Supporting Information

Micro-environment Triple-Responsive Hyaluronic Acid Hydrogel Dressings to Promote Antibacterial, Collagen Deposition, and Angiogenesis for Diabetic Wound Healing

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S1 Materials and methods

S1.1 Preparation of the OHA

The preparation of oxidized hyaluronic acid (OHA) followed the previously reported procedure with minor modifications.[1] Briefly, hyaluronic acid (HA) (2 g) was dissolved in 100 mL deionized water (DW). Next, $NaIO_4$ (1.064 g) was added to the HA solution, which was then stirred at room temperature in darkness. After 24 h of reaction, the reaction was terminated by glycol (1.5 mL). The product, OHA, was dialyzed in DW for 5 days in a dialysis tube (MWCO: 8kDa-14 kDa) to remove unreacted reactants and freeze-dried for 48 h. The oxidation degree (OD) of the OHA was determined by a hydroxylamine hydrochloride method.[1] Briefly, 0.05 g OHA was dissolved in hydroxylamine hydrochloride solution (0.35 M, 25 mL) with methyl orange (0.005 %). Then, 0.1 M NaOH was added to titrate the above solution (hydroxylamine hydrochloride can react with aldehyde group of OHA to generate oxime, with release of HCl). The oxidation degree (OD) of OHA was calculated as equation:

$$OD_{OHA}\,(\%) = (0.001 \times V_t \times C_0 \times 379.32) / (2 \times m_0) \times 100\% \quad (1)$$

Where V_t is the consumed volume of NaOH solution (mL), C_0 is the concentration of NaOH (mol/L) and m_0 is the weight of OHA.

S1.2 Preparation of the OHA-APBA

EDC (0.96 g) and NHS (0.60 g) were added to the OHA solution (1 g, 100 mL) and stirred for 0.5 h until fully dissolved. 3-APBA (0.69 g) was added to the reaction system for 24 h at room temperature in darkness. The OHA-APBA sample was then dialyzed in DW (MWCO = 8-14 kDa), followed by freeze-drying for subsequent utilization. The

degree of substitution (DS) of the OHA-APBA sample was obtained by ^{1}H NMR in D_2O (ADVANCE III 400 MHz, Bruker, Germany). The DS of the OHA-APBA were calculated as equation:[2]

$$DS_{APBA}\,(\%) = A_{(\text{APBA of OHA-APBA})} / A_{(\text{methyl of HA})} \times 3/4 \times 100\% \quad (2)$$

Where $A_{(\text{APBA of OHA-APBA})}$ and $A_{(\text{methyl of HA})}$ are the integrated intensities of benzene ring and methyl (-$NHCOCH_3$) in the spectra of OHA-APBA, respectively.

S1.3 Preparation of the HAAD

The preparation of adipic dihydrazide grafted HA (HAAD) followed the previously reported procedure with minor modifications.[3] Firstly, EDC (0.82 g) and NHS (0.60 g) were added to HA solution (1 g, 100 mL) and stirred for 0.5 h. Then, ADH (3.5 g) was added to the reaction system. After pH adjustment to 3-5 with hydrochloric acid, the system was stirred in darkness at room temperature for 24 h. The HAAD sample was dialyzed in DW (MWCO=8-14 kDa) and freeze-dried for later use. The HAAD was characterized by ^{1}H NMR in D_2O and its DS was calculated using the following equation:[3]

$$DS_{ADH}\,(\%) = A_{(\text{methylene of HAAD})} / A_{(\text{methyl of HA})} \times 3/4 \times 100\% \quad (3)$$

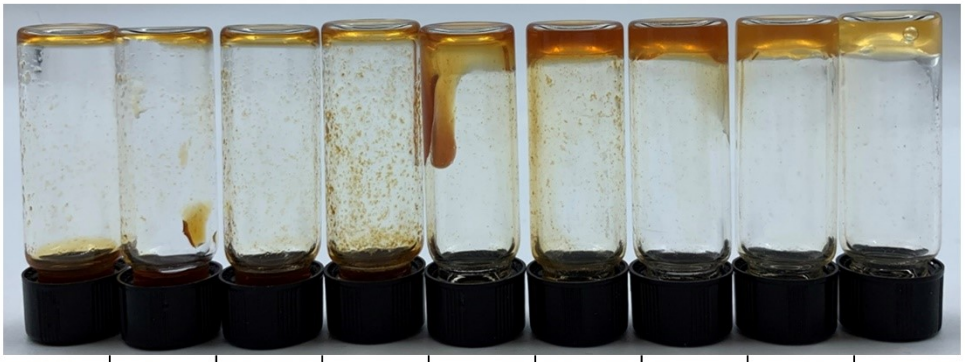
Where $A_{(\text{methylene of HAAD})}$ and $A_{(\text{methyl of HA})}$ are the integrated intensities of methylene (-CH_2CH_2-) and methyl (-$NHCOCH_3$) in the spectra of HAAD, respectively.

S1.4 Establishment of diabetic mice model

All animal protocols in this study were approved by the Animal Ethics Committee for Clinical Research and Animal Trials of the First Affiliated Hospital of Sun Yat-sen

University (approval no. [2023]144) and met the requirements of the National Research Council's Guide for the Care and Use of Laboratory Animals. Male C57BL/6J mice (20~25 g) were acclimatized for 1 week before any treatment. To establish type 1 diabetes, C57BL/6 mice fasted 16 h were injected with streptozotocin (STZ) in the abdomen, with a dose of 50 mg/kg for 5 consecutive days. STZ solution was freshly prepared in cold citric acid-sodium citrate buffer (0.1 M sodium citrate, pH 4.5). After 3 days of continuous measurement with a blood glucose meter, the blood glucose concentration was >300 mg dL^{-1} (16.65 mmol L^{-1}) and the model was successful.

S2 Supporting figures



OHA-APBA	9	8	7	6	5	4	3	2	1
HAAD	1	2	3	4	5	6	7	8	9

Fig. S1 Picture of the hydrogels formed with different volume ratios of OHA-APBA and HAAD solution.

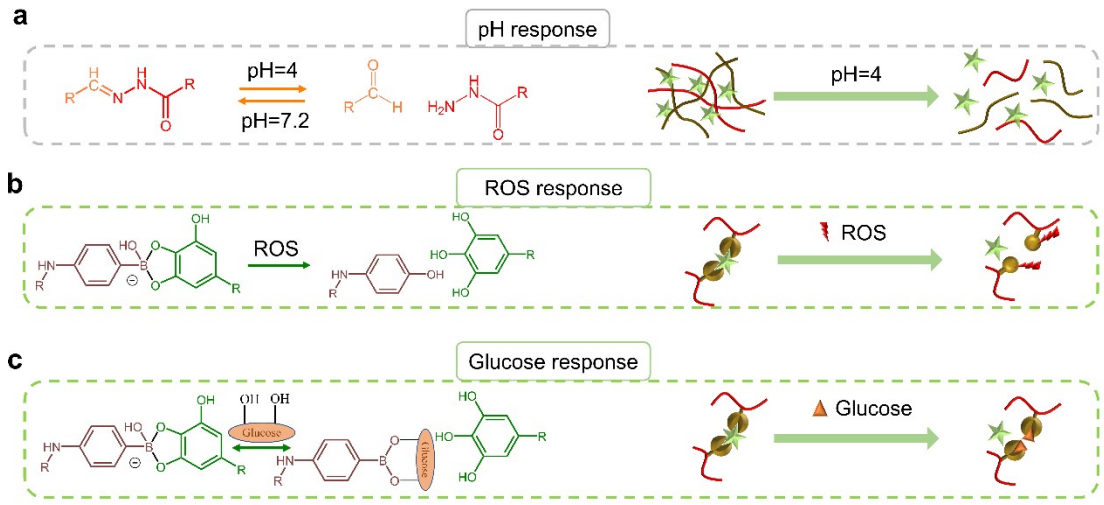


Fig. S2 Illustration of the responsive release mechanism of the OAH@TA hydrogel under pH (a), ROS (b) and glucose (c) conditions.

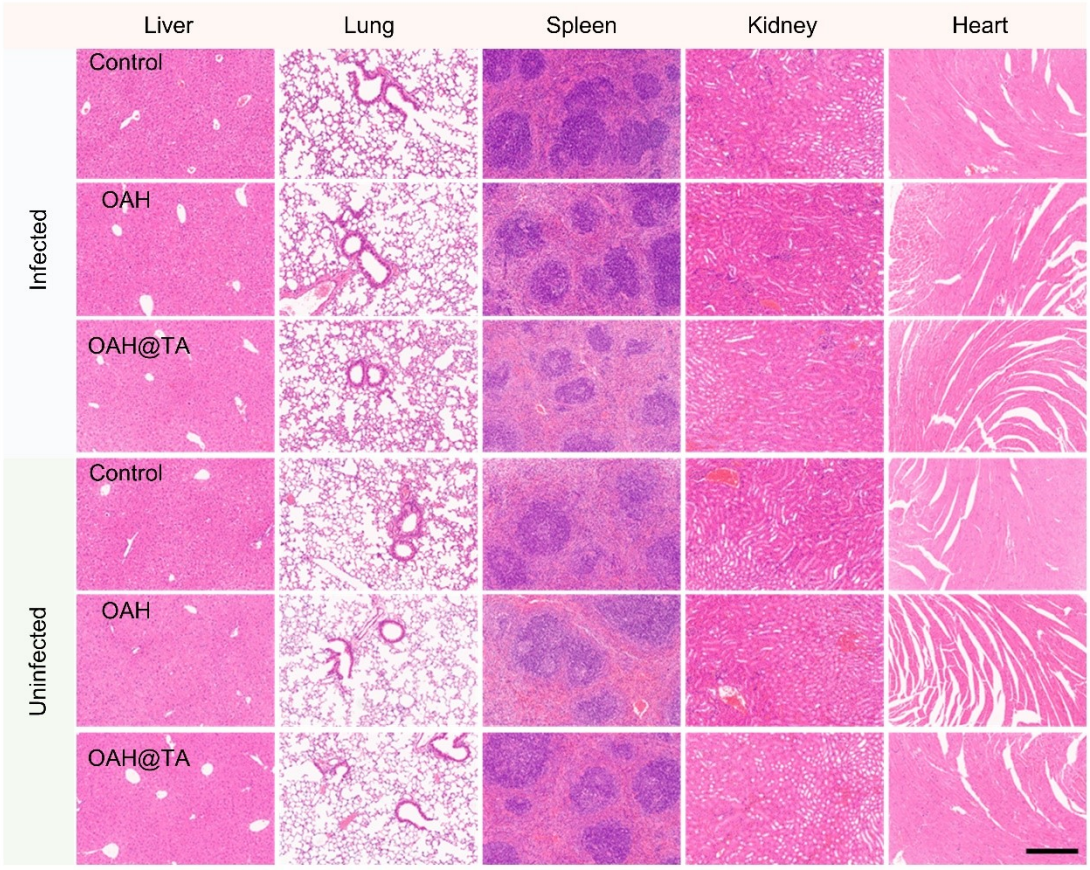


Fig. S3 The H&E staining of main organs in different groups (Scale bar = 300 μm).

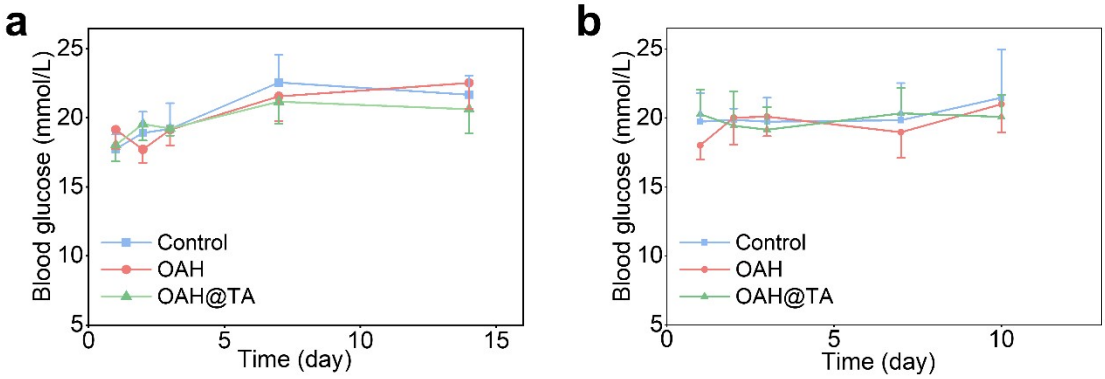


Fig. S4 Blood glucose levels of infected diabetic mice (a) and uninfected diabetic mice (b).

Reference:

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3. T. Zhou, J. Ran, P. Xu, L. Shen, Y. He, J. Ye, L. Wu and C. Gao, *Carbohydr Polym*, 2022, 292, 119667.