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Starch-regulated adhesive hydrogel with controllable separation property for painless dressing change

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Supporting information

2.5 Water content and swelling properties

HAVS-0, HAVS-1, HAVS-2 and HAVS-3 (radius: 7mm, thickness: 2mm) were weighed and freeze-dried for 48 hours to measure water content. The formula of water content (M) was defined as:

$$M(\%) = \frac{W_0 - W_1}{W_0} \times 100$$

W_0 and W_1 were the weight of hydrogel before and after drying. To analyze the swelling properties of the hydrogels, four HAVS hydrogel samples (radius: 7mm, thickness: 2mm) were weighed and soaked in deionized water at 25 °C. The weight of the hydrogel sample was measured at fixed intervals until the swelling was equilibrated. The formula of swelling degree (G) was defined as:

$$G(\%) = \frac{M_1 - M_0}{M_0} \times 100$$

M_0 was the initial weight of the hydrogel, and M_1 was the weight of the hydrogel at different time points.

2.6 Mechanical measurement

The universal testing machine (INSTRON, USA) was used to measure the mechanical properties of hydrogels at room temperature. Samples were prepared in a rectangular shape (length: 50 mm, width: 10 mm) with a thickness of 2 mm. The speed of tensile test was constant at 50 mm/min. For compression test, the hydrogel was prepared as cylinder (diameter: 15 mm, thickness: 20 mm) and the compressive speed was fixed at 20 mm/min. The cyclic compressive tests were conducted for thirty loading-unloading cycles at compressive rate of 20 mm/min and strain of 50% without intervals between consecutive cycles. All samples were retested three times.

2.7 Adhesion strength test

The adhesion property of the HAVS hydrogel was evaluated by a lap-shear test. The porcine skin was cut into 3 cm×1 cm rectangle and immersed into PBS before use. Then the hydrogel was applied onto the surface of porcine skin and another skin was put onto the hydrogel surface. The adhesive area was 1 cm×1 cm. And the adhesion strength of the hydrogel to aluminum sheet and glass were tested by the same method. The adhesive strength was calculated by the equation:

$$Adhesive\ strength = \frac{F}{A}$$

F (N) was the force when the overlapped skins were peeled off and A (m^2) was the overlapped area. In addition, the adhesion

reversibility between the hydrogel and porcine skin was quantified using a standard 180° peeling test. One side of the hydrogel was covered with a black conductive adhesive backing to prevent the elongation along the peeling direction, and the other side was tightly attached to the porcine skin surface. During the peeling process, deionized water was sprayed on the interface between the hydrogel and porcine skin to quantify the change of adhesion energy. After the first peeling test, the water on the surface of the hydrogel was gently wiped off, and the hydrogel was again attached to the surface of the porcine skin for another 20 min, prior to the second test. The adhesion energy was calculated as:

$$Adhesion\ energy = \frac{F}{W}$$

F (N) was the value of peeling force from plateau, and W (m) was the width of the sample. The test speed was constant at 100 mm/min, and all samples were repeated three times.

2.8. Antibacterial activity

The antibacterial activities of hydrogels were evaluated through disc method by using *S. aureus*, *E. coli* and *C. albicans* as model bacteria. Hydrogel samples with the diameter of 5 mm were immersed alternately in water and 75% ethanol and then sterilized under UV light for 30 min. 100 μL of 10^8 CFU/mL bacterial suspensions was coated onto agar plate

surface and spread it evenly with a spreader. The sterilized hydrogel sample was gently placed on an agar plate and the plate was sealed. The agar plates were placed at 37 °C (*S. aureus*, *E. coli*) or 28 °C (*C. albicans*) for 24 h, respectively, and then the growth of bacterial colonies around the samples was observed.

The antibacterial activities of HAVS hydrogels against *S. aureus*, *E. coli* and *C. albicans* were also evaluated by colony formation counting method. 500 μL of bacterial suspension (10^8 CFU/ml) was added in sterilized liquid LB medium (*S. aureus*, *E. coli*) or PDA medium (*C. albicans*). 1 g of sterilized hydrogel was added to the inoculated medium, and co-cultured with the bacteria in a 37 °C or 28 °C incubator for 24 hours. The bacteria suspension without hydrogel was used as the blank group. Afterward, the cultured bacterial suspension was diluted 8 times, and 100 μL of the diluted bacterial suspension was evenly spread on the agar plate, and then the plate was sealed. After incubation at 37 °C or 28 °C for 24 h, the colony-forming units (CFU) on the agar plates were counted and the growth of bacteria on each agar plate was photographed. Each group was tested more than three times, and the antibacterial activity was calculated by the following equation:

$$\text{Antimicrobial activity (\%)} = \frac{\text{cell count of the control} - \text{cell count of the}}{\text{cell count of the control}} \times 100\%$$

For morphological observation, the bacterial scanning electron microscope samples were prepared by the filter paper wrapping method. The co-culture solution of bacteria and hydrogel was centrifuged at 12000 r·min^{-1}, and the bacterial sediment was collected and transferred to filter paper bags. After that, the bacteria were fixed with 2.5 wt% fresh glutaraldehyde, dehydrated gradient with absolute ethanol solution and then dried naturally. Bacterial morphologies were observed by field-emission scanning electron microscopy (SEM, JEOL JSM 7800F).

2.9 Cytotoxicity test

The MTT method was used to evaluate the HAVS hydrogels cytotoxicity with the mouse fibroblast cells (L929 cells). L929 mouse fibroblasts were cultured in complete growth medium consisted of 89% DMEM, 10% fetal bovine serum and 1% penicillin-streptomycin. First of all, L929 cells were seeded in 96-well plate at a density of 1×10^4 cells/well, and then the sterile hydrogel (30 mg) was introduced into the wells. 200 μL of PBS was added to the outer circle to prevent the cell fluid from drying out. After being co-incubated for 24 h at 37 ^{o}C and 5% CO_2, the hydrogel and medium were removed. Then 20 μL of

MTT solution (5 mg/mL, filtered through a microporous membrane) was added into each well and incubated for 6 h. Eventually, the medium was replaced by 150 μL of DMSO and the optical density (OD) at 490 nm was measured by enzyme-linked detector (BIOBASE-EL10A). The cell viability was calculated according to a previous study. Cells seeded into culture without hydrogel served as the control group. All the experiments were carried out in quintuplicate.

2.10 In Vivo Wound Healing

The skin full-thickness model of SD rat was used to evaluate the effect of hydrogels on wound healing. All rats (female, approximately 240 g) were randomly divided into 5 groups. After being anesthetized with isoflurane, the dorsal area of rats was totally depilated and full-thickness wound (15 mm×15 mm) was established on the upper back of each rat. The HAVS hydrogel dressings were attached to the wound surfaces as the experimental groups and the rat with bare wounds was used as the control group. After five days of operation, the wound HAVS hydrogel dressing was removed. Then the wound area was measured and photographed on 5^{th}, 10^{th} and 15^{th} respectively. The areas of wounds were measured with Image-Pro Plus 6.0 software, and the formula for calculating the wound closure (%) was as follows:

$$Wound\ closure\ (\%) = \frac{A_0 - A_n}{A_0} \times 100\%$$

Where A_0 was the initial wound area and A_n was the wound area at day n (n = 5, 10 or 15). To evaluate skin regeneration in that area, the wound area tissue from rats collected on 15th was cut for histological study. Hematoxylin-Eosin (H&E) staining was performed on the skin sections, and the stained sections were observed by microscopy.

Table S1. The specific component of HAVS hydrogels

Sample	AAc (g)	HEMA (g)	[VBIm]Br (g)	MAS (g)	H_2O (g)	APS (g)
HAVS-0	1.50	1.30	0.52	0	6.0	0.02
HAVS-1	1.50	1.30	0.52	0.12	6.0	0.02
HAVS-2	1.50	1.30	0.52	0.24	6.0	0.02
HAVS-3	1.50	1.30	0.52	0.36	6.0	0.02

Table S2 The specific component of [VBIm]Br gradient antibacterial test.

Sample	AAc (g)	HEMA (g)	[VBIm]Br (g)	H_2O (g)	APS (g)
HAV-1	1.50	1.30	0.17	6.0	0.02
HAV-2	1.50	1.30	0.35	6.0	0.02
HAV-3	1.50	1.30	0.52	6.0	0.02
HAV-4	1.50	1.30	0.69	6.0	0.02

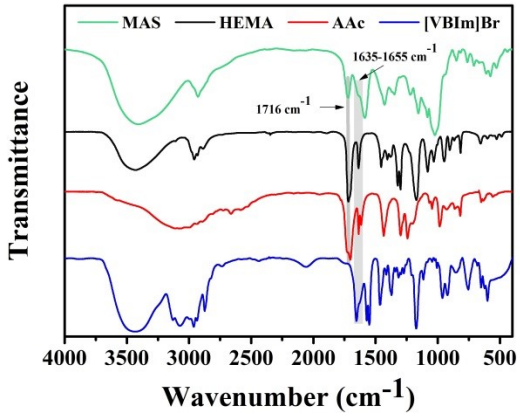

Fig. S1 FT-IR spectra of MAS, HEMA, AAc and [VBIm]Br.

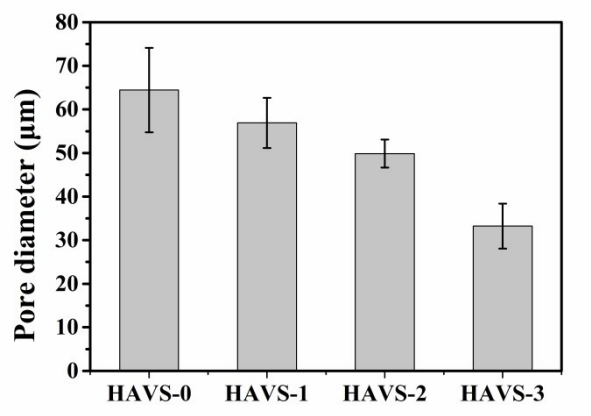

Fig. S2 Pore size distribution of the HAVS hydrogels via Image-Pro Plus 6.0

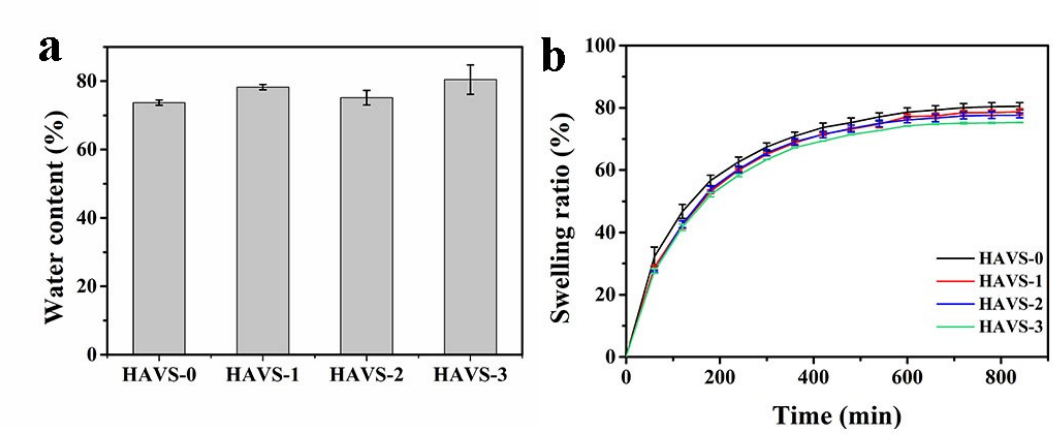

Fig. S3 (a) Water content and (b) swelling rate of HAVS hydrogels (n=3).

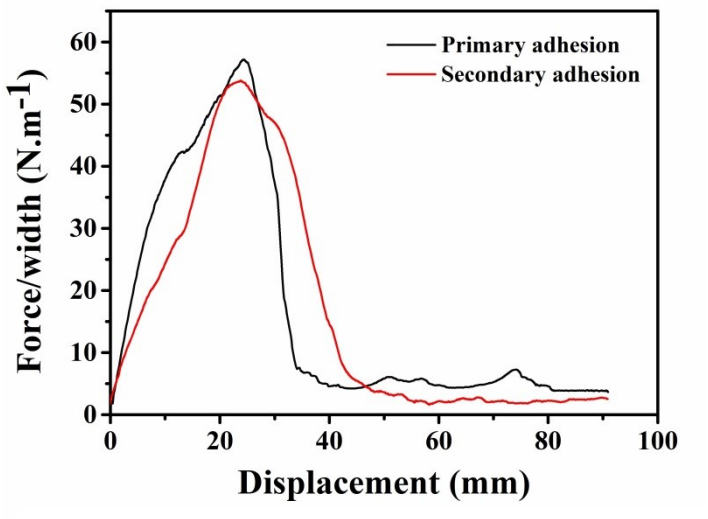

Fig. S4 Curves of the peeling force for hydrogel adhered porcine skin under deionized water.

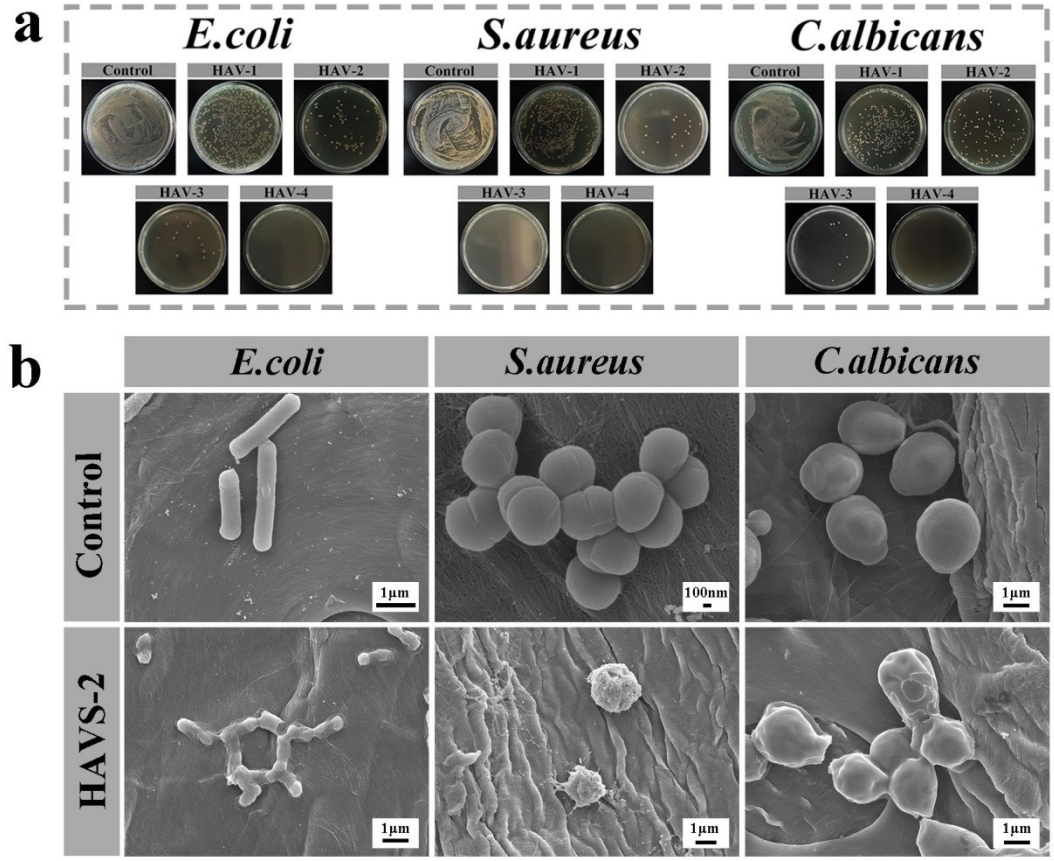

Fig. S5 (a) Photographs of plate-coated of *E. coli*, *S. aureus* and *C. albicans* treated with HAV hydrogels; (b) SEM images of *E.*

coli, *S. aureus* and *C. albicans* before and after treatment using the HAVS-2 hydrogel.

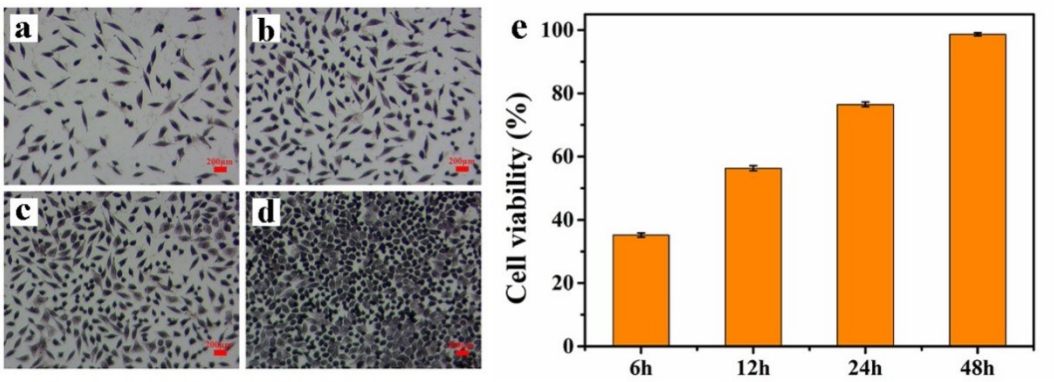

Fig. S6 The microscope images of L929 mouse fibroblasts co-cultured with HAVS-2 hydrogel at (a) 6h (b) 12h (c) 24h and (d) 48h; (e) the activity value of L929 mouse fibroblasts at different time points.

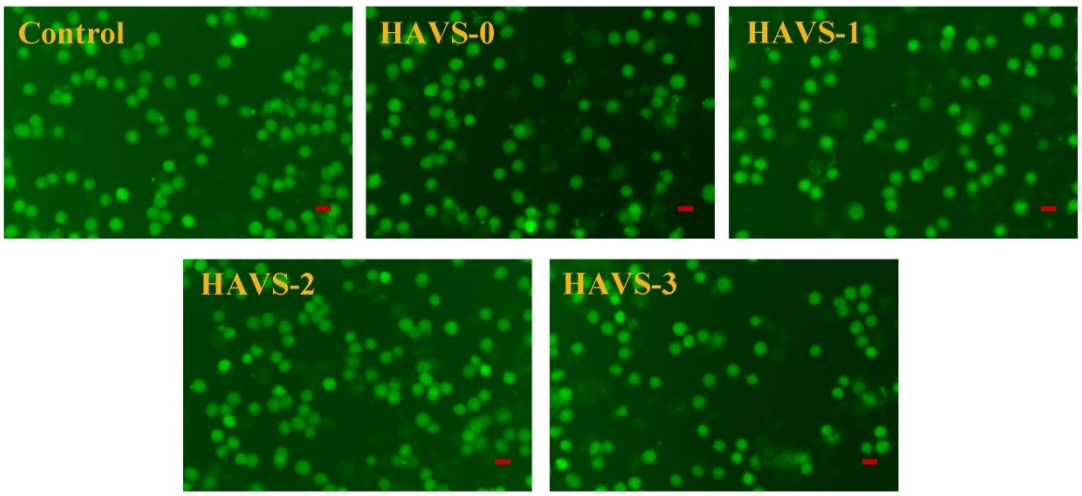

Fig. S7 Live/Dead staining of L929 cells after treated with HAVS hydrogels for 24 h (Scale: 500 μm).