The evolution of fat grafting: from plastic surgery to regenerative medicine

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Abstract

Autologous fat grafting has been around for over a century. However, only recently has its regenerative potential been discovered. Adipose tissue contains adipose tissue-derived stem cells (ADSC), a mesenchymal stem cell type. These cells are likely to be responsible for the regenerative potential of adipose tissue. ADSC are able to differentiate into multiple lineages of the mesoderm and secrete a whole variety of cytokines and growth factors that are known to play a role in normal wound healing. However, the exact mechanism by which ADSC could cause tissue regeneration is not known. It is though that ADSC contribute to tissue regeneration by paracrine means, or by taking part in tissue remodeling them self. This report will give an overview of autologous fat grafting, how it started and how it has evolved over time, its clinical use and its future potential, and the underlying mechanisms that could possible explain its regenerative potential.
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1. Introduction

Adipose tissue, a tissue formerly thought of as merely a storage place for fat, has now proved to be a powerful tool in reconstructive and cosmetic surgery. Adipose tissue has been used as filling material for over a century, starting of by filling depressed scars\(^1,2\). Autologous fat grafting is currently also being used for a variety of cosmetic and reconstructive procedures, such as breast augmentations\(^3,4\), or filling up wrinkles\(^5,6\). The discovery was made that mesenchymal stem cells are residing in this type of tissue, making its regenerative potential apparent\(^7,8,9\).

There are a variety of reasons why adipose tissue and its adipose tissue-derived stem cells (ADSC) could be a good tool for regenerative therapy. First and foremost being that ADSC are very similar to mesenchymal stem cells (MSC) found in bone marrow and like bone marrow-derived MSC (BMSC), ADSC can differentiate into many lineages of the mesoderm, like bone, cartilage, fat, and muscle\(^10,11,12\). However, ADSC are easier to harvest, in a less invasive manner and adipose tissue contains more stem cells per cubic centimeter, compared to bone marrow\(^13,14\), making it a more favorable source for stem cell harvesting.

Adipose tissue is further more recognized as a major endocrine organ of the human body, containing multiple cell types, releasing numerous kinds of cytokines and growth factors\(^5\). ADSC, in particular, are known to secrete a variety of factors, which might help explain it’s regenerative potential\(^16,17,18\). However, ADSC their ability to differentiate into multiple cell types could also suggest the contribution of ADSC to tissue regeneration\(^10,11,12\). To this date, the mechanism by which adipose tissue and ADSC contribute to tissue regeneration is not known.

This report will give an overview of autologous fat grafting, how it started and how it has evolved over time, with the aim of finding a possible explanation for the underlying mechanism by which ADSC are though to contribute an promote regeneration. Together with what is known about normal wound healing, this review will come up with it’s own mechanism, based upon existing theories.
2. Autologous fat grafting

2.1 Fat
Fat (or adipose tissue) in the human body is generally known as a source of energy, in the form of triglycerides. It also stores fat-soluble vitamins, isolates heat, works cushioning and is also recognized as a major endocrine organ\textsuperscript{15}. Adipose tissue is not only comprised out of adipocytes. It also contains pre-adipocytes, endothelial cells, fibroblasts and adipose-derived stem cells (ADSC). These cells are known to be capable of differentiating into many lineages and have since been studied as a potential regenerative source\textsuperscript{8-10}.

2.2 Plastic surgery discovering regenerative potential of adipose tissue
Gustav Neuber was the first to publish his findings regarding the use of autologous fat transplantation. Neuber successfully treated a depressed (indented) facial scar using autologous fat grafting, on a 20-year-old female. The graft was small and was taken out the upper arm of the patient. Neuber noticed a decrease in resorption by reducing the size of the graft particle. Performing autologous fat transplants over a certain size, failed to work\textsuperscript{1-2}. However, the technique quickly fell out of favor due to the tendency of the fat to resorb, form cysts and eventually almost be fully replaced by fibrous tissue. Results following fat grafting varied greatly, and not everybody was convinced of its potential as a cosmetic filler\textsuperscript{20}. Ersek, for example, found autologous fat transplantation to be disappointing\textsuperscript{21}. However, after Coleman developed a new technique for fat harvesting and placement, preserving the fragile cells and showing long-term fat graft survival\textsuperscript{22, 23}, Ersek altered his techniques and subsequently reported promising results\textsuperscript{24}. These findings suggest that ADSC could possibly be a promising therapeutic source for reconstructive and regenerative therapy\textsuperscript{5, 25-27}.

Although the Coleman technique takes into considerations the vulnerable and fragile nature of the adipocytes, some researchers consider the cells that survive the procedure to be responsible for the regeneration process that occurs after fat grafting. This fraction is likely to consist of pre-adipocytes and ADSC, for they are more likely to survive the traumatic event of liposuction. Pre-adipocytes and ADSC are more resilient and tougher compared to the lipid-filled mature adipocytes. They are also able to survive much longer without nutrition\textsuperscript{27, 28} and need much lower oxygen levels compared to mature adipocytes\textsuperscript{29}. Some researchers are therefore convinced that the ADSC are responsible for regeneration and rejuvenation effects seen after grafting. Other cells that compose the adipose tissue, mainly the adipocytes, are likely to act as a matrix or niche for the ADSC that reside in the adipose tissue\textsuperscript{20}.

Even though it is now known that grafted adipose tissue is able to survive over a long period of time\textsuperscript{23, 24, 30, 31}, fat grafting still remains unpredictable and large variations between graft survival can still be found between patients, even when performed according to the Coleman technique. Though, this might be due to variability of the number of ADSC present in grafted fat\textsuperscript{6}. However, due to all of the success cases that have resulted from autologous fat grafting, efforts are being made to make autologous fat grafting even more reliable and efficient. Researchers are investigating new methods to increase graft survival, such as, adding insulin\textsuperscript{32}, adding platelet rich plasma\textsuperscript{33, 34} or fibroblast growth factor\textsuperscript{35}, restoring membrane integrity and minimizing mechanical
damage\textsuperscript{36, 37}. All of which do show some degree of improvement in fat graft survival, in the model that is has been investigated on. Of course one should always consider the side effect of a potential pretreatment of the adipose tissue, when treating humans. Not pretreating the harvested adipose tissue might therefore still be the better option.

\textit{Fat harvesting and placement}

Since Coleman developed and reported about his new technique for autologous fat grafting, his technique has been widely used to this day. The fat grafting procedure consists of three main steps: fat harvesting, fat processing and preparation, and fat transplantation/placement. Adipose tissue is normally harvested through liposuction from the abdomen, thigh or knee\textsuperscript{3, 22}. The technique developed by Sydney Coleman uses a blunt cannula. The blunt cannula enables the surgeon to perform liposuction and simultaneously avoid as much damage to the tissue as possible. It reduces the risk of damaging underlying tissue structures of the patient, such as nerves and blood vessels, and simultaneously also reduces the damage to cells, preserving more usable fat cells for transplantation. After harvesting (liposuction) the syringe containing the lipoaspirate is placed into a centrifuge. To prepare the adipose tissue for transplantation, the usable fraction must be separated from cellular debris, blood and other cell types. This is done through centrifugation of the lipoaspirate. After centrifugation the aspirate separates into three main layers. The top layer mainly consists of oil (triglycerides) from ruptured adipocytes; the middle portion consists primarily of usable fatty tissue and the third layer is blood and water. The top layer is decanted and the bottom layer drained leaving only the middle layer. Excess oil is removed by placing an absorbent material (ea. wick) at the top of the remaining layer of cells. This produces adipose tissue that is usable for transplantation.

Here after small incisions (2-mm) are made, on the patient, at the graft side. A blunt cannula is inserted through these incisions, till it reaches the designated plane. While withdrawing the cannula, small amounts of fatty tissue are injected in the pathway of the retreating cannula. This way the fatty tissue falls into the cannula tracks, causing the host tissue to collapse around it\textsuperscript{22}.

Though it is not proven that the Coleman technique increases graft survival by reducing damage to the fatty cells, graft survival rates do improve when following the Coleman technique compared to results obtained by previous performed techniques\textsuperscript{21, 23, 24}.

\textit{Complications}

Most common complications that occur through autologous fat grafting are mainly technique related and therefore avoidable. The most common complication is grafting too little or too much adipose tissue. Too little tissue will not give improvement and injecting too much adipose tissue into a specific spot could cause isolation of fatty tissue through the excess surrounding adipose tissue. The transplant is thereby not receiving sufficient nutrition, resulting in necrosis. This may cause the fatty tissue to resorb, form oil cysts and eventually induce fibrosis, all making the procedure less effective\textsuperscript{4, 20, 22, 38}.

Another common problem are irregularities after fat grafting. This can be caused by faulty placement, migration of the transplanted tissue or even the intrinsic nature of the patient’s body. However, chances of irregularities occurring decrease when an experienced surgeon performs the technique\textsuperscript{4, 22}.
Something that also needs to be considered, are the thin patients with limited adequate donor sites available for fat grafting. Especially the ones that already have experienced liposuction. These patients might not be suitable for autologous fat grafting, depending on the size of the procedure and the BMI of the patient. For example, patients with a low BMI (under 18.5) might not be suitable for breast augmentation, because there are not sufficient autologous donor sites available. However, this same patient can be suitable for autologous fat grafting for filling up a small scar, where a smaller amount of transplanted material is needed.

Naturally, as with many other procedure, autologous fat grafting can cause swelling, edema, induration and in some cases, although not common, even infection. After the procedure, care should be directed at trying to minimize the swelling, avoiding migration and prevent infection by keeping the wound clean. However, most infections are due to non-sterile fat grafting equipment, and care should therefore be taken when performing the procedure.

Furthermore, surgeons should always be very cautious when inserting and removing the cannula, even when the cannula is blunt, to avoid creating intravascular emboli. Also, underlying structures such as nerves, glands and blood vessels can get seriously damaged during the procedure, causing severe and or permanent injuries. Fortunately, events like these are extremely rare.

Mentioned here were only a number of complications that could arise from autologous fat grafting. These are considered to be very common and or very dangerous complications that could occur from autologous fat grafting.

2.3 Types of stem cells
The stem cells found in adipose tissue (ADSC) are a type of mesenchymal stem cells and are considered to be multipotent or adult stem cells. The term stem cells means undifferentiated cells with self-renewal capacity. These cells can either differentiate into other type of cells or self-renew. There are multiple types of stem cells found in different types of tissues. In general, stem cells can be classified in three main groups: totipotent, pluripotent and multi-potent stem cells. Totipotent stem cells are able to differentiate into all embryonic and extra-embryonic (yolk sack, placenta) cell types. Pluripotent stem cells are able to give rise to all cell types of the embryo and multi-potent stem cells can differentiate into a limited number cell lineages.

Studies suggest that human embryonic stem cells (hESC) could possibly be a powerful tool for regenerating a range of damaged tissues, treat a variety of medical conditions, and assist in the development of new therapies and drugs. However, use and destruction of human embryos lies very sensitive to a great number of people and is subject to great ethical debate. Together with problems like teratoma formation, low quantities and chances of rejection, causes hESC research therefore to be very limited.

However, multi-potent stem cells do represent a promising source for tissue engineering. Friedenstein and colleagues were the first to discover that bone marrow contains mesenchymal stem cells. These cells are able to generate bone, cartilage, adipose tissue, tendon, muscle and even neuronal tissue. However, mesenchymal stem cells are misrepresented as stem cells, for they do not contain self-renewal capacity and are therefore not “immortal”. Mesenchymal stem cells can therefore also be referred
as mesenchymal stromal cells. Most other tissues have their own stem cell pool, of which most do classify as stem cells (e.g., skin and muscle)\textsuperscript{48,49}. However, bone marrow is not the ideal source for collecting mesenchymal stem cells. Not only does it require a very invasive and painful procedure to harvest from patients, the quantity of stem cells that can be collected from this bone marrow is very limited. In addition, the cells also need to be cultured for several weeks to generate more quantities, making this whole procedure expensive and thereby also changing the biology of the stem cells\textsuperscript{50}.

\textit{Adipose derives stem cells}

On the other hand, stem cells found in adipose tissue are very similar to the bone marrow-derived mesenchymal stem cells (BMSC). Adipose tissue-derived stem cells (ADSC) also have the potential to differentiate into a variety of lineages of mesodermal tissues, such as bone, cartilage, fat, and muscle\textsuperscript{10,11-13}. Adipose tissue, however, is easier to harvest, less invasive and yields more stem cells (100 to 1000 times more) per cubic centimeter compared to bone marrow\textsuperscript{13,14}. Making it a more favorable source for MSC harvesting, then bone marrow. Because ADSC are also a type of MSC, these cells are also being misrepresented as stem cells, and can therefore also be called adipose derived-stromal cells\textsuperscript{48,49}. 
3. The Evolution of autologous fat grafting

As mentioned before, autologous fat grafting has been around for over a century. Neuber was the first to report about his findings using autologous fat grafting. Although a very important discovery, certain complications caused the technique to quickly fall out of favor. With the appearance of silicone in the 1940-1950s attention was redirected from autologous fat toward silicone as a filling material.

The introduction of liposuction in the early 1980s renewed the interest in autologous fat grafting. Unfortunately, the same complications, mainly being fat resorption, kept occurring. This problem was overcome by Coleman’s new technique in the 1990s. Coleman invented a technique for fat harvesting and placement, to preserve the fragile nature of adipocytes. Coleman initially used this new technique for autologous fat grafting on patients to rejuvenate their face and noticed long-lasting results.

At first liposuctions were used as cosmetic fillers. Autologous fat transplant could provide long-lasting, natural-appearing structural changes and was the safest and most ideal substance for soft-tissue augmentation, if and when performed according to the Coleman technique, and by an experienced surgeon. Later on researchers noticed adipose tissue its regenerative capacity, and started exploring this phenomena. Till this date, the exact mechanic details by which adipose tissue facilitates regeneration/repair have not been described. However, most researchers consider the ADSC that reside in the adipose tissue, to be the main contributor to the regeneration process that occurs after autologous fat transplantation.

Since the discovery of ADSC, researchers are focusing on developing cell therapies for regenerative purposes. Though autologous fat grafting has already been used for numerous procedures, for both reconstructive as cosmetic purposes, such as the rejuvenation of the skin, breast augmentation, treating damaged resulting from radio therapie, treatment of scars caused by thermal injury, and treatment of depressed scars.

Recent studies are also focusing on the underlying mechanism of adipose tissue, their ADSC and their role in tissue regeneration. By understanding the mechanism, the medical field strives to improve existing therapies and treatments, and also come up with new ways to treat other types diseases, afflictions or traumas.
4. Scarring

Autologous fat grafting has shown to contribute to regeneration and wound healing. However, as mentioned before, the mechanism by which it does so is not known. To better understand the possible role of ADSC and their role in regeneration, this chapter describes the original wound healing and scarring process. In the discussion we come back to this mechanism in regards of the ADSC.

4.1 Wound healing

Wound healing depends on two major phenomena: re-epithelization (replication and movement of the epidermal cells) and, the formation and contraction of granulation tissue (consisting of small vessels, fibroblasts, myofibroblasts and inflammatory cells). The formation and contraction of granulation tissue is essential for the maintenance of tissue continuity and reduction of wound size, eventually producing a scar. The adult skin, for example, consists of the epidermis, dermis and an underlying thick layer of subcutaneous tissue called the hypodermis. The skin serves as the protective barrier against the outside world. Damage to the skin must therefore be rapidly and efficiently mended, often by a temporary clot composed of cross-linked fibrin and extra cellular matrix (ECM) proteins such as vitronectin, fibronectin and thrombospondin. Not only does this clot form a barrier against invading microorganisms, it also serves as a matrix and reservoir of growth factors for invading cells, during the later stages of the healing process. However, the clot is only temporarily. To complete the healing process, the clot will have to be replaced primarily with the ECM protein, collagen (which also composes most of the original skin) and the epidermal edges have to migrate towards each other, restoring the integrity of the skin, resulting in scar formation.

Although scar tissue is comprised of the same ECM proteins (mainly collagen) as the tissue that it has replaced, the orientation of collagen is different. In normal tissue the collagen fibers are orientated in an open basketweave-like network, whereas fibrous tissue is characterized by a higher degree of alignment. Because of this formation the tissue cannot function as it could before. Burn scars, for example, are less elastic, do not contain hair follicles and sweat glands as normal skin tissue does. Scar formation in the heart, caused by a myocardial infarction, loses muscular power and could eventually cause heart failure. Understanding the mechanism involved in the formation of scar tissue is therefore important for therapeutic intervention.

4.2 Mechanism

Upon injury, cytokines and growth factors such as basic fibroblast growth factor (bFGF), transforming growth factor beta (TGF-β), and platelet-derived growth factor (PDGF) are released from damaged ECM, dying cells, activated platelets and macrophages. These cytokines and growth factors are known to play part in the healing process. When the damaged tissue turns ischemic, bone marrow cells (BMC) are triggered to mobilize from the bone marrow to the afflicted tissue and contribute to the regeneration and healing process. Epithelial progenitor cells (EPC), mobilized from the bone marrow, have demonstrated to help with neovascularization in mice and are also know to mobilize in humans upon stimulation (injury/trauma). Even non-hematopoietic MCS have shown to mobilize upon stimulation in mice and rat models, and suggests that they contribute to the regeneration of the lost tissue.
EPC and MSC release numerous cytokines and growth factors that might play a role in tissue repair and regeneration. EPC secrete vascular endothelial growth factor (VEGF), PDGF, granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF)\(^\text{18}\), among others. MSC have been found to release VEGF, IL-6, monocyte chemo-attractant protein-1 (MCP-1), bFGF and hepatocyte growth factor (HGF)\(^\text{19}\). Suggesting that EPC and MSC mediate tissue regeneration and repair by recruiting inflammatory cells and stem/progenitor cells.

Burn injuries for example cause proteins in the afflicted area to coagulate, blocking the blood flow to this area, resulting in ischemia\(^\text{78}\). The body reacts to this by sending EPC, from the bone marrow to the afflicted area\(^\text{74}\), where EPC differentiate into endothelial cells, contributing to the formation of blood vessels\(^\text{79}\). Severe burns, however, show a delayed release of EPC\(^\text{80}\), slowing down the revascularization, which is essential for the healing process. As a result, these burn wounds could turn into hypertrophic scars, with a changed microcirculation\(^\text{80-82}\). Proper blood flow in burned tissue (and other types of wounds) is important in the wound healing process, for low oxygen leads to an up regulation of TGF-β1, which plays and major role in scar formation\(^\text{83, 84}\).

TGF-β1 is an important regulator of cell proliferation, collagen production and when strongly upregulated, can cause fibrosis\(^\text{85, 86}\). Hypoxia induces the upregulation of TGF-β1, which causes the downregulation of certain matrix metalloproteinases (MMP) proteins. These MMP regulate the remodeling of the ECM, degrading it upon proper stimulation\(^\text{87, 88}\).

**Myofibroblast**

It was previously thought that contraction of the scar tissue was mainly due to collagen shortning\(^\text{59, 61}\). However, some observations indicated that it is the granulation-tissue cells that are important in force generation\(^\text{99}\). In particular the myofibroblasts, cells with both fibroblast and smooth muscle (SM) cell characteristics. Myofibroblasts seem to have an important role in generating tension during wound healing and pathological contraction\(^\text{20 90, 91, 92}\).

Myofibroblasts are generated by differentiation of fibroblasts. Trauma/damage to the connective tissue/microenvironment causes the fibroblast to evolve into a proto-myofibroblast\(^\text{93}\). The proto-myofibroblast are present in early granulation tissue, 2 to 4 days after injury and is characterized by the formation of stress fibers (consisting of β and γ actins). The proto-myofibroblast then proceeds to differentiate in to the myofibroblast through growth factors and ECM component stimulation\(^\text{59}\). The Myofibroblasts express myosin and α-SM actin, an actin isoform that is typically found in vascular SM cells\(^\text{94, 95}\). It’s whole contractile apparatus, organized as bundles of microfilaments, generates force to the surrounding ECM. Both the α-SM actin expression and collagen type I production in these cells are regulated by TGF-β1. This force generation will then eventually result in ECM reorganization and wound contraction\(^\text{96, 91, 93}\). Myofibroblast regulation is still not well understood, involving not only growth factors, but also cytokines, matrix components and other cell signals\(^\text{59, 93}\).

When re-epitheliazation has been completed, massive apoptosis of myofibroblasts and vascular cells causes the granulation tissue to slowly disappear. This evolves the
granulations tissue into a poorly cellularized scar, consisting of fibroblasts, extracellular matrix (mainly collagen) and small vessels\textsuperscript{96}. However, in some cases the wave of myofibroblasts apoptosis is lacking. In these cases the granulation tissue can evolve into hypertrophic scars, still containing a high number myofibroblasts. These hypertrophic scars contain an inappropriate amount extracellular matrix deposition, which may continue for several years, obliterating the architecture of the parenchyme or connective tissue \textsuperscript{59, 97}. 


5. Hypotheses regarding the underlying mechanism

Although autologous fat grafting has been around for over a century, 120 years of research has not been able to clarify the exact mechanism underlying the regenerative capacity of adipose tissue. However there are a lot of speculations and hypotheses circulating around. This chapter will give an overview of some of those hypotheses and possible mechanisms.

5.1. Adipocyte-committed ADSC embedded in, or activated by the ectopically transplanted fat

One plausible hypothesis comes from the observations made by Rigotti and his colleagues. They had compared the ultrastructure of mammary lesions before and after fat grafting, in humans. Before the fat transplantation most of the adipocytes in the aspirate were seriously damaged through centrifugation, and there were no mature or differentiated pre-adipocytes present in the recipient radiotherapy-damaged tissue. Rigotti and colleagues found that the mammary radio-lesions were regenerating, after fat transplantation. They found new adipocyte formation and many adipocyte precursors at different stages of differentiation, in the recipient tissue. Although Rigotti and his colleagues point was to prove that it was the ADSC that are the main contributors to the healing process and not the fragile adipocytes of whom most are destroyed in the fat grafting procedure, these results led other authors to consider that the stem cells that cause the regeneration might not come from the lipoaspirate, but from the patients recipient tissue itself. Mazzola and colleagues discuss whether adipocyte-committed ADSC come from the transplant or are already locally present in the recipient tissue and subsequently get activated by the ectopically transplanted fat. The question remained if the ADSC enriched aspirate is the main contributor to regeneration, or if the ADSC enriched aspirate functions as an atypical ectopic niche by releasing trophic factors, such as cytokines, growth factors and pro-angiogenic factors to activate endogenous stem cells. Considering that ADSC are able to differentiate into multiple cell lineages and that fat is a major endocrine organ, the ADSC enriched aspirate could be both the main contributor to the regeneration process and also serve as an atypical ectopic niche for endogenous ADSC.

5.2. ADSC revascularization and tissue remodeling

A potential mechanism by which ADSC may contribute to regeneration is by improving the blood supply. Several studies, including both Valina et al. and Schenke-layland et al., have already demonstrated an improved cardiac function after a myocardial infarction, through transplantation of ADSC, in animal models (pig and rat), suggesting the contribution of ADSC to the improvement myocardial perfusion. Sultan and colleagues hypothesized about a possible mechanism for adipose tissue to change a burn scar. They used a thermally injured murine model to try and determine the mechanism by which ADSC might contribute to the healing process of a burn wound and observed a more advanced revascularization at the burn site in fat-grafted mice, compared to saline-treated mice. In parallel, the pro-vasculogenic genes, vascular endothelial growth factor (VEGF) and stromal cell-derived factor-1 (SDF-1), were significantly elevated together with the anti-apoptotic BCL2 gene, in the fat-grafted...
animals. On the other hand, the pro-apoptotic BAX gene showed a decreases expression in the fat grafted animals, compared to the saline-grafted animals\textsuperscript{98}, confirming their observations.

The early revascularization, in the fat-grafted mice, proved to be important in the prevention of fibrosis. Improving revascularization in this area, improves oxygenation, which could protect the wound form TGF-\(\beta\)1 up-regulation. In turn, this would improve the wound healing process, and thereby scar quality and texture\textsuperscript{80}. Indeed, significantly less fibrosis and scarring was observed in fat grafted mice, compared to the saline grafted controls. Reduced expression of fibrotic factors Col1a1, MMP9, TIMP-1 and TGF-\(\beta\)1, confirmed these results, leading to the conclusion that human adipose tissue indeed helps to accelerate the revascularization of burn wounds in the mice, thereby decreasing fibrosis, resulting in less scarring\textsuperscript{98}.

**Neovascularization and tissue remodeling upon ADSC activation**

Factors and cytokines released upon injury contribute to the healing process. One study demonstrates that bFGF, EGF, PDGF, and TGF-\(\beta\) significantly improve endogenous ADSC proliferation, migration and network formation. The authors demonstrated the therapeutic potential of the soluble factors (bFGF, EGF, PDGF, and TGF-\(\beta\)) for various ischemic conditions. Administration of these factors, in vitro and in vivo (mice), induced adipose remodeling and neovascularization, through the activation of inter alia ADSC\textsuperscript{99}. Their research suggests that human ADSC seem to preferentially differentiate in to either adipocytes and/or vascular endothelial cells. This suggest that ADSC not only have a paracrine function in wound recovery, by secreting cytokines and growth factors, but may also play role in tissue remodeling.

**Contribution of ADSC in improved myocardial perfusion post infarction**

As mentioned before, Valina and colleagues demonstrated a possible role for ADSC in restoring cardiac function after myocardial infarction in pigs. ADSC were administrated into an acute infarcted myocardium, where they differentiated into endothelial and vascular smooth muscle cells, thereby improving tissue remodeling, left ventricle function and eventually myocardial perfusion. Upon histologic analysis, the group identified the donor ADSC at the site of infarction. These cells were distributed within and around the circulatory system and expressed endothelial and smooth muscle markers, such as the von Willebrand factor, \(\alpha\)-SM actin and desmin, confirming the role of ADSC in restoring cardiac function\textsuperscript{56}. These results suggest the contribution of ADSC in both revascularization and tissue remodeling.

**Contribution of ADSC in bone formation/remodeling**

Human ADSC have demonstrated to be able to form bone in vivo, using the supportive scaffolds, hydroxyapatite/tricalcium phosphate (HA-TCP) matrix\textsuperscript{100} or by exposing the ADSC to either recombinant human bone morphogenic protein-2 (BMP2) or adenovirus containing BMP-2 cDNA\textsuperscript{101}. Human ADSC were contributing to the bone formation in severe combined immunodeficiency (SCID) mice, which was confirmed through histological analysis, revealing cells that stained positive for human nuclear antigens\textsuperscript{100, 101}. These findings are supported by a clinical report from 2004, describing a successful treatment of a widespread calvarial (skullcap) defect of a 7-year-old girl, who had sustained this injury after severe trauma to the head. The calvarial defects were repaired.
two years after the initial injury, by using ADSC. ADSC were processed and applied to the calvarial defect, for treatment. CT-scans, two years after the injury, showed new bone formation and calvarial repair\textsuperscript{102}. Though, it was not proven that the ADSC differentiated into bone, it does support the idea of ADSC as a potential therapeutic treatment for bone defects.

\textit{Other contributions of ADSC to tissue remodeling}

Some studies suggest the involvement of ADSC in the regeneration of neuronal tissue in rats, where the ADSC were pre-treated with azacytidine for neural differentiation\textsuperscript{103}. Other studies demonstrate the involvement of ADSC in the regeneration of cartilage in nude mice (with or without pre-treating the ADSC with chondrogenic media)\textsuperscript{104} and skeleton muscles in rabbits\textsuperscript{105}.

Thus by administrating certain factors, or pre-treating the ADSC with certain factors, one can direct these cells to differentiate into certain types of cells, thereby contributing to tissue regeneration. Although pre-treatment isn’t always necessary, for it has already been shown that ADSC differentiate into endothelial cells when exposed to endogenous growth factors and cytokine\textsuperscript{99, 106}. These results, suggest that ADSC could take part in both the accelerated revascularization process and in tissue regeneration/remodeling.
6. Clinical use

There are numerous applications for which adipose tissue can be used. From reconstructive surgery and cosmetic surgery to therapeutic treatments, treating and repairing damaged tissue and or organs. This chapter summarizes clinical applications for which adipose tissue is already being used or has been used.

6.1. Fillers

The use of fillers for replacing lost tissue has been around for over a century. Paraffin was the first injectable filling agent (1830), however was later dropped as a filling material due to serious complications\(^\text{107}\). Silicone has been used as a cosmetic filler since the 1940s. As a liquid injectable filler, silicone shares a lot of similarities with paraffin. Like paraffin, silicone is a clear, inert and oily substance, however silicone gives similar complications as paraffin and has therefore also been recently dropped as a filler\(^51,52,108\). Bovine collagen and hyaluronic acid are Food and Drug Administration (FDA) approved and up to this date the most common filling materials used in cosmetic procedures\(^108,109\).

Another good candidate for a filling material is adipose tissue, one of the first clinical applications for which adipose tissue was used for. Adipose tissue is easily available. Every human does possess some, if not a lot, adipose tissue. Furthermore because it can be harvested from the patients itself, rejection is not an issue, whereas collagen is harvested from animals and carries a small chance of rejection. These characteristics make adipose tissue a good candidate for filling material for reconstructive\(^6,20,110\), or cosmetic purposes\(^22\). Some of the most common clinical applications for adipose tissue in reconstructive and cosmetic surgery are summarized below.

- Plastic and reconstructive surgery of the breast\(^110-115\)
  - Cosmetic breast enlargement
  - Breast reconstruction after e.g. surgical removal of the breast
  - Correction of Poland’s syndrome
- Maxillo-facial (jaws and face) surgery\(^6,20,22,116-119\)
  - Rejuvenation of the face
  - Reconstruction of locally excised tissue
  - Treatment of scars
- Reconstruction of the gluteal-trochanteric region (Buttocks)\(^120\)
  - Cosmetic filling

6.2. Reconstruction and regeneration

*Burn scar Treatment*

Autologous fat grafting has been used to treat a whole variety of conditions, not the least being burn injuries. Scars that result form deep burns are fibrotic scars with abnormal texture, pliability, color and thickness\(^121\). These can result in serious deformities when left untreated. Treating burn scars include silicone gels, pressure dressing\(^122,123\), corticosteroids\(^124\) and now possibly autologous fat grafting\(^57,98\). Treating patients with hypertrophic burn scars with autologous adipose tissue reduces scar tissue formation, improves scar quality, skin texture and thickness. New patterns of collagen deposition,
dermal hyperplasia and local hypervascularity were observed in the treated area, similar to the original skin tissue\textsuperscript{57}.

**Radiation damage treatment**

Radiation therapy, an oncologic treatment, does not discriminate between cancerous and healthy tissue. Damage caused by radiotherapy could develop in a pathologic condition like radiodermatitis, with the possibility that it later on develops into subcutaneous fibrosis and in critical cases in radio-necrosis. Radiolesions seem to be caused by an altered blood flow\textsuperscript{27}. Treatment of these lesions often entails invasive surgical removal\textsuperscript{125}. One study has demonstrated a low-invasive, highly effective therapeutic approach for resolving the late side effect of radiation treatment. Rigotti and colleagues have shown great clinical improvement of degenerative chronic radiolesions, by transplanting autologous lipoaspirates under the fibrotic skin. This treatment can hopefully prevent invasive surgical interventions by breaking the vicious cycle of developing vascular lesions, causing ischemia, causing hyperpermeability, causing fibrosis, which then increases the ischemia\textsuperscript{27}.

6.3. **Autologous fat grafting to face and neck**

**Dysphonia treatment**

Another condition were fat grafting has demonstrated to be an effective treatment is in the treatment of Dysphonia (disorders of the voice), like glottic insufficiency (space between your vocal folds). Glottic insufficiency makes a person sound wheezy. Trying to correct wheezy sound by straining your vocal cords, could lead to vocal injury. Treating glottic insufficiency with structural fat grafting, a minimal invasive procedure, showed significantly improvement\textsuperscript{126, 127}.

**Rhinoplasty**

Rhinoplasty or reconstructive and cosmetic surgery to the nose can also benefit from autologous fat grafting. Saddle nose deformity (loss of height of the nose, because of a collapsed bridge) can be corrected through adipose tissue. Other conditions where fat grafting can be beneficial in respect to rhinoplasty is camouflaging cartilage, bone irregularities and atrophy or scarring of the nose, to provide a better skin texture. When grafting fat at the level of the nasal valve, it might even improve airway obstruction\textsuperscript{20, 128}.

**Frey syndrome**

Frey syndrome is a condition that is characterized by profuse sweating and facial flushing and can be developed after a parotidectomy, in which the parotid gland is removed (partially or whole). Autologous fat grafting has demonstrated to relieve the symptoms that are associated with post parotidectomy Frey syndrome (PFPS). It can also fill the indentation left behind form the parotid gland excision, creating a more aesthetic look\textsuperscript{20}.

**Scars**

As mentioned before adipose tissue can help treat scars, to obtain a better aesthetic result. Examples are, scars caused by congenital malformations (like a cleft lip), scars caused by trauma, surgical procedures or scars caused by post surgical complications, e.g. tracheostomy and Pharyno-cutaneous fistulae. In the case of the cleft lip, autologous fat grafting can reduce scarring and even increase the upper lip volume. The same is true
for other (depressed) scars\textsuperscript{20}. Fat grafting has already been described as a treatment for depressed acne scars\textsuperscript{129} and a variety of scars caused by other means.

This chapter has described that autologous fat grafting not only contributes to the avoidance of scar tissue formation, but also seems to contribute to healing/correcting of already existing scars. Autologous fat injections/transplantations in already existing scars cause a morphological reduction of the scar, giving it a more aesthetic look\textsuperscript{20, 27, 57}. These results suggest that ADSC contribute to both avoidance of scar tissue formation as to the healing of already formed scar tissue. However, histological examination of treated scars is needed to support these speculations.
7. **Discussion/conclusion**

Autologous fat grafting appear to have great regenerative and wound healing potential. ADSC are considered to be the cells that are responsible for regeneration, through either paracrine function or by taking part in tissue remodeling them self. They contribute to revascularization, which is crucial for proper wound healing, thereby improving and accelerating the wound healing process\(^6, 98, 99\).

ADSC secrete a number of cytokines and growth factors that have been summarized in table 1. This secretion profile is very similar to that of the bone marrow-derived mesenchymal cells\(^{16}\), which also display similar regenerative function.

### Table 1: Cytokines and Growth factors secreted by ADSC

<table>
<thead>
<tr>
<th>Angiogenic</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>16, 17</td>
</tr>
<tr>
<td>HGF</td>
<td>16, 17</td>
</tr>
<tr>
<td>PDGF</td>
<td>18</td>
</tr>
<tr>
<td>TGF-β</td>
<td>17</td>
</tr>
<tr>
<td>Hematopoietic</td>
<td></td>
</tr>
<tr>
<td>Flt-3 ligand</td>
<td>16</td>
</tr>
<tr>
<td>G-CSF</td>
<td>16</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>16</td>
</tr>
<tr>
<td>M-CSF</td>
<td>16</td>
</tr>
<tr>
<td>MCP-1</td>
<td>19</td>
</tr>
<tr>
<td>Pro-inflammatory</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>16</td>
</tr>
<tr>
<td>IL-8</td>
<td>16</td>
</tr>
<tr>
<td>IL-11</td>
<td>16</td>
</tr>
<tr>
<td>LIF</td>
<td>16</td>
</tr>
<tr>
<td>TNF-α</td>
<td>16</td>
</tr>
</tbody>
</table>

ADSC secrete nearly all the growth factors that take part in normal wound healing. Which leads me to conclude that ADSC indeed have a major paracrine function in regenerating tissue. These cytokines and growth factors are known to play an important role in wound healing and can therefore be responsible for the accelerated and improved revascularization of damaged tissue. Based on studies demonstrating the participation of ADSC in regenerating blood vessels\(^99, 106\), I am also convinced that endogenous, but also auto-secreted (secreted by ADSC), factors cause ADSC to differentiate in endothelial cells and contribute to the vessel formation. Therefore ADSC might also have an auto regulatory function, causing them to differentiate and participate in tissue remodeling; next to their paracrine function.

**Mechanism hypothesis**

Taking all information into consideration, the following hypothesis emerged: Autologous transplanted ADSC contribute to an accelerated and improved revascularization by
secreting cytokines and growth factors that activate the endogenous system to repair and heal damaged tissue. EPC are mobilized to the damaged tissue and start the formation of the blood vessels. The transplanted ADSC also take part in the formation of the new blood vessels, by differentiating into endothelial cells. These two functions of ADSC combined cause an accelerated revascularization. Through this accelerated and improved revascularization, oxygen levels in the damaged tissue are kept up to standard. In normal wound healing, hypoxia causes an upregulation of TGF-β1. TGF-β1 could then induce the differentiation of pro-myofibroblast into myofibroblasts. These myofibroblasts are mainly responsible for producing inappropriate amounts of ECM and creating contractile force that would normally help to close the wound. Upregulation of TGF-β1 also causes a down regulation of certain MMP. These MMP regulate the ECM balance by degrading ECM upon stimulation. This could eventually result in a buildup of collagen in the damaged tissue, also known as fibrosis, which is a main characteristic of scar tissue. The excessive amount of collagen causes it to align, resulting in a dense and tight formation, thereby obliterating the tissues original structure and reducing its function. Thus by accelerating and improving revascularization, hypoxia is avoided, which in turn avoids the upregulation of TGF-β1. This avoids the induction of myofibroblast differentiation and the down regulation of MMP, thereby limiting the collagen production and degrading excessively produced collagen. When the wound has been repaired, the damaged tissue will have preserved more of it's original structure and thereby preserved more of its original function. The remaining scar will also have a better aesthetic result, compared to normal wound healing. Figure 1 gives an overview of the suspected mechanism.

Thus by accelerating revascularization, scar tissue formation can be avoided, through the actions of ADSC. Although this only shows ADSC contribution to vessel formation, other studies have demonstrated that ADSC do take part in the tissue regeneration. By administrating certain factors, or pre-treating the ADSC with specific factors, one can direct these cells to differentiate into certain cell lineages, thereby contributing to tissue regeneration. Pre-treated ADSC seem to contribute to bone, cartilage, cardiac muscle and skeleton muscle, in vivo. These results were confirmed through histologic analysis, demonstrating the expression of typical cell markers that are corresponding with the type of tissue that they were rebuilding. However, there little evidence supporting the theory that ADSC can differentiate into other types of tissues in vivo, without any pre-treatment. Though we can't exclude the possibility that it does. ADSC might therefore contribute to the healing and regeneration of other types of tissues in a similar manner.

Conclusion
Discoveries made up to this point, suggest that ADSC can be a powerful tool in tissue engineering with a prosperous future ahead. Autologous fat grafting is already being used in the clinic for reconstructive and cosmetic procedures, in both avoiding scar formation as healing of existing scar tissue. Furthermore, ADSC show promising potential in a variety of future treatments. ADSC could possibly treat bone loss or heal fractures in the nearby future; already having shown positive results in a severe case of calvarial defect. Other potential future applications are: treatment of myocardial infarctions, regeneration of skeleton muscle and cartilage and possibly contributing to the treatment of neurological disorders.
Figure 1. Wound healing mechanism, with and without transplanted ADSC.

A. 1) Upon injury, fibroblasts (FB) in the skin differentiate into pro-myofibroblast (P-MF). 2) The damage to the tissue causes hypoxia. 3) Blood platelets (BP), macrophages and damaged extracellular matrix (ECM) secrete cytokines and growth factors, supporting the healing process. 4) Hypoxia in the damaged tissue induces the mobilization of endothelial progenitor cells (EPC) from the bone marrow, causing them to secrete a variety of angiogenic, hematopoietic, and pro-inflammatory factors. EPC differentiate into epithelial cells and contribute to the regeneration of the blood vessels. 5) Meanwhile, the hypoxia causes an upregulation of the TGF-β1, causing it to 6) induce the differentiation of P-MF to MF. MF produce ECM (collagen). 7) TGF-β1 inhibits matrix metalloproteinase protein (MMP) expression, thereby inhibiting the degradation of excessive ECM, which leads to 8) fibrosis.

B. Adipose-derived stem cells (ADSC) interfere at step four of the process. 4) Autologous transplanted ADSC secrete also a variety of angiogenic, hematopoietic, and pro-inflammatory factors, and are also able to differentiate into epithelial cells (EC), contributing to revascularization. 5) The microcirculation system gets regenerated and 6) cause the oxygen levels to rise again, 7) downregulating and avoiding further upregulation of TGF-β1, thereby lifting the inhibitory effect on MMP, causing it to degrade excessively produced ECM.
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53. Timothy A, et al. Adipose-derived stem and progenitor cells as filler in plastic and


103. Erickson GR, et al. Chondrogenic potential of adipose tissue-derived stromal cells in vitro and


