Stem Cells and Healing: Impact on Inflammation

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Significance: The number of patients with nonhealing wounds has rapidly accelerated over the past 10 years in both the United States and worldwide. Some causative factors at the macro level include an aging population, epidemic numbers of obese and diabetic patients, and an increasing number of surgical procedures. At the micro level, chronic inflammation is a consistent finding.

Recent Advances: A number of treatment modalities are currently used to accelerate wound healing, including energy-based modalities, scaffoldings, the use of mechano-transduction, cytokines/growth factors, and cell-based therapies. The use of stem cell therapy has been hypothesized as a potentially useful adjunct for nonhealing wounds. Specifically, mesenchymal stem cells (MSCs) have been shown to improve wound healing in several studies. Immune modulating properties of MSCs have made them attractive treatment options.

Critical Issues: Current limitations of stem cell therapy include the potentially large number of cells required for an effect, complex preparation and delivery methods, and poor cell retention in targeted tissues. Comparisons of published in-vitro and clinical trials are difficult due to cell preparation techniques, passage number, and the impact of the micro-environment on cell behavior.

Future Directions: MSCs may be more useful if they are preactivated with inflammatory cytokines such as tumor necrosis factor alpha or interferon gamma. This article will review the current literature with regard to the use of stem cells for wound healing. In addition the anti-inflammatory effects of MSCs will be discussed along with the potential benefits of stem cell preactivation.

SCOPE AND SIGNIFICANCE
The purpose of this review is to provide the reader with a brief understanding of the causative factors for nonhealing wounds and to understand how stem cells may play a therapeutic role. The review covers some basic definitions of stem cells but focuses primarily on the use of mesenchymal stem cells (MSCs). The authors reviewed relevant papers over the past 10 years that were published in English; however, this is not an exhaustive review. A review of important animal and clinical trials are described in which MSCs are used to heal wounds and/or to improve the quality of healing. The immune modulating features of MSCs are described, in particular the anti-inflammatory effects that MSCs have on the wound healing process.

TRANSLATIONAL RELEVANCE
As in any other area of scientific advancement, therapeutic approaches for wound healing involving MSCs are based on a number of critical prior scientific streams from related fields. Biomedical engineering research has led to the development of scaffoldings that can mimic a natural endogenous dermal structure.
These advances allow for a more efficient delivery of stem cells into the wound environment. The advancements in stem cell research highlighted potential therapeutic options, including the ability of stem cells to directly differentiate into specific cells as well as their ability to provide necessary cues for the recruitment of different cell types needed in the regenerative process. Basic science research in the area of fibrosis and scar formation highlighted the importance of chronic inflammation and the negative impact of an over active repair process. Investigators studying various organ systems report similar unifying biochemical processes involving tissue repair and regeneration. Fetal healing research led to the understanding that a limited inflammatory response could lead to scarless healing. Using a systems biology approach, the MSC has been targeted as an ideal candidate to assist in the healing process. The basic science research in each of the previously mentioned scientific areas has an immediate potential bench to bedside application.

CLINICAL RELEVANCE

Currently, there are numerous therapeutic options for the treatment of nonhealing wounds but very little supporting evidence. Despite the growing number of treatments, there is only a 50–60% healing rate in most clinical applications with equally high rates of recidivism. The process of tissue repair and scar formation has negative clinical implications in heart, lung, liver, and brain tissue, in addition to dermal wound healing. As observed in fetal healing, the optimal regenerative healing environment is possible when inflammation is minimized but not absent. Systemic treatments aimed at global immune suppression can improve healing, but the quality of healing may be compromised through weakened scar formation. MSCs can “sense” the degree of inflammation in the microenvironment and respond by release of growth factors and cytokines to reduce the inflammatory process using real-time biochemical cues. If effective, reducing inflammation to an appropriate level to allow healing to proceed should also result in improved tensile strength and scar quality, thereby reducing recidivism.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Wound healing: normal process and factors impeding healing

Wound healing requires the successful completion of an orchestrated series of tightly controlled biochemical and cellular events to achieve healing. Overlapping phases of hemostasis, inflammation, proliferation, and remodeling are common to wounds of all etiologies. A chronic wound develops when a wound fails to heal within an expected time frame and fails to achieve functional closure. There are many factors that impede healing, including co-morbid clinical conditions, aging, poor tissue perfusion, malnutrition, unresolved pressure to the surface of the wound, immune suppression, malignancy, infection, obesity, and a number of medications. The usual patient with a nonhealing wound has a combination of several of the factors mentioned earlier, making any one therapeutic option unlikely to succeed. One common thread with almost all nonhealing wounds is a persistent inflammatory state. Macrophages, known to mediate inflammation, influence healing in a positive way through increasing angiogenesis, decreasing bacterial loads, phagocytosing debris, and providing matrix deposition. If, however, a persistent inflammatory state develops in which the macrophages are dysregulated and become skewed toward a type I inflammatory phenotype, there is an increase in inflammatory cytokine release, increased protease secretion, and a reduction in endogenous local growth factors, impeding progress toward wound repair and regeneration. Current therapeutic approaches for patients with nonhealing wounds start with medical stabilization of the patient, including the correction of any and all possible underlying co-morbid conditions listed earlier. Standard of care also includes debridement of any nonviable tissue and the use of dressings that provide a moist healing environment. Both synthetic and biological scaffolding have been implanted into the wound bed in an effort to recreate a favorable environment for native cells to infiltrate and accelerate the healing process. Applying external strain to the wound, as occurs in nature, has also been used over the past decade with increasing regularity. While each of these components are required to achieve healing, in nature, they proceed simultaneously instead of in the sequential method in which clinicians currently apply them. This concept was summarized nicely in a paper by Gurtner et al. (Fig. 1). Another potential explanation for the nonhealing wound is the presence of intrinsically dysfunctional or senescent cells that are incapable of responding to normal biochemical signals. These findings have led investigators to consider the use of cell-based therapy as a solution for the chronic nonhealing wound. Allogeneic cells have been used for the treatment of venous ulcers...
and diabetic foot ulcers with good success. Initially thought to be replacements for autologous skin grafts, biological skin substitutes are now known to act in a paracrine fashion, stimulating the wound to progress toward healing. Worldwide economic pressures lead to an increasing focus on not only clinical outcomes but also achieving these outcomes in a cost-effective patient-centered manner. A more regenerative healing process should also theoretically lead to decreased wound recidivism compared with the usual process of tissue repair, which is traditionally marked by scar formation.

Epidermal cellular therapies versus stem cells: strengths and weaknesses

Coverage for wounds with autologous split thickness skin grafting has been available since the late 1800s. The procedure is limited, however, by the size of healthy available skin, the time it takes for engraftment, and the healing of the donor site. Cultured epidermal cell sheets, developed in the 1970s, were an initial attempt to overcome some of these limitations. These sheets were costly and time consuming to create, limiting their clinical utility. Cadaveric skin, a true allograft, can provide immediate coverage but is ultimately rejected. Cadaveric dermal tissues and synthetic dermal substitutes have provided clinicians with more options in recent years but still require skin grafting for ultimate closure. Cultured bilayer and single-cell biological dressings have emerged, each with randomized controlled trial data to support their efficacy in nonhealing wounds. Despite these advances, overall healing rates continue to remain at 50–70%, and wound recurrence is high regardless of wound etiology. Since stem cells have great proliferative capacity and the ability for self-renewal as well as the ability to differentiate into a number of different tissue types, stem cells became a desirable therapeutic option for nonhealing wounds.

While fetal or embryonic stem cells demonstrate extensive proliferative capacity, self-renewal, and totipotency, or the ability to differentiate into all cell types, due to ethical and logistical issues, most research and development is now predominantly focused on adult stem cells. Adult stem cells are multipotent, indicating that they can differentiate into a number of cell types but not all cell types, and have proliferative capacity limited to the lifespan of the organism. Studies in bone marrow-derived stem cells have provided the greatest clinical safety and efficacy experiences among all adult stem cell sources. In the bone marrow, two stem cell types have been clinically developed. The first, hematopoietic stem cells (HSCs) give rise to all blood components. These cells have been clinically developed for bone marrow transplantation, but the CD34+ sub-population, which can give rise to endothelial cells, has recently been investigated in clinical trials to speed wound healing, predominantly due to its potential for enhanced angiogenic activity. The remaining part of this review will focus on adult MSC, as in addition to their pro-angiogenic capabilities, these cells can modify host inflammatory responses, thereby having the potential to reduce time spent in unproductive inflammatory response by switching to pro-regenerative activities.

Figure 1. Potential therapies for reducing scar formation during wound repair. To manipulate wound repair to become more regenerative than scar forming, strategies include the use of biomimetic scaffolds, the manipulation of the mechanical environment (e.g., negative pressure wound therapy to increase healing) or the electrical environment, the administration of small molecules, the use of gene-therapy approaches, and the use of cell-based strategies (including administration of epithelial stem cells.) All of these elements have been demonstrated to have an effect on in vitro and in vivo models of wound healing as single-agent therapies. In theory, many of these elements could be combined to recreate a receptive environment (or soil) to promote regeneration. Combining these with the appropriate stem cells (or “seed”) will undoubtedly alter the result of wound healing in humans. (Reprinted with permission from Gurtner et al.) To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound
MSCs give rise to tissues of mesenchymal origin. Sources of adult stem cells include sites rich with high vascular flow and proliferative and pro-regenerative cellular activity such as the bone marrow, umbilical cord, and amniotic fluid. In the marrow, the endosteal and vascular niches serve as the microenvironment for maintaining HSCs; growing evidence indicates that MSCs and HSCs co-regulate activities of each other, coordinating the function of the marrow and, as a consequence, both the inflammatory and pro-regenerative cells released from that reposito.

Adipose-derived MSCs are notably different from previously mentioned sources, as they are isolated from tissue, which is considerably less vascularized and is rich in the secretion of pro-inflammatory growth factors. Clinical studies demonstrate that cells isolated from bone marrow, cord blood, and adipose tissue differ in their ability for proliferation, differentiation, and gene products. For instance, cord blood MSC are unable to differentiate well into adipose tissue, but are highly proliferative; bone marrow and adipose MSC proliferate at similar rates; however, all have different genomic patterns and products that vary with cell passage and individual. These variabilities in cell source may provide an insight on the variable efficacy observed in patient response to these cellular therapies. There is also still controversy in the field over which surface markers define stem cell status.

Studies in small animal models suggest that stem cells not only improve wound healing when applied alone but also synergize with scaffolds. When adipose-derived MSC were loaded on biological scaffoldings, there was enhanced viability when compared with direct topical application, suggesting a simultaneously multi-modal product, that is, scaffold-loaded stem cells, can augment stem cell efficacy. In addition to the options of retrieving MSCs directly from the patient or from a young donor, it is also possible to indirectly develop terminally differentiated cells, such as fibroblasts, from either patient or donor to obtain mesenchymal-like stem cells. Terminally differentiated cells can be reprogrammed to dedifferentiate into a pluripotent cell, via viral transfection, plasmids, synthesized RNAs, or small molecules. Using these induced human pluripotent stem cells (iPSCs), investigators have recently coaxed human iPSCs to take on the phenotype of MSCs, helping to attenuate critical limb ischemia in a mouse model. There is a low transfection rate and still many unanswered questions as to the immunogenicity of these recently developed cell lines.

The safety of MSCs has been evaluated in a recent meta-analysis and at this time appear to be safe for clinical use. Badavias and Falanga used subcutaneous injections of bone marrow along with topical application of MSCs in an early human study and demonstrated improved healing. Falanga et al. subsequently demonstrated improved healing in both human and diabetic mouse models with MSCs using a fibrin spray to apply the cells topically. Javazon demonstrated improvement in wound healing as well as decreased pain in a diabetic mouse model using a topical application of stem cells. The proposed mechanism of action includes differentiation into epidermal and dermal cells, improved vasculogenesis, immune modulation, and paracrine signaling pathways. Limitations to successful therapy, however, include cell delivery methods (IV, topical/spray, scaffold-loaded, or subcutaneous injection), cell viability, heterogeneity in MSC preparations, and inconsistent micro-environmental cues. In one of the only two reported randomized clinical trial using MSCs, Dash et al. treated ischemic and diabetic ulcers and noted both wound healing and decreased pain. This study utilized intramuscular injections of MSCs and demonstrated an increase in immature cells, vascularity, and reticulin fibers that were not seen in control cases. Jain et al., however, found no significant improvement in healing using topically applied and subcutaneously injected MSCs in patients with lower extremity ulcers. Wu et al. noted both MSC differentiation into epidermal cell lines, in addition to increases in angiogenesis via paracrine signaling in a diabetic mouse model. There appears to be bi-directional cross-talk between MSCs and macrophages. This relationship can potentially be manipulated to enhance a more regenerative wound micro-environment. One of the proposed mechanisms of action for this includes a paracrine-like reprogramming of inflammatory type 1 macrophages to a type 2 anti-inflammatory, pro-regenerative phenotype. Alternatively activated macrophages have been shown to increase levels of the anti-inflammatory cytokine II-10 while simultaneously lowering pro-inflammatory cytokines such as II-1b, II-17.

It is known that aging reduces both the rate and quality of healing in adults. The number of MSCs also decline with advancing age in humans. Using a mouse model, Lee et al. demonstrated that the tensile strength of a healed wound in an aged animal could be improved to the level of a young animal with the use of MSCs. In this
study, a lower number of MSCs were used than in previous reports, and the authors theorize that preactivation with interferon gamma (IFG) might have induced increased potency of the cells. In addition, Lee et al. blocked the positive effect of MSCs on improved tensile strength by chemically depleting the macrophages in both the young and aged animals, further supporting the importance of MSC/macrophage interactions (Fig. 4). Investigators have found improved scar formation, increased tensile strength and vascularity in a number of animal models. The same group, using an incisional wound mouse model, further evaluated cytokine levels from treatments using fibroblasts, nonactivated MSCs, and activated MSCs.* The authors noted that the presence of transforming growth factor β3 (TGF/β3), a growth factor typically found in scarless healing, is enhanced and prolonged with treatment of activated MSCs when compared with naive MSCs or fibroblasts.

Inflammation and the impact of preactivation

As stated earlier, chronic inflammation is one of the hallmarks of a chronic nonhealing wound. Wound healing usually progresses through hemostasis, inflammation, proliferation, and, finally, a remodeling phase. The initial platelet plug that forms results in a temporary provisional matrix for cells to invade and transform into a mature granulating bed. Neutrophils, mast cells, and monocytes arrive in the matrix through a process that is enhanced by vasodilation and a marked increase in permeability secondary to mast cell histamine release. Monocytes differentiate into macrophages and due to their plasticity can be influenced by the microenvironment to polarize toward either a classical or an alternately activated phenotype. While neutrophils and macrophages are crucial for defense, in the absence of infection, the reactive oxygen species (ROS) and other inflammatory mediators released by these cells can retard healing. The pro-inflammatory conditions surrounding the nonhealing wound have been compared with the micro-environmental conditions noted in cancer. In fact, tumors have been referred to as wounds that do not heal. While inflammation is necessary to initiate wound healing, it is equally important that micro-environmental signals are generated for the termination of this phase. Therefore, researchers are now able to apply lessons learned from modulating inflammation in cancer to nonhealing wounds. In addition, many chronic conditions share the pathophysiology of inflammation, tissue repair, and, ultimately, fibrosis. The effect of MSCs on inflammation has potential therapeutic implications for a myriad of clinical conditions.

After prolonged myocardial ischemia, cells undergo necrosis, apoptosis, or can remain in a hibernating or stunned state. These concepts have been applied to the processes noted in chronic wound healing. After myocardial infarction, cells express damage-associated proteins (DAMPs) that attract and activate various components of the immune system. Attempts at pharmacological therapy to lower inflammation have resulted in poor healing, scar formation, and, ultimately, heart failure. These findings led investigators to research alternative methods for reducing inflammation without compromising healing (Fig. 5). There are several molecules and pathways that have been implied in MSC-dependent immunomodulation, including indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), nitric oxide (NO), TGF/1, and interleukin 10 (IL-10). IV MSCs were found to decrease myocardial infarction size in a mouse model, despite the fact that the majority of cells were filtered by the lung. After trapping in the lungs, MSCs up-regulated the expression of the anti-inflammatory protein tumor necrosis factor inducible gene siRNA6 (TSG-6).
Confirmation of this mechanism of action was proved by achieving similar outcomes when IV infusions of purified TSG-6 were utilized. In another organ system, the allergic response to inflammatory airway disease was reduced by the use of human iPSCs in a mouse model. A reduction in nasal eosinophilia, levels of Th-2 cytokines, and serum IgE levels were appreciated using human iPSCs in this trial. In ophthalmology, both early surgical inflammatory and delayed immune...
responses were blunted through IV MSCs in a mouse model of both auto and allograft corneal transplantation. Allografted animals demonstrated significantly lower levels of inflammatory cytokines during the surgical-induced early inflammatory period and the delayed allorejection time frame when MSCs were used (Fig. 6). Interestingly, as in the myocardial infarction studies, the MSCs were found to be trapped in the lung. The trapped MSCs were also found in this study to secrete TSG-6. IV-MSCs had no effect on small interfering RNA (siRNA) TSG-6 knockdown mice while IV injection of recombinant TSG-6 was equally as effective in preventing both early and late rejection of corneal allografts, further confirming the paracrine nature of the MSC response. In another trial, MSCs given to mice just before inducing sepsis using a cecal perforation model reduced mortality and preserved critical organ function.

MSC can respond to differential levels of inflammation by correspondingly altering both function and phenotype. At low exposures of IFG, a highly inflammatory cytokine found in wounds and other sites of tissue injury, MSC can up-regulate surface expression of major histocompatibility complex Class II and adopt the ability to present antigen and promote an inflammatory response.

At higher doses of IFG, MSC become more immunosuppressive, releasing greater amounts of indoleamine 2,3-dioxygenase, Cox-2, and PGE-2. We observed increased immunosuppressive potential of IFG activated MSC when compared with naïve MSC in reducing the mortality of ongoing graft versus host disease and in preventing mortality when given early in the course of the disease. Reduction of early inflammatory responses in wounds combined with regenerative signals may be beneficial in triggering a more rapid course of early repair and regeneration in adult wound healing, as this process more closely resembles fetal healing, in which wounds heal by regeneration without contracture or scar. MSC effects are largely paracrine in nature. IFG activation appears to augment this paracrine function by increased MSC production of cytokines, chemokines, and growth factors. We propose that such activation may provide a new and more potent method of accelerating tissue regeneration.

**TAKE-HOME MESSAGES**

- Inflammation is a necessary component of the normal wound healing process but if unchecked and persistent, can lead to a nonhealing wound.
- There are many macro and micro-level confounding variables that contribute to a nonhealing wound. This helps explain why therapies that target a single process or factor often fail to improve healing in clinical trials.
- MSCs can provide the right biochemical cues depending on how they sense the wound bed micro-environmental signals. This individualized approach could help reduce inflammation and, therefore, tissue repair with its expected high scar tissue levels.
- Preactivation of MSCs might offer a method of improving the potency of these cells without the need for additional cell numbers.
- There is an increasing awareness that the clinical benefit from MSCs is through paracrine functioning and not terminal differentiation of the cells.
- The use of scaffodings and other methods may improve cell viability and durability, which is currently a major limitation for stem cell use.

**SUMMARY**

Wound healing is a complex process that requires an orchestrated biochemical series of events to occur to achieve tissue closure. The process of healing in most adult tissues results in significant scar formation as a part of the normal repair process. Fetal healing, in which tissue is replaced without scar formation, is known to proceed with minimal inflammation. Current devices and dressings, therefore, have targeted inflammation as a method to emulate the fetal healing environment. Clinicians currently start with moist dressings and then move through a series of treatment options of increasing complexity if there is either an absent or less than adequate healing response. This sequential treatment method fails to capture the natural complex processes that are simultaneously proceeding in the *in-vivo* setting. The wound care industry has generated an enormous amount of products, dressings, devices, and, more recently, scaffodings and cell-based therapies but to date, only 50–70% of wounds heal within randomized controlled trials. Stem cells offer a novel therapeutic option that differs dramatically from current treatment options. MSCs can be deployed within scaffodings, topically or by injection, to sense the microenvironment and influence the healing process in a more regenerative manner through paracrine functioning. With pre-activation of MSCs, it is thought that fewer cells are required and that their paracrine effects are heightened. There are those that raise questions
about safety, but many patients have received stem cell therapy and meta-analysis reports confirm that assumption. The next big thing for wound care will be to create off the shelf, allogeneic, activated MSCs that can be delivered in a biological scaffold to promote a more regenerative healing process. It should follow, that with less scar and increased tensile strength that recidivism will decrease. As payment for healthcare begins to focus on outcomes, clinicians will need to achieve quality healing, low recurrence, and patient-centered outcomes in a cost-effective manner. It may prove that the initial expense of stem cell therapy has long-term cost-effective outcomes.

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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