Supporting Information

of

Anti-swelling, antithrombotic and antibacterial zwitterionic hydrogel coatings with sandwich structure on polymer substrates

Zhang Jing[a,*], Tang Xuying [a], Wu Minmin [a], Feng Jie [a], Zhang Qinggang[b,*]

[a]College of Materials Science and Engineering, Zhejiang University of Technology, Hangzhou, Zhejiang, 310014, P. R. China

[b]Heart Center, Department of Cardiovascular Medicine, Zhejiang Provincial People's Hospital (Affiliated People's Hospital, Hangzhou Medical College), Hangzhou, Zhejiang, 310014, P. R. China

[*]Corresponding authors: Email：

zhangjing@zjut.edu.cn (J. Zhang), zqgzd2003@163.com (Qg.Zhang)

Experimental Section

Contact angle test:

The water contact angle of bare substrates and hydrogel-coated substrates were tested by contact angle measurement system (Dataphysics OCA35, Germany). The surfaces of the samples were purged with N_2 first. Then 4.0 μL of water droplets was put on the surfaces of the samples in the air, and the contact angle was measured between the water droplets and the surfaces of the samples.

Characterizations of hydrogel coatings：

Energy dispersive spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific) were used to analyze the surface chemistry of the bare

silicone substrate, pSBMA/FDA hydrogel-coated silicone and pCB/pSBMA/FDA hydrogel-coated silicone. The morphology of the hydrogel coatings was observed by field-emission scanning electron microscopy (FEI Nova Nano SEM 450). All hydrogel coatings were lyophilized with a freeze dryer prior before test.

In vitro anti-bacterial adhesion test:

Escherichia coli (*E. coli*, Gram-negative) and Staphylococcus aureus (*S. aureus*, Gram-positive) were used to study the anti-bacterial adhesion performances of the hydrogel coatings. *E. coli* and *S. aureus* were incubated for 10 ~ 12 h at 37 ^{o}C on a Luria-Bertani (LB, OXOID) agar plate. One colony of each type of bacteria was placed in 40 mL LB and diluted to the optical density of ~ 0.1 (*E. coli*) and ~ 0.05 (*S. aureus*) at 600 nm after being shaken (the corresponding bacterial densities were 2.53×10^7 CFU/mL and 1.14×10^7 CFU/mL for *E. coli* and *S. aureus*, respectively). Bare substrates and hydrogel-coated substrates were sterilized in 75% ethanol solution, rinsed with PBS, and placed in a 12-well sterilized plate for anti-bacterial adhesion test. Subsequently, 3 mL of prepared bacterial suspension solution and in order to trigger GOx to degrade glucose 100 μL glucose solution was added into the well and cultured at 37 ^{o}C for an appropriate time under 120 rpm shaking. Bare silicone, pSBMA/FDA hydrogel-coated silicone, pSBMA/FDA@GOx hydrogel-coated silicone and pCB/pSBMA/FDA@GOx hydrogel-coated silicone were washed 3 times with sterilized PBS and stained with LIVE/DEAD BacLight Survival Kit (Thermo Fisher Scientific Inc., NY) in the dark for 10 min, and then rinsed again with sterilized PBS. The samples were observed with an inverted fluorescence microscope (Carl Zeiss Inc., Germany).

Anti-protein adhesion test of hydrogel coatings:

The bare silicon, pSBMA hydrogel-coated silicone, pSBMA/FDA hydrogel-coated silicone and pCB/pSBMA/FDA hydrogel-coated silicone samples (5 mm × 5 mm × 3 mm) were placed in a 24-well plate, and 1 mL HRP-IgG were added into each plate. After being placed in a constant temperature incubator at 37 ^{o}C for 12 h, the samples were taken out and divided into two groups and soaked in phosphate buffered saline (PBS) for 0.5 h and 3 h, respectively. Then the samples were put into a 24-well plate

after washed by PBS and added with 1 mL of 0.1 M citrate-phosphate buffer (pH = 5) containing o-phenylenediamine (1 μg/mL) and hydrogen peroxide (0.03%). After 15 min, 2 M H_2SO_4 was added to stop the reaction. The supernatant was collected and the optical density (OD) was detected by a microplate reader (SpectraMax M2) at 492 nm.

Particle Size Measurement:

Particle size measurement using nanoparticle size and Zeta (Brookhaven). GOx, FDA and FDA@GOx are all prepared in water at a concentration of 1 mg/mL.

In vitro cytotoxicity and hemocompatibility

200 mg bare silicone or pCB/pSBMA/FDA@GOx hydrogel-coated silicone were incubated in 2 mL of complete 1640 medium (containing 10% fetal bovine serum (FBS)) for 1 day, 4 days, and 7 days at 37 °C, respectively. L929 cells at a density of 5 × 10^3/well and a certain concentration gradient of leachate (12.5 mg/mL, 25 mg/mL, 50 mg/mL, 100 mg/mL) were incubated for 24 h, respectively. All cultures were then removed and 100 μL of fresh medium containing MTT solution (5 mg/mL) was added and incubated for 3 h. Then all solutions were taken out, 100 μL of dimethyl sulfoxide (DMSO) was put in each well, and the optical density (OD) of each well was recorded at 492 nm. The calculation of cell viability as follows:

$$Cell\ \ viability = \frac{OD_{treated} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100\% \quad (2)$$

Where ODblank, ODtreated, and ODcontrol are the optical density values of background wells, sample wells in the presence of leachate and control wells in complete medium, respectively.

The hemocompatibility of the hydrogel coatings was tested according to previous research.36 In short, fresh rat erythrocytes were diluted to 10 vol% with PBS. 0.5 mL of the leachate of the hydrogel coatings at different concentrations (2 mg/mL, 4 mg/mL, 8 mg/mL) was incubated with 0.5 mL of diluted rat erythrocytes for 4 h at 37 oC. After incubation, the solution was centrifuged at 3000 rpm for 5 min. The supernatant (100

μL) was transferred to a new 96-well plate and measured at OD 545. PBS and Triton groups were set as control.

Table 1. Atom percentage results of silicone, pSBMA/FDA hydrogel coated silicone and pCB/pSBMA/FDA hydrogel coated silicone.

Samples	Elements (atom%)			
	C	O	S	Si
Silicone	0		0	
pSBMA/FDA hydrogel coated silicone	54.72	42.15	3.13	0
pCB/pSBMA/FDA hydrogel coated silicone	54.41	45.29	0.30	0

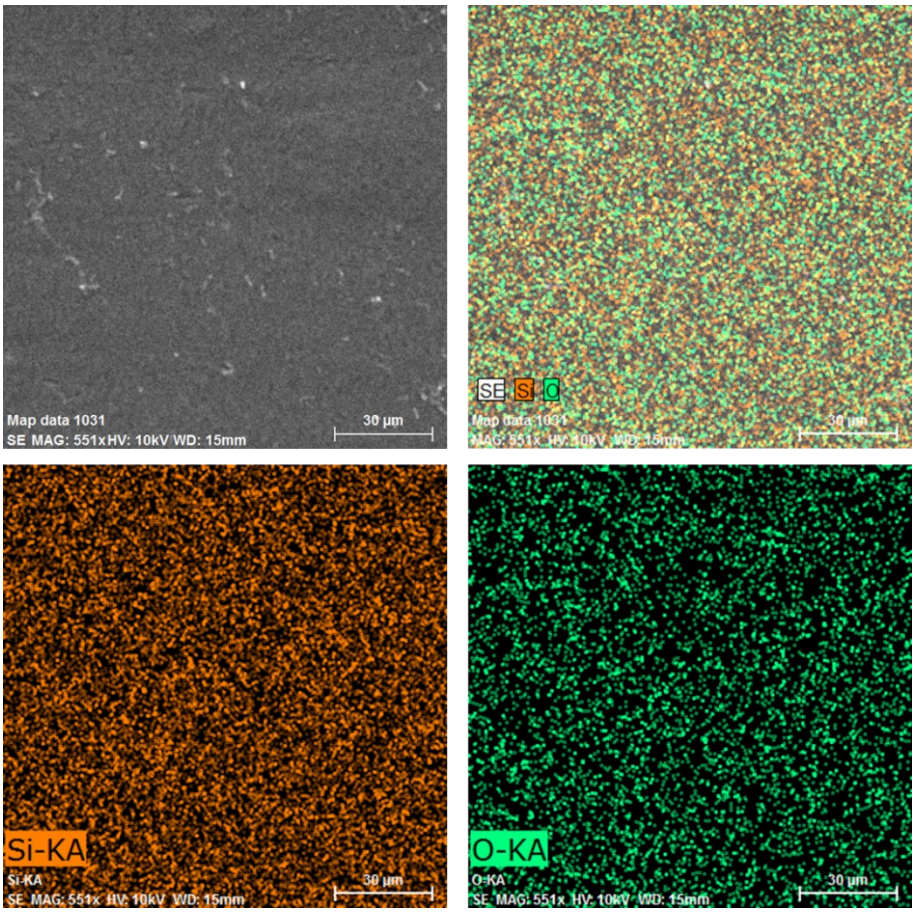

Figure S1. The EDS elemental analysis of bare silicone.

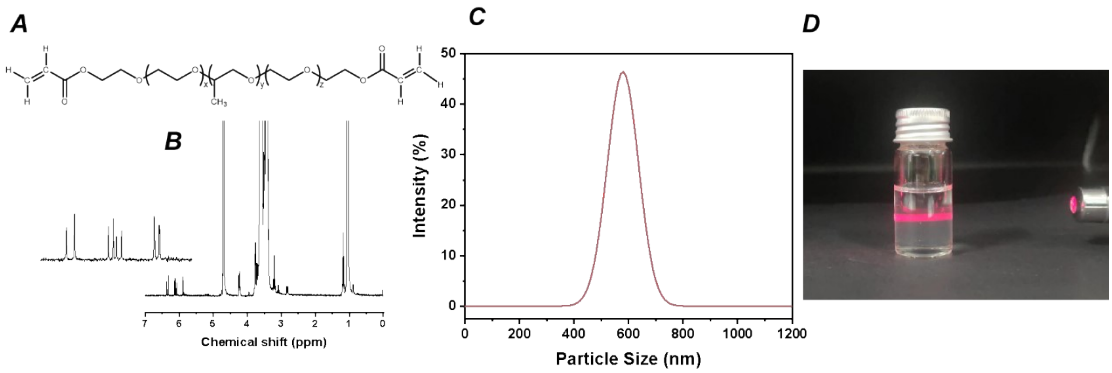

Figure S2. (A) Structural formula of FDA. (B) ^{1}H NMR of FDA; (C) particle size distribution of FDA; (D) Tindal effect of FDA micelles under laser irradiation;

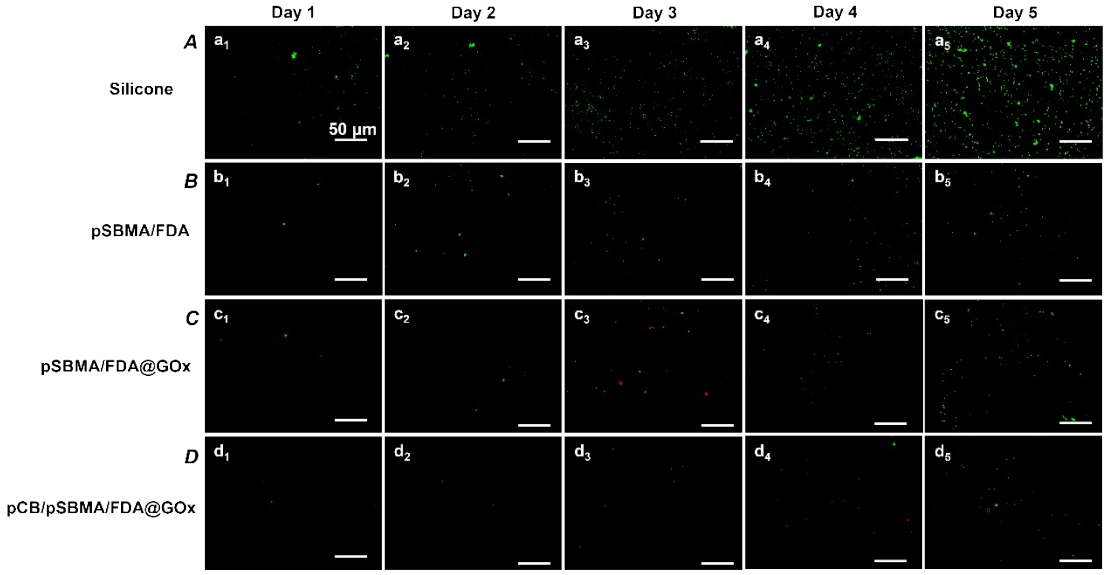

Figure S3. Adhesion of S. aureus on bare silicone (A), pSBMA/FDA hydrogel-coated silicone (B), pSBMA/FDA@GOx hydrogel-coated silicone (C) and pCB/pSBMA/FDA@GOx hydrogel-coated silicone surfaces(D).

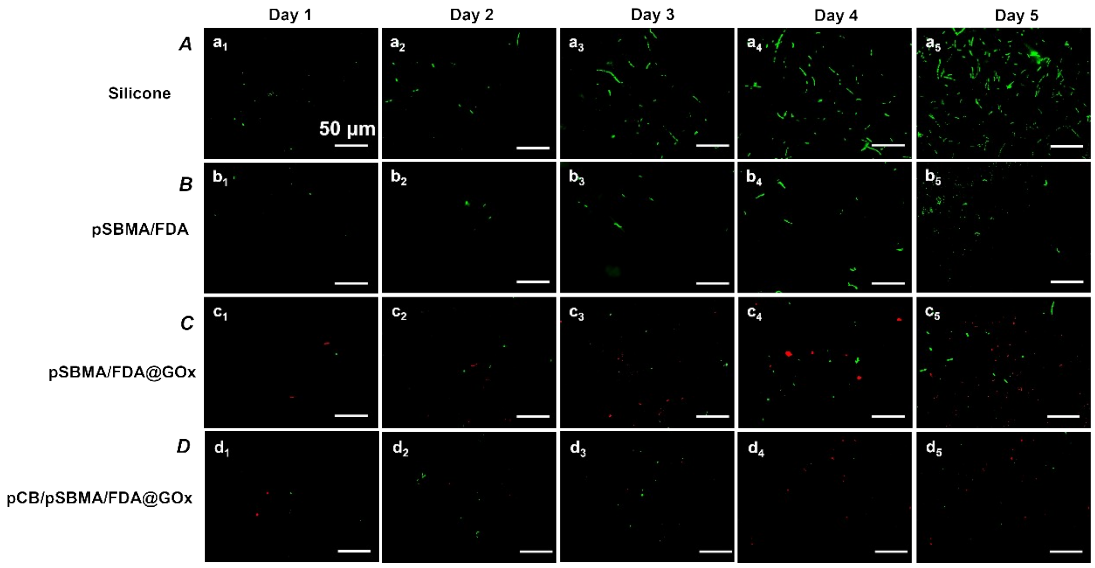

Figure S4. Adhesion of E. coli on bare silicone (A), pSBMA/FDA hydrogel-coated silicone (B), pSBMA/FDA@GOx hydrogel-coated silicone (C), and pCB/pSBMA/FDA@GOx hydrogel-coated silicone surfaces (D).