

The Potential of Adipose Derived Regenerative Cells for Non-Healing Wounds

BACKGROUND

The complex nature of wound healing carries with it implicit vulnerabilities such that, under certain circumstances, progression through the different phases of healing can be protracted or even arrested resulting in development of a chronic wound. By definition, a chronic wound is a wound that does not heal within the initial 3 month period¹ and affects greater than 6.5 million patients in the USA alone². Complicating circumstances that cause a delay or obstruction of the natural wound healing response include peripheral vascular disease, spinal cord injury, complications associated with burns, diabetes, obesity and prior exposure to irradiation. In such settings, there is evidence that cells within the wound that are responsible for healing exhibit functional impairment. Consequently, delivery of supplemental cells with the capacity to promote healing has the potential to treat chronic wounds.

ADIPOSE TISSUE AS A REGENERATIVE CELL SOURCE

The concept that adipose tissue contains cells with broad potential for regenerative medicine had its beginnings in a paper in the journal *Tissue Engineering* published in 2001³. In this work the authors placed cells obtained from human adipose tissue in culture and showed that the cultured cell population possesses a key characteristic of stem cells; it can differentiate into cells of multiple

lineages. This study is the most cited paper in the history of *Tissue Engineering* having been cited over 900 times; it is still among the journal's most frequently downloaded papers. The following year the same research team published a follow-up study that proved that the property of multilineage differentiation was possessed by single cells within the cell population thus proving that there are stem cells in human adipose tissue⁴. The cultured cell population is usually described in the literature as Adipose-Derived Stem Cells (ADSCs). ADSCs have many properties in common with a related cell type cultured out of bone marrow that is generally referred to as Mesenchymal Stem Cells or Marrow Stromal Cells (MSCs). For example, both cell types can differentiate along osteogenic, adipogenic, chondrogenic, and myogenic lineages⁴⁻⁶ and express similar, though not identical, cell surface markers⁷.

As research into adipose stem cells continued

it became apparent that the cell population used to start the cultures had properties that could be valuable in the clinic. First, it contains stem cells that can be grown up in culture to generate ADSCs. In fact, adipose tissue is the richest known source of adult stem cells which usually comprise around 1-2% (and as much as 5%) of the starting population obtained from adipose tissue^{8,9}. This compares very favorably with age-matched marrow in which MSCs usually comprise only 0.0004% of cells¹⁰. Second, the cell population used to start ADSC cultures also contains other cells that have potentially useful properties including endothelial progenitor cells, tissue resident macrophages, pericytes endothelial cells, and smooth muscle cells. For this reason we refer to this non-cultured cell population as Adipose-Derived Stem and Regenerative Cells (ADRCs). A table of the average composition of the cell population is shown in **Table 1**.

Table 1: Composition of Adipose-derived regenerative cells

Cell Type	Average Frequency
Endothelial cells	7%
Smooth muscle cells	9%
Blood cells	22%
Tissue Macrophages	23%
Other (CD34+/CD31-/CD45-)	38%

Table 2. Summary of Published Preclinical Efficacy Data

Authors	Model	Treatment	Comments
Kim <i>et al</i> , 2007 ¹¹	Full thickness, 7 mm wound in nude mice	Cultured human cells in collagen gel	↑ epithelialization, accelerated closure, ↓ inflammation
Nambu <i>et al</i> , 2009 ¹²	Full thickness skin wound in diabetic (db/db) mice	Non-cultured autologous cells in atellocollagen matrix	↑ granulation tissue, capillary density, and epithelialization
Blanton <i>et al</i> , 2009 ¹³	Full thickness wounds in pigs	Cultured autologous cells in platelet-rich plasma	↑ capillary density and wound score
Altman <i>et al</i> , 2008 ¹⁴	Full thickness wounds in nude mice	Cultured cells on silk fibroin scaffold	↑ closure, epithelialization, and capillary density
Fu <i>et al</i> , 2007 ¹⁵	Full thickness wound in minipigs	Adipose cell extract delivered directly to wound	↑ epithelialization, and capillary density
Nambu <i>et al</i> , 2007 ¹⁶	Full thickness wounds in mitomycin C-treated mice	Cultured autologous cells on atellocollagen scaffold	↑ granulation tissue, capillary density
Amos <i>et al</i> , 2009 ¹⁷	Full thickness skin wound in diabetic (db/db) mice	Cultured human cells injected directly into wound	↑ rate of wound closure
Ebrahimian <i>et al</i> , 2009 ¹⁸	Full thickness wound in normal and irradiated mice	Intravenous or direct injection of cultured cells into the wound	↑ rate of closure and viscoelasticity in both normal and irradiated mice; effect greater than with marrow cells
Kim <i>et al</i> , 2008 ¹⁹	Chronic UV exposure in hairless mice	Direct injection of cultured cells	↓ Histologic damage and fibrosis
Park <i>et al</i> , 2008 ²⁰	Full thickness wounds in mini-pigs	Direct injection of cultured cells	↓ Histologic damage and fibrosis
Lu <i>et al</i> , 2008 ²¹	Random pattern ischemic skin flap in mice	Direct injection of cultured cells into the pedicle	↑ flap viability and capillary density
Uysal <i>et al</i> , 2009 ²²	Ischemia-reperfusion in random pattern skin flap	Injection of cultured autologous cells into the flap	↑ flap viability and capillary density

ADRCs are a population of cells that can be extracted from adipose tissue by a process of washing, enzymatic digestion, and filtration.

PUBLISHED PRECLINICAL REPORTS OF FRESHLY-ISOLATED AND CULTURED ADIPOSE-DERIVED REGENERATIVE CELLS IN WOUND HEALING

A number of investigators have used ADRCs (fresh or cultured) in wound healing studies. While most of these studies have applied cells obtained by placing the cells in culture following their isolation from the tissue, several studies have reported that the freshly isolated cells, prior to culture are also capable of mediating wound healing. This data is summarized in **Table 2**. All investigators report improved wound healing parameters including increased wound closure rate, increased granulation tissue, and improved wound vascularity and perfusion.

UNPUBLISHED CLINICAL EXPERIENCE

In the following reports patients received autologous freshly-isolated cells to treat wounds that were refractory to prior therapy.

Patient 1: Data from Sadanori Akita, Nagasaki University Hospital, Nagasaki, Japan

Patient Characteristics: A 90 year old Japanese woman presented with a non-healing sacral ulcer with exposed sacral bone (**Figure 1a**). The patient had received radiotherapy for gynecologic malignancy ~40 years before presentation. The wound arose in an area within the irradiated field and was refractory to conventional therapy for >4 years. *Treatment:* ADRCs for therapy were isolated from approximately 200 mL of autologous adipose aspirated from the patient's abdomen.

During processing the wound was debrided and the exposed sacral bone was reduced. Following processing ~85% of the cells were injected into the sub-dermis surrounding the wound bed using a 1 mL syringe with a 2" long 22G needle. Injections were performed radially around the wound bed as shown by the markings in **Figure 1b**. The remaining 15% of cells were loaded onto a collagen sponge (Terudermis, Olympus Corp) and placed directly on the wound **Figure 1b**.

Outcome: Within two weeks there was evidence of healing with pinkening at the periphery of the wound (**Figure 1c**). Healing continued to progress such that the wound was completely closed with a slightly hypertrophic scar by day 192 (**Figure 1f**). The wound remains closed at one year follow-up (data not shown).

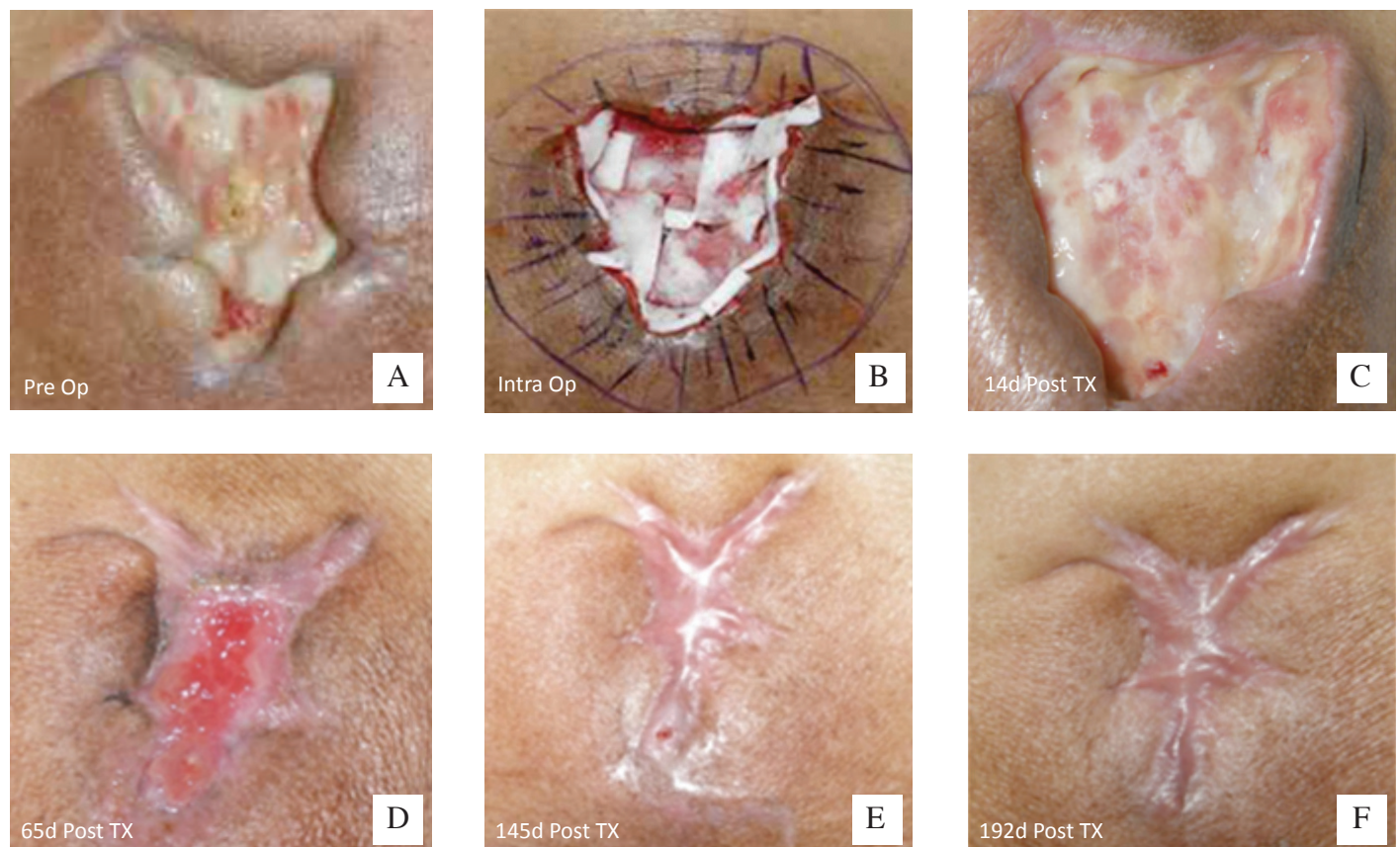


Figure 1. Progression of Treatment and Healing of Sacral Ulcer (Patient 1)

Patient 2: Data from Sadanori Akita, Nagasaki University Hospital, Nagasaki, Japan

Patient Characteristics: 52 year old Japanese woman with a non-healing ulcer in the neck secondary to irradiation (**Figure 2**). Ulcer was refractory to conventional therapy for 4 years.

Treatment: ADRCs for therapy were isolated from approximately 200 mL of autologous adipose tissue aspirated from the patient's abdomen. During processing the wound was debrided. Cells were injected subcutaneously around the wound using a 1 mL syringe with a 2" long 22 G needle. Injections were performed radially around the wound bed as for Patient 1. Additionally, ADRC-enriched fat was used to rebuild the loss of soft tissue in the subcutaneous space.

Outcome: The wound was completely closed by day 72 (**Figure 2**).

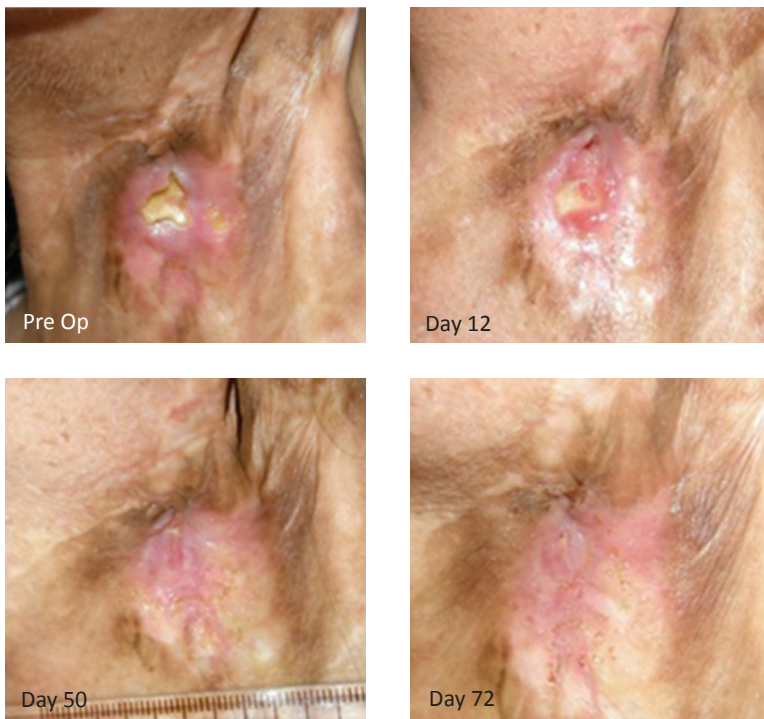


Figure 2. Progression of Treatment and Healing of Neck Ulcer (Patient 2)

Patient 3: Data from Sadanori Akita, Nagasaki University Hospital, Nagasaki, Japan

Patient Characteristics: 67 year old Japanese woman with a non-healing ulcer in the breast secondary to irradiation (**Figure 3**). Ulcer was refractory to conventional therapy for >5 years.

Treatment: ADRCs for therapy were isolated from approximately 200 mL of autologous adipose tissue aspirated from the patient's abdomen. During processing the wound was debrided. Cells were injected subcutaneously radially around the wound.

Outcome: The wound was completely closed by day 72 (**Figure 3**).



Figure 3. Progression of Treatment and Healing of Breast Ulcer (Patient 3)

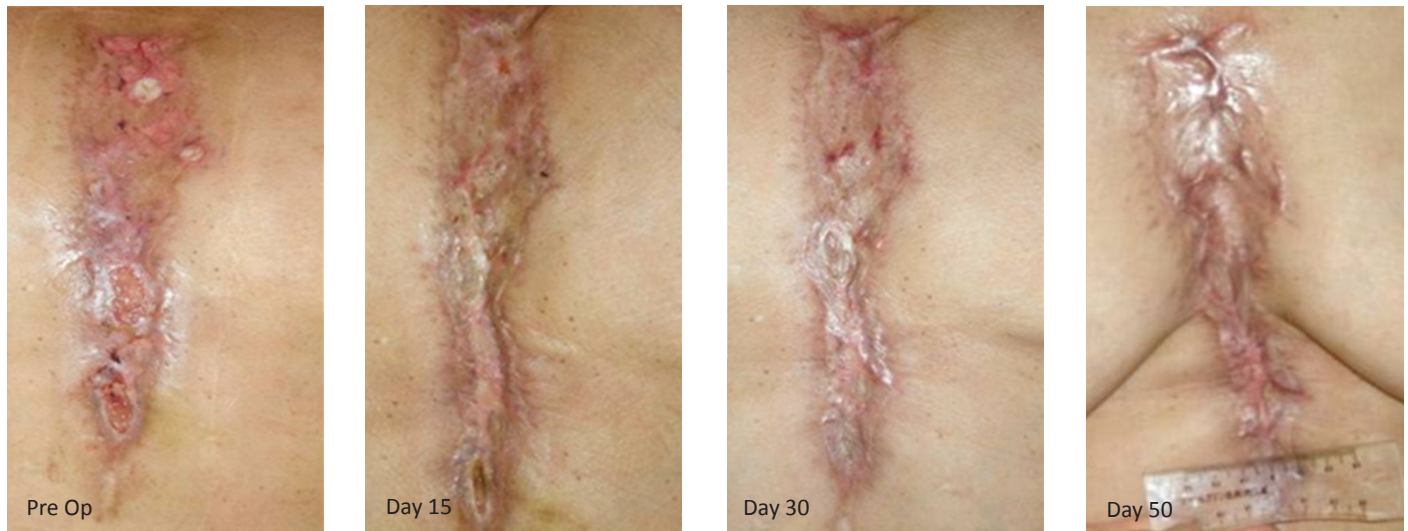


Figure 4. Progression of Treatment and Healing of Sternal Wound (Patient 4)

Patient 4: Data from Sadanori Akita, Nagasaki University Hospital, Nagasaki, Japan

Patient Characteristics: 68 year old Japanese woman with a non-healing ulcer on the chest and upper abdomen secondary to coronary artery bypass surgery in 2000. Skin necrosis and keloid formation were present since 2008 (**Figure 4**). Ulcer was refractory to conventional therapy for >5 years.

Treatment: ADRCs for therapy were isolated from approximately 200 mL of autologous adipose tissue aspirated from the patient's thighs. During processing the wound was debrided. Cells were combined with undigested adipose tissue and injected subcutaneously around the wound.

Outcome: The wound was completely closed by day 50 (**Figure 4**).

Patient 5: Data from Claudio Calabrese, University of Florence Careggi, Florence, Italy

Patient Characteristics: 65 year old Italian woman with a non-healing sternal ulcer secondary to irradiation for lymphoma (**Figure 5**). Ulcer was refractory to conventional therapy for >8 years.

Treatment: ADRCs for therapy were isolated from approximately 180 mL of autologous adipose tissue aspirated from the patient's abdomen. During processing the wound was debrided. Cells were injected subcutaneously around the wound as marked in **Figure 5a**.

Other parameters of wound dressing were not reported by the investigator.

Outcome: The wound was completely closed by day 84 (**Figure 5b**).

SUMMARY

In summary, adipose tissue is a rich source of stem and regenerative cells that is easily accessible and yields high quantities of clinical grade cells. These ADRCs have been investigated at the basic science, preclinical and early clinical phases with very exciting results. There seems to be a clear alteration of the wound healing response, especially in patients with radiation based chronic wounds. While many more questions are still to be answered, this therapy is currently helping patients with no other viable treatment options.

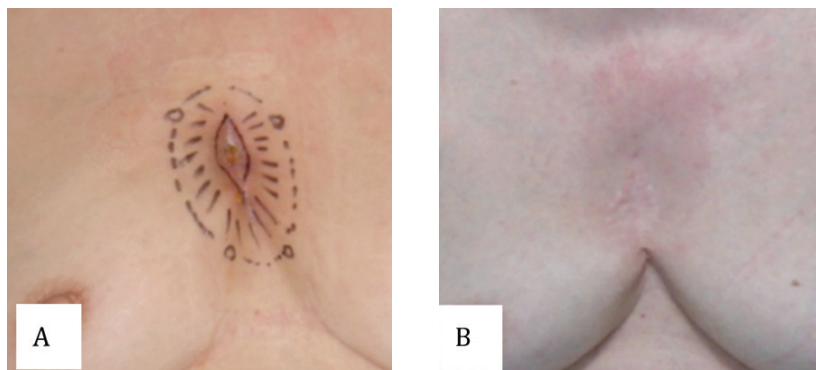


Figure 5. Progression of Treatment and Healing of Sternal Ulcer (Patient 5)

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