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Supporting Information

Decellularized Scaffold-based Poly(ethylene glycol) Biomimetic Vascular Patches Modified with Polyelectrolyte Multilayer of Heparin and Chitosan: Preparation and Vascular Tissue Engineering Applications in a Porcine Model

Huimin Gao[1,†], Pengpeng Hu[1,†], Gaoqi Sun[1], Lei Wang[1], Yu Tian[1], Hong Mo[1,*], Cheng Liu[2,*], Jun Zhang[1], Jian Shen[1,*]

[1]Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, Jiangsu Key Laboratory of 3D Printing Equipment and Manufacturing, National and Local Joint Engineering Research Center of Biomedical Functional Materials, Jiangsu Engineering Research Center for Biomedical Function Materials, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, China

[2]Medical School, Nanjing University, Nanjing 210093, P. R. China

[†] These authors contributed equally to this work.

*Authors to whom all correspondence should be addressed

E-mail Addres**s: 07198@njnu.edu.cn**

or liucheng80@126.com

or jianshen_nj@163.com

1. Preparation of PEM-modified PEG/DCS (Hep-CS-*n*/PEG/DCS)

Glacial acetic acid (0.4 g) and chitosan (0.2 g) were added to deionised water (100 mL) to obtain a chitosan solution (2.0 g/L). Similarly, heparin sodium (0.2 g) was added to deionised water (100 mL) to obtain a heparin solution (2.0 g/L).

The CS/PEG/DCS vascular patch was prepared by soaking PEG/DCS in PBS for 30 min and then in chitosan solution for 15 min. The physically adsorbed chitosan on CS/PEG/DCS was removed by washing it three times with PBS. Likewise, PEM-modified vascular patches Hep-CS-*n*/PEG/DCS (*n* = 3, 4, 5, 6, 7) were obtained by dipping them alternately in chitosan and heparin solutions. The final deposition solution corresponding to *n* is listed in Table S1.

Table S1. The final deposition solution corresponding to *n* for Hep-CS-*n*/PEG/DCS.

n	3	4	5	6	7
the final deposition	chitosan	heparin	chitosan	heparin	chitosan

2. Determination of PEG content

In this experiment, the coating rate of PEG on DCS was calculated using the weight increase method as follows:

$$CR = \frac{m_1 - m_0}{m_0} \times 100\%$$

where CR is the coating rate of PEG. m_1 is the quality of PEG/DCS after purification and drying, and m_0 is the quality of DCS.

Table S2 PEG content on DCS surface

The quality of the DCS (g)	The quality of the PEG/DCS (g)	the coating rate of PEG on DCS (%)
0.0245	0.0426	73.88
0.0213	0.0370	73.71
0.0221	0.0382	72.85
0.0252	0.0437	73.41
0.0281	0.0486	72.95
0.0287	0.0498	73.52

3. XPS analysis for PEG/DCS and Hep-CS-5/PEG/DCS

PEG/DCS and He-Ch-5/PEG/DCS vascular patches were measured using the D/max 2500VL/PC X-ray Photoelectron Spectroscope (Japan) at a focused monochromatised Al-Kα (150 W, 500 μm radiated area) radiation of 1486.6 eV. The diameter of their radiated area was 500 μm, and the residual pressure inside the analysis chamber was in the 10^{-8} Pa range. The curves were recorded at a constant pass energy of 20 eV.

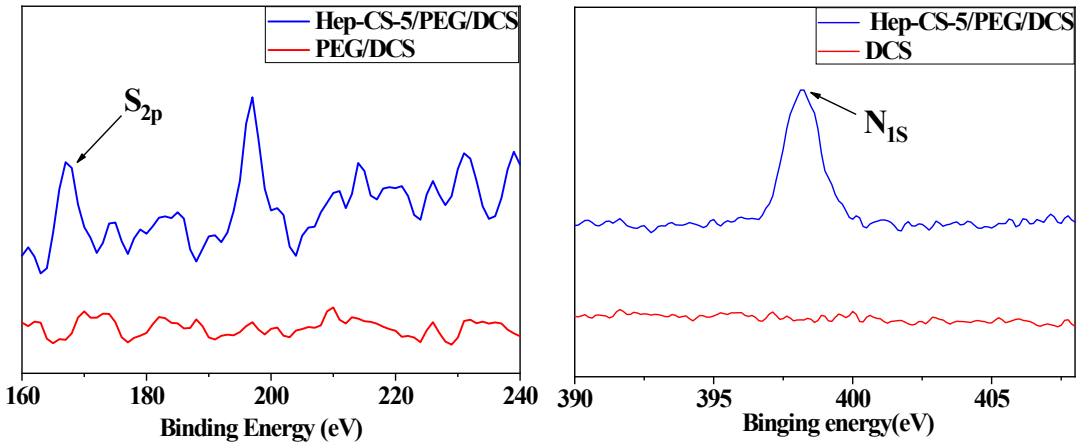

Figure S1. XPS patterns for DCS, PEG/DCS and Hep-CS-5/PEG/DCS

4. Surface amino group density

Each vascular patch was soaked in acid orange II (pH = 3, 5×10^{-4} M) and shaken in a shaking incubator for 12 h. Then, the vascular patch was washed with HCl (pH = 3) five times to remove the acid orange II dye physically adsorbed onto the surface. The vascular patch was dried in vacuum for 2 h, soaked in NaOH (pH = 12), and then shaken for 30 min to desorb the dye. The absorbance of the resultant desorption solution was measured at 485 nm using the BioTek Synergy 2 ELISA Reader (BioTek Company, U.S.). The density of amino groups on the vascular patch surface was determined according to the standard curve of acid orange II.

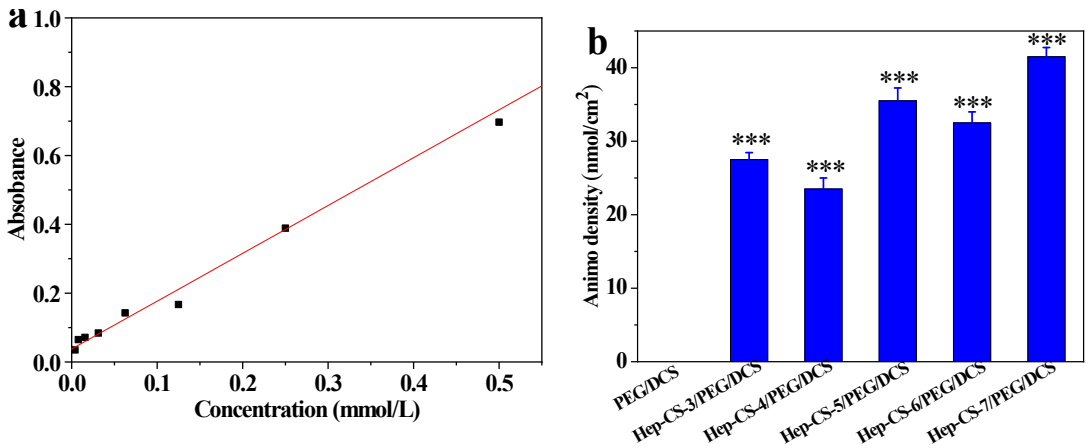

Figure S2. (a)The standard curve of acid orange II, (b) the amino group density on the vascular patch surfaces. (n = 3, *p < 0.05, **p < 0.01 and ***p < 0.001 versus PEG/DCS).

5. Water contact angle measurements

Each vascular patch was cut into a wafer (diameter: 1 cm). The static water contact angle was measured using the DSA 100 contact angle apparatus (Krüss Co., Germany)

at 25 ℃. All measurements were performed three times.

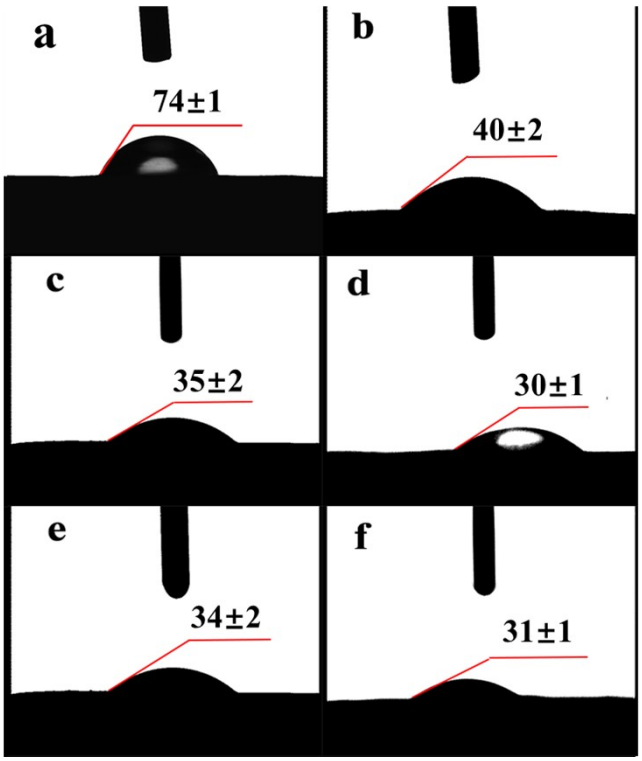

Figure S3. Static contact angle images: (a) PEG/DCS, (b) He-CS-3/PEG/DCS, (c) Hep-CS-4/PEG/DCS, (d) Hep-CS-5/PEG/DCS, (e) Hep-CS-6/PEG/DCS, (f) Hep-CS-7/PEG/DCS.

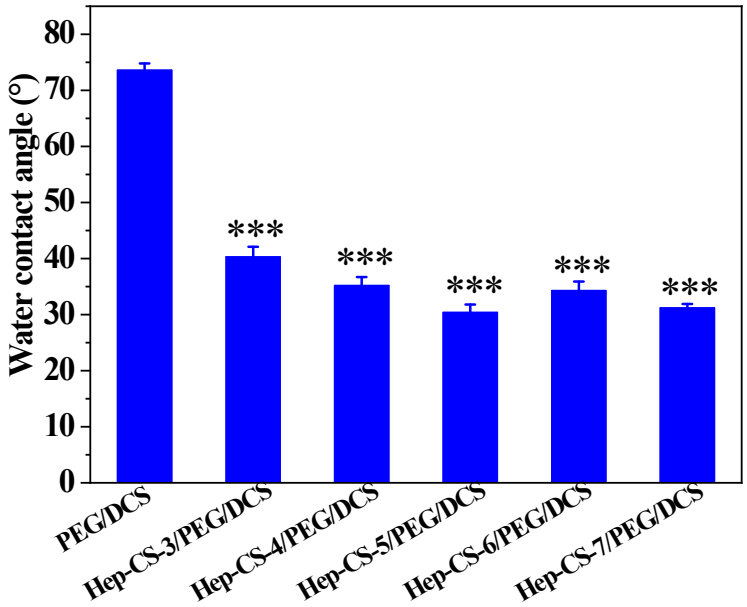

Figure S4. Water contact angles (n = 3, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus PEG/DCS).

6. Hemolysis analyses of the vascular patches

Table S3 Hemolysis rates of the vascular patches

Samples	Hemolysis rate (%)
Blank	0.89 ± 0.14
PEG/DCS	1.75 ± 0.23
Hep-CS-3/PEG/DCS	0.98 ± 0.12
Hep-CS-4/PEG/DCS	0.91 ± 0.14
Hep-CS-5/PEG/DCS	0.83 ± 0.10
Hep-CS-6/PEG/DCS	0.86 ± 0.09
Hep-CS-7/PEG/DCS	0.90 ± 0.13

7. Morphologies of RBCs

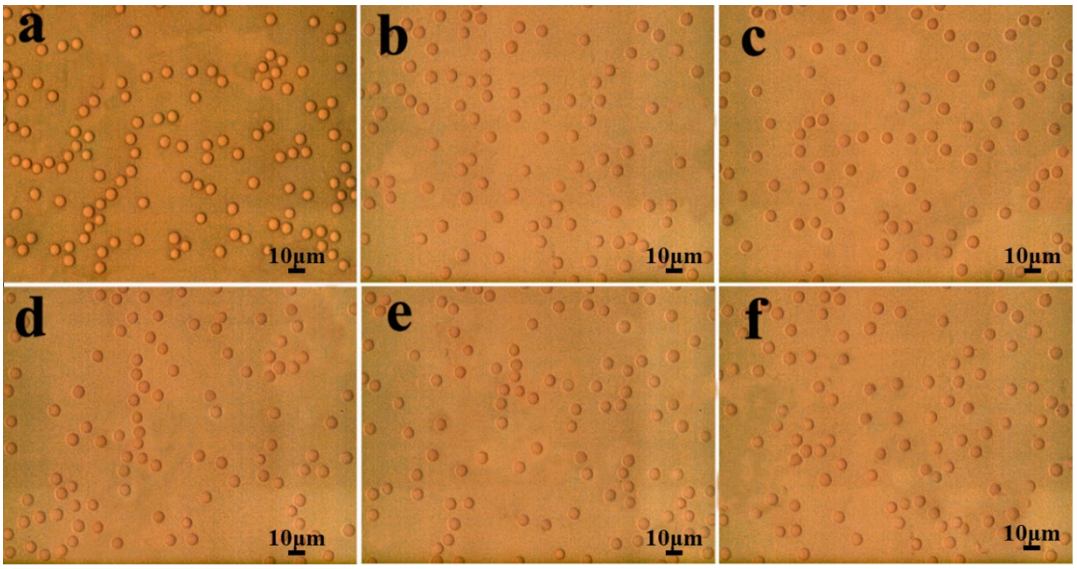

Figure S5. Morphologies of RBCs exposed to (a) the control (normal saline), (b) Hep-CS-3/PEG/DCS, (c) Hep-CS-4/PEG/DCS, (d) Hep-CS-5/PEG/DCS, (e) Hep-CS-6/PEG/DCS, (f) Hep-CS-7/PEG/DCS.

8. *In vitro* blood compatibility

Each sample was washed with PBS and soaked in normal saline (10 mL) at 37 ℃ for 24 h to obtain a sample solution.

APTT: The sample solution was mixed with PPP (0.1 mL), and the mixture was added to a haemagglutinin cup. The cup was incubated at 37 ℃ for 5 min, and then the APTT reagent (0.1 mL) was added and cultured for 5 min. Then, $CaCl_2$ (0.1 mL, 0.025 M) was added to the cup. The coagulation time was measured as APTT. Normal saline (10 mL) was used as the control. Each test was performed in triplicate.

Thrombin time (TT): The sample solution was mixed with PPP (0.2 mL), and the mixture was added to a haemagglutinin cup. The cup was incubated at 37 ℃ for 3 min, and then the TT reagent (0.2 mL) was added to the cup at 37 ℃. Then, the coagulation time was measured as TT. Normal saline (10 mL) was used as the control. Each test was performed in triplicate.

Prothrombin time (PT): The sample solution was mixed with PPP (0.1 mL), and the mixture was added to a haemagglutinin cup. The cup was incubated at 37 ℃ for 3 min, and the PT reagent (0.2 mL) was added to the cup at 37 ℃. Then, the coagulation time was measured as PT. Normal saline (10 mL) was used as the control. Each test was performed in triplicate.

Each sample was washed with PBS and placed into a well of a 96-well plate at 37 ℃. Then, PPP (0.1 mL) and $CaCl_2$ (0.1 mL, 0.025 M) were added to the well. The optical density (O.D.) was measured at 405 nm using the BioTek Synergy 2 ELISA Reader. The test frequency was one time per minute. The mixture of PPP (0.1 mL) and $CaCl_2$ (0.1 mL, 0.025 M) was used as the control. Each test was performed in triplicate.

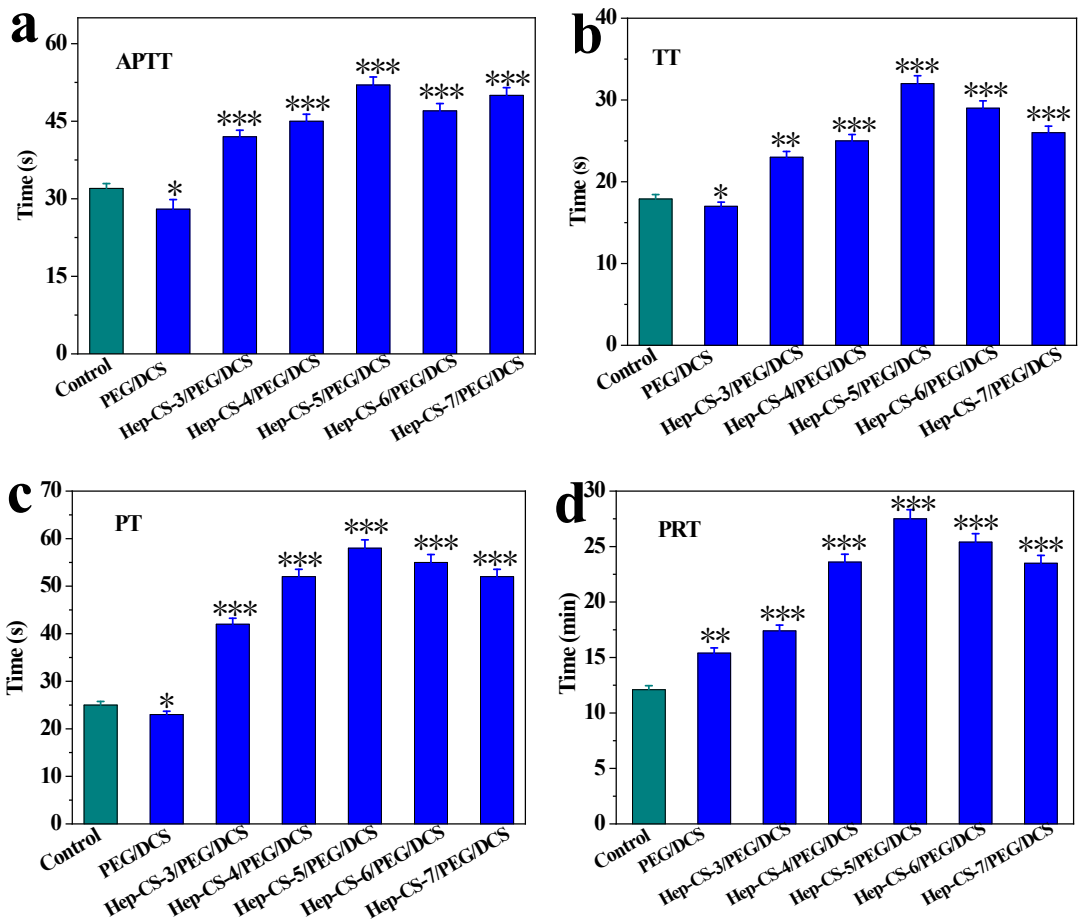

Figure S6. *In vitro* coagulation times: (a) APTT, (b) TT, (c) PT; (d) the plasma recalcification time (PRT). (n = 3, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus the control).

9. *In vitro* cytotoxicity

EPCs were used to measure cytotoxicity through the MTT assay, and the extract of each sample was obtained in accordance with the ISO 10993-12 standard. Foetal bovine serum was mixed with EBM-2 cell culture medium (1:9, v/v) to form an extraction medium. Each sample (surface area: 2 cm^2) was extracted in the extraction medium at 37 ℃ for 24 h to obtain a test extract. Trypsin (0.25 wt%) was added to EPCs in the exponential growth phase to obtain a cell suspension (1×10^4 cells/mL). The cell suspension (100 μL) was added to a well of a 96-well plate and incubated in 5% CO_2 humid atmosphere at 37 ℃ until the cell proliferation rate reached 80%–90%. Then, the

culture medium was removed, and the test extract (100 μL) was added. The resultant mixture was incubated in 5% CO_2 humid atmosphere at 37 ℃ for 12 or 24 h. Then, the test extract was removed, and tetrazolium salt (MTT) solution (20 μL) was added. The resultant mixture was incubated in 5% CO_2 humid atmosphere at 37 ℃ for 4 h. Then, the MTT solution was removed, and dimethyl sulfoxide (150 μL) was added. The resultant mixture was shaken at 37 ℃ for 10 min. The O.D. was measured at 490 nm using the BioTek Synergy 2 ELISA Reader. All measurements were run eight times.

The degree of cytotoxicity was determined as follows:

$$RGR = \frac{D_t - D_b}{D_{nc} - D_b} \times 100\%$$

where RGR is the relative growth rate of the cells, and D_t, D_b, and D_{nc} are the O.D. of the test sample, positive control, and negative control, respectively. The positive control was MTT solution, and the negative control was the extraction medium.

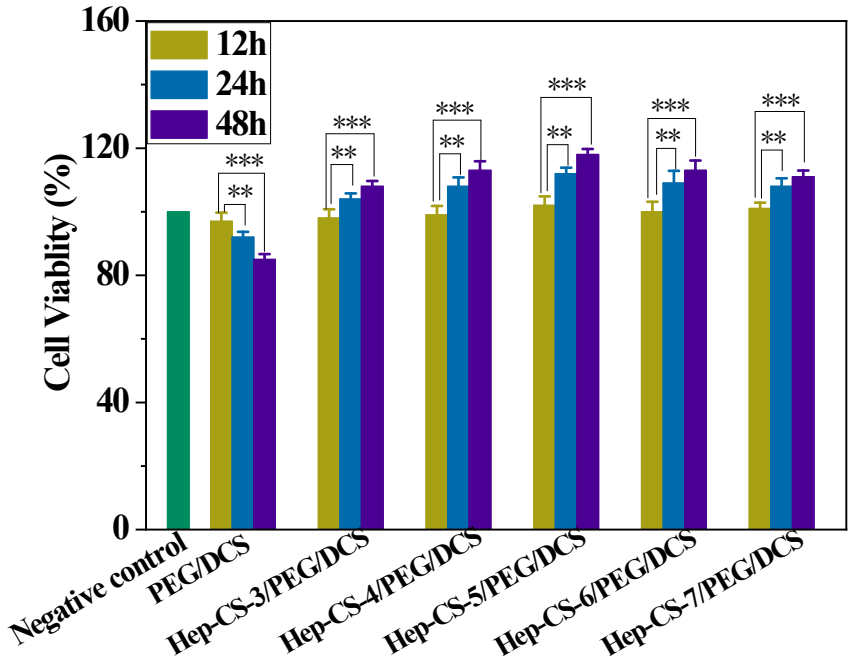

Figure S7. Viabilities of EPCs incubated in extracts of the materials. (n = 3, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$).

10. *In vivo* implantation of a vascular patch

These patches were used to cover the wounds on the left carotid arteries of small suckling pigs and then were sutured.

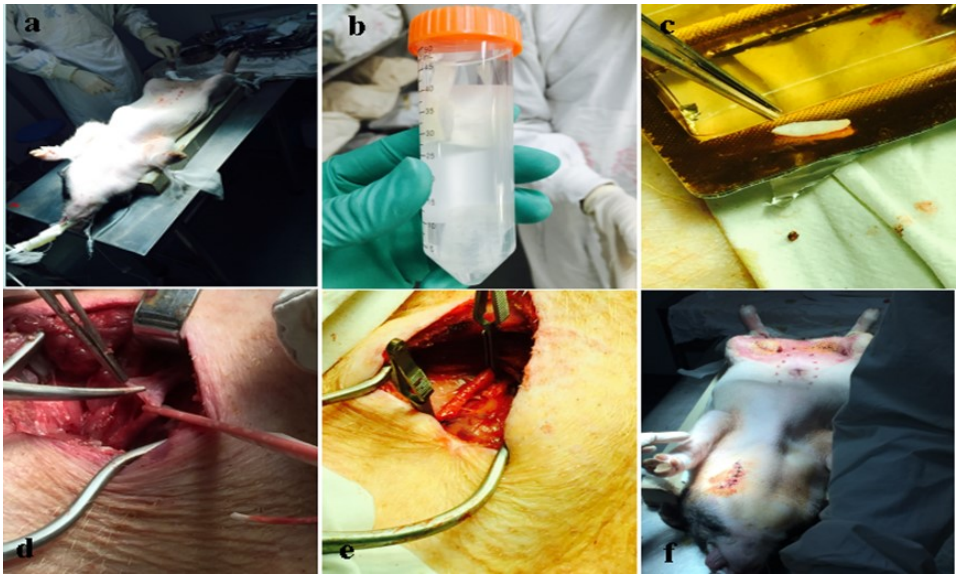

Figure S8. Surgical images of *in vivo* implantation

11. Antibacterial property tests of chitosan

Table S4 Diameter of antibacterial ring for *Staphylococcus aureus* of each vascular patch

Samples	Diameter of antibacterial ring (mm)
Blank	0
PEG/DCS	11.06
Hep-CS-3/PEG/DCS	28.50
Hep-CS-4/PEG/DCS	28.10
Hep-CS-5/PEG/DCS	30.94
Hep-CS-6/PEG/DCS	30.28
Hep-CS-7/PEG/DCS	34.60

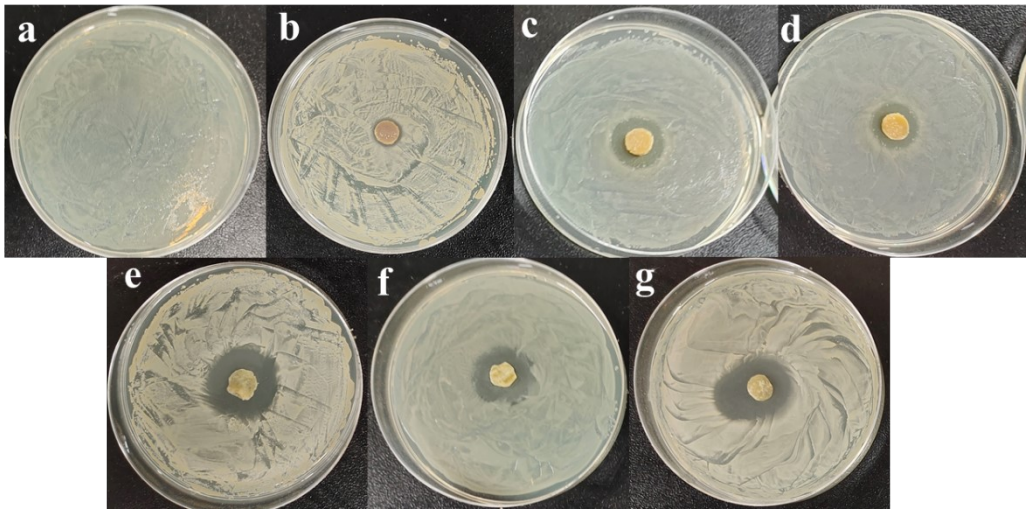

Figure S9. Images of antibacterial ring diameter of each vascular patch: (a) blank, (b) PEG/DCS, (c) Hep-CS-3/PEG/DCS, (d) Hep-CS-4/PEG/DCS, (e) Hep-CS-5/PEG/DCS, (f) Hep-CS-6/PEG/DCS, (g) Hep-CS-7/PEG/DCS.

The antibacterial effect of each vascular patch on *Staphylococcus aureus* is shown in Table S4 and Figure S9. With an increase in chitosan content, the diameter of the antibacterial ring gradually increased. The results show that chitosan has high antibacterial activity.