

# Stem Cell Therapy in Wound Healing

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## ABSTRACT

Wound healing is a complex restorative process of the altered cutaneous tissue, which is impaired by numerous local and systemic factors, leading to chronic non-healing lesions with few efficient therapeutic options. Stem cells possess the capacity to differentiate into various types of cell lines. Furthermore, stem cells are able to secrete cytokines and growth factors, modulating inflammation and ultimately leading to angiogenesis, fibrogenesis, and epithelization. Because of their paracrine activity, these cells are able to attract other cell types to the base of the wound, improving the formation of new skin layers. Mesenchymal stem cells derived from the adipose tissue, bone marrow, and placenta, offer numerous ways of implementation. The process of harvesting, growing, and administering stem cells depends on the site and type of the cells, but recent trial results showed improvement of wound healing independent of the administration site. Bioengineered skin substitutes are validated for treatment of chronic wounds with direct application on the skin surface. These offer physical scaffolding for the migrating cells and promote secretion of growth factors, thus facilitating rapid wound healing. Obtaining further clinical data is essential, but stem cell therapy may become a first-line therapeutic choice for the treatment of non-healing chronic wounds.

**Keywords:** stem cell therapies, non-healing wounds, bioengineered skin

## INTRODUCTION

Chronic impaired wound healing is still a challenging therapeutic task for health-care experts. It involves a vast interdisciplinary approach from surgical, medical, dermatological, diabetic, general practitioner specialist, and nursing staff for long-term success. Aging and various medical conditions create a physiopathological ground for impaired wound healing, leading to a long and exhausting restorative process, which often remains unsuccessful and greatly affects the quality of life of these patients.<sup>1,2</sup> As the aging population is growing worldwide, it is expected that the burden of chronic wounds will increase significantly in the next few decades.<sup>3</sup> Approximately 1% of the population suffers from non-healing wounds, generating tremendous costs for healthcare systems.<sup>4,5</sup> Despite the multitude of recently developed therapeutic options for these patients, the results are still unsatisfactory. Multiple clinical and preclinical trials are under development worldwide for the assessment of novel therapeutic methods in chronic wound healing.<sup>6,7</sup>

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As a result of translational medicine research progresses, stem cells have emerged as a viable alternative for the treatment of this complex pathology for enhancement of tissue regeneration.<sup>8</sup> Stem cell therapy has already been intensely studied in organ damage repair and regeneration in numerous diseases.<sup>9,10</sup> One of the most promising results was obtained in the field of cardiovascular diseases, namely recovery in the post-myocardial infarction phase and critical limb ischemia.<sup>11–13</sup> The major drawback of stem cell-based treatment is represented by the difficulties of adequate stem cell population selection, delivery, and the prevention of immune and tumor responses.<sup>14</sup>

The aim of this clinical update is to summarize the current applications of stem cell therapies in the treatment of chronic wounds.

## **PATHOPHYSIOLOGY OF IMPAIRED WOUND HEALING**

Regeneration and healing of the altered cutaneous tissue is a complex, well-organized process. After platelet aggregation and clot formation in the hemostasis phase, the inflammatory process is activated by chemokines, which attract neutrophils, lymphocytes, and monocytes that release inflammatory cytokines and growth factors.<sup>15</sup> Chronic wounds exhibit a prolongation of the inflammatory phase, which ultimately leads to failure of the healing process due to alteration of the extracellular matrix.<sup>16</sup> The proliferative and resolute phase of wound healing is characterized by angio- and fibrogenesis, with collagen formation and re-epithelization. Chronic wounds are usually defined by deficient vascularization, with reduction of angiogenesis and formation of fibrotic and epithelial cells.<sup>17</sup> All stages can be altered by aging and by the presence of local factors, such as infections, ischemia, radiation, traumas, toxins, as well as systemic factors such as diabetes, cancers, drugs, neuropathies, smoking, or alcoholism.<sup>18</sup>

## **THE ROLE OF STEM CELLS IN WOUND HEALING**

Given their pluripotent and self-renewing characteristics, stem cells are able to differentiate into different types of mature functional tissues.<sup>19</sup> Their potential in the epithelial healing of chronic wounds lies in their capacity to secrete cytokines that promote angiogenesis, cell proliferation, and tissue regeneration. The development of bioengineered tissues impregnated with stem cells stays at the basis of this novel chronic wound care therapy.<sup>20</sup> One of the most important factors that affect the success

of stem cell therapy is represented by the selection of stem cell populations.

### **Mesenchymal stem cells**

Mesenchymal stem cells (MSC) can be isolated from various types of tissues including bone marrow, umbilical cord blood, and adipose tissue, and possess the ability of good adherence to plastic materials.<sup>21</sup> These stem cells can be delivered topically or systemically.<sup>22</sup> In addition to their ability to differentiate into various cell lines and to promote angiogenesis, MSCs present potent immunomodulatory and immunoregulatory characteristics, thus coordinating the inflammatory process, reducing scarring, and modulating fibrosis. This is achieved by releasing multiple chemokines that act as mediators.<sup>23,24</sup> The efficiency of MSCs in the treatment of critical limb ischemia has already been proven safe and efficient.<sup>25</sup> Currently there is no evidence of any clinical benefit regarding the tissue origins of stem cells used in wound healing.

### **Adipose stem cells**

Adipose stem cells (ASC) are pluripotent MSCs, which can be extracted from adipose tissue, are easily harvested by liposuction or minimal-invasive surgical excision and can be preserved for up to six months. Another advantage of adipose tissue over other extraction sites of MSCs comes from the fact that a large number of stem cells can be yielded from this type of tissue compared to other sites (e.g., 40-fold higher compared to bone marrow) and can be transplanted to autologous or allogeneic hosts.<sup>26</sup> Autologous ASC transplantation is well tolerated and it is proven to be safe in terms of malignant transformation.<sup>27</sup> Given the rich amount of easily accessible fat tissue in the body and their angiogenic and paracrine potential, ASCs are widely used MSCs in wound healing research.<sup>28,29</sup> ASCs improve wound healing by the secretion of mediators such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), fibronectin, and collagen I, stimulating angiogenesis and smaller scar formation.<sup>30</sup> Because of their paracrine effect, ASCs stimulate other cells and recruit stem cells to form fibroblasts, keratocytes, and endothelial cells, and down-regulate the inflammatory response, therefore leading to improved epithelization.<sup>18,31</sup> Numerous studies have analyzed the effect of ASCs in different types of chronic wound healing, with positive histological and clinical results in skin cancer-related wounds.<sup>32</sup> Ischemic and irradiation wounds are also an important topic in chronic wound

healing, with different results so far.<sup>33</sup> Another broadly researched application of ACSs is represented by diabetic wounds. Recent preclinical studies suggest that ASCs combined with artificial skin enhances diabetic wound healing through the secretion of growth factors and new vessel formation in diabetic rats. An improvement was observed in capillary formation, fibroblast proliferation, epithelization, and wound contraction compared to control groups.<sup>34–36</sup> Encouraged by the positive preclinical results, many ongoing clinical trials are being carried out, but few results have been published so far about wound healing. However, the results of other applications of ASCs (e.g., osteogenic defect healing and Crohn's disease fistulas) are promising.<sup>37,38</sup>

### Bone marrow stem cells

Bone marrow is one of the most frequently used sites for stem cell extraction. These cells play an important role in each phase of wound healing, thus they are targeted as viable therapeutic alternatives in chronic wound healing.<sup>39</sup> Bone marrow-derived stem cells also hold the property to adhere to plastic materials and can be applied directly on the surface of the wounds, injected in the edges of the wound, or administered systemically.<sup>40</sup> Randomized controlled clinical trials have reported positive outcomes with topical and intramuscular delivery of bone marrow-derived MSCs and autologous biograft in chronic diabetic wounds. Significant contraction in wound size ( $7.26 \pm 1.41 \text{ cm}^2$  vs.  $2 \pm 0.98 \text{ cm}^2$ ,  $p < 0.001$ ) was observed at 12 weeks, with prolonged pain-free walking distance ( $38.33 \pm 17.68 \text{ m}$  vs.  $284 \pm 212 \text{ m}$ ,  $p < 0.001$ ).<sup>41</sup> Another study, which used bone marrow-derived stem cells applied with a polymer spray on surgical excision chronic wounds, has demonstrated the efficiency of this therapy, leading to the repair of the epithelial surface in eight weeks.<sup>42</sup> An enhancement of the vascularity and growing of the dermal layer was observed in a study in which the researchers had injected bone marrow-derived stem cells in the edges of the wound in patients with diabetic foot. This was accompanied by a reduction in wound size.<sup>43</sup> Autologous bone marrow-derived stem cells have led to complete wound healing in a study that included patients with leg ulcers that lasted longer than one year despite conventional treatment.<sup>44</sup> In a randomized controlled trial where intramuscular bone marrow-derived stem cell were administered for diabetic patients with critical limb ischemia and foot ulcers, there was a significantly higher healing rate and pain-free walking distance recorded during the 24-week follow-up period.<sup>45</sup>

### Placental and embryonic tissue

Placental tissue is an excellent source of stem cells, with pluripotent differentiation and immunomodulatory capacity, which makes it a suitable alternative for the bioengineering of skin substitutes that can be used for the treatment of chronic wounds.<sup>46</sup> Furthermore, growth factors and extracellular matrix that are essential for the natural wound healing process are also secreted by the placenta.<sup>47</sup> Preclinical investigations are carried out to determine the safety and efficiency of this method. Bioengineered skin substitutes are available from the human neonatal fibroblast-dermis, which promotes cell migration and, through a paracrine effect and secretion of growth factors, enhances epithelization. It also offers physical scaffolding for migrating cells into the base of the wound, helping the recovery and healing of chronic ulcers. Randomized trials have proved the efficacy of these skin substitutes in chronic diabetic ulcers, with significantly higher wound closure rates compared to controls at 12 weeks (30% vs. 18.3%,  $p = 0.02$ ).<sup>48,49</sup> Another bioengineered skin substitute is derived from neonatal foreskin, and it is approved for clinical use, being based on a bilayer structure of fibroblasts and keratocytes that facilitates the migration of monocytes to the wound base and the secretion of growth factors such as PDGF, fibroblast growth factor (FGF), or granulocyte colony-growth factor.<sup>50</sup> These factors enable cell integration and allow vascular ingrowth, thus increasing and accelerating chronic wound healing. The published results of recent clinical trials are encouraging and have proved the efficacy of this treatment option in patients with diabetic foot ulcers and venous leg ulcers.<sup>51,52</sup> A median of 61 days was recorded until complete wound healing vs. 181 days in the control group ( $p = 0.003$ ) in patients with venous leg ulcer.<sup>53</sup>

### PROSPECTS OF STEM CELLS IN WOUND HEALING

The origin of stem cells, the process of harvesting, preservation, and manipulation of cell lines, as well as their use is still under strict regulations both in Europe and in the United States, and they raise ethical controversies in terms of research and clinical use.<sup>54</sup> Another limitation of stem cell therapy is linked to the huge costs of manufacturing, resourcing, and preservation of cell lines. Promising results of current stem cell therapies are encouraging investments in this flourishing research field, which leads to the emergence of new preclinical and clinical trials, which may transform it into a widely accessible therapeutic method.

## CONCLUSIONS

Despite many current therapeutic options, chronic wounds still remain a great concern for many healthcare professionals, and the number of these patients is expected to grow in the future due to the aging population and increasing incidence of diabetes. Stem cell therapy holds the potential of clinical improvement for these patients, due to its capacity of tissue regeneration, and direct and paracrine activity. A considerable number of stem cell types are used in preclinical and clinical studies with encouraging results, but further trials are needed to validate this therapeutic option.

## CONFLICT OF INTEREST

Nothing to declare.

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