Supplementary material

Synergistic wound repair effects of a composite hydrogel for delivering tumor-derived vesicles and S-nitrosoglutathione†

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† Electronic supplementary information (ESI) available.

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Contents

Section 1

Experimental Section

1. The swelling rates of hydrogels

The experimental method refers to one previous report. [1] The swelling rates of hydrogels were detected in 37 ℃ PBS. The swelling rate of the GSNO/tEVs/GG-cl-Im-Ba hydrogels was computed with the following equation: Swelling rate (%) =(Wt-W0)/W0*100, where Wt refers to the wet weights of the hydrogels at different times, and W0 refers to the dried weights after freeze-drying.

2. Cell adhesion test

25 μL fibronectin solution (50 μg/mL) was added to the bottom of the 96-well plate, and the orifice plate was gently shaken to spread the solution evenly, and they were placed in a sterile operating table to dry at room temperature overnight. On the next day, DMEM medium containing 2% BSA was added to the 96-well plate and sealed at 37 ℃ for 1 h, and then the plate was washed with PBS and dried for use. After co-incubation of L929 cells with macrophages, L929 cells were digested with trypsin and washed with PBS. After suspension cells in serum-free medium, L929 cells were inoculated into transwell cells treated with fibronectin at the density of 2×10^5 cells per well for 20 min. The specimen was washed with PBS for three times to remove unadherent cells, and then incubated with DMEM complete medium and 20 μL MTT for 4 hours. Subsequently, the absorbance of 570 nm was detected by microplate reader (Multiskan FC, Thermo Fisher, USA).

3. *In vivo* biocompatibility evaluation

To assess the biocompatibility of tEVs, the blood samples of the mice were collected. The whole blood samples were adopted for routine blood tests (Tecom, TEK-VET3, China) and the serum separated by centrifuging was evaluated for organ function via blood biochemical analysis (Biobase, Chemray 800, China).

4. Hemolysis assay

To isolate and collect red blood cells (RBCs), fresh rat blood was diluted by physiological saline and centrifuged at 3,000 rpm for 10 min. RBCs were washed several times until the supernatant was clear. After that, the RBCs were diluted with physiological saline to obtain 2% (V/V) erythrocyte suspension before use. GG-cl-Im-Ba hydrogels were dissolved in physiological saline at different concentrations (5%-20%, V/V) and a suspension of RBCs was added. The mixtures were maintained at 37 °C for 3 h in a thermotank, after which they were centrifuged and the supernatant of each sample was collected. Distilled water and physiological saline were used as the positive and negative controls, respectively. The hemolysis rate was calculated by the following equation:

$$\text{Hemolysis rate (\%)} = (A_{sample} - A_{negative}) / (A_{positive} - A_{negative}) \times 100$$

Where A_{sample}, $A_{negative}$, and $A_{positive}$ represented the absorbance of test samples, negative control, and positive control at 540 nm (Multiskan FC, Thermo Fisher, USA), respectively.

5. Biocompatibility of GG-cl-Im-Ba hydrogel

The experimental method refers to one previous report.[2] The prepared GG-cl-Im-Ba

hydrogel was injected subcutaneously directly. After 4 days, the surrounding tissues were sectioned and H&E staining was used to evaluate the biocompatibility of GSNO/tEVs/GG-cl-Im-Ba hydrogel *in vivo*.

6. ROS expression at the site of refractory diabetic wounds

The expression of ROS was detected by the reactive oxygen species assay kit (Beyotime, S0033S), DCFH-DA was diluted with serum-free medium at a ratio of 1:800, and the diluent was injected around the wound area and reacted in darkness for 20 min. The small animal imaging system (OV100, Olympus, Japan) was used to detect ROS expression under the excitation wavelength of 488 nm.

Section 2

Supplementary Figures

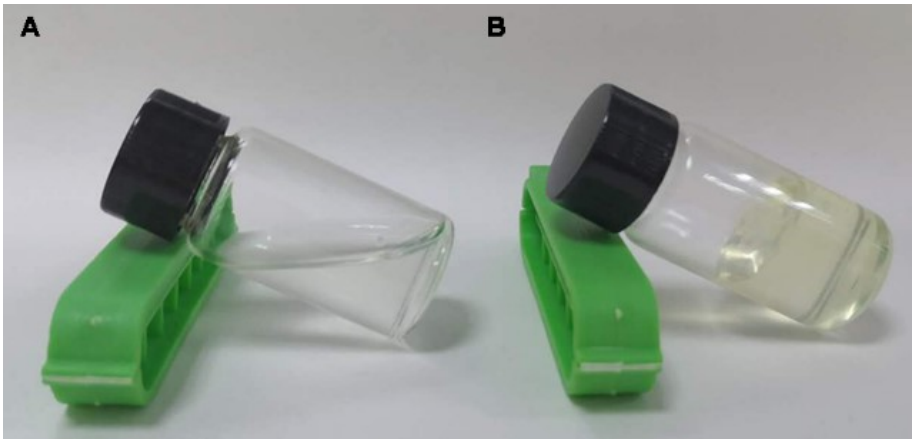

Fig. S1 Photographs of GG solution before crosslinking and hydrogel. (A) Free GG solution. (B) tEVs/GSNO/GG-cl-Im-Ba hydrogel.

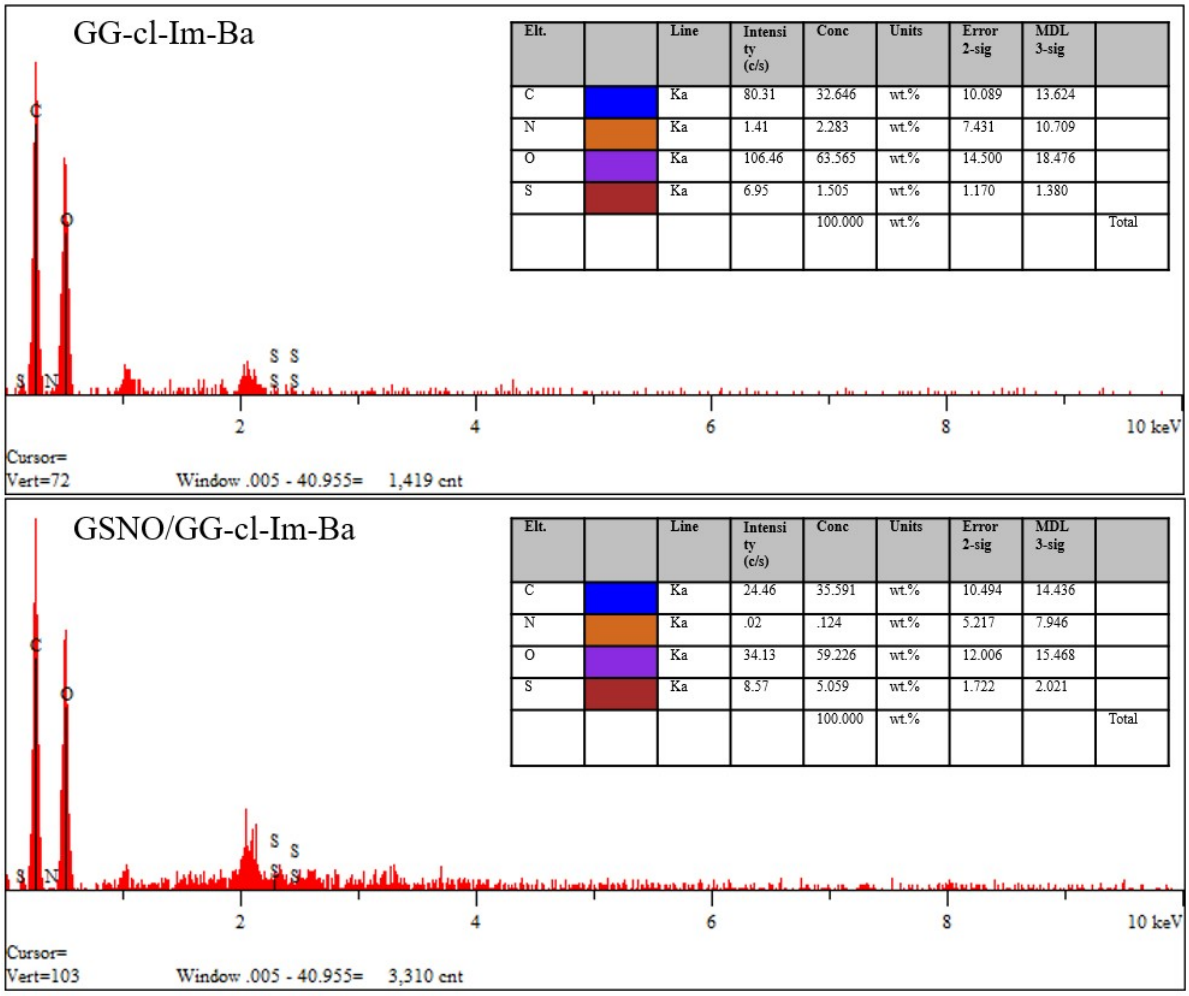

Fig. S2 SEM-EDX of GG-cl-Im-Ba and GSNO/GG-cl-Im-Ba hydrogel.

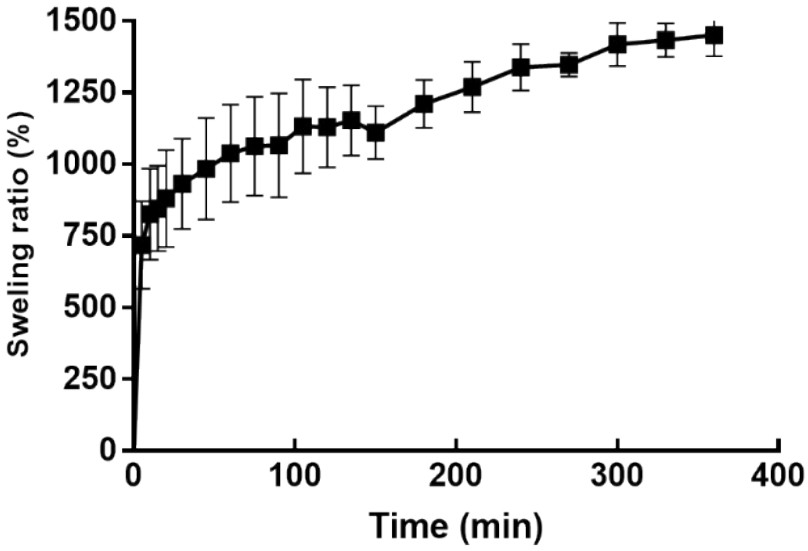

Fig. S3 The weight change (swelling) of hydrogel immersed in PBS solution at different time.

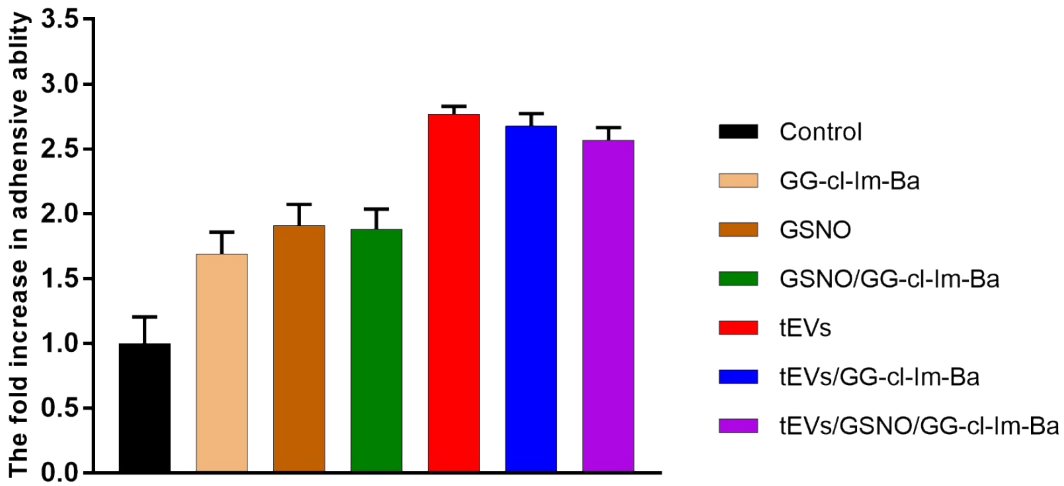

Fig. S4 L929 cells adhesion propertied after treatment of different formulations.

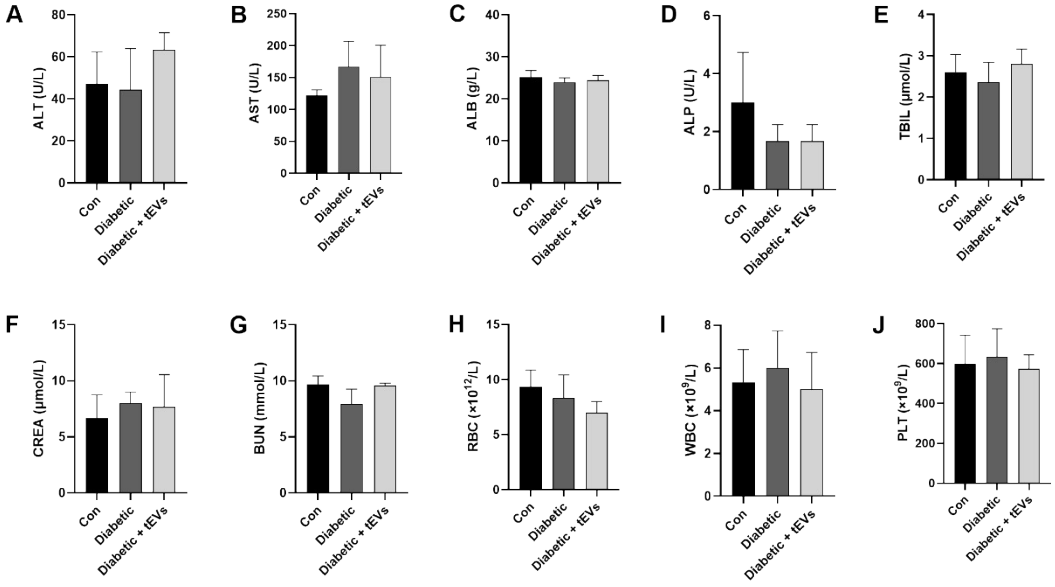

Fig. S5 Biocompatibility of tEVs. The blood serum biochemical parameters (A-G) and blood routine examination (H–J) of the mice.

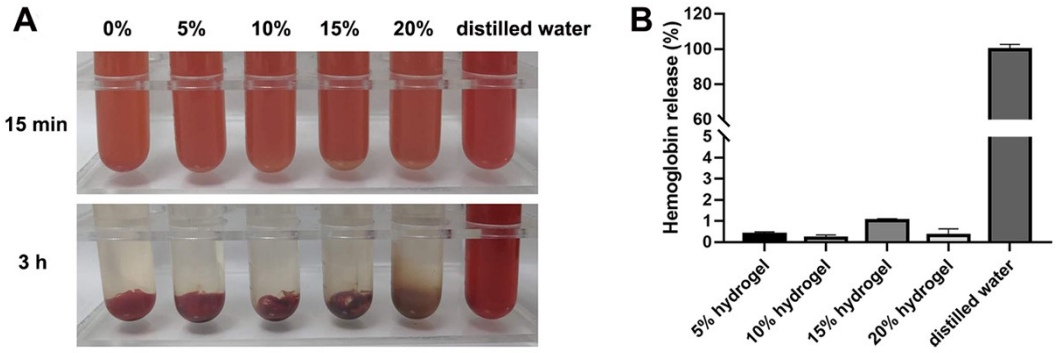

Fig. S6 Hemolysis evaluation of the tEVs/GSNO/GG-cl-Im-Ba hydrogels. (A) Photos of hydrogels hemolysis in each group at different times; (B) Histogram of Hemolysis rates.

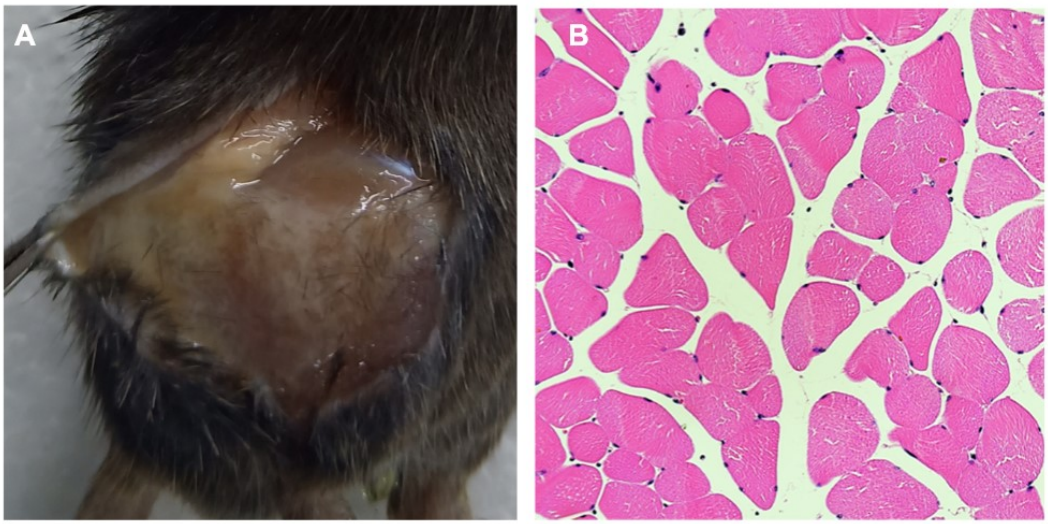

Fig. S7 (A) Hydrogel appearance under the skin of diabetic mice after 4 days. (B) H&E staining of the muscular tissue around the hydrogel.

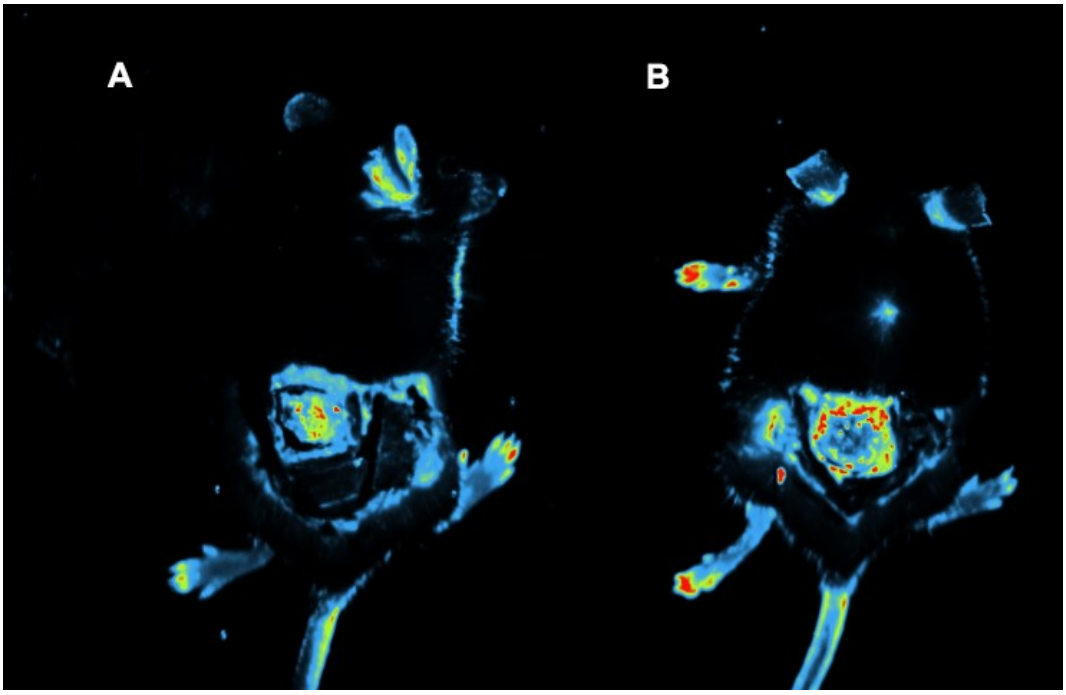

Fig. S8 Detection of ROS level in wound area. (A) Fluorescence imaging of wound area in normal mice for its ROS level evaluation. (B) Fluorescence imaging of wound area in diabetic mice for its ROS level evaluation.

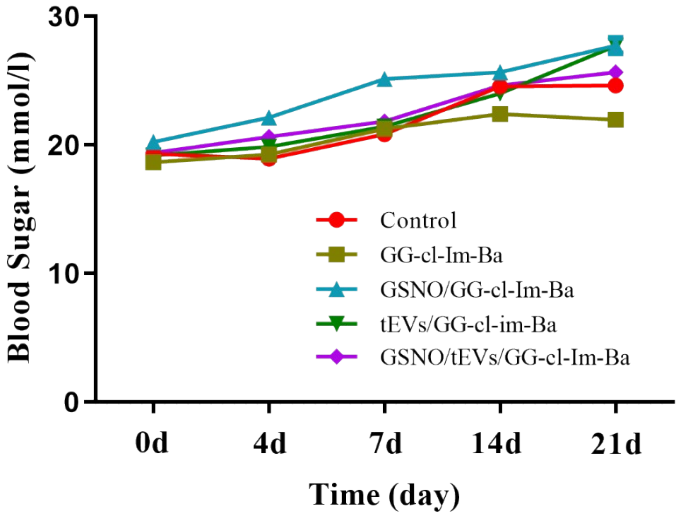

Fig. S9 Blood glucose levels of mice in different groups.

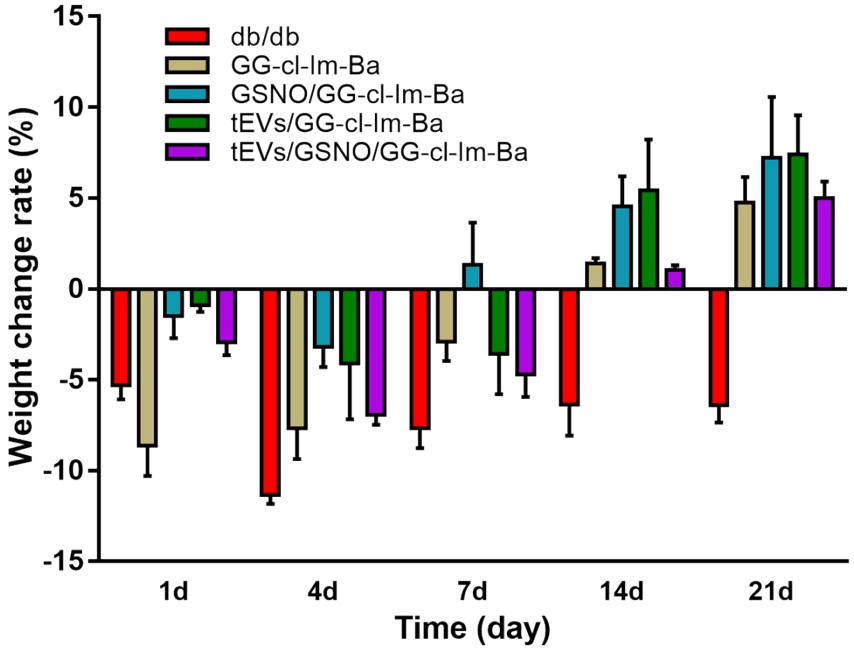

Fig. S10 The rate of body weight changes at 1, 4, 7,14 and 21 days post-wounding compared with the pre-wound weight.

Section 3

References

1. J. Liu, M. Qu, C. Wang, Y. Xue, H. Huang, Q. Chen, W. Sun, X. Zhou, G. Xu and X. Jiang, *Small*, 2022, **18**, e2106172.
2. Q. Dang, C. Liu, Y. Wang, J. Yan, H. Wan and B. Fan, *Carbohydrate polymers*, 2016, **136**, 516-526.