

Flexible, high strength and multifunctional polyvinyl alcohol/MXene/polyaniline hydrogel enhancing skin wound healing

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1. Materials and Methods

1.1. Antibacterial activity of the hydrogels in vitro and vivo

The hydrogel discs were put into the 48-wells of plates, 10 μ L of bacterial suspension (in PBS, 10^6 CFU mL^{-1}) was added to the hydrogel surface and incubated for 3 h. The control group, 10 μ L of bacterial suspension was directly diluted with PBS. The inhibition effect of 808 nm light irradiation on MXene for bacteria was studied. The hydrogel discs were put into the 48-wells of plates, 10 μ L of bacterial suspension (in PBS, 10^6 CFU mL^{-1}) was added to the hydrogel surface and 808 nm light on the material for 20 min. 1 mL of PBS was employed to re-suspend bacterial survivors. Add 100 μ L of bacterial dilution to solid medium and culture for 24 hours. The colony-forming units (CFU) on the solid medium were counted after 24 h. Following, 100 μ L of bacterial dilution added to the 96-well plates and tested with a microplate reader at 590 nm, and calculate the killing ratios.

$$\text{Kill (\%)} = \frac{OD_{Con} - OD_{Hydrogels}}{OD_{Con}} \times 100$$

The BALB/c mice (male, 6-8 weeks old) were used to employed in vivo antibacterial models. The E. coli was selected for in vivo antibacterial experiment due to it is not pathogenic to animals. The hydrogel discs were put into the 48-wells of plates, 10 μ L of bacterial suspension (in PBS, 10^6 CFU mL^{-1}) was added to the hydrogel surface, meanwhile the on the hydrogel discs with bacterial suspension lighted 808 nm for 20 min as the 808 nm light group, and incubated for 3 h in an incubator. The control group, 10 μ L of bacterial suspension was directly diluted with PBS. Following, 100 μ L of bacterial suspension was injected subcutaneously into mouse and the mouse was sacrificed after three days. Subsequently, the mouse subcutaneous tissues were crushed and resuspended in 1 mL of PBS respectively. The 100 μ L of PBS were spread onto the solid medium. The CFU were counted after incubation for 24 h.

1.2. Cytocompatibility and cell attachment

All the hydrogel discs were prepared under sterile conditions. The cells were seeded in 48-well plates (2×10^4 cells/well). The hydrogel discs were put into the wells of plates after 6 h. After 1 day

of incubation, the medium and the hydrogel discs were removed. The cells in the well plates were resuspended with PBS and the cell suspension was transferred into a 48-well plate. Each well was added dyeing working solution and incubated at room temperature for 30 min. A fluorescence microscope (EVOS FL Auto, Thermo Fisher Scientific, USA) was used to observe the cell status.

1.3. In vitro promotion of cell proliferation, migration and angiogenic activity

Cell proliferation on the hydrogels were evaluated using the MTT method. Cells were seeded onto the hydrogel discs in 96-well plates (2×10^3 cells/well) for 1, 2 and 3 days. After, MTT was added, then dimethyl sulfoxide was employed to dissolve the formazan. The absorbance at 570 nm was measured with a microplate reader and cell viability was calculated. Monolayer cells were formed after 24 h. A 1000 μ L pipette plastic tip was used to scratch the monolayer adherent cells. After, the hydrogel slices were put into the wells to contact the scratches. Cells were cultured using the medium containing 0.1% FBS for 24 h. After incubation for 24 h, the medium, the hydrogel and cell debris were removed. Photos of cell scratches were taken using an inverted microscope. Besides, the effect of ES on cell proliferation, covers were designed for 100mm dishes for ES of the hydrogels. Rectangular card slots were made in the dish, and NIH3T3 cells were inoculated after placing them in the hydrogel. After 1 day of culture, electrical pulses (1 Hz, 0.05 mA for 30 min; the polarity conversion was performed after 15 min) were applied with an electrostimulator. The angiogenic activity were tested by HUVECs cells. The hydrogel was immersed in complete medium for 10 days as the conditional medium. The cells were seeded in 48-well plates (2×10^4 cells/well). Cells were cultured using the medium containing 0.1% FBS for 12 h. The ratio of conditioned medium and matrix was 1:1 (v/v) and mixed and distributed to 48-well plates cultured for 1 h at 37 °C, evenly. Then the cells were distributed to the substrate surface cultured for 8 h. Photos of angiogenesis numbers were taken using an inverted microscope. The Image J was used to quantitatively calculate the angiogenesis numbers.

1.4. Histocompatibility of the hydrogels

Briefly, hydrogels were cut into square about 1 cm² for use. Fresh blood was taken from the eyeballs of mouse and prepared to a saline suspension. Then, the hydrogels were immersed in the blood suspension at 37 °C for 4 h. Physiological saline solution was employed as a negative control, and deionised water was employed as a positive control. After, the supernatant was centrifuged and collected for detection with a microplate reader.

1.5. Skin wound healing model

In brief, the mice were randomly divided into seven groups, namely the control, PVA, PM, PMP1, PMP2, PMP3 and PMP4 hydrogel group. There were 6 mice in each group. After the mice were anesthetized, use scissors to create a wound about 1 cm diameter and 0.5 mm deep on the back of the mouse. The control group was not treated after modeling. The hydrogel discs were placed onto the injury region as the experimental group. The wound was observed and photographed to record wound area. The wound area was measured using ImageJ software. The formula of wound healing (WH) was decided as follows:

$$WH (\%) = \frac{Area_0 - Area_n}{Area_0} \times 100$$

The “Area₀” represent the initial area of the wound and the “Area_n” represent the day 4, day 10 and day 14 after modeling of the wound, respectively.

1.6. Histology and immunohistochemistry analysis

In order to evaluate histomorphology of regenerative skin tissue, all the slices were stained with hematoxylin-eosin. In order to evaluate the inflammatory response, the slices on the 6 day were stained with immunohistochemical staining. Immunohistochemical slices were stained with IL-6 (Proteintech, 66146-1-Ig) and TNF-α (Proteintech, 60291-1-Ig).

1.7. Immunofluorescence Staining

In order to evaluate cytokine expression, the fixed and slices on the 14 day were reacted with primary anti-VEGF antibody (Proteintech, 19003-1-AP). In order to evaluate neovascularization, the fixed and slices on the 10 and 14 day were reacted with alpha smooth muscle actin (Proteintech,

14395-1-AP). In order to evaluate collagen deposition, the fixed and slices on the 6 and 14 day were reacted with primary antibodies vimentin (Proteintech, 60330-1-Ig) and collagen I (Proteintech, 67288-1-Ig). All of the slices were observed and photographed under an inverted fluorescence microscope. Photos were analyzed with an Opera Phenix (PerkinElmer Inc., UK)

2. Results and discussion

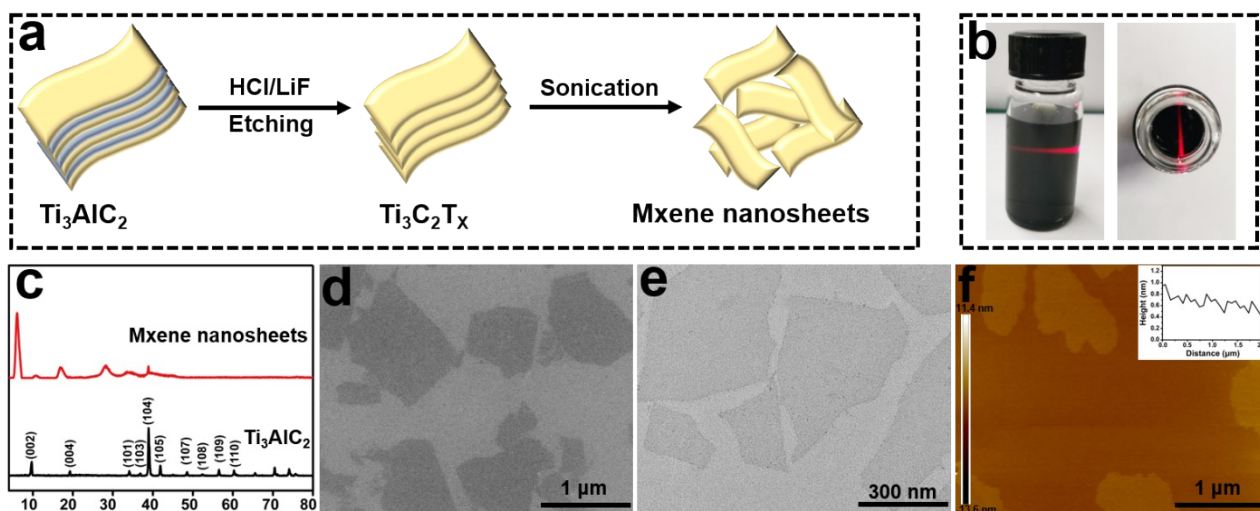


Fig. S1 (a) Schematic preparation process of MXene nanosheets.(b) Tyndall scattering effect of MXene nanosheet solution. (c) XRD patterns of Ti_3AlC_2 and MXene nanosheets. (d) SEM images of the MXene nanosheets. (e) TEM images of the MXene nanosheets. (f) AFM images and the corresponding height profiles of the MXene nanosheets.

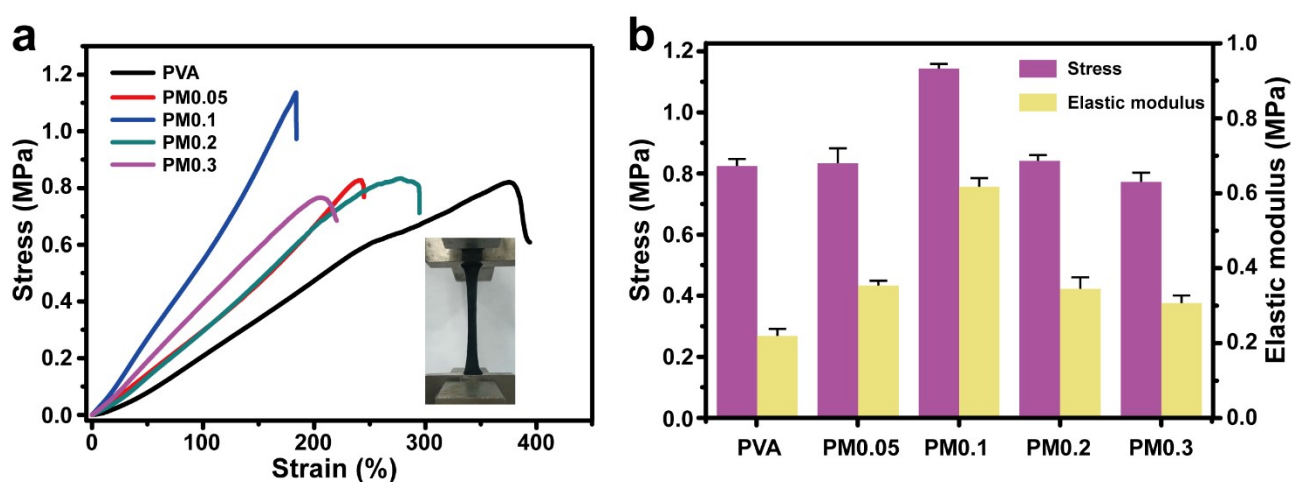


Fig. S2 (a) Tensile stress-strain curves of PVA/MXene hydrogel containing different amounts of MXene nanosheets. (b) Tensile strength and elastic modulus of PVA/MXene hydrogel containing different amounts of MXene nanosheets.

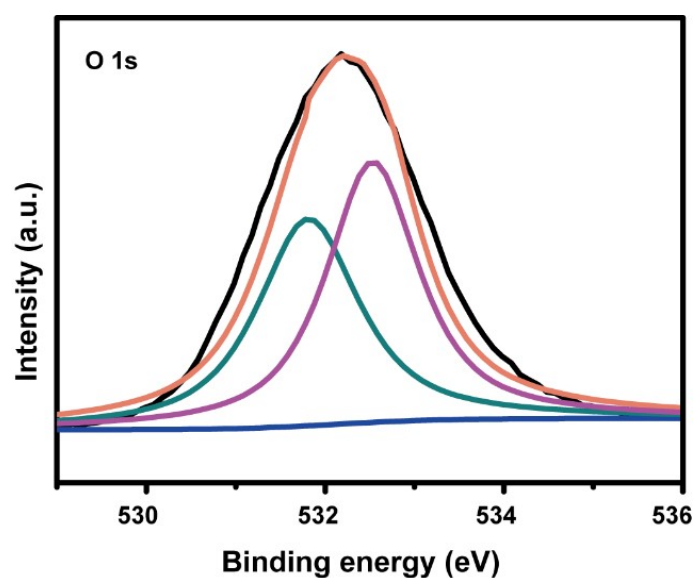


Fig. S3 The O 1s spectra of XPS survey of the PMP hydrogel.

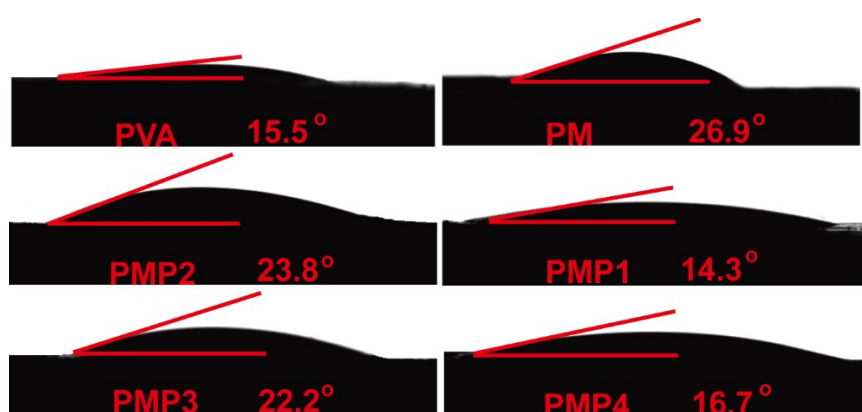


Fig. S4 The water contact angle of PMP hydrogels.

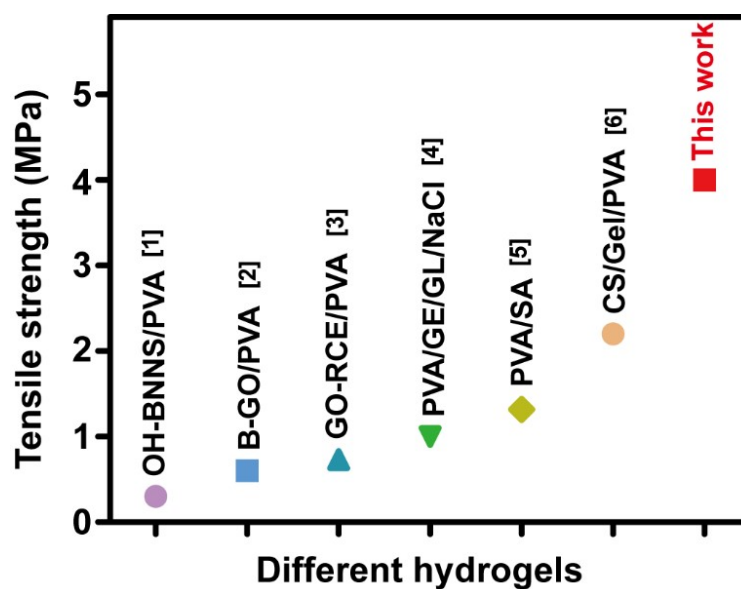


Fig. S5 Compare of tensile strength between PMP3 and other hydrogels.

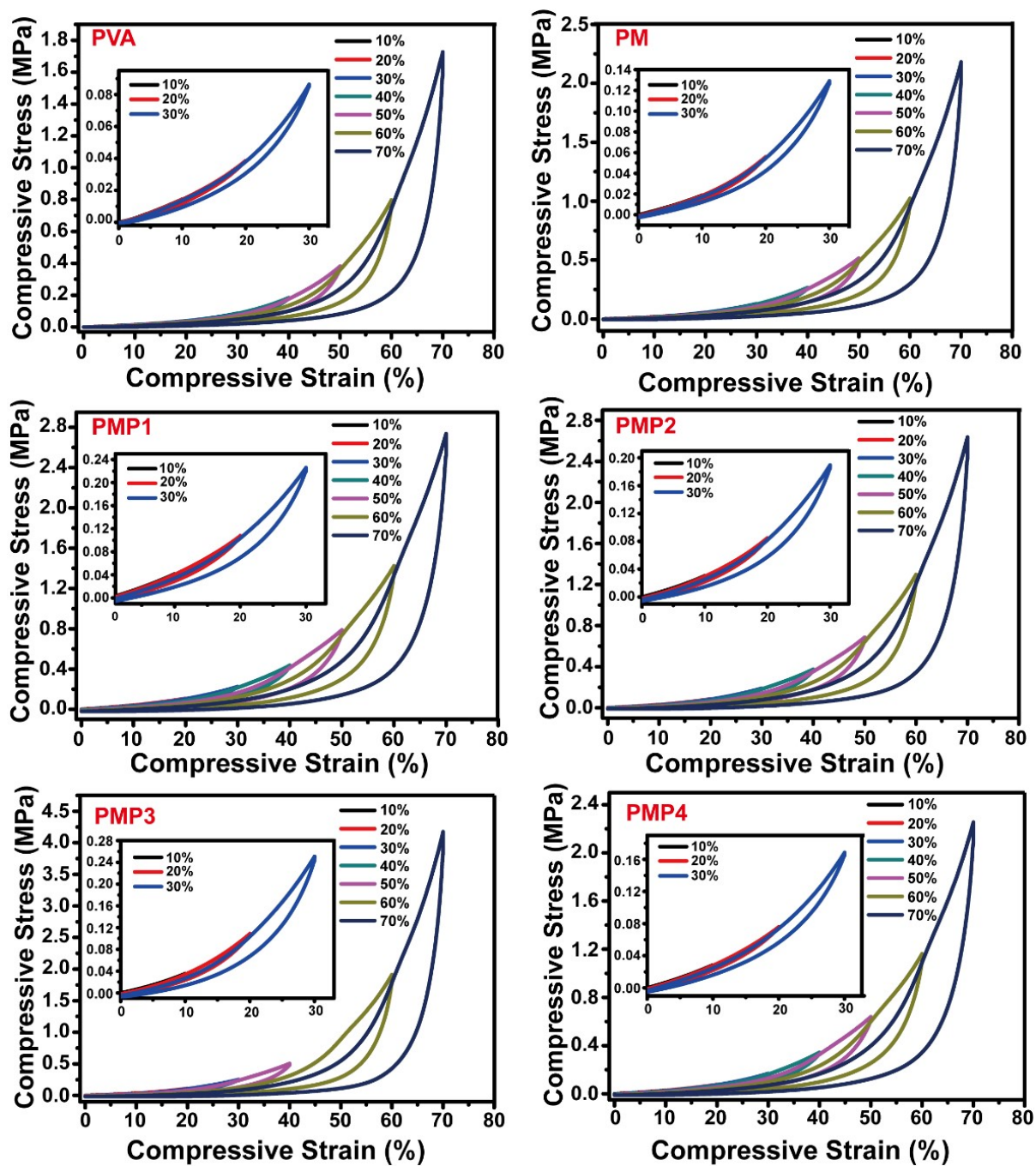


Fig. S6 Compression rebound curves of PMP hydrogels under the 10%-70% strain.

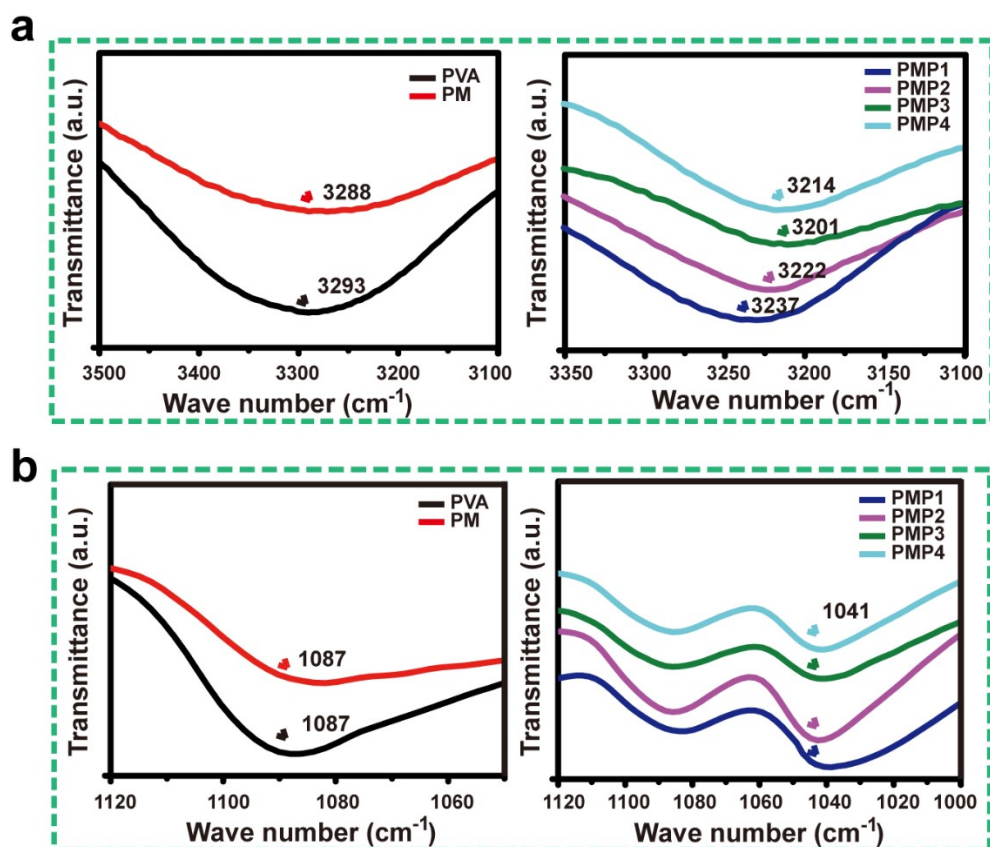


Fig. S7 (a) FTIR stretching variations of O-H. (b) FTIR stretching variations of C-O.

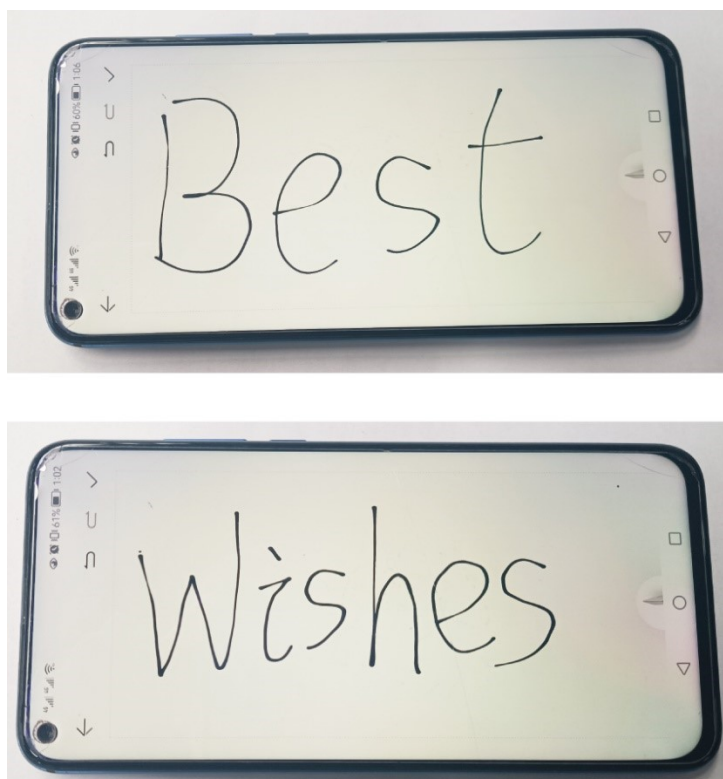


Fig. S8 Writing words by using the PMP3 hydrogel pen.

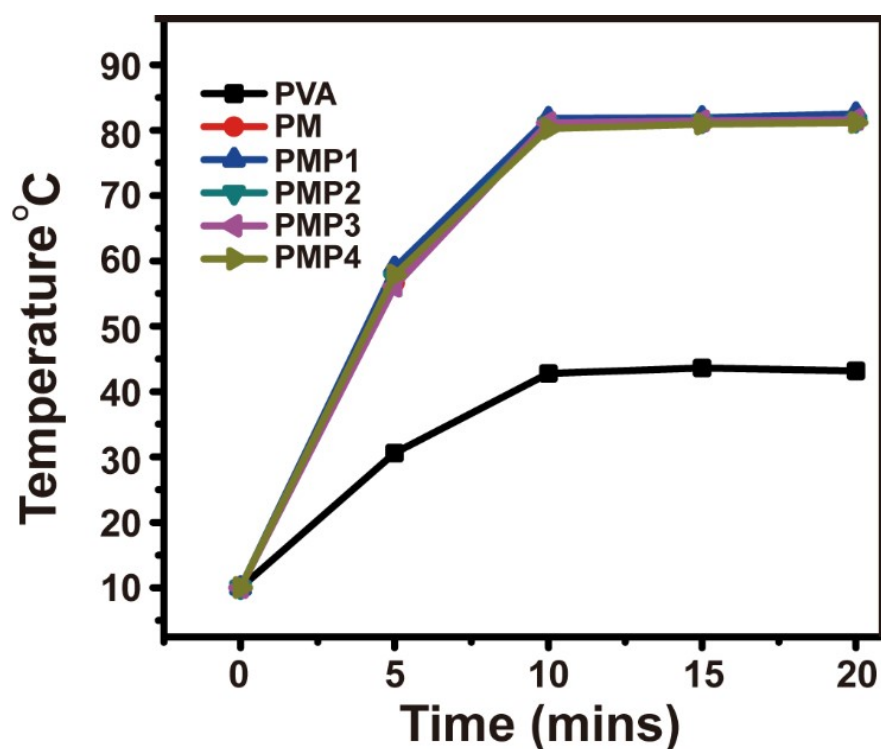


Fig. S9 Light heating curves of the hydrogels.

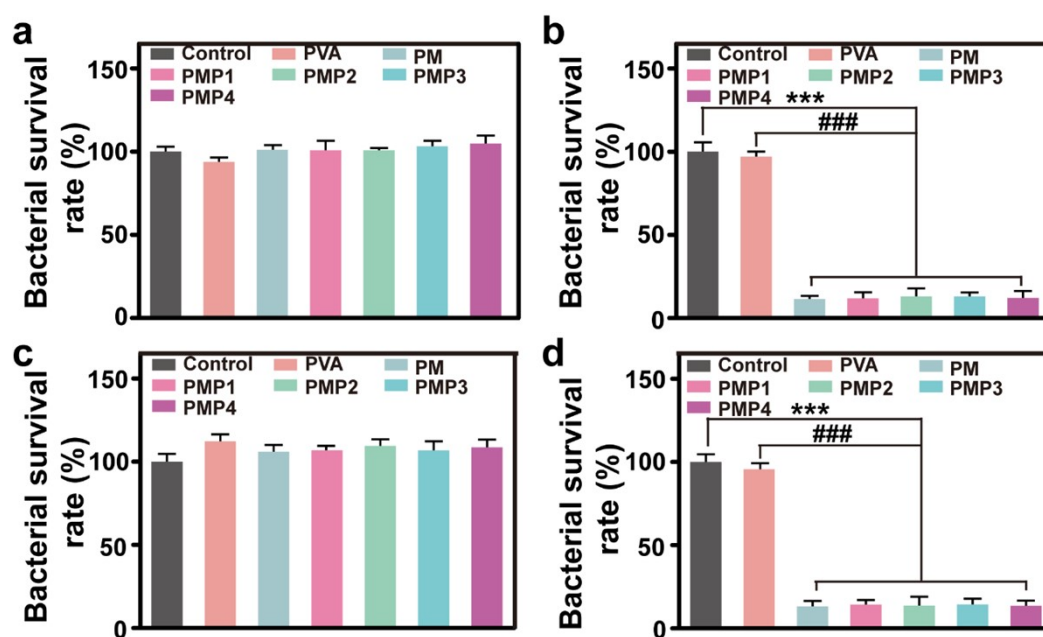


Fig. S10 (a) Antibacterial activity of the hydrogels against *S. aureus* in vitro (b) Antibacterial activity of the hydrogels against *S. aureus* with NIR light in vitro. (c) Antibacterial activity of the hydrogels against *E. coli* in vitro (d) Antibacterial activity of the hydrogels against *E. coli* with NIR light in vitro. *** $p < 0.001$ for PVA, PM, PMP1, PMP2, PMP3 and PMP4 vs Control group, ### $p < 0.001$ for PM, PMP1, PMP2, PMP3 and PMP4 vs PVA group.

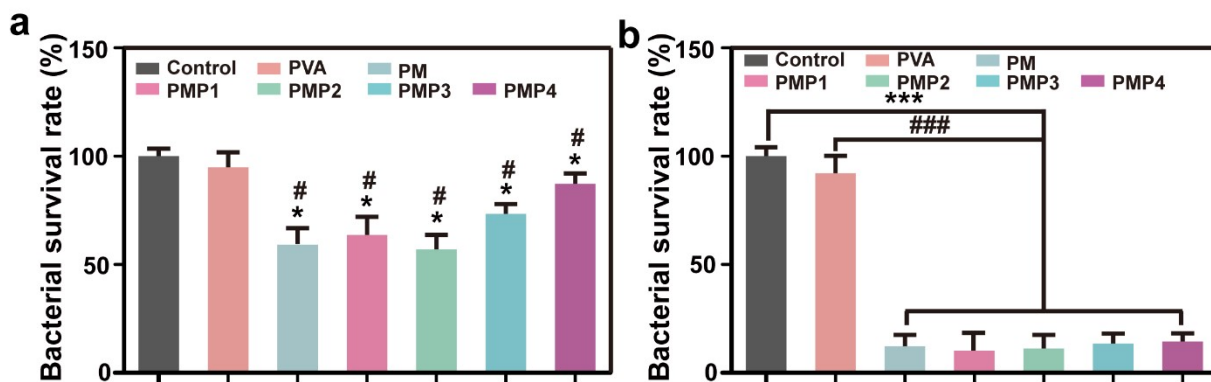


Fig. S11 (a) Antibacterial activity of the hydrogels against *E. coli* in vivo (b) Antibacterial activity of the hydrogels against *E. coli* with NIR light in vivo. *** $p < 0.001$ for PVA, PM, PMP1, PMP2, PMP3 and PMP4 vs Control group, ### $p < 0.001$ for PM, PMP1, PMP2, PMP3 and PMP4 vs PVA group.

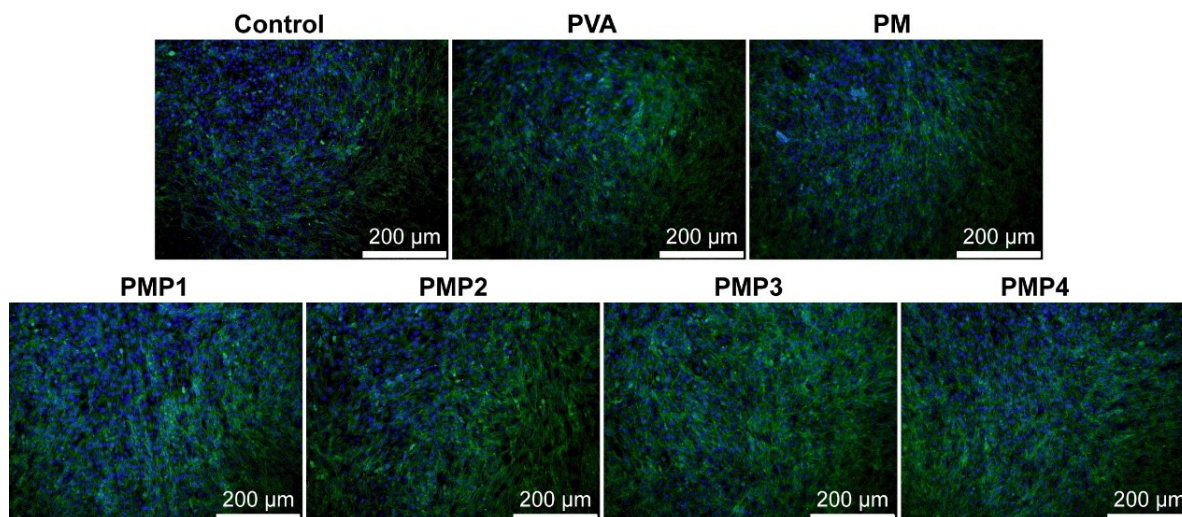


Fig. S12 Cell adhesion and morphology after contact with the hydrogels on 48 h

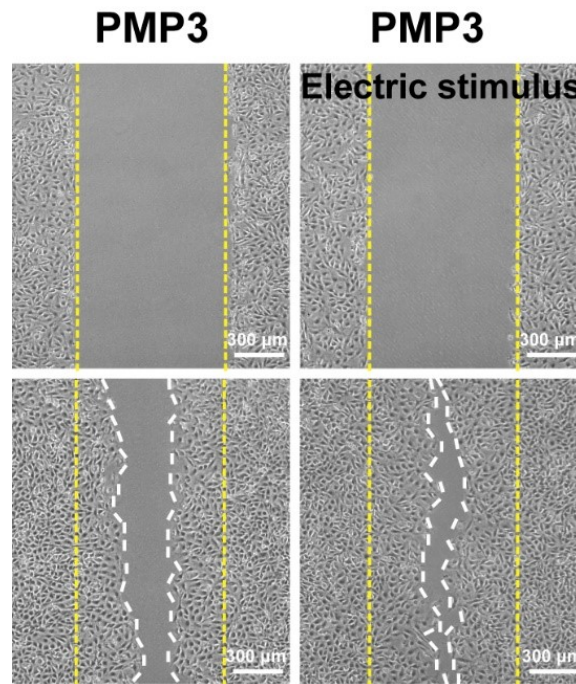


Fig. S13 Cell migration after contact with the hydrogels with the electric stimulus.

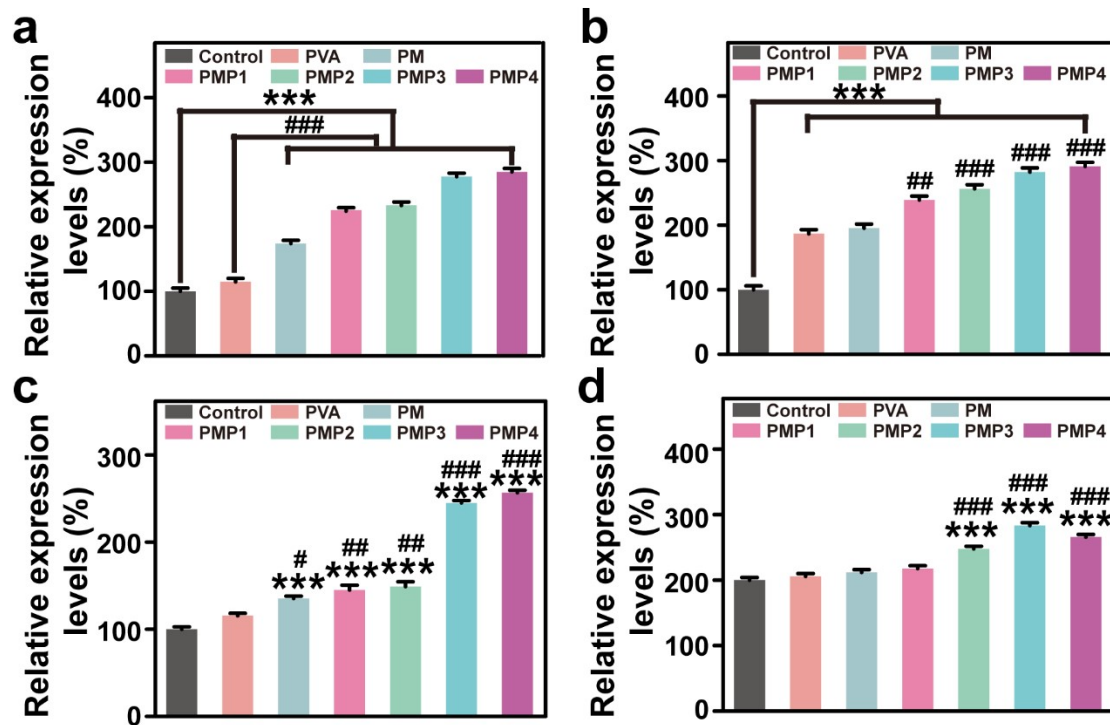


Fig. S14 Quantified analysis of the relative percentage of area coverage by (a) VEGF (b) α -SMA (c) Vimentin (d) Collagen. *** $p < 0.001$ for PVA, PM, PMP1, PMP2, PMP3 and PMP4 vs Control group. ## $p < 0.01$, ### $p < 0.001$ for PM, PMP1, PMP2, PMP3 and PMP4 vs PVA group.

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