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Adipose Derived Stem Cell

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KEYWORDS

Adipose Derived Stem Cells; Cell Surface Characterization; Mesenchymal Stem Cells; Stromal Cells; Autologous Fat.

ABSTRACT:

Adipose-derived stem cells (ADSC) are increasingly popular due to their potential in wound healing and tissue engineering. Stem cell therapy has limited application, due to the need to maintain cell viability and function as well as safety concerns. It has been increasingly reported that the effects of ASC are largely due to the paracrine effects of secreted factors, which can be accumulated in the conditioned medium (CM). Our aim was to conduct a systematic review of the literature to summarize the scientific work that has been done on ADSC to date. Conclusion show that ADSC can encourage the growth of active tumor cells. Therefore its use must be done carefully. It is recommended that further research is needed to identify potential complications and other problems that may arise from the use of ADSC.

INTRODUCTION

Fat is often associated with a condition or disease that has a negative impact on a person's quality of life. On the other hand, this component has many benefits and is currently the subject of extensive research. Essentially, fat tissue plays physiological roles such as energy storage and metabolism, thermoregulation, and hormone metabolism. Additionally, adipose-derived stem cells act as mesenchymal cells with multipotent differentiation capabilities, proving useful in various pathological conditions.

Autologous fat is an ideal material for augmentation in plastic and reconstructive surgery. Currently, plastic surgeons widely use autologous fat grafting for reconstructive and aesthetic procedures (Moustaki et al., 2017). This technique is valuable in contouring, augmentation, rejuvenation, therapy for radiation damage, treatment of capsular contracture of the breast, scar tissue therapy, post-traumatic deformity, congenital anomalies, burns, and soft tissue defects. The popularity of this grafting technique stems from its ability to provide replacement volume and improve tissue quality at a relatively low cost, with low donor site morbidity. It is easily accessible, available in many healthcare centers, and boasts biocompatibility aspects (Shih et al., 2020). However, it's important to note that fat is intolerant of hypoxia and ischemia, leading to a high respiration rate. The isolation of mesenchymal stem cells from fat tissue through a combination of autologous fat and fat transplantation adipose-derived stem/stromal cells (ADSC) is a promising strategy to overcome these challenges (Moustaki et al., 2017).

Adipose-derived stem cells are mesenchymal cells found in fat tissue that can differentiate into various types of cells along germ lineages, including osteocytes, adipocytes, neuronal cells, vascular endothelial cells, cardiomyocytes, pancreatic beta cells, and hepatocytes (Shukla et al., 2020). Previous studies have examined the effects of fat grafting enriched with ADSCs, showing that this technique can improve graft sustainability compared to traditional fat grafting (Trojahn Kølle et al., 2012). This literature review aims to provide insight into the role of ADSC in fat grafting, with the hope of increasing the knowledge of clinicians and enabling its application in daily practice.

RESULT AND DISCUSSION

Adipose Tissue

Adipose tissue is generally classified into two groups, namely brown adipose tissue (BAT), which is thermogenically active, and white adipose tissue (WAT), responsible for energy storage (Hoppela et al., 2018). In humans, BAT is distributed in the cervical, supraclavicular, axillary, paravertebral, mediastinal, and upper abdominal areas. In contrast to BAT, WAT is the dominant adipose tissue in humans, predominantly

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located in visceral or subcutaneous regions. White adipose tissue used in autologous fat grafting is generally subcutaneous (Shih et al., 2020).

White adipose tissue comprises two main components: mature adipocytes and the stromal vascular fraction (SVF). SVF is a heterogeneous cell population consisting of endothelial cells, smooth muscle cells, pericytes, leukocytes, fibroblasts, mast cells, preadipocytes, and multipotent ADSC (Shih et al., 2020). The cellular components of SVF account for almost 50% of the number of cells in fat tissue, while adipocytes account for 90% of the total tissue volume (Khouri & Khouri, 2017). On the other hand, the extracellular matrix connects adipocytes and contributes to the formation of fat lobules in adipose tissue (Shih et al., 2020).

2.2 Adipogenesis

Adipocytes originate from ADSC, and their development occurs in two main phases. The first phase is determination, where ADSC develops into the adipocyte lineage, becoming preadipocytes. At this stage, preadipocytes cannot differentiate into other cell types. The second phase is the terminal differentiation phase, where lipid accumulation occurs in the cytosol of preadipocytes, and they develop into mature adipocytes (Shih et al., 2020). As age increases, the capacity of preadipocytes to differentiate into functionally mature adipocytes decreases (Cartwright et al., 2007). The size of mature adipocytes varies in diameter, ranging from 50 to 150 μm, and these cells can last up to 10 years. Mature adipocytes are vulnerable and have poor tolerance to mechanical trauma and ischemia, while preadipocytes have minimal metabolic activity and are 20 times smaller than mature adipocytes, with higher tolerance to trauma and ischemia (Shih et al., 2020).

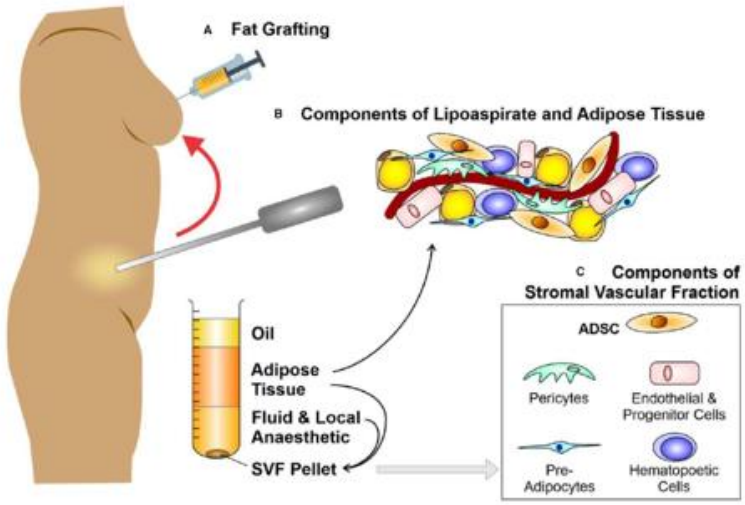

Figure 1. Liposuction, Fat Grafting, and Fat Tissue Components

(A) Fat grafting after liposuction of subcutaneous fat from the abdominal area. (B) Lipoaspirate components, which are divided into the fat layer (removed), fat tissue, and infranatant (consisting of blood, plasma, and local anesthetic). (C) Fat tissue components and stromal vascular fraction (SVF) can be reinserted to enhance fat grafting. The next process is as follows: collagenase digestion and centrifugation allow for SVF isolation (Shukla et al., 2020).

Fat tissue is rich in blood vessels, with each adipocyte bound by a capillary network that allows efficient exchange of metabolic products. Due to the high density of capillaries and low oxygen consumption, the skin's fatty tissue has the highest tissue partial oxygen tension (ptO2: 40-60 mmHg) compared to other internal organs (Shih et al., 2020).

Initially, adipose tissue was considered an inert substance that only plays a role in energy consumption. However, recent research shows that adipose tissue has significant proliferative and regenerative potential, primarily driven by adipose-derived stem cells (ADSC). Adipose tissue contains adult stem cells in the highest percentage compared to cells from other tissues, even surpassing the spinal cord. There are 4,500 ADSCs per millimeter of fat, whereas bone marrow only has 100 –

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1,000 stem cells per millimeter of bone marrow (Shih et al., 2020).

The regenerative effect of ADSC is derived from cytokines and growth factors secreted paracrine, with the highest secretion occurring during hypoxic conditions. The secreted factors include angiogenic cytokines like vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), fibroblast growth factor 2, basic fibroblast growth factor, and hematopoietic cytokines such as granulocyte colony-stimulating factor. These factors impact stem cell differentiation, induce angiogenesis, stimulate tissue remodeling, and trigger wound healing (Shih et al., 2020).

Mature adipocytes have the ability to differentiate into ADSC, which can then differentiate into adipocytes or other cell types, such as osteocytes. ADSCs are the main cell population responsible for regenerating adipocytes and have the ability to undergo multilineage differentiation into adipocytes and vascular endothelial cells, as well as other tissues like bone, cartilage, striated muscle, nerves, and skin. A small part of ADSC, known as the adipose-multilineage-differentiating stress-enduring (Muse) cell, has higher potential and is believed to contribute to the ability of ADSCs to differentiate into endodermal and ectodermal-derived tissues such as skin and nerves. The density of ADSCs in tissue also contributes to the regenerative potential of adipose tissue (Shih et al., 2020). The broad regenerative and angiogenic properties of ASCs mean that these cells can be utilized in fat grafting (Bellini et al., 2017).

Deep Adipose Tissue Fat Grafting

Autologous fat grafting, or the surgical transfer of fat tissue from one part of the body to another, was first introduced by the German surgeon Gustav Adolf Neuber in 1893. He successfully transplanted fat from the upper arm to fill atrophic scar tissue on the face. However, after this success, some reported failures in grafting, making this technique not yet widely applied (Agarwal & Mistry, 2017; Shih et al., 2020; Strong et al., 2015).

The problem that arises from autologous fat transfer is the unpredictable rate of fat resorption. The resorption rate of graft adipose tissue is reported to vary widely, from 20% to 90%, resulting in a reduction in implant volume (Bellini et al., 2017; Doornaert et al., 2019). This necessitates repeat procedures and may lead to poor aesthetic results (Shih et al., 2020).

Revascularization Graft

During the grafting of adipose tissue, the tissue is transferred in an avascular condition and must undergo revascularization to survive. Revascularization is largely controlled by perivascular ADSCs, which release angiogenic factors when ischemia occurs (Shih et al., 2020). After the surgical transfer of non-vascularized autologous fat tissue, the graft initially obtains nutrition through plasma diffusion from the surrounding host tissue over several days. Within 48 hours post-implantation, new blood vessels begin to provide nutrition to the graft (Mashiko & Yoshimura, 2015). Revascularization occurs centripetally, starting from the periphery and moving towards the center fat droplet (Bellini et al., 2017). In severe ischemia, most adipocytes in the regenerative and necrotic areas die within the first 24 hours, leading to the release of inflammatory and injury-related factors (Shih et al., 2020).

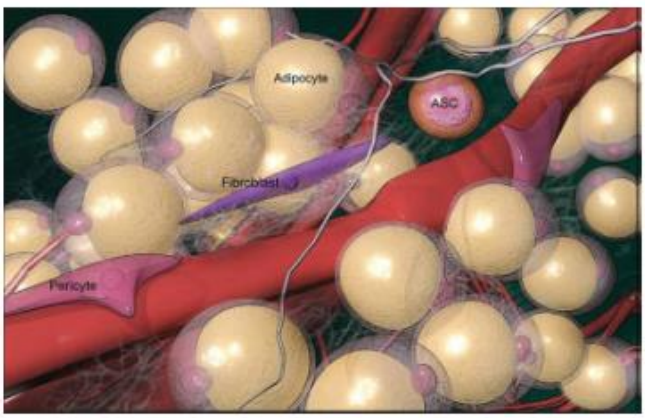

Figure 2. Adipose-derived stem cell in the perivascular area. (Shih et al., 2020)

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Among the cellular components of adipose tissue, mature adipocytes are the most susceptible to ischemia and die first. If ischemia continues, vascular endothelial cells and blood-derived cells will also die. Adipose-derived stem cells are relatively resistant to ischemia and can survive up to 3 days in severe ischemic conditions. During this time, ADSCs undergo proliferation and adipogenesis, triggering angiogenesis in response to signals from dying cells around them to facilitate the repair process and restore damaged tissue (Eto et al., 2012; Mashiko & Yoshimura, 2015). Revascularization in the regeneration zone is enhanced by ADSCs in the first 3 days after transplantation. Adipogenesis from ADSCs replaces dead adipocytes in the regenerative zone up to 3 months after grafting. Additionally, phagocytosis of dead adipocytes takes several weeks to months depending on tissue size. Therefore, the volume of grafted fat remains unchanged during the first 4 weeks despite significant adipocyte death (Mashiko & Yoshimura, 2015; Yoshimura et al., 2011).

To achieve sufficient revascularization of the graft, each fat graft droplet (G) must interact with the capillary recipient area (R) in a 1:1 ratio to form a successful fat-recipient (GR) complex. If there are more fat droplets than the recipient capillary area, inadequate angiogenesis may result in fat resorption and necrosis. This concept, introduced by Khouri, is known as principal stoichiometry in fat grafting (Abu-Ghname et al., 2019; Khouri & Khouri, 2017). Ideally, there should be optimal interaction between the graft and recipient, good vascularization, and optimal oxygen tension for the recipient tissue (Khouri & Rheault, 2021; Mashiko & Yoshimura, 2015).

Response to Damage and Mechanical Stress

When adipose tissue is damaged, degenerative changes occur, triggering the release of inflammatory cytokines and injury-related cytokines to accelerate wound healing. Adipose-derived stem cells, stimulated by this process, undergo proliferation and secrete secondary factors such as aHGF and VEGF to regenerate adipose tissue and suppress fibrosis during the first week of injury. Bone marrow progenitor cells are also recruited and collaborate with ADSCs to repair adipose tissue (Mashiko & Yoshimura, 2015).

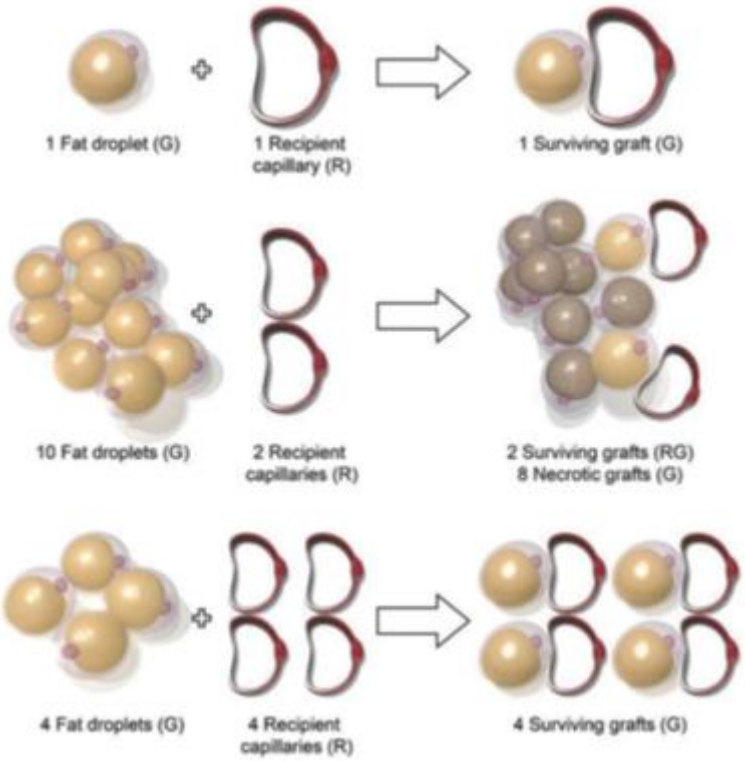

Figure 3. Principle Stoichiometry for fat grafting.

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Each fat droplet (G) must bind to the vascular supply of the recipient capillary (R) in a 1:1 ratio to achieve adequate revascularization. All fat tissue that does not meet the 1:1 principle will not survive. (Shih et al., 2020) Mechanical forces, both exogenous and endogenous, can affect cellular function, survival, and growth of adipose tissue (Mashiko & Yoshimura, 2015). Various fat grafting techniques exert different mechanical stresses on adipocytes, and previous research shows that cell viability and adipocyte functionality differ between these techniques (Strong et al., 2015). Physical interactions with the extracellular matrix of adipose tissue can also influence the properties of stem cells, and external tissue expansion can stimulate reversible adipogenesis, resulting in enlarged adipose tissue (Mashiko & Yoshimura, 2015; Shih et al., 2020).

Life Sustainability Graft

Currently, three theories explain how graft fat persists after vascular surgical implantation, and research suggests that each theory may play a role in the survival process. These theories are survival theory graft, replacement theory graft, and host cell replacement theory (Doornaert et al., 2019).

The survival theory of graft, first described by Peer et al., states that after surgical transfer, graft fat initially survives through nutrient diffusion from plasma until neovascularization of the recipient area is formed (Doornaert et al., 2019). Only fat cells with an adequate supply of new blood can survive (Pu, 2016). Smaller graft volumes can provide better survival than larger volumes because smaller volumes achieve better diffusion and perfusion (Shih et al., 2020).

The replacement theory of graft states that very few donor adipocytes survive the grafting process. Most of the grafted adipocytes are replaced by donor adipose-derived stem cells (ASCs) that are simultaneously internally transferred. The stromal vascular fraction (SVF) from the donor is responsible for adipogenesis and angiogenesis (Doornaert et al., 2019). Providing additional SVF and ADSC to graft fat can increase graft retention. Additionally, grafts with higher density have a higher ADSC concentration, resulting in a higher survival rate than those with lower density (Shih et al., 2020).

According to the host cell replacement theory, no graft cells survive, and all these cells are replaced by recipient cells. Graft cells undergo necrosis and are replaced with fibrous tissue, new fat cells, and blood vessel growth from the recipient tissue. Thus, the integrity and area of the recipient are the main determinants of graft survival (Doornaert et al., 2019) (Shih et al., 2020).

The acute regenerative and adipogenic phase of graft fat loss is completed in 3 months, followed by a "chronic" stabilization process that can last up to the next 9 months. The duration of this remodeling process can reach up to one year. During the “chronic” phase, fat absorption continues without compensatory ADSC regeneration. Any dead adipocytes remaining in the central regenerative or necrotic zone will undergo resorption, fibrogenesis, or form oil cysts (Mashiko & Yoshimura, 2015).

The rate of resorption depends on the diameter of the lipid droplet. Droplets <8mm in diameter can be absorbed relatively quickly and replaced by fibrosis. On the other hand, droplets with a diameter of >8mm are absorbed more slowly, and oil cyst permanent formation occurs before these large droplets can be completely absorbed, causing chronic inflammation and calcification over time (Mashiko & Yoshimura, 2015; Shih et al., 2020).

The balance between the rate of lipid resorption, necrosis, and adipocyte replacement plays an important role in determining the final volume (Mashiko & Yoshimura, 2015; Pu, 2016). If the graft volume is small, lipid resorption and adipocyte regeneration are completed within a 3-month period, and the final graft volume will not change significantly after 3 months. However, if the graft volume is large, many large droplets may remain incompletely absorbed within 3 months, leading to continued absorption without compensatory adipogenesis for up to a year. This process causes a clinically significant volume reduction. The final achievable volume of the graft is influenced by various factors, including the microenvironment of the graft, vascularization, processing techniques, and postoperative care (Mashiko & Yoshimura, 2015). In recent decades, several methods for preparing the recipient site have been proposed, and the surgical results of fat grafting have shown improvement. One approach is to leverage adipose-derived stem cells (Mashiko & Yoshimura, 2015; Shih et al., 2020).

Adipose-Derived Stem/Stromal Cells

1) Characteristics Adipose-derived Stem Cells

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Adipose-derived stem cells are cells that can adhere to plastic media under standard culture conditions. These cells are characterized by CD34+, CD31-, and CD45- as cell surface markers, and they possess multipotent differentiation capabilities into mature bone, cartilage, and fat. In adults, stem cells uniquely differentiate into various more specific cell types to repair damaged cells, maintain tissue integrity, and uphold cell homeostasis with normal growth and healing rates (Shukla et al., 2020).

Mesenchymal stem cells, including those derived from adipose tissue (ADSCs), have the potential for use in tissue regeneration. Similar to mesenchymal stem cells from bone marrow, ADSCs can differentiate into various cell types such as fat, cartilage, bone, skin, muscle, endothelium, and nerve-like cells when exposed to certain induction factors. ADSCs offer the advantage of being 500 times more abundant in fat tissue than in bone marrow, with approximately 5 x 10^5 ADSCs obtainable from 400-600 grams of fat. Moreover, ADSCs are easier to obtain and less invasive (Shukla et al., 2020).

These cells produce a variety of chemokines, cytokines, and growth factor proteins, contributing to the paracrine effects crucial for tissue regeneration. ADSCs can trigger tissue regeneration by modulating biological and molecular control signals, promoting angiogenesis and lymphangiogenesis, suppressing local immune or inflammatory responses, and reducing fibrogenesis (Shukla et al., 2020).

2) Isolation of Adipose-derived Stem Cells

The isolation of adipose-derived stem cells from human lipoaspirate is influenced by several factors, including interpatient variability, collection location, collection system, lipoaspirate storage duration, cell isolation method, degree of collagenase digestion, and centrifugation protocol. Recent evidence suggests that ADSCs originate from perivascular cells localized around blood vessels. Additionally, the number of isolated ADSCs correlates with blood vessel density in adipose tissue. Harvesting fat from the superficial layer, obtained through liposuction, may increase ADSC recovery compared to conventional suction-assisted liposuction, which extracts fat from deeper layers. Liposculpture using a smaller-diameter cannula may provide advantages in harvesting superficial fat layers with less risk of skin irregularities (Trivisonno et al., 2014).

Compared with fat harvested using a conventional 3 mm diameter cannula, adipose tissue aspiration with a microcannula (≤ 2 mm) may offer advantages in harvesting the superficial fat layer and improving ADSC isolation yield. In lipoaspirate collected by microcannula infiltration, a higher fraction of vascular cells capable of forming capillary-like structures was observed in the basement membrane matrix assay, expressing CD31 (Trivisonno et al., 2014).

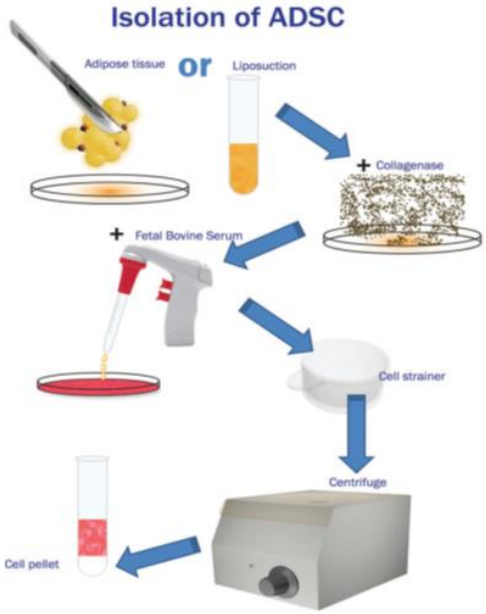

Figure 4. Retrieval process of adipose-derived stem cells.

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After enzymatic digestion, the enzyme effect can be neutralized by fetal bovine serum (FBS), and the mixture is filtered through a cell strainer. Cell pellets will gather after centrifugation and separate from the supernatant. (Naderi et al., 2017)

It is important to note that the use of ADSCs is still controversial due to the lack of a clear explanation regarding ADSC purification and culture protocols, as well as the use of sub-total or whole stromal vascular fraction (SVF). Safety concerns arise, especially regarding the potential of stimulating cancer growth in areas previously affected by cancer, attributed to the secretion of pro-angiogenic factors like VEGF-A. Additionally, chronic calcification in the graft area poses challenges for monitoring cancer in the future (Shukla et al., 2020). The addition of components, such as the collagenase process, raises questions about potential changes in fat, suggesting it may resemble a modified therapeutic product rather than a straightforward autologous tissue transfer (Raposio & Ciliberti, 2017).

3) Work mechanism Adipose-derived Stem Cells in Tissue Regeneration

Adipose-derived stem cells possess the ability for adipogenic differentiation, capable of inducing restoration effects on tissue contour and volume. This is thought to occur through the direct differentiation of ADSCs into adipocytes, or via the paracrine effect of ADSCs influencing local stem cells to differentiate into adipocytes, resulting in the formation of new fat in the graft area (Shukla et al., 2020).

Nie et al. demonstrated that intradermal administration of ADSCs facilitates wound closure in mice by increasing epithelialization and granulation tissue deposition. The accelerated wound healing is attributed to the differentiation of ADSCs into epithelial and endothelial cells, thereby enhancing regeneration and angiogenesis (Nie et al., 2011). ADSCs can be administered topically, intravenously, and intramuscularly to trigger wound healing (Kim et al., 2019).

Tissue perfusion improves after ADSC injection due to paracrine induction of angiogenesis or repair of existing vascular structures. The release of angiogenic factors from ADSCs triggers revascularization and wound healing. Key proteins involved include insulin-like growth factor (IGF), platelet-derived growth factor (PDGF)-bb, fibroblast growth factor (FGF), transforming growth factor (TGF) beta, interleukins (IL)-6, -8, stromal-related protein, matrix metalloproteinase (MMP), monocyte chemoattractant protein (MCP)-1, stromal cell-derived factor (SDF)-1, and vascular-related proteins such as vascular endothelial growth factor (VEGF)-A, -C, and -D (Shukla et al., 2020).

Angiogenic lymph factors secreted by ADSCs play a role in lymphangiogenesis, repairing or restoring lymphedema in damaged tissue. ADSCs counteract lymphatic fluid stasis by inhibiting TGF-beta 1, leading to the expression of podoplanin, Prox-1, and the growth factor VEGF-C. Proteins detected in ADSCs, distinguishing them from other mesenchymal stem cells, exhibit pro-lymphangiogenic effects (Shukla et al., 2020).

The antioxidant effect of ADSCs provides a regenerative effect by mitigating cellular damage caused by free radicals, hypoxia, and the effects of reperfusion after ischemia. Key growth factors involved are PDGF-AA, HGF, IL-12, G-CSF, and IGFBP, mediated by pigmented epithelial-derived growth factor and superoxide dismutase. ADSC-induced cytokines also modulate immune responses and inflammation. Adipose-derived stem cells inhibit the proliferation of T cells and B cells through pathways mediated by NFκB. The secretion of IL-6 and IL-8 acts as an attractant for monocytes and macrophages, playing a role in triggering the wound healing process (Shukla et al., 2020).

Extracellular vesicles are a heterogeneous population of cell particles ranging from nano to micro sizes, enclosed by a membrane. These vesicles are crucial for intracellular communication and consist of microvesicles, exosomes, and other extracellular vesicle populations. Various cell types continuously release extracellular vesicles into the extracellular environment, containing proteins, peptides, RNA, lipids, and DNA fragments that act locally or circulate through the bloodstream to modulate cellular responses in distal areas through paracrine signals (Shukla et al., 2020).

Fat tissue macrophages release exosomes containing specific miRNAs, facilitating glucose intolerance and insulin resistance in mouse populations (Wu et al., 2018). Adipocytes communicate with fat tissue macrophages through these extracellular vesicles, directly delivering fat to macrophage-like cells in fat tissue. This has implications for the pathology of obesity, as lean mice release at least 1% of lipid content per day via exosomes

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ex-vivo. Additionally, an increase in these vesicles in the plasma is observed along with an increase in BMI (Amosse et al., 2018). Based on these findings, it is known that the ADSC secretome has been proven to trigger wound healing and nerve regeneration. Therefore, exosomes are used in engineering a specific extracellular vesicle delivery system (Shukla et al., 2020).

Benefit Adipose-derived Stem Cell

1) Breast Augmentation

Initially, flaps were used for breast reconstruction. The flap consists of muscle, fatty tissue, fascia, dermis, and skin. Subsequently, autologous fat transplantation became a more widely used method in breast reconstruction. However, there are several considerations, such as the safety and effectiveness of fat grafting (lipoaspirate) to the breast (Krumbeck et al., 2013).

Recently, the concept of fat grafting has been further developed into fat grafting enriched with stem cells. However, this again raises questions about the safety of stem cells given to the breast. It remains a matter of debate whether the total amount in the transferred tissue or the concentration of transferred stem cells, which may differ in fat grafts and flaps, has a positive or negative influence on the rate of recurrence, metastatic spread, or initiation of the cancer cascade. However, if stem cells are not added, it can be assumed that the lipoaspirate contains the same number of stem cells as the same volume of adipose flap tissue (Krumbeck et al., 2013).

After a total mastectomy, fat grafting is considered a surgical option, which is always possible. However, it is not recommended for the contralateral breast. Oncologic follow-up should be performed at an experienced healthcare center. For aesthetic indications and patients with congenital breast deformities, fat grafting is considered a surgical option if the patient has a low risk of developing cancer. Women undergoing this procedure should be less than 35 years old and have no personal or family history of cancer. Genetic testing for BRCA I/II is highly recommended, as is preoperative imaging of the breast. After the procedure, it is recommended to carry out control for one year and undergo a radiological examination (Krumboeck et al., 2013).

It is important to remember that every woman has a risk of experiencing breast cancer in her life. In American women, the lifetime risk is greater than 12.5% with a mean annual incidence of 85–125/100,000 women depending on the ethnic group. In patients with a positive family history, the risk of developing breast cancer increases significantly. The number of first-degree relatives with breast cancer influences the lifetime risk (Krumboeck et al., 2013; Metcalfe et al., 2010).

2) Facial Rejuvenation

In the lower eyelid area, dermal melanocytosis, fine lines, skin atrophy, dryness, and loss of subcutaneous fatty tissue are early signs of aging. This is a common condition reported by patients seeking aesthetic treatment. In addition to adding volume over the orbicularis muscle and improving skin laxity, fat injection in the periorbital area can improve pigmentation. Indications for micro-amount fat transfer in the lower eyelid area are limited due to the high risk of overcorrection and lumping (Surowiecka et al., 2021). General indications for lipofilling lower eyelids include a history of failure of hyaluronic acid (HA) filler and mesotherapy. Nano fat transfer and fat emulsion are used primarily to remove dark circles on the lower eyelids. The administration of SVF can be combined with emulsion fat transfer, so it can provide an increase in volume and minimal tissue elevation. Most clinicians use 27 G sharp needles for intradermal and subdermal nanofat injections. Another technique uses a 23 G sharp needle to inject a smaller portion of the graft, followed by deeper volume restoration with a 20 G sharp needle (Azzam et al., 2020; Tonnard et al., 2013). Surowiecka et al. do not filter emulsified fat and use a 21 G blunt cannula. Using one of the above entry points (zygomatic arch), they performed regular subdermal tissue filling with the same injection depth, and no hematomas were reported. The emulsified fat is applied deeper over the orbicularis muscle, and the SVF is injected into the subdermis using the same, but more superficial, entry point. They used an average of 1.5 mL of emulsified fat and 1.5 mL of SVF per site (Surowiecka et al., 2021).

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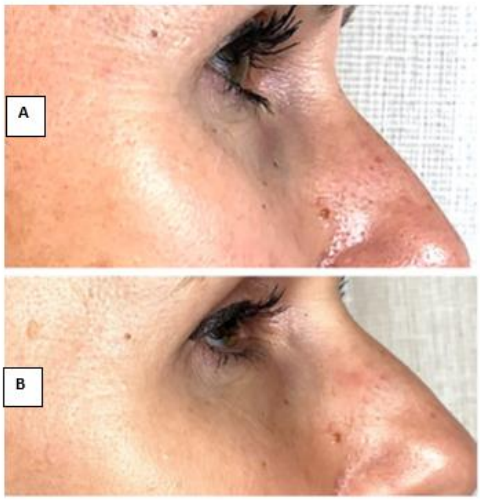

Figure 5. Clinical photos before and after correction

A 56-year-old female patient. Photo (A) is before the procedure, and (B) is 3 months after the administration of emulsified fat 1.5 mL and 1 mL stromal vascular fraction (Surowiecka et al., 2021).

Tonnard et al. wrote a case report covering three patients. In detail, they carried out high-negative-pressure liposuction procedures using standard liposuction tools. In the first group, a standard 3mm Mercedes-type liposuction cannula with large side holes (2x7mm) was used. The second and third groups used a multiport 3 mm cannula with sharp side holes of 1 mm diameter. After washing with saline and filtering, there was no further processing for lipoaspirate group one (so-called macro fat) and group two (microfat). In group three, lipoaspirate was mechanically classified after the washing stage. Emulsification was carried out by repeatedly moving the fat in two layers with a syringe of 10 ml connected to each other using a female-to-female Luer-Lock connector. After 30 repetitions, the fat turned into an emulsion. At the end of the fragmentation process, the fat became liquid and appeared whitish. After this emulsification, the liquid fat was filtered again using sterile nylon cloth, and the effluent was collected in a sterile container. This process was carried out to remove connective tissue remnants that could obstruct the fine needle. This effluent is called nanofat (Tonnard et al., 2013).

The first patient was a 41-year-old woman with fan-shaped vertical rhytids and photoaging skin in the decollete area. Rhytides were treated by injecting microfat 6 ml using a 23G needle with a longitudinal injection technique. Additionally, the surface area of the decollete was treated with a superficial subdermal injection of 12 ml of nanofat, using a 27G needle, and the fan-shaped technique repeatedly. In the first week after injection, the corrected area was visibly overcorrected but gradually became normal with results 3 months after correction (Tonnard et al., 2013).

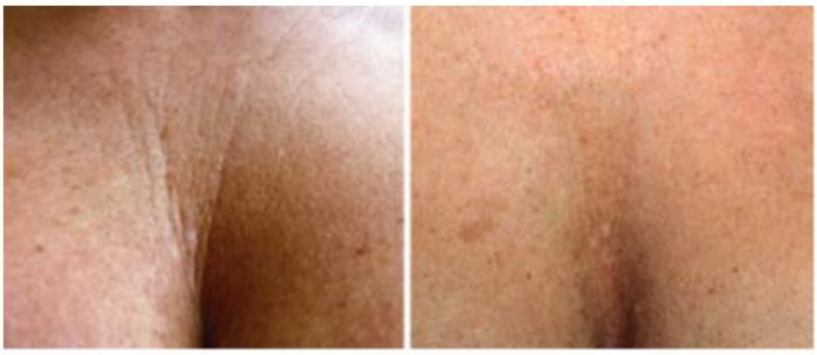

Figure 6. Clinical photo of skin photo aging

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Photo (A) shows fan-shaped vertical rhytides of the decollete area. Photo (B) is three months after injecting 6 ml of microfat on rhytides and 12 ml of nanofat into the superficial subdermis throughout the decollete area. Actinic and rhytides seem to be improving (Tonnard et al., 2013).

The second patient is a 33-year-old woman with moderate prominence in the lower eyelid fat compartment and a prominent eyelid-cheek border. The skin of the lower eyelid appears highly pigmented, extending along the nasojugal groove. This pigmentation has been present since childhood. To correct this case, Tonnard et al. performed lower eyelid blepharoplasty with fat draping along the lower and lateral orbital rim, accompanied by intradermal infiltration using nanofat to improve pigmentation (Tonnard et al., 2013).

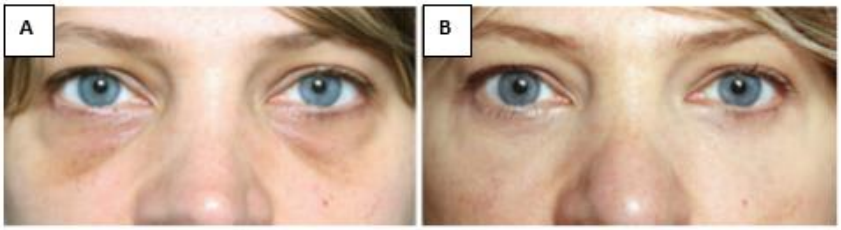

Figure 7. Clinical photo of the patient's eyelid blepharochalasis and dark color on the lower eyelid

Photo (A) is before the procedure, and (B) is 7 months after fat redraping, blepharoplasty, and intradermal injection of 1.6 ml of nanofat in the lower eyelid and pigmented nasojugal groove (Tonnard et al., 2013).

The third patient from the case report of Tonnard et al. is a 61-year-old woman who desires perioral rejuvenation, especially correction of vertical rhytides of the upper and lower lips. They performed microfat grafting of rhytides accompanied by nanofat grafting throughout the perioral region and cheeks. The procedure required injecting 4 ml of microfat with a 23G needle (sharp needle intradermal fat grafting technique) into rhytides, and 6 ml of nanofat which was injected intradermally into the skin of the upper and lower lips, as well as both cheeks (Tonnard et al., 2013).

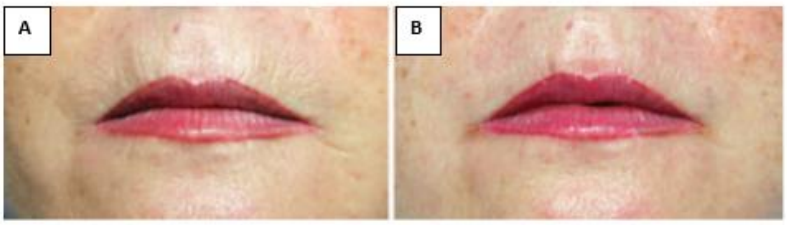

Figure 8. Clinical photos of patients with perioral rhytides

Photo (A) before the procedure, and photo (B) 7 months after injection. The quality of the skin in photo (B) appears to be better. (Tonnard et al., 2013)

The most common side effect of lipofilling is swelling that can last up to a month after the procedure. Additionally, there is the potential for graft migration, calcification, cyst formation, ischemia, necrosis, and vision loss. Vascular complications are rare, but if they occur, they can cause blindness. Although most cases of blindness are reported after intramuscular injection in the glabella and nasal areas, practitioners should also be careful in the lower eyelid area. In-depth knowledge of anatomy is mandatory, and it is recommended to use blunt cannulas with a large diameter (Surowiecka et al., 2021).

3) Lipoatrophy Face

Facial lipoatrophy is a problem that accompanies several congenital and acquired diseases. Lupus erythematosus profundus and scleroderma in saber stroke often cause facial lipoatrophy. This condition is often the manifestation of the disease most complained about by people. Facial lipoatrophy is also seen in patients with human immunodeficiency virus (HIV) infections and Parry-Romberg syndrome (progressive hemifacial

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atrophy or idiopathic hemifacial atrophy), which involves not only facial lipoatrophy but also bone defects. So far, there is no medical treatment to correct facial lipoatrophy (Yoshimura et al., 2008).

Lipo Injection has been used to correct lipoatrophy with moderate success. On the other hand, microsurgical tissue transfer has become the standard surgical treatment to reshape the facial deformities seen in Parry-Romberg syndrome. However, the surgical procedure leaves prominent scars on the face and donor area. Autologous Lipo Injection is a promising treatment for augmentation of soft tissue because it does not leave incision scars or complications related to foreign bodies (Yoshimura et al., 2008).

In the technique cell-assisted lipotransfer (CAL), fat that has a low ADSC content is converted into fat that is rich in ADSC by supplementation using cells isolated from adipose tissue. Yoshimura et al. used this principle for facial soft tissue augmentation. Before suctioning, the abdominal wall is infiltrated with saline solution along with diluted epinephrine (0.001%). Adipose tissue was suctioned using a cannula with an inner diameter of 2.5 mm and a conventional machine for liposuction. In the CAL group, about half of the liposuction aspirations collected were used for SVF isolation. The stromal vascular fraction was isolated from adipose and fluid from aspiration liposuction. The adipose portion of the aspirate liposuction was processed with 0.075% collagenase in buffered saline for 30 minutes on a shaker with a temperature of 37°C. Mature adipocytes and connective tissue were separated from ADSC-containing SVF by centrifugation (800 x g, 10 min), and then rinsed three times with buffer saline. The liquid portion was centrifuged (800 x g, 5 min), and the pellet was resuspended in hypotonic water to lyse erythrocytes. The cell processing procedure takes approximately 90 minutes. During the processing period, the other half of the lipoaspirate is taken as graft material. The surgery was performed in a sterile operating room, and cell isolation was performed in a sterile cell processing room. During the procedure, the patient is under general anesthesia (Yoshimura et al., 2008).

The adipose part of the aspiration liposuction was centrifuged at 700 x g for 3 min without washing and placed in a metal jar (500 mL) placed in water and crushed ice. The stromal vascular fraction is freshly isolated from the adipose part and the fluid is added to the graft material. After mixing and waiting 10 to 15 minutes for the cells to stick to the aspirated fat, the added fat cells are then put into a syringe. Yoshimura et al. used an 18 G needle (25 or 60mm long) for lipoinjection, which is inserted in several layers (subcutaneous fat layer and muscle layer) and in several directions to achieve diffuse distribution of the graft material. The amount of adipose tissue transplanted is determined by try-on overcorrection of about 20% (Yoshimura et al., 2008).

Subcutaneous hemorrhages are seen in some parts of the face and resolve within 1 to 2 weeks. Patients generally recover from postoperative swelling in around 4 weeks. The transplanted adipose tissue was gradually absorbed during the first 2 months postoperatively. Volume only experienced minimal changes, and all patients showed cosmetic improvement (Yoshimura et al., 2008).

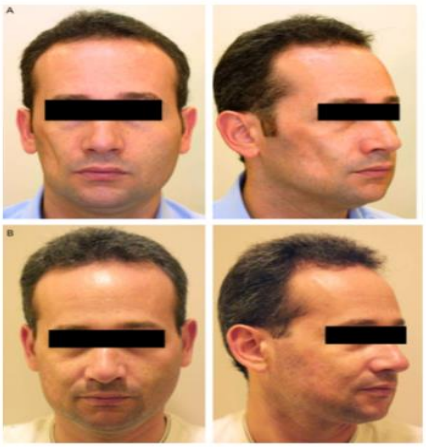

Figure 9. Clinical photo of a patient with lipoatrophy

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The patient is a 35-year-old man who has grade 4 lipoatrophy. He was diagnosed with Parry-Romberg syndrome at the age of 20 years. Photo (A) before the procedure, and (B) 13 months after the procedure using 110 ml CAL (Yoshimura et al., 2008).

Wound Healing

Wound healing is a complex but tightly regulated biological process. The skin must regenerate after damage. Under normal conditions, this process involves cell migration, cell proliferation, and extracellular matrix deposition/remodeling. However, the wound healing process is disrupted in certain conditions, such as burns, chronic wounds, and extensive loss of skin tissue. If a disturbance occurs, an approach is needed to speed up the healing process (Hassanshahi et al., 2019).

Giving stem cells is a promising approach, not only accelerating the healing process of acute wounds but also improving the healing process of chronic wounds. The clinical application of stem cells for wound healing is hampered by several factors, including ethical issues, the risk of stem cell tumorigenicity, and the risk of cell rejection by the host immune system. Therefore, considering all the above concerns, the application of potential cell sources that have the least limitations and have optimal regenerative capacity may result in better therapeutic approaches for wound healing. Among the various types of stem cells studied, ADSCs appear to be a type of cell that has the potential to overcome the limitations mentioned previously and is still classified as clinically safe and effective for accelerating the wound healing process (Hassanshahi et al., 2019).

Evidence from animal studies suggests that local administration of ex vivo expanded ADSCs into skin lesions of various etiologies can improve wound healing outcomes. However, these studies have differences regarding the method of taking ADSCs, culture conditions, and the method of administration (Luck et al., 2021). To date, there is only one clinical trial evaluating the effectiveness of local administration of ADSCs for healing skin wounds. In this prospective cohort study, autologous ADSCs were isolated using the Celution system and administered to arterial ulcers without expansion in culture, leading to wound repair (Marino et al., 2013). Other studies have shown that intramuscular injection of autologous ex vivo expanded ADSCs is an effective therapeutic option for critical limb ischemia that cannot be revascularized and for its ulcer sequelae (Bura et al., 2014). Regional ADSC administration may only be relevant for lesions secondary to peripheral vascular disease (Luck et al., 2021).

One of the unique properties of ADSCs in accelerating wound healing is the cellular plasticity of these stem cells. These cells can differentiate into fibroblasts, which have morphological similarities to fibroblasts, express vimentin and fibronectin, and have heat shock proteins. In addition, ADSCs can differentiate into keratinocytes expressing representative markers for epidermal keratinocytes and into epithelial cells expressing integrins, desmoglein 3, cytokeratin 5, cytokeratin 14, cytokeratin 19, and cytokeratin 6. Adipose-derived stem cells also have the potential to differentiate into endothelial cells, as several studies have shown the incorporation of ASC-differentiated endothelial cells into the walls of newly formed blood vessels (Hassanshahi et al., 2019).

The main mechanism that plays a role in wound healing is through paracrine secretion of factors that encourage differentiation and proliferation of stem and neighboring cells. With some secretions of growth factors, including FGF-2, IGF-1, HGF, VEGF, and TGF-β1, in paracrine action, ADSCs are able to improve the wound healing process by recruiting endogenous cells and inducing the proliferation of fibroblasts and keratinocytes. Neovascularization is also stimulated at the injury site due to the presence of anti-inflammatory cytokines. This paracrine property of ADSCs is also demonstrated in post-radiation wound healing. Stem cells are able to induce neovascularization in the wound bed by producing keratinocyte growth factor and VEGF to the surrounding cells (Hassanshahi et al., 2019).

Adipose-derived stem cells have also been proven to have anti-apoptotic and antioxidant properties which can improve the wound healing process by having antioxidant effects, for example, heat shock proteins and free radicals which can be captured in ischemic conditions so that pre-existing fibroblasts can be protected from oxidative stress. Furthermore, ADSCs have considerable resistance to the cytotoxic effects of bacterial strains such as Staphylococcus, Streptococcus pyogenes, and Escherichia coli, which could make ADSCs a good cell-based therapy option for the treatment of infected wounds (Hassanshahi et al., 2019).

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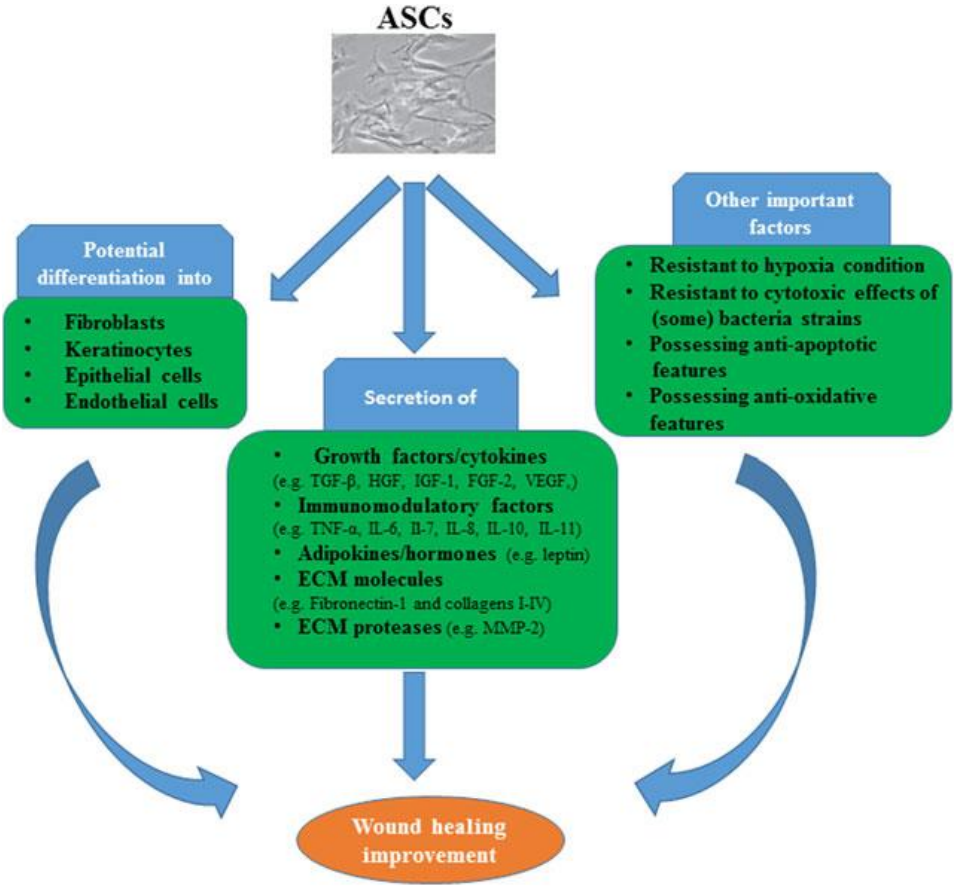

Figure 10. Regenerative mechanism of adipose-derived stem cells in accelerating wound healing

These stem cells can speed up the wound healing process in several ways, namely by differentiating into other types of cells after transplantation; through their secretory profile that triggers biological processes, including proliferation, cell differentiation, angiogenesis, and inflammation; as well as by having various cellular and molecular characteristics such as resistance to (some) toxins originating from bacteria, hypoxic conditions, apoptosis

Coronary heart disease

In research conducted by Leobon et al., ADSCs showed the ability to differentiate into cardiomyocytes (Léobon et al., 2009). Several studies also show that there is increased angiogenesis and vasculogenesis in the cardiovascular system, not only because of the differentiation potential of ADSCs but also based on their paracrine effects (Cai et al., 2009; Wang et al., 2009). In addition, myocardial regeneration can be triggered by inducing myogenic and angiogenic mechanisms. The MyStrom Cell trial, which is used for treatment of ADSC in chronic ischemic heart disease and pre-treatment with VEGF, suggests that ADSCs are a safe choice to improve cell survival (Qayyum et al., 2017).

Cerebral Ischemic Disease Adipose-derived stem cells can be a consideration for therapy during post-stroke recovery that focuses on increasing blood vessel density, limiting infarction damage, and improving neurological function to reduce patient morbidity. Reduction of infarct area upon administration of ADSC is associated with increased neurogenesis and vasculogenesis (Hutchings et al., 2020). Different studies report that administration of ADSC to mice that have experienced a stroke can lead to behavioral recovery. Stimulated angiogenesis is limited to the infarct area and is related to the elimination of necrotic brain tissue, while vascularization is the basis for striatal neurogenesis (Thored et al., 2007).

Ischemic Limb Disease Giving ADSC to mice with ischemic limb disease was able to increase blood flow

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and capillary density (Hutchings et al., 2020). Adipose-derived stem cell (hASC) was administered to a mouse model of ischemic limb disease, and there was an increase in capillary density through paracrine activity that improved muscle recovery (Kishimoto et al., 2016). By inducing specific gene expression patterns precisely and well controlled, the angiogenic effect was increased. ADSC therapy may be possible. For example, inducing v-myc expression in hADSCs can lead to greater proliferation and migration potential as well as greater secretion of VEGF (Shevchenko et al., 2013).

Allograft Adipose-derived stem cells can be used to increase autotransplant vascularization, increase tolerance, and reduce toxicity (Hutchings et al., 2020). Manavella et al. demonstrated that neovascularization can be enhanced in grafted ovarian tissue following the secretion of VEGF and differentiation of ADSCs into blood vessels (Manavella et al., 2019). Further studies on cryopreservation and transplantation of ovarian tissue showed faster and better deoxygenation and revascularization of the graft. Based on this study, it appears that increased follicle survival and reduced apoptosis (Dolmans et al., 2019).

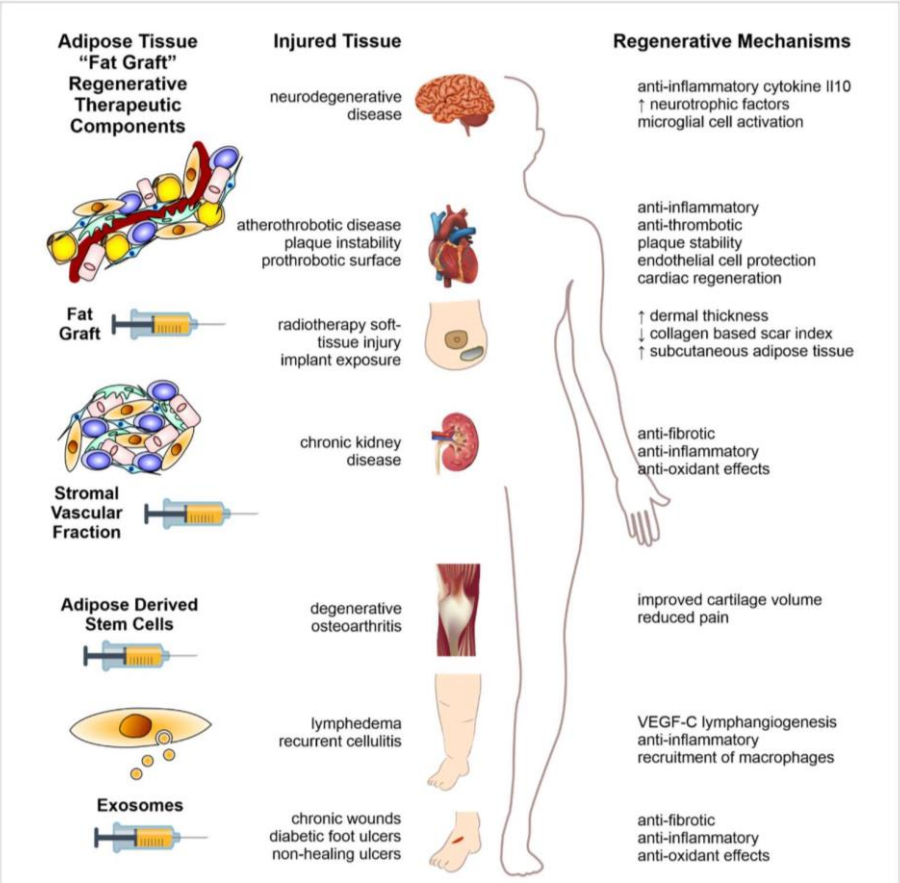

Figure 11. Fat tissue therapy in various diseases. (Shukla et al., 2020)

Limitations of Adipose-derived Stem Cell The potency and efficacy of ADSCs can be influenced by several factors including donor gender, age, body mass index, and chronic diseases. This requires consideration before clinical application. Donor specificities such as age and gender can influence the potential of ADSCs including the speed/capacity of cell proliferation and differentiation, as well as their anti-apoptotic ability. Stem cells isolated from infants have a different morphology and a higher capacity to trigger angiogenesis and osteogenesis, when compared with older subjects (Hassanshahi et al., 2019).

Tissue sources and isolation methods to obtain maximum ADSC results also vary between women and men. Adipose-derived stem cells from female adipose tissue

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were most effectively isolated when degraded overnight with 0.2% collagenase, and 1-hour incubation with 0.2% collagenase was more effective for isolating ADSCs from male adipose tissue. However, significantly higher ADSC yields were obtained from female adipose tissue than from male adipose tissue. This suggests that ADSC production and proliferation may depend on the presence of androgens and estrogens because estrogen and androgen receptors are expressed by mesenchymal stem cells. Androgens have been shown to have an inhibitory effect on stem cell function, and estrogens are able to stimulate cytokine production as well as growth factor in mesenchymal stem cells (Hassanshahi et al., 2019).

In addition to age and gender, body mass index, and donor chronic diseases may also contribute to donor-specific differences. Increased body mass index negatively affects the proliferation and differentiation potential of ADSCs. Body mass index is an important criterion in selecting ADSC donors for clinical applications. Chronic diseases such as diabetes can also alter the potential of ADSCs, especially for wound healing, although previous studies have shown that autologous ADSCs accelerate the wound healing process in diabetic pig models and in patients with diabetes (Irons et al., 2018; Marino et al., 2013). Preclinical studies in mice show that diabetes significantly reduces production of growth factors from ADSCs such as HGF, VEGF, and IGF-1, and changes the intrinsic properties and functions of ADSCs (Marino et al., 2013).

The composition of acute wound fluid and chronic wound fluid differs significantly. This affects the application of ADSC. Unlike acute wound fluid, which stimulates ADSC proliferation, chronic wound fluid has an inhibitory effect on ADSC proliferation and migration. Cells treated with chronic wound fluid were shown to have increased expression of the growth factors VEGF, FGF2, and the protease MMP-9 in contrast to ADSCs treated with acute wound fluid. The production of pro-inflammatory cytokines IL-1β, IL-6, and IL-8 also increased significantly in cells treated with chronic wound fluid compared to acute (Hassanshahi et al., 2019).

Some of the limitations of ADSC applications that are still faced include the lack of definite guidelines regarding culture techniques, safety and quality of in vitro cell expansion, risk of infection, as well as functional and genetic instability. Preclinical studies are also hampered by the short lifespan of ADSCs (Hassanshahi et al., 2019). Reporting of related complications is quite minimal because clinical use is still limited. Some complications that can arise are generally similar to fat graft complications, such as edema, redness, loss of volume, tingling, and bruises. Other complications that may occur include hematoma, cellulitis (which can be treated with antibiotics), fibrosis, oil cyst, and calcification. Postoperative subcutaneous bleeding and edema can reportedly last up to four weeks (Yoshimura et al., 2008). These multipotent cells also have a risk of cellular transformation or tumorigenesis (Tabit et al., 2012). Because ADSCs are progenitor cells, patients with a history of tumors should not receive this therapy. In one study, ADSCs given together with human prostate cancer cells in mice could increase the rate of tumor progression (Prantl et al., 2010). Another study with a breast cancer model found that ADSCs were able to trigger the growth of active tumor cells, but not resting cells (Zimmerlin et al., 2011).

CONCLUSION

Therapy using ADSC is one of the important advances in the field of medicine and provides new hope in terms of soft tissue repair. However, although ADSC appears superior to autologous fat transfer, especially in terms of post-mastectomy breast reconstruction and formation of new blood vessels, studies have shown that ADSC can promote active tumor cell growth. Therefore, its use must be done carefully. Much further research is still needed to identify potential complications and other issues that may arise from the use of ADSC.

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