Supporting Information

Asymmetric Porous Hydrogel Encapsulating Vulcanized Molecular Brushes with Anti-bacterial Adhesion, Anti-infection, and Pro-healing Properties towards Infected Wound Treatment

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Materials

Styrene (Tianjin Yongda Chemical Reagent Co., LTD.) was purified by passing through a basic alumina column. Cuprous bromide (CuBr; Aladdin, 99.99%) was purified by washing sequentially with acetic acid and ethanol, filtration and drying, and was stored under nitrogen before use. Polyvinyl alcohol 1799 (PVA-1799, degree of alcoholysis 98~99 mol%), dimethyl sulfoxide (DMSO), and phosphate-buffered saline (PBS; 0.01 M, pH 7.4) were purchased from Macklin (China). N,N-dimethylformamide (DMF; Aladdin, AR), tetrahydrofuran (THF; Aladdin, AR), triethylamine (Aladdin, AR), *N,N,N',N',N''*-pentamethyldiethylenetriamine (PMDETA; Aladdin, AR), tetraethyl orthosilicate (TEOS; Aladdin, AR), dichloromethane (Aladdin, HPLC), 2-bromoisobutyryl bromide (BiBB; Aladdin, AR), elemental sulfur (S_8; Aladdin, AR), and carbon disulfide (CS_2; Aladdin, AR) were used as received. Cell Counting Kit-8 (CCK-8) was purchased from MedChemExpress (America). Fetal bovine serum (FBS) was purchased from Bovogen (Australia). Calcein AM/PI double staining kit was purchased from Bestbio (China). Dulbecco's modified eagle medium (DMEM) was purchased from Gibco (America). L929 fibroblasts were purchased from Procell (China). The male Sprague Dawley (SD) rats (160-220 g) were purchased from Guangdong Medical Lab Animal Center (China).

Sample preparation

SiO_2-*g*-PS molecular brushes were synthesized following our previous work.[1, 2] SiO_2-*g*-PS was mixed with commercial elemental sulfur (S_8) and sealed in a glass vial.

Thereafter, the mixture underwent a stepwise heat treatment process in a tube furnace under N_2 atmosphere, including 200 °C for 2 h and 280 °C for another 4 h with a heating rate of 2 °C min^{-1}.[3] After that, SiO_2-*g*-*s*PS nanoparticles were obtained followed by washing with CS_2 for three times. SiO_2/*s*PS was prepared according to the above heat treatment process with the mixture of silicon oxide, linear PS polymers and elemental sulfur.

7.2 g of PVA-1799 was added into 40 mL of DMSO, and the mixture was stirred overnight at 60 °C. The solution was poured into molds after cooling down to room temperature, followed by immersing in 1.2 L deionized water for 2 days. The as-obtained exogels were completely frozen in fridge at -20 °C for 2 days and freeze-dried for 2 days to obtain the cryogels. The APH were obtained by soaking the cryogels in PBS buffer for 1 day for rehydration. The preparation procedure of VMB@APH and SiO_2/*s*PS@APH similar with that of APH, except that of SiO_2-*g*-*s*PS or SiO_2/*s*PS nanoparticles were added into of DMSO respectively and dispersed under ultrasonic for 2 hours before adding PVA-1799. The parameters for preparing the precursor solutions of different samples were listed in Table S1.

Material characterization

The nanomorphology was visualized with a scanning electron microscopy (SEM, S-4800) and a transmission electron microscopy (TEM, Tecnai G2 Spirit). X-ray photoelectron spectroscopy (XPS) were investigated using a Thermo ESCALAB 250. Thermogravimetric analysis (TGA) curves were conducted on a PE Pyris1instrument.

The Fourier transform infrared (FT-IR) spectra of VMB and SiO_2-*g*-PS were measured with an Equinox 55 FT-IR spectroscopy (Bruker, Germany), and the FT-IR spectrum of the asymmetric porous cryogel encapsulating VMB was measured with a Nicolet iS50 FT-IR spectroscopy with an attenuated total reflectance attachment (Thermo scientific, America). The mechanical properties of samples were investigated by a universal mechanical testing machine (Kobe, China) at room temperature. For tensile tests, the dumbbell-shaped samples (2 mm width) were stretched at a speed of 30 mm min^{-1}. Cyclic tensile test was performed for 20 continuously repeated loading-unloading cycles by reaching 20% of tensile strain at the speed of 20 mm min^{-1}. For compressive tests, cylinder-shaped samples (12 mm diameter and 7 mm height) were compressed at a speed of 1 mm min^{-1} and tests were ended when the compressive strain reached 85%. Cyclic compressive test was performed on a cylinder-shaped sample (14 mm diameter and 11 mm height) for 50 continuously repeated loading-unloading cycles by reaching 30% of compressive strain at the speed of 3 mm min^{-1}. For fracture energy characterization, the notched samples with a 5-mm pre-crack and the notchless samples were stretched at a speed of 2 mm min^{-1}. The fracture energies of samples were calculated according to the reported literature.[4]

Evaluation of anti-bacterial adhesion and antibacterial performances

The dried cryogels were sterilized by ethylene oxide and rehydrated with sterile PBS buffer before the tests. Subsequently, the products were cut into tablets (7 mm in

diameter), sterilized in 75% ethanol for 30 minutes, and further washed by sterile PBS buffer for three times.

To evaluate sample's performance on the anti-bacterial adhesion, 100 μL of *Staphylococcus aureus* (*S. aureus*) suspension (10^7 CFU mL^{-1}) was seeded onto the loosely porous or the densely porous surfaces of APH, and cultured in an incubator at 37 °C. After 16 hours, the APHs were washed twice with PBS carefully to remove the unattached bacteria, followed by immersing in 2.5% glutaraldehyde for 4 hours and sequentially dehydrating with gradient ethanol. The as-obtained samples were frozen in liquid nitrogen and freeze-dried for 1 day before SEM imaging.

To evaluate sample's antibacterial performance, the samples were immersed in 100 μL of *S. aureus* suspension (10^7 CFU mL^{-1}) and cultured in an incubator at 37 °C for 9 hours. Subsequently, the suspension was diluted and seeded on agar plates. After 18 hours of culturing, the antibacterial ratios of different samples were calculated according to equation (1):

$$Antib\text{acterial ratio} = \frac{\text{CFU of APH} - \text{CFU of sample}}{\text{CFU of APH}} \times 100\% \quad (1)$$

Cytocompatibility evaluation

The dried cryogels were sterilized using ethylene oxide and rehydrated with sterile PBS buffer before testing. Subsequently, the products were cut into tablets (7 mm in diameter), washed by sterile PBS buffer, and further sterilized under ultraviolet light. L929 fibroblasts were seeded in the wells (5×10^4 cells per well) with samples and cultured in 500 μL of complete culture medium (DMEM with 10% fetal bovine serum)

in an incubator at 37 °C with 5% CO_2. After 1 and 5 days, culture medium of each well was removed and washed with PBS buffer. For the CCK-8 assay, 500 μL of complete culture medium and 50 μL of CCK-8 reagent were added to each well and incubated for 2 hours. The absorbance (*A*) of the medium was measured using a microplate reader (Epoch2, Biotek, America) at 450 nm. Blank complete culture medium without cells nor samples was served as the blank group. The cell viability was calculated according to equation (2):

$$\text{Cell viability} = \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}} \times 100\% \quad (2)$$

For the live/dead assay, 200 μL of calcein AM/PI double staining agent was added to each well and incubated at 4 °C for 15~20 min. The cells were washed with PBS buffer and observed using a laser confocal microscope (TCS SP8, Leica, Germany).

For cell morphology assay, 1000 μL of complete culture medium was added into 10 mg of sample and incubated at 37 °C for 1 day to prepare the conditioned culture medium. L929 fibroblasts were seeded in the wells (5×10^4 cells per well) and cultured with complete culture medium (DMEM with 10% fetal bovine serum) in an incubator at 37 °C with 5% CO_2 for 1 day. Subsequently, the culture medium was replaced by the conditioned culture medium. After 1 day of culture, L929 fibroblasts were fixed with 4% paraformaldehyde for 10 minutes, followed by permeabilized and closed with 0.5% Triton X-100/1% NBS-PBS for 20 minutes. The cytoskeleton and cell nucleus were stained by Actin-Tracker Green and 4’,6-diamidino-2-phenylindole (DAPI), respectively. Cell morphology was observed by a laser confocal microscope (AE31E, Motic, China).

In vitro cell adhesion test

All the dried cryogels were sterilized by ethylene oxide and rehydrated by sterile PBS buffer before the tests. The as-obtained VMB@APHs were cut into tablets (10 mm in diameter), sterilized in 75% ethanol for 30 minutes, and further washed by sterile PBS buffer for three times. L929 fibroblasts were seeded on the loosely porous and the densely porous surfaces of VMB@APHs (5×10^4 cells per well) and cultured in 500 μL of complete culture medium in an incubator at 37 °C with 5% CO_2 for 2 days. Subsequently, VMB@APH were washed with PBS buffer carefully. 500 μL of calcein AM/PI double staining agent was added to the wells and incubated at 37 °C for 30 minutes. The L929 fibroblasts adhered on VMB@APHs were observed using a laser confocal microscope (AE31E, Motic, China).

In vivo wound healing

Animal surgery plan was approved by the Research Ethics Committee of Guangdong Provincial People's Hospital (KY2023-415-02). Male Sprague Dawley rats (160-220 g, Guangdong medical laboratory animal center, China) were used in the study.

All the dried cryogels were sterilized by ethylene oxide and rehydrated by sterile PBS buffer before the tests. The hydrogels were cut into tablets (10 mm in diameter), sterilized in 75% ethanol for 30 minutes, and further washed by sterile PBS buffer for three times. Before the surgery, rats were anesthetized with pentobarbital sodium solution (3 wt.% in normal saline, 1.3 mL kg^{-1} for each rat) and their dorsal area was depilated and disinfected. Three full-thickness circular wounds (10 mm in diameter)

were created on the back of each rat by 10-mm skin biopsy punch. Subsequently, 100 μL of *S. aureus* suspension (10^7 CFU mL^{-1}) was added into the wound. After seeding for 2 hours, the wound received no hydrogel dressing treatment was the control group, and the wounds treated with APH or VMB@APH were the experiment groups. All the wounds were covered with medical adhesive tapes. On day 5 and day 10, rats were anesthetized by isoflurane and the wound sizes were recorded via digital camera. On day 15, rats were humanely euthanized, and their wound sites were harvested in full layer in conjunction with surrounding tissues. The tissues were immersed in 4% paraformaldehyde solution for 2 days, embedded in paraffin, and stained with hematoxylin and eosin staining, Masson trichrome staining, and immunohistochemistry staining images of cluster of differentiation 68 and interleukin-6 by Wuhan Servicebio Technology Co., Ltd.

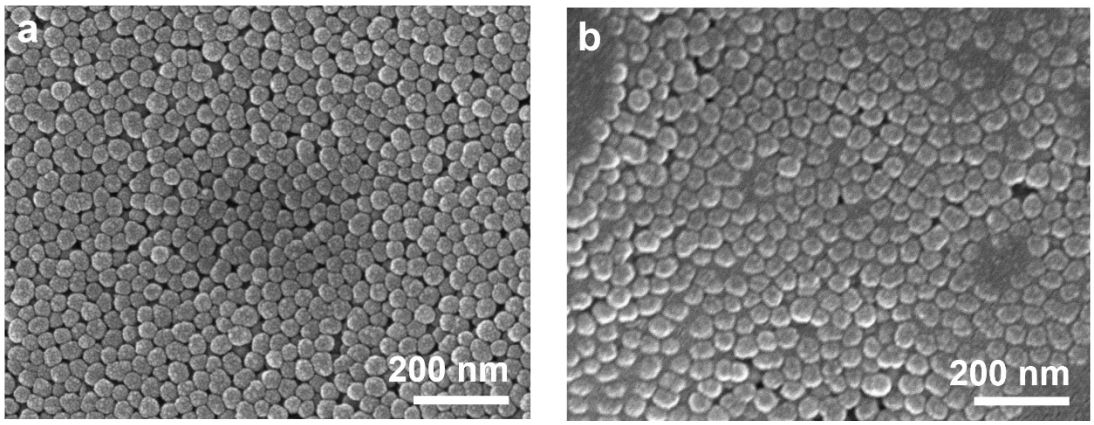

Figure S1. SEM images of (a) SiO_2-Br and (b) SiO_2-*g*-PS.

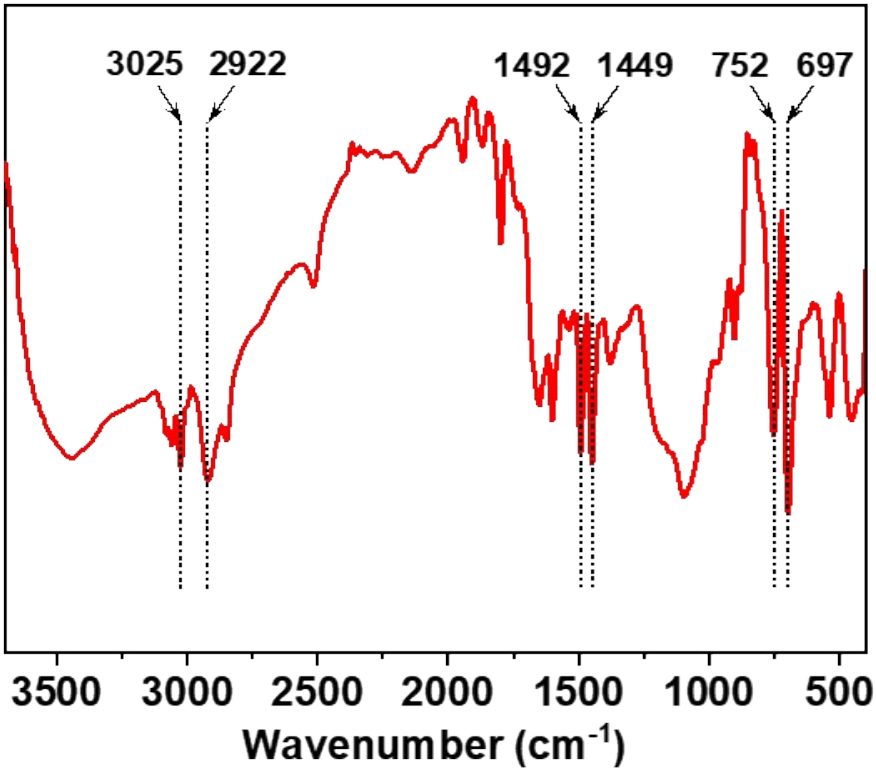

Figure S2. FT-IR spectrum of SiO_2-*g*-PS.

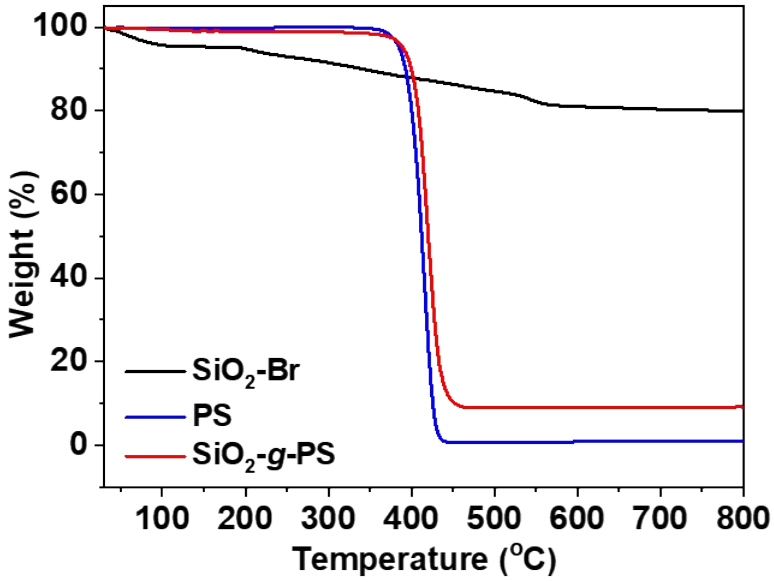

Figure S3. TGA curves of SiO_2-Br, PS, and SiO_2-*g*-PS.

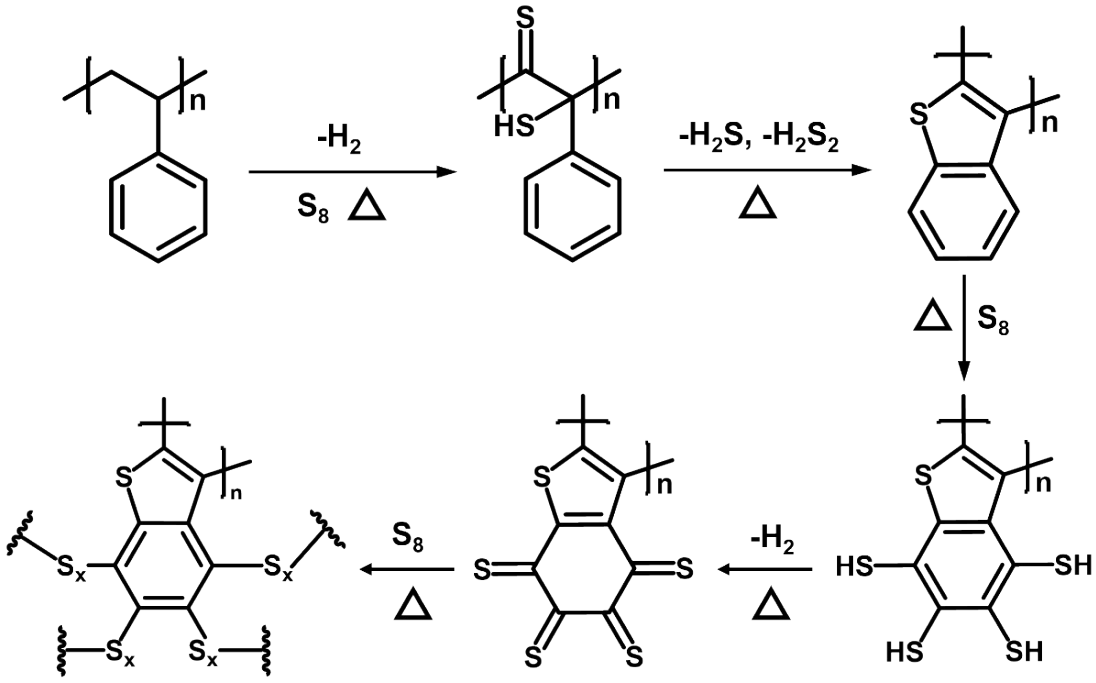

Figure S4. The possible reaction mechanism of polystyrene vulcanization.

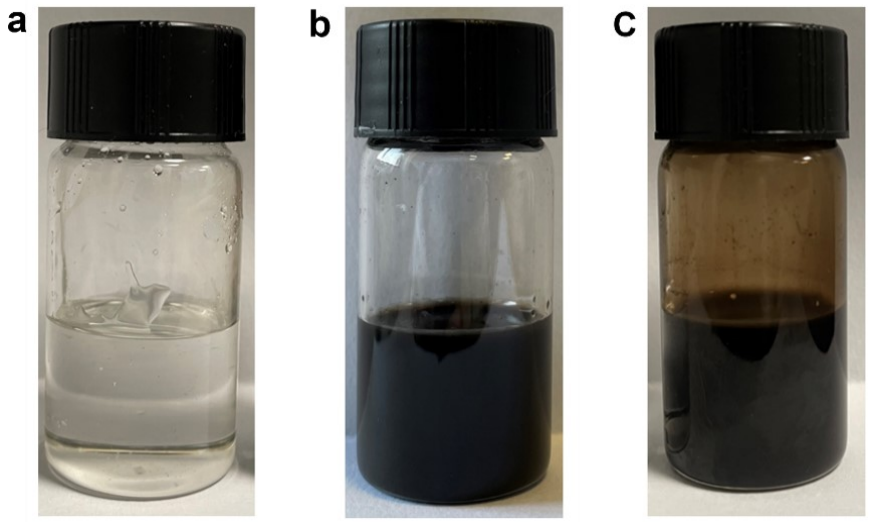

Figure S5. Digital photos of (a) PVA/DMSO solution, (b) VMB/DMSO dispersion, and (c) PVA/DMSO solution with dispersed VMB.

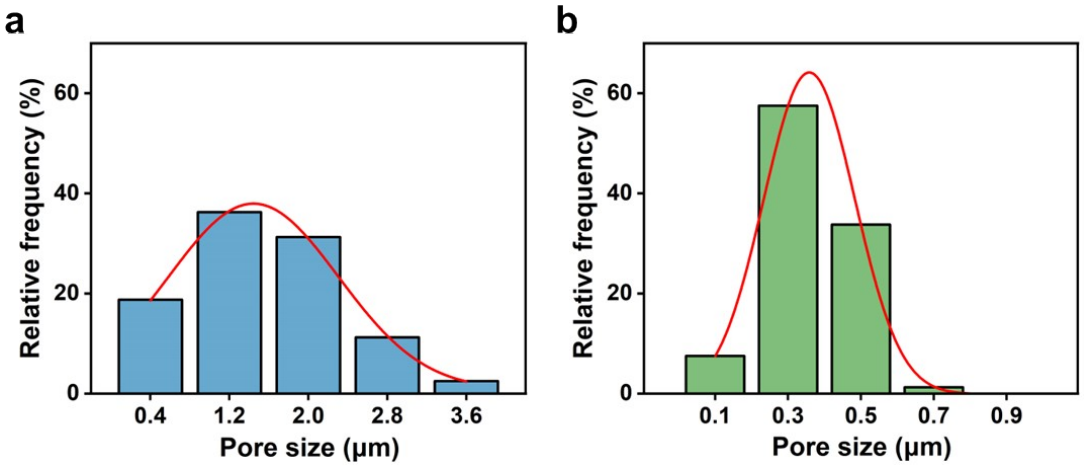

Figure S6. Pore size distributions for (a) loosely porous and (b) densely porous surfaces of the asymmetric porous cryogel encapsulating VMB. Noted that samples' pore sizes are obtained from their SEM images by measuring the maximum pore diameters of 80 randomly selected pores.

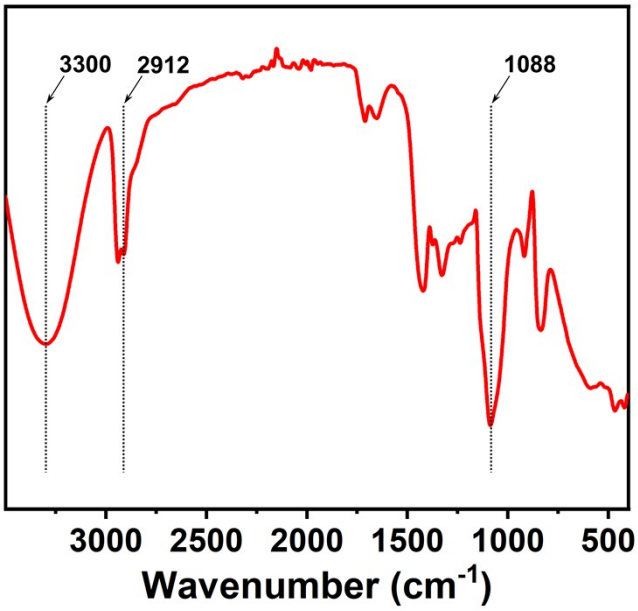

Figure S7. FT-IR spectrum of the asymmetric porous cryogel encapsulating VMB .

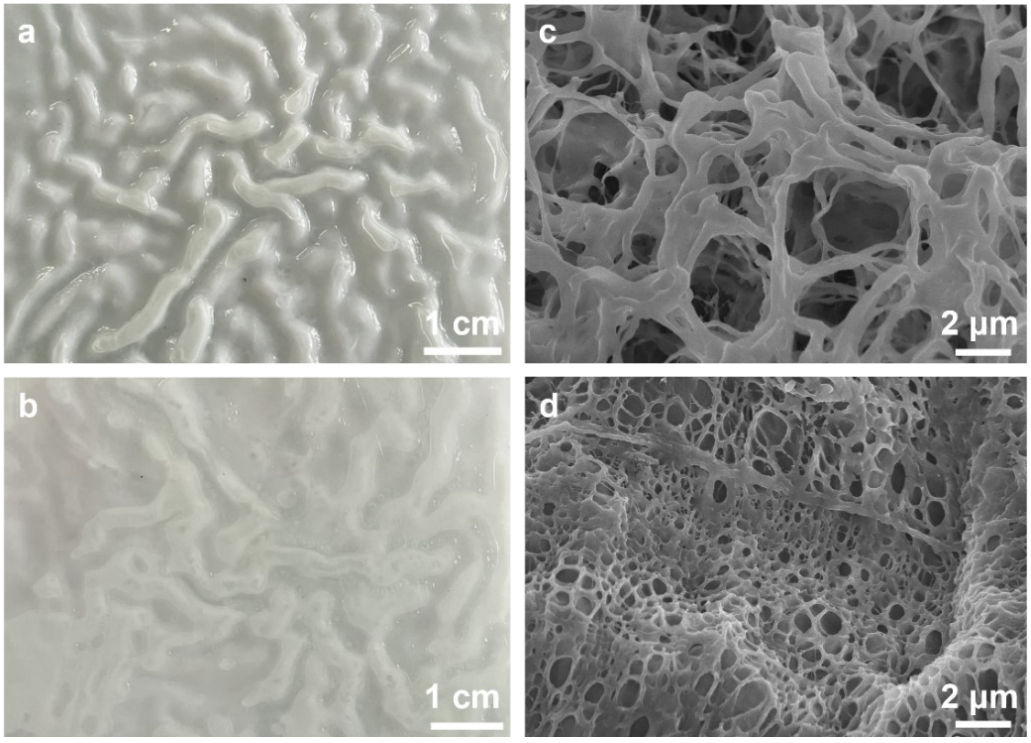

Figure S8. Digital photos of (a) the loosely porous surface and (b) the densely porous surface for the APH. SEM images of (c) the loosely porous surface and (d) the densely porous surface for the asymmetric porous cryogel.

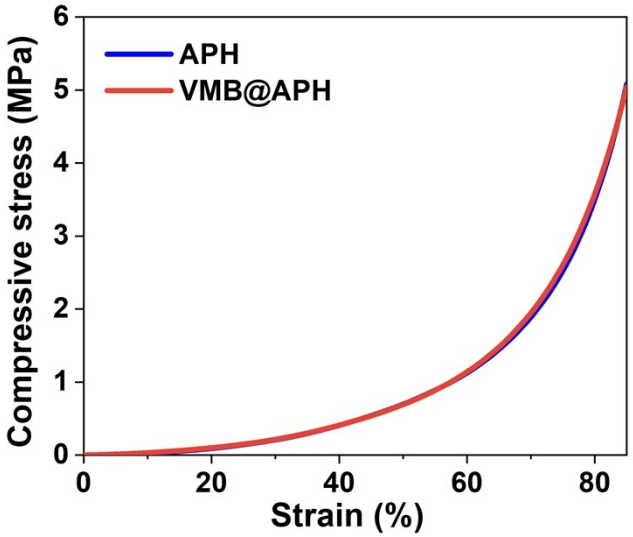

Figure S9. Compressive stress-strain curves of APH and VMB@APH.

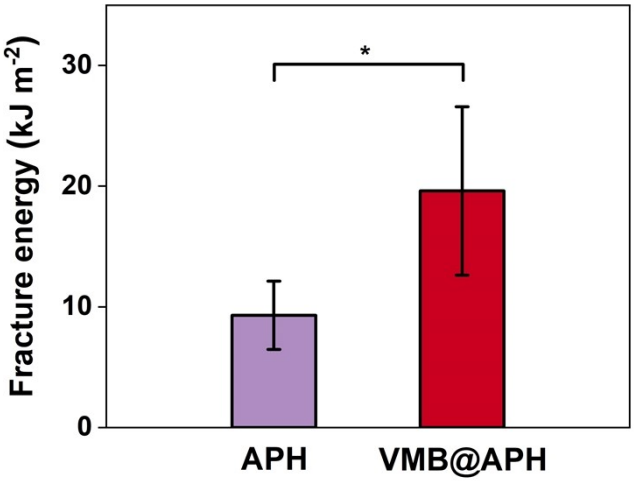

Figure S10. Fracture energies of APH and VMB@APH (n = 4; Student's t test; * p < 0.05; error bars = SD; data are presented as mean values ± SD).

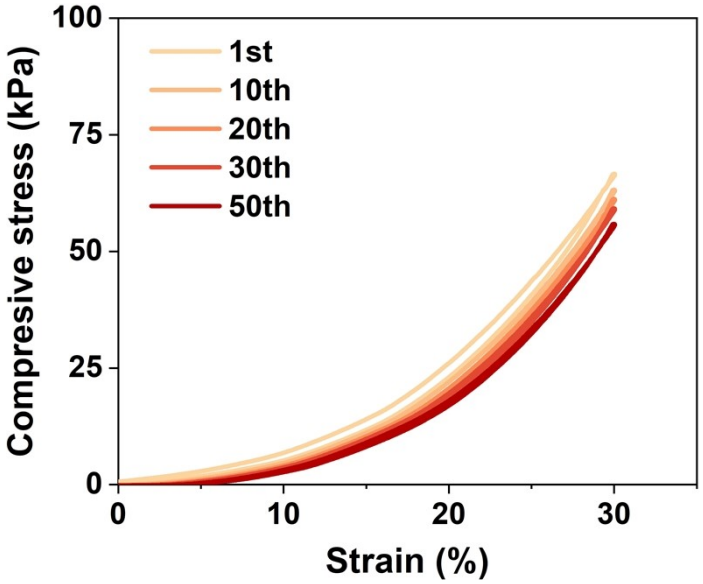

Figure S11. Cyclic compressive loading-unloading curves of VMB@APH.

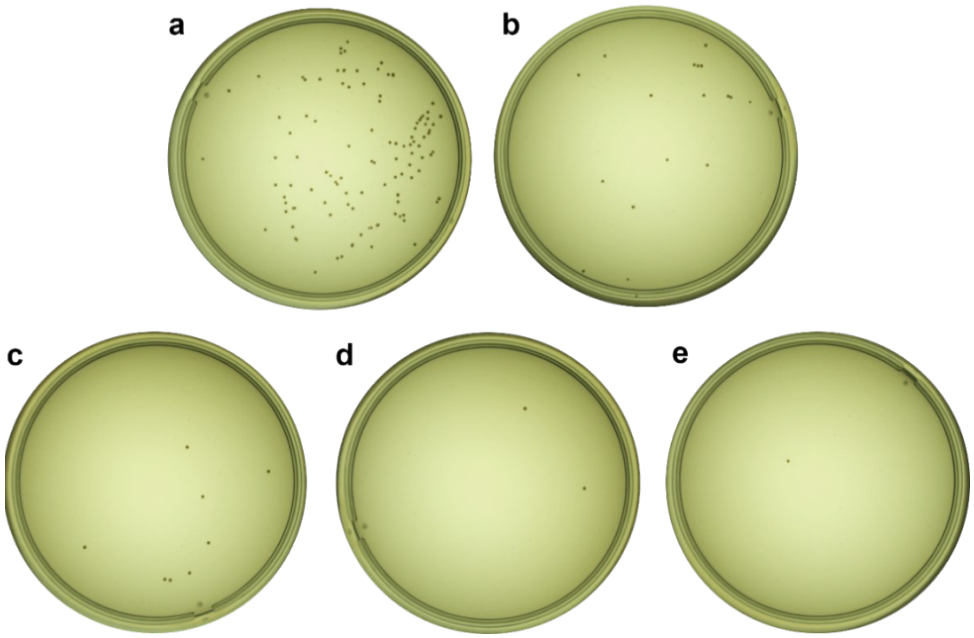

Figure S12. Digital photos of colonies of *S. aureus* after treatments with (a) APH, (b) VMB@APH-0.8%, (c) VMB@APH-1.7%, (d) VMB@APH-3.3%, and (e) VMB@APH-5.0%.

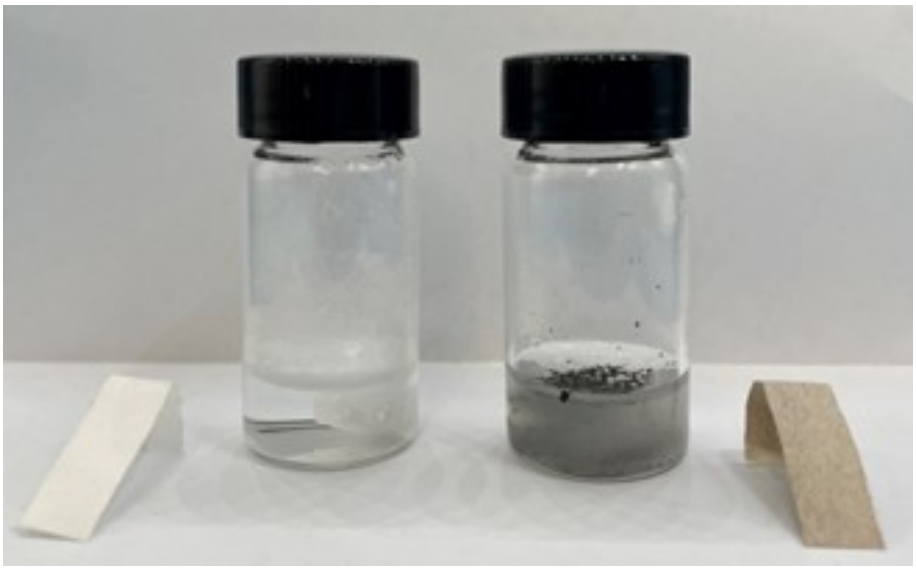

Figure S13. Lead(II) acetate test strip after 5 hours of exposure to cysteine solutions with dispersed SiO_2-*g*-PS (left) and VMB (right).

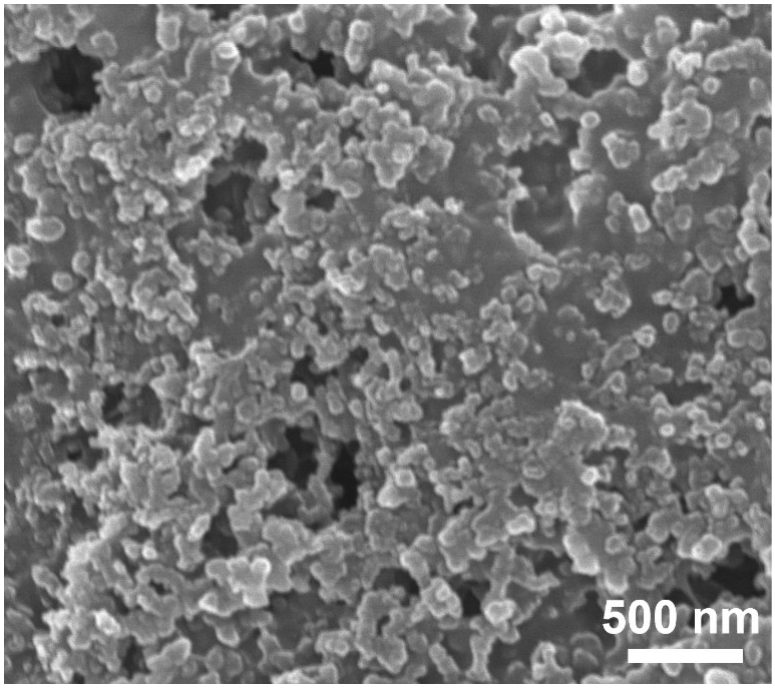

Figure S14. SEM image of SiO_2/*s*PS.

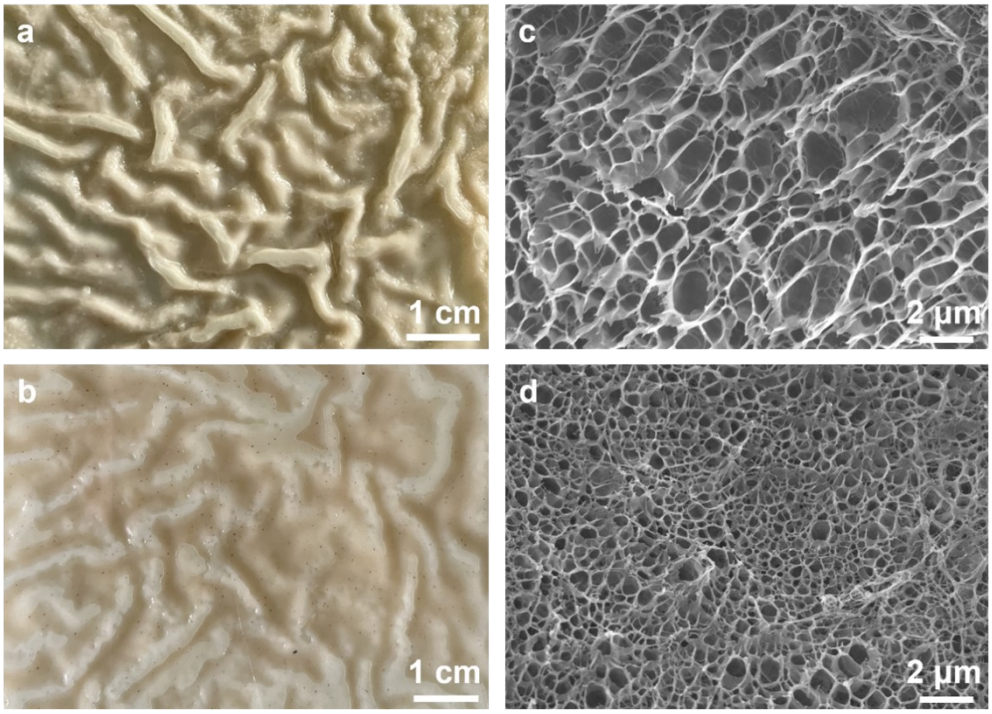

Figure S15. Digital photos of (a) the loosely porous surface and (b) the densely porous surface for the SiO_2/*s*PS@APH. SEM images of (c) the loosely porous surface and (d) the densely porous surface for the asymmetric porous cryogel encapsulating SiO_2/*s*PS.

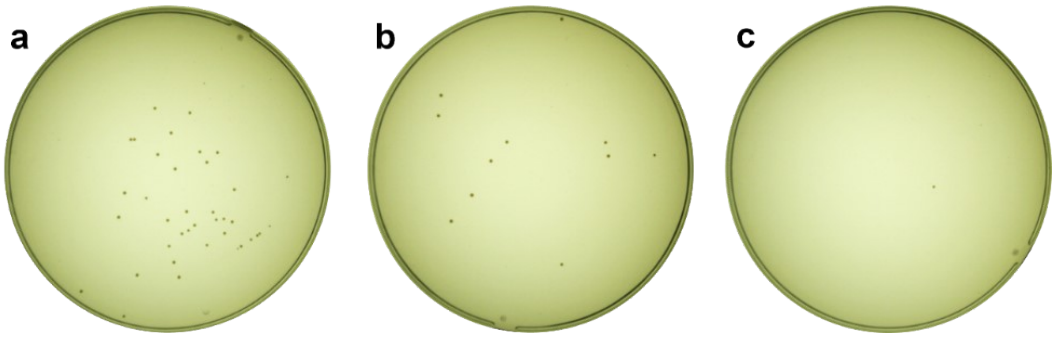

Figure S16. Digital photos of the viable bacterial colonies of (a) APH, (b) SiO_2/*s*PS@APH, and (c) VMB@APH.

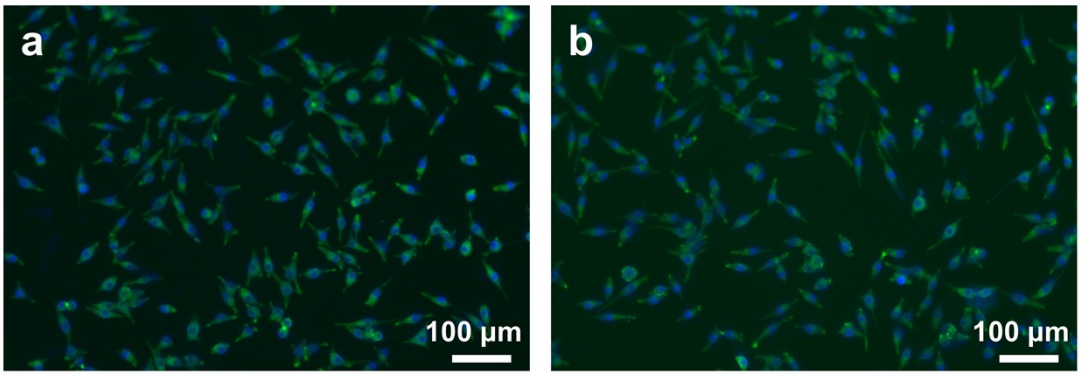

Figure S17. Morphologies of L929 fibroblasts cultured in conditioned culture media of (a) APH and (b) VMB@APH for 1 day.

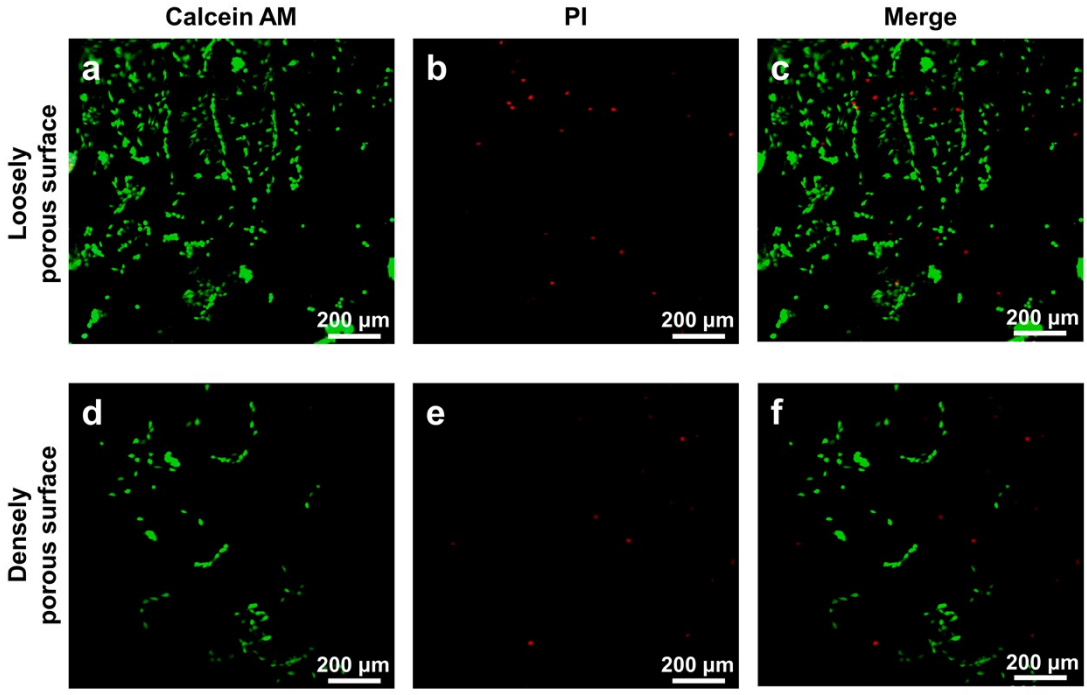

Figure S18. Fluorescence images of L929 fibroblasts cultured on (a-c) the loosely porous surface and (d-f) the densely porous surface of VMB@APH for 2 days.

Table S1. Parameters for preparing the precursor solutions of different samples.

Sample	*m*(SiO_2-*g*-*s*PS) (mg)	*m*(SiO_2/*s*PS) (mg)	*V*(DMSO) (mL)	*m*(PVA-1799) (g)
VMB@APH-0.8%	60	0	40	7.2
VMB@APH-1.7%	120	0	40	7.2
VMB@APH-3.3%	240	0	40	7.2
VMB@APH-5.0%	360	0	40	7.2
SiO_2/*s*PS@APH	0	240	40	7.2

References

[1] J. Wu, S. Liu, J. Huang, Y. Cui, P. Ma, D. Wu, K. Matyjaszewski, *Macromolecules* **2021**, *54*, 2992.

[2] W. Mai, Y. Zuo, C. Li, J. Wu, K. Leng, X. Zhang, R. Liu, R. Fu, D. Wu, *Poly. Chem.* **2017**, *8*, 4771.

[3] J. Wu, J. Huang, Y. Cui, D. Miao, X. Ke, Y. Lu, D. Wu, *Adv. Mater.* **2023**, *35*, 2211471.

[4] W. Liang, W. He, R. Huang, Y. Tang, S. Li, B. Zheng, Y. Lin, Y. Lu, H. Wang, D. Wu, *Adv. Mater.* **2022**, *34*, 2108992.