Review Article

Adipose-derived mesenchymal stem cells in wound healing: A clinical review

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Abstract

The aim of this article was to review clinical studies on the use of adipose derived mesenchymal stem cells in the treatment of chronic wounds. A search on PubMed was performed on April 30th, 2014 to identify the relevant clinical studies. We reviewed 13 articles that reported the use adipose-derived stem cells in the treatment of different types of wounds. Adipose-derived stem cells have the potential to be used in the treatment of chronic wounds. However, standard methods for isolation, storage and application of these cells are needed. New materials to transfer these stem cells to injured tissues should be investigated.

Introduction

Wound healing is a well-organized physiological process, which involves inflammation, cellular proliferation, and remodeling. Normal wound healing requires coordination of cellular migration, cellular proliferation, and extracellular matrix deposition processes. External or internal factors such as diabetes, radiotherapy, and infection cause delayed or impaired wound healing by interrupting above-mentioned processes.

Regenerative medicine is mainly focused on clinical use of stem cells. Stem cells can divide indefinitely and differentiate to various specialized cells. Multipotent stem cells are essential for tissue regeneration and repair of injured tissues in adult life. We will come closer to a clinically feasible stem cell-based treatment if our knowledge of stem cell biology expands with new studies.

Adipose tissue is used in plastic surgery since the ends of 19th century. Recently, it was found that adipose tissue is rich in mesenchymal stem cells and has an important potential to be used in regenerative treatments (1). Despite promising results from preclinical studies, there is not much progress in clinical applications of adipose-derived mesenchymal stem cells in wound healing (2). The aim of this review is to provide basic understanding of stem cells and to review clinical studies on adipose-derived mesenchymal stem cells and wound healing.

Adipose Derived Mesenchymal Stem Cells

Stem cells were first defined in embryonic life. However, later studies demonstrated that there are tissue resident stem cells that can replicate and differentiate to specialized cells. Recently, adipose tissue attracts the attention of researchers as a potential source of stem cells (3). Adipose tissue consists of several different cell types. Among these are adipocyte progenitor cells, fibroblasts, pericytes, stromal cells, mesenchymal stem cells, endothelial progenitor cells and even hematopoietic stem cells (4-7). Bone marrow is used as the main autologous stem cell source in adults. However, adipose tissue has several advantages over bone marrow. First, adipose tissue is abundant in humans. Second, adipose tissue can be easily obtained with liposuction, which is less painful compared to bone marrow.

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marrow aspiration. Third, the number of stem cells that can be isolated from adipose tissue is higher than that isolated from bone marrow (5,6).

Adipose tissue-derived stem cells express cell markers specific to mesenchymal stem cells. Among these are CD34, CD44, CD106, CD117, and STRO-1. But, they do not carry hematopoietic lineage markers (CD45, CD14, CD16, CD61) and endothelial cell markers (CD31, CD144, von Willebrand factor). Adipose-derived mesenchymal stem cells can differentiate into osteogenic, condrogenic, myogenic, and adipogenic lineage cells when cultured in vitro (2,8).

The reason why mesenchymal stem cells are present in adipose tissue is not clear. There are two theories. First theory proposes that bone marrow-derived circulating stem cells reside to adipose tissue. The similarities between bone marrow-derived and adipose tissue-derived mesenchymal stem cells support this theory. Second theory suggests that stem cells in adipose tissue are in fact pericytes. Pericytes are present in microvessels as a support cell. While they express some of the mesenchymal stem cell markers (CD44, CD73, CD90, CD105), they do not express endothelial and hematopoietic cell markers. Similar to mesenchymal stem cells, pericytes attach to the culture dish, proliferate in culture and differentiate into bone, cartilage and adipocytes (2).

Isolation of Stem Cells from Adipose Tissue

Adipose tissue obtained with surgical procedures is diced into small pieces. Following enzymatic digestion with collagenase, stromal vascular fraction is obtained. Stromal vascular fractions contain several cell types including pre-adipocytes, endothelial cells, smooth muscle cells, pericytes, fibroblasts, mesenchymal stem cells, hematopoietic stem cells, endothelial progenitor cells, erythrocytes, T-lymphocytes, B lymphocytes, monocytes, and macrophages (2). Stromal vascular fraction is also used in clinical application. But the effectiveness of this technique is limited due to the low percentage of stem cells in the stromal vascular fraction (9).

Mesenchymal stem cells are isolated by culturing stromal vascular cells in cell culture. These cells are injected subcutaneously or directly applied over the wounds. It should be noted that intravascular applications of mesenchymal stem cells carry significant risks (10).

Preclinical Studies

Adipose tissue-derived mesenchymal stem cells can contribute wound healing by differentiating into cells that are involved in wound healing or by secreting several growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), keratinocyte growth factor, platelet-derived growth factor, insulin-like growth factor 1, EGF, stromal cell-derived factor 1.

Altman et al. applied acellular dermal matrix including human adipose-derived stem cells over wounds in atonic mice. They found that stem cells did not enter systemic circulation and were present in wound tissue at 2 weeks. Adipose-derived stem cells increased microvascular network and increased healing rate (11). Similar results were reported by Nambu et al. in a diabetic wound model in mice. Stem cells isolated from adipose tissue of mice were applied in streptozotocine induced diabetic mice (12). Wound healing rate increased, and skin biopsies demonstrated increased capillary density after adipose tissue-derived stem cells application.

Kim et al. cultured adipose tissue-derived stem cells with human dermal fibroblasts. They found that adipose tissue derives stem cells increased type I collagen synthesis, Type I and Type III collagen and fibronectin mRNA levels in fibroblasts (13).

Ebrahimian et al. demonstrated that adipose-derived stem cells that injected to wound tissue secretes VEGF, incorporate into capillary walls and express endothelial phenotype (14). They showed increased capillary density and perfusion after stem cell application.

Hong et al. compared the effectiveness of bone marrow and adipose tissue-derived stem cells in a rabbit ear wound model (15). They found that adipose-derived but not bone marrow-derived stem cells increased granulation tissue. Furthermore, they showed that adipose-derived stem cells get fibroblast phenotype after topical application, proliferate in the wound and recruit endothelial cells.

The survival of transplanted stem cells in the wound tissue is critical in the success of these applications. Kim et al. found that low-level laser increases the effectiveness of stem cells by decreasing apoptosis in stem cells (16).

Diabetics compose a significant portion of patients with chronic wounds. Adipose derives stem cells that isolated from diabetic mice have lower proliferation and migration potential (17). They also secrete less
growth factors (17). Thus, stem cells isolated from diabetic patients may have lower healing potential (18). Therefore, autologous stem cell transplantation may be unsuccessful in diabetics.

Data Acquisition
A search on PubMed was performed on April 30th, 2014 to identify the original studies that used adipose-derived stem cells for wound healing in a clinical setting. We used the following keywords (“adipose-derived stem cells” AND “wound OR ulcer”). 355 articles were found. Non-English language articles (n=21) were excluded. Titles and abstracts of remaining articles were evaluated. Full text of articles deemed to be potentially relevant (n=23) were obtained and reviewed. References of review articles and clinical studies were also skimmed for additional articles. Thirteen articles were identified. However, one of the two papers by Akita et al. was excluded since, both papers reported the same patient (19,20).

Clinical Studies
12 articles were included in this review (Table 1). Adipose-derived stem cells were used in the treatment of chronic radiation injury (19-21), chronic wounds in patients with peripheral arterial disease (18,22,23), facial skin necrosis (12,24,25), and complex perianal fistula (9,26).

Chronic radiation injury
Akita et al. described the first case of treatment of chronic radiation injury with autologous adipose tissue-derived cell suspension. An 89 years old woman, who developed a sacral ulcer (5 cm × 10 cm) 40 years after receiving radiotherapy for uterine cancer, underwent liposuction. Out of 250 ml liposuction material, 5 ml of cell rich fraction was obtained, which contained $3.8 \times 10^7$ cells. Following sharp debridement of the wound bed, the wound was covered with artificial dermis, which was soaked by autologous adipose-derived stem cells, together with angiogenic and mitogenic factor of bFGF. The wound was healed at postoperative day 82 (19). The treatment protocol included FGF in addition to stem cells.

Rigotti et al. injected cell mixture (60-80 cc) derived from autologous lipoaspirates of 20 patients with radiotherapy-induced skin lesions (fibrosis, atrophy, skin retraction, ulcer) (21). Patients received 1-6 treatments (median: 2 treatments) based on the severity of the symptoms. Patients were followed between 18 and 33 months. They observed improvement in LENT-SOMA scores in 19 (95%) patients. Ulcer was present in 8 patients and wound healing was achieved in all of them. Post treatment skin biopsies revealed new blood vessel formation.

Diabetic foot ulcers
Han and co-workers used cells isolated from lipoaspirates in the treatment of diabetic foot ulcers (27). 54 patients with non-healing ulcers despite 6 weeks of standard treatment were randomized into control (n=26) and treatment (n=28) groups. Abdominal fat tissue obtained by liposuction was digested with collagenase and filtered to prepare a cell mixture. This cell mixture was mixed with fibrinogen and thrombin and applied over the wounds, $4 \times 10^6 - 8 \times 10^6$ cells were applied. Patients in the control group received fibrinogen and thrombin without cells. Two patients in the treatment group were excluded due to development of infection. After 8 weeks, while complete healing was observed in all patients (100%) in the treatment group, only 16 (62%) patients in the control group showed complete healing. Complete healing time was 33.8±11.6 days in the treatment group and 42.1 ± 9.5 days in the control group. The authors reported that they did not observe any complication related to the application of cells isolated from lipoaspirate on wounds.

Lee et al. injected autologous adipose-derived stem cells in gastrocnemius muscle of 15 patients with peripheral arterial disease (18). Of these patients, 3 had diabetes mellitus (18). Details of this study will be given in the following section on peripheral arterial disease. Two patients had non-healing ulcer, and complete healing was achieved in both of them. The third patient had necrosis on his foot. The necrosis did not improve with stem cell treatment, and he eventually underwent minor amputation. New collateral development was observed in 2 (66.7%) patients. Lee et al. also found that proliferation potential of adipose-derived stem cells isolated from diabetic patients was lower than non-diabetic controls.

Chronic wounds in patients with peripheral arterial disease
Peripheral arterial disease is one of the leading causes of chronic ulcers of lower extremity. Lee et al. injected autologous adipose-derived stem cells in gastrocnemius muscles of 12 patients with thromboangiitis obliterans (18). Under spinal anesthesia, totally, $3 \times 10^8$ stem cells
Table 1. The studies investigated the effectiveness of adipose-derived stem cells in the treatment of wounds

<table>
<thead>
<tr>
<th>Reference</th>
<th>Author</th>
<th>Publication date</th>
<th>n</th>
<th>Cell source</th>
<th>Dose and route of administration</th>
<th>Follow-up duration</th>
<th>Treatment outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic radiation injury</td>
<td>Rigotti et al.</td>
<td>2007</td>
<td>20</td>
<td>SVF</td>
<td>60-80 cc/intralesional injection 1-6 applications</td>
<td>18 to 33 months</td>
<td>Wound healing: 8/8 (100%) Complete wound closure in 81 days</td>
</tr>
<tr>
<td>19</td>
<td>Akita et al.</td>
<td>2010</td>
<td>1</td>
<td>SVF</td>
<td>3.8×10^7 cells/5 ml, periwound injection and soaked into artificial dermis</td>
<td>18 months</td>
<td></td>
</tr>
<tr>
<td>Diabetic foot ulcer</td>
<td>Han et al.</td>
<td>2010</td>
<td>54</td>
<td>SVF</td>
<td>4×10^8 or 8×10^8 cells combined with fibrinogen and thrombin, Topical application</td>
<td>2 months</td>
<td>100% healing in treatment group, 62% healing in control group</td>
</tr>
<tr>
<td>18</td>
<td>Lee et al.</td>
<td>2012</td>
<td>3</td>
<td>Cultured ADSC</td>
<td>3×10^6 cells (total) intramuscular injections at 60 points</td>
<td>6 months</td>
<td>Wound healing: 2/4 (100%) Collateral development: 2/5 (66.7%)</td>
</tr>
<tr>
<td>Chronic wounds in patients with peripheral arterial disease</td>
<td>Lee et al.</td>
<td>2012</td>
<td>12</td>
<td>Cultured ADSC</td>
<td>3×10^7 cells (total), intramuscular injections at 60 points</td>
<td>6 months</td>
<td>Wound healing: 4/7 (57.1%) Collateral development: 8/10 (80%)</td>
</tr>
<tr>
<td>23</td>
<td>Marino et al.</td>
<td>2013</td>
<td>10</td>
<td>SVF</td>
<td>5 mL (3×10^6 cell/ml) periwound injections</td>
<td>3 months</td>
<td>Wound healing: 6/10 (60%)</td>
</tr>
<tr>
<td>22</td>
<td>Bura et al.</td>
<td>2014</td>
<td>7</td>
<td>Cultured ADSC</td>
<td>108 cells, intramuscular injections at 15 points</td>
<td>6 months</td>
<td>Wound healing: 4/7 (57%)</td>
</tr>
<tr>
<td>Facial skin necrosis</td>
<td>Sung et al.</td>
<td>2011</td>
<td>2</td>
<td>SVF</td>
<td>3 ml, intralesional injection and wound coverage</td>
<td>8-10 days</td>
<td>Wound healing: 2/2 (100%)</td>
</tr>
<tr>
<td>25</td>
<td>Jo et al.</td>
<td>2013</td>
<td>4</td>
<td>SVF and cultured ADSC</td>
<td>5×10^5-1×10^6 cells, 2-5 applications</td>
<td>1-11 months</td>
<td>Wound healing: 4/4 (100%)</td>
</tr>
<tr>
<td>Complex perianal fistula</td>
<td>Garcia-Olmo et al.</td>
<td>2009</td>
<td>G1:25 G2:24</td>
<td>Cultured ADSC</td>
<td>G1:fibrin glue G2:ADSC: 20×10^6 cells, if the fistula not closed at week 8, a second injection was made (40×10^6 cells)</td>
<td>2 months</td>
<td>Complete closure of fistula G1:4/25 (16%) G2:17/24 (71%)</td>
</tr>
<tr>
<td>9</td>
<td>Garcia-Olmo et al.</td>
<td>2009</td>
<td>SVF: 4 ADSC: 4</td>
<td>SVF vs. cultured ADSC</td>
<td>SVF: 5.5-176×10^6 cells ADSC: 3.5-30×10^6 cells</td>
<td>2 months</td>
<td>Complete closure of fistula SVF 1/4 (25%) ADSC 3/4 (75%)</td>
</tr>
<tr>
<td>29</td>
<td>Herreros et al.</td>
<td>2012</td>
<td>G1:64 G2:60 G3:59</td>
<td>Cultured ADSC</td>
<td>G1: cultured ADSC G2: fibrin glue+ADSC G2: fibrin glue ADSC: 20×10^6 cells, if the fistula not closed at week 12, a second injection was made (40×10^6 cells)</td>
<td>12 months</td>
<td>Fistula closure rate: G1: 57.1% G2: 52.4% G3: 37.3%</td>
</tr>
<tr>
<td>30</td>
<td>de la Portilla et al.</td>
<td>2013</td>
<td>24</td>
<td>Allogeneic ADSC</td>
<td>20×10^6 cells, if the fistula not closed at week 12, a second injection was made (40×10^6 cells)</td>
<td>6 months</td>
<td>Complete closure of fistula tract: 9/16 (56.3%)</td>
</tr>
</tbody>
</table>

ADSC: Adipose derived stem cells, SVF: Stromal vascular fraction

were injected at 60 different points. Adipose-derived stem cells were isolated from the liposuction material of patients and expanded in vitro before intramuscular injection. Before stem cell therapy, three patients
had resting pain, 7 patients had non-healing ulcer, and 2 patients had foot necrosis. Clinical improvement was observed in 7 of 12 (58.3%) of patients. Post treatment angiography at 6 months showed new collateral development in 8 of 10 patients. They also observed reduced pain score and increased walking distance (18). Adverse events included mild fever (n=1), headache (n=1), flu-like symptoms (n=1), and pain at the injection sites (n=2).

Marino et al. used stem cells extracted from autologous fat in the treatment of lower extremity chronic ulcers in 10 patients with peripheral arterial disease. In this study, a commercially available device (Celution system, Cytori Therapeutics, Inc. San Diego, CA) was used to isolate stem cells from adipose tissue. Ten patients received stem cell injections (15 × 10^5 cells/5 ml) at the edges of the ulcer and the remaining ten patients served as controls. Complete healing was achieved in 6 of 10 patients treated with stromal vascular fraction. The authors did not provide any information about the outcome of the patients in the control group.

In a recent Phase I trial, Bura et al. evaluated the feasibility and safety of intramuscular injection of cultured ADSC in patients with critical limb ischemia. Adipose tissue was harvested by liposuction of abdominal fat in 11 patients. All patients had Rutherford III-6 peripheral arterial disease. Two patients were excluded due to contamination of liposuction material, and 2 others were excluded due to amputation before the adipose-derived stem cell injection. In remaining 7 patients, after 2 weeks of cell culture, stromal vascular fraction (10^4 cells) was injected into gastrocnemius muscles and anterior compartment of ischemic leg. Injections were made at 15 sites in each muscle using a standard grid. Trans-cutaneous partial oxygen pressure increased in 5 patients and reduced in 1 patient. Three patients (43%) underwent major amputation. Lower extremity ulcers tended to improve in patients who did not undergo amputation. The authors reported no adverse event related to adipose derived stem cell treatment.

**Facial skin necrosis**

Sung et al. used ADSC for the treatment of nasal skin necrosis secondary to filler injection in 2 patients. Stromal vascular fraction was prepared from fat tissue obtained by abdominal liposuction. Stromal vascular fraction were both injected into the lesions at the subcutaneous and dermal levels and used for wound coverage. Complete epithelization was achieved 8 and 10 days after injection.

Jo et al. used adipose derived stem cell for the treatment of facial skin defects in 4 patients. The patients had small full-thickness skin defects on the facial area. Three of the wounds were on the nasal tip. Lipoaspirates, which was taken from the patient’s inner thigh or abdomen, was used for adipose-derived stem cell isolation. They used both stromal vascular fraction and cultured adipose-derived stem cells up to 6th passage. Adipose-derived stem cells (5 × 10^5-1 × 10^6 cells) were injected 2-5 times with 10-20 day intervals between injections. All patients healed in 1 or 11 month after using stem cell local injections and topical application.

**Complex perianal fistula**

Garcia-Olmo et al. compared the effectiveness of fibrin glue with and without adipose-derived stem cells in patients with complex perianal fistula (26). Adipose-derived stem cells were expanded in cell culture after isolation from lipoaspirate. Adipose-derived stem cells (20 × 10^6) were injected into fistula walls. If the fistula did not heal at 8 weeks, a second injection of adipose-derived stem cells (40 × 10^6) was performed. Out of 24 patients receiving stem cell therapy, 17 (71%) showed fistula healing while only 4 (16%) out of 25 patients treated with fibrin glue healed. Furthermore, they found that the quality-of-life was higher in patients receiving ADSC therapy. However, in a recent Phase III study from the same group, the effectiveness of 20 × 10^6 stem cells alone (n=64), stem cells with fibrin glue (n=60) and fibrin glue alone were compared in the treatment of perianal fistula (28). Healing rates for each group were 39.1%, 43.3%, and 37.3% at 6th months, and 57.1%, 52.4%, and 37.3% at 1 year, respectively (16). There was no statistically significant difference between the groups.

In another study, Garcia-Olmo and colleagues compared the effectiveness of stromal vascular fraction and expanded adipose-derived stem cells in the treatment of enterocutaneous fistula in patients with Crohn’s disease (9). They found that while 3 of 4 patients who received adipose-derived stem cells had cure, only 1 of 4 patients who received stromal vascular fraction had cure. This study suggests that expanded adipose-derived stem cells are more effective than stromal vascular fraction (9).

Later, the same group investigated the effect of allogeneic adipose-derived stem cells in the treatment of complex perianal fistula in Crohn’s disease (29). Lipoaspirates obtained from healthy donors were used for stem cell isolation. Adipose-derived stem cells were
expanded in culture, and 20 million stem cells were injected into one draining anal fistula in 24 patients. If the fistula did not close at week 12, a second injection with $40 \times 10^6$ cells was made. Patients were followed for 24 weeks. Eight patients were dropped out due to various reasons. Nine of 16 patients (56.3%) who completed the study achieved closure of external opening of the treated fistula at week 24. Complete fistula closure was achieved in 6/14 (30%) patients. This is the only study that used allogeneic stem cells.

**Conclusion and Future Projections**

The number of clinical studies investigating the effectiveness of adipose tissue-derived stem cells is limited. ADSCs were used in the treatment of chronic radiation injury (19–21), chronic wounds in patients with peripheral arterial disease (18,22,23), facial skin necrosis (12,24,25), and complex perianal fistula (9,26). There are other areas that ADSC therapy may be useful. Among these are the control of scar formation and burn wound healing. Wound maturation, which mainly is composed of control of scar formation, is an important part of the wound healing process. Scar formation is closely related to the inflammatory process during the wound healing (30). It is advocated that ADSCs may reduce scar formation with their anti-inflammatory and immunosuppressive effect (31). In a pig study, Yun et al. injected ADSCs into scars formed after healing of full-thickness skin defects. This study showed that, usage of ADSC is not only reduced the scar size but also improved the scar color and pliability. In the clinical application, Khouri et al. reported burn scarring release with lipofilling (32).

Another potential application of stem cells is burn wound healing. There are several experimental studies showing effectiveness of stem cells on burn wound healing (33). The promising preclinical research on animal models has validated that stem cells enhance burn wound healing. Stem cell therapy has been used for burn treatment in limited numbers of clinical studies. Natesan et al. showed that mesenchymal stem cells can be isolated from discarded burned skin, and these cells can be used for wound repair and skin regeneration therapies (34). Rasulov et al. showed that allogeneic bone marrow stem cells increased healing in a patient with extensive third degree burn with 30% TBSA (35). Autologous cultured keratinocytes bearing epidermal stem cells were showed to cover large surface of the burn wound in the treatment of seven major burn patients (36).

Adipose-derived stem cells have the potential to be used in the treatment of chronic wounds. However, standard methods for isolation, storage, and application of these cells are needed. New materials to transfer these stem cells to injured tissues are also investigated. Future studies should aim to develop methods to track transplanted stem cells in the wounds.

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**References**


