Combination therapy of hydrogel and stem cells for diabetic wound healing

Huang JN et al. Combination therapy for diabetic wound healing

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Abstract
Diabetic wounds (DWs) are a common complication of diabetes mellitus; DWs have a low cure rate and likely recurrence, thus affecting the quality of patients’ lives. As traditional therapy cannot effectively improve DW closure, DW has become a severe clinical medical problem worldwide. Unlike routine wound healing, DW is difficult to heal because of its chronically arrested inflammatory phase. Although mesenchymal stem cells (MSCs) and their secreted cytokines can alleviate oxidative stress and stimulate angiogenesis in wounds, thereby promoting wound healing, the biological activity of MSCs is compromised by direct injection, which hinders their therapeutic effect. Hydrogels form a three-dimensional network that mimics the extracellular matrix, which can provide shelter for stem cells in the inflammatory microenvironment with reactive oxygen species in DW, and maintains the survival and viability of stem cells. This review summarizes the mechanisms and applications of stem cells and hydrogels in treating DW; additionally, it focuses on the different applications of therapy combining hydrogel and stem cells for DW treatment.

Key Words: Combination therapy; Mesenchymal stem cells; Hydrogel; Diabetic wound; Cells delivery; Wound healing

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Core Tip: Diabetic wounds (DWs) are a common diabetes mellitus complication with a low cure rate and likely recurrence. Although stem cell therapy is suitable for DW
healing, simple transplantation methods, such as intravenous, subcutaneous, intramuscular, and local injection, are not conducive to cell survival, thus resulting in compromised efficacy. To improve the outcome of stem cell therapy, researchers have designed different types of hydrogels for stem cell delivery to ensure cell viability and paracrine functions. Herein, we discuss the current roles and applications of hydrogel and stem cell combination therapy for DW treatment.

**INTRODUCTION**

Diabetes mellitus (DM) is a significant global public health burden because of its high incidence and mortality rates\(^1\). In 2019, 1.5 million people died of DM\(^2\). Diabetic wounds (DWs) are one of the most concerning complications of DM and affect up to 25% of diabetic patients\(^3\). In addition to causing patient suffering, DW has a low cure rate and high amputation rate, and thus, it places a long-term burden on society\(^4\).

DW is difficult to heal because its healing process is unlike that of normal wounds. Normal wound healing typically includes three phases: inflammation, proliferation, and remodeling. Various cells, growth factors, and cytokines play important roles in each phase to ensure smooth wound healing progress\(^6\). Owing to the elevated levels of reactive oxygen species (ROS), impaired immune function, and cellular dysfunction in the DW microenvironment, the healing stage stagnates in the inflammatory phase\(^6\). In addition, the peripheral arterial disease leads to a lack of blood perfusion and hypoxia within wounds, thereby increasing ROS release\(^5\). ROS also induces the expression of extracellular matrix (ECM) degradation enzymes that degrade ECM, thus precluding the normal matrix-cell interaction required for wound healing and prolonging the inflammation phase of DW healing\(^5\).

DW healing remains a clinical challenge because of several complications in the DW microenvironment, including oxidative stress, chronic inflammation, and angiogenic dysfunction\(^7\). Current clinical treatments (standard care) involve glycemic control, offloading, debridement, and infection management, which are painful and insufficient for curing DWs\(^6\). Therefore, new approaches for improving DW healing must be
developed. The application of functional hydrogel dressings or scaffolds is a promising advanced therapy[9].

Hydrogels are three-dimensional (3D) networks with high water content and have been intensively studied because they can be functionalized and have good biocompatibility. Several studies have shown that hydrogels provide a moist environment, contribute to cell migration and tissue regeneration, and promote wound healing[10]. Therefore, hydrogels are considered ideal dressings for DW[11]. Furthermore, hydrogels provide antioxidant, antibacterial, proangiogenic, and proliferative functions owing to the sustained release of bioactive agents encapsulated in hydrogels. Stem cells are bioactive agents that promote wound healing and are effective in skin regeneration[12].

Stem cells possess self-renewal and differentiation abilities, and are essential for post-injury skin repair[13]. Thus, stem cell therapy has become a promising new approach for treating DW. Local injection of the cell suspension or stent implantation stimulates neovascularization, accelerates wound closure, prevents wound contracture and scar formation, and ultimately improves wound healing[14]. However, the outcome of stem cell therapy is hindered by the poor bioactivity of stem cells and, thus, the low amounts of secreted cytokines in the hyperglycemic inflammatory microenvironment of DW. Effective stem cell delivery remains a challenge[15].

To achieve better healing outcomes, combining hydrogel and stem cell treatment is one of the most promising therapies for DW[16]. Although various reviews on stem cell therapy or hydrogel therapy for DW have been reported, reviews on combined therapy are limited. Herein, we review the mechanisms of DW therapy combining hydrogel and stem cells, and focus on pre-clinical studies of therapy combining hydrogel and stem cells for DW.

FUNCTIONAL HYDROGELS FOR DW TREATMENT
Wound dressings play an essential role in DW[2]. Hydrogels have become appealing and promising among various wound dressings owing to their high moisture retention,
biocompatibility, and similarities to living tissues. Hydrogels accelerate wound healing by maintaining gas exchange in the wound, reducing pain by absorbing exudates, preventing infection, and maintaining a moist environment for cell migration. In addition, hydrogels have been used as delivery systems to minimize drug toxicity and improve drug delivery efficiency. Functional hydrogels, such as antioxidants, immune regulation, and vascularization hydrogels, have been designed according to the wound microenvironment of DW.

DWs are often accompanied by oxidative and antioxidant imbalance in vivo. Hydrogels are designed to alleviate excessive oxidative reactions. Self-antioxidant materials, such as 2-hydroxyethyl methacrylate and polyvinyl alcohol, can directly act on wounds; additionally, gel-loaded antioxidant drugs, such as curcumin, or bioactive substances can be used to achieve antioxidant effects. These materials act as reducing agents.

Because the inflammatory phase has an active defense response to external stimuli, the inflammatory response aids in cleaning the wound during the healing process. However, in chronic wounds, such as DW, owing to repeated tissue damage, cytokines continue to recruit immune cells to the wound, thereby resulting in an excessive inflammatory response and blocked healing. Therefore, the inhibition of excessive immune responses is also considered. Hydrogels, such as sodium alginate and zwitterionic hydrogels, can provide a protective microenvironment for wounds and regulate the transformation of macrophages between pro-inflammatory and anti-inflammatory. Meanwhile, anti-inflammatory drug-loaded hydrogel dressings have a local sustained-release effect. Therefore, responsive hydrogels that can change their properties according to environmental clues to achieve sustained release of entrapped drugs are also desirable.

Angiogenesis is essential for tissue regeneration, whereas the formation of healthy blood vessels is hindered by various microenvironment conditions in DWs. Therefore, promoting blood vessel formation is conducive to DW healing. Studies have shown that some hydrogel materials, such as chitosan and hyaluronic acid, regulate the
activity and distribution of cytokines or growth factors\textsuperscript{[24]}. These materials simulate the microenvironment of the extracellular matrix, thereby promoting tissue formation. Bioactive components, including the epidermal growth factor (EGF) and vascular EGF (VEGF), can also be encapsulated by hydrogels, which can promote the regeneration of blood vessels\textsuperscript{[25]}.

In general, the mechanism of hydrogels in DW is relatively clear and positively affects DW healing.

**CURRENT STUDIES OF MESenchymAL STEM CELLS FOR DW HEALING**

In addition to selecting different hydrogel materials, drugs, and biological factors, using stem cells to treat DW is desirable. Stem cells can asymmetrically replicate and differentiate into different cell types\textsuperscript{[26]}. With the unlimited replication capacity, they can provide numerous “sister” stem cells\textsuperscript{[15]}. Furthermore, because stem cells secrete pro-regenerative cytokines, stem cell therapy, which treats diseases or injuries by administering stem cells into damaged tissues, has been used as an intervention to DW\textsuperscript{[27]}. Stem cells used for wound healing and tissue regeneration include embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs)\textsuperscript{[15]}.

Allogeneic, xenogeneic, and autologous MSCs have been widely used in skin regeneration and wound healing owing to their significant proliferation, migration ability, and long-term self-renewal potential\textsuperscript{[28]}. Considering the impaired function of MSCs derived from patients with diabetes and the risk of tissue rejection, allogeneic MSCs are more widely used\textsuperscript{[29]}. MSCs that are locally injected into wounds are involved in various stages of wound healing. They reduce inflammatory responses through immunomodulation and growth factor production\textsuperscript{[15]}, accelerate neovascularization and epithelialization, and stimulate collagen synthesis\textsuperscript{[30]}, thereby accelerating wound healing\textsuperscript{[30]}. Additionally, clinical studies have demonstrated the efficacy of MSCs in treating diabetic ulcers\textsuperscript{[30]}. For example, injecting allogeneic MSCs into the dermis-epidermal junction\textsuperscript{[31]} or
subcutaneous and intramuscular tissue around wounds\cite{32} facilitated DW healing in patients.

The potential benefits of MSC therapy have been demonstrated in several studies. Although simple transplantation methods, such as intravenous, subcutaneous, intramuscular, and local injection of MSCs, have achieved some pre-clinical and clinical success\cite{5}, MSC performance still has numerous limitations. Premature senescence and apoptosis of MSCs transplanted in DW are some of the biggest limitations\cite{33,34}. Owing to hyperglycemia caused by DM, DW generates a chronic inflammatory microenvironment and accumulates advanced glycation end products, which is not conducive to the survival of stem cells\cite{35} and increases the degradation of growth factors secreted by the effector cells, thus compromising efficacy\cite{36}. After implantation, the delivery mechanisms must be optimized to ensure cell viability, paracrine function, and differentiation function, which in turn ensures MSC therapy outcomes.

Abundant evidence has shown that using hydrogels to deliver MSCs improves DW healing. Hydrogels are ideal carrier systems for stem cells because they produce a relatively uniform distribution of transplanted cells and retain high-water content, close to that of the native tissue, thus improving the retention and survival of stem cells at transplantation sites. Transplanted stem cells can exert their functions through paracrine signals and differentiate into the various cell types required in healthy tissues (Figure 1).

**APPLICATIONS OF COMBINATION THERAPY OF HYDROGEL AND STEM CELLS FOR DIABETIC WOUND HEALING**

As previously discussed, although stem cell therapy has promising potential for DW healing, the delivery system is one of the biggest obstacles to its therapeutic efficacy. Traditional injection of MSCs always results in low cell viability and transient engraftment, whereas using advanced biomaterial scaffolds (such as films, nanofibers, and hydrogels)\cite{13} to maintain cellular viability, proliferation, and differentiation has received considerable attention\cite{37}. Hydrogels have physical and biological
characteristics similar to those of natural tissues\textsuperscript{[36]}; this renders them as ideal candidates for SCs delivery. Inspired by the encouraging outcomes of hydrogels on DW healing and their function as a carrier system for drugs, the efficacy of MSCs has been improved with hydrogels\textsuperscript{[37]}. For a successful clinical application of the therapy, the optimal hydrogel composition for cell delivery must be considered, and appropriate application methods to ensure stem cell viability and promote DW healing must be designed. Currently, the most common application methods of hydrogels and stem cell combination therapy for DW healing are divided into hydrogel sheets, \textit{in situ} forming hydrogels, and hydrogel microspheres (MS) (Figure 2).

**HYDROGEL SHEETS**

Applying hydrogel sheets on wounds is a convenient stem cell delivery method, wherein hydrogels are typically pre-formed in molds, with stem cells seeded onto or inside hydrogels. Rustad \textit{et al.}\textsuperscript{[38]} seeded MSCs onto collagen-pullulan hydrogels, and significantly accelerated wound healing and skin appendage recovery in mice within 11 d. The amount of microangiogenesis was approximately doubled in wounds treated with MSC-seeded hydrogel sheets compared with those treated with MSCs injection. Given that the biomimetic hydrogel provides a functional niche to augment the regenerative potential of MSCs, the implanted MSCs differentiated into dermal fibroblasts, pericytes, and endothelial cells, which contribute to wound healing\textsuperscript{[38]}. In another study, Guo \textit{et al.}\textsuperscript{[39]} demonstrated the improved retention and survival rate of MSCs in hydrogel sheets when transplanted into mouse hearts compared to cell suspension alone. Cells were observed inside the hydrogel sheets for over 9 d in ICR mice.

\textit{In vitro} culturing of stem cells within hydrogels was found to promote cell adhesion and enhance stem cell functions by supporting normal phenotype maintenance and empowering the transdifferentiation capacity into specific skin lineages compared with the immediate transplantation of stem cell-seeded hydrogels\textsuperscript{[40]}. da Silva \textit{et al.}\textsuperscript{[41]} pre-cultured adipose-derived mesenchymal stem cells (ADSCs) in hyaluronic acid-based
sponge hydrogel in neurogenic/standard media for 14 d before transplantation onto the DW of mice. Wounds treated with pre-cultured ADSCs spongy hydrogels improved wound closure rates compared to the untreated control and acellular spongy hydrogel groups after healing for 4 w. The hydrogel sheet promoted the polarization of M1-type macrophages to the M2 type (anti-inflammatory) and improved successful neoinnervation.

Because of the high concentration of inflammatory cytokines in the DW microenvironment, which impairs the activity of MSCs and degrades growth factors secreted by stem cells, single functional hydrogel sheets may not be sufficient for DW healing. To be more suitable for DW treatment, hydrogel sheets that inhibit inflammatory responses or protease activity are more effective. Ahmed et al. studied the wound healing efficacy of bone marrow-derived MSCs (BMSCs) delivered by nitric oxide (NO)-releasing hydrogels on diabetic rabbits. As an endogenous molecule, NO increased angiogenesis and improved immune responses during acute infections. NO-releasing hydrogels increased the viability and proliferation of BMSCs under oxidative stress. In addition to improving collagen deposition and promoting re-epithelialization and angiogenic activity, the NO-releasing hydrogel with BMSC treatment upregulated the expression of growth and cytoactive factors for DW healing within 16 d.

In addition to traditional manufacturing technology, 3D bioprinting builds special structures layer-by-layer according to a predetermined computer model that better fits the skin’s architecture and geometry, providing hydrogel sheets with more complex structures. Xia et al. developed curcumin-incorporated 3D bioprinting gelatin methacryloyl (GelMA) to seed ADSCs and promote DW healing within 21 d. Curcumin encapsulation in 10% GelMA hydrogel exhibited inhibitory effects on ROS generation and ADSCs apoptosis, and living cells were detected after scaffolds embedded with ADSCs were implanted into the back of nude mice for 21 d. Further, the scaffold increased the amount of collagen deposition and induced angiogenesis in DW. In addition to 3D bioprinting, multifunctional hydrogel sheets with complex 3D structures
can be produced by folding or weaving microfiber-shaped hydrogels\(^ {47}\). Hydrogel sheets can also be easily functionalized, such as the thermally responsive release of stem cells or drugs\(^ {48}\) for oxidative stress resistance, antibacterial activity, and other functions.

Because stem cells can be cultured separately and the hydrogel sheet is easy to handle, combination therapy with hydrogel sheets and MSCs is easily transformed into a clinical setting\(^ {49}\). According to a clinical report, Ravari et al\(^ {50}\) applied BMSCs along with platelets, fibrin glue, and bone marrow-impregnated collagen matrix onto wounds, which resulted in the complete wound closure in three of eight patients with aggressive, refractory DWs within 4 wk of treatment. Additionally, topical administration of placenta-derived mesenchymal stem cells in a sodium alginate hydrogel completely healed diabetic foot ulcers\(^ {51}\). However, this clinical case report must be evaluated further because of the limited sample size of the report. Although functionalizing or changing shapes is very convenient, hydrogel sheets must be pre-formed before application. Because hydrogel sheets are not conducive to long-term storage and the bonding between the sheets and wound surface is limited, \textit{in situ} forming hydrogels have attracted attention.

**IN-SITU FORMING HYDROGEL**

\textit{In situ} forming hydrogels are another mainstream application of combination therapy, with stem cells suspended in the precursor solution before application\(^ {52}\). After the mixed precursor solution is injected into the wound site, the hydrogel-containing stem cells are formed \textit{in situ} on wound beds \textit{via} chemical bonds\(^ {53}\). Compared with hydrogel sheets, injectable hydrogels are more flexible in their application; this flexibility allows them to adapt to complex-shaped wounds and fit closely\(^ {54}\). Eke et al\(^ {55}\) designed a precursor solution composed of methacylated gelatin (GelMA) and methacylated hyaluronic acid (HAMA) containing ADSCs, which can be crosslinked within 40 s of ultraviolet (UV) irradiation to form hydrogels \textit{in situ}. Reportedly, the hydrogel promoted cell proliferation, and \textit{in vivo} studies revealed a three-fold increase in
vascularization for the ADSC-loaded hydrogel group compared to the hydrogels without cells.

However, because UV irradiation may induce chromosomal and genetic instability\[^{56}\], UV-crosslinked hydrogels on exposed wounds negatively affect cell viability and differentiation\[^{57}\], which is detrimental to wound healing. Owing to its high biocompatibility and specificity\[^{58}\], enzymatic crosslinking has received considerable attention\[^{59}\]. Yao \textit{et al}\[^{52}\] developed a gelatin-hydroxyphenyl (GH) hydrogel with the dual enzyme crosslinking of horseradish peroxidase (HRP) and galactose oxidase (GalOx), and the hydrogel encapsulated with BMSCs achieved gelation within 5 min at the wound site. The GH hydrogel provides a friendly 3D microenvironment for BMSCs, thereby improving the transplanted cells' survival and accelerating wound closure\[^{52}\].

Given that frequently studied natural hydrogels, such as gelatin, collagen, or hyaluronic acid, contain a single component of ECM, their potential to provide the optimum microenvironment for stem cell proliferation and differentiation is limited\[^{60}\]. ECM maintains the original components of the native tissue and is considered an ideal scaffold for tissue regeneration\[^{61}\]. Chen \textit{et al}\[^{62}\] developed an ECM-derived hydrogel from a human decellularized adipose tissue matrix (HDAM) to deliver ADSCs to DW. The hydrogel was prepared \textit{via} pepsin digestion and pH neutralization. The paracrine activity of ADSCs encapsulated in the hydrogel was enhanced, whereas the secretion of the hepatocyte growth factor increased, thus promoting neovascularization during wound healing\[^{62}\]. Compared with the untreated control, local ADSC injection, and acellular hydrogel groups, treatment with ADSC-hydrogel composites accelerated wound closure in diabetic mice and restored cutaneous appendages within 14 d\[^{62}\].

For better DW healing outcomes, specific materials are co-entrapped inside the hydrogel for hemostasis and anti-inflammatory properties, and the stem cell viability in the hydrogel can reach an ideal state by optimizing its mechanical strength. Xu \textit{et al}\[^{63}\] encapsulated MSCs in an injectable hydrogel system of GelMA and catechol-modified chitosan (Chi-C) cross-linked with dithiothreitol (DTT) to repair full-thickness DW. Chi-C has a good hemostatic effect, and zinc ions were introduced into the hydrogel to
enhance angiogenesis. The cell adhesion, proliferation, and differentiation potency of umbilical cord-derived mesenchymal stem cells (UMSCs) in vitro were well maintained in GelMA with optimal stiffness. At the same time, the hydrogel-UMSC combined treatment promoted DW healing by inhibiting the inflammatory factors TNF-α and IL-1β in vivo, with a wound closure rate of 92.2% within 14 d. Compared with the untreated control, local UMSCs injection, and acellular hydrogel groups, collagen deposition was significantly abundant on day 7, whereas the most vascular regeneration with the earliest hair follicle formation was found on day 14[63].

Dispersive MSCs are usually loaded inside hydrogels. Recently, 3D MSCs spheroids were found to possess better differentiation potential than dispersive MSCs[64], which exhibited enhanced vascularization and anti-inflammatory effects[65], thereby promoting wound closure[66]. Yang et al[67] combined injectable thermosensitive chitosan/collagen/β-glycerophosphate (β-GP) hydrogels with 3D MSC spheroids, rapidly converted to a gel by physical cross-linking at body temperature, and then completely covered the wound surface and fitted to any shape of the wound bed. Compared with the local 2D monolayer MSC injection and 2D monolayer MSC-encapsulated hydrogel groups, angiogenic factors were much higher for wounds treated with 3D MSC spheroid-encapsulated hydrogel (almost 3-fold), and neovascularization was enhanced, thereby achieving complete re-epithelialization within 3 wk of implantation[67].

Although in situ forming hydrogels adapt to complex-shaped wounds and fit tightly, thus enabling flexible use at the wound bed, the bulk hydrogel formed at the wound site produces poor tissue infiltration and, thus, low stem cell survival. Compared with in situ forming hydrogels, hydrogel microspheres have a larger specific surface area and more specific functions, thus playing an essential role in the medical field.

HYDROGEL MICROSPHERES

Hydrogel microspheres exhibit good dispersion and stability in physiological environments with a high drug-loading capacity[68]. Their drug-carrying[69] and
bioactive factors\cite{70} are highly effective in DW treatment. We previously demonstrated that antibiotic and growth factor separately loaded alginate/CaCO\textsubscript{3} microspheres prepared using microfluidic technology sustainably released drugs and exhibited pH sensitivity. These microspheres were embedded in the regenerated tissue and functioned as scaffold materials. They improved wound healing with thicker granulation tissue and stimulated angiogenesis, ideally meeting the requirements of different stages of wound healing\cite{71}. Lei et al\cite{70} developed biohybrid agarose microspheres conjugated with a basic fibroblast growth factor (bFGF), which achieved local growth factor delivery, stimulated angiogenesis, and enhanced wound healing in diabetic mice.

Inspired by drug delivery microspheres, the special geometry of hydrogel microspheres is conducive to the diffusion of nutrients and wastes\cite{72}. Microspheres that deliver stem cells can release stem cells, thereby promoting proliferation and differentiation and enhancing the formation of integrated functional tissues\cite{73}. Stem cell-loaded microspheres have been applied in various tissue systems, including cartilage\cite{74}, bone\cite{75}, bone marrow\cite{72}, and brain tissue systems\cite{76}. Intracerebral implantation of stem cells using microspheres in the rat brain improves stroke treatment\cite{76}, Mao et al\cite{72} demonstrated that microgel encapsulation sustained MSC survival after intravenous injection in mice and enhanced the immunoregulatory capacity of MSCs in a bone marrow transplantation model.

Considering that our previous study demonstrated that hydrogel microspheres act as scaffolds and gradually integrate into regenerated skin tissue, we designed gelatin microspheres encapsulated with ADSCs from rats (rADSC/MS) with an ideal mechanical strength and degradation rate that matches tissue regeneration to improve DW healing\cite{77}. Gelatin microspheres promoted the adhesion and proliferation of fibroblast cells and maintained the viability of encapsulated rADSCs. Slowly released exosomes from rADSCs were eventually internalized by HUVECs, which suggested a potential exosome mechanism for improving wound healing. The implanted rADSC/MS gradually integrated into the regenerated skin tissue, thus facilitating the
arrangement of neat collagen fibers. Compared with the untreated group and the MS group, rADSCs embedded in rADSC/MS promoted M2 macrophage polarization and recovery of peripheral nerves, formed larger blood vessels, and eventually generated a dermis close to normal tissue within 14 d (Figure 3)[77].

Previous studies have demonstrated that hydrogels provide a functional niche for MSCs, which enhances MSCs regeneration potential and promotes wound healing. Pre-clinical studies on the combined treatment of DWs with hydrogels and stem cells are summarized in Table 1.

**CONCLUSION**

This review discussed the benefits associated with therapy combining hydrogels and MSCs for DW healing. Researchers have explored different application methods for stem cell delivery with hydrogels, including hydrogel sheets, in situ forming hydrogels, and hydrogel microspheres. In addition to providing a friendly microenvironment for stem cells, this strategy enhances the adhesion between the dressing and wound, and facilitates the function of stem cells, ultimately benefiting vascular and neural regeneration in DW. Furthermore, hydrogel microspheres have the advantages of a larger specific surface area, more uniform dispersibility, and more specific functions; additionally, they can effectively deliver various types and functions of cells into the wound. Therefore, hydrogel microspheres loaded with stem cells are expected to play an important role in clinical practice.

Therapy combining hydrogels and MSCs has shown great potential for DW healing. However, the plasticity of MSCs has led to their double-sidedness for clinical applications. Although the multi-differentiation ability provides them with good application prospects, it increases the risk of tumorigenicity[78]. As a solution, cell-free treatments, such as exosomes and artificial cell products derived from the MSCs secretome, have attracted recent interest. Exosomes and secretomes retain the paracrine factors of stem cells[7]. Although extensive studies have explored the combination therapies of hydrogels and MSCs for DW healing, additional work is required to
optimize parameters, such as the storage and transport stability of cells, and avoid their tumorigenic and immunogenic risks. Further improvement and testing of this technology in vivo will also contribute to the clinical transformation of combination therapy.

**Figure 1** Therapy combining hydrogels and mesenchymal stem cells promotes diabetic wound healing. Mesenchymal stem cells (MSCs) in hydrogels are long-lastingly present in the wound and regulate wound healing. These cells release exosomes, growth factors and cytokines, reduce the levels of interleukin-1 and tumor necrosis factor-α and other pro-inflammatory cytokines to modulate the inflammatory response, enhance angiogenesis via increasing vascular endothelial growth factor and hepatocyte growth factor, and promote fibroblast and keratinocyte migration. MSCs can also be transdifferentiated into other cell types to increase wound closure. MSCs: Mesenchymal stromal cells; IL-1: Interleukin-1; TNF-α: Tumor necrosis factor-α; VEGF: Vascular endothelial growth factor; HGF: Hepatocyte growth factor.

**Figure 2** Three application methods of hydrogels and mesenchymal stromal cells combination therapy for diabetic wound healing. A: Hydrogel sheets pre-formed before application; B: In situ forming hydrogels injected at the wound for sol-gel transition; C: Hydrogel microspheres applied onto the diabetic wound. MSCs: Mesenchymal stem cells.

**Figure 3** Representative Masson’s Trichrome-stained wound sections for blank, gelatin microspheres, and gelatin microspheres encapsulated with ADSCs from rats (rADSC/MS) groups on days 3 and 14. “M” represents gelatin microspheres.
Table 1 Summary of studies regarding therapy combining hydrogels and stem cells for diabetic wound healing

| Stem cells information (types, dosage) | Hydrogel composition | Hydrogel types | Application methods | Animal | Wound size (diameter), efficiency location | Full re-epithelialization | Outcome |
|----------------------------------------|----------------------|----------------|---------------------|--------|--------------------------------------------|--------------------------|---------|
| Umscs from human, xenogeneic, 1 × 10⁶ | Self-assembled nanoparticle | Self-assembled nanoparticle hydrogels with easy biomimetic functionalization | Cells were encapsulated into the in-situ injectable hydrogels | NOD/SCID mice dorsal | 8 mm, 10 d | Accelerated wound healing inhibiting inflammation promoting angiogenesis |
| βmscs from N-carboxyethyl chitosan (N-allogenic, 2 × 10⁵ chitosan)/hyaluronic acid-aldehyde (HA-ALD) hydrogel | Hemostasis and antimicrobial hydrogels | Cells were encapsulated into the in-situ injectable hydrogels | STZ induced diabetic rats | 5 mm, foot 12 d | Promoted healing; secretion growth factor rbmscs modulated inflammatory environment inhibiting |

15 / 22
Adscs from Gellan gum- Vascularization
human, hyaluronic acid (GG- hydrogels
xenogeneic, 3 HA) spongy
× 10^3 hydrogels

Cells were STZ induced 9 mm, 4 wk
seeded onto diabetic mice dorsal
the top of
spongy-like
hydrogel
sheets

Accelerated skin wound
induced th
phase switch
inflammatory
proliferative
presented
epidermis w
number
Bmcs from SNAP-loaded rabbits, chitosan-PVA allogenic, 1 x hydrogel

Vascularization of hydrogels

Cells were Alloxan 20 mm, 14 d intra-monohydrate dorsal dermally induced injected and diabetic topically rabbits covered with hydrogel sheets

Augmented wound decreased inflammation expression expression VEGF and promoted angiogenesis forming capillaries improving proliferative keratinocyte basal layer; the num intraepidermal fibers is regenerated epidermis
| Adscs from | Curcumin- | Antioxidant | Cells were STZ induced 15 mm, 21 d | Promoted microvascular healing; | microvascular vessel remodeling. | microvascular remodeling. |
|-----------|-----------|-------------|----------------------------------|-------------------------------|-------------------------------|--------------------------|
| human, xenogeneic, $5 \times 10^5$ | incorporated 3D hydrogels | gelatin | encapsulated diabetic nude dorsal into mice | hadscs apoptosis | increased thickness of collagen sheets | skin appendages regeneration |

| Adscs from | Human | Intact ECM-derived | Cells were KK/Upj-Ay/J 8 mm, 14 d | Accelerated wound closure and skin regeneration | skin appendages restoration |
|-----------|-------|-------------------|----------------------------------|---------------------------------------------|--------------------------|
| human, xenogeneic, $2.5 \times 10^5$ | adipose tissue matrix | (hdam) hydrogel | living tissues | injectable hydrogels | cutaneous appendages |
Umscs from Gelatin methacrylate Vascularization Cells were Diabetic mice 8 mm, 14 d human, (gelma) and chitosan- hydrogels mixed with (db/db) dorsal xenogeneic, 5 catechol (Chi-C) the in-situ injectable hydrogels

Promoted the healing process inhibiting expression of IL-1β to inflammation. Accelerated angiogenesis, epithelialization, promoted deposition, induced repair of skin appendages such as hair.
Pdscs from Chitosan/collagen/β-
human, glycerophosphate (β-
xenogeneic, 1 GP) hydrogel and ph-
responsive hydrogels × 10^6

Thermosensitive 3D spheroids were encapsulated in the in-situ injectable hydrogels

Diabetic mice 7 mm, 3 wk dorsal

Accelerated closure by angiogenesis paracrine effect of hydrogel environment for the accelerated proliferation paracrine secretion.

Adscs from Gelatin hydrogel rats, allogenic, 5 × 10^6

Adaptive Injectable STZ induced 8 mm, 14 d hydrogel microspheres microspheres with degradation rates well-matched to

diabetic rats dorsal

M2 m polarization deposition, angiogenesis
| Cells | Source | Adipose-, Diacrylate Hydrogel | Adipose-, (PEG)-Gelatin Hydrogel |
|-------|--------|-----------------------------|---------------------------------|
| Cells | Genetically Encapsulated | Diabetic Mice 6 mm, 15 d | Mixed with (db/db) Dorsal |
| Adipose cell from Polyethylene glycol human, (PEG)-gelatin-based hydrogels | Vascularization | | |
xenogeneic, 3 hydrogel $\times 10^3$

the *in-situ*

injectable hydrogels
closure; encapsulated attached and well inst hydrogel, improved cell retention, reduced inflammatory infiltration, enhanced neovascularization.

\(^1\)Wound size (side length × side length).

UMSCs: Umbilical cord-derived *mesenchymal stem cells*; BMSCs: Bone marrow-derived *mesenchymal stem cells*; ADSCs: Adipose-derived *mesenchymal stem cells*; PDSCs: Placenta-derived *mesenchymal stem cells*. 
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