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REVIEW

Stem cells and chronic wound healing: state of the art

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Abstract: Currently available treatments for chronic wounds are inadequate. A clearly effective therapy does not exist, and treatment is often supportive. This is largely because the cellular and molecular processes underlying failure of wound repair are still poorly understood. With an increase in comorbidities, such as diabetes and vascular disease, as well as an aging population, the incidence of these intractable wounds is expected to rise. As such, chronic wounds, which are already costly, are rapidly growing as a tremendous burden to the health-care system. Stem cells have garnered much interest as a therapy for chronic wounds due to their inherent ability to differentiate into multiple lineages and promote regeneration. Herein, we discuss the types of stem cells used for chronic wound therapy, as well as the proposed means by which they do so. In particular, we highlight mesenchymal stem cells (including adipose-derived stem cells), endothelial progenitor cells, and induced pluripotent stem cells. We include the results of recent in vitro and in vivo studies in both animal models and human clinical trials. Finally, we discuss the current studies to improve stem cell therapies and the limitations of stem cell-based therapeutics. Stem cells promise improved therapies for healing chronic wounds, but further studies that are well-designed with standardized protocols are necessary for fruition.

Keywords: stem cells, chronic wounds, cell therapy, wound healing

Introduction

Chronic wounds represent a significant burden both financially and in terms of lost quality of life, affecting both individual patients and the health-care system as a whole. In North America alone, management of complex wounds, which include chronic wounds, pressure ulcers, and nonhealing surgical wounds, carries an annual price tag of US$10 billion. Worldwide, the global wound care market is projected to surpass US$22 billion per year by 2020.[1] Whereas acute wounds are expected to eventually heal, chronic wounds are defined by a physiologically impaired healing response. Current wound management strategies remain unable to adequately treat chronic wounds, resulting in a vigorous pursuit of novel therapies, including those utilizing stem cells. The inherent regenerative capabilities of stem cells make them ideal targets for addressing the unmet clinical needs associated with chronic wounds, as well as expanding our understanding of the wound healing process. Though numerous clinical trials have measured the effects of stem cells in wound healing, most have been in the context of treating a more systemic illness such as critical limb ischemia (CLI); wound healing is instead often relegated to secondary outcome measures, making analysis of their clinical efficacy more difficult. This review will cover the clinically relevant sources of stem cells for chronic wound therapy, the means by which they modulate

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wound healing, and the results of recent in vitro and in vivo studies conducted in animals and humans. Both published and ongoing clinical trials will be discussed, in addition to where the field is headed. While this review focuses specifically on stem cells as they relate to chronic wound therapy, the reader is directed to the review by Li and Fu[2] for a more in-depth discussion of stem cell mechanisms involved in physiological wound healing.

Stem cell sources for regenerative medicine

Stem cells are defined by their ability to self-renew and differentiate into multiple cell types. They are categorized as either multipotent or pluripotent based on the variety of cell lineages to which they may give rise. Multipotent cells are equivalent to adult stem cells, with the ability to differentiate into several different lineages. Pluripotent cells are embryonic stem cells (ESCs), with an even greater capacity for differentiation, able to form all of the functional cell types of an organism. Various stem cell populations have been the subjects of significant research efforts. The most clinically relevant stem cell populations include mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), and induced pluripotent stem cells (iPSCs).

Mesenchymal stem cells

Under suitable conditions, MSCs have the ability to differentiate into bone, fat, cartilage, and muscle. Their ability to adhere to polystyrene tissue culture plastic remains a crude but effective means of isolating what is now understood to be a very heterogeneous population of progenitor cells. In addition to the aforementioned characteristics, the International Society for Cell Therapy has included cell surface expression in its guidelines for defining MSCs: $CD73^+$, $CD90^+$, $CD105^+$, $CD11b/14^-$, $CD19/CD73b^-$, $CD34^-$, $CD45^-$, and $HLA\text{-}DR^-$.[3] $CD271^+$ has also been described as the most specific marker for bone marrow-derived mesenchymal stem cells (BM-MSCs) and adipose-derived stem cells (ASCs).[4] However, consensus has yet to be reached with regard to their antigen expression profile.[5] The inconstant nature of surface marker expression as these cells are removed from their native environment and expanded ex vivo merely worsens the surrounding controversy.[6] Lack of standardization can thereby impede our ability to draw comparisons across studies. Despite these shortcomings, MSCs are understood to play a significant role in coordinating the wound healing response as it advances through phases of inflammation, proliferation, and remodeling.[7] In addition to their capacity for self-renewal, MSCs have immunomodulatory effects at the local environments into which they are transplanted,[8,9] supporting native cells via various paracrine mechanisms that promote cell survival, migration, and proliferation.[10,11] This regenerative potential has garnered significant interest for applying MSCs in clinical interventions.

Within the larger group of cells termed MSCs, there are several described subpopulations, differentiated largely by their tissue of origin, but also in terms of sometimes-controversial differences in phenotype. MSCs reside in all mesenchymal tissues, a few of which are more practical for clinical purposes. BM-MSCs are one of the most studied populations. However, harvest of these cells necessitates painful bone marrow aspiration. This, in addition to the scarcity of BM-MSCs (1 in 10,000 mononuclear bone marrow cells)[12] has shifted favor toward the more accessible and abundant ASCs. With regards to cell-surface expression, the International Fat Applied Technology Society together with the International Society for Cell Therapy distinguish ASCs from MSCs by their positivity for CD36 and negativity for CD106 in culture.[13] ASCs are generally harvested as part of lipoaspirate within the stromal vascular fraction (SVF), which itself has been used for regenerative wound therapy.[14] The SVF contains a diverse population of cells, including endothelial, hematopoietic, and pericytic lineages, as well as MSCs. ASCs are believed to account for up to 3% of total cells within isolated SVF, which is orders of magnitude higher than the corresponding proportion of MSCs in bone marrow.[15]

The relative dearth of MSCs in bone marrow aspirate has also led to the utilization of bone marrow mononuclear cells (BMMNCs) in clinical studies, which demonstrate their potential for accelerating wound healing. BMMNCs are also a heterogeneous group, thought to contain EPCs and MSCs in addition to an array of growth factors and cytokines, together promoting angiogenesis.[16,17] Amniotic fluid, placental tissue, and umbilical cord (including Wharton's jelly and cord blood) are further sources of MSCs. Peripheral blood is also considered by some to be a source of MSCs following mobilization by granulocyte-colony stimulating factor (G-CSF) injection, though these mobilized cells would generally be classified as BMMNCs or peripheral blood mononuclear cells (PBMNCs). Recently, Li et al[18] described a coculture system that routinely produces MSCs from peripheral blood without mobilization. Despite sharing the MSC moniker, populations from varying sources differ in terms of cell surface markers and differentiation efficiency, though they maintain their overall multipotent characteristics.

While research has often focused on addressing the issues associated with a heterogeneous and poorly characterized cell population, the improved wound healing associated with MSC administration may in fact depend, at least in part, on multiple cell types working in concert. Rodriguez-Menocal et al[19] demonstrated that whole bone marrow is more effective at stimulating angiogenesis relative to cultured bone marrow cells or MSCs, resulting in faster wound healing. A clinical study comparing BMMNCs to MSCs corroborated these findings in patients with chronic wounds.[20] Similarly, BMMNCs demonstrate superior osteogenic and angiogenic differentiation potential compared with isolated $CD34^+$ cells.[21] Administering heterogeneous groups of stem cells may allow for communication between different cell types, facilitating improved tissue regeneration, as is seen with coculture of MSCs together with EPCs.[22]

Applied to chronic wound therapies, both MSCs[23,24] and ASCs[25] have demonstrated an ability to improve wound healing in experimental diabetic models. Findings from in vitro experiments have elucidated a number of ways that BM-MSCs promote tissue regeneration, including production of growth factors, cytokines, collagens, and matrix metalloproteinases.[26,27] Their direct interactions with other cells, such as keratinocytes, also accelerate wound healing.[28] Despite their ability to differentiate into various cell types, the mechanism of action of MSCs is largely paracrine in nature.[29] For example, ASC-conditioned media can stimulate the migration of vascular endothelial cells, fibroblasts, and keratinocytes, suggesting that the secretome can promote wound healing even in the absence of the cells themselves.[30]

Endothelial progenitor cells

Recruited from the bone marrow, EPCs circulate in the blood expressing hematopoietic and endothelial surface markers, localizing to sites of tissue injury and ischemia.[31] At the wound bed they contribute to vasculogenesis, the process by which new vessels are formed from circulating progenitor cells. Like MSCs and ASCs, EPCs represent another heterogeneous population, with variations in classification that impede standardization of their clinical applications. The definition of EPCs overlaps with PBMNCs and BMMNCs (Figure 1), which may lead to confusion. In terms of clinical applications, EPCs are harvested from the peripheral blood (except Wettstein et al[32] who harvested from bone marrow), often following G-CSF mobilization from the bone marrow, and are commonly enriched for CD34 positivity. The angiogenic potential of these cells has made them promising targets for treating chronic wounds with underlying ischemic pathologies.

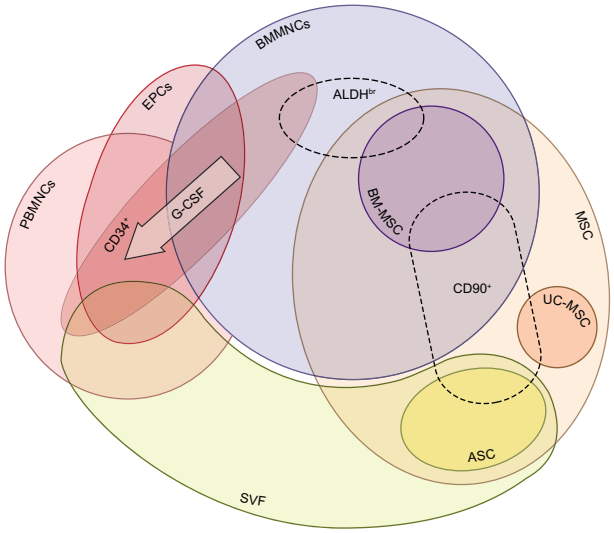

Figure 1 Stem cell populations administered in clinical trials.
Note: Dashed lines represent more ambiguously characterized populations.
Abbreviations: ALDH^{br}, aldehyde dehydrogenase bright cell; EPC, endothelial progenitor cell; ASC, adipose-derived stem cell; BMMNC, bone-marrow mononuclear cell; BM-MSC, bone-marrow mesenchymal stem cell; SVF, stromal vascular fraction; PBMNC, peripheral blood mononuclear cell; G-CSF, granulocyte-colony stimulating factor; UC-MSC, umbilical cord mesenchymal stem cell.

Induced pluripotent stem cells

In the search for regenerative cell therapies, ESCs provide an excellent early possibility given their pluripotent nature. However, ethical concerns regarding embryological tissue usage have limited their applications. Furthermore, when used in adults, these cells would be allogeneic, potentially resulting in immune-mediated rejection. In 2007, the first human iPSCs were produced in vitro.[33,34] iPSCs circumvent the aforementioned barriers to ESC use in that they are derived from adult somatic cells, allowing for autologous tissue generation without need of posttransplant immunosuppressive therapy. While the first human iPSCs were derived from adult fibroblasts, more recent advances have allowed for faster and more efficient production from ASCs,[35] demonstrating further potential of MSCs in regenerative medicine.

iPSCs may overcome current limitations in wound healing therapies, but their use is not without risk. A common concern associated with cell-based therapies is that in utilizing cells with a nearly unlimited ability to self-renew and regenerate, there is the potential for malignant transformation. Reverting cells from a fully differentiated to a pluripotent state requires the use of reprogramming factors. Despite demonstrating reduced tumorigenicity over previous viral integration methods,[36] alternative adenoviral, plasmid-based, and recombinant protein-based strategies[37,38] continue to rely on protooncogenic reprogramming factors.[39] More studies are thus needed to

establish a safety profile for iPSC interventions. As such, there are currently no clinical trials utilizing iPSCs underway in the US. However, worldwide, their first use in a clinical trial began in 2014. While this Japanese study is aimed at treating age-related macular degeneration, the findings from this study may hopefully assuage initial concerns allowing for further clinical study, including iPSC use in chronic wound therapy.

Interactions between stem cells and chronic wounds

Chronic wound characteristics

Physiological wound healing occurs within a microenvironment conducive to tissue repair; high levels of growth factors and mitigated degradative enzymes promote the functionality of fibroblasts, keratinocytes, and vascular endothelial cells, which are key instigators of wound healing.[40] Conversely, the chronic wound bed is an environment of unabated inflammation, low mitogenic activity, excessive matrix metalloproteinases, extracellular matrix degradation, reduced angiogenesis, and premature fibroblast senescence, resulting in an overall delayed time to healing.[41–44] While chronic wounds have a variety of causes such as pressure, diabetes, and peripheral arterial disease, the majority of chronic wounds share at least some of these pathological mechanisms.[45] Additionally, failed reepithelialization may perpetuate these processes.[46] Chronic wounds demonstrate a pathological level of underhealing. The use of stem cells in addressing these lesions is geared toward restoring the wound's ability to heal, either by supplanting ineffective healing mechanisms or by augmenting muted physiological processes.

Inflammation

One of the proposed advantages of stem cell therapies is that their immunomodulatory effects can shift the wound equilibrium away from degradation toward tissue synthesis. In diabetic and venous ulcers for example, the cellular infiltrate and extracellular matrix demonstrate a lower $CD4^+$ (T-helper)/$CD8^+$ (cytotoxic) T-cell ratio relative to acute wounds, as well as increased B-cells and plasma cells.[47] This proinflammatory state can be reversed through BM-MSC suppression of T-lymphocytes[48] and B-cells.[49] However, the effects of chronic wounds on stem cells may impair their ability to modulate the wound microenvironment. For example, relative to acute wound fluid, chronic wound fluid is less chemotactic to ASCs and inhibits rather than stimulates their proliferation.[50]

Infection

The chronic inflammatory state is in part related to bacterial colonization. Interestingly, not only do MSCs decrease inflammation at the wound bed, they also enhance bacterial clearance and improve survival in sepsis via secretion of the antimicrobial peptide LL-37.[51,52] However, there may be limitations to this antimicrobial effect. Long-term bacterial colonization of chronic wounds is often facilitated by polysaccharide biofilms, which may negatively impact MSCs. Exposure to biofilm-conditioned media as well as isolated soluble biofilm factors alone are both sufficient to impair MSC migration and differentiation while promoting apoptosis.[53] These findings suggest that chronic wounds provide a suboptimal environment for transplanted stem cells, which may thus impede the utility of stem cell therapy in chronic wounds, at least in those with ongoing bacterial colonization with biofilm forming organisms.

Hypoxia

MSCs have a high tolerance for oxidative stress in vitro, which suggests that they are ideally suited to treating ischemic pathology, promoting tissue regeneration, and reducing reactive oxygen species burden.[54] ASC survival and ensuing tissue regeneration in nonvascularized fat grafts via adipogenesis and angiogenesis suggests this phenomenon also applies in vivo.[55] Additionally, MSCs may promote wound healing in response to hypoxia, increasing paracrine secretion of transforming growth factor-β_1, which in turn can restore fibroblast wound healing functionality inhibited under hypoxic conditions.[56] Despite tolerance, and even activation of stem cells in hypoxic environments, byproducts of tissue hypoxia may be detrimental to their regenerative capabilities. As an example, elevated lactate levels, as are found in chronic wounds, are associated with gene expression in MSCs associated with inflammation and apoptosis.[57]

Fibroblasts

Fibroblasts are fundamental to the wound-healing cascade, but in the diabetic wound environment they display decreased proliferation and migration.[47,58] However, restoration of fibroblast function is possible via paracrine signals from MSCs, such as those elucidated by Shabbir et al.[59] Exosomes secreted by MSCs are taken up by fibroblasts from both normal and diabetic wounds leading to increased cellular migration, with a greater increase observed in the chronic wound fibroblasts.[59]

Cytotoxicity

Patients undergoing chemotherapy experience suboptimal wound healing. Paclitaxel was found to be more cytotoxic to ASCs compared to fibroblasts,[60] suggesting patients

undergoing chemotherapy may demonstrate a diminished response to stem cell-based therapies. Conversely, in an off-label trial, plerixafor was found to be better than G-CSF at mobilizing hematopoietic stem cells in diabetic patients, despite being indicated for lymphoma and multiple myeloma.[61] Of note, however, is that the patients in this trial were not undergoing chemotherapy for cancer treatment, and thus they do not represent the traditional patient sample receiving this drug. Radiation is another cancer-related treatment with numerous side effects, including chronic ulcer formation. Using a rat model, Huang et al[62] demonstrated that ASCs have the ability to accelerate healing of these ulcers. Impairments in wound healing vary greatly across patients undergoing cancer therapy. This variability may lead to a wide variety of wound healing responses to stem cells, necessitating further study.

Stem cells harvested from patients with chronic disease

Further potential hurdles to autologous stem cell use in chronic wound therapy relate to the quality and quantity of cells harvested from patients with systemic disease. Systemic disease may be linked to depletion of angiogenic precursor cells both in the bone marrow and peripheral circulation.[63] In vitro experiments by Cianfarani et al[64] found ASCs isolated from diabetic mice to have lower proliferative and migration potential, muted stem cell surface marker expression, and less paracrine secretion of cytokines involved in wound healing, including vascular endothelial growth factor-A, hepatocyte growth factor, and insulin-like growth factor-1. Compared to ASCs harvested from nondiabetic mice, this also translates into a blunted improvement in diabetic wound healing.[64] Human ASCs harvested from ischemic limbs of diabetic patients also display muted functionality.[65] Similarly, bone marrow cells harvested from chronic wound patients and EPCs from diabetic patients demonstrate reduced growth in culture and diminished stem cell potency relative to healthy controls.[66,67] In stratifying patients receiving BMMNCs for CLI based on clinical outcome, Altaner et al[68] found that patients who responded to therapy have higher CD44 and CD90 MSC expression and greater interleukin-4 and interleukin-6 secretion. However, the diabetic status of the patients in this trial did not correlate with response,[68] which is surprising given recent studies showing diabetes impairs the ability of ASCs to promote neovascularization (via subpopulation depletion).[69] Not all studies have shown that chronic systemic disease impairs stem cell functionality. Smadja et al[70] noted that BM-MSCs harvested from CLI patients and healthy controls demonstrate similar proangiogenic effects when transplanted into CLI-induced mice. Increasing age and BMI are risk factors for chronic disease. Therefore, it is understandable that they also correlate negatively with stem cell yield and proliferative capacity.[71–73] Overall, the effects of long-term exposure to chronic pathophysiology on endogenous stem cell properties is likely quite variable, with corresponding differences in response to autologous cell therapy between patients. It is unfortunate that the patients most in need of stem cell-based wound therapies are also the least likely to have an optimal progenitor population to draw from.

Review of the clinical evidence

To the authors' knowledge, no systematic review has been conducted that includes all types of stem cells used to manage chronic wounds. Several meta-analyses of stem cell therapies of CLI exist, but other forms of chronic wounds are omitted. Inconsistencies across clinical trials are often cited as making statistical comparisons difficult, if not impossible. Our own findings echo this sentiment, with variations between studies that include (but are not limited to) type of stem cell; method of cell harvest, purification, expansion, and administration; number of cells administered; graft immunophenotype; targeted pathophysiology; nebulous outcome measures (eg, complete wound closure vs improved wound healing vs time for given reduction in wound area); and follow-up time. We have identified 45 published trials measuring the effects of stem cell therapies on chronic wound healing, the majority of which are early Phase I trials assessing safety and efficacy, without sample randomization or placebo control. Many patients with wounds refractory to treatment over months to years may be considered to provide fairly adequate internal historical controls, though the inherent decrease in validity precludes their incorporation into meta-analysis. The difficulty of conducting randomized controlled trials has also spurred some investigators, such as Lu et al,[20] to use contralateral ischemic limbs within the same patient as an internal control. Because of the overall small number of true randomized trials, these have sometimes been included in meta-analyses. This practice is likely to introduce confounding given the importance of paracrine-mediated MSC functionality.[10] Given the incredible variability in trial designs that include stem cell-based wound therapy, our literature search was systematic (Figure 2, Tables S1–S3), but statistical meta-analysis was not performed. The full list of relevant studies can be found in Table 1.

The type of cell used in chronic wound therapy varies widely across clinical trials, with different degrees of overlap

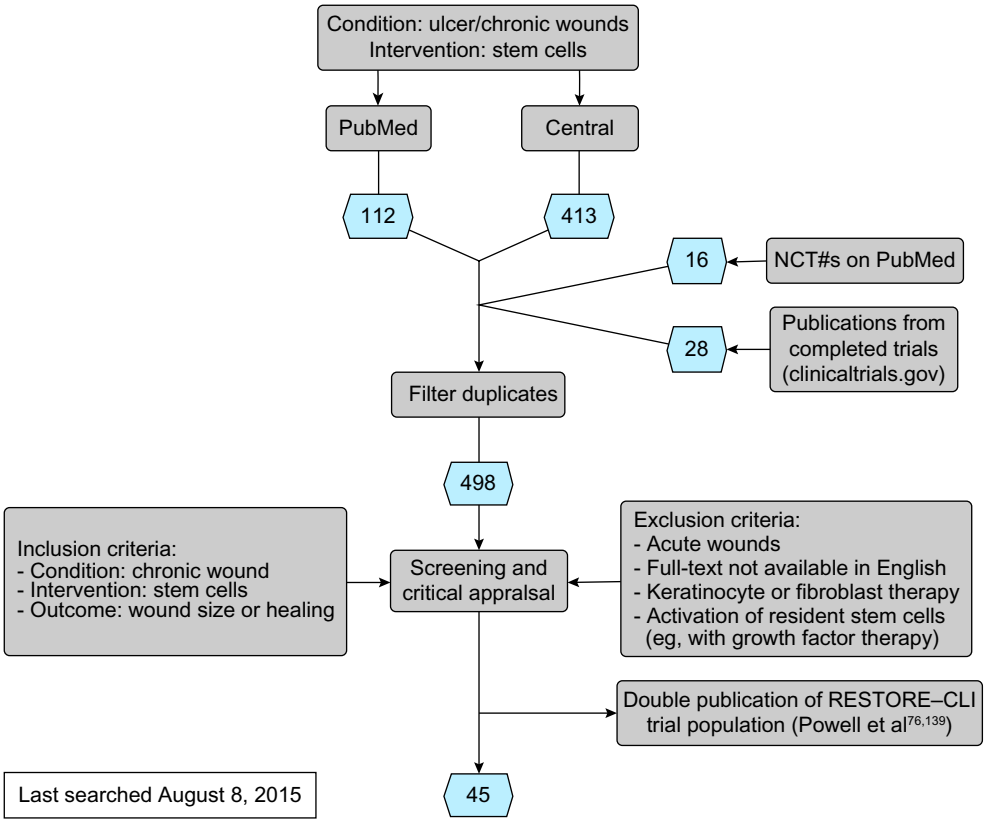

Figure 2 Literature search flowchart.
Abbreviations: NCT, National Clinical Trial; CLI, critical limb ischemia.

between them, further complicated by often-controversial classification standards. We have attempted to categorize the cell types used in the 45 trials identified as being relevant to the use of stem cells in chronic wound therapy (Figure 1, Table 1). BMMNCs are a frequently used source of progenitor cells for clinical use comprising a heterogeneous population that includes MSCs and EPCs. Numerous study protocols, including Huang et al,[74] harvest cells originally localized to the bone marrow, but which have been mobilized by G-CSF into the peripheral blood (characterizing them as PBMNCs), circumventing the need for more invasive bone marrow aspiration. Within a given cell population, certain studies also include populations enriched for particular markers, such as aldehyde dehydrogenase bright cells ($ALDH^{br}$), $CD90^{+}$, or $CD34^{+}$ cells.[75–77] MSCs are another population frequently used in clinical trials, sourced from various tissues including bone marrow, adipose tissue, and the umbilical cord. SVF represents an additional collection of progenitor cells, including MSCs and EPCs.[78]

Of the 45 selected trials, only 17 include independent placebo controls (3 additional studies relied on internal controls) (Table 1). To date, the JUVENTAS trial is the largest published randomized controlled trials utilizing stem cells in wound healing. Results from this study indicate that there is no statistical significance between the placebo and treatment groups.[79] The fact that this study categorized major amputations as nonhealing ulcers is likely to have skewed outcomes defined as complete wound healing relative to other studies, which traditionally exclude these patients from analysis. However, the measurements of wound area reduction, which did exclude patients who underwent amputation, also demonstrated a clinically insignificant advantage of BMMNC administration.

The two largest ongoing trials, NCT01245335 and NCT02099500, predict enrollments of 200 or more each, and utilize cells from bone marrow and adipose tissue, respectively. NCT01245335 is a double-blinded, randomized, placebo-controlled trial. Though it does not specifically include wound healing as an outcome, patients must have Rutherford Category 5 peripheral artery disease to qualify for enrollment, which is defined as minor tissue loss (nonhealing ulcer, focal gangrene with diffuse pedal ischemia).[80] Given that the primary outcome is improvement in Rutherford classification, this study will likely provide valid data as to the effect of bone marrow stem cells on wound healing in a much larger population than previously

Table 1 Published studies

Author	Graft	Cell type	Ulcer type	Route of administration	Sample size (Control)	Controlled trial	$P < 0.05$	Results
Holzinger et al[119]	Autologous	APC (PBMNC)	Ischemic, venous	Direct	PAD: 21; Venous: 12 (30)	Y	*	Complete wound healing in 29 of 33 ulcers at 4.6 ±1.9 weeks vs 17 of 30 ulcers in control group at 8.1±1.2 weeks ($P<0.01$)[a]. Direct application: topical
Huang et al[74]	Autologous	APC (PBMNC)	Diabetic/ischemic	IM	18 (18)	Y	*	Complete wound healing in 14 of 18 limb ulcers (77.8%) vs 8.9% (7 of 18) in control ($P=0.016$).
Ozturk et al[120]	Autologous	APC (PBMNC)	Diabetic/ischemic	IM	9 (8)	Y	*	Complete wound healing in 6 of 9 patients vs no change in control group ($P<0.01$)[a].
Losordo et al[77]	Autologous	APC (PBMNC-CD34$^+$)	Ischemic	IM	7 (7)	Y		Loss to follow-up in 4 treatment group patients and 5 controls at final timepoint prevented conclusion from being drawn.
Mohammadzadeh et al[121]	Autologous	APC (PBMNC)	Ischemic	IM	7 (14)	Y		Improved wound healing: wound area (cm^2) change over 3 months: treatment: 14.2±4.1 → 8.50±6.15 vs control: 15.8±17.0 → 18.16±23.20; $P=0.290$ for wound areas at follow-up.
Szabo et al[122]	Autologous	APC (PBMNC)	Ischemic	IM	8 (7)	Y		Complete wound healing in 2 and partial healing of 4 wounds at 3 months (vs 1 healed in control group). Complete wound healing in 3 of 8 in treatment group at 2 years.
Wettstein et al[32]	Autologous	APC (BM-CD34$^+$)	Pressure	Direct	3 (3)	(Y)		Wound volume decrease: 48%±3% (vs 40%±6% on the sham side) *Direct application: injection*
Wang et al[96]	Autologous	APC (PBMNC)	Ischemic	IM (+PNS IV)	27/25 [±PNS] (0)	N	"*"	Ulcer area improvement greater in PBMNC + PNS group vs PBMNC alone ($P=0.004$) at 16 weeks. No statistically significant difference in terms of number of patients with healed ulcers 8 of 27 (+PNS, 29.6%) vs 4 of 25 (–PNS, 16.0%; $P=0.244$).
Kawamoto et al[123]	Autologous	APC (PBMNC-CD34$^+$)	Ischemic	IM	unknown (0)	N		Improved wound healing: ulcer size significantly smaller at week 12 ($P=0.001$) than at baseline with no difference between 3 dosing groups. 17 patients included in study; number with chronic wounds not included.
Kinoshita et al[124]	Autologous	APC (PBMNC-CD34$^+$)	Ischemic	IM	TAO: 7 PAD: 3 (0)	N		Complete wound healing in 7 of 7 (TAO) and 2 of 3 (PAD) patients by week 52. Remaining PAD ulcer healed by week 208. No ulcer recurrence by week 208.
Mutirangura et al[125]	Autologous	APC (PBNM-CD34$^+$)	Ischemic	IM	6 (0)	N		Complete wound healing in 4 ulcers/amputation stump.
Tanaka et al[126]	Autologous	APC (PBMNC-CD34$^+$)	Diabetic	IM	5 (0)	N		Complete wound closure at average of 18 weeks

(*Continued*)

Table 1 (*Continued*)

Author	Graft	Cell type	Ulcer type	Route of administration	Sample size (Control)	Controlled trial	$P < 0.05$	Results
Bura et al[127]	Autologous	ASC	Ischemic	IM	6 (0)	N		Decreased ulcer number and size.
Lee et al[128]	Allogeneic	ASC	Ischemic	IM	9 (0)	N		Wound healing in 6 of 9 patients (66.7%).
Marino et al[129]	Autologous	ASC	Ischemic	Direct	10 (0)	N		Complete wound healing in 6 of 10 cases and decrease in diameter and depth in all cases. *Direct application: injection*
Cervelli et al[14]	Autologous	e-SVF	Mixed	Direct	10 (10)	Y	*	Improved wound healing: 97.9%±1.5% reepithelialisation (vs 87.8%±4.4% in control group; $P<0.05$). *Direct application: injection*
Dash et al[130]	Autologous	BM-MSC	Diabetic, ischemic (TAO)	IM + Direct	DFU: 3 (3); TAO: 9 (9)	Y	*	BuergerImproved wound healing: 97.9%±1.5% reepithelialisation (vs 87.8%±4.4% in control group; $P<0.05$). Direct application: injection's disease: 71% decrease in ulcer surface area (vs 23% in control group) at 12 weeks ($P<0.001$). Diabetic foot: 73% decrease as compared to a 30% decrease in control ($P<0.001$). *Direct application: injection + topical*
Gupta et al[131]	Allogeneic	BM-MSC	Ischemic	IM	7 (6)	Y		Complete wound healing in 6 of 7 patients in treatment group vs 6 of 6 in control.
Das et al[132]	Allogeneic	BM-MSC	Ischemic	IA	4 (0)	N		Improved wound healing ranging from complete healing to approximately 70% reduction in the ulcer area.
Falanga et al[93]	Autologous	BM-MSC	Diabetic, venous	Direct	6 (0)	N		Complete wound healing (n=1), 40% mean reduction in area (n=4), no change (n=1). A strong direct correlation was found between the number of cells applied (greater than 1×10^6 cells/cm^2 of wound area) and the subsequent decrease in chronic wound size (P=0.0058). *Direct application: fibrin polymer spray*
Ravari et al[133]	Autologous	BM-MSC	Diabetic	Direct	8 (0)	N		Complete wound healing in 3 out of 8 at 4 weeks (significantly reduced wound in remaining 5). *Direct application: injection + topical*
Vojtassak et al[134]	Autologous	BM-MSC	Diabetic	Direct + biograft	1 (0)	N		Decrease in wound size, increased vascularity and thickness of dermis at wound bed at 29 days. *Direct application: injection + topical*
Wang et al[135]	Allogeneic	UC-MSC	Dermatomyositis	IV	1 (0)	N		Improvement in chronic non-healing skin ulcers.
Li et al[136]	Autologous	BMMNC	Ischemic	IM	19 (17)	Y	*	Improved wound healing (>50% decrease in wound area) in 5 of 19 at 6 months (26% vs control 0 of 17 [0%]; P=0.047).

Walter et al[137]	Autologous	BMMNC	Ischemic	IA	40 (21)	Y	*	Improved wound healing (ulcer area [cm^2] 3.2±4.7 to 1.89±3.5; P=0.014 vs placebo, 2.92±3.5 to 2.89±4.1; P=0.5) at 3 months.
Jain et al[138]	Autologous	BMMNC	Diabetic	Direct	25 (23)	Y	"*"	Complete wound healing in 40% of cases (vs 29.2% in control group; $P<0.05$ – though 3 patients in treatment group [30% of this group's healed cases] and 1 control patient [14.3% of healed wounds in this group] had a skin graft). Improved wound healing: average wound area reduction of 17.4% at 2 weeks (vs 4.84% in control group; $P<0.05$). Difference at 12 weeks not statistically significant (36.4% treatment vs 27.32% control group; $P>0.05$). *Direct application: injection + topical*
Powell et al[76]	Autologous	BMMNC-CD90$^+$	Ischemic	IM	29 (16)	Y	"*"	Time to failure longer in treatment group vs control ($P<0.0001$). Treatment failure events: 13 of 29 in treatment group vs 14 of 16 in control group (P=0.0098). *Wound healing not considered independently.*
Powell et al[139]	Autologous	BMMNC-CD90$^+$	Ischemic	IM	13 (6)	Y		Complete wound healing in 31% of patients at 12 months (vs 13% in control group; $P>0.05$).
Barc et al[140]	Autologous	BMMNC	Ischemic	IM	14 (15)	Y		Complete wound healing in 5 of 14 cases (8 of 14 showed improvement vs 3 of 15 in control group; P=0.06[a]).
Teraa et al[79]	Autologous	BMMNC	Ischemic	IA	51 (50)	Y		No treatment-related trends in terms of wound healing observed postinjection at 6 or 12 months, including between high and low dose groups. Complete wound healing in 19 out of 51 (37%) at 6 months (vs 14 out of 50 [28%]; RR =1.33; 95% CI, 0.75–2.35). Ulcer area tended to decrease in both groups without a difference between the groups (difference in ulcer area at 6 months –0.1 cm^2; 95% CI, –6.1 to 5.9)
Franz et al[141]	Autologous	BMMNC	Ischemic	IA	8 (0)	N		Complete wound healing in 5 of 8 patients at 3 months.
Kirana et al[89]	Autologous	BMMNC vs BMMNC-CD90$^+$	Diabetic/ischemic	IM & IA	12 / 10 (0)	N		Complete wound healing in 10 of 12 (83%) BMMNC group patients vs 8 of 10 in CD90$^+$ enriched group; (P=0.47). No statistical difference between routes of administration.

(*Continued*)

Table 1 (*Continued*)

Author	Graft	Cell type	Ulcer type	Route of administration	Sample size (Control)	Controlled trial	$P < 0.05$	Results
Klepanec et al[88]	Autologous	BMMNC	Ischemic	IM vs IA	IM: 13 IA: 14	N		Improved wound healing in 27 of 37 cases at 6 months. No difference between routes of administration.
Matoba et al[84]	Autologous	BMMNC	Ischemic (include TAO)	IM	PAD 43; TAO 28 (0)	N		Improved wound healing – median area (cm^2) decreased after 6 months ($P<0.001$): PAD: 3.5 (range 0–77) → 0.0 [# patients with ulcers 43 →17] TAO: 1.8 (range 0–35) → 0.0 [# patients with ulcers 28 →15] Wounds remained at the smaller size at 2-year follow-up.
Napoli et al[142]	Autologous	BMMNC	Ischemic	IA	7 (0)	N		Improved wound healing (≥1 cm decrease of the ulcer) in 6 of 7 patients (at 6–12 months).
Perin et al[75]	Autologous	BMMNC vs ALDH^{br}	Ischemic	IM	BMMNC: 4 ALDH^{br}: 2 (0)	N		No significant changes from baseline noted in ischemic ulcer grade.
Procházka et al[143]	Autologous	BMMNC	Ischemic	IM	42 (0)	N		Complete wound healing in 28 of 42 patients at 90 days.
Ruiz-Salmeron et al[144]	Autologous	BMMNC	Diabetic/ischemic	IA	10 (0)	N		Complete wound healing in 9 of 10 patients at 12 months.
Sarasua et al[145]	Autologous	BMMNC	Pressure	Direct	22 (0)	N		Complete wound healing in 19 of 22 patients (86.36%) after an average of 21 days. During a mean follow-up of 19 months, none of the resolved ulcers recurred. *Direct application: topical*
Takagi et al[146]	Autologous	BMMNC	Systemic sclerosis	IM	11 (0)	N		Complete wound healing in 9 of 11 patients at 2-year follow-up. *Skin grafting was performed over ulcers in addition to IM injection in all but one case*
Dubsky et al[147]	Autologous	BMMNC or PBMNC	Diabetic/ischemic	IA	31 PTA: 30 (23)	Y	*	Complete wound healing in 84% of patients (BMMNC and PBMNC groups combined) at 12 months (vs 57.7% in PTA group and 44.4% in control; $P=0.042$).
Dubsky et al[148]	Autologous	BMMNC or PBMNC	Diabetic	IM	25 (18)	Y	*	Complete wound healing in 14 of 25 (56%) combined treatment group patients at 6 months (vs 3 of 18 [16.7%] in control group; $P=0.0093$).

Lu et al[20]	Autologous	BMMNC vs BM-MSC	Diabetic/ischemic	IM	BMMNC: 11 BM-MSC: 11 ("21")	(Y)	(*)	Ulcer healing rate (defined as number of patients with healed ulcers/total patients in group) higher in BMMNC group vs BM-MSC and control group: Complete wound healing in 6 of 11 BM-MSC group vs 2 of 21 in control; *P*=0.006 at 4 weeks. Complete wound healing in 11 of 11 BMMNC group vs 11 of 21 in control; *P*=0.001 at 12 weeks. Complete wound healing in 10 of 11 BM-MSC group vs 5 of 11 in BMMNC; *P*=0.022 at 6 weeks. Complete healing of all ulcers occurred at 8 and 12 weeks in BM-MSC and BMMNC groups, respectively.
Huang et al[74]	Autologous	BMMNC vs PBMNC	Ischemic	IM	BMMNC: 74 PBMNC: 76 (0)	N		No difference in ulcer healing between the different cell therapies.
Lasala et al[150]	Autologous	BMMNC + BM-MSC	Ischemic	IM	8 (0)	N		Significant improvement or complete wound healing in 6 of 8 patients.
Jimenez et al[151]	Autologous	Hair follicles	Mixed	Direct	6 ("6")	(Y)	(*)	Improved wound healing: 27.1% reduction of ulcer area within experimental square at 18 weeks (vs 6.5% in control square; *P*=0.046). *Direct follicular graft transplant*

Notes: [a]When not stated in original publication, clinical outcomes were analyzed with Fisher's exact test to calculate *P*-value. (Y), Internal control used eg, different area of wound or contralateral limb; (*), statistically significant but used internal control; "*", statistical significance not directly related to improved wound healing in cell therapy group vs control.

Abbreviations: Y, yes; N, no; ALDH^{br}, aldehyde dehydrogenase bright cell; APC, angiogenic progenitor cell; ASC, adipose-derived stem cell; BMMNC, bone-marrow mononuclear cell; BM-MSC, bone-marrow mesenchymal stem cell; DFU, diabetic foot ulcer; e-SVF, enhanced stromal vascular fraction; IA, intra-arterial; IM, intramuscular; IV, intravenous; PAD, peripheral arterial disease; PBMNC, peripheral blood mononuclear cell; PBNM, peripheral blood non-mobilized cells; PNS, *Panax notoginseng* saponins; PTA, percutaneous transluminal angioplasty; TAO, thromboangiitis obliterans (Buerger's disease); UC-MSC, umbilical cord mesenchymal stem cell.

studied. While NCT02099500 addresses the relative scarcity of published clinical trials using ASCs for chronic wound therapy and includes a large sample size, this trial lacks a placebo control arm. Overall, with regard to large studies involving placebo controls, 4 of 11 ongoing trials with an enrollment ≥50 do not have a placebo or sham control arms (NCT02099500, NCT01903044, NCT02089828, and NCT01456819).

Comparisons of published studies with ongoing or unpublished studies (Table 2) demonstrate a shift in focus (Figure 3). The relative increase in studies utilizing ASCs coincides with a more recent appreciation for the higher yield

Table 2 Unpublished studies

NCT Number	Recruitment	Enrollment	Diabetes	Ischemia	Venous	Pressure	Hypertensive	Intervention
NCT01595776	Completed	8		✓				Autologous APC/ PBMNC
NCT00919516	Completed	49		✓				Autologous BMMNC
NCT00883870	Completed	20		✓				Allogeneic BM-MSC
NCT00221143	Completed	15			✓			Autologous APC
NCT01065337	Completed	30	✓	✓				Autologous BMMNC
NCT00616980	Completed	28		✓				Autologous APC
NCT00523731	Completed	6		✓				Autologous APC
NCT00392509	Completed	20		✓				Autologous BMMNC vs $ALDH^{br}$
NCT00468000	Completed	86		✓				Autologous BMMNC ($CD90^{+}$ enriched)
NCT01232673	Completed	96	✓	✓				Autologous BMMNC
NCT00872326	Completed	20	✓	✓				Autologous BMMNC
NCT00371371	Completed	160		✓				Autologous BMMNC
NCT00282646	Completed	40		✓				Autologous BMMNC
NCT00535548	Completed	3				✓		Autologous APC
NCT00677404	Completed	20		✓				Autologous BMMNC
NCT00797056	Completed	32		✓				Autologous APC
NCT01584986	Completed	22		✓				Autologous APC
NCT01480414	Completed	20		✓				Autologous BMMNC
NCT01408381	Completed	38		✓				Autologous BMMNC
NCT00955669	Completed	40	✓	✓				Autologous BMMNC vs BM-MSC
NCT02287831	Active, not recruiting	30	✓	✓				Allogeneic UC-MSC
NCT01745744	Active, not recruiting	33		✓				Autologous ASC
NCT01049919	Active, not recruiting	152		✓				Autologous BMMNC
NCT01472289	Active, not recruiting	15		✓				Autologous BMMNC
NCT01245335	Active, not recruiting	210		✓				Autologous BMMNC
NCT01751282	Active, not recruiting	66	✓	✓	✓	✓	✓	Autologous BMMNC in fibrin spray
NCT01305863	Active, not recruiting	60		✓				Device: ASC coated ePTFE vascular graft
NCT02394886	Recruiting	5	✓	✓				Allogeneic ASC
NCT01484574	Recruiting	126		✓				Allogeneic BM-MSC
NCT01932021	Recruiting	10					✓	Autologous adipose tissue graft
NCT02474381	Recruiting	60	✓					Autologous APC
NCT02454231	Recruiting	38		✓				Autologous APC vs BMMNC
NCT02099500	Recruiting	200		✓				Autologous ASC
NCT02092870	Recruiting	25	✓	✓	✓	✓	✓	Autologous ASC
NCT01937416	Recruiting	10	✓	✓				Autologous BMMNC

(*Continued*)

Table 2 (*Continued*)

NCT Number	Recruitment	Enrollment	Diabetes	Ischemia	Venous	Pressure	Hypertensive	Intervention
NCT01572376	Recruiting	30				✓		Autologous BMMNC
NCT01456819	Recruiting	50		✓				Autologous BMMNC ± BM-MSC
NCT02089828	Recruiting	50		✓				Autologous $CD34^+$ enriched vs PBMNC
NCT02304588	Recruiting	20	✓	✓				Autologous MSC
NCT01833585	Recruiting	10		✓				Autologous PBMNC
NCT02145897	Recruiting	60		✓				Autologous SVF vs ASC
NCT01916369	Recruiting	9		✓				CTX DP (Human neural stem cell product)
NCT01686139	Not yet recruiting	10	✓	✓				Allogeneic BM-MSC
NCT01558908	Not yet recruiting	15		✓				Allogeneic Endometrial-MSC
NCT01353937	Not yet recruiting	27	✓	✓				Autologous APC
NCT02375802	Not yet recruiting	12				✓		Autologous ASC
NCT02477540	Not yet recruiting	10		✓				Autologous BM-MSC
NCT01903044	Not yet recruiting	60		✓				Autologous BMMNC

Notes: Results of clinicaltrials.gov search showing ongoing trials or unpublished studies pertaining to stem cell therapies in patients with chronic wounds, verified within the last 4 years.

Abbreviations: $ALDH^{br}$, aldehyde dehydrogenase bright cell; APC, angiogenic progenitor cell; ASC, adipose-derived stem cell; BMMNC, bone-marrow mononuclear cell; BM-MSC, bone-marrow mesenchymal stem cell; SVF, enhanced stromal vascular fraction; PBMNC, peripheral blood mononuclear cell; UC-MSC, umbilical cord mesenchymal stem cell; ePTFE, expanded polytetrafluoroethylene.

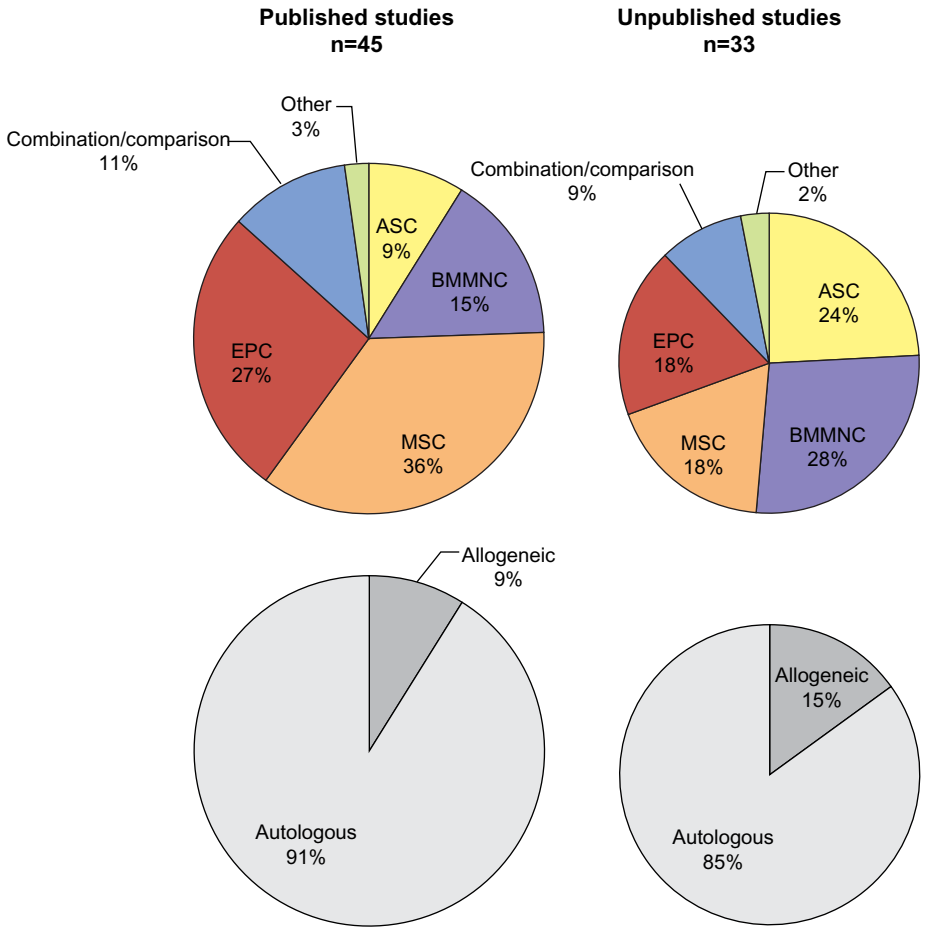

Figure 3 Relative proportions of stem cell population use in analyzed published and unpublished clinical studies.

Note: Chart area is proportional to sample size.

Abbreviations: ASC, adipose-derived stem cell; EPC, endothelial progenitor cell; MSC, mesenchymal stem cell; BMMNC, bone marrow mononuclear cell.

of readily accessible stem cells existing within adipose tissue. Harvesting these cells from what is generally classified as biohazardous waste following liposuction may allow patients to forgo painful bone marrow aspiration. Additionally, BMMNCs appear to be favored in ongoing or unpublished trials, potentially due to several studies documenting the superiority of more heterogeneous cell transplants.[19–21] The number of studies and their respective sample sizes receiving allogeneic cells is also greater among ongoing trials, reflecting an understanding that autologous stem cell potency may be blunted in patients with chronic systemic illness. Conversely, allogeneic cells have been shown to significantly enhance diabetic wound healing.[81]

Taken together, the published studies demonstrate that stem cell therapies can indeed lead to healing of chronic wounds resistant to traditional therapy. Meta-analyses corroborate this impression; most recently Liew et al[82] calculated an odds ratio of 2.90 (95% confidence interval [CI], 1.44–5.82) when comparing stem cell therapies with control treatment for complete ulcer healing. However, the 2015 meta-analysis by Liu et al[83] suggests that this benefit may wane with longer follow-up times. While there is sufficient evidence to support the belief that stem cells improve chronic wound healing in clinical trials, the limited number of placebo control groups and inconsistent means of reporting wound healing (eg, using median wound area[84] rather than complete wound closure) prevents us from establishing the true extent of this benefit. In our literature search, 9 of the 17 placebo-controlled trials showed a statistically significant improvement in wound healing with cell therapy. Interestingly, these studies include an array of methods to administer cells (intramuscular, intra-arterial, and direct application) and cell populations (PBMNCs, SVF, BM-MSCs, and BMMNCs). This suggests that many tissues and extraction methods offer means of reliably harvesting cells that can augment the healing process.

Improving stem cell yield, efficacy, and lifespan – current and future techniques

Unfortunately, though MSCs have demonstrated an ability to improve wound healing, their lifespans are short in vitro, reducing the efficacy of ex vivo expansion. Furthermore, they demonstrate suboptimal engraftment, survivability, and retention at the wound when transplanted,[85,86] with several of the underlying mechanisms discussed previously. Many studies rely on intradermal or intramuscular injection to administer stem cells in suspension. Though technically simple, there is a relative loss of therapeutic efficacy, potentially caused by subsequent anoikis in the absence of cell–matrix attachment or shear forces during the injection.[87] When compared, stem cells delivered intramuscularly and intra-arterially demonstrate no significant difference in terms of improved wound healing.[88,89] Conversely, intravenous administration of stem cells is uncommon in chronic wound therapy because of cell entrapment in the pulmonary vasculature (the pulmonary "first-pass" effect).[90] Stem cell localization to the wound bed is notably impaired in chronic (but not acute) wounds partly due to downregulated stromal cell-derived factor 1 (a chemokine attracting MSC to wound bed) secondary to uncontrolled inflammation.[91] The resulting decrease of ASC migration to the wound bed further elaborates on the difficulties of systemic stem cell therapy for chronic wound healing, necessitating consideration when developing mechanisms of administration for translational medicine. Advances in stem cell surface modification offer a potential solution to this problem by targeting cells to specific tissues.[92]

Poor cell engraftment and survivability are problematic considering the dose–effect relationship observed by Falanga et al,[93] though the number of administered cells has not universally been shown to correlate with response.[77] Defining dose in heterogeneous cell populations can also be difficult, but perhaps subpopulation composition may be less relevant (given BMMNC:MSC ratios resulted in similar clinical improvements).[94] Efficacy is most likely related to a minimum required dose,[93] while the lack of consensus may be related to inconsistent methodologies (eg, wound type, cell type, harvest, expansion, and administration). Increasing stem cell potency with adjuvants such as platelet-rich plasma or *Panax notoginseng* saponins are possible alternatives for decreasing the minimum required dose.[95,96]

Means of prolonging transplanted cell lifespan are now heavily sought, as MSCs must survive to influence healing. Numerous possibilities have arisen, such as hyperoxic and pan-caspase pretreatment, which reduces MSC apoptosis in ischemic microenvironments.[97] Hypoxic preconditioning has also been shown to increase paracrine secretion by MSCs.[98] Mohanty et al[99] showed that small molecule-induced prion protein upregulation also results in increased lifespan and yield of MSCs in culture, as well as improved engraftment. Gene therapy provides further opportunity, such as protein kinase G1α overexpression via adenovirus vector to promote MSC survival.[100] Low-level light irradiation is another recent tool for increasing stem cell wound healing potency.[101]

Endogenous wound healing pathways provide further means by which to optimize MSC survival. In the presence of proapoptotic cytokines (FasL, ubiquitous in chronic

wound microenvironments), endothelial growth factor (EGF) molecules tethered to growth scaffolds demonstrated an ability to improve MSC survival via activation of the EGF-receptor.[102] Surface-tethered EGF generated a superior MSC response relative to saturating concentrations of soluble EGF,[103] supporting the use of other endogenous mediators such as the matrix protein Tenascin-C combined with biosynthetic scaffolds to enhance survival of transplanted MSCs.[104] Such combinations may reduce the inflammatory response to the scaffolds themselves.[105]

Scaffolds are valuable additions to stem cell-based wound therapy as they provide an external niche for transplanted cells outside of the hostile wound environment, while still allowing them to facilitate wound healing. In an excisional wound model, Rustad et al[87] showed both a faster time to complete wound closure and a return of skin appendages in wounds treated with a biomimetic pullulan–collagen hydrogel scaffold seeded with MSCs. Moreover, this hydrogel led to longer MSC viability, increased engraftment efficiency, and enhanced angiogenesis. Clinically, Yoshikawa et al[106] used a composite graft of BM-MSCs incorporated into a collagen sponge to successfully treat decubitus ulcers refractory to artificial skin grafting. ASCs embedded in silk fibroin scaffolds and fibrin gels have demonstrated a similar ability to accelerate wound healing in vivo.[107,108] Autologous MSCs applied with a fibrin spray system also resulted in some improvement in patients with chronic ulcers.[93] Scaffolds offer a viable means for enhancing stem cell engraftment and survivability. It is therefore likely that their incorporation into cell-based therapies will increase markedly in the near future.

Clinical implementation of cell-based therapies has opened a new frontier in the development of biomedical devices aimed at optimizing current therapies.[109] The fundamental risk of contamination associated with cell products has spurred the development of closed systems for harvesting and/or culturing cells.[110] Widespread clinical use also necessitates scalable technologies, such as tissue bioreactors.[111] Increased automation of stem cell harvest, isolation, and expansion will allow for more standardized therapies, and subsequently more generalizable results. Novel approaches to cell population characterization such as kinome analysis may also improve clinical efficacy, or at least provide a better measure of prognosis.[112]

Promoting stem cell yield, survival, and efficacy at the wound bed are worthy goals. However, it is also possible that truly successful chronic wound therapy requires a deeper understanding of how the various types of stem cell therapies can modulate systemic pathophysiology. For example, in type 2 diabetes, inhibiting the local proinflammatory phenotype at the wound bed may be insufficient to completely restore wound healing; restoration of an anti-inflammatory M1/M2 macrophage equilibrium is required to allow for physiological wound healing.[113]

Limitations

Some of the limitations of stem cells in wound therapies have already been discussed, such as phenotypic drift in culture, heterogeneity of cell populations, and the variable quality of cells depending on their source. Beyond barriers to therapeutic efficacy, there are also potential risks, as is the case with any medical intervention. The possibility of malignant transformation exists whenever stem cells are transplanted. While this may be a greater risk with pluripotent iPSCs, study of the more commonly used multipotent MSCs has generated less of a concern. While malignant transformation has been observed in long-term culture,[114] the larger body of evidence suggests that the risks of malignant transformation are low, especially prior to MSCs undergoing senescence.[115] Follow-up to one of the original studies thought to demonstrate spontaneous MSC transformation has since shown a small number of malignant cells to be the culprit.[116] Furthermore, the beneficial immunomodulatory properties of stem cells are also not without theoretical risks. MSC immunomodulation and homing to different target organs can increase risks of opportunistic or disseminated infections, as well as susceptibility to malignancy.[117,118] Finally, transplant of biological material also carries risks of directly transmitting infectious agents.[117] Overall, clinical trials demonstrate that stem cell applications to wound healing are safe, but physicians must continue to reevaluate the risks and benefits of their use as the results of more long-term follow-up studies are published.

Conclusion

Stem cells can provide the next step in advancing wound care, particularly for chronic wounds resistant to current therapies. Meta-analyses consistently show that stem cells provide a safe and effective means for promoting chronic wound healing. However, the statistically similar improvement observed in both trial arms of the large JUVENTAS study offers a solid reminder of the importance of placebo controls for measuring the true efficacy of stem cell therapy. As development of commercial devices for stem cell therapies increases, standardization of protocols will allow for greater study validity. Therapies can then be fine-tuned and catered to specific pathologies, whereas currently the different stem cell

populations and routes of administration provide only roughly comparative results across several head-to-head studies. As we move forward, stem cells are likely to become a common tool available to clinicians for wound management, but clinical practice must follow evidence of safety and efficacy, so frequent reevaluation of the literature will be critical as new therapies are described.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary Materials

Table S1 CENTRAL search strategy

#1	Pressure ulcer* or decubitus ulcer* (Word variations have been searched)
#2	Leg ulcer* (Word variations have been searched)
#3	Skin ulcer* or cutaneous ulcer* (Word variations have been searched)
#4	Varicose ulcer* or varicose or venous hypertension ulcer* or hypertension ulcer* or venous hypertension or venous stasis or stasis ulcer* or venous stasis ulcer* or stasis (Word variations have been searched)
#5	Foot ulcer* or foot* or plantar ulcer* or plantar (Word variations have been searched)
#6	Carotid ulcer* (Word variations have been searched)
#7	Diabetic foot or foot ulcer* or diabetic ulcer* or diabetic foot ulcer* (Word variations have been searched)
#8	Chronic wound* (Word variations have been searched)
#9	Vasculit* and ulcer (Word variations have been searched)
#10	(ischemi*) near ulcer* (Word variations have been searched)
#11	MeSH descriptor: [Ulcer] explode all trees
#12	(ulcer*) near/3 (healing or size or area or number or reduc*) (Word variations have been searched)
#13	(wound*) near/3 (healing or size or area or number or reduc*) (Word variations have been searched)
#14	{or #1-#13}
#15	MeSH descriptor: [Stem Cell Transplantation] explode all trees
#16	MeSH descriptor: [Stem Cells] this term only
#17	MeSH descriptor: [Hematopoietic Stem Cells] explode all trees
#18	MeSH descriptor: [Mesenchymal Stromal Cells] explode all trees
#19	MeSH descriptor: [Bone Marrow Cells] explode all trees
#20	MeSH descriptor: [Induced Pluripotent Stem Cells] explode all trees
#21	(mononuclear or endothelial or mesenchymal) near/3 cell*:ti,ab,kw (Word variations have been searched)
#22	(stem or progenitor or precursor or therap*) near/3 cell*:ti,ab,kw (Word variations have been searched)
#23	((embryo* or fetal or foetal or umbilical or marrow or cord) near/5 cell*):ti,ab,kw (Word variations have been searched)
#24	(BM-MNC* or PB-MNC* or AT-MSC*):ti,ab,kw (Word variations have been searched)
#25	(adipose or adipose derived) near/5 (stem cell* or cell*):ti,ab,kw (Word variations have been searched)
#26	{or #15-#25}
#27	#14 and #26

Abbreviations: BM-MNC, bone marrow mononuclear cell; PB-MNC, peripheral blood mononuclear cell; AT-MSC, adipose tissue-derived mesenchymal stem cells.

Table S2 PubMed search strategy

	Search Query
#1	Search (pressure ulcer*[Title/Abstract] OR decubitus ulcer*[Title/Abstract])
#2	Search (skin ulcer*[Title/Abstract] OR cutaneous ulcer*[Title/Abstract])
#3	Search leg ulcer*[Title/Abstract]
#4	Search (varicose ulcer*[Title/Abstract] OR varicose[Title/Abstract] OR venous hypertension ulcer*[Title/Abstract] OR hypertension ulcer*[Title/Abstract] OR venous hypertension[Title/Abstract] OR venous stasis[Title/Abstract] OR stasis ulcer*[Title/Abstract] OR venous stasis ulcer*[Title/Abstract] OR stasis[Title/Abstract])
#5	Search (foot ulcer*[Title/Abstract] OR foot*[Title/Abstract] OR plantar ulcer*[Title/Abstract] OR plantar[Title/Abstract])
#6	Search carotid ulcer*[Title/Abstract]
#7	Search (diabetic foot[Title/Abstract] OR foot ulcer*[Title/Abstract] OR diabetic ulcer*[Title/Abstract] OR diabetic foot ulcer*[Title/Abstract])
#8	Search chronic wound*[Title/Abstract]
#9	Search (vasculit*[Title/Abstract] AND ulcer[Title/Abstract])
#10	Search ischemic ulcer*[Title/Abstract]
#11	Search (#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10)
#12	Search Stem Cell Transplantation[MeSH Terms] OR Stem Cell Transplantation[Title/Abstract]
#13	Search Stem Cells[MeSH Terms] OR Search Stem Cells[Title/Abstract]
#14	Search Hematopoietic Stem Cells[MeSH Terms] OR Hematopoietic Stem Cells[Title/Abstract]
#15	Search Mesenchymal Stromal Cells[MeSH Terms] OR Mesenchymal Stromal Cells[Title/Abstract]
#16	Search Bone Marrow Cells[MeSH Terms] OR Bone Marrow Cells[Title/Abstract]
#17	Search (mononuclear cells[Title/Abstract] OR mesenchymal stem cells[Title/Abstract] OR bone marrow transplantation[Title/Abstract] OR adult stem cells[Title/Abstract] OR iPS cells[Title/Abstract] OR adipose derived stem cell[Title/Abstract])

(*Continued*)

Table S2 (*Continued*)

	Search Query
#18	Search (BM-MNC*[Title/Abstract] OR PB-MNC*[Title/Abstract] OR AT-MSC*[Title/Abstract] OR ASC[Title/Abstract] OR iPS[Title/Abstract])
#19	Search (#12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18)
#20	Search (#11 AND #19)
#21	Search clinical trial[Publication Type]
#22	Search (#20 AND #21)

Abbreviations: iPS cells, induced pluripotent stem cells; BM-MNC, bone marrow mononuclear cell; PB-MNC, peripheral blood mononuclear cell; AT-MSC, adipose tissue-derived mesenchymal stem cells; ASC, adipose-derived stem cell.

Table S3 clinicaltrials.gov search strategy

Conditions	Wound OR "Chronic wound" OR Ulcer OR Gangrene OR Ischemia OR Peripheral Vascular Diseases
Interventions	Stem cells OR MSC OR ASC OR mononuclear OR iPS
Results	697 studies found of which 48 were found to be relevant to chronic wound healing with stem cell-based therapies and verified within last 4 years (if incomplete)

Abbreviations: iPS cells, induced pluripotent stem cells; MSC, mesenchymal stem cells; ASC, adipose-derived stem cell.