

Supporting Information

Multifunctional coatings that mimic the endothelium: surface bound active heparin nanoparticles with in-situ generation of nitric oxide from nitrosothiols

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The supporting information includes: Materials and methods (M1- M3), Table S1, Figure S1-S3.

Materials and methods:

M1. Quantification of heparin density and releasing profile.

The amount of immobilized heparin on NP-immobilized surface and reference samples was determined by a TBO assay. First, the samples were incubated in 5 ml freshly prepared solution of 0.04 wt% TBO in aqueous 0.01M HCl/0.2 wt% NaCl under static conditions at 37°C for 4 h and rinsed three times with DI water. During this period, Hep-TBO complex was formed on the samples surface, then dissolved and released by 5 ml of 80% ethanol/0.1M NaOH mixture (v/v=4/1) solution. After that, 150 µl supernatant was added to a 96-well plate and the absorbance was measured at 530 nm by a microplate reader (Quant, Bio-tek instruments Inc.) The amount of immobilized heparin can be obtained with calibration curve. Blanked SS-Dopa surface

was used as comparison. For calibration curve preparation, 2 ml of 0.04 wt% TBO was firstly added to 2 ml of a known concentration heparin solution and incubated at 37°C with gently shaking for 4 h, the Hep-TBO complex spontaneously formed and precipitated in the mixture. Then the mixture was centrifuged at 3500 rpm for 10 min, the supernatant was removed and the precipitate was carefully rinsed twice with aqueous 0.01M HCl/0.2wt% NaCl. Finally, 5 ml of 80% ethanol/0.1M NaOH mixture (v/v=4/1) solution was added to dissolve the precipitate and the absorbance was measured at 530 nm. For quantitative characterization of the heparin release, the samples were immersed in PBS at 37 °C and shaken (60 rpm) for 1, 3, 5, 7, 10, 14, 21 and 28 days in an airtight centrifuge tube. The release medium at each time point was collected and the amount of heparin released was measured by using the same procedure based on a standard curve preparation. There were six parallel samples in each group.

M2. Quantitative characterization of amine density

The Acid Orange II (AO II) test was used here to determine the surface amine density after Hep-Cys/PEI nanoparticles immobilization. In detail, 0.5mM AO II solution was prepared by dissolving 8.75mg AO II powder in 50ml HCl (pH=3) solution. Then the samples were incubated in 1ml of AO II solution with gentle shaking at 37°C for 6 h; subsequently, samples were rinsed three times with pH=3 HCl for 5 min each. The samples were then immersed into 1 ml NaOH solution dissolved in DI water, pH=10, and shaken for 15 min at 37°C to dissolve the absorbed AO II on the sample surfaces. Ultimately, 150µl of supernatant was transferred to a 96-well plate and the absorbance of the supernatant was determined in a microplate reader at 485 nm; the amine density on the sample surfaces was evaluated by a standard calibration curve. There were six parallel samples in each group, and SS-Dopa was set as blank control. For standard curve preparation, 0.5mM AO II solution in 10mM NaOH was prepared and serial twofold dilutions with 10mM NaOH were made; the absorbance of the known concentration AO II solutions was then measured in a microplate reader at 485 nm.

M3. Stent Morphology Examination and Balloon Expansion Tests

The surface morphology of the cardiovascular stents coated with polydopamine and NP5 were examined by scanning electron microscopy (SEM, Quanta 200, FEI, Holland) before balloon expansion. The modified stent was mounted onto an angioplasty balloon and the balloon was dilated from 1.65 mm to 3.0 mm (diameter) at a pressure of 8 atm. The post-expansion stents were examined by SEM.

Table S1. Elemental composition of NP5 during dynamic release determined by XPS.

Time	C(%)	N(%)	O(%)	S(%)
0 day	62.84	12.06	23.13	1.97
1 day	63.04	11.03	24.07	1.86
3 day	62.74	11.86	23.56	1.80
7 day	64.09	9.69	24.31	1.91
14 day	67.33	8.53	22.35	1.79
28 day	68.12	8.41	21.86	1.61

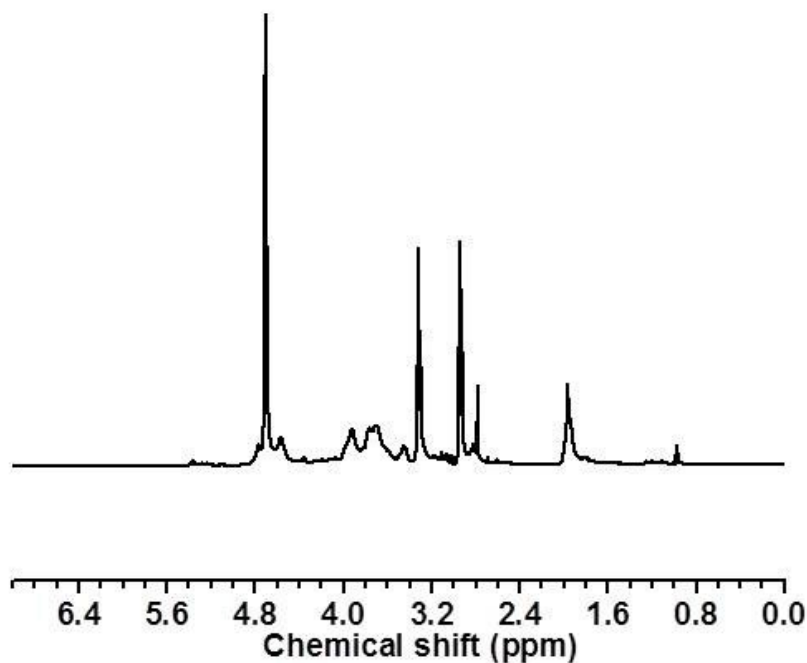


Figure S1. The chemical shift of cystamine-modified heparin ($^1\text{H-NMR}$).

Notes: The successful modification of heparin with cystamine was demonstrated with the peaks at approximately 2.9 and 3.3 ppm (Hep-Cys).

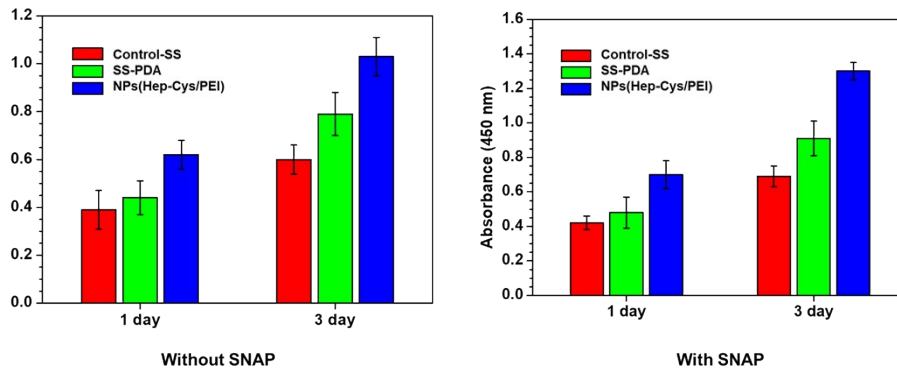


Figure S2. Cell viability of endothelial cells (ECs) on different surfaces after cultured for 1 day and 3 days.

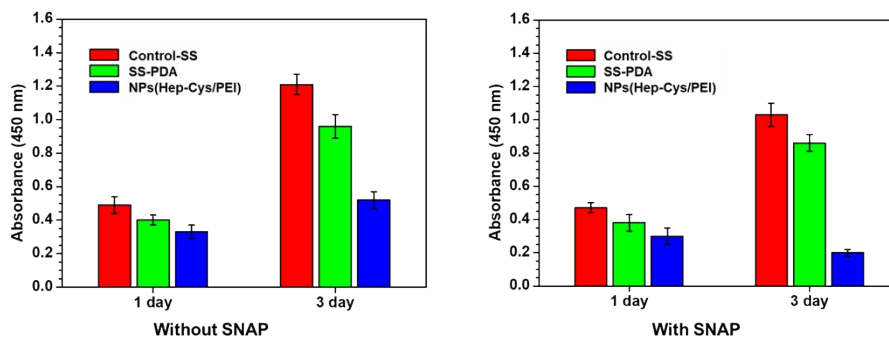


Figure S3. Cell viability of smooth muscle cells (SMCs) on different surfaces after cultured for 1 day and 3 days.