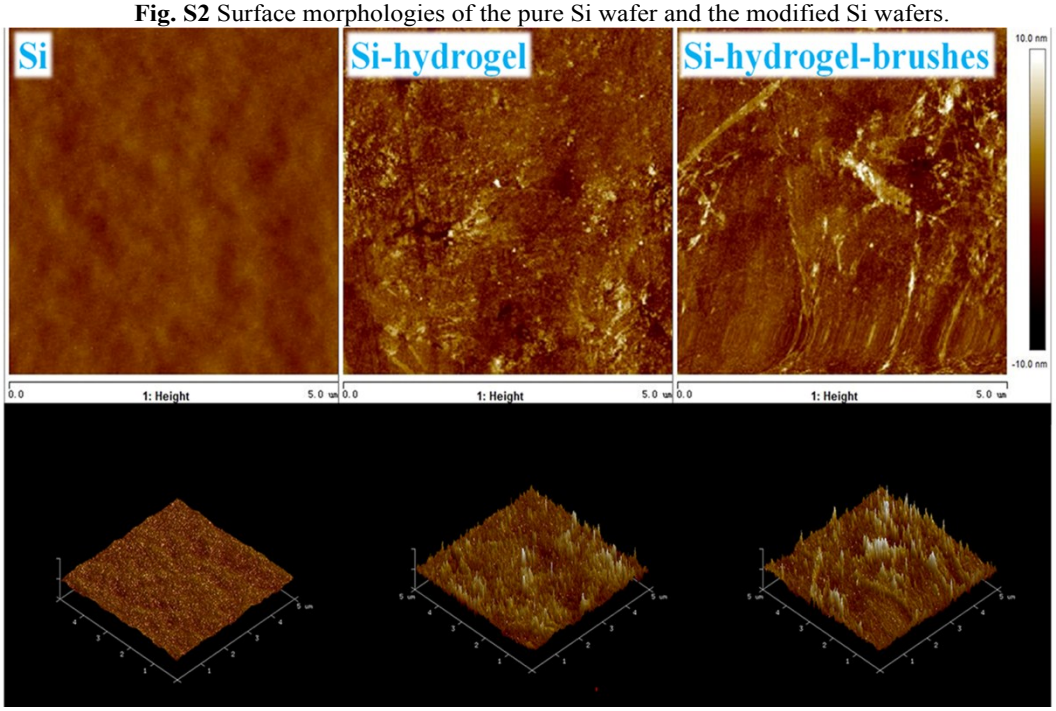

Fig. S3 AFM images of the pure Si wafer and the modified wafers.

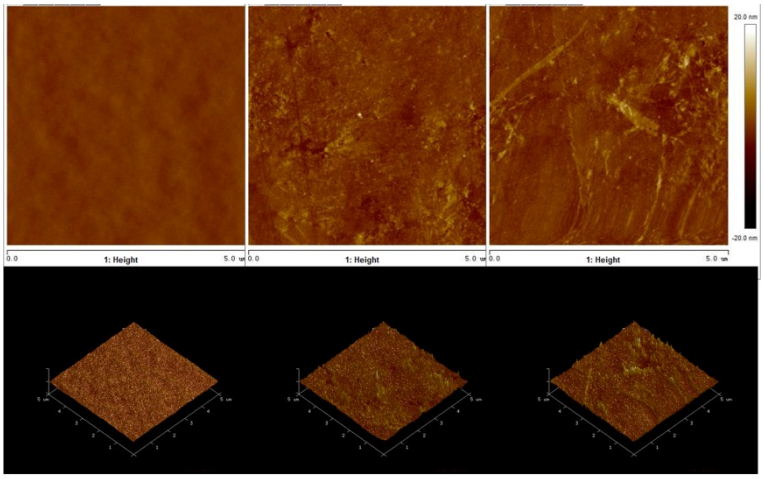

Fig. S4 AFM images of the pure Si wafer and the modified wafers with a scale bar of 20 nm.