COMPREHENSIVE INVITED REVIEW

Stem Cells in Skin Wound Healing: Are We There Yet?

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Significance: Cutaneous wound healing is a serious problem worldwide that affects patients with various wound types, resulting from burns, traumatic injuries, and diabetes. Despite the wide range of clinically available skin substitutes and the different therapeutic alternatives, delayed healing and scarring are often observed.

Recent Advances: Stem cells have arisen as powerful tools to improve skin wound healing, due to features such as effective secretome, self-renewal, low immunogenicity, and differentiation capacity. They represent potentially readily available biological material that can particularly target distinct wound-healing phases. In this context, mesenchymal stem cells have been shown to promote cell migration, angiogenesis, and a possible regenerative rather than fibrotic microenvironment at the wound site, mainly through paracrine signaling with the surrounding cells/tissues.

Critical Issues: Despite the current insights, there are still major hurdles to be overcome to achieve effective therapeutic effects. Limited engraftment and survival at the wound site are still major concerns, and alternative approaches to maximize stem cell potential are a major demand.

Future Directions: This review emphasizes two main strategies that have been explored in this context. These comprise the exploration of hypoxic conditions to modulate stem cell secretome, and the use of adipose tissue stromal vascular fraction as a source of multiple cells, including stem cells and factors requiring minimal manipulation. Nonetheless, the attainment of these approaches to target successfully skin regeneration will be only evident after a significant number of *in vivo* works in relevant pre-clinical models.

SCOPE AND SIGNIFICANCE

FROM A PHYSIOLOGICAL perspective, effective skin wound healing still represents a major concern for global healthcare, as the currently available skin substitutes and alternative therapeutics lead to unsatisfactory results. This problematic affects a wide range of patients with various wound types resulting from burns, traumatic injuries, and diabetes, where delayed healing and scarring is a reality. In the past few years, new insights into the wound-healing process triggered the development of more sophisticated strategies that take advantage of specific performers such as artificial extracellular matrix (ECM)-like matrices, growth factors, and primarily stem cells.

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TRANSLATIONAL RELEVANCE

Endogenous stem cells are vital players in the well-coordinated cell-signaling cascades of wound healing. From a therapeutic perspective, their mobilization to the wound site has been suggested; however, insights into their mechanism of action are particularly difficult to attain. This has been hampering clinically relevant outcomes, thus supporting the exploitation of the therapeutic action of exogenous stem cells. Whether these can act as building blocks and/or potent secretome units is deeply dependent on the cell source and on the administration strategies. Both effects have been shown to significantly impact wound healing, targeting wound re-epithelialization, hair follicle (HF) formation, and neovascularization.

CLINICAL RELEVANCE

Despite all the pre-clinical studies using exogenous stem cells in a wound-healing context, translation into clinical trials is fairly recent. The majority of these focus on the use of bone marrow mesenchymal stem cells (BM-MSCs) mostly to treat chronic wounds. Alternatively, adipose stem cells (ASCs) potential to treat burn wounds and diabetic foot/venous ulcers is currently being evaluated, addressing variables such as cell number, administration mode, and wound area. The knowledge acquired from these trials is expected to lead the development of hybrid constructs as "engineered regenerative platforms" to actively encourage skin wound regeneration.
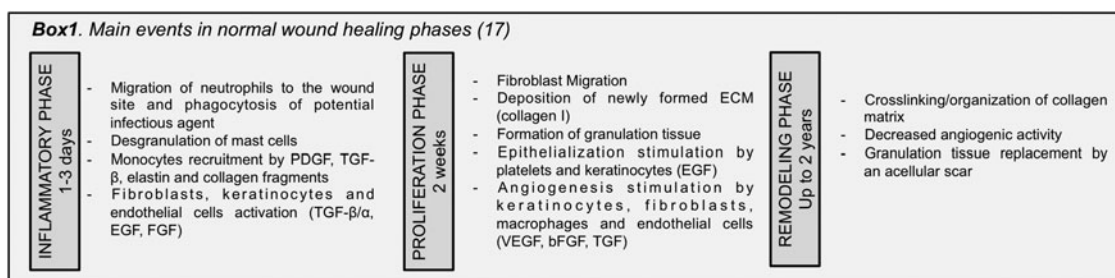
Stem cell involvement in wound-healing phases

Wound healing is a complex dynamic response to a physical trauma that comprises three overlapping phases: inflammatory, proliferative, and remodeling, extensively reviewed elsewhere[1,2] (Box 1 for a short overview of the main events). Their progression depends on a well-coordinated interplay of cell-signaling events at the wound site and surrounding tissues,[2] in which endogenous stem cells are vital players. These include HF bulge[3–5] and dermal sheath stem cells (DSCs),[6] as well as mesenchymal stem cells (MSCs) such as BM-MSCs[7–9] and specu-

latively ASCs,[10] as very little is known in terms of its endogenous role during wound healing. These cells are located within distinct skin niches that are divided mainly in epidermal and dermal niches.[11]

During both embryonic development and adult homeostasis, epidermis and HF are distinct compartments formed by independent stem cell populations. HF, in particular, does not contribute to interfollicular epidermis maintenance.[3] However, several authors revealed[3–5] that after wounding, HF bulge stem cells rapidly migrate toward the interfollicular epidermis, generating short-lived transient-amplifying cells that promote re-epithelialization. Contrarily, DSCs surrounding HF units ensure dermal papilla cell maintenance and are involved in dermal repair by acquiring a myofibroblastic phenotype.[6] Despite the compartmentalized structure of skin, paracrine signaling and cell–matrix communication are determinant to modulate an efficient wound-healing response.[12] Details on the internal mechanisms of action within skin stem cell niches in a wound-healing scenario were recently reviewed elsewhere,[11–14] and they are not the focus of this review.

In this context, the mechanism of action of endogenous stem cells outside the niche is particularly difficult to understand. The disclosure of their contribution in skin wound healing has been hampered by the use of rodent models, mechanistically different from humans, as well as by the lack of more sophisticated cell-tracking methodologies. Moreover, the role of BM is controversial due to its complex set of phenotypes and location, far from the cutaneous wound site.[9] Nonetheless, it is known that BM-MSCs contribution to cutaneous wound healing is substantially greater than the previously recognized sub-population CD45+, which is composed by BM hematopoietic cells and antigen-presenting fibroblasts.[15] Additional evidence showed that the contribution of BM-MSCs goes beyond early inflammation, contrary to what was initially believed.[16] These cells are maintained in the newly formed dermis playing a substantial role in the formation of new connective tissue through the production of collagen I and III.[7,15] More recently,

Box1. Main events in normal wound healing phases (17)

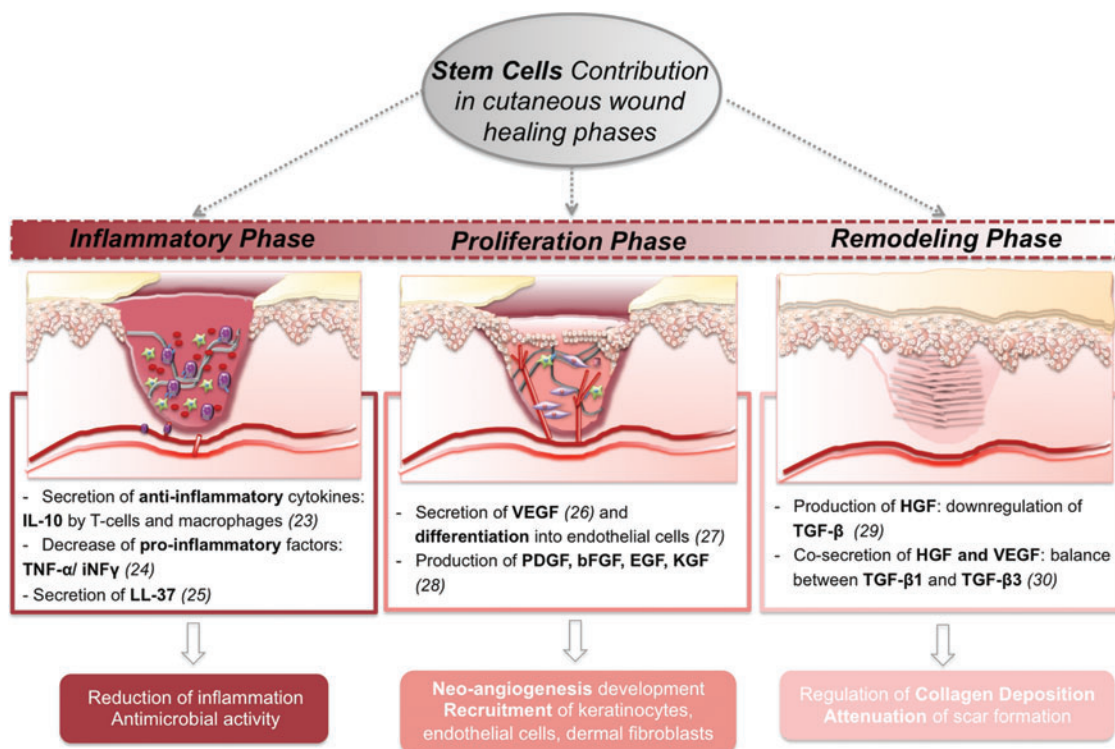| INFLAMMATORY PHASE 1-3 days	PROLIFERATION PHASE 2 weeks	REMODELING PHASE Up to 2 years
- Migration of neutrophils to the wound site and phagocytosis of potential infectious agent - Desgranulation of mast cells - Monocytes recruitment by PDGF, TGF-β, elastin and collagen fragments - Fibroblasts, keratinocytes and endothelial cells activation (TGF-β/α, EGF, FGF)	- Fibroblast Migration - Deposition of newly formed ECM (collagen I) - Formation of granulation tissue - Epithelialization stimulation by platelets and keratinocytes (EGF) - Angiogenesis stimulation by keratinocytes, fibroblasts, macrophages and endothelial cells (VEGF, bFGF, TGF)	- Crosslinking/organization of collagen matrix - Decreased angiogenic activity - Granulation tissue replacement by an acellular scar

Figure 1. Schematic overview of stem cell potential in the wound-healing scenario, along with their different mechanisms of action and potential outcome to target different stages, such as inflammation, proliferation, and remodeling. IL-10, interleukin 10; TNF-α, tumor necrosis factor alpha; INFγ, interferon gamma; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; KGF, keratinocyte growth factor; HGF, hepatocyte growth factor; TGF-β, transforming growth factor beta. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

further indications related to MSCs recruitment from bone marrow to participate in wound healing were demonstrated based on fetal microchimerism.[9] However, the role of other endogenous MSCs in skin wound healing remains unknown. Therefore, despite the indications provided by exogenous application of ASCs on the regulation of blood vessels formation through a crosstalk with endothelial cells,[10] studies that address its endogenous role for skin hemostasis/healing are still missing.

Despite this limited knowledge, local stem cell mobilization[17,18] has been proposed as an answer to the low engraftment rate of transplanted exogenous stem cells.[19] Nonetheless, it is important to highlight that these mechanisms have been studied in rodents and that the translational settings toward humans might represent a long leap for a clinical situation. Thus, the most followed approach for exploiting the therapeutic action of stem cells in the wound healing has been the application of exogenous MSCs at the wound site and their potential has been reviewed by several authors.[14,20,21] They can regulate specific events that occur along the different phases of the wound-healing cascade, as reviewed by Maxson *et al.*[22] and summarized in

Fig. 1,[23–30] opening distinct possibilities toward an effective healing.

Stem cells as building blocks and secretome units (771/8796)

Building blocks

Autologous skin substitute application in large full-thickness wounds/burns is hampered by the scarcity of biological material.[31] To counter this, different MSCs have started being used to replace dermal fibroblasts (DFbs).[32–34] Tonsil-derived MSCs in a collagen-based dermal-epidermal construct promoted epidermal stratification, cornification, and pigmentation comparable to human dermal fibroblasts (hDFbs), after transplantation in immunocompromised rats.[32] A similar outcome was found when the same cells were incorporated in a decellularized dermis and transplanted into a laser injury in humanized skin graft cultures.[33] However, unlike ASCs,[35] few studies detail the isolation procedures and expansion potential of tonsil-derived MSCs, hindering, for now, their positioning as a valid stem cell source. Epidermal-like cells and adipocytes generated from human adipose stem cells (hASCs) obtained from the debridement of burned

patient's wounds[34] were used to recreate the epidermal and hypodermal layers of a skin analogue where the hDFbs of the dermal-like layer are replaced by hASCs. Still, *in vivo* data are needed to validate such constructs for cutaneous wound healing.

The works described earlier[32–34] represent the few stem cell-based alternatives to three-dimensional (3D) organized dermo-epidermal substitutes, highlighting the question whether it is necessary to recreate a 3D substitute with the classical organization of the skin tissue to improve wound healing. Considering the different number of works using stem cells either injected at the wound borders[15,36] or randomly incorporated within biomaterial-based matrices,[37–42] there is skepticism regarding the need for 3D organization. Nonetheless, this discrepancy can be correlated with the limitations of MSC differentiation toward epithelial and endothelial lineages,[43] as well as to the deficient purity/stability of epidermal stem cells cultures.[43] Stem cells from distinct sources, including MSCs, have shown a strong effect in HF formation.[37,39,44,45] In our recently published work,[37] the enhanced HF formation appears to be associated with keratinocyte growth factor (KGF) overexpression resulting from a direct interaction between resident keratinocytes (KCs) and transplanted hASCs cell sheet constructs. MSCs mechanisms that improve HF formation are, however, still elusive.[45] The identification of transplanted MSCs in the neoepidermis[27,42,46] has been supporting the hypothesis of transdifferentiation into epidermal cells. However, despite the set of *in vitro* studies,[47–49] this hypothesis is still poorly explored. Transdifferentiation of MSCs into endothelial cells after transplantation into cutaneous wounds is also supported by few works showing mouse[27,40] and human BM-MSCs-derived endothelial cell integration in the neo-vasculature. Interestingly, these findings were also associated to a pericytic phenotype of undifferentiated transplanted cells also described by others[45] and in accordance to the reports that associate MSCs with perivascular niches and close/common origin with pericytes.[50,51]

Despite being able to act as building blocks, stem cells rich secretome (discussed in the next section "Secretome units") appears to be the major tool to modulate functional regeneration (Fig. 2).
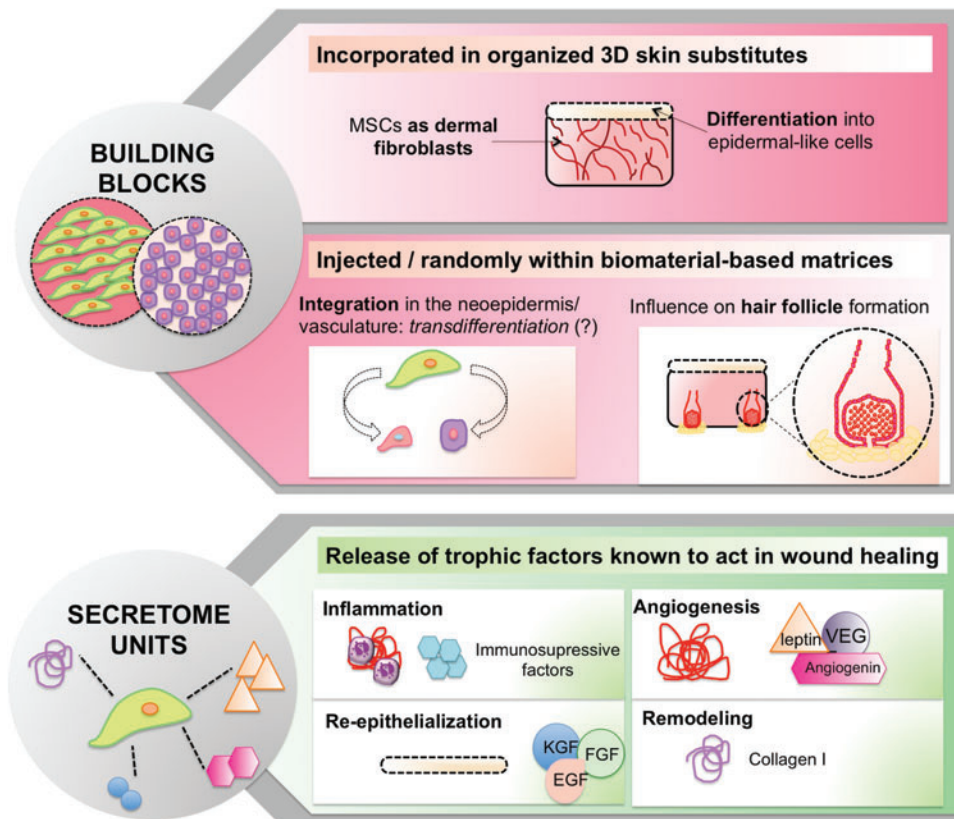

Figure 2. Schematic perspective of stem cells as powerful tools in skin wound-healing improvement. Special highlight provided on their contribution as building blocks and as potent secretome units at different responses levels. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Secretome units

MSCs secretome has been analyzed to assess stem cell mechanisms of action during the wound-healing process. BM-MSCs[52] and hASCs[53] secretome is immunosuppressant, modulating secretion of factors such as interleukin (IL)-6/10 and tumor necrosis factor alpha, as well as dendritic cell functions. Curiously, BM-MSCs-conditioned medium (CM) improved macrophage recruitment by potentiating secretion of macrophage inflammatory protein-1alpha/beta and erythropoietin.[26] BM-MSCs,[26,54] hASCs,[55] and umbilical cord mesenchymal stem cells[56] were also shown to release KGF, fibroblast growth factor 2, and epidermal growth factor. These promoted KCs migration in vivo, significantly influencing wound re-epithelialization.[26,55,56] Likewise, the impact on wound angiogenesis/vascularization at the wound site was also associated to vascular endothelial growth factor (VEGF) secreted by mouse bone marrow mesenchymal stem cells[26] and hASCs.[55] Other important regulators of angiogenesis, namely angiogenin, VEGF-A, and leptin, were also identified in hASCs, human bone marrow mesenchymal stem cells, DSCs, and dermal papilla cells (DPCs) secretome.[54] While angiogenin and VEGF-A secretion levels were comparable within MSCs populations, DSCs and DPCs released a significantly higher amount of leptin. These angiogenic features were confirmed in excisional[26,57] and chronic wounds[58] healing, and they can be maximized by playing with the oxygen concentration, as discussed in section "Hypoxia." MSCs secretome can further act in the remodeling phase of cutaneous wound healing. hASCs-CM is shown to promote the synthesis of collagen I by DFbs,[55,59,60] thus influencing wound closure. This effect on DFbs was also observed when human amniotic fluid MSCs-CM[61,62] was administered in a mouse excisional wound, suggesting their action via the transforming growth factor beta (TGF-β)/SMAD2 pathway.[62]

Overall, these data demonstrate that MSCs from different sources have a potent secretome that is capable of influencing different cells involved in the wound-healing process, modulating their activation, migration, and proliferation.

Clinical placement

Clinical cases and trials

Despite all pre-clinical wound-healing studies, translation of stem cell-based strategies in the clinical settings is still reduced. Most of the clinical trials/case studies comprise the use of BM and adipose tissue (AT) MSCs, to treat various wound types, namely chronic wounds as recently revised by Maxson et al.[22] BM-MSCs, still considered the gold standard for several applications, have a history of successful healing of chronic wound ulcerations,[63,64] despite reported poor cellular engraftment. Therefore, new delivery strategies such as the use of a fibrin spray are currently under trial (NCT01751282). Alternatively, three different

Table 1. *Key registered clinical trials/case studies of stem cell-based strategies for skin wound healing*

Cell type	Design	Wound type	Time frame	Status/outcome	Reference
Allogeneic BM-MSCs	Injection: test of four different doses	Second-degree burns (<20% TBSA)	No less than 52 weeks	Not yet recruiting	NCT02104713[a]
Autologous BM-MSCs	Forty injections of CD34+ cells	Chronic critical limb ischemia, diabetic foot	≈17 weeks	Therapy resulted in 79% limb salvage	NCT01232673[a,36]
Autologous BM-MSCs	BM-MSCs in fibrin spray	Non-healing	24 weeks	Ongoing	NCT01751282[a]
hASCs	Multiple injections within and periphery of the wound	Diabetic foot venous/pressure ulcer	≈10 weeks	Recruiting	NCT02092870[a]
hASCs	Injection (3–8×10⁶cells) and incorporation in PRP matrix	Burns	Not defined	Ongoing	2012-001596-36[b]
hASCs	Injection and spraying (4.5×10⁶ cells)	Burns	Not defined	Ongoing	2009-016365-29[c]
SVF	Injection	Venous ulcers	Not defined	Not yet recruiting	[d]
Autologous lipoaspirate	Injection	Diabetic/venous stasis wounds	52 weeks	Unknown	NCT00815217[a]
Autologous hair-follicles	20 hair grafts/2×2 cm² ulcers	Chronic leg ulcers	18 weeks	Ulcer area reduction, enhanced epithelialization, neovascularization, dermal reorganization	[66]
Follicular epidermal stem cells	Fully differentiated autologous epidermal equivalent	Recalcitrant vascular leg ulcers	—	Healing and complete closure	[67]

[a]Identifier on US clinical trials website: http://clinicaltrials.gov/results for: stem+cells+wounds.
[b]Identifier on EU clinical trials website: http://clinicaltrialsregister.eu/results for: stem+cells+wounds.
[c]Identifier on EU clinical trials website: http://clinicaltrialsregister.eu/results for: burn+mesenchymal stem cells.
[d]Australian Clinical trials website: http://australianclinicaltrials.gov.au/.
BM-MSCs, bone marrow mesenchymal stem cells; TBSA, total body surface area; hASCs, human adipose stem cells; PRP, platelet-rich plasma; SVF, stromal vascular fraction.

registered clinical trials (Table 1) using hASCs to treat burn wounds diabetic foot/venous ulcers are being undertaken. Variables such as cell number (NCT02092870), administration mode, including incorporation in platelet-rich plasma (2012-001596-36) or spraying (2009-016365-29) versus injection, and site of administration (NCT02092870) are being addressed.

In a different perspective, other tissues/fractions such as lipoaspirate and stromal vascular fraction (SVF) that fit "minimal manipulation" criteria, possibly with the rationale of particularly tackling the lack of vasculature of venous ulcers, are being tested (Box 1). In the same context, autologous scalp HF grafts were directly transplanted into chronic leg ulcers[65] as healing units. Although yielding promising results, limitations such as sample size, absence of stratification in the whole ulcer area, and difficult follow-up due to fibrotic healing in some patients, among others, were also identified.[65]

Interestingly, with the exception of BM-MSCs delivered in fibrin, none of the most recent trials has tested the combination of stem cells with biomaterial-based matrices. Apart from an earlier trial that confirmed that autologous epidermal equivalents engineered from outer root sheath KCs (EpiDex™) were safe and as effective as a standard mesh graft in the treatment of recalcitrant vascular leg ulcers,[66] new skin analogues have not reached clinical trials/case studies. However, artificial dermis with non-cultured autologous hASCs and exogenous basic fibroblast growth factor (bFGF) showed a high degree of success in the healing of patients who suffered chronic radiation injuries.[67]

Overview of regulatory aspects for clinical application

Stem cell-based products (apart some exceptions) are advanced therapy medicinal products (ATMPs). These products imply "substantial manipulation" of tissues/cells, resulting in biological/physiological function alteration.[68] Cell products with substantial or minimal manipulation are distinguished by their regulatory systems. *In vitro* cultivation of isolated cells represents substantial manipulation, while the simple isolation of BM mononuclear cells or SVF for an autologous approach enters the minimal manipulation category. Regulatory requirements for these minimally manipulated products are similar to the ones for blood banking products. This allows their preparation in suitable transfusion or blood banking facilities.[69]

The ATMP regulation has been differently managed worldwide; the EU and the United States have implemented new systems for the regulation of ATMPs that intend to merge, as much as possi-

ble, the regulatory requirements worldwide.[70] In the EU, the European Medicines Agency (EMA) established a Committee for Advanced Therapies (CAT) (in accordance to 1394/2007). The CAT has the responsibility to analyze each ATMP application submitted to EMA. The approval process also requires the Committee for Medicinal Products for Human Use feedback, whose decision is subsequently formalized by the European Commission that binds all EU member states. In the United States, applications are directed to the Food and Drug Administration (FDA), the Office of Cellular, Tissue, and Gene Therapies, and the Center for Biologics Evaluation and Research; the review process is conducted. Nonetheless, FDA has very much adopted a case-by-case analysis of ATMPs with little product "class" guidance to instruct compliance.

Maximization of stem cell potential in wound healing

Despite all the hype on stem cells' therapeutic application for cutaneous wound healing, it is clear that there is still significant room for improvements. A new trend of the field has been following strategies that can target mechanistic features of a skin wound-healing microenvironment to maximize the performance of stem cell-based approaches.

Hypoxia

In most of the human tissues, the oxygen (O_2) concentration that cells sense is significantly lower than the atmospheric concentration, or normoxia (20–21%, v/v), and it varies from 14% in highly perfused tissues to 1% or less in the bone marrow.[71] Moreover, it is widely known that a wounded tissue is hypoxic due to the disruption of the blood vessels and increased O_2 consumption by the local cells.[72] Because "hypoxia" denotes a state where the O_2 concentration to which a cell is subjected is lower than that at the cell native site or niche, it is a relative term. Under the scope of this review, hypoxia refers to O_2 concentration lower than the atmospheric one.

The O_2 concentration is an integral part of the stem cells' niche[71]; BM-MSC, in particular, are subjected to physiologic O_2 concentrations between 1% and 3%.[73] hASCs can face from 5% to 9%, but some controversy regarding these values can be found.[74] Cells respond to O_2 variations mainly through the action of the hypoxia-inducible factor (HIF)-1α.[75,76] This highly conserved protein is degraded at atmospheric O_2 concentrations[77] but stable in hypoxic conditions, promoting the activation of target genes[76,77] such as the angiogenesis- and the wound healing-related genes VEGF, platelet-derived growth factor, TGF-β3, TGF-β1, stromal cell-derived factor 1, and bFGF.[75,78–83]

Despite the limited attention given to hypoxia culture, an increasing number of works demonstrate that MSCs cultured under low O_2 concentrations have improved proliferation, clonogenicity, survival, and, notably, an enhanced secretome.

Hypoxia and wound healing. As referred in section "Stem cells as building blocks and secretome units (771/8796),", the major effect of stem cells on skin healing has been associated to paracrine signaling either directly or through their CM. Thus, considering the impact of hypoxic conditions in the nature of their secretome, it is likely that their healing potential can be modulated under those conditions. Chen et al.[84] demonstrated that hypoxic culture (2% O_2) increases the expression and secretion of bFGF, VEGF-A, and IL-6/8 in mouse BM-MSCs, subsequently inducing proliferation and migration of KCs, fibroblasts, endothelial cells, and monocytes. This was also confirmed in vivo after topical application of CM from hypoxia-cultured BM-MSC in full-thickness excisional wounds. In comparison to the use of CM from BM-MSC cultured in normoxia, increased cell proliferation, neovascularization, as well as recruitment of inflammatory macrophages were observed. Increased amounts of VEGF and bFGF in the CM of BM-MSC cultured in hypoxia were suggested to stimulate those processes that will, ultimately, lead to enhanced skin wound healing, although increased skin contraction was also observed. While the involvement of VEGF and bFGF was similarly reported by Lee et al.[85] using CM of hASC cultured in hypoxic conditions (2% O_2), contrarily to what is stated by the authors, the effect of neutralizing antibodies to VEGF and bFGF was not reflected in the reduced wound area, and it was most likely directly correlated with rodent skin contraction. Interestingly, Frazier et al.[86] analyzed the secretome of hASCs cultured in hypoxia (5% O_2) and observed that low oxygen conditions may favor a fibrosis-inhibiting, regeneration-promoting immunoregulatory role of hASCs in wound healing. In addition, the use of a splinted wound imprinting control region mouse model, where the effect of wound contraction in wound closure is eliminated,[87] confirmed enhanced wound closure in animals treated with CM of amniotic fluid-derived MSC (AF-MSCs) cultured in hypoxia (1% O_2) versus normoxia. CM of hypoxic AF-MSCs CM contained elevated levels of VEGF and TGF-β1 that induced hDFb migration in vitro. This effect was reversed by the inhibition of TGF-β/ SMAD2 and PI3K/AKT pathways, suggesting that wound-healing enhancement by AF-MSC hypoxia CM happens, at least partially, via increased fibroblast recruitment and migration.

Overall, these works show how culture in hypoxia can alter the MSC secretion patterns, likely to have a paracrine effect on other cells and tissues and impact the wound-healing process at different stages. Hypoxia culture seems to be able to modulate inflammatory cell recruitment due to strong secretion of immunomodulatory molecules such as programed death ligand-1 and indoleamine 2,3-dioxygenase,[74] which will positively impact the inflammatory phase of wound healing. Furthermore, increased secretion of VEGF and bFGF in the hypoxic CM strongly suggests that the proliferative wound-healing stage is affected by the consequent increase in neo-angiogenesis by paracrine signaling[75,83] independently of the MSC source. In opposition, the fibrosis-inhibiting signaling might be dependent on both the O_2 concentration and the cell type, as a balance between TGF-β1 and TGF-β3 is needed to achieve skin regeneration rather than fibrosis and scarring (Fig. 3). However, to effectively harness this potential of hypoxic-cultured stem cells, appropriate ways to deliver cells to the wound site will have to be developed. As shown in Table 1, there is a multitude of strategies to deliver cells to the wound site, ranging from injection to spraying or incorporation in a matrix. An incorrect strategy may not translate the full potential of the cells. Therefore, for each particular clinical setting, specific approaches must be defined to allow hypoxic-cultured cells to maintain a favorable secretome that results in improved healing or regeneration.

AT stromal vascular fraction. As described in section "Overview of regulatory aspects," despite the attractiveness of hASCs for cutaneous healing,[88] their use is associated with complex regulatory issues.[68] In opposition, the use of SVF, a heterogeneous mixture of 30–40% of ASCs and endothelial cells, smooth muscle cells, pericytes, fibroblasts, mast cells, and preadipocytes,[89] enters the "minimal manipulation" category, facilitating clinical applications. The SVF unique composition has been posed as beneficial to enhance the ASCs regenerative potential, offering unique benefits in cutaneous wound-healing applications.[55,90] However, few clinical studies directly compare the administration of cultured hASCs versus SVF and additional data are still needed to reinforce the current pre-clinical findings.

Current data show not only an effect of SVF on collagen synthesis[91] but also its strong angiogenic potential.[92,93] Very recently, Klar et al. suggested that SVF possesses similar percentages of stromal and endothelial cells, demonstrating that when in-
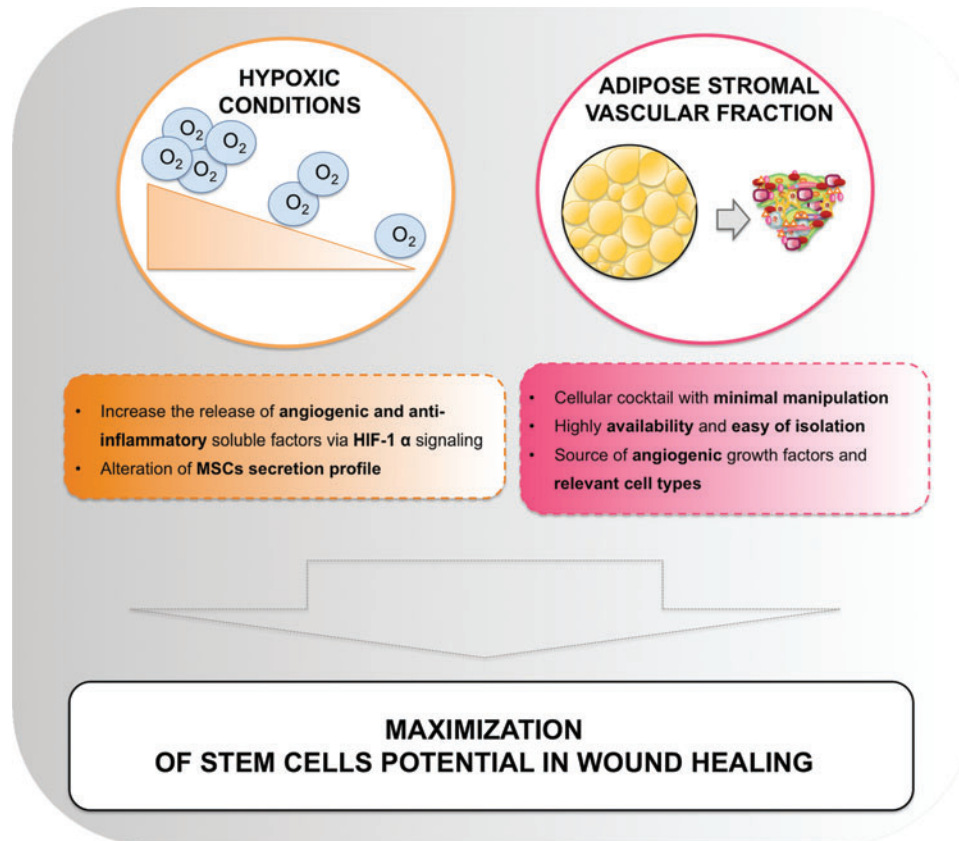
Figure 3. Overview of strategies to maximize stem cell application/potential in the wound-healing context. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

corporated in 3D fibrin or collagen, SVF cells were capable of *de novo* formation of microvascular networks, causing a rapid anastomosis with the host vasculature and a sustained epidermal coverage.[92] Moreover, when applied to burn wounds, increased VEGF production and reduced inflammation was observed.[93] Likewise, in a scenario of diabetic wounds, VEGF and bFGF expression was also increased,[94] accelerating the wound-healing process.

The several clinical studies using SVF to treat several conditions of very distinct diseases reviewed elsewhere[95,96] suggest that this stromal cell fraction is safe and can be efficiently transplanted in either an autologous or allogeneic manner. However, as mentioned earlier, to our knowledge only one trial is addressing the effectiveness of autologous SVF under the scope of skin wound healing in the treatment of venous ulcers.

FUTURE TRENDS

A new era in skin wound healing and regeneration has risen in the past few years having stem cells as central players. MSCs from bone marrow, AT, AF, and umbilical cord matrix are among the most explored. Their role is being discussed from two intimately connected viewpoints: as *building blocks* of the newly formed tissue due to their (trans)differentiation ability and as potent secretory units that can modulate the wound-healing cell-signaling pathways. However, while transdifferentiation mechanisms are poorly dissected, the interest in MSCs secretome has been increasingly rising. The spatiotemporal regulation of MSCs secretome in the context of the different wound types is, nevertheless, not achieved.

This uncertainty, as well as the reduced control of the artificial hybrid microenvironments that are being proposed, is greatly hampering the translation of stem cell-based strategies. Moreover, preclinical validation using closer to human models comprises a crucial step to support this translation. The mouse skin full-thickness wound model is the most used animal model, which, regardless of its contribution and convenience, presents limitations resulting from healing dissimilarities between humans and rodents.[97] While in rodents the wound closure occurs mainly via contraction, human skin is tethered to subcutaneous tissues and wounds heal by granulation tissue formation and re-epi-

thelialization. Due to the anatomic similarities with the human skin as well as to the identical mechanisms of healing, the porcine model has been described as the most suitable pre-clinical model in the cutaneous wound context.[98]

Advances in material science opened up the possibility of specifically tailoring a microenvironment to modulate cell behavior,[99] namely by incorporating cell-adhesion motifs,[100] cell-signaling elements,[101–104] and genetic encoding sequences.[105–107] However, the sophistication of the designed matrices is still not satisfactory. Likewise, the understanding/control of biomechanical mechanisms at the wound site through mechanically tailored materials, minimizing scar tissue formation, has been underexplored.[108] Thus, the sustained knowledge acquired from the studies on the wound-healing processes/events is expected to lead the development of hybrid constructs as "engineered regenerative platforms" to actively encourage skin wound regeneration. Sophisticated techniques that allow combining stem cells with a maximized potential, within a matrix capable of mimicking different aspects of the natural ECM, will certainly have a significant impact. In this context, 3D biofabrication appears as a breakthrough technology in the field of tissue engineering that offers advantages such as the automated creation of hybrid structures that differently combine living and non-living materials in a pre-defined 3D micro-organization.[108–110] Biofabrication strategies for skin regeneration/repair are limited to the creation of skin grafts consisting of layered fibroblasts and KCs embedded in collagen,[111,112] and more recently AF-MSCs within fibrin-collagen.[113] So far, major limitations have been associated to the materials' features that have to comply not only with the fabrication requirements but also with cellular phenotypic demands. But ultimately, the design of *engineered regenerative platforms* in which the placement of different cellular units is within a specific microenvironment, potentially biofunctional and mechanically adjusted, will lead the path to regenerate skin with compartments, such as HF and other appendages.

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> **TAKE-HOME MESSAGES**
>
> - Endogenous stem cells are recruited to the wound site, triggering specific responses in the different healing phases but approaches that are capable of precisely controlling cell recruitment are yet to be defined.
> - Exogenous stem cells from distinct sources have demonstrated potential therapeutic action, often limited due to poor cellular engraftment and low transdifferentiation ability.
> - Despite all the studies conducted at a research level with exogenous stem cells, this trend has just recently started being translated into clinical trials.
> - Alternative approaches to maximize stem cell potential that goes beyond a single cell's injection are a major demand to tackle reduced engraftment and to tailor their secretome.
> - The use of hypoxia and the exploration of SVF have been proposed to maximize stem cell potential.
> - Cultured MSCs in hypoxia have shown increased proliferation, survival, and, more importantly, a healing-driven secretome.
> - Minimal cell manipulation to reduce regulatory issues has been posed as an advantage of SVF, whose angiogenic potential and enhanced effect on collagen production are being reported.
> - Sophisticated techniques that allow combining maximized stem cells within a matrix that is capable of mimicking different aspects of the natural ECM will lead the path to regenerate skin.

AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

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veloping scaffold-free approaches for Tissue Engineering. His research interests have been centered in proposing new strategies to promote the vascularization of engineered tissues and in maximizing the angiogenic potential of different stem cells. **Alexandra Pinto Marques, PhD,** is principal investigator at 3B's Research Group. She has been integrating knowledge in the field of biomaterials and tissue engineering as a way to define innovative strategies to improve the functionality of skin tissue-engineered constructs. She has also paid particular attention to the vascularization hurdle by exploring the potential of endothelial cells obtained from different sources.

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Abbreviations and Acronyms

3D	= three-dimensional
AF-MSC	= amniotic fluid-derived MSC
ASC	= adipose stem cell
AT	= adipose tissue
ATMP	= advanced therapy medicinal product
bFGF	= basic fibroblast growth factor
BM-MSC	= bone marrow mesenchymal stem cell
CAT	= Committee for Advanced Therapies
CM	= conditioned medium
DFb	= dermal fibroblast
DPC	= dermal papilla cell
DSC	= dermal sheath stem cell
EGF	= epidermal growth factor
EMA	= European Medicines Agency
FDA	= Food and Drug Administration
hASC	= human adipose stem cell
HF	= hair follicle
HGF	= hepatocyte growth factor
HIF	= hypoxia inducible factor
IL	= interleukin
INFγ	= interferon gamma
KC	= keratinocyte
KGF	= keratinocyte growth factor
MSC	= mesenchymal stem cell
PDGF	= platelet-derived growth factor
PRP	= platelet-rich plasma
SVF	= stromal vascular fraction
TBSA	= total body surface area
TGF-β	= transforming growth factor beta
TNF-α	= tumor necrosis factor alpha
VEGF	= vascular endothelial growth factor