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Hydrogel-Based Strategies to Advance Therapies for Chronic Skin Wounds

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Abstract

Chronic skin wounds are the leading cause of nontraumatic foot amputations worldwide and present a significant risk of morbidity and mortality due to the lack of efficient therapies. The intrinsic characteristics of hydrogels allow them to benefit cutaneous healing essentially by supporting a moist environment. This property has long been explored in wound management to aid in autolytic debridement. However, chronic wounds require additional therapeutic features that can be provided by a combination of hydrogels with biochemical mediators or cells, promoting faster and better healing. We survey hydrogel-based approaches with potential to improve the healing of chronic wounds by reviewing their effects as observed in preclinical models. Topics covered include strategies to ablate infection and resolve inflammation, the delivery of bioactive agents to accelerate healing, and tissue engineering approaches for skin regeneration. The article concludes by considering the relevance of treating chronic skin wounds using hydrogel-based strategies.

Contents

1. INTRODUCTION

Chronic skin wounds are a critical problem that is reaching epidemic proportions; they are estimated to affect 20–60 million people worldwide by 2026 (1). Unlike acute wounds, which heal after a certain period of time, chronic skin wounds heal slowly (in 8 weeks or more) or not at all. Chronic wounds can lead to long-term hospitalization, which entails a high burden on the health care system due to medical costs associated with wound care products, surgery, and physician and nursing resources. Medical assistance does not prevent serious complications such as foot amputation, morbidity, and mortality, as no efficient therapies have been developed. In fact, the 5-year mortality rate of chronic skin wounds is comparable to or worse than that of some common types of cancer, including prostate, breast, and colon cancers (2).

The etiology of nonhealing chronic skin wounds is variable, and although not completely understood, it has allowed classification into ulcers of pressure and of venous/arterial or diabetic origin (3). Venous and pressure ulcers commonly affect elderly people, and their prevalence is due to an increase in the elderly population, who are likely to develop chronic venous disease and venous hypertension (venous ulcers) or to be bedridden (pressure ulcers) (3). Diabetes and associated comorbidities that result from hyperglycemia, such as obesity, peripheral vascular disease, atherosclerotic disease, and peripheral neuropathy, have been associated with diabetic foot ulcerations and arterial ulcers (3). A detailed investigation of the pathophysiology of chronic skin wounds reveals that different cellular and molecular mechanisms are impaired in wound healing (**Figure 1**). Wound healing should occur following a coordinated sequence of phases (hemostasis, inflammation, proliferation, and remodeling), but in chronic skin wounds the process halts at the inflammatory phase. Patients with vascular impairment have less blood flow to the site of injury, resulting in an impaired immune response and great vulnerability to infection (4–6). Once infected, the wound microenvironment becomes proinflammatory due to the high levels of mediators secreted by the recruited immune cells (neutrophils and macrophages) (7, 8). Nevertheless, the inability of the immune cells to eradicate infection contributes to a persistent state of inflammation that is believed to be what prevents wound healing from progressing to the proliferative phase (7, 8). Other factors are also known to contribute to the poor healing of chronic wounds. Blood circulation is destabilized in chronic skin wounds not only because of inadequate blood supply to pressure and venous/arterial ulcers but also because of impaired angiogenesis arising from the decrease in angiogenic molecules (2, 9). Re-epithelialization is weakened, possibly because of the decreased proliferation and migration of keratinocytes (10). The integrity of the extracellular matrix (ECM) is likewise affected by excessive degradation associated to the unbalanced ratio of metalloproteinases (MMPs) and tissue inhibitor metalloproteinases (TIMPs) (11). Neuropathy in patients with diabetes also affects the release of neuropeptides and neurotrophic factors known to regulate important mechanisms in wound healing (12).

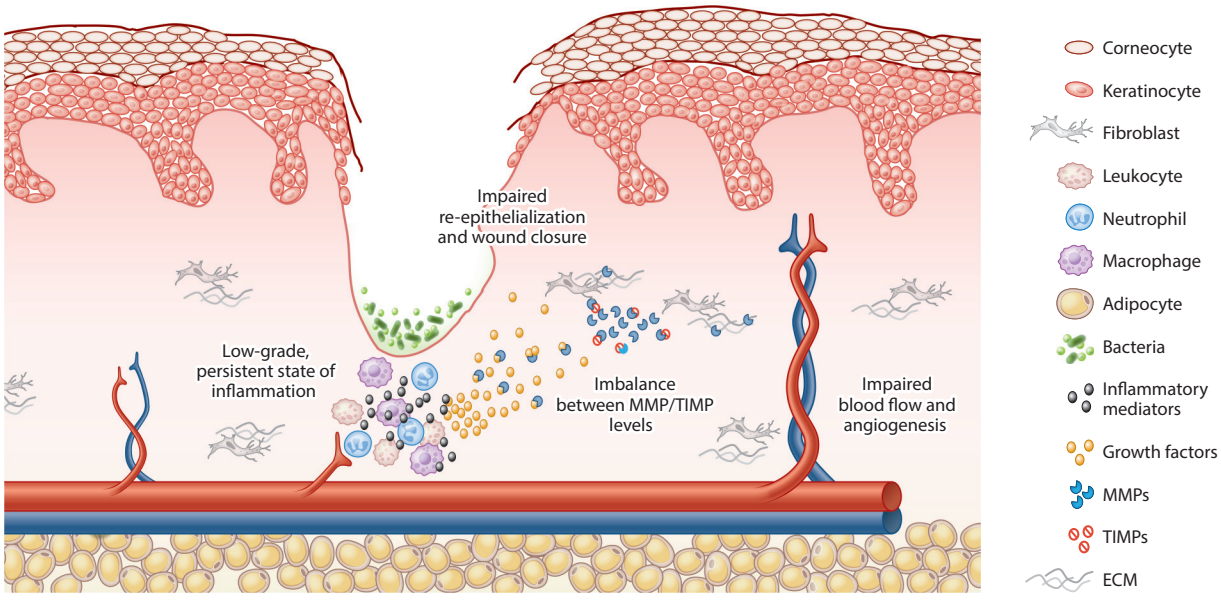

Figure 1

Pathophysiology of chronic skin wounds. A breach in the skin creates susceptibility to incidental microorganism colonization. The wound environment, characterized by necrotic skin tissue and superficial debris, becomes prone to biofilm formation, resulting in infection. Neutrophils and macrophages are recruited to the wound site, releasing different chemical mediators that are not sufficient to eradicate infection. Inflammation persists in the wound to fight the infection, leading to a state of chronic inflammation that—in combination with other factors consistent with impaired re-epithelialization, reduced blood flow and angiogenesis, and imbalanced levels of MMPs and TIMPs—hinders the progression of healing from the inflammatory to the proliferative phase. Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of MMP.

Traditional treatments for chronic skin wounds are based on daily wound management: debridement to remove necrotic and/or infected tissue, off-loading to release pressure in the wound, and application of skin dressings to absorb wound exudate or to provide a moist environment (13). Advanced treatments include topical administration of growth factors (e.g., Regranex® gel; Smith & Nephew, London, UK), microbicidal skin dressings (e.g., Aquacel® Ag; ConvaTec, Reading, UK), ECM derivative–based skin substitutes (e.g., Integra®; Integra LifeSciences, Plainsboro, NJ), and growth factor– or cell-based skin tissue–engineered substitutes (e.g., Graftskin®/Apligraf®; Organogenesis, Canton, MA) (14, 15). However, the overall successful outcome that has been reported is accelerated wound closure, rather than skin regeneration (16). Fast wound closure is critical for any type of wound, especially for nonhealing chronic wounds, but the complex problems associated with impaired healing cannot be dissociated from re-epithelialization. Often, re-epithelialization is achieved, but the dermis and surrounding tissues are still wounded leading to very high recurrence rates (17). This may be because the mechanisms that aid the healing of chronic skin wounds are not fully understood. Therefore, advances in the field of biomaterials and tissue engineering (TE) may lead to improved therapies for debilitating chronic skin wounds.

In this article, we review hydrogel-based approaches that are under investigation for the treatment of chronic skin wounds, followed by the rationales for targeting infection and inflammation. We discuss considerations regarding the quality of healing and ultimately skin regeneration

achieved with bioactive agent–releasing systems and TE strategies. We present challenges and opportunities and offer concluding remarks about the usefulness and limitations of the approaches under development for the treatment of chronic skin wounds.

2. HYDROGEL-BASED SYSTEMS

A 1962 study by Winter (18) gave rise to one of the main principles of wound management, namely that a moist wound environment is necessary for faster and better healing (setting aside assumptions such as enhanced microbial proliferation in moist wounds). Since then, hydrocolloids, gels, and hydrogels have been used in wound management to provide a moist environment and aid in debridement (14). A hydrocolloid is defined as a colloid system composed of hydrophilic polymers (colloid particles) that can form viscous dispersions and/or gels in water. The colloid particles can move freely in solution; at concentrations below a critical polymer concentration they show Newtonian behavior, whereas above the critical polymer concentration the polymer coils overlap and interpenetrate, leading to an increase in viscosity and non-Newtonian behavior (19). A gel is defined as a soft, solid, or solid-like material with two or more components, one of which is a liquid found in high quantities. Solid-like gels do not have an equilibrium modulus; in other words, the storage modulus exhibits a pronounced plateau on the order of seconds, and the loss modulus is considerably lower than the storage modulus in the plateau region (20). Hydrogels are composed of hydrophilic polymer chains that absorb large quantities of water (more than thousands of times their dry weight) without dissolving owing to the presence of cross-links (21). Gels and hydrogels provide additional moisture to wounds, as the hydration mediated by hydrocolloid dressings depends on the absorption of fluids from the wound (22). A significant advantage of hydrogels over gels is their stability due to the cross-linked polymeric network, which facilitates handling during wound management. By contrast, the loose structure of gels does not allow their complete removal from the wound, which may lead to wound infection.

In relation to other systems, hydrogels offer great advantages such as the incorporation of bioactive agents and/or cells due to mild processing conditions. The incorporated bioactive molecules can be then delivered in a more prolonged mode, which represents a great advantage in relation to their topical administration (23). Depending on the ultimate goal, hydrogel properties (composition, sensitivity to wound stimuli, etc.) can be tailored to deliver specific mediators aiming to ablate infection (antiseptics, antibiotics) and resolve inflammation (anti-inflammatories and antioxidants), critical issues in chronic wounds. Moreover, hydrogels can be used to deliver bioactive molecules known to accelerate healing, or to support and maximize the therapeutic potential of skin or stem cells promoting an increase in vessel density and re-epithelialization as well as new ECM production and maturation, ultimately aiming to achieve full skin regeneration (**Figure 2**).

2.1. Targeting Infection and Inflammation

The first step of wound management involves debriding the wound to remove necrotic and/or infected tissue. At this stage, it is important to avoid incidental bacterial contamination and biofilm formation to limit infection and at the same time reduce the persistent inflammation characteristic of chronic skin wounds.

The incorporation of antiseptics in hydrogels has been proposed as a way to avoid contamination and colonization with incidental microorganisms during wound management. Silver nanoparticles, despite their toxicity, are the most common antiseptic used for this purpose (24–26). Alternative antiseptics, such as povidone iodine (27), iodine (28), cefazolin (29), chlorhexidine (30), polyhexamethylene biguanide (31), octenidine (32), zinc oxide (33), hydrogen peroxide (34), and

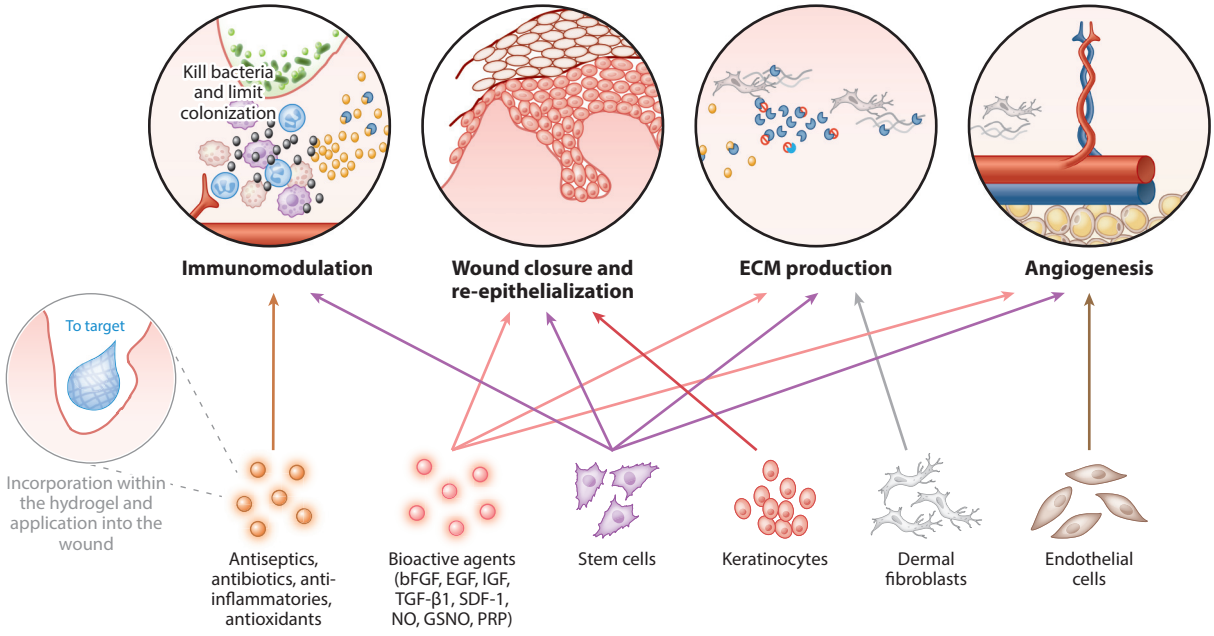

Figure 2

Hydrogel-based strategies for the treatment of chronic skin wounds. The rationale of these strategies involves the incorporation of specific mediators to ablate infection (antiseptics, antibiotics) and resolve inflammation (anti-inflammatories, antioxidants, and stem cells), to accelerate healing (bioactive molecules and cells), and to promote skin regeneration (bioactive molecules and cells). Abbreviations: bFGF, basic fibroblast growth factor; ECM, extracellular matrix; EGF, epithelial growth factor; GSNO, nitrosoglutathione; IGF, insulin-like growth factor; NO, nitric oxide; PRP, platelet-rich plasma; SDF, stromal cell–derived factor.

antimicrobial peptides (35), have been explored. More sophisticated systems involving the incorporation of liposomes or nanocapsules in hydrogels have been employed in order to ensure more prolonged and sustained release of antiseptics as well as improved tolerance. For example, Repithel® (Mundifarma, Basel, Switzerland), composed of a liposomal hydrogel containing 3% povidone iodine, has demonstrated more targeted release and greater efficacy at lower concentrations in comparison with conventional povidone iodine–releasing products (36).

Hydrogels containing antibiotics, including vancomycin (37), tetracycline (38), ciprofloxacin (39), and gentamicin (40), may be used if antiseptics are not sufficient to treat severely infected wounds. This approach enables localized delivery of the antibiotic at the wound site so as to overcome the drawbacks of a systemic delivery such as secondary undesirable responses due to high dosages. A significant challenge of antibiotic-containing hydrogels involves modulating the release of the antibiotic in order to maximize its efficacy within safe dosage limits. Cyclodextrins (41) or microspheres (42) have been proposed to promote more sustained release of antibiotics; an exciting possibility involves the use of stimuli-responsive hydrogels that can release antibiotics upon a stimulus such as high levels of thrombin or bacteria-producing β-lactamase (43, 44). Nonetheless, the risk of developing resistance to antibiotics remains a matter of debate among clinicians. This risk, together with the need to match the antibiotic with the type of colonizing microorganism, has hindered development and the market availability of antibiotic–hydrogel systems.

Approaches that do not involve the incorporation of antiseptics or antibiotics into hydrogels, thereby circumventing adverse effects or resistance, rely on hydrogels' intrinsic properties provided by chemical moieties with antimicrobial activity incorporated into the polymer backbone.

For example, quaternary ammonium groups are cationic surfactants that interfere with the anionic phospholipid membrane of bacteria due to electrostatic interactions that result in cell lysis (45, 46). Although such approaches seem promising, whether they are sufficient to eliminate chronic wound infections remains to be demonstrated.

Although the most prominent property of hydrogels is their high water uptake capability, some hydrogels also affect immune cell recruitment, phenotype, and activation (47–49), which is a useful property in targeting the persistent inflammatory environment in chronic skin wounds. A pullulan–collagen hydrogel enhanced recruitment of neutrophils and T cells and decreased recruitment of macrophages in excisional wounds in mice (47). Similarly, reduction of proinflammatory M1 macrophages was observed in full-thickness wounds in diabetic mice treated with *N*-isopropylacrylamide hydrogels (**Figure 3**) (48). In another study, a hydrogel

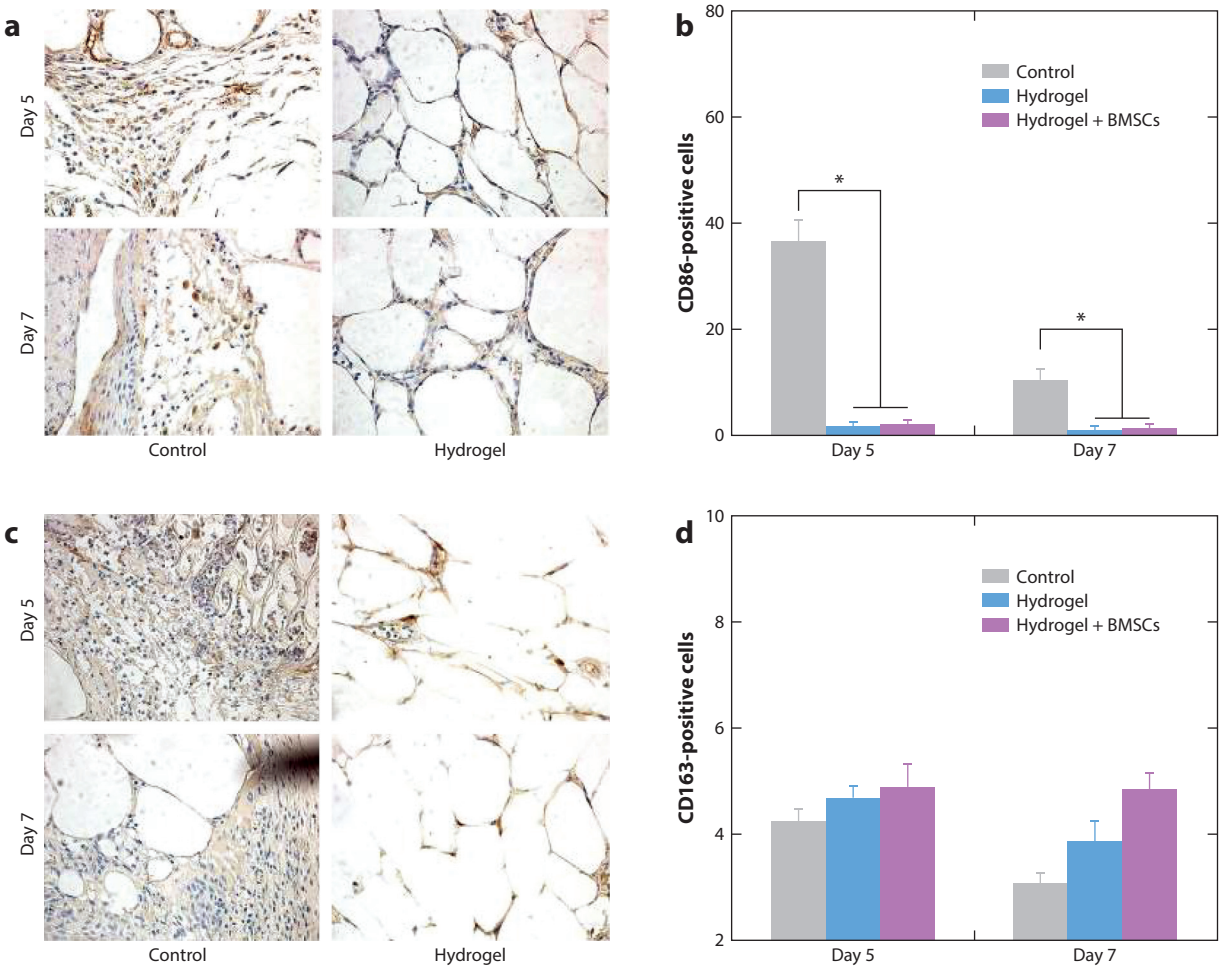

Figure 3

Anti-inflammatory effect of *N*-isopropylacrylamide hydrogel in diabetic murine wounds. Immunohistochemical detection of (*a*) CD86- and (*c*) CD163-positive cells at wound sites on days 5 and 7 in control and hydrogel groups, respectively. (*b*,*d*) Quantification ($n = 5$, $^*p < 0.05$). Abbreviation: BMSC, bone marrow stromal cell. Adapted from Reference 48 with permission. The images are available for reuse under a Creative Commons Attribution 4.0 International License (CC-BY 4.0).

consisting of star-shaped poly(ethylene glycol) (PEG) and glycosaminoglycan (GAG) derivatives reduced inflammation in wounds in diabetic mice by sequestering inflammatory chemokines such as monocyte chemoattractant protein 1, interleukin-8, macrophage inflammatory protein (MIP)-1α, and MIP-1β (49). Despite these findings, the anti-inflammatory properties attributed to hydrogels arise mainly from additives, such as the macrophage recruitment agent sphingosine-1-phosphate agonist (SEW2871) (50), phenolic plant extracts (51–54), honey (55), or antimicrobial peptides (56, 57). While the incorporation of SEW2871 into hydrogels promotes the preferential recruitment of *M2* macrophages to the wound site (50), other additives reduce oxidative stress mainly through the scavenging of reactive oxygen species (51–57). Despite the significant increase in superoxide dismutase (SOD) and catalase levels observed in diabetic rat wounds treated with gellan gum–chitosan hydrogels that release a phenolic plant extract (54), the exact anti-inflammatory and immunomodulatory mechanisms of most of the additives incorporated in hydrogels have not been fully elucidated. Moreover, although some hydrogels are known to affect the inflammatory environment of skin wounds, clinical outcomes in chronic wounds are not as striking as expected. This is probably because there exist additional, key factors in these wounds beyond what is currently known.

2.2. Delivery of Bioactive Agents to Foster Repair

Skin wound healing is a complex and intricate process involving myriad cells and chemical mediators with autocrine, paracrine, and endocrine functions. While the signaling cascade during acute wound healing is fairly well known, chronic wounds are known to have unbalanced levels of many of these mediators, which has motivated the delivery of bioactive agents as a strategy to overcome the stalled inflammatory process. Regranex, which combines a gel (not a hydrogel) with platelet-derived growth factor (PDGF), is the first and only bioactive agent–releasing system to be approved by the US Food and Drug Administration. This gel reduces the healing time of diabetic neuropathic ulcers by nearly 6 weeks compared with placebo by stimulating fibroblast proliferation and increasing granulation tissue formation (58).

Additional strategies combining other growth factors or cytokines at various dosages within different hydrogels have been proposed (**Table 1**). GAG hydrogels have been used for this purpose, due to their natural ability to bind tissue/ECM biochemical mediators (59–64). This strategy enables one to combine the properties of the GAGs with their ability to sequester and bind growth factors, which are then gradually released in situ while the hydrogel degrades. Growth factors that have been incorporated in hydrogels include fibroblast growth factor (FGF), epithelial growth factor (EGF), keratinocyte growth factor, insulin-like growth factor, stromal cell–derived factor 1, vascular endothelial growth factor (VEGF), and PDGF (59–62, 65–76). Given the role of these factors in acute skin wound healing, it is not surprising that results reported from studies focused on accelerated wound closure, enhanced re-epithelialization, increased ECM deposition, and angiogenesis/neovascularization. Some authors have suggested that enhanced healing is mediated by the effect of growth factors on cell migration (62) and cell proliferation (65, 66, 69, 70, 72, 74, 76). As the mechanisms at work are far from clear, the immediate challenge is to understand how the dosage or the use of diabetic animals (62, 66, 68, 70, 72) has influenced these responses. A parallel approach has focused on the release of plasmids encoding for specific growth factors from hydrogels to maximize the effect of the growth factor. Angiogenesis was targeted in diabetic wounds using a DNA vector encoding VEGF, even in the absence of proangiogenic factors (77). Despite the enhanced granulation tissue formation, no significant effect on angiogenesis was demonstrated. An enhanced healing response has been observed for other bioactive agents, such as SOD, nitrosoglutathione, nitric oxide (NO), interleukins, MIP-3α, ions,

Table 1 Strategies of hydrogels incorporating bioactive agents for the healing of full-thickness skin wounds

Bioactive agent	Hydrogel	Outcome	Animal, wound size	Reference(s)
FGF-2 (0.5 μg)	Chitosan	Improved wound closure, re-epithelialization, granulation tissue formation, and capillary formation (relative to S)	Diabetic mice, 100 mm^2	71
FGF-2 (20–50 μg)	Gelatin	Improved re-epithelialization and vessel formation; reduced scarring; adjusted balance between tissue proliferation and apoptosis (relative to H)	Diabetic mice, 78.5 mm^2	72
FGF-2 (2–20 μg)	Chondroitin 6-sulfate and heparan sulfate	Accelerated wound closure and epidermis/dermis thickening; improved vascularization (relative to S and H)	Diabetic mice, 200 mm^2	59
FGF-2 (20 μg)	PVA, gelatin, and chitosan	Accelerated wound closure, epidermal outgrowth, and re-epithelization; faster transition from the inflammatory to the maturation phase; enhanced collagen deposition, myofibroblasts, and vessel formation (relative to $^{-}$C)	Healthy rats, 400 mm^2	73
FGF-2 or aFGF (1.6 μg)	Heparin poloxamer	Improved wound closure, granulation tissue formation, re-epithelialization, and blood vessel density (relative to BA)	Healthy mice, 28 mm^2	60
EGF (5 mg)	PVA and alginate	Accelerated wound closure; improved re-epithelialization and vessel formation (relative to S, H, and BA)	Diabetic rats, 169 mm^2	74
EGF (25–50 mg)	PLA	Enhanced wound closure, granulation tissue formation, collagen deposition, and vessel formation (relative to S and H)	Healthy rats, 400 mm^2	75
EGF (0.1 μg)	PEG and heparin	Advanced granulation tissue formation, capillary formation, and re-epithelialization (relative to S and BA)	Healthy mice, 150 mm^2	61
EGF (NA)	Sodium carboxymethyl chitosan	Accelerated wound closure and re-epithelialization (relative to S and H)	Diabetic rats, 314 mm^2	76
	Gelatin	Accelerated wound closure (relative to $^{-}$C,$^{+}$C and H) and re-epithelialization (relative to $^{-}$C); reduced granulation tissue formation (relative to $^{-}$C)	Healthy rats, 177 mm^2	65
KGF (0.023 nM)	Fibrin	Enhanced re-epithelialization and granulation tissue formation (relative to S and BA)	Diabetic mice, 100 mm^2	66
PDGF-BB (4 μg)	Alginate sulfate	Enhanced density of hair follicles, blood vessels, and collagen fibers; reduced inflammation (relative to S and H)	Healthy rats, 78.5 mm^2	67
VEGF (0.1 or 1 μg)	Desulfated heparin derivatives and star-shaped PEG	Enhanced granulation tissue formation, vessel formation; increased cellularity (relative to H)	Diabetic mice, NA	62

(*Continued*)

Table 1 (*Continued*)

Bioactive agent	Hydrogel	Outcome	Animal, wound size	Reference(s)
VEGF (20 μg), IL-10 (2 μg)	Hyaluronic acid, gelatin, and PEG	Enhanced re-epithelialization, vessel formation; led to less occlusion and death of blood vessels and fewer epidermal rete ridges (relative to H)	Healthy horse, 225 mm^2	68
VEGF plasmid (250 μg)	Hyaluronic acid–MMP	Enhanced granulation tissue formation (relative to H)	Healthy mice, 28 mm^2	77
IGF (5 μg)	PVA	Reduced wound size (relative to H); increased granulation tissue formation (relative to H, BA, and BA in methylcellulose gel)	Healthy rats[a], 210 mm^2	69
			Steroid-treated rats, 50 mm^2	70
IL-8 (100 ng), MIP-3α (200 ng)	Gelatin	Facilitated cell infiltration into the wound area; accelerated wound healing; enhanced re-epithelialization/neovascularization; increased collagen deposition (relative to S and H)	Diabetic mice, 393 mm^2	78
PRP (NA)	PDLLA-PEG-PDLLA:PLEL	Led to faster wound closure, better re-epithelialization, collagen formation, and vascularization (relative to S and H)	Healthy rats, 400 mm^2	88
	Gelatin	Increased epithelialization lengths, superior capillary formation, and wound contraction prevention (relative to S, H, and BA)	Healthy mice, 28–50 mm^2	89
	Collagen	Accelerated wound healing; enhanced vessel formation and hair and sweat gland formation (relative to S and H)	Healthy rats, 50 mm^2	90
PRP (0.1 mL)	Purilon (Coloplast, Humlebæk, Denmark)	Accelerated wound closure; enhanced release of VEGF; increased number of blood vessels over time (relative to S, H and BA)	Healthy mice, 50 mm^2	91
SDF-1 (20 ng)	Alginate	Accelerated wound closure; enhanced infiltration of endothelial cells (relative to H)	Healthy mice, 28 mm^2	92
SDF-1 (10 μg)		Accelerated wound closure with low evidence of scarring, but not vascularization (relative to S and PBS)	Healthy Yorkshire pigs, 20 mm^2	93
SDF-1 (0.5 μg), SEW2871 (2.5–10 μg)	Gelatin	Enhanced recruitment of MSCs and macrophages, especially M2 macrophages (relative to PBS)	Healthy mice, 50 mm^2	50
SDF-1 (0.5 μg), SEW2871 (2.5 μg)		Enhanced recruitment of MSCs and macrophages, especially M2 macrophages; enhanced gene expression of anti-inflammatory cytokines (relative to PBS)	Diabetic mice, 50 mm^2	94

(*Continued*)

Table 1 (*Continued*)

Bioactive agent	Hydrogel	Outcome	Animal, wound size	Reference(s)
SDF-1 (40 ng)	PPCN	Accelerated wound closure, improved granulation tissue formation, and epithelial maturation; had the highest density of perfused blood vessels (relative to H and BA)	Diabetic mice, 28 mm^2	95
GSNO (100 μm)	Pluronic F-127	Accelerated wound closure, re-epithelialization, and organized granulation tissue, especially when applied in the proinflammatory phase of wound healing (relative to H)	Healthy rats, 10–40 mm^2	79, 80
		Decreased wound size, reduced cellular density, and accelerated progression of the inflammatory phase (relative to H)	Healthy rats, 50 mm^2	81
GSNO (61 nmol)	PAA	Increased angiogenesis; collagen fiber organization; and TGF-β, IGF-1, SDF-1, and IL-10 gene expression (relative to H)	Healthy mice, 28 mm^2	82
NO (NA)	PVA	Thickening of granulation tissue and scar (relative to H)	Diabetic mice, 177 mm^2	83
SOD (1.6 mg)	Chitosan, heparin, and poly(γ-glutamic acid)	Accelerated wound healing by promoting wound closure and collagen deposition (relative to $^{-}$C)	Diabetic rat, 78.5 mm^2	84
DFO (NA)	PVA and chitosan	Higher expression of angiogenesis-related cytokines mediated by interference from the required prolyl-hydroxylase cofactors by acting as a Fe^{2+} chelator and upregulating the expression of hypoxia-inducible factor 1α (relative to H)	Diabetic rats, 177 mm^2	85
Copper (0.04 nmol)	PPCN	Accelerated wound closure rates; enhanced angiogenesis, collagen deposition, and re-epithelialization (relative to PBS)	Diabetic mice, 28 mm^2	86
Boron (16 mg)	Carbopol	Enhanced wound healing rate and histopathological scores (relative to S and H)	Diabetic rats, 28 mm^2	87

[a]Ischemic skin flap.

Abbreviations: a-, acidic; BA, bioactive agent control; $^{-}$C, gauze negative control; $^{+}$C, Comfeel (Coloplast) positive control; DFO, desferrioxamine; EGF, epithelial growth factor; FGF, fibroblast growth factor; GSNO, nitrosoglutathione; H, hydrogel control; IGF, insulin-like growth factor; IL, interleukin; KGF, keratinocyte growth factor; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; NA, not available; NO, nitric oxide; PAA, poly(acrylic acid); PBS, phosphate-buffered saline; PDGF, platelet-derived growth factor; PDLLA-PEG-PDLLA:PLEL, poly(D,L-lactide)–poly(ethylene glycol)–poly(D,L-lactide); PEG, poly(ethylene glycol); PPCN, poly(PEG citrate-*co*-*N*-isopropylacrylamide); PRP, platelet-rich factor; S, no treatment/sham control; SDF, stromal cell–derived factor; SEW2871, sphingosine-1-phosphate agonist; SOD, superoxide dismutase; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

and platelet-rich plasma (PRP), independently of the animal model, hydrogel, and type and dosage of the bioactive agent (**Table 1**) (50, 78–95). The only modes of action that have been addressed are the purported in vitro angiogenic potential of PRP-releasing hydrogels (88) and the ability of desferrioxamine-releasing hydrogels to interfere with the required prolyl-hydroxylase cofactors by acting as an Fe^{2+} chelator and upregulating the expression of hypoxia-inducible factor 1α (85).

The findings described above indicate a need to standardize protocols in order to better understand the quality of healing. Without such standardization and further research on the mechanisms that contribute to enhanced healing, other aspects of treatment, such as modulation of the amount and rate at which each bioactive agent is released from the hydrogel, are trivial.

2.3. Tissue Engineering Approaches Aiming at Regeneration

Tissue regeneration and the full restoration of tissue function are the ultimate goals of the TE field, especially for chronic wounds, given the poor quality of healing achieved with current approaches. Therefore, the use of advanced cell-based therapies as living responsive systems has the potential to significantly advance wound management in terms of clinical efficacy.

2.3.1. Skin substitutes. The goal of skin regeneration has been pursued primarily through the development of bilayered epidermal–dermal substitutes that were expected to integrate with host skin, thereby re-establishing fully functional skin tissue. Tissue-engineered skin substitutes, beginning with Graftskin/Apligraf, have long been used for the treatment of chronic skin wounds and have shown clinical evidence of enhanced healing in comparison to the standard of care (96). Because these substitutes are yet not able to fully regenerate the injured skin, different hydrogel-based systems composed of keratinocytes and/or fibroblasts have also been explored. Although many attempts at creating independent dermis-like (97–99) or epidermis-like (100, 101) hydrogel-based systems have been made, few epidermal–dermal skin equivalents have been proposed, and these were explored only in an acute wound healing context (101, 102). These studies reported faster recruitment of CD45 leukocytes, earlier blood vessel formation, and accelerated wound closure (101), as well as enhanced cell proliferation, suppressed immune response, and increased neovascularization and neodermis deposition (102). Despite these general improvements, the outcomes did not seem to improve upon those obtained with current commercially available skin substitutes.

2.3.2. Stem cell–based strategies. A critical limitation of stem cell therapies is the limited retention time of the transplanted cells. Hydrogels are an excellent option as delivery vehicles, as they increase the length of time that stem cells reside in a wound (**Table 2**) (48, 103–115). This property arises from the ability of some hydrogels to promote cell adhesion and to empower stem cell function by supporting the maintenance of their normal phenotype (23, 116, 117). These features are reinforced by in vitro preculture of stem cells within hydrogels, as demonstrated by the presence of transplanted cells in the wound for periods longer than 11 days post transplantation (**Figure 4**) (118, 119). Additional issues include controversial results regarding stem cell transdifferentiation into specific skin lineages (118, 119). Alternative hydrogel-based TE approaches that are under development rely on the therapeutic potential of undifferentiated stem cells (120), which refers to stem cells' angiogenic potential (48, 105–113, 118, 119, 121, 122), immunoregulatory/privileged capacity (48, 104, 106–108), regenerative secretome (48, 104, 112–115, 121, 122), and homing potential (110, 111, 114) (**Table 2**). In the context of chronic wounds, each of these

Table 2 Stem cell–laden hydrogel strategies for the healing of full-thickness skin wounds

Cell type (number)	Hydrogel	Preculture?	Outcome	Animal, wound size	Reference
hBMSCs (400,000)	PEG–gelatin	No	Accelerated wound closure; improved re-epithelialization, vascularization, and granulation tissue formation (relative to S); reduced infiltration of polymorphonuclear and mononuclear cells (relative to H); higher infiltration of M1 and M2 macrophages, but not a higher M2/M1 macrophage ratio (relative to S)	Healthy rats, 50 mm^2	108
mBMSCs (50,000)	NIPAM	No	Accelerated wound closure and re-epithelialization; improved granulation tissue formation, vessel formation, and ECM secretion; reduced wound contraction (relative to S and H); reduced inflammation (relative to S)	Diabetic mice, 50 mm^2	48
hASCs (20,000)	Adipose ECM, methylcellulose	No	Accelerated wound closure and re-epithelialization; improved vessel formation; minimum scar formation (relative to H)	Healthy rats, 169 mm^2	109
ds hASCs (100,000)	PEG–collagen–fibrin	No	Dermal matrix deposition, epithelial margin progression, and reduced wound contraction (relative to H)	Nude rats, 176 mm^2	110
mASCs (250,000)	Pullulan–collagen	No	Accelerated wound closure and increased vascularity due to the recruitment of provascular circulating BM-MPCs (relative to H)	Healthy mice, 28 mm^2	111, 112
rASCs (1,000,000)	Pluronic F-127	No	Accelerated wound closure; enhanced vascularization, cell proliferation, and regeneration of granulation tissue; enhanced mRNA expression of VEGF and TGF-β1 (relative to S, H, and C)	Diabetic rats, 64 mm^2	113
mBMSCs (1,000,000)	PAA-cross-linked poly-NIPAM	No	Accelerated wound closure; improved epithelial cell proliferation and re-epithelialization; reduced inflammatory responses; enhanced collagen deposition, tissue remodeling, and secretion of TGF-β1 and bFGF (relative to S, H, and C)	Healthy mice, 100 mm^2	114
hASCs (2,000,000)	Chitosan and gelatin	No	Higher capillary density and higher stem cell number in the wound (relative to H and C)	Healthy mice, 28 mm^2	115

(*Continued*)

Table 2 (*Continued*)

Cell type (number)	Hydrogel	Preculture?	Outcome	Animal, wound size	Reference
ASCs (2,500,000)	PEG and hyaluronic acid	No	Accelerated wound closure; enhanced re-epithelialization and neodermis and vessel formation; inhibited inflammatory mediators (relative to S, H, and C)	Diabetic rats, 95 mm^2	104
mASCs (300,000)	PEG and gelatin	No	Accelerated wound closure; decreased inflammatory cell infiltration; enhanced vessel formation (relative to S, H and C)	Diabetic mice, 28 mm^2	105
pASCs and mASCs (1,000,000)	Gelatin	No	Accelerated wound closure (relative to H)	Healthy pigs and mice, 100 mm^2	106
rBMSCs (2,500,000)	Chitin	No	Accelerated wound repair characterized by enhanced granulation tissue formation and vessel formation; better viability of the implanted cells (relative to S, H, and C)	Healthy rats, ~314 mm^2	107
mASCs (1,000,000)	Elastin	NA	Accelerated wound closure and re-epithelialization (relative to S); increased viability of transplanted cells (relative to C) and vascularization (relative to S, H, and C)	Healthy mice, 50 mm^2	121
hAFSs (5,000,000)	Heparin-conjugated hyaluronic acid	NA	Improved wound closure, re-epithelialization, vascularization, and ECM production (relative to S and H)	Healthy mice, 400 mm^2	12
mBMSCs (250,000)	Pullulan–collagen	Yes (24 h)	Accelerated wound closure and vascularization; higher engraftment of stem cells in the wounds and stem cell differentiation in vivo (relative to C)	Healthy mice, 28 mm^2	119
hASCs (200,000–500,000)	Gelatin	Yes (3 days)	Accelerated wound closure; enhanced epidermal recovery and vascularization; stem cell differentiation in vivo (relative to C)	Healthy mice, 100 mm^2	118
hASCs (1,000,000)	Gellan gum and hyaluronic acid	Yes (2 days)	Increased epidermal thickness; enhanced ECM formation (relative to S)	Healthy mice, 113 mm^2	126
hASCs (300,000)		Yes (14 days)	Faster transition from the inflammatory to the proliferative phase, thicker and more mature epidermis, enhanced neoinnervation, and increased ECM formation (relative to S and H)	Diabetic mice, 64 mm^2	103

Abbreviations: AFS, amniotic fluid–derived stem cell; ASC, adipose stem cell; bFGF, basic fibroblast growth factor; BM-MPCs, bone marrow–derived mesenchymal progenitor cells; BMSC, bone marrow–derived mesenchymal stem cell; C, cells only; ds, debrided skin; ECM, extracellular matrix; h-, human; H, hydrogel control; m-, mouse; mRNA, messenger RNA; NA, not available; NIPAM, *N*-isopropylacrylamide; p-, pig; PAA, poly(acrylic acid); PEG, polyethylene glycol; r-, rat; S, sham control; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

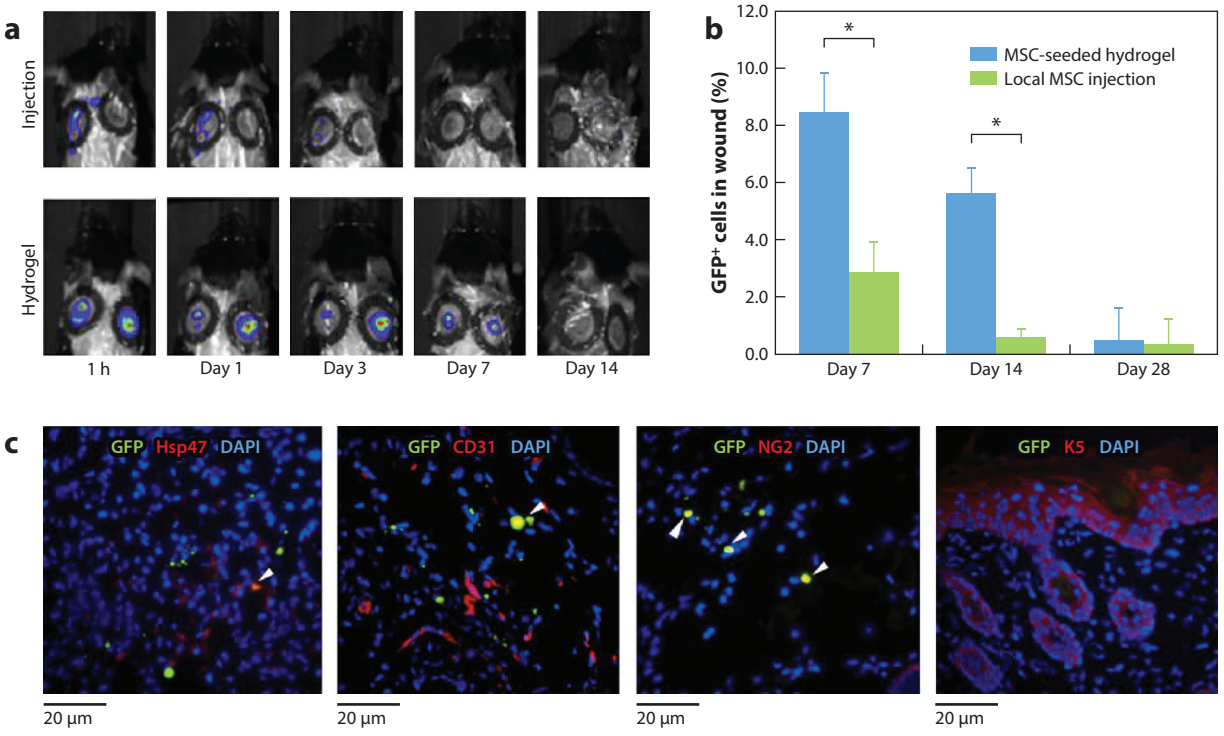

Figure 4

MSC-laden pullulan–collagen hydrogel for the treatment of wounds evidencing stem cell engraftment. (*a*) Bioluminescence imaging showing luciferase-expressing mMSCs in the wounded area. (*b*) Quantification of the GFP-labeled mMSCs by flow cytometry (*$p < 0.05$). (*c*) Colocalization of GFP-labeled mMSCs with Hsp47 (fibroblast marker), NG2 (pericyte marker), and CD31 (endothelial cell maker) (*yellow*, *arrowheads*), but not with K5 (keratinocyte marker). Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; GFP, green fluorescent protein; MSC, mouse mesenchymal stem cell. Adapted from Reference 119 with permission.

features could significantly affect healing. In fact, one of the most remarkable outcomes that has been reported after implantation of stem cell–laden hydrogels in skin wounds is enhanced vessel formation (48, 103–113, 118, 119, 121, 122), critically impaired in chronic wounds. Higher levels of angiogenic growth factors (104, 112–115, 121, 122) and progenitor cells (111) in wounds treated with stem cell–laden hydrogels seem to indicate that stem cells can support an angiogenic environment. Therefore, the capacity of stem cells to release angiogenic mediators, as has been extensively demonstrated in vitro (104, 111–115, 121, 122), and to recruit circulating progenitor cells (111) may be crucial for further exploration of chronic wound treatment. Additionally, the stem cell secretome has been connected to other regenerative features, such as improved re-epithelialization of diabetic excisional wounds, although increased levels of FGF, transforming growth factor (TGF)-β1, and EGF detected in vitro were not confirmed in wounds treated with stem cell–laden hydrogels (48). Reduced levels of immune cells (including macrophages, T lymphocytes, polymorphonuclear cells, and mononuclear cells) (103, 105, 108), a higher ratio of anti-inflammatory to proinflammatory (M2/M1) macrophages (103), and reduced levels of inflammatory mediators (e.g., tumor necrosis factor α) (104) have also been detected in wounds treated with stem cell–laden hydrogels. Thus, the impact of these strategies on the resolution of the inflammatory stage of chronic wounds may be highly relevant.

Overall, hydrogel-based strategies capable of acting as suitable supports that can maximize stem cells' therapeutic potential have the potential to target the different stages of wound healing in general and perhaps to overcome critical problems associated with nonhealing wounds, such as deficient angiogenesis and persistent inflammation. Nevertheless, the exact mode of action of the systems under development has yet to be demonstrated.

2.3.3. Endothelial cell–based strategies. Ischemia and impaired angiogenesis are responsible for loss of tissue viability and necrosis, which significantly affect healing (2, 9). Therefore, fostering blood perfusion through a network of capillaries is critical for the treatment of chronic wounds. Hydrogels that naturally have angiogenic features, such as fibrin-based hydrogels (123), or that present binding sites for endothelial cell receptors, such as collagen-based (122–125) and hyaluronic acid–based (126–129) hydrogels, have been selected for use in the development of such networks in combination with endothelial cells. One of the most successful approaches to promote fast vessel formation upon implantation has been prevascularization of the constructs in vitro. Vascularized hydrogels have been obtained in vitro by culturing endothelial cells alone or in combination with mural cells, such as pericyte precursor cells, stem cells, or fibroblasts (130–134). Adipose stromal vascular fraction (SVF)-derived endothelial and perivascular cells incorporated in fibrin–collagen type I hydrogels form a vascular-like network in vitro that, after transplantation into full-thickness wounds in immune-deficient rats, anastomosed to the host vasculature within 4 days (**Figure 5**) (123).

Early vascular cells derived from human induced pluripotent stem cells reprogrammed either from a healthy donor or from a patient with type 1 diabetes also showed the capacity to prevascularize hyaluronic acid hydrogels. The constructs thus obtained accelerated wound closure, granulation tissue formation, macrophage infiltration into the hydrogel, and higher vessel density and blood perfusion of wounds in diabetic rats, indicating rapid integration with the host vasculature (129). The effect of endothelial cells goes beyond their ability to vascularize hydrogels that then anastomose with the host tissue (125, 126). The CD248-expressing cell fraction isolated from the stromal vascular fraction (SVF), known for its high level of angiogenic gene transcripts, within pullulan–collagen gels promoted faster re-epithelialization in addition

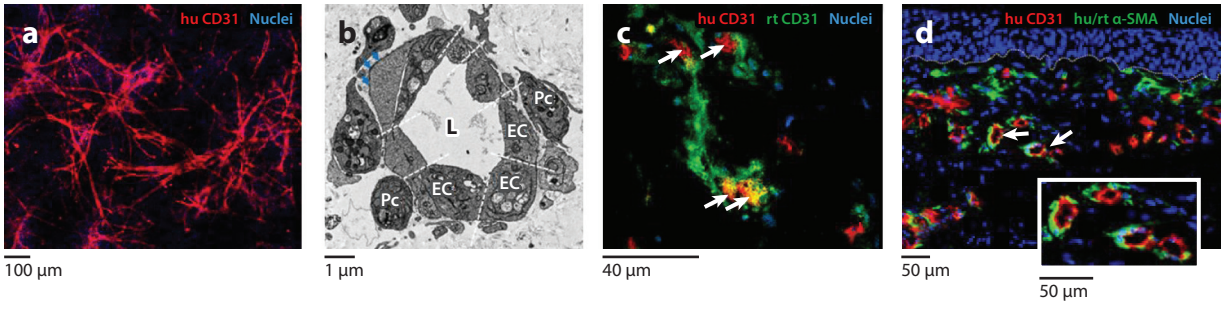

Figure 5

Stromal vascular fraction–derived endothelial and perivascular cells precultured in fibrin–collagen type I hydrogels for the treatment of rat wounds evidencing in vitro tubular-like structure formation and in vivo anastomosis with the host vasculature. (*a*,*b*) Interconnected bioengineered capillary formation after 21 days of in vitro culture, detected by (*a*) fluorescence microscopy after immunostaining the endothelial cells with CD31 (*red*) and staining the nuclei with Hoechst (*blue*) and (*b*) transmission electron microscopy after cross-sectioning evidencing the presence of a central lumen (L) surrounded by multiple endothelial cells (EC), covered by pericytes (Pc). The deposition of basement membrane (*blue arrows*) was also detected. (*c*,*d*) Anastomosis (*arrows*) of human capillaries with rat capillaries (*c*) 4 days and (*d*) 7 days post transplantation, identified by the colocalization of human-specific CD31 (endothelial marker) with rat-specific CD31 and α-smooth muscle actin (α-SMA; pericyte marker). Adapted from Reference 123 with permission.

to enhanced presence of vascularity of full-thickness wounds in mice (125). These results agreed with our results showing enhanced vessel density and re-epithelialization of murine full-thickness wounds treated with gellan gum–hyaluronic acid spongy-like hydrogels incorporating human adipose stem cells (hASCs) and microvascular endothelial cells (126).

Overall, vascularized hydrogels integrate rapidly with the host vasculature, making them suitable for addressing the need to foster early perfusion of chronic wounds. However, the long-term stability of chimeric vessels has not been comprehensively evaluated; in the case of failure, healing might be compromised. Moreover, the role of endothelial cells in skin wound healing goes beyond the establishment of a new microcirculation, reinforcing the need to determine the exact correlation between vascularization and re-epithelialization in the healing of chronic wounds.

2.3.3. Schwann cell–based strategies. Despite the importance of the cutaneous peripheral nervous system in the perception of pain and in prompting the release of neuropeptides and neurotrophic factors that regulate important healing mechanisms (12), its regeneration after wounding has been neglected. Given that neuropathic skin is associated with the emergence, recurrence, and impaired healing of chronic wounds in diabetic patients, regeneration of damaged nerves and consequent restoration of neuromodulator levels are major requirements. Schwann cells are key cellular players in the treatment of neuropathy, as they are responsible for nerve repair (135). So far, the use of Schwann cells for skin wound healing is reduced to the incorporation of mouse cells into a human skin tissue–engineered substitute. The enhanced nerve fiber migration and myelin sheath formation thus obtained led to a recovery of nerve function, as demonstrated by a current perception threshold similar to that of normal skin for large and myelinated Aβ-sensory fibers (136). Despite these promising results, strategies using human Schwann cells are limited by the challenges of isolating and expanding the appropriate number of cells for clinical applications. Stem cells have been considered as an alternative cell source, but their differentiation from dermal skin–derived precursor (137) or from human stem cells (138) remains a challenge. In fact, hASCs conditioned to Schwann cell–like phenotype and incorporated within hyaluronic acid–based spongy-like hydrogels failed to promote neoinnervation in diabetic murine wounds, in contrast to unconditioned hASCs (**Figure 6**). This finding was explained by a more controlled transition from the inflammatory to the proliferative phases, which possibly shows an indirect relation between undifferentiated stem cells with innervation rather than its integration in the injured nerve fibers (139). Other studies have also shown a direct effect of neuromediators on inflammatory cytokine expression and the inflammatory infiltrate of diabetic wounds (140, 141), which is likely to be a key nexus to be explored in future research to promote the neoinnervation of chronic wounds.

In sum, given the importance of Schwann cells to repair damaged nerves and the limited availability of Schwann cells from human sources, efforts to improve the differentiation of stem cells toward a Schwann-like phenotype is likely to lead to a breakthrough in terms of the neoinnervation of chronic wounds. Nonetheless, a better understanding of neuromediator mechanisms in wound healing is critical to advance strategies that target neoinnervation, possibly in the short term.

3. CONCLUDING REMARKS AND FUTURE PROSPECTS

The treatment of chronic skin wounds is complex and challenging due to their pathophysiology, which results in the impaired functioning of different cells and in unbalanced levels of critical biochemical healing mediators. Biomedical engineers have made significant progress in understanding that the healing of chronic wounds needs to be addressed according to each wound's

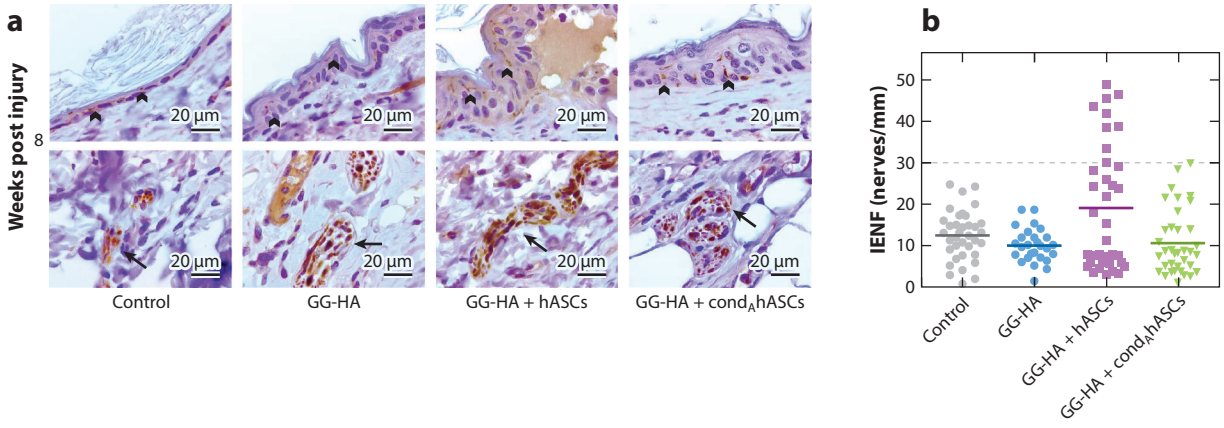

Figure 6

hASC-laden HA-based spongy-like hydrogels for the treatment of diabetic murine wounds showing enhanced neoinnervation. (*a*) Identification of PGP9.5-immunostained nerve endings (*arrowheads*) and Remak bundles (*arrows*). (*b*) Quantification of IENF 8 weeks after transplantation of HA-based spongy-like hydrogels (GG-HA), hASCs containing HA-based spongy-like hydrogels (GG-HA + hASCs), and conditioned hASCs containing HA-based spongy-like hydrogels (GG-HA + cond$_A$hASCs). Control represents nontreated wounds. Abbreviations: GG, gellan gum; HA, hyaluronic acid; hASC, human adipose stem cell; IENF, intraepidermal nerve fibers. Adapted from Reference 139 with permission.

pathophysiology and beyond its primary cause. Thus, devised therapies need to be specific and adjusted accordingly. Hydrogel-based systems offer tremendous potential for several reasons:

1. Hydrogels have long been recognized as the standard treatment for necrotic and sloughy wounds. The high water content of hydrogels is responsible for their unique ability to immediately cool a wound surface, providing a soothing effect, and promote autolytic debridement.
2. Hydrogels offering sustained and/or stimuli-responsive delivery of antiseptics or antibiotics represent a promising strategy to target severely infected wounds with higher efficacy. Antimicrobial strategies to circumvent adverse effects or antibiotic resistance are under development, but whether they are sufficient to eliminate chronic wound infections remains to be demonstrated.
3. Hydrogels that can modulate the local inflammatory environment or that incorporate antioxidants or anti-inflammatories have been extensively explored. Although this strategy exemplifies a way to direct a therapy toward a specific stage of wound healing, clinical outcomes for some of these products are not impressive. Thus, there is a need to better understand the hallmark of chronic skin wounds—inflammation—in order to achieve improved results.
4. Hydrogels incorporating bioactive agents involved in the different healing stages have great potential to improve wound healing, but standardized protocols are needed in order to better understand the quality of the healing and to determine the amount and rate at which each bioactive agent should be released. Preclinical data have been obtained in acute wound models that, in the best cases, emulate the primary causes of chronic skin wounds rather than the nonhealing mechanisms.
5. Hydrogels combined with different types of cells have been proposed as the future of advanced therapies for improved wound healing, with the goal of reaching full skin regeneration:

- Hydrogels incorporating primary cells such as keratinocytes and fibroblasts do not seem to offer other advantages beyond the current skin substitutes. Other primary cells, such as endothelial cells and Schwann cells that are known to improve, respectively, the neovascularization and neoinnervation of chronic wounds, could be a way to further advance these systems, but difficulties regarding accessibility to human tissue and isolation/expansion to clinically relevant numbers remain.
- Stem cell–laden hydrogels, which surmount the challenges posed by primary cells and the still-controversial (trans)differentiation of stem cells, have been proposed as an alternative therapy that maximizes stem cells' immunoregulatory, regenerative, secretory, and homing potential. They may be able to overcome critical markers of nonhealing wounds, such as deficient angiogenesis and persistent inflammation. However, their exact mode of action has yet to be demonstrated. Moreover, autologous approaches can be compromised in many patients due to possible cellular alterations, such as those caused by high glucose levels and aging.

To date, hydrogel-based strategies developed for the treatment of skin wounds have presented many challenges but also new opportunities. Each chronic wound has a different etiology that needs to be recognized as unique, which is a prerequisite for a specific approach targeting the wound's particular pathophysiology. Tailor-made therapies have been already adopted for different types of acute wounds and will become increasingly relevant for chronic wounds.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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