

Multiple dynamic crosslinked multifunctional hydrogels with glucose/pH dual-responsive adipose-derived stem cells-exosomes-releasing for diabetic wound healing

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Abstract

Background: Diabetic wounds feature a high-glucose and acidic microenvironment that impairs macrophage polarization and healing. Adipose-derived stem cell-derived exosomes (ADSC-exos) show therapeutic potential but suffer from rapid clearance. This study aimed to develop a smart hydrogel for glucose/pH-responsive ADSC-exos release.

Methods: A dual-responsive hydrogel (HAP/OCS/PEG/Ag-E) was fabricated via dynamic triple cross-linking. Characterization included rheometry, mechanical tests, and microscopy. In vitro macrophage polarization was assessed via flow cytometry and Western blot. A diabetic mouse wound model evaluated healing rates, histology, angiogenesis, and inflammation. Proteomics and pathway inhibition studies explored mechanisms. Statistical analysis used t-tests and ANOVA.

Results: The hydrogel exhibited excellent self-healing, adhesion, and controlled ADSC-exos release under high-glucose/acidic conditions. It promoted M2 macrophage polarization, reduced pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α), and accelerated wound healing with enhanced angiogenesis and collagen deposition. Mechanistically, the hydrogel suppressed the Notch/NF- κ B/NLRP3 signaling pathway.

Conclusion: The smart hydrogel facilitates diabetic wound healing through microenvironment-responsive ADSC-exos release and Notch/NF- κ B/NLRP3 pathway inhibition, offering a promising strategy for chronic wound treatment.

Keywords: Glucose/pH dual responsive hydrogels; ADSC-exos; Macrophage; Chronic diabetic wound; Inflammation

Highlights

- We propose a glucose/pH dual-responsive adipose-derived stem cells exosome (ADSC-exo)-releasing hydrogel therapy and provide a potential mechanism for alleviating the inflammatory response in diabetic wounds.
- We successfully developed a smart hydrogel delivery system that responds to the high glucose and acidic microenvironments, enabling controlled release of ADSC-exos under pathological conditions.
- The hydrogel reduced the release of inflammatory cytokines and alleviated the inflammatory response via the Notch/NF- κ B/NLRP3 signaling pathway.

Background

Diabetic wounds, which are commonly located in the extremities, are often difficult to treat. These wounds are the most common non-traumatic cause of extremity amputation [1, 2], with a toe amputation ratio of ~14%–24% [3, 4]. How to effectively promote the healing of diabetic wounds is a major challenge that must be urgently addressed in medical research [5–7]. The high-glucose microenvironment of diabetic wounds impairs the conversion of macrophages from the M1 to M2 phenotype, and M1 macrophages

continue to infiltrate and secrete large amounts of pro-inflammatory cytokines [8, 9]. The hyperinflammatory state in the surrounding tissues attracts many activated neutrophils, whose phagocytosis and degranulation functions become activated. These neutrophils secrete additional myeloperoxidase into the extracellular space, which leads to the production of hypohalous acids, especially hypochlorous acid, resulting in the formation of a low-pH microenvironment at the wound site [1, 10]. Moreover, the high-glucose environment associated with diabetic wounds favors bacterial outgrowth, with lactic and acetic acids production during wound-related

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metabolism, further exacerbating the acidic environment in these wounds [11, 12]. The high-glucose and low-pH microenvironment associated with diabetic wounds leads to impaired proliferation, differentiation, and migration of repair-related cells; hinders local angiogenesis and circulation; and impedes extracellular matrix (ECM) synthesis, ultimately resulting in suboptimal healing of these wounds [13, 14].

Multiple reports support the beneficial role of exosomes from mesenchymal stem cells (MSC-exos) in tissue repair [15–17]. They are nanometers in size and immune-tolerated, exerting various biological functions through transferring messenger ribonucleic acids (mRNAs), and micro RNAs (miRNAs), and other molecules to recipient cells [17, 18]. MSC-exos ameliorate bronchial dysplasia and restore lung function by inhibiting proinflammatory M1 macrophages and enhancing the effects of anti-inflammatory M2 macrophages [19]. Wang *et al.* reported that MSC-exos also exert inhibitory effects on the inflammation-related Notch pathway [20]. Our study [21] showed that ADSC-exos, a subtype of MSC-exos in cutaneous tissue, increased M2 polarization of macrophages and reduced the release of inflammatory cytokines in a high-glucose environment *in vitro*. However, when ADSC-exos were injected into diabetic wounds *in vivo*, their anti-inflammatory effects were limited because of rapid clearance of the body, moreover, prolonged diabetic wound healing further limited the potential therapeutic benefit of ADSC-exos [22–24].

In recent years, hydrogels have attracted researchers' interest owing to their excellent hydrophilicity, biocompatibility, and 3D porous structure [25–27], whose structure is similar to that of the ECM, enabling optimization of the wound microenvironment to promote healing [28, 29]. Moreover, the use of hydrogels as delivery systems for exosomes is expected to enable MSC-exos to efficiently exert long-lasting biological effects through controlled-release delivery [26, 30–33]. However, hydrogel dressings that can intelligently release MSC-exos in response to the pathological wound microenvironment while exhibiting multifunctional properties to promote diabetic wound healing has barely been reported.

In this study, we designed a multiple dynamic crosslinked multifunctional hydrogels with glucose/pH dual-responsive ADSC-exosomes-releasing for diabetic wound healing. The hydrogel was engineered through dynamic triple cross-linking involving Schiff base, phenylboronic ester, and thiol-Ag⁺ interactions, integrating antibacterial, anti-inflammatory, tissue-adhesive, and self-healing properties. Hyaluronic acid was modified with adipic dihydrazide and 3-aminophenylboronic acid to yield hyaluronic acid-phenylboronic acid conjugate (HAP), enabling pH/glucose-responsive crosslinking via Schiff base and phenylboronic ester bonds. Oxidized chondroitin sulfate (OCS) introduced aldehyde groups for additional Schiff base crosslinking, while its ortho-dihydroxy groups facilitated borate ester formation with HAP. This dual dynamic network selectively dissociates in the hyperglycemic and acidic diabetic wound microenvironment, triggering hydrogel swelling for controlled ADSC-exos release.

However, hydrogel dressings based on dynamic dual cross-linking between a Schiff base and phenyl borate ester are extremely prone to structural collapse. Furthermore, the strong susceptibility to bacterial infection is another characteristic of diabetic wounds. Therefore, we introduced 4-arm PEG-thiol to generate a dynamic Ag⁺-based triple

cross-linking network in the hydrogel, which conferred long-term stability, along with antibacterial properties and superior mechanical performance (Figure 1a). The hydrogel exhibited excellent self-healing capability, injectability, adhesion, and biocompatibility properties. Moreover, it was shown to accelerate wound healing in a mouse model of diabetic wounds by reducing the inflammatory response, promoting vascular reconstruction, and enhancing re-epithelialization and collagen deposition. Mechanistic findings revealed that the hydrogel promoted the polarization of macrophages to the M2 phenotype by releasing exosomes that inhibit Notch/NF- κ B/NLRP3 signaling to ameliorate the inflammatory response in diabetic wounds (Figure 1b). This study demonstrated the potential application of this multifunctional hydrogel with smart release of ADSC-exos as a novel highly promising strategy for the clinical treatment of chronic diabetic wounds.

Methods

Evaluation of the dual responsiveness of the hydrogels to pH and glucose

The fluorescent dye rhodamine was used as a model drug for the preliminary evaluation of the dual responsiveness of the hydrogels to pH and glucose. Specifically, the PEG/Ag and HAP/OCS/PEG/Ag hydrogels were first loaded with equal concentrations of rhodamine and then placed in low-pH (pH = 5.5) and high-glucose (33 mM glucose) solutions. The hydrogel solutions were then placed in a shaking incubator at 37°C for 1 h, and hydrogel structural integrity and rhodamine release from the hydrogel were observed. A camera was used to record the aforementioned process.

Proteomic analysis

Label-free LC-MS/MS was used for proteomic analysis of the control group and HAP/OCS/PEG/Ag-E hydrogel group ($n = 5$). After protein extraction and trypsin digestion, liquid chromatography mobile phase A was used to dissolve the peptides; then, the peptides were separated by a NanoE-lute ultra-performance liquid chromatography system and injected into the capillary ion source for ionization analyses by tims-TOF Pro mass spectrometry. The MaxQuant search engine (v.1.5.2.8) was used to process the MS/MS data. KEGG online tools were used to annotate the differentially expressed proteins, which were subsequently matched with annotations in the database using KEGG Mapper. The GO annotation proteome was screened with the UniProt-GOA database (<http://www.ebi.ac.uk/GOA/>). P -values < .05 were considered to indicate statistical significance.

Statistical analysis

All the data were analysed using SPSS 17.0 software, and the results are presented as the mean $\pm$ standard deviation (SD). Student's t -test was used for comparisons between two groups, and statistical significance was analysed via one-way variance (ANOVA) one-way ANOVA with Tukey's multiple comparison test. $P < .05$ was considered to indicate statistically significant results.

Results

Hydrogel preparation and composition

In this study, a dual pH/glucose-responsive hydrogel dressing (HAP/OCS/PEG/Ag) was constructed by dynamic dual

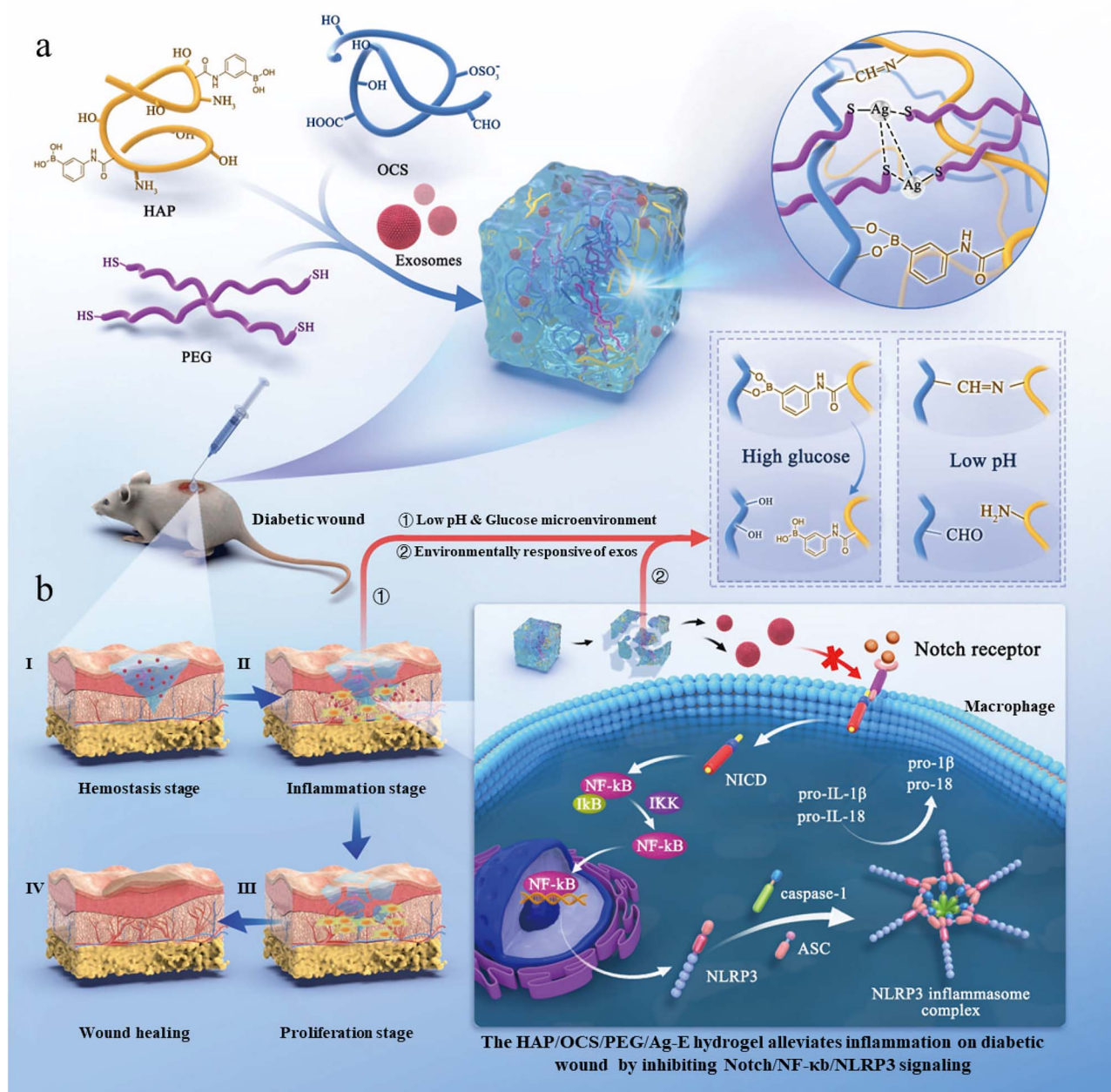


Figure 1. Schematic illustration of the fabrication and application of the HAP/OCS/PEG/Ag-E hydrogel for diabetic wound management. **(a)** Synthetic route of the HAP/OCS/PEG/Ag-E hydrogel. **(b)** Mechanistic insights into the impacts of the HAP/OCS/PEG/Ag-E hydrogel on macrophages during diabetic wound healing. PEG polyethylene glycol, E exosomes, Ag argentine, -CHO aldehyde group, -OH ortho-dihydroxy group, -HOOC carboxyl group, -H₂N amino group, NLRP3 NOD-like receptor family pyrin domain containing 3)

cross-linking via Schiff base and phenyl borate ester bonds in HAP and OCS, respectively, supplemented by a third dynamic cross-linking network involving 4-arm PEG-thiol and Ag⁺. Exosomes (E) were then loaded onto the hydrogel to obtain the HAP/OCS/PEG/Ag-E hydrogel dressing. The characterization results of ADSCs and ADSC-Exos are presented in Figure S1a–e and Figure S2a–c. To assess how the pH/glucose dual-responsive release of exosomes affects diabetic wound healing, we fabricated a non-responsive PEG/Ag hydrogel dressing using 4-arm PEG-thiol and Ag⁺. Subsequently, exosomes (E) were loaded into this hydrogel to prepare the PEG/Ag-E hydrogel dressing. As observed via laser confocal microscopy, the exosomes were encapsulated within the

HAP/OCS/PEG/Ag-E hydrogel (Figure S2d). The compositions of all four hydrogels are shown in Figure 2a.

Rheological and swelling properties of the hydrogels

We evaluated the rheological properties of the four hydrogels (Figure 2b). The storage modulus (G') of all hydrogels was significantly higher than the loss modulus (G''), confirming their elastic nature. Compared to the PEG/Ag hydrogel (G' of ~600 Pa), the HAP/OCS/PEG/Ag hydrogel had a higher modulus ($G' > 1500$ Pa) due to the introduction of the second cross-linking network. Notably, the introduction of

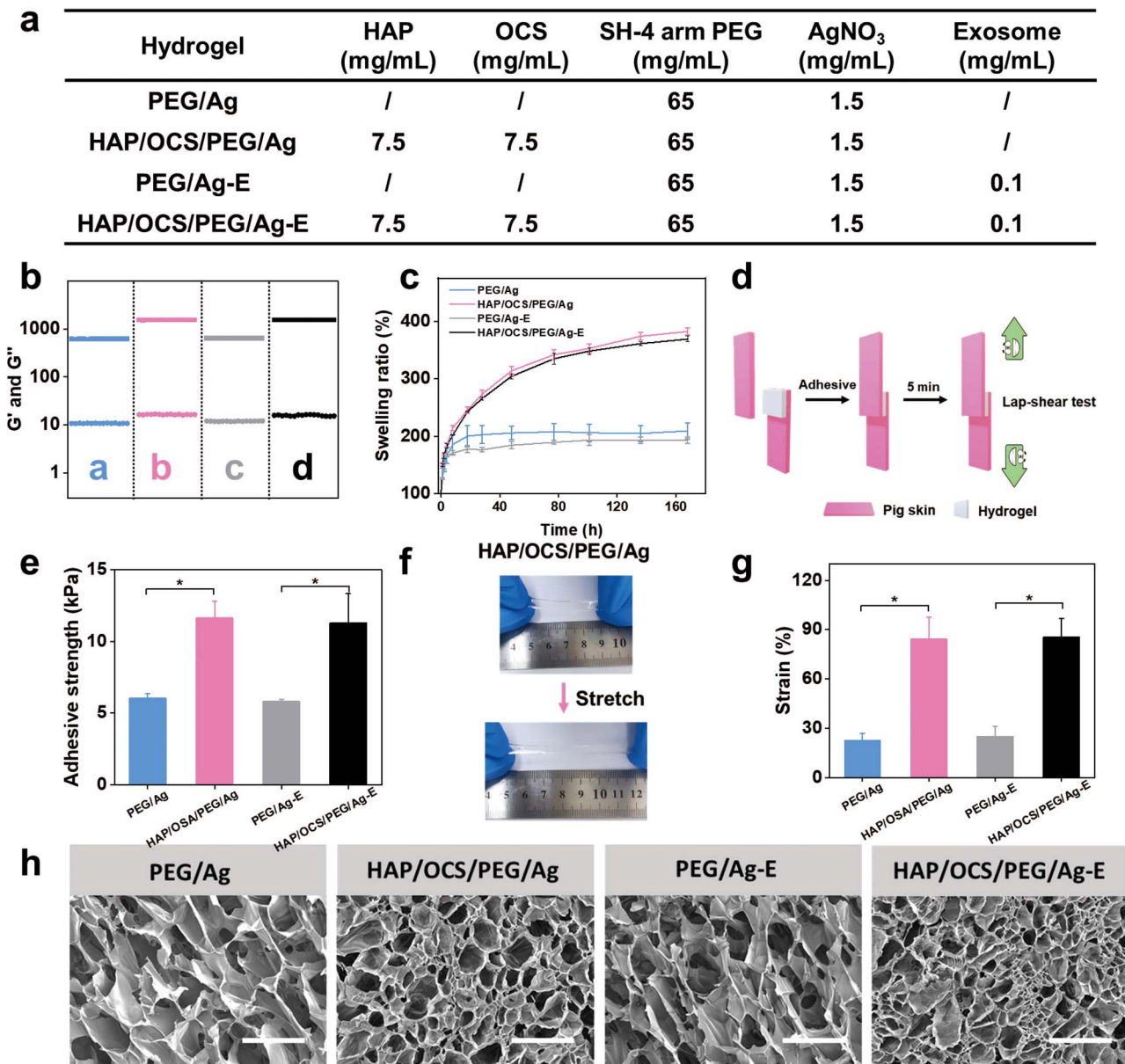


Figure 2. Basic performance characteristics of the hydrogel. (a) Hydrogel composition. (b) Hydrogel rheological behavior. (c) Hydrogel swelling properties. (d) Schematic diagram for evaluating the tissue adhesion of hydrogels under lap shear conditions. (e) Tissue adhesion performance of the hydrogels. (f) Visual demonstration of the hydrogels' tensile properties. (g) Tensile properties of the hydrogels. (h) Morphology of the hydrogels; scale bar = 300 μ m. Data are expressed as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. P values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (E Exosomes)

exosomes did not affect the rheological properties of these two hydrogels.

The PEG/Ag-series hydrogels have favorable water absorption capacity and can thus maintain a moist microenvironment, absorb wound exudate, and prevent infections in the wound. As depicted in Figure 2c, the incorporation of hydrophilic polysaccharides—for instance, HA and CS—led to the HAP/OCS/PEG/Ag hydrogel exhibiting a higher equilibrium swelling ratio ($\sim 400\%$) and a longer equilibrium swelling period (>100 h) compared with the PEG/Ag hydrogel. The latter has an equilibrium swelling ratio of 200% and an equilibrium swelling period of <10 h. This helps maintain a moist microenvironment and facilitates exudate absorption during the early stage of wound healing.

Importantly, exosomes loading did not affect the swelling properties of either hydrogel.

Evaluation of tissue adhesion, mechanical performance, and microstructure of the hydrogels

Sufficient tissue adhesiveness is essential for hydrogel wound dressings to maintain adherence to wound surfaces. A swine skin lap shear test was used to evaluate the tissue adhesiveness of the four hydrogel types (Figure 2d). As shown in Figure 2e, the strength of adhesiveness between the PEG/Ag-series hydrogels and skin was ~ 5 kPa, attributable to the hydrophilicity of PEG, covalent cross-linking between thiol groups in the hydrogel and tissue extracellular proteins during *in situ* gelation, and the entanglement of polymer chains.

After introducing the dual cross-linking secondary network involving the Schiff base and the phenyl borate ester in HAP and OCS, respectively, to the hydrogel, the tissue adhesiveness of the HAP/OCS/PEG/Ag and HAP/OCS/PEG/Ag-E hydrogels increased to 11–12 kPa. This increase was mainly due to the formation of dynamic Schiff base interactions between aldehyde groups in the hydrogel and amino groups on the tissue surface. HAP/OCS/PEG/Ag-E showed greater tissue adhesiveness than commercially available fibrin glue (~5 kPa), making it suitable for chronic diabetic wounds.

Frequent movement and stretching of the skin necessitate greater extensibility and mechanical performance of hydrogel dressings. Therefore, we evaluated the tensile properties of the hydrogels through macroscopic observations of stretching and quantitative tensile. First, the HAP/OCS/PEG/Ag hydrogel was highly stretchable (Figure 2f). Further quantitative stretch tests showed that the stretch ratios of HAP/OCS/PEG/Ag and HAP/OCS/PEG/Ag-E exceeded 80%, while the PEG/Ag-series hydrogels had poor tensile properties (stretch ratio <30%, Figure 2g). The stretchability of the HAP/OCS/PEG/Ag and HAP/OCS/PEG/Ag-E hydrogels significantly exceeded human skin (50%), suggesting that the HAP/OCS/PEG/Ag hydrogel wound dressing can satisfy wound healing requirements in terms of stretchability.

Interconnected large-pore structures within the hydrogel can support cellular penetration, facilitating wound healing. We characterized the microscopic appearance of the four hydrogels. The PEG/Ag-series hydrogels showed uniform but incomplete large pore structures, likely due to the low cross-linking density of the polymer network (Figure 2h). After the introduction of the dual cross-linking secondary network involving the Schiff base and the phenylboronic ester in HAP and OCS, respectively, the HAP/OCS/PEG/Ag and HAP/OCS/PEG/Ag-E hydrogels demonstrated complete and dense large pore structures capable of enhancing cellular infiltration and material exchange. In addition, the microscopic appearance of the hydrogels was consistent with the modulus results.

Evaluation of hydrogel self-healing and dual responsiveness to pH and glucose

Skin stretching, frequent movement, and infiltration of external environmental factors can physically damage hydrogel dressings. Therefore, effective self-healing is necessary to maintain an intact barrier, thereby prolonging dressing lifespan. The four hydrogels' dynamic cross-linking networks enable self-healing through single/multiple reversible bonds. Under alternating step strains (600% to 1%), the G' of the PEG/Ag hydrogel decreased from ~600 Pa to ~170 Pa. This led to G'' being greater than G' , indicating network disruption (Figure 3a). However, switching to low strain (1%) allowed the rapid recovery of the hydrogel to its original modulus, with G' being greater than G'' . This demonstrated that the dynamic cross-linking between thiol- Ag^+ in the hydrogel could quickly restore the network structure to achieve rapid self-healing. Three consecutive strain cycles showed consistent G'/G'' recovery, confirming repeatable self-healing. After incorporating dynamic dual cross-linking-encompassing both Schiff base and phenyl borate ester linkages-into the hydrogel, the HAP/OCS/PEG/Ag hydrogel exhibited a more stable G' value under low strain. This indicates that it has superior self-healing capabilities compared to the PEG/Ag

hydrogel (Figure 3b). Building on these findings, we further assessed the macroscopic self-healing performance of the HAP/OCS/PEG/Ag hydrogel (Figure 3c). This was done by bisecting both rhodamine-stained and unstained hydrogel samples, then bringing the cut surfaces into contact without external intervention for 1 min. Healed hydrogels retained >65% strain capacity, nearing the original 80% (Figure 3d). This efficiency stems from synergistic thiol- Ag^+ coordination, Schiff base, and phenyl borate ester networks (Figure 3e).

Dual responsiveness to glucose and pH, attributed to dynamic dual cross-linking involving Schiff base and phenyl borate ester in HAP and OCS respectively, allows the hydrogel to intelligently respond to high-glucose and low-pH microenvironment associated with chronic diabetic wounds, achieving on-demand exosome release. Rhodamine-loaded PEG/Ag and HAP/OCS/PEG/Ag hydrogels were respectively placed in high-glucose and low-pH environments (Figure 3f). The PEG/Ag hydrogel maintained structural integrity over an extended period, and the rhodamine payload was not released to a significant degree. In the HAP/OCS/PEG/Ag hydrogel, destruction of the internal dynamic cross-linking network collapsed under high glucose and low pH (Figure 3g), triggering rapid swelling and even partial dissolution. Under these conditions, the hydrogel's rhodamine payload was rapidly released into the external environment.

Furthermore, we measured the concentration of released ADSC-exos in different environments. ADSC-exos release quantification showed both hydrogels exhibited sustained release in PBS (pH 7.4, glucose-free) with comparable cumulative concentrations (Figure 3h). Acidic conditions (pH 5.5) significantly enhanced exosome release versus physiological pH (Figure 3i), due to pH-triggered phenyl borate ester cleavage. This pH-responsive behavior correlates with enhanced drug release in diabetic wounds (Figure 3i), highlighting HAP/OCS/PEG/Ag-E hydrogel's therapeutic potential. Hyperglycemic conditions (33 mM glucose) induced glucose-dependent release kinetics (Figure 3j): Schiff base-mediated structural changes in HAP/OCS/PEG/Ag-E hydrogels progressively amplified ADSC-exos release. These results validate the hydrogel's dual pH/glucose responsiveness for enhanced diabetic wound repair.

Hemocompatibility and cytocompatibility of the hydrogel

Hemostasis, the first stage of wound healing, requires hemocompatible wound dressings. In this study, a hemolysis assay was used to evaluate the hemocompatibility of the four hydrogels. The four hydrogels were incubated with mouse red blood cells (RBCs) at 37°C for 1 h (Figure S3a and b), all hydrogels produced pale-yellow supernatants similar to PBS controls, with intact RBC morphology. In contrast, Triton X-100 caused bright red supernatant due to complete hemolysis and erythrocyte rupture. Quantitative analysis showed hydrogel hemolysis ratios <3% (vs. PBS 0% and Triton X-100 100%), confirming excellent hemocompatibility (Figure S3c).

Hydrogel wound dressings must be biocompatible, and cytocompatibility testing is the most commonly used method to evaluate the biocompatibility of biomaterials *in vitro*. Fibroblasts are important cellular components of the wound healing process. Therefore, in this study, fibroblasts (L929 cells) were placed in direct contact with the four hydrogels

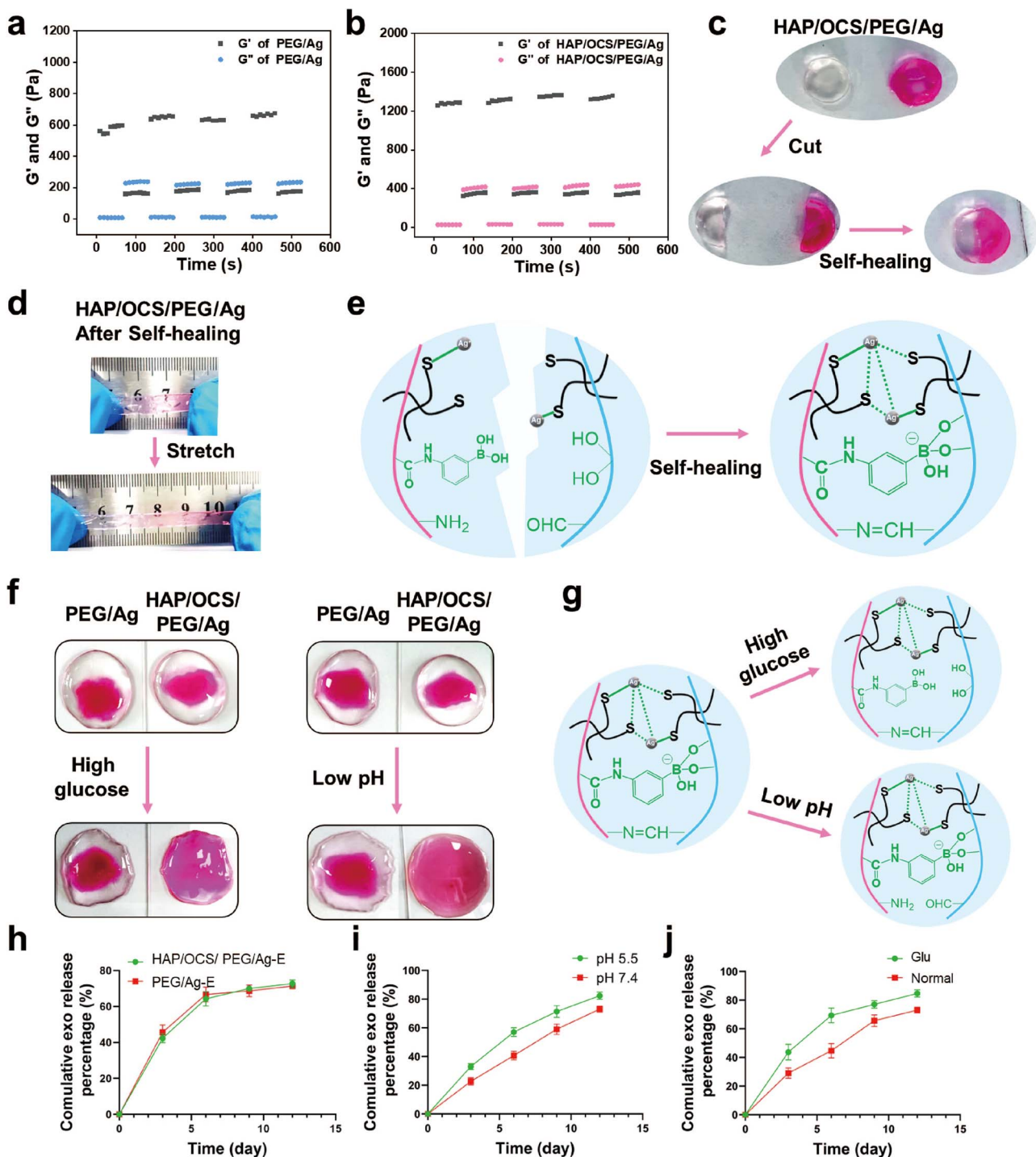


Figure 3. The self-healing properties and dual responsiveness of the dual dynamic network hydrogel to pH and glucose. The rheological properties of the PC hydrogel when the alternate step strain was switched from 1% to 600%. (a) PEG/Ag hydrogel. (b) HAP/OCS/PEG/Ag hydrogel. (c) Visual demonstration of the self-healing capability of the HAP/OCS/PEG/Ag hydrogel. (d) Visual demonstration of the self-healing capability of the hydrogel. (e) Schematic diagram of the self-healing mechanisms of the hydrogel. (f) Visual demonstration of the responsiveness of the hydrogel to pH/glucose. (g) Schematic diagram of the mechanism underlying the response of the hydrogel to pH/glucose. (h) Exosome release profiles of ADSCs in the HAP/OCS/PEG/Ag-E and PEG/Ag-E hydrogels. (i) pH and (j) glucose responsive exosome release. Data are expressed as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. P -values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

to evaluate their cytocompatibility. After 1 day of direct contact with the hydrogel, the viability of L929 cells in the four hydrogel groups was greater than 90% (Figure S3d). Further dead/live staining showed that the cell density of L929 cells in contact with the four hydrogels was similar to

that of the control group and the high glucose group, and most L929 cells maintained a spindle shape (Figure S3e). The Ki67 immunofluorescence (IF) assay showed that the HAP/OCS/PEG/Ag-E group contained the largest number of Ki67-positive cells (Figure S3f). This finding confirms that the

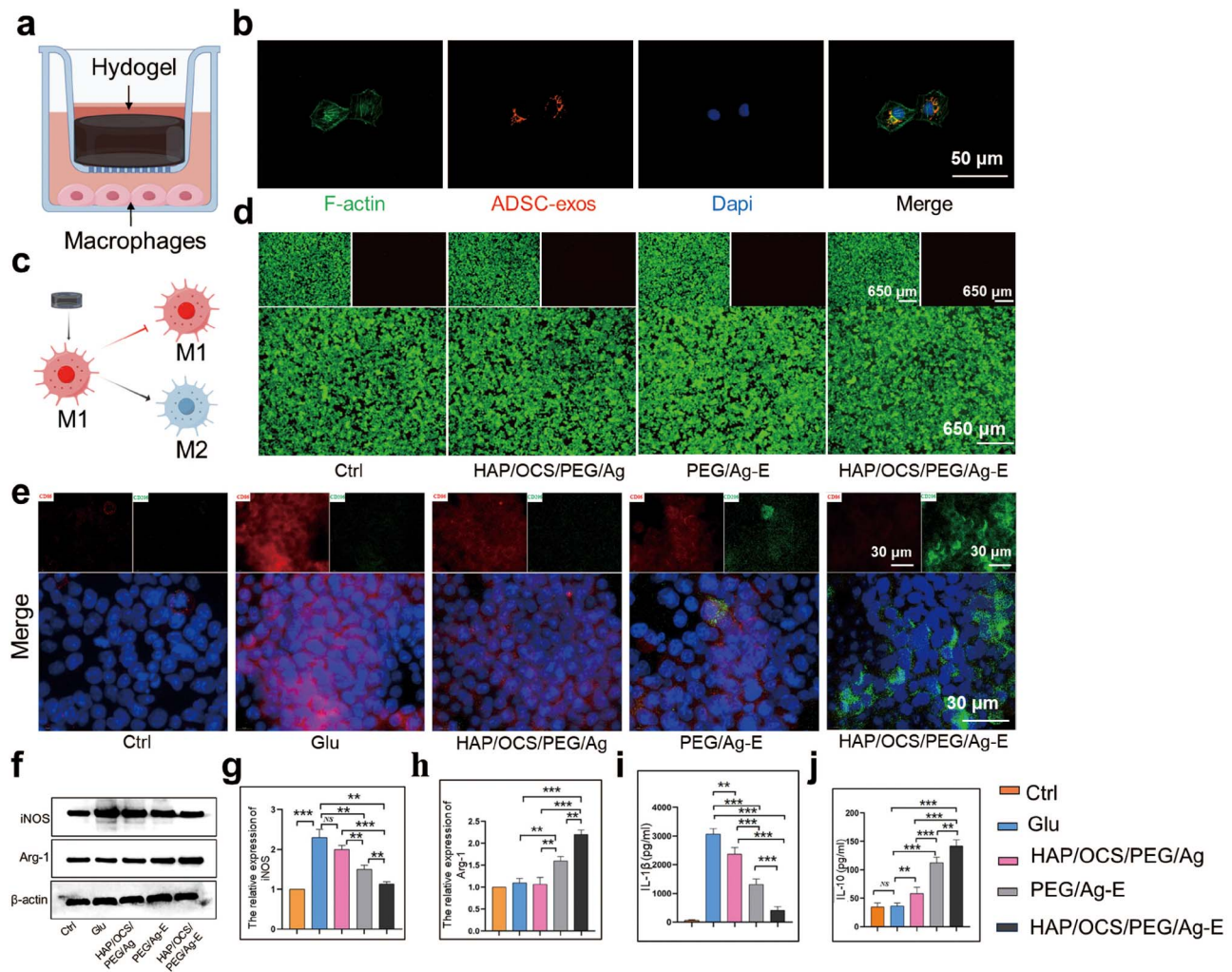


Figure 4. Impacts of the PEG/Ag-series hydrogels on macrophage polarization. (a) Schematic diagram showing performance in the Transwell assay. (b) Representative IF images of PKH26-labeled exosomes in macrophages. Scale bar = 100 μm. (c) Exosome-loaded hydrogels promote the conversion of macrophages from the M1 phenotype to the M2 phenotype. (d) Representative live/dead staining images of macrophages in each group. Scale bar = 650 μm. (e) IF image showing the proportions of CD86-positive and CD206-positive macrophages in each group. Red fluorescence represents the M1 macrophage phenotype marker CD86, green IF represents the M2 macrophage-specific protein marker CD206, and blue fluorescence represents the nucleus. Scale bar = 30 μm. (f) Western blot results showing iNOS and Arg-1 expression in each group. (g and h) Quantitative statistical analysis of the iNOS and Arg-1 data. (i and j) IL-1β and IL-10 levels measured via ELISA. Data are presented as mean ± SD and statistical significance was analysed via one-way ANOVA with Tukey's multiple comparison test. *P*-value: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *Con* control, *Glu* glucose, *M1* classically activated macrophage, *M2* alternatively activated macrophage, *E* exosomes

HAP/OCS/PEG/Ag-E group has a strong ability to promote cell proliferation.

Effects of the hydrogels on macrophages

Macrophages are highly plastic cells. During the wound healing process, they play a crucial role in resolving inflammation throughout the repair process via M1/M2 phenotypic transition [34–36]. Macrophage responses to hydrogels were assessed using Transwell chambers (Figure 4a and c). Exosomes exert their biological effects upon internalization by the recipient cell [37, 38]; accordingly, ADSC-exos labeled with the fluorescent dye PKH26 and macrophages cocultured for 24 h, after which exosome uptake by macrophages was investigated (Figure 4b). To simulate a diabetic environment *in vitro* [39], we cultured the macrophages under normal conditions as controls, while under high-glucose conditions as high-glucose

controls, and the macrophages with different hydrogels under the high-glucose conditions as the experimental groups.

After 3 days of culture, we assessed the viability of the macrophages [40]. The results showed that minimal dead cells were present in each group, with no differences in cell viability among groups (Figure 4d). Dual immunohistochemistry for CD86⁺ (M1)/CD206⁺ (M2) revealed significantly lower CD86⁺/CD206⁺ ratios in the HAP/OCS/PEG/Ag-E group versus others (Figure 4e). Flow cytometry confirmed this group had fewer M1 (CD86⁺) macrophages than high-glucose controls and more M2 (CD206⁺) macrophages than the other experimental groups (Figure S4). Inducible nitric oxide synthase (iNOS) and arginase 1 (Arg-1) serve as markers for M1 and M2 macrophages, respectively [41, 42]. Western blot results showed that, compared with the high-glucose control group, the exosome-loaded group exhibited reduced iNOS expression and increased Arg-1 levels. The HAP/OCS/PEG/Ag-E group showed the lowest iNOS and

highest Arg-1 levels (Figure 4f), confirmed by quantitative analyses (Figure 4g and h).

IL-1 β is a proinflammatory cytokine secreted by M1 macrophages [43], whereas IL-10 is an anti-inflammatory cytokine from M2 macrophages [44]. Enzyme-linked immunosorbent assays confirmed that compared with the PEG/Ag-E, the HAP/OCS/PEG/Ag-E group had significantly lower concentrations of IL-1 β in the cell supernatant and markedly greater protein expression of IL-10 (Figure 4i and j). These results indicate that both the PEG/Ag-E and the HAP/OCS/PEG/Ag-E hydrogels promote macrophage polarization toward the M2 phenotype and reduce inflammatory cytokine release. This can be attributed to the strong anti-inflammatory effects of ADSC-exos in the hydrogel. The glucose/pH-responsive group's enhanced M2 polarization and anti-inflammatory efficacy stem from Schiff base/phenylboronic ester dual cross-linking, enabling microenvironment-responsive ADSC-exos release. This mechanism ensures optimal exosome bioactivity in diabetic conditions.

The hydrogels promote diabetic wound healing

Next, the *in vivo* efficacy of the hydrogels was assessed. Streptozotocin-induced diabetic mice [45] with fasting blood glucose exceeding 16.7 mmol/L via tail vein puncture [46] received 1 cm-diameter full-thickness dorsal wounds. Control mice used Tegaderm dressings, while experimental groups received PEG/Ag-series hydrogels. Ten days after treatment with PKH67-labeled HAP/OCS/PEG/Ag-E, exosome fluorescence remained detectable in the wound tissues (Figure S2e). As shown in the wound images (Figure 5a), the wound area gradually decreased in all groups. By day 7, the HAP/OCS/PEG/Ag-E group exhibited significantly faster healing versus others, achieving near-complete closure by day 10 (Figure 5a and i). Non-responsive exosome hydrogels promoted healing, but environment-responsive hydrogels enhanced efficacy through increased ADSC-exos release. Hematoxylin and eosin (H&E)-stained day 14 wounds revealed the HAP/OCS/PEG/Ag-E group had shortest wound length and most complete epithelialization (Figure 5b), confirming superior healing capacity.

The formation and arrangement of collagen stand as key indicators for assessing wound healing. Sufficient collagen deposition facilitates repair processes, while excessive deposition alongside disorganized collagen fibers can lead to scar hypertrophy, thereby hindering healing. In comparison with other groups, the HAP/OCS/PEG/Ag-E group exhibited the most orderly collagen arrangement and the highest collagen content (Figure 5c). Both H&E and Masson staining validated the effectiveness of the HAP/OCS/PEG/Ag-E hydrogel in wound healing. Blood vessels support healing by providing nutrients and oxygen [47, 48]. Blood perfusion analysis using imaging devices [49] revealed gradual perfusion increase across groups over time (Figure 5d and k). The exosome-loaded hydrogel groups exhibited superior angiogenesis, with HAP/OCS/PEG/Ag-E group showing the highest vascularization due to microenvironment-responsive exosome release. α -SMA/CD31 [50, 51] co-IF at day 7 post-injury demonstrated that environmentally responsive hydrogels induced more intact vascular lumens with significantly higher density than those in other groups (Figure 5e-h and j).

Anti-inflammatory effects of hydrogels on diabetic wounds

Previous studies confirm that after tissue injury, M1 macrophages are initially recruited to wounds, attracting inflammatory cells to clear necrotic tissue and pathogens. During later healing stages, macrophages transition predominantly to the M2 phenotype, promoting tissue repair [52–54]. However, in chronic diabetic wounds, M1/M2 transition is impaired, leading to prolonged inflammation [8, 55, 56]. Persistent inflammation inhibits angiogenesis, resulting in poor granulation tissue development, delayed epithelialization, and slower wound healing [57–59].

We analysed macrophage polarization and inflammation *in vivo*. Day 3 wound specimens were subjected to IF staining for macrophage markers: F4/80 (pan-macrophage), CD86 (M1), and CD206 (M2). Diabetic controls showed abundant CD86⁺F4/80⁺ M1 macrophages. The HAP/OCS/PEG/Ag-E group exhibited significantly fewer M1 macrophages (Figure 6a and h) and predominant M2 polarization (Figure S5a and b) versus other groups. IL-6 and TNF- α levels were assessed via immunohistochemistry on day 3. All exosome-loaded hydrogels reduced inflammatory cytokine expression versus controls (Figure 6b), confirming exosome-mediated anti-inflammatory effects.

Furthermore, wounds treated with the environmentally responsive hydrogel exhibited significantly lower levels of inflammatory cytokines compared to other groups. Quantitative analyses confirmed that this group had the lowest IL-6 and TNF- α expression (Figure 6b–d). These findings indicate that the hydrogel efficiently releases exosomes in response to the wound's high-glucose/low-pH microenvironment, thereby exerting potent anti-inflammatory effects in diabetic wounds. Western blot analysis demonstrated that the HAP/OCS/PEG/Ag-E group more effectively reduced iNOS expression in M1 macrophages and enhanced Arg-1 expression in M2 macrophages versus other treatments (Figure 6e–g). Collectively, these data demonstrate that the environmentally responsive hydrogel substantially ameliorates the wound's inflammatory microenvironment and promotes M2 macrophage polarization, primarily due to ADSC-exos' efficient release.

Proteomic analysis validates adipose-derived stem cells exosomes anti-inflammatory effects

Day 7 post-injury skin tissues underwent LC-MS/MS proteomics [60, 61] ($n = 5$ /group: control vs. HAP/OCS/PEG/Ag-E). Quality control ensured data reliability (Figure 7a and b). Analysis of 4445 proteins identified 479 differentially expressed (272 $\uparrow$;207 $\downarrow$), visualized via volcano plot (Figure 7d). Cytoplasmic (35.46%) and extracellular (14.79%) localization suggested exosome involvement (Figure 7c). Heatmaps [62] revealed HAP/OCS/PEG/Ag-E-mediated suppression of inflammatory proteins during remodeling (Figure 7f). GO/KEGG analysis [63, 64] highlighted enrichment in immune defense pathways, including NF- κ B signaling and sugar metabolism (Figure 7e and h). These findings collectively demonstrate ADSC-exos' potent anti-inflammatory bioactivity through targeted pathway modulation.

Protein–protein interaction (PPI) analysis [65] revealed correlations between NF- κ B-related proteins and four others,

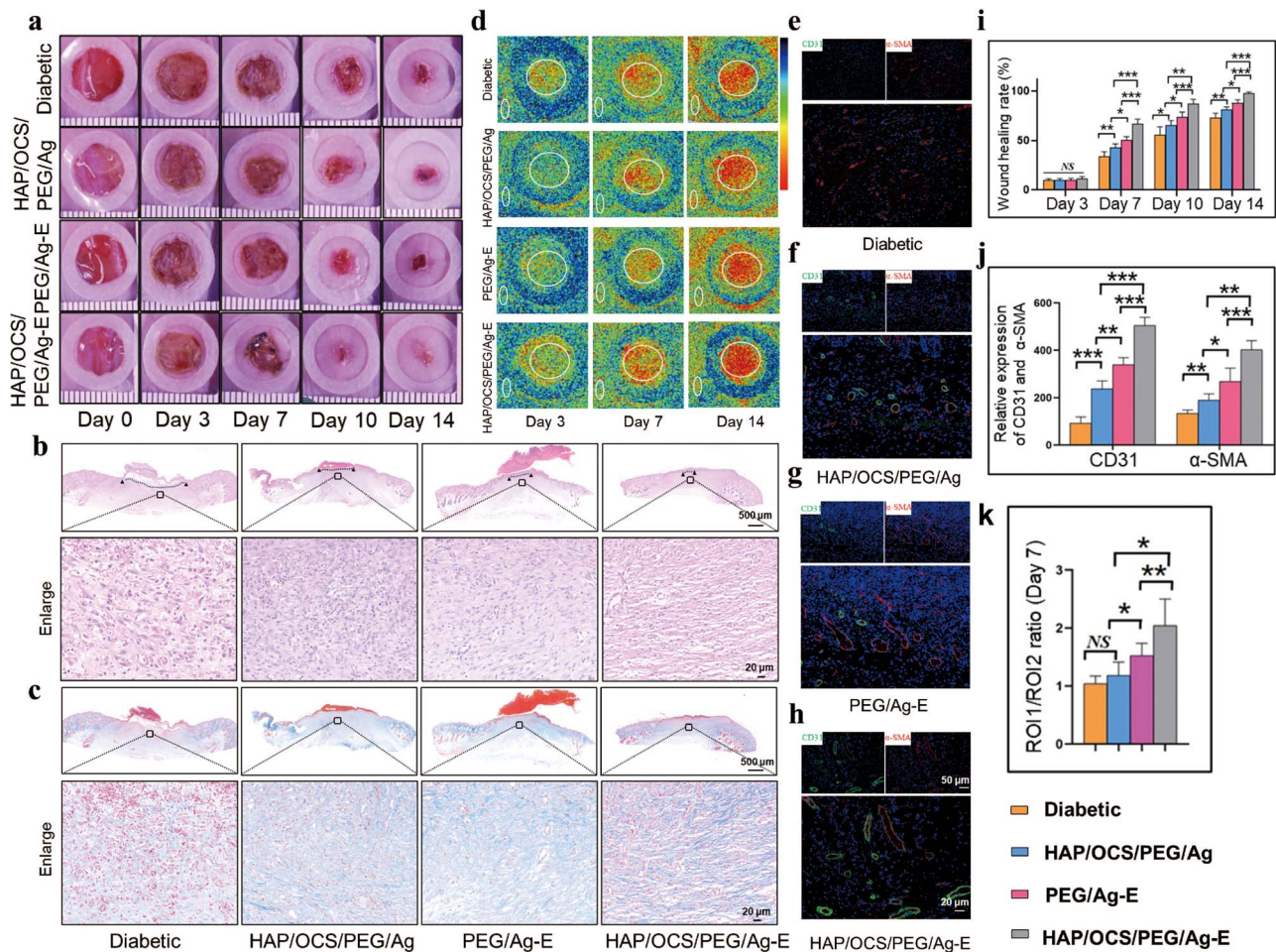


Figure 5. Influence of PEG/Ag-series hydrogels on wound healing in diabetic mice. (a) Digital images of wound regions treated with Tegaderm dressings or PEG/Ag-series hydrogels on days 0, 3, 7, 10, and 14. Scale bar = 1 cm. (b and c) Representative H&E and Masson staining outcomes of wound tissues in each group on day 14. Scale bar = 500 μ m (upper); scale bar = 20 μ m (lower). (d) Typical laser Doppler perfusion images of each group on days 3, 7, and 14. (e–h) Representative images of CD31 and α -SMA IF staining of wound tissue on day 7. Scale bar = 50 μ m, upper; scale bar = 20 μ m, lower. (i) Analysis of the wound closure rate in each group at each time point. (j) Quantitative analysis of CD31 and α -SMA co-localization in wound tissue on day 7 post-injury. (k) Results of the quantitative analysis of ROI1/ROI2 in each group. Data are presented as mean $\pm$ SD and statistical significance was analysed via one-way ANOVA with Tukey's multiple comparison test. P -value: * P < 0.05, ** P < 0.01, *** P < 0.001

with NLRP3 emerging as a key player (Figure 7g). Previous studies demonstrate upregulated NLRP3 inflammasome expression in chronic diabetic wounds, co-occurring with elevated IL-1 β /IL-18 levels [66–68]. We propose that macrophage activation triggers NF- κ B signaling, subsequently activating the NLRP3 inflammasome complex and amplifying proinflammatory cytokine production, thereby exacerbating the wound's inflammatory microenvironment. The HAP/OCS/PEG/Ag-E hydrogel likely exerts anti-inflammatory effects via suppression of this NF- κ B/NLRP3 axis, as validated in subsequent mechanistic studies.

As a classic upstream regulatory pathway of NF- κ B, Notch signaling exerts detrimental effects on late-stage diabetic wound healing. Proteomics revealed that the HAP/OCS/PEG/Ag-E hydrogel suppressed NF- κ B pathway activity and exhibited NF- κ B/NLRP3 interactions. We subsequently validated the Notch/NF- κ B/NLRP3 axis. Notch signaling was activated under high glucose, while the HAP/OCS/PEG/Ag-E hydrogel suppressed Notch-related proteins (NICD, Hes-1, Hey-1), confirming Notch pathway

inhibition (Figure 7i and j). Using RBPJ $^{-/-}$ myeloid cells (Notch $^{-/-}$ group) and wild-type controls, we observed that both HAP/OCS/PEG/Ag-E and Notch $^{-/-}$ groups exhibited reduced NF- κ B activation (p-I κ B α , p-P65) and NLRP3/Caspase-1 expression versus controls (Figure 7k–n). NF- κ B inhibitor (BAY11-7082) controls further demonstrated that the hydrogel decreased NLRP3/Caspase-1 levels (Figure 7o and p) and pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6; Figure 7q–s), while elevating anti-inflammatory IL-10 (Figure 7t). These data conclusively demonstrate that the HAP/OCS/PEG/Ag-E hydrogel alleviates inflammation through Notch signaling blockade, thereby suppressing NF- κ B/NLRP3 activation and inflammatory cytokine release.

Discussion

At present, there are >537 million diabetic patients worldwide, and the incidence of diabetic wounds is ~8.1%. Due to the high-glucose and low-pH microenvironment associated with diabetic wounds, repair-related cell proliferation,

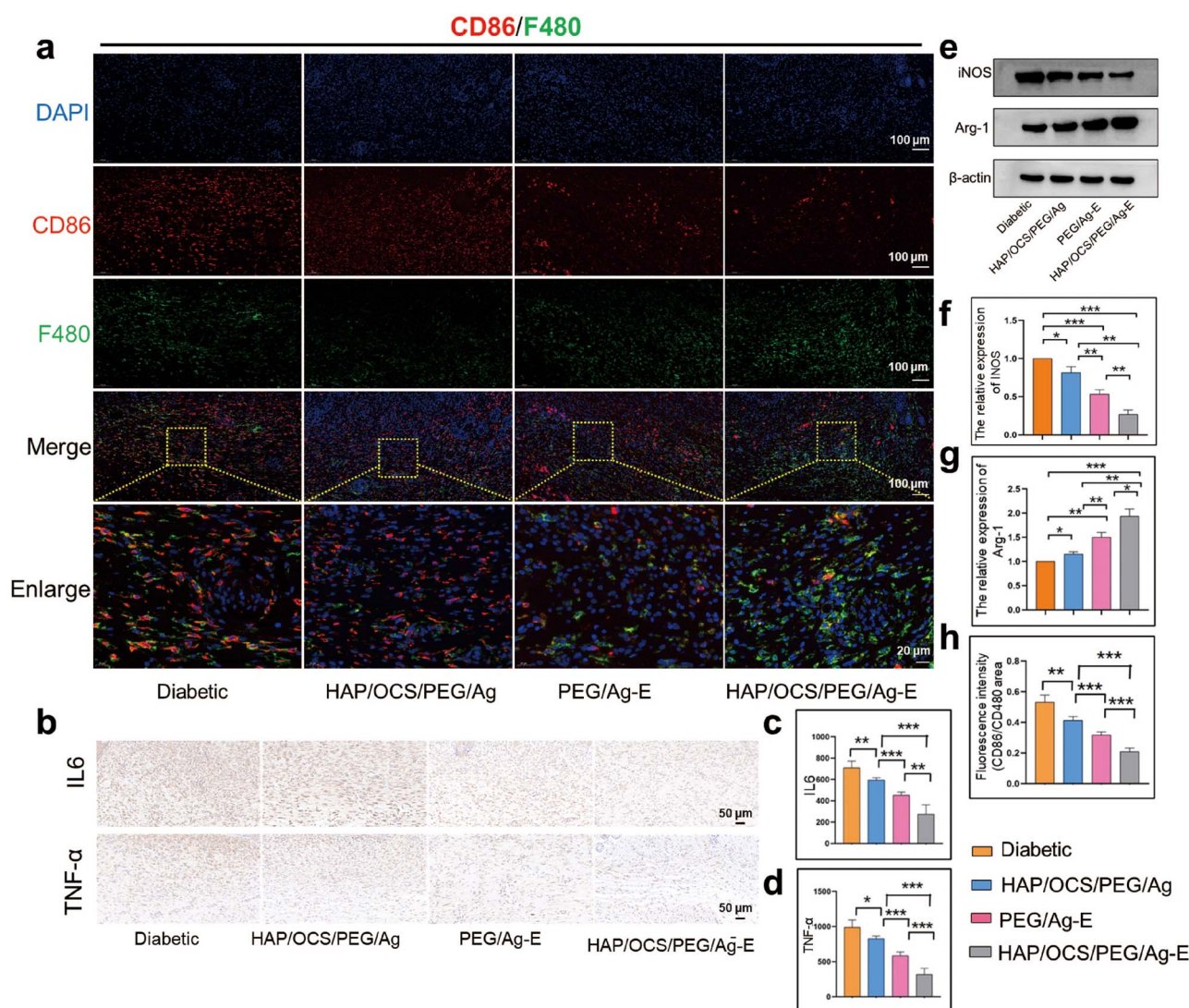


Figure 6. The influence of PEG/Ag-series hydrogels on wound inflammation in diabetic mice. **(a)** Representative IF staining image of CD86/F4/80 cells at 3 days post-injury. Scale bar = 100 μ m; enlarged scale bar = 20 μ m. **(b)** Representative images of immunohistochemical staining of IL-6 and TNF- α on day 3 of wound tissue healing scale bar = 50 μ m. **(c and d)** Statistical analysis showing the relative expression of IL-6 and TNF- α . **(e)** Western blot results showing iNOS and Arg-1 expression in each group. **(f and g)** Quantitative statistical analysis of iNOS and Arg-1 levels. **(h)** Statistical analysis of the relative expression of CD86/F4/80. Data are presented as mean $\pm$ SD and statistical significance was analysed via one-way ANOVA with Tukey's multiple comparison test. *P*-value: **P* < 0.05, ***P* < 0.01, ****P* < 0.001

differentiation, and migration are impaired, thereby hindering local angiogenesis and circulation and impeding ECM synthesis, ultimately resulting in the suboptimal healing of these wounds [13, 14]. This places a heavy burden on families and society. As key components of the innate immune system, macrophages are abnormally activated and mediate inflammation and oxidative stress in diabetic wounds [3]. Studies have confirmed that the number of M1 macrophages in diabetic wounds is significantly increased, and these cells release proinflammatory factors, which modulate angiogenesis and reduce ECM deposition. This abnormal inflammatory environment weakens the ability of M2-polarized macrophages to exert anti-inflammatory effects and promote wound repair. Reduced secretion of anti-inflammatory factors further exacerbates the inflammatory imbalance, ultimately leading to impaired wound healing [4]. Therefore, promoting M2-type polarization and functional recovery of macrophages is critical for the healing of diabetic wounds.

ADSC-exos can promote M2 phenotypic transformation of macrophages in high-glucose environments *in vitro* and reduce inflammatory factor release. However, their therapeutic efficacy upon injection into diabetic wounds remains limited. The reason for this finding is that, on the one hand, prolongation of the healing process limits the function of exosomes with a short half-life; on the other hand, hostile high-glucose/low-pH microenvironments impairing exosome bioactivity, ultimately hindering diabetic wound treatment. In this study, an intelligent dual-responsive exosome delivery system based on a dynamic triple cross-linking network of Schiff bases, phenylboronic esters, and 4-arm PEG-thiol with Ag⁺ was prepared for the treatment of chronic diabetic wounds. This system specifically responds to pathological high-glucose and acidic microenvironments via Schiff base/phenylboronic ester dual-crosslinking, enabling controlled ADSC-exos release under diabetic conditions. Compared to non-responsive hydrogels,

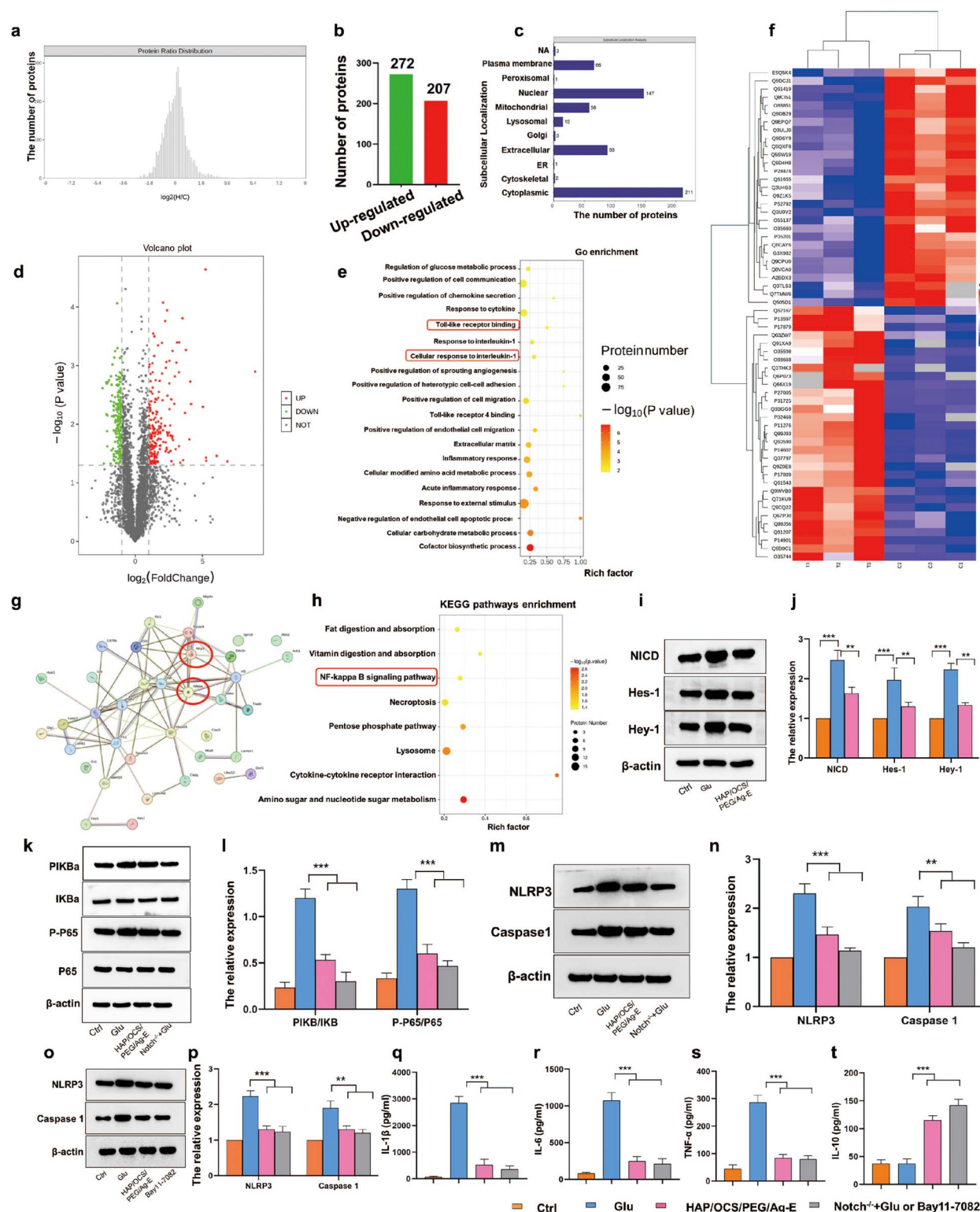


Figure 7. Proteomic analyses of the biological effects of the control and HAP/OCS/PEG/Ag-E hydrogels. The HAP/OCS/PEG/Ag-E hydrogel relieved inflammation by activating the notch/NF- κ B/NLRP3 pathway. (a) The number of differentially expressed proteins and normal distribution of the phenotypes of the samples. (b) The differentially expressed proteins between the two groups were quantified with a histogram. (c) Classification of subcellular proteins in the exosome group. (d) Volcano plot showing the differentially expressed proteins between the two groups (red dots: upregulated; green dots: downregulated). (e) GO annotation showing the different biological roles of exosomes in the hydrogel. (f) Heatmap representation of cluster analysis of the HAP/OCS/PEG/Ag-E hydrogel. (g) The protein-protein interaction results indicated that there was a strong interaction between NF- κ B and NLRP3. (h) KEGG pathway enrichment analysis suggested that differentially expressed proteins were enriched in pathways related to the cellular inflammation process, such as the NF- κ B signaling pathway. (i and j) The hydrogel regulated the expression of proteins related to the notch pathway, further indicating its ability to relieve inflammation through notch pathway activation. (k and l) The relevant proteins of the NF- κ B pathway in each group. (m and n) The levels of the relevant proteins NLRP3 and Caspase-1 in each group. (o and p) Changes in the protein expression of NLRP3 and Caspase-1 in each group following inhibition of the NF- κ B pathway. (q-t) The IL-1 β , IL-6, TNF- α , and IL-10 levels in each group were measured via ELISA. Data are presented as mean $\pm$ SD and statistical significance was analysed via one-way ANOVA with Tukey's multiple comparison test. P -value: * P < 0.05, ** P < 0.01, *** P < 0.001

the microenvironment-responsive hydrogel achieves localized exosome enrichment, thereby exerting enhanced anti-inflammatory effects and accelerating diabetic wound healing.

Concretely speaking: this intelligent dual-loaded hydrogel is constructed based on a dual dynamic crosslinked network formed by HAP and OCS: On one hand, the Schiff base bonds ($-C=N-$) confer pH responsiveness, exhibiting significantly accelerated hydrolysis rates in the acidic microenvironment (pH 5.0–6.5) of diabetic wounds; on the other hand, the phenylboronate ester bonds ($B-O-C$) provide glucose sensitivity, undergoing competitive dissociation when wound glucose concentration exceeds 7 mM. The synergistic action of these dual bonds reduces crosslinking density, triggering hydrogel network hydrolysis and subsequent exosome release. Thereby significantly elevating the local exosome concentration at the wound site to effectively modulate the inflammatory response, accelerate wound healing, and synergistically promote vascularization, collagen remodeling, and inflammation regulation, ultimately improving the healing quality of diabetic wounds.

The importance of the Notch signaling pathway in cell fate determination and development is well established. Studies demonstrate that macrophage-specific Notch signaling blockade improves late-stage wound repair in type II diabetes. Our group previously elucidated Notch signaling's role in burn-induced oxidative stress, sepsis, and inflammation. In this study, we explored the HAP/OCS/PEG/Ag-E hydrogel's regulatory effects on macrophage function at molecular, cellular, and systemic levels, revealing its suppression of Notch signaling-related proteins. GO analysis identified enriched differentially expressed proteins involved in immune defense and inflammatory processes, while KEGG pathway analysis highlighted NF- κ B signaling as a key inflammatory pathway. Protein–protein interaction (PPI) analysis revealed NF- κ B-NLRP3 correlations (Figure 7g). Activation of the NLRP3 inflammasome complex triggers IL-1 β cleavage/release, which amplifies TNF- α , IL-8, and IL-18 production, exacerbating the inflammatory cascade—a hallmark of chronic refractory wounds. Supporting this, NLRP3-deficient mice exhibit reduced M1-associated cytokines (e.g. TNF- α) and elevated M2-associated IL-10. NLRP3 pathway inhibition enhances VEGF, chemokines, and angiogenesis factors, promoting neovascularization and granulation tissue formation. Our findings confirm that the microenvironment-responsive hydrogel accelerates diabetic wound healing via Notch/NF- κ B/NLRP3 axis suppression, alleviating inflammation and stimulating angiogenesis. This system optimizes ADSC-exos' therapeutic potential, offering novel strategies for clinical management of chronic diabetic wounds.

Although this hydrogel demonstrates promising therapeutic potential, its clinical translation still faces challenges in scalable production stability (e.g. dynamic crosslinking quality control) and cost-effectiveness. Integrating 3D printing technology to fabricate customized dressings or employing nanoengineering to optimize material composition may serve as effective strategies to overcome current limitations. Future research could explore: combining microfluidics to precisely regulate exosome loading efficiency; incorporating artificial intelligence predictive models to optimize dynamic crosslinking parameters, and developing biodegradable alternatives to mitigate long-term risks. These directions will advance smart hydrogels toward clinical utility.

Conclusions

We successfully developed a dual-responsive hydrogel delivery system that efficiently responds to the high-glucose and acidic microenvironment of chronic diabetic wounds. The HAP/OCS/PEG/Ag-E hydrogel effectively promotes diabetic wound healing through controlled ADSC-exos release. This hydrogel demonstrates optimized self-healing properties, injectability, and tissue adhesion—critical characteristics for clinical wound management. Notably, the hydrogel exhibits antimicrobial activity and high biocompatibility. Within a diabetic full-thickness cutaneous wound model, the dual-responsive HAP/OCS/PEG/Ag-E hydrogel notably promoted the M1-to-M2 macrophage polarization shift, mitigating tissue inflammation, fostering angiogenesis, and speeding up wound healing. At the mechanistic level, the hydrogel inhibited the release of inflammatory cytokines through the Notch/NF- κ B/NLRP3 signaling pathway—this was confirmed by the decreased expression of pathway-related proteins and the weakened inflammatory cascades.

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Author contributions

Yue Zhang (Data curation [lead], Methodology [lead], Writing—original draft [lead]), Meng Li (Funding acquisition [lead], Project administration [lead], Writing—original draft [equal]), Shijie Song (Data curation [equal], Investigation [lead], Methodology [lead], Writing—original draft [equal]), Fenghui Hei (Conceptualization [equal], Investigation [equal], Methodology [equal]), Shaoyang Ma (Data curation [equal], Formal analysis [equal], Visualization [equal]), Jiao Cao (Data curation [equal], Investigation [equal], Supervision [equal]), Baolin Guo (Data curation [lead], Supervision [lead], Writing—review & editing [lead]), Dahai Hu (Project administration [lead], Writing—review & editing [lead]).

Supplementary data

Supplementary data is available at *Burns & Trauma Journal* online.

Ethics approval and consent to participate

All animal procedures were carried out in accordance with the principles of ARRIVE and approved by the Ethics Committee of Fourth Military Medical University.

Conflicts of interest

The authors declare no competing financial interest.

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Data availability

The authors declare that all the data supporting the findings of this study are available within the article and supplementary information and from the corresponding authors upon request. Source data are provided with this paper.

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