Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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A novel self-healing triple physical cross-linked hydrogel for antibacterial dressing

Dongrun Li[1, 2], Xu Fei*[1], Kang Wang[3], Longquan Xu[1], Yi Wang[3], Jing Tian*[3], Yao Li*[2]

2.4 Water content and swelling properties of the PAAMVs

Four hydrogel samples (radius: 5 mm, thickness: 2 mm) were weighed and then freeze-dried for 24 hours to measure water content. The formula for water content (M) is defined as:

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

W_0 and W_1 are the weight of the hydrogel before and after drying, respectively. In order to analyze the swelling behavior of PAAMV hydrogels, four hydrogel samples (radius: 8 mm, thickness: 4 mm) were immersed in deionized water at 25 °C, and the weight of the hydrogel samples was measured at intervals until the swelling equilibrium was reached. The moisture on the surface of the hydrogel was gently absorb by using filter paper and was weighted immediately. The swelling rate (G) formula is calculated as:

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

M_0 and M_1 are the weights of the hydrogel before swelling and after swelling equilibrium, respectively.

2.5 The protein adsorption experiment of PAAMVs

In this experiment, bovine serum albumin (BSA) was used as the model protein, and the adsorption performance of PAAMVs was determined by Bradford method. The prepared hydrogels pieces (50 mg) were placed in a 24-hole tissue culture plate, and each sample was set with three parallel samples. All samples were immersed in 75% ethanol for 1 h and soaked in PBS buffer (pH=7.4) for 2 h. After UV irradiation for 0.5 hours, the samples were infiltrated in 1 mL BSA (10 mg/mL) solution and cultured in a constant temperature shaker at 37 °C for 24 h. The adsorption capacity can be obtained by calculating the concentration difference of BSA before and after adsorption. The absorbance of BSA solution was determined at 595 nm by UV spectrophotometer, and the concentration was determined using a BSA calibration curve of the same wavelength. The consistence of BSA in the solution before and after adsorption was calculated by BSA standard curve of the same wavelength. The protein adsorption capacity was calculated by the following formula:

$$Adsorbed\ BSA\ (mg/g) = \frac{C_0 - C_a}{w}V$$

Where C_0 is the BSA concentration (mg/mL) before adsorption, C_a is the concentration (mg/mL) of BSA after adsorption, w is the initial weight (g) of hydrogel and V is the initial volume of BSA solution (mL).

2.6 Mechanical properties of PAAMVs

All tensile and compression tests were tested with an electronic universal testing

machine. In the tensile test, the initial distance of the sample (length: 40 mm, width: 20 mm, thickness: 10 mm) between the two fixtures was 20 mm, and the tensile speed was constant at 5 mm/min. For the compression test, the cylindrical sample (diameter: 30 mm, thickness: 20 mm) was tested with a 20 mm clamping distance, and the strain rate was constant at 2 mm/min. All mechanical experiments were carried out in air at 25 °C.

2.7 Self-healing performance test of PAAMVs

An optical microscope was applied to observe and record the self-healing process of the hydrogel. A columnar hydrogel sample (diameter: 20 mm, length: 8 mm) was sliced into two pieces, and then the two separated hydrogels were put together promptly at 25 °C without any extra pressure, observed for 24 h under a 10x microscope. The disc shaped (diameter: 50 mm, thick: 4 mm) and long strip (10 cm) hydrogel samples were cut in half, half of them was immersed in methylene blue solution, and the filter paper was used to wipe off the excess dye solution. The other half was not processed, and then immediately put together. After 24 hours of healing, the stretching or knotting stretching conditions were observed. In addition, in order to test the mechanical properties of the self-healing hydrogel, a long strip (length: 40 mm, width: 17 mm, thickness: 7 mm) was cut in half, and then the two separated samples were put back together immediately. Without any other stimulation or pressure, the cut hydrogel self-heal at 25 °C for 24 h, and the healed hydrogel was tested with the electronic universal testing machine. The initial clamp spacing was 20

mm, and the strain rate in the air was 5 mm/ min. The self-healing efficiency of strain (ε_h%) and self-healing efficiency of stress (λ_h%) can be calculated using following equations.

$$\varepsilon_h\% = \frac{\varepsilon_b}{\varepsilon_{b0}} \times 100\%$$

where, ε_b is the elongation ratio of the healed samples and ε_{b0} is the elongation ratio of the pristine hydrogel.

$$\lambda_h\% = \frac{\lambda_b}{\lambda_{b0}} \times 100\%$$

where, λ_b is the breaking strength of the healed samples and λ_{b0} is the breaking strength of the pristine hydrogel.

2.8 Antibacterial performance test of PAAMVs

The antimicrobial properties of PAAMVs were measured by the disc method. *E. coli*, *S. aureus* and *C. albicans* were used to assess the antibacterial effect of the hydrogels. 100 μL of 10^8 CFU/mL bacterial suspension was coated on the surface of the LB or PDA plates, and then round sterile samples with a diameter of 1 cm (soaked in water and 75% ethanol alternately and sterilized by ultraviolet for 30 minutes) was placed on the surface of the culture medium. After incubating at 37 °C for 24 h, the growth of bacteria around the sample were observed.

The antibacterial activities of PAAMV hydrogels against *S. aureus*, *E. coli* and *C. albicans* were evaluated by colony counting method. The PAAMV0, PAAMV1, PAAMV2 and PAAMV3 hydrogels (1 g) were alternately soaked in water and 75% ethanol for 30 min and then sterilized under ultraviolet light for 30 minutes. 500 μL of

S. aureus and *E. coli* (10^8 CFU/mL) were inoculated into 30 mL of sterilized liquid LB medium, and 500 μL of *C. albicans* (10^8 CFU/mL) was inoculated into 30 mL of sterilized liquid PDA medium, and then the sterilized PAAMV hydrogels were placed in the above-mentioned LB and PDA liquid medium. *S. aureus* and *E. coli* were cultivated at 37 °C with 120 rpm for 24 hours, and *C. albicans* was cultivated at 28 °C with 120 rpm for 24 hours. The obtained bacterial solutions were diluted to be 10^{-6} times of the original concentration and 100 μL of the diluted bacterial suspension were spread on the solid medium. After culturing for 24 hours at 37 °C (*E. coli* and *S. aureus*) or 28 °C (*C. albicans*), the number of colony forming units (CFU) were counted and recorded, respectively. The experiment was repeated three times and took the average value. A bacterial suspension without hydrogel was served as a control.

The bacterial scanning electron microscope samples were prepared by the filter paper wrapping method. The sterile PAAMV3 hydrogels (1g) were cultured with *E. coli*, *S. aureus* and *C. albicans* for 6 hours, respectively. 8 mL bacterial solution was centrifuged in a 10 mL centrifuge tube at 12000 $r \cdot min^{-1}$ for 5 min to collect bacterial precipitates. 2.5 wt% fresh glutaraldehyde was added to suspend and fix the bacteria for 2 hours, and then washed with deionized water for three times (centrifugation to discard the supernatant each time, adding deionized water and blowing the bacteria with a straw). After centrifugation, the concentrated bacterial solution was transferred to 1 cm × 4 cm filter paper package, immediately sealed with stapler, dehydrated with 10%, 20%, 30%, 50%, 70%, 80%, 95% and 100% ethanol respectively (each step was about 10 minutes). The filter paper package was dried in the ventilated kitchen and

observed by scanning electron microscope (JEOL-JSM-7800F). Bacterial suspension without hydrogel was used as a control.

2.9 Cytotoxicity test of PAAMVs

The cytotoxicity assessment of PAAMV hydrogels were performed by the MTT method. Generally, L929 mouse fibroblasts were fostered in a conventional growth medium containing 89% DMEM, 10% FBS, and 1% penicillin-streptomycin at 37 °C and 5% CO_2. Sterile PAAMV hydrogels (30 mg) were placed in a 96-well plate and then 100 μL of cell suspension (1×10^4 cells/mL) were inoculated in the well. The cells were cultured on the hydrogels for 48 hours, and the medium was removed. MTT was yellow and could be reduced to blue-violet by living cells. 5 mg MTT was dissolved in 1 mL cell culture medium to prepare 5 mg/mL MTT solution. 20 μL MTT solution and 150 μL medium were added to each well, and then the well plate was put into the incubator at 37 °C with 5% CO_2 for 6 hours to form formazan. The medium was removed, and 150 μL DMSO was added to each well and shaken for 15 minutes to dissolve formazan. The optical density (OD) of formazan was quantified by enzyme-linked detector (BIOBASE-EL10A) at 490 nm. As the control group, the cell suspension (1×10^4 cells/mL) was inoculated into empty culture plate without adding PAAMV hydrogel samples. All the experiments were made in quintuplicate.

2.10 Cell scratch test and rat wound healing test of PAAMVs

In order to analyze the wound healing ability of hydrogel in vitro, cell scratch

test was carried out. L929 mouse fibroblasts were placed into 6-well plate with 2×10^4 cells per well. After 24 hours of incubation at 37 °C with 5% CO_2, L929 mouse fibroblasts covered the whole well plate. The supernatant of each pore was removed and the sterile pipette gun head was used to mark each orifice plate. After that, fresh DMEM medium and 3 mg hydrogel were added to well plate, and the cell migration was observed under the condition of 5% CO_2 37 °C for 12 hours and 24 hours respectively.

For rats wound healing test, twenty female SD rats were randomly divided into 5 groups: control group (without hydrogel dressing), PAAMV0, PAAMV1, PAAMV2 and PAAMV3 hydrogel groups, repeat 4 experiments for each group. All rats anesthetized with isoflurane were shaved the back hair and created a full-thickness wound (2 cm each in length and width). Next, the sterile PAAMV hydrogel dressings were sutured on the wound with a surgical thread, and the rats with bare wounds were used as a control group. After three days of operation, the PAAMV hydrogel dressing on the wound of rats was removed, and wounds were taken and photographed on 0^{th} days, 3^{th} days, 6^{th} days, 9^{th} days, 12^{th} days and 15^{th} days. Rats were sacrificed 15 days after surgery, samples were processed and skin tissue samples were cut for histological study. After staining with hematoxylin-eosin (H&E), the structure of the sample was observed with an optical microscope.

Table S1. Feed ratio of PAA-MGA and PAAMV hydrogels

Sample	AAc (g)	MGA (mg)	[VBIm]Br (g)	$AlCl_3\cdot6H_2O$ (mg)	KOH (mg)	H_2O (mL)	APS (mg)

PAA-MGA	3	100	0	0	70	12	8
PAAMV0	3	100	0	151	70	12	8
PAAMV1	3	100	0.5	151	70	12	8
PAAMV2	3	100	0.75	151	70	12	8
PAAMV3	3	100	1	151	70	12	8

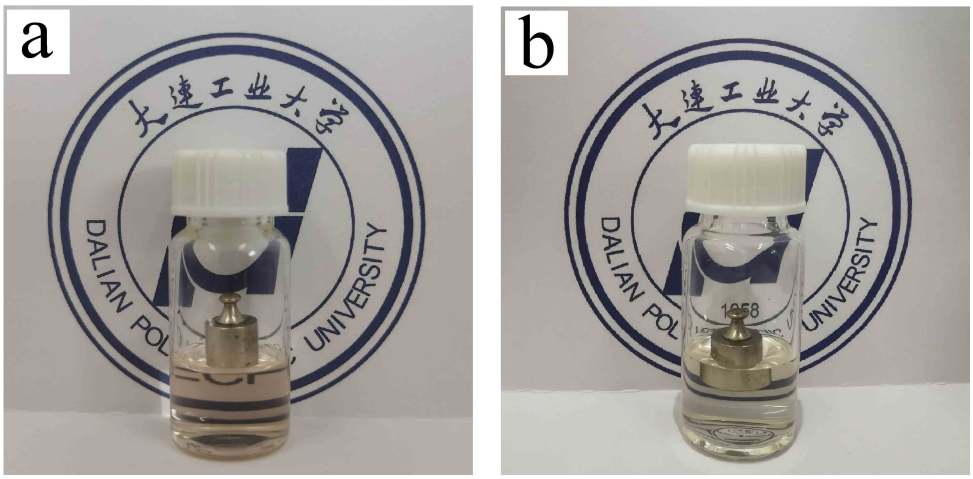

Fig. S1 Photographs of (a) PAAMV0 hydrogel pressure test with a 5 grams weight, (b) PAA-MGA hydrogel pressure test with a 5 grams weight

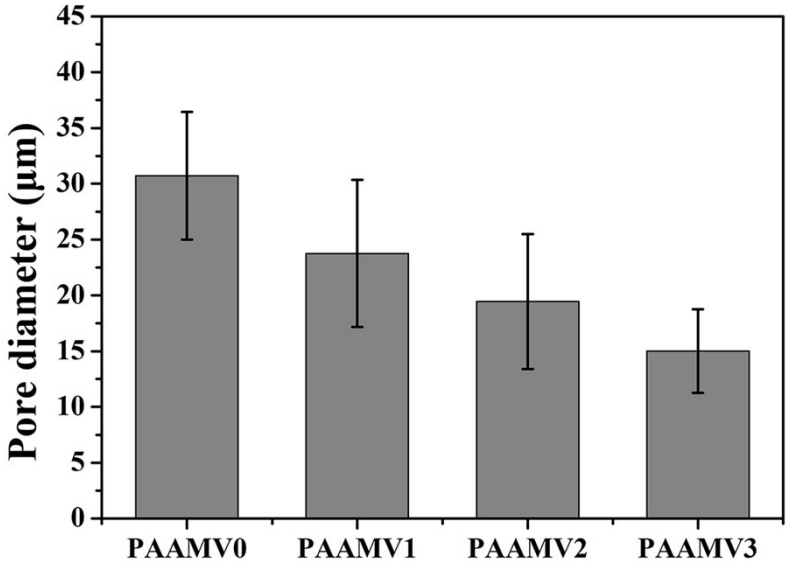

Fig. S2 Pore size distribution of the hydrogels via employing Image-Pro Plus 6.0

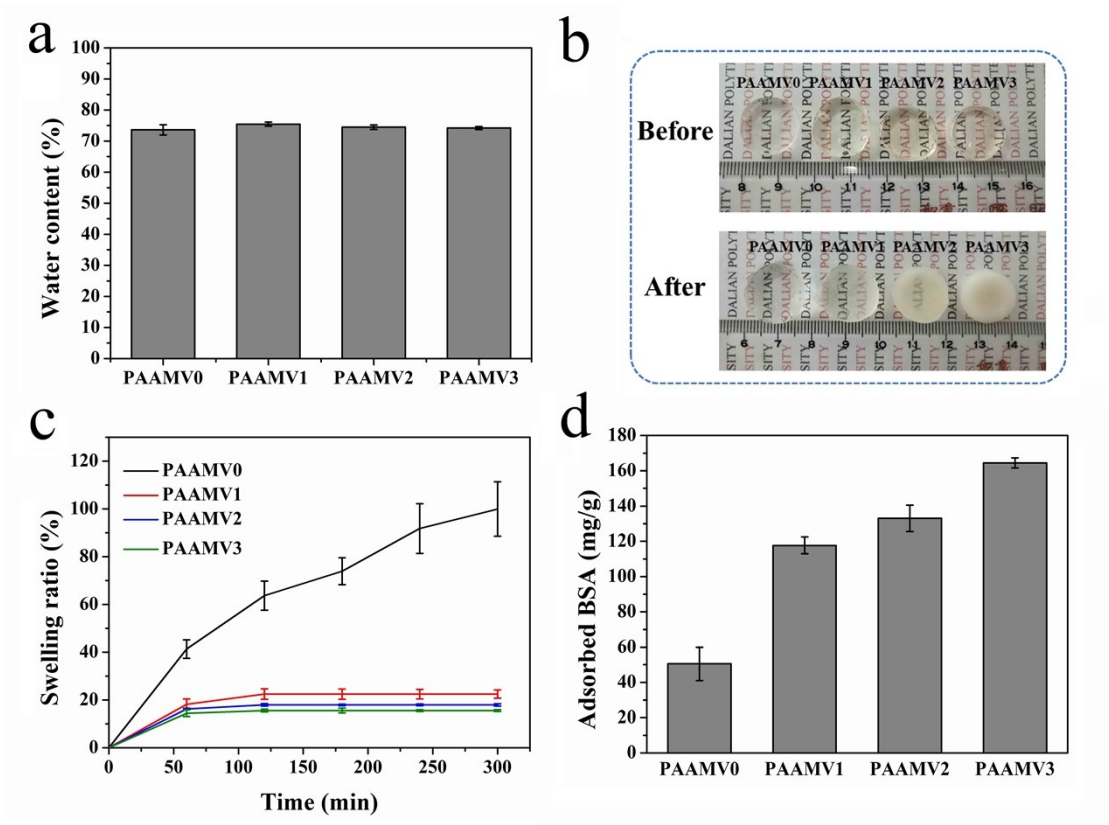

Fig. S3 (a) Water content (n=3), (b) photographs of PAAMV hydrogels before and after swelling for 5 hours at room temperature (25 °C) (n=3), (c) swelling rate of PAAMV hydrogels at different time (n=3), (d) BSA adsorption capacity of PAAMV hydrogels (n=3)

Fig. S4 Photographs of tensile and pressure test for PAAMV3 hydrogel after swelling

Fig. S5 Photographs of the bent PAAMV3 hydrogel

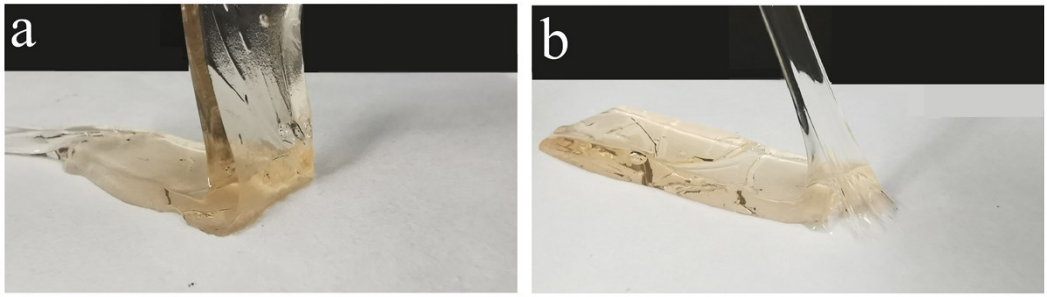

Fig. S6 The adhesion test photographs of (a) PAA-Al^{3+} hydrogel and (b) PAAMV0 hydrogel

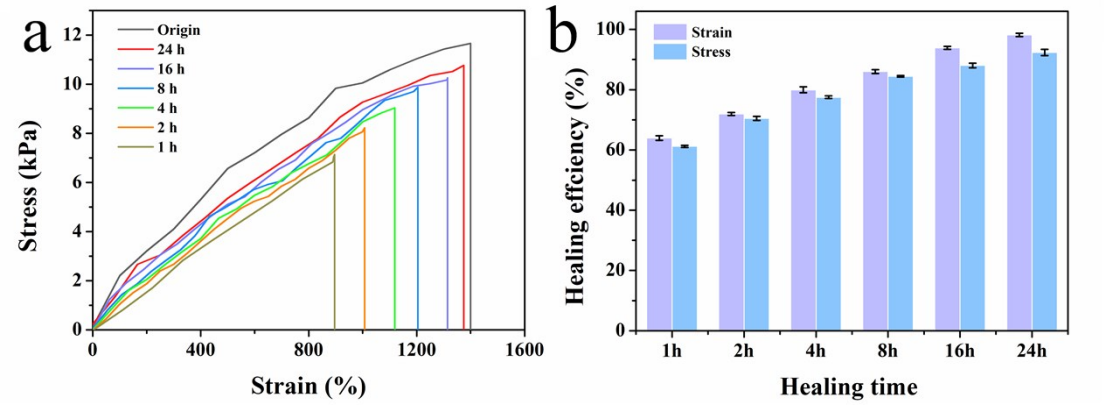

Fig. S7 (a) The stress-strain curves and (b) corresponding self-healing efficiency of the PAAMV3 hydrogel healed at 25 °C for various self-healing time

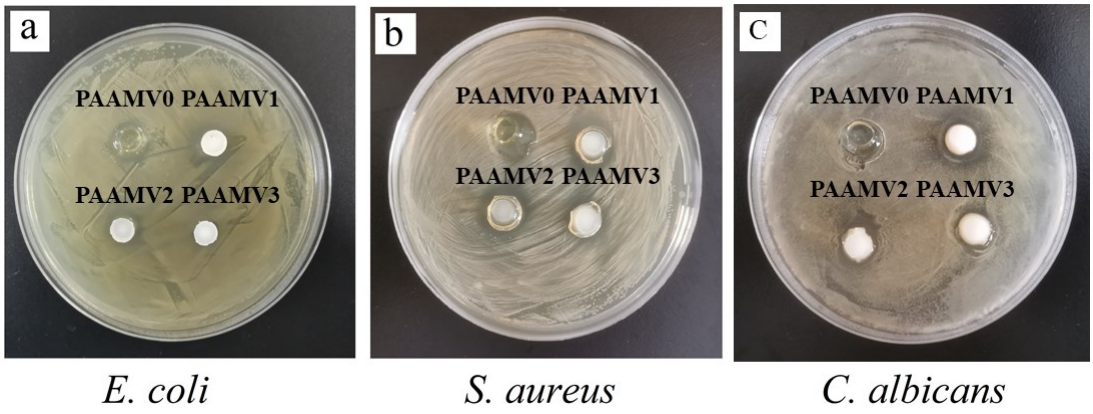

Fig. S8 Antibacterial results of PAAMV hydrogels by the disk method against (a) *E. coli*, (b) *S. aureus* and (c) *C. albicans*

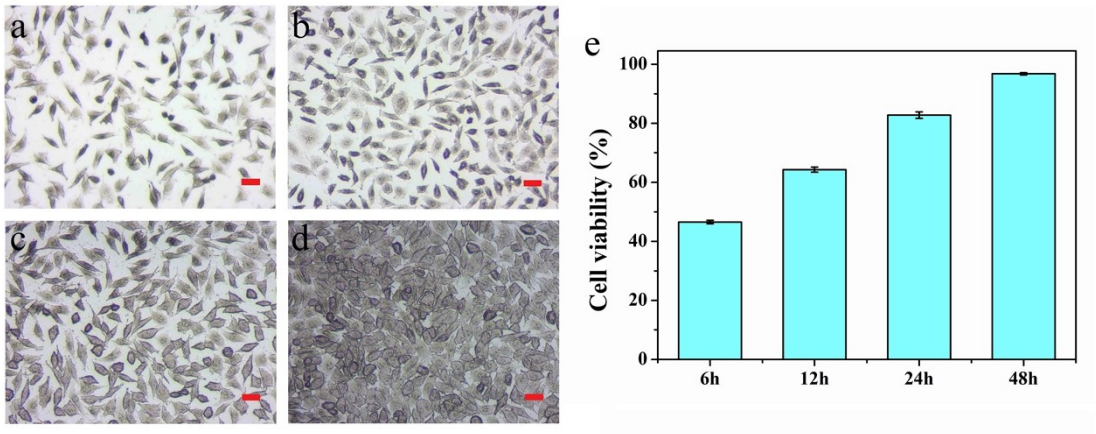

Fig. S9 The cell morphology of L929 mouse fibroblasts co-cultured with PAAMV3 hydrogel under 10X microscope was observed at (a) 6h, (b) 12h, (c) 24h and (d) 48h, scale bar: 100 μm, (e) the activity value of L929 mouse fibroblasts at different time points (n = 5)

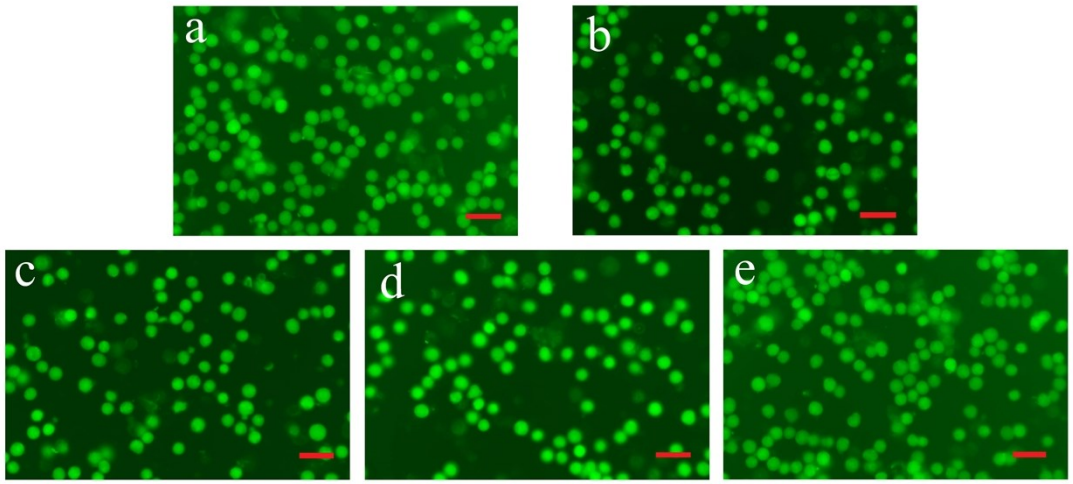

Fig. S10 Fluorescence micrographs of 24 h staining live/dead L929 cells on (a) control, (b) PAAMV0, (c) PAAMV1, (d) PAAMV2, (e) PAAMV3 hydrogels, respectively