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Autologous Adipose Stem Cells Use for Skin Regeneration and Treatment in Humans

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Abstract
There is growing evidence that adipose stem cells contribute to the restoration of tissue vascularization, and have a potentially large therapeutic effect in the field of regenerative medicine. The purpose of this study was to assess the clinical effectiveness of lipoaspirate transplantation on the treatment of skin lesions in humans. This study began with surgical procedures in 2009 followed by a follow-up plan for 4 months. Twenty clients underwent therapy for skin lesions and follow-up. Adipose derived stem cells can promote human dermal fibroblast, proliferation, and re-epithelialization of cutaneous wounds, rejuvenation of the aging skin and related skin lesions. These stem cells replenish dying cells, and have the capacity to regenerate new tissues. Adverse events including pain, swelling and allergy were minimal. All participants expressed their satisfaction of the results. This surgical procedure is a low-invasive therapeutic approach that can resolve the problem of depressed skin, skin lesions, and wrinkles. Adopting this procedure decreases the cost of skin care, and improves client's long-term outcome. Furthermore, it facilitates cell-mediated skin repair and regeneration.
Keywords: Adipose stem cells, Lipoaspirate, skin diseases.

1. Introduction

Stem cell therapy offers a reasonably high potential for skin regeneration exposed to an injury or a disease. The functional stem cell unit is present throughout all layers of the skin and through its physical and chemical cues enables skin regeneration.[1] Adipose tissue has been used recently to harvest progenitor stem cells that carry these cues. The process of obtaining a considerable amount of adipose tissues sufficient to use in skin regeneration is highly appealing due to its relative availability and accessibility.[2]

However, further studies are required to define precisely the adipocyte role in skin homeostasis, especially among clients with different disorders such as burn. This study aimed to assess the clinical effectiveness of lipoaspirate transplantation on the treatment of skin lesions in humans.

2. Background

Research on adult stem cells began nearly six decades ago when researchers discovered that bone marrow stem cells (BMSC) contain at least two types of cells;[3] hematopoietic and the non-hematopoietic stem cells.[4] Since then, using stem cells has developed significantly, and tremendous potentials were determined in treating different conditions that affect body systems, including heart, gut, bone marrow, skeletal muscle, and skin.[5] For instance, using mesenchymal stem cells (MSC) is becoming more realistic in burn treatment and the results are encouraging.[6] Resources of MSCs include tissues, such as adipose tissues, umbilical blood and skin tissues. MSCs extracted from adipose tissue represent a very promising, easy, abundant and cost-effective source.[7] Adipose tissue offers adequate amount to perform as many procedures as required over the bone marrow source.[8,9] It also contains a higher percentage of healing cells than BMSCs.[10] Adipose tissue contains a large number of stromal stem cells, which have CD45, CD31, CD105 and surface phenotype from fibroblast like colonies proliferate (e.g., CFU F).[5] The stromal cells can differentiate into several lineages including osteogenic, chondrogenic, adipogenic, and neurogenic lineages.[3] Secreted cytokines from ASC have shown both to promote fibroblast migration during wound healing and to up-regulate neovascularization in animal models.[11,12] Although recently identified, those multipotent cells exhibit significant potential for numerous applications in skin repair.

The isolation of ASCs can be performed from the stromal vascular fraction (SVF) of a homogenized fat tissue. The multipotent cells are closely associated with perivascular cells and can maintain the potential to differentiate into multiple cell phenotypes, such as smooth muscle, endothelium adipose tissue, cartilage, and bone.[13] Detailed immunohistological studies have demonstrated that stem cell markers (e.g., STRO-1, Wnt5a, SSEA1) are

differentially expressed in capillaries, arterioles, and arteries within the adipose tissue.[14] This expression suggests that the ASCs may be vascular stem cells at diverse stages of differentiation. Adipogenic and angiogenic pathways appear to be regulated concomitantly, and adipocytes excrete multiple cytokines that induce blood vessel formation including vascular endothelial-derived growth factor (VEGF) and fibroblast growth factor-2 (FGF2).[14,15]

Additionally, cell surface expression of platelet-derived growth factor receptor beta (PDGFRβ) has been linked to the putative mural stem cells.[16] Reciprocal crosstalk between endothelial cells and ASCs may regulate blood vessel formation. Immature adipocytes have also been shown to control hair follicle stem cell activity through PDGF signaling.[15]

ASCs promote human dermal fibroblast proliferation by direct cell to cell contact, and by induced paracrine activation, which accelerates the re-epitheliazation of cutaneous wounds significantly.[17,18] The ability of adult stem cells to alter tissue microenvironment via secretion of soluble factors contributes considerably to tissue repair compared with their multipotential differentiation. In addition, adult stem cells can modulate the immune and inflammatory responses that promote wound healing.[19] The transplantation of autologous ASCs on the surface of deep burn wound decreases inflammatory cell infiltration in the wound, and accelerates the formation of new vessels and granulation tissue.[18] Labeled ASCs were observed in the epidermis, hair follicles, sebaceous glands, blood vessels and dermis in full thickness wound. Adipose stem cells derived via the extracellular dermal matrix can survive after in vivo engraftment.[20] They can further differentiate spontaneously along the vascular endothelial, fibroblastic, and epidermal epithelial lineages; thus, significantly improving wound healing.[21]

These stem cells are characterized by plasticity, which refers to the ability of stem cells isolated from a single type of tissue to convert into cells of similar and different embryonic germ layer.[22] Plasticity of ASCs toward cells of both mesodermal lineages and non mesodermal lineages were shown in their differentiation into chondrocytes, osteoblasts, myocytes, and cells of ecto- and endodermal origins.[23] This issue adds further to the preferable use of ASC over bone-marrow stem cells. These characteristics make the ASC superior to BMSC for regeneration applications, especially in skin wounds and problems.[15]

Although autologous ASCs in skin regeneration achieves higher rates of success, costs less and has an easy-to-access reservoir,[15] the efficacy of therapy differs substantially based on a comprehensive medical consultation. Adult stem cells therapy for skin damage should be customized individually for each client.

It would certainly be a mistake to underestimate the complexity of stem cell, biological behavior and the clinical applications. Treatment of skin problems with ASC is promising, but requires further exploration. This study offers an inquiry in this area.

3. Materials and Methods

3.1 Sample

A sample of 20 participants, 8 men (40%) and 12 women (60%), in the age of 20 to 65 years was self selected. They visited a private plastic clinic and were suffering from different skin problems. The participants were approached in the clinic while waiting for their turn, and the study purpose, procedure, potential benefits and risks were all explained to them. They were provided with a brochure that explains all information needed to participate in this study. The researchers' contact details were also included so that they may contact and exclaim about any inquiry. Participants then signed a consent form to participate in the study, and provided their contact details, and the plan to enroll in the study sample commenced then. All participants were contacted, interviewed for eligibility, and were informed at the beginning of the procedure that they may leave at any time during the study. Once enrolled in the study, the full procedure was planned and implemented according to the participant's convenience.

All participants were assessed for their facial skin problems. They had the following problems: browning secondary to burn, wrinkles under the eyes and at the corner of the mouth, nasolabial grooves and marionette lines, wrinkles between the eyebrows, horizontal forehead furrows, and irregularities from acne marks.

3.2 Procedure

This study was divided into five related phases performed under aseptic conditions. These steps are in chronological arrangement as follows:

1) Collection and storage of lipoaspirate (liposuction)

Stromal stem cells were isolated through lipoaspirate from several regions of the body including hip, thigh, and abdominal regions. At least 300 ml of lipoaspirate were collected into a sterile container to isolate uncultured stem cells in significant numbers; this varies between clients. The actual adipose tissue volume used for digestion after washing steps was nearly two thirds of the collected lipoaspirate volume (i.e. 200ml). If overnight storage of

lipoaspirate is required, it was stored at room temperature.[11]

2) Lipoaspirate washing

It was necessary to wash the lipoaspirate extensively in order to remove the majority of erythrocytes and leukocytes. To achieve this target, the following steps were performed under aseptic conditions:

a. Nearly 300 ml of lipoaspirate were placed into a sterile medium bottle.
b. The adipose tissue was allowed to settle above the blood fraction.
c. Blood was removed using a sterile 25 ml pipette.
d. An equivalent volume of HBSS with antibiotics and fungi zone was added.
e. The mixture was shaken vigorously for 5-10 seconds.
f. The HBSS was then removed carefully.
g. The above washing steps (4 to 6) were repeated three times. Medium from the final wash became clear and ready for the next step.

3) Collagenase digestion

Dispersion of adipose tissue is achieved by collagenase digestion. This enzyme has an advantage over other enzymes in that it can disperse adipose tissue efficiently while maintaining high cell viability. The following steps were followed to achieve collagenase digestion:

a. Collagenase solution was made up just prior to digestion. Powdered collagenase was added to HBSS to achieve a final concentration of 0.2%. The required amount of collagenase was dissolve into 40 ml of HBSS, and then the remaining working volume was filtered and sterilized. Antibiotics and fungi zone were added.
b. The washed adipose tissue was mixed with collagenase solution and incubated at 37 ^{o}c on a shaker for 1 to 2 hrs. The culture flasks were shaken manually vigorously for 5-10 seconds every 15 min.
c. On completion of the digestion period, the consistency of the digested adipose tissue becomes soup like.
d. FBS was added to obtain a final concentration of 10% to stop collagenase activity.

4) Separation of the stromal-vascular fraction

The ability of lipid-filled adipocytes to float after digestion is used to separate them from the stromal vascular fraction (SVF). The SVF predominantly contains erythrocytes, leukocytes endothelial cells and stromal stem cells. Erythrocytes were removed first using the red blood cell lysis buffer.

5) Injection of pellet

The pellet resulting from the process of washing and digestion were injected into the subcutaneous layer of the skin. The results were then observed on the clients.

After the transplantation procedure has finished, clients were followed for 4 months in a bimonthly visits. The progress was observed and all inquiries made by them were answered. No major adverse events were reported by any of the clients.

4. Ethical considerations

All the experimental protocols were approved by the Institutional Review Board of Zarqa University, Jordan. The procedure of the study explained fully by the researchers and the participants were all informed that they can withdraw at any time without giving any reason.

5. Results

Therapy with ASCs and growth factors released by the synergistic effect of platelets and white blood cells rejuvenated the skin from inside in all clients. This therapy played a role in stopping bleeding and repairing damaged blood vessels and cells in the body. It also led to increased collagen production, growth and neogenesis of epithelial cells and vascular endothelial cells, and promotion of wound healing. In addition, injecting ASCs and platelets growth factors has increased collagen and hyaluronic acid production. Hyaluronic acid and collagen are commonly used in injection therapy to treat wrinkles as both are frequently seen in areas of proliferation and migration which allow for cellular and fibrous barriers to be penetrated.[24] Thus, the presence of both materials is important for morphogenesis and tissue repair.

In this study, clients indications for injecting the material were as follows: skin browning secondary to burn; wrinkles under the eyes, nasolabial grooves and marionette lines, wrinkles between the eyebrows, horizontal forehead furrows and wrinkles in the corner of the mouth; and irregularities from acne marks. The procedure took on average 15 minutes and treatment efficacy was immediate to nearly all participants. The injection procedure was repeated four times during the four month period (i.e. once every fourth week). Low pain level was reported by 50% of clients during the first day after the therapy session. Allergy was not reported by any of the clients.

The following changes were all observed in clients, but with variations:

a. Cell growth, new generation and repair of blood vessels, collagen production
b. Growth and new generation of vascular endothelial cells
c. Growth and neogenesis of epithelial cells and vascular endothelial cells, promotion of wound healing.
d. Tissue repair, cell growth, collagen production, hyaluronic acid production
e. Promotion of epithelial cells growth, angiogenesis, promotion of wound healing

These variations were mainly related to age, general skin health, the size, and nature of skin problem. Generally, younger clients exhibited higher rates of healing. Although wrinkles in old aged (>60 year-old) required longer period of time to decrease, the results were encouraging even among them. The following two cases were chosen randomly to represent findings in this study:

5.1 Case One:

A 40 years old woman had first and second degree facial skin burns, and a total burned area of 30%. She was injected with autologous stem cells onto the surface of the thermal facial burn and was followed-up by the researchers every second week (i.e., 2, 4, 6, 8, 10, 12, 14 and 16) from the treatment day. The follow-up notes determined the progress and observation of any adverse events. These notes confirm high tempo of neoangiogenesis and re-epithelialization of brown zones on the face, and accelerated rehabilitation on this patient. Figure 1 illustrates the pretreatment and the post treatment (end of week 16) with improvement in the browning of the skin and the overall skin condition.

5.2 Case Two:

A woman aged 55 years with numerous wrinkles around her eyes was injected with autologous stem cells. The results showed that using these stem cells as fillers of wrinkles, which did not involve the injection of any foreign substance, gave facial rejuvenation. Figure 2 illustrates the pretreatment and the post treatment at the end of week 16. There was a significant improvement on the wrinkles that were developing progressively on this client.

Generally, all follow-up notes made by the researchers determined that the injected sites had experienced no adverse events, and increased speed of change to more youthful look. Almost all clients in this study experienced significant improvement in the appearance of the wrinkles.

6. Discussion

Findings in this study indicate that injecting autologous adipose tissue-derived stem cells and fat injection is very promising treatment to depressed areas and scars in the face. It has been reported in similar types of treatment that adverse events, time consumption and the results usually represent very challenging issue and decrease client satisfaction. The procedure adopted in this study, however, represented a reasonably satisfying one to clients.

Using autologous adipose tissue injections produced a significant decrease in deep nasolabial grooves, wrinkles on the forehead, wrinkles on the neck, wrinkles on the lips, and dark circles under the eyes. Some of these have, in fact, disappeared totally from the face. There has been a systematic progressive regeneration exhibited within the ultra-structural of the target tissue, including neovessel formation. Clinical outcomes led to a systematic improvement or remission in all evaluated clients. In addition, brown discoloration secondary to abnormal skin condition decreased significantly in almost all clients with this condition.

The Adipose-derived stem cells can accelerate cutaneous healing in the skin leading to decreased or diminished grooves and wrinkles within the treated areas of the face. Similarly, findings reported that ASCs promoted healing of full thickness wounds in mice.[18]

Therapeutic use of ASCs depends largely on the possession of a multilineage differentiation potential, which can be attractive candidates for clinical applications to use in skin repair or regeneration.[23] Seeding adipose-derived stem cells onto a dermal substitute improves skin regeneration and tissue integration by increasing vascularity and collagen synthesis. Further studies are necessary to achieve complete epithelialization with the use of adipose-derived stem cells.[23]

Furthermore the use of autologous ASCs decreased the possibility of T-cell mediated immune response.[23] Autologous ASCs are easily obtained through lipoaspiration compared with the BMSC that requires a much greater effort and volume per person.[15] BMSC have low immunogenicity and immunoregulatory actions that are obtained through suppressing the functions of immune cells, including T cells.[25] Although ASCs have similar characteristics as BMSC, allogeneic ASCs are not affected by the host immune response, and cannot accelerate wound healing like autologous ones. ASCs have also been functionally characterized by their ability to undergo lineage differentiation, and provide a lower immune-expression characteristic.[26]

Wound healing and clinical remarks of the decreased or disappeared wrinkles at injection places on the face. Although limited in number of participants and procedural control, this study provides evidence of the positive results autologous ASC transplantation may have on various skin problems.

7. Conclusion

Findings in this study support the use ASCs autologous transplantation and fat injection to regenerate skin. The ASCs have demonstrated superior results over BMSC in many areas. Generally, the use of autologous ASCs in wound healing and skin regeneration is a promising area for the future in cell-based medicine. However, results in this study need to be retested further using a bigger sample and different skin conditions and purposes of injections and transplantations.

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Figure 1. Case One: Woman had first and second degree facial skin burns (Before). The effect of autologous adipose stem cells injections (After).

Figure 2. Case Two: the pictures show a woman with numerous wrinkles around her eyes (Before), and the effect of autologous adipose stem cells injections (After).

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