Supporting Information

Wound microenvironment self-adaptive all-in-one hydrogel for rapid healing of the diabetic wound

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Supplementary experimental section

Materials

Gelatin (Gel) was provided by Shanghai ABCONE Biotechnology Co., Ltd. (China). Polyvinyl alcohol (PVA) and L-arginine were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (China). 3-Aminophenylboronic acid (M-APBA), Metformin was obtained from Energe Chemical Co., Ltd. (China). Dialysis Membranes were provided by Beijing Solarbio Science & Technology Co., Ltd. (China). DSPE-PEG_{2000}-PBA was purchased from Xi'an Ruixi Biological Technology Co., Ltd. (China). Lecithin and Cholesterol were purchased from Shanghai Macklin Biochemical Co., Ltd. (China). Streptozotocin was purchased from Dalian Melonepharma Biochemical Co., Ltd. (China). L(+)-ascorbic acid was obtained from Beijing Innochem Co., Ltd. (China). All other chemicals were purchased from Shanghai Aladdin Biochem Technology Co., Ltd. (China).

Synthesis of hydrogel@lipo

The Gel/PBA solution (1 mL), liposome (5 mg), and 10% w/v PVA solution were mixed. After a few seconds, the hydrogel@lipo was formed.

Release behavior of hydrogel@lipo

Hydrogel@lipo were separately immersed in PBS with or without glucose and incubated in a 37°C incubator. The samples were retrieved at different time points, and centrifuged, and the supernatant was analyzed using a microplate reader. Subsequently, fresh solutions were added, and the samples were returned to the constant temperature incubator for further incubation.

To further confirm the load and release behavior of hydrogel@lipo, fluorescent rhodamine B was encapsulated in the liposomes. The hydrogel@lipo was immersed in PBS with/without glucose for 24 h, and the fluorescence intensity of the hydrogel@lipo was observed by fluorescence microscope.

The extraction traction process of different composite hydrogel extraction solution

The distinct composite hydrogels were subjected to freeze-drying and subsequently underwent radiation sterilization for a duration of 12 h. Following three rounds of PBS

washing for each composite hydrogel, the Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 g/L glucose was individually added, allowing for a 24 h soaking period. The final solution was then filtered using a 0.22 μm filter for the purpose of sterilization and subsequently stored at 4°C.

Cytocompatibility

CCK-8, live/dead staining, hemolysis experiments, and cytoskeletal staining were employed to illustrate the favorable biocompatibility of the hydrogel extraction solution. The HUVECs were seeded into a 96-well plate and incubated for 24 h until the cell density reached 50% confluence. Subsequently, the culture medium was replaced with a hydrogel extract, and the cells were further incubated for 24 h. Cell toxicity was assessed according to the instructions provided in the Cell Counting Kit-8 (CCK-8) manual. The assessment of green fluorescence expression in diverse composite hydrogels was conducted through live/dead staining assays utilizing HUVECs, NIH 3T3, and RAW 264.7 cells. In the case of cytoskeletal staining, NIH 3T3 cells were cultivated in a 12-well plate and treated with the hydrogel extraction solution for a duration of 12 h. Following fixation with 4% paraformaldehyde, the cells were subjected to FITC-Phalloidin and DAPI staining. For the blood compatibility assessment, erythrocyte suspensions were derived from fresh mouse ocular blood, exposed to the hydrogel extraction solution for 30 minutes, and subsequently documented through photography. Absorbance measurements were conducted using a multifunctional enzyme marker. Hemolysis rate calculation formula: HR (%) = $[(A_h-A_n)/(A_w-A_n)] \times 100\%$ (A_h: sample group; A_n: NaCl; Aw: water).

Cell migration assay

The cell migration ability was evaluated through scratch wound healing and transwell assays. In the scratch wound healing assay, NIH 3T3 cells were seeded into a 6-well plate. A vertical scratch was created on the cell surface using a 200 μL pipette tip, followed by PBS washing. The culture was supplemented with the hydrogel extraction solution, and photographs were taken at different times. For the transwell assay, 200 μL of HUVECs suspension was introduced to the upper chamber of the transwell, while

the lower chamber received the hydrogel extraction solution for a 24 h incubation period. Afterward, the cells were fixed using 4% paraformaldehyde for 15 minutes and stained with 0.5% crystal violet. The upper chamber cells were gently removed using a cotton swab, and the chambers were positioned on slides for microscopic observation. Quantitative analysis was performed using Image J software.

Cell proliferation assay

HUVECs were cultured in 12-well plates and incubated for 24 h. Subsequently, they were co-cultured with the hydrogel extraction solution for 24 h. The proliferation of HUVECs was evaluated using the EdU-555 cell proliferation kit.

Tube formation assay

The experimental materials were pre-chilled beforehand, and all procedures were conducted within ice boxes. Upon thawing of the matrigel, 40 μL of matrigel was dispensed into each well of a 96-well plate. Following solidification at room temperature, HUVECs suspensions were introduced into each well for subsequent incubation. Photographic documentation was carried out at different times, and quantitative analysis was conducted using Image J software.

Flow cytometry detection

Fresh mouse skin tissue was promptly collected and assessed within a 24 h window. The skin tissue was subjected to cutting, digestion, and subsequent filtration, resulting in the generation of a cell suspension. This suspension was meticulously washed, followed by centrifugation and the removal of the supernatant. A Red Blood Cell Lysis Buffer was introduced. The cell suspensions were then subjected to incubation with CD86 (FITC) and CD206 (APC) antibodies. This approach facilitated the detection and subsequent evaluation of immune cells within the mouse skin tissue.

Western blot detection

Protein expression levels of CD31, collagen I, and IL-1β in tissues were assessed through western blot analysis. For the western blot assay, the skin tissue must be rapidly frozen in liquid nitrogen immediately upon removal. The initial step involved weighing the skin tissue, followed by grinding with the addition of RIPA buffer. Subsequent centrifugation at 4°C was performed, and the resulting supernatant was quantified for

protein content. Configuration of the separation gel and concentrate gel ensued, with subsequent sampling for SDS-PAGE (10%) carried out until effective separation of the target proteins was achieved. Post SDS-PAGE, the membrane was transferred and subsequently incubated at 4°C for 1-2 h. Consecutive incubations were carried out using primary and secondary antibodies. Following this, a chromogen solution was introduced, and exposure photographs were captured. Ultimately, the data were analyzed utilizing Image J software.

Supplementary figure captions

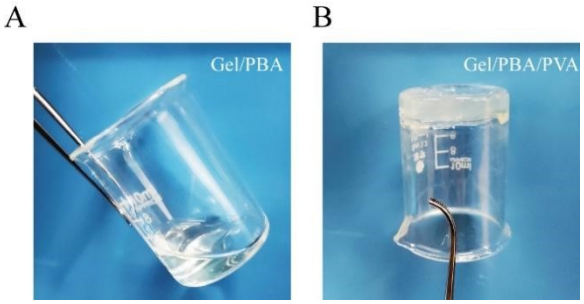

Fig. S1 Photographs of (A) Gel/PBA and (B) Gel/PBA/PVA.

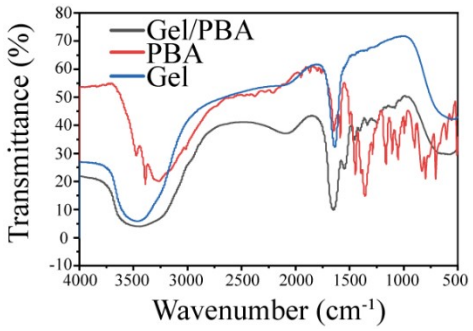

Fig. S2 FTIR spectra of the Gel/PBA, Gel, and PBA.

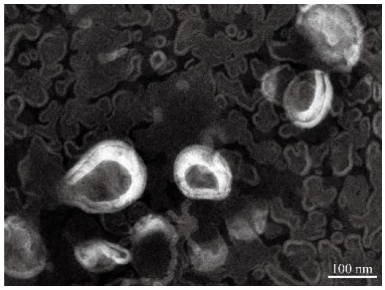

Fig. S3 TEM images of the blank liposome, scale bar: 100 nm.

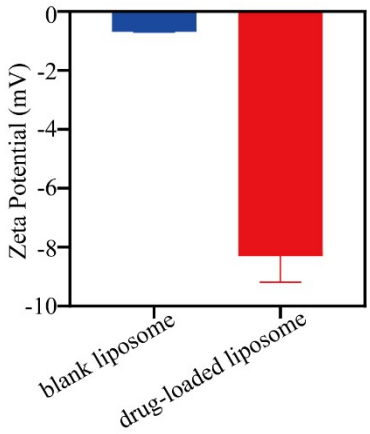

Fig. S4 Zeta potential of blank liposome and drug-loaded liposome.

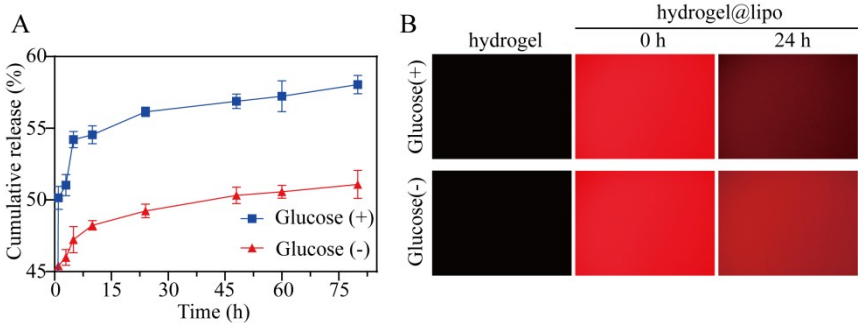

Fig. S5 (A) Liposome release behavior of hydrogel@lipo in PBS with/without glucose. (B) Fluorescence images of hydrogels treated with different buffers.

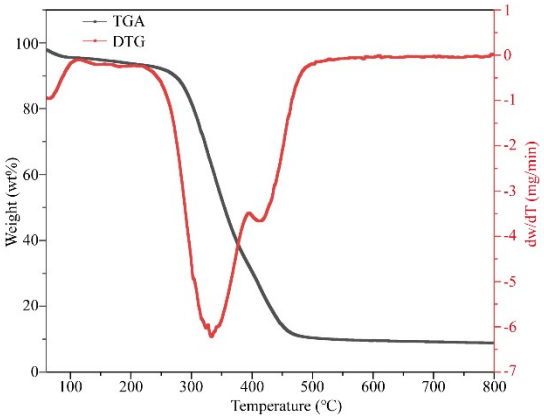

Fig. S6 TGA and derivative thermogravimet (DTG) curves of hydrogel@lipo.

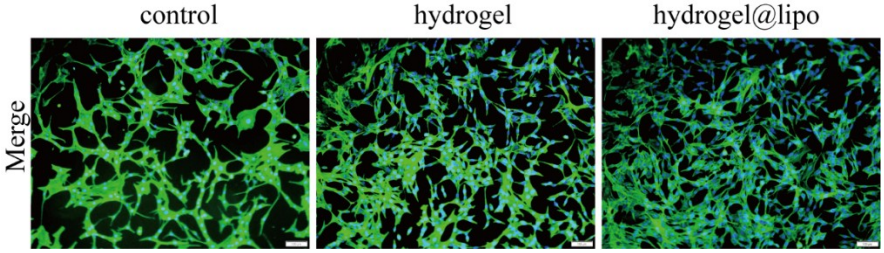

Fig. S7 Cytoskeleton staining assay (green: FITC-Phalloidin; blue: DAPI), scale bar:

100 μm.

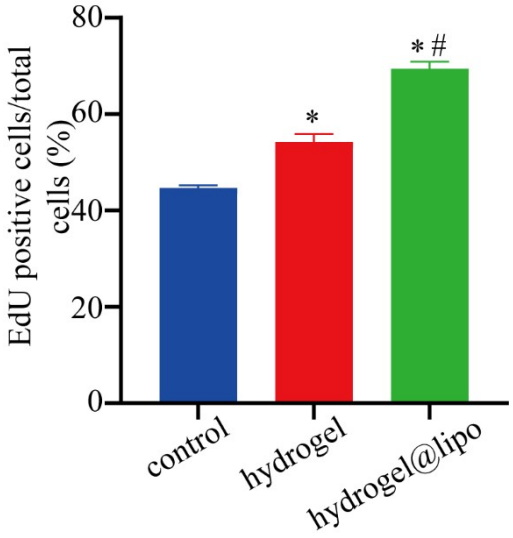

Fig. S8 Quantitative analysis of EdU positive HUVECs. *P <0.05 vs control; $^{\#}P <$ 0.05 vs hydrogel.

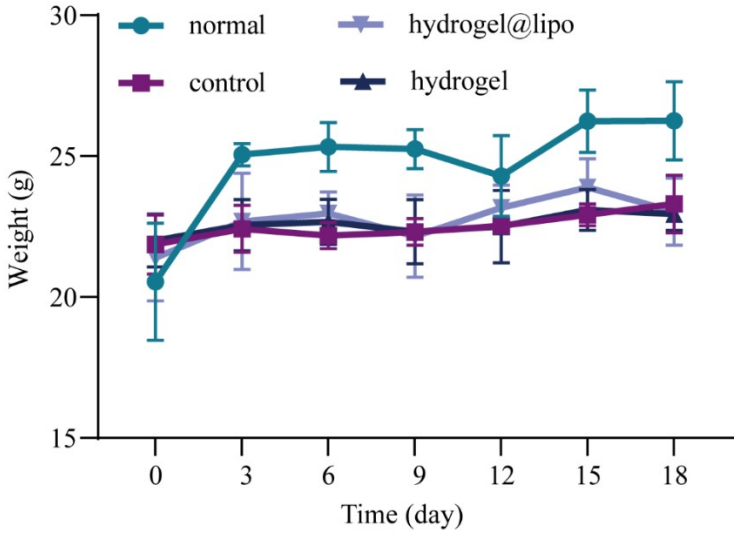

Fig. S9 Body weight changes of different groups of mice before and after modeling.

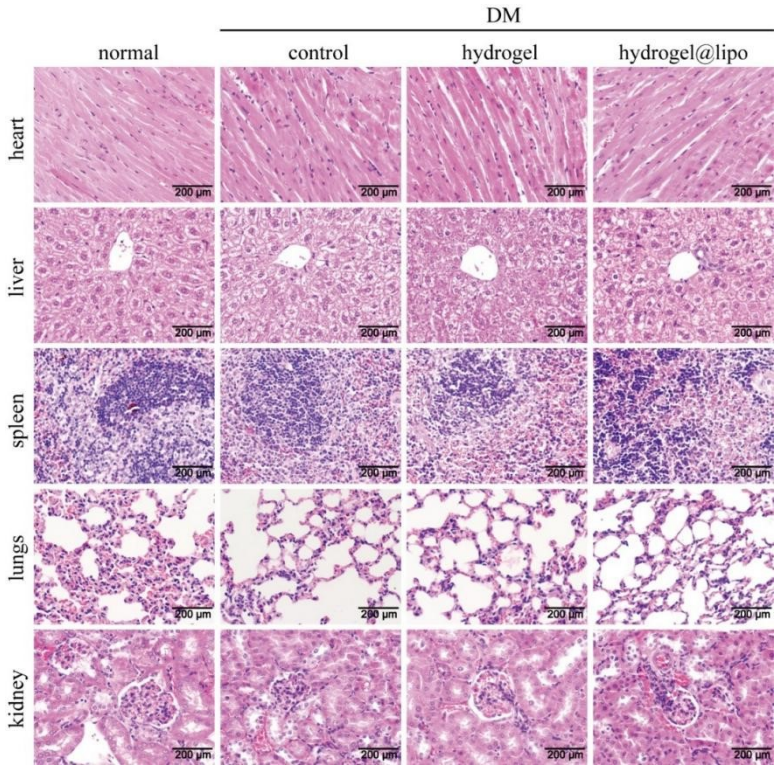

Fig. S10 H&E staining of heart, liver, spleen, lung, and kidney, scale bar: 200 μm.

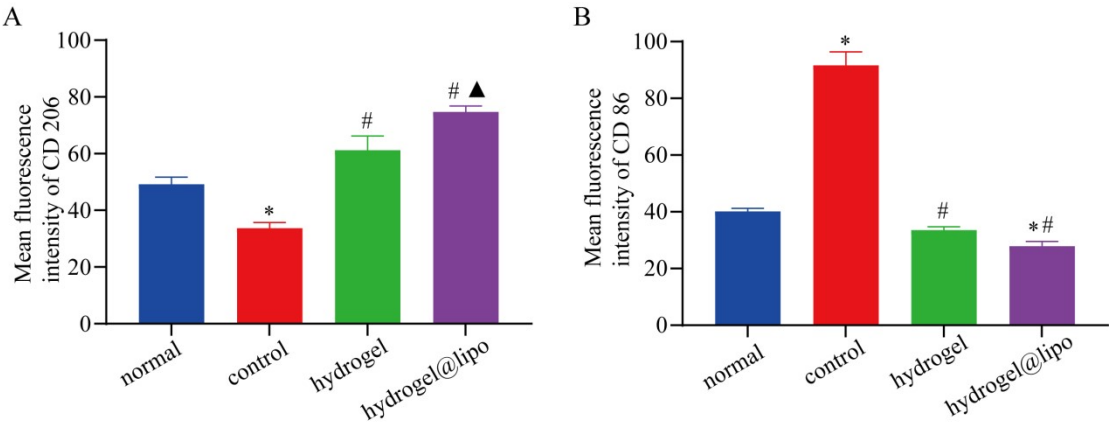

Fig. S11 (A, B) Mean fluorescence intensity of CD206 and CD86. *$P < 0.05$ control vs normal; $^{\#}P < 0.05$ vs control; $^{\blacktriangle}P < 0.05$ vs hydrogel (n=3).

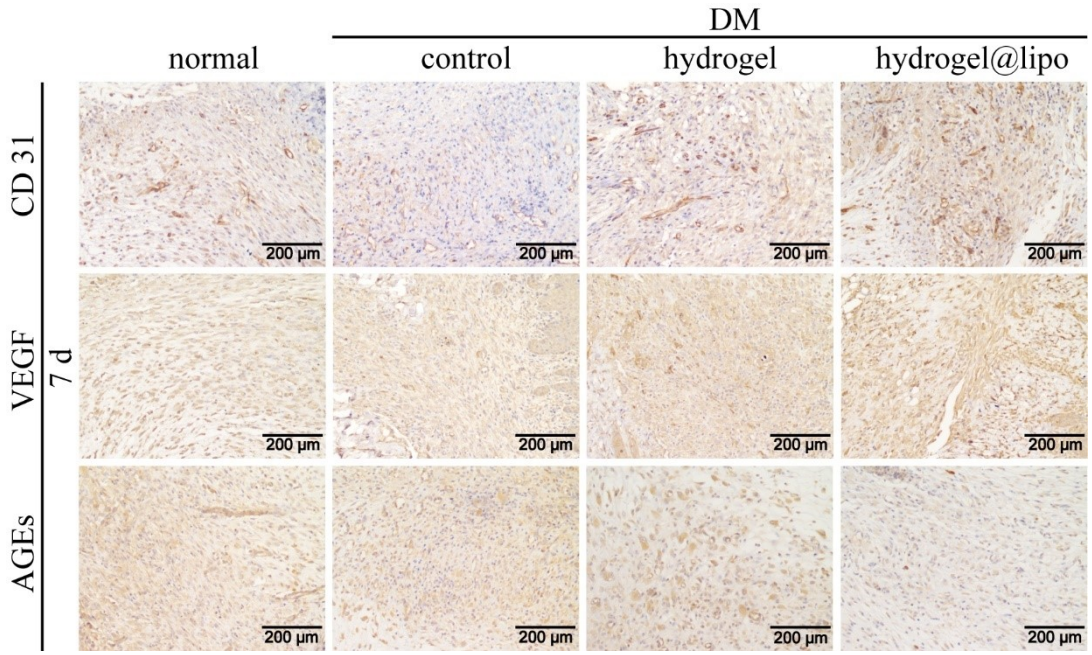

Fig. S12 Immunohistochemical staining images of CD31, VEGF, and AGEs in wound skin tissue of mice on day 7, scale bar: 200 μm.

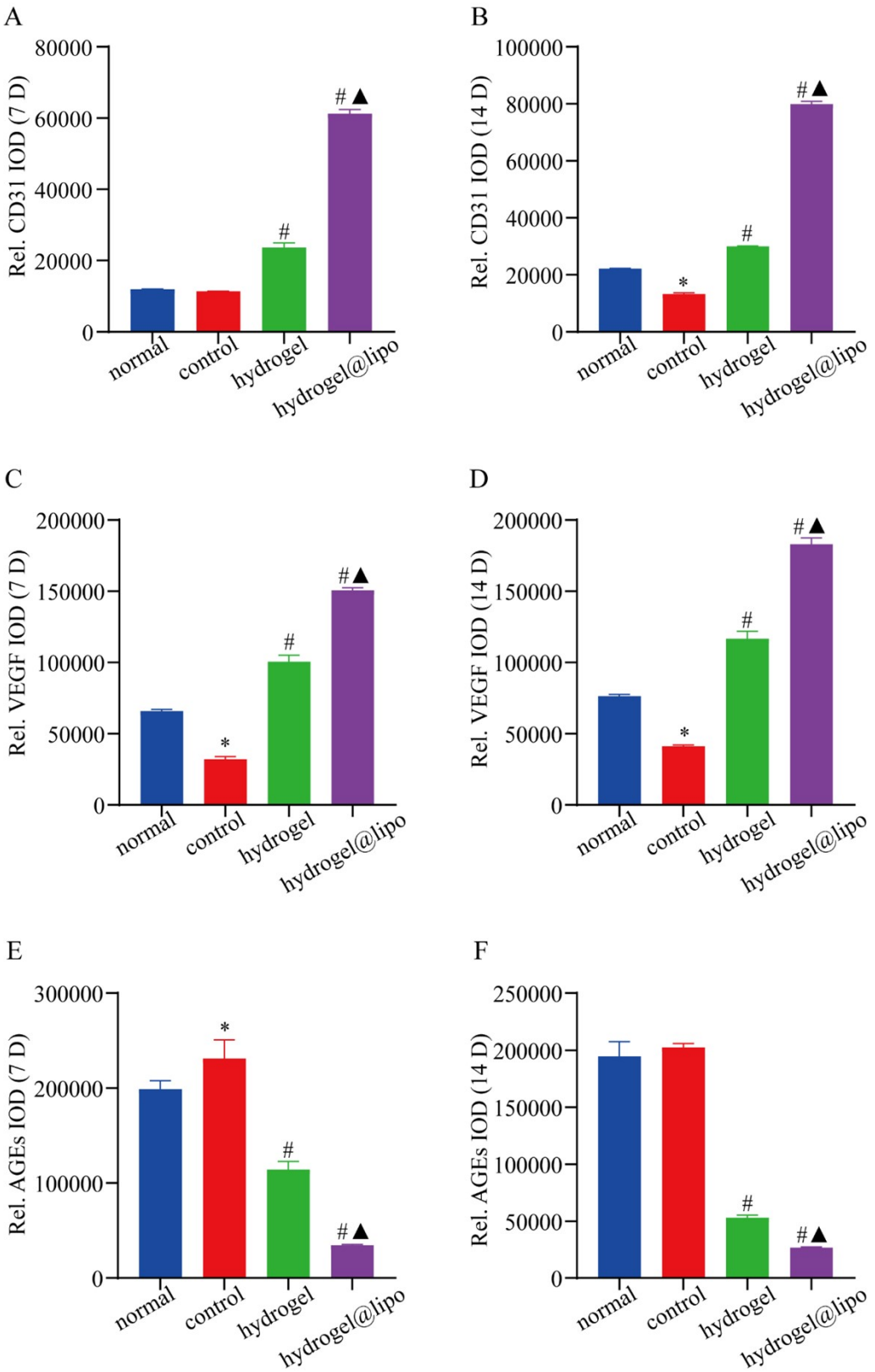

Fig. S13 Quantitative analysis of (A, B) CD31, (C, D) VEGF, and (E, F) AGEs integrated optical density on days 7 and 14. *P <0.05 control vs normal; $^{\#}P$ <0.05 vs control; $^{\blacktriangle}P$ <0.05 vs hydrogel (n=3).

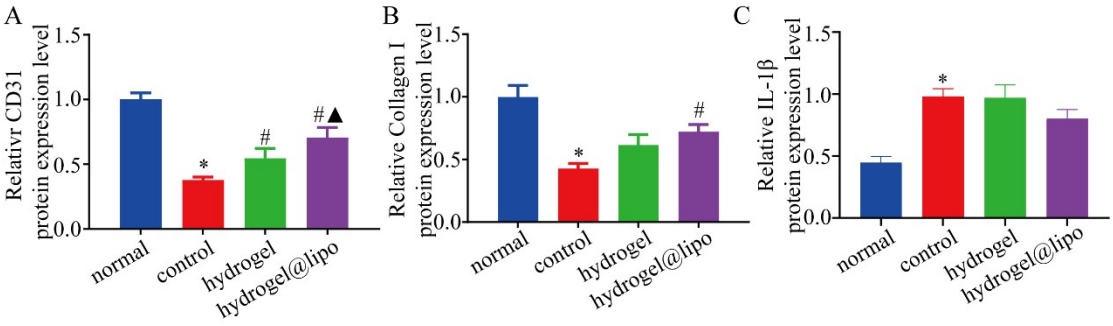

Fig. S14 Quantification of the signal intensity of (A) CD31, (B) collagen I, and (C) IL-1β on days 14 mice trauma skin tissue in western blot analysis. *P < 0.05 control vs normal; $^{\#}P$ < 0.05 vs control; $^{\blacktriangle}P$ < 0.05 vs hydrogel (n=3).

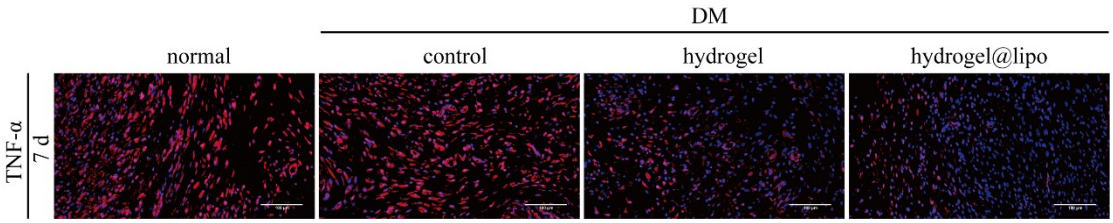

Fig. S15 Immunofluorescence assay for TNF-α in mice traumatized skin tissue on days 7, scale bar: 100 μm.