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Review

ADIPOSE-DERIVED STEM CELLS AS A NOVEL ANTI-AGING THERAPY IN COSMETIC SURGERY: A CONCISE REVIEW

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ABSTRACT

Facial aging is a complex process and often poses a challenge for plastic and aesthetic surgeons. Injectable fillers and autologous adipose tissue are widely used for the restoration of soft tissue volume. However, lipotransfer currently lacks standardization and its unpredictable reabsorption rate remains an unresolved issue. Adipose-derived stem cells (ASCs) are multipotent mesenchymal stem cells that could be responsible for the main rejuvenation capabilities of fat grafts. Currently, ASCs are administered in conjunction with autologous fat, while injectable vehicles such as hyaluronic acid, or other injectable biomaterials, are under study for the treatment of facial aging with promising results. Indeed, bioengineered fillers are at a very early stage of development, and could be further developed as semi-permanent fillers with biological properties of engrafted adipose tissue. ASCs could emerge as an effective and novel anti-aging therapeutic agent. However, extensive analysis of their actual effectiveness and mechanism of action are required.

1. Introduction

In recent years, aging populations, increased demand for a youthful appearance and development of minimally invasive therapeutic options have led to an increase in cosmetic surgery. In the U.S. alone, 14.6 million cosmetic procedures were performed in 2012—a 5% increase from 2011. Furthermore, minimally invasive facial surgeries showed the highest increase.[1] Consequently, the interest in regenerative medicine applied to the field of facial rejuvenation has increased. Facial aging presents a challenging problem for plastic and aesthetic surgeons; it is a multifactorial, multistep process that involves structural and volumetric changes in the skin, muscles, skeleton, and adipose tissue.[2] Facial tissue descent is caused not only by gravity, but also by the reabsorption and repositioning of the facial adipose system.[3-6] Injectable fillers and autologous adipose tissue have been popular choices for the augmentation of lost soft facial tissue.[7] Recently, the rejuvenating properties of adipose tissue were highlighted by studies that described improved texture and elasticity of the skin, normalization of altered pigmentation, and amelioration of scars following fat grafting.[8-10] Although lipofilling is widely used by plastic surgeons for the treatment of facial aging, there is a lack of standardized harvesting and processing procedures.[11] Moreover, the main unresolved issue with autologous fat transfers is the unpredictable reabsorption rate of the engrafted fat, which can range from 30% to 70%.[12, 13] Graft survival is dependent on the vascular supply of oxygenated blood; only adipose tissue within a 2-mm distance from an artery will survive, while the remaining tissue will likely undergo necrosis and fibrous transformation.[14] The discovery of adipose-derived stem cells (ASCs), a resident population of adult mesenchymal stem cells (MSCs) within the adipose tissue, prompted studies on new therapeutic strategies.[15] ASCs are multipotent mesenchymal stem cells that display regenerative capacity by differentiating into cells of mesenchymal, ectodermal, and endodermal lineages, and by the paracrine release of growth factors.[16-22] ASCs are thought to mainly be responsible for the rejuvenation capabilities of fat grafts, and their use showed lower reabsorption rate due to improved angiogenesis and reduced inflammatory response.[23] Currently, strategies using ASCs with autologous fat transfer, injectable vehicles such as hyaluronic acid (HA), or other injectable biomaterials, are under study for the treatment of the aging face with promising results. [24-26] This concise review aims to provide the readers with background on soft tissue augmentation, as well as ASC biology, to

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better enable understanding of emerging strategies for the treatment of facial aging.

2. The aging face

The physical characteristics of the face change with time and represent aging. The skin and all of the tissues of the face are responsible for the observed senescence.[27] Smoking, sun damage, genetics, bone reabsorption, tissue atrophy, and genetics may all accelerate the aging process.[28] Over time, rhytides start appearing on the face. They are initially visible only dynamically and eventually become visible even in static conditions, when the mimic muscles are at rest. Furthermore, the natural folds (the nasojugal and nasolabial folds) deepen because of tissue descent due to gravity-related decrease of ligamentous attachment of the face and volume loss in the facial adipose system.[29] The loss of bone prominences also appears with aging, rendering comprehensive rejuvenation challenging.[29] In youth, the rounded fullness of the face can be viewed as a "base up triangle," with the cheeks forming the base. However, it shifts to a "base down triangular" configuration in elderly patients.[30] The upper face (brow, forehead, temple, and upper lids) usually shows the very first signs of aging: frontal rhytids appear, brows drop down, and palpebral ptosis may occur.[31] Aging usually causes a dissipation of fat around the orbital rim, associated with bone reabsorption rather than gravitational dropping. Therefore, traditional blepharoplasty focuses on fat transfer to prevent the bony look and to improve the senescence-related enophthalmos, which may exacerbate the palpebral ptosis.[32,33] In the midface, infraorbital hollowness, ptosis of the malar fat pad with the loss of the malar prominence, and thinning of the tear trough skin, leading to its deepening, are very common with aging.[33] Even the perioral region may eventually show signs of aging. The lips usually get thinner and lose their tone because of subcutaneous fat atrophy, causing fine wrinkles that deepen with time.[34] Furthermore, marionette line and nasolabial folds increase in depth following volume and projection loss in the lips.[35] Rhytidectomy alone usually cannot address these issues; lipofilling is, therefore, widely adopted as an adjuvant treatment in order to fill the prejowl sulci, marionette lines, and lips with satisfactory results.[7]

3. Cadaveric studies have demonstrated that the facial adipose system can be separated into the deep and superficial layers.[6,36] Both layers are further partitioned into multiple compartments by a complex network of fibrous membranes containing blood vessels.[37-39] These septal boundaries arise from the superficial fascia and penetrate the deep dermal layer, thereby providing the skin with mechanical support and protecting its vascular supply.[40,41] The loss of volume in each compartment is a crucial feature of the aging face; some compartments consequently shift downward, and others may even migrate, leading to an alteration of facial contours.[40,42] Not all compartments undergo the same changes. Therefore, precise anatomical knowledge of the facial adipose system is essential to accurately direct the injection of fat grafts.

4. Volume restoration in any facial rejuvenation treatment is as important as the traditional lifting and excisional-based techniques. Surgical planning must, therefore, be tailored to individual patient needs and resuspension of deep tissues should be combined with the preservation and restoration of the facial adipose system in order to obtain a healthier and younger face.[6] Additionally, macroscopic changes in facial skin follow ultrastructural changes.[35] Actinic elastosis is one of the main lesions of aging skin. Histological examination usually shows mononuclear inflammatory infiltration in the dermis along with elastic fiber degeneration (elastosis). Over time, the aging skin loses its ability to retain water molecules and, therefore, its elasticity, because of the loss of the extracellular matrix fibers, such as oxytalanic elastic fibrils and type I, III, and IV collagen oligosaccharidic polymers. The elastic fibers of the papillary and reticular dermis appear disorganized, with decreased deposition of newly formed fibers, contributing to the loss of tone and elasticity. Tissue atrophy and epidermal thinning correspond with the loss of the original contour of the face. Electron microscopy of the dermis shows an irregular pattern of large caliber elastic fibers dispersed into a network of collagen fibers, which may be assembled in dense bundles in some areas.[11]

3. Soft Tissue Augmentation: Background

Fat Grafting

The first study on fat grafting dates back to 1893, when Neuber filled scars with autologous fat.[43] His technique was refined by Bruning as an injectable procedure in 1911, and Hollander reported the successful treatment of patients affected by lipoatrophy of the face after fat grafting in 1912.[44,45] Autologous fat transfer became popular, given its abundance, low cost, autologous nature, and biocompatibility.[7] However, this popularity receded with time due to the inconsistent and technique-dependent results caused by the unpredictable reabsorption rates. Lipofilling regained popularity in the 1970s thanks to the work of Fisher.[46] Since then, advances in harvesting, processing, and injecting procedures have resulted in the wide use of autologous fat transfer by plastic surgeons. Graft retention remains extremely variable, and is inversely related to the size of the graft.[24] Presumably, the physiology of fat grafts is the same as that of other tissue grafts; it first survives via nutritional diffusion from the recipient vascular bed until vascularization occurs. However, only the portion of the graft close to the vascular bed of the recipient receives enough nourishment and survives. The central portion of the grafts undergoes a fibrotic involution because of adipocyte apoptosis. Only the tissues within a 2-mm distance from an artery receive vascular supply adequate for survival.[14] Various mechanisms such as mechanical disruption of cells, lipid-induced membrane damage, and others that have not yet been identified, could also contribute to graft survival.[24]

In order to maximize graft survival, less traumatic procedures were studied and the size of the fat graft was decreased.[47] In 1997, Coleman reported a new technique for autologous fat transfer: the "lipostructure technique".[48,49] He suggested distributing small deposits of fat through multiple access points at various subcutaneous depths. Decreasing the size of the grafts could increase the amount of fat adjacent to the recipient vascular bed, thereby improving survival. To date, no consensus exists regarding the best technique to maximize survival or anticipate the amount of reabsorbed graft.

Soft-tissue Filler

The American Society of Plastic Surgeons reported that over 1.5 million soft tissue filler injections were performed in the U.S. alone in 2010 — a 200% increase with respect to the past decade.[50] Paraffin, rubber, latex, and silicone were the first materials to be used as soft-tissue fillers since the beginning of the 20th century.[51] Most of these are now banned; however, the number of available synthetic and natural soft tissues fillers, such as hydroxyapatite, polylactic acid (PLA), collagen, HA, and biopolymers [polymethylmethacrylate (PMMA), and dextran], has greatly increased corresponding with their widespread use.[52]

Temporary soft tissue fillers (i.e. collagen, HA) typically have reabsorption rates between 6 and 12 months. Repeated injections are therefore needed for a lasting cosmetic result.[26] PLA and hydroxyapatite have a longer lifespan, ranging from 12 to even 18 months. Biopolymers are permanent fillers, which typically last much longer, but exhibit some level of reabsorption over time.[53]

Collagen forms 70-80% of human connective tissue.[54] Prior to the advent of HA, it was the gold standard for facial rejuvenation.[55] Bovine, human, or porcine collagen could be used; however, given the lower incidence of hypersensitivity and immune reactions, the use of human collagen gained more prominence.[55-57]

HA is the most widely used injectable filler, since it is a naturally occurring polysaccharide of the connective tissue (especially the human dermis), and elicits minimal immune and allergic responses.[58,59] Currently, the Food and Drug Administration (FDA) has approved only ten HA-based fillers, which have a lifespan of up to 12 months and very few adverse effects.[59] The soft tissue augmentation that follows HA injection initially corresponds to the volume of HA deposits and to the physiological ability to attract water molecules.[54] Wang *et al.* demonstrated that HA also has the ability to stimulate the activity of dermal fibroblasts *in vivo*.[60]

Facial rejuvenation procedures currently represent the main use of soft-tissue fillers. However, studies investigating their application in the field of tissue regeneration have been reported. They may act as 3D scaffolds in cell-based therapies. Stem cells or dermal fibroblasts may be efficiently vehicled by these biomaterials, enhancing the integration with native cells.[33,61] Moreover, soft-tissue fillers may promote neovascularization by providing structural support to the newly formed vessels.[24]

Indication

Plastic surgeons widely use autologous fat grafting for a variety of pathological conditions, including the field of breast reconstruction and restoration of soft facial tissue after trauma or tumor resection, congenital deformities, Parry-Romberg syndrome, scleroderma, orbital and periorbital surgery, facial plasty, burns, and scars.[62-74] Rejuvenation of the face is another highly-prevalent use of fat transfer; the forehead, zygomas, lips, nose, chin, and mandible are the facial units where injections are usually performed.[75-79] Moreover, the idea that the most agreeable cosmetic outcomes come from the combination of facial lifting and lipofilling, in "lift-and-fill" rhytidectomy, is gaining popularity.[35]

Although soft-tissue fillers were initially created for facial rejuvenation, they are increasingly used for treatment of the nasolabial fold, correction of volume deficiencies, improvement of facial contours, treatment of facial wrinkles (marionette line, periocular, perioral, and glabellar wrinkle), lip augmentation, and nose remodeling.[78-81] Dermal fillers are also applied, albeit less commonly, for the treatment of scars, chin shaping after implant positioning, and earlobe treatment.[82]

Soft-tissue fillers work best when the volume needed to correct the defect is small. The material is implanted in the deep dermis or the superficial fatty tissue, depending on the type of filler used and the anatomical site to be treated.[83] Fat grafts are better suited for wider volume restoration, when the graft may be injected in the superficial or deep layer of the facial adipose system.

The treatment of the perioral region is one example. Autologous fat transfer is performed only in the deeper layer, in order to correct the deflation of the area, while the white roll is best addressed by using bioengineered fillers like HA.[35] Fat transfer in the white roll could cause asymmetric results as a consequence of lip movement.[84] Given the unpredictable reabsorption of fat grafts, such asymmetries may become more obvious as the graft gets closer to the skin surface.

Advantages, Drawbacks, and Complications

Autologous fat and soft-tissue or dermal fillers adequately address deflation, wrinkles, and contour defects of the aging face. They are readily available and tolerated by most patients, as they are minimally invasive procedures helping to maintain a youthful appearance. However, fat graft procedures are easy, cost effective, and use autologous tissue, with no risk of immune or allergic reactions. The biocompatibility of the fat grafts adapts to the growth of the face over time, resulting in a permanent cosmetic outcome.[85] Furthermore, recent studies have indicated that autologous fat not only provides volume replacement but may also offer dermal regeneration of thinned skin.[39,40] This rejuvenating and regenerative effect is not clearly understood. The long-lasting improvement after fat grafting may be explained by the structural and ultrastructural changes that occur in the skin and subcutaneous tissue.[86, 87] Skin regeneration mediated by autologous fat could be regarded as a necessary component for global rejuvenation, and current bioengineered fillers do not have similar properties.

The main drawback of fat grafting is the inability to accurately predict outcome, as reabsorption may vary from 30 to 70%.[12,13] Observation, physical examination, and digital pictures are often the only tools to assess the results. Laser scanners, 3D photography, and magnetic resonance imaging (MRI) present novel strategies to objectively assess the retention rate of the grafts.[88]

Dermal fillers have some limitations: immunologic reactions, the possibility of material migration, and the need for allergy testing prior to use in some fillers.[89] Certain fillers are also expensive, which is related to their overall temporary nature and the need for multiple injections in order to maintain the outcome.[56]

Soft-tissue fillers and fat grafts are considered safe and effective; both have a very low incidence of complications, which are usually mild and transient.[90] A common complication is an asymmetric outcome, which could be due to poor technique, improper treatment, or defect/filler mismatch.[53] Local edema, erythema, inflammation, and bruising are also relatively common immediate/early complications as they are a result of the trauma caused by injections.[81] None of these complications affect the final result, however, and usually resolve within 5 to 10 days. Dermal fillers are very rarely visible, but may appear as a bluish discoloration caused by improper depth of injection.[91] Infections and cellulitis are infrequent and responsive to antibiotics.[55] Rare, but severe, complications

include skin necrosis, anaphylactic reactions (dermal fillers), blindness, and even death. [53,54,57] Furthermore, dermal fillers generate foreign body granulomas in 0.1% of patients; these usually form at least 2 months after treatment and persist for at least two months.[80] Uncommon complications of fat grafting include fibrosis, oil cysts, and calcification.[92] Finally, fat graft hypertrophy is also reported, as the graft can mimic abdominal fat over time.[47]

4. Potential Role of ASCs as Innovative Adjunction in Anti-aging Treatments

Biological and Metabolic Properties of ASCs

ASCs are a plastic-adherent, multipotent cell population, which show differentiation potential towards the adipogenic, osteogenic, and chondrogenic lineages.[93] ASCs reside within the "stromal vascular fraction" (SVF) of the adipose tissue. The SVF comprises a heterogeneous population of cells (preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages, and lymphocytes) obtained after enzymatic digestion of adipose tissue.[94] ASCs account for 30% of the total SVF cells.[95]

ASCs are commonly characterized by their immunophenotype: they express the superficial markers CD13, CD29, CD34, CD44, CD73, CD90, CD105, CD166, and MHC I, and lack the expression of CD14, CD31, CD45, CD133, and HLA-DR.[96-98] ASCs display greater proliferative capacity and similar senescence when compared to bone marrow-derived stem cells (BMSCs).[94] ASCs are considered to be stable in long-term culture, and their therapeutic properties are not related to donor age.[99]

In vitro studies have demonstrated ASC multipotency and plasticity; they have the potential to differentiate not only into cell lineages of mesodermal origin, but also into ectodermal and endodermal lineages.[16-22,93-95] Furthermore, *in vivo* experiments testing the capability of ASCs to effectively promote regenerative processes have shown that a variety of tissues and organs could be engineered from ASCs.[100]

ASCs are currently being investigated as a therapeutic strategy for a diverse group of pathological conditions.[95,101] Their regenerative potential includes angiogenic, antioxidative, and immunomodulatory properties.[102, 103]

ASCs act by two main mechanisms: differentiation into required lineage pathways (also modulating the "stem cell niche" of the host) or secretion of paracrine factors.[104] Nie *et al*. demonstrated, in an *in vivo* animal model, spontaneous ASC differentiation into a vascular endothelial phenotype that lead to newly formed capillaries.[105] Traktuev reported that ASCs could be found around newly formed blood vessels as pericytes, providing vascular stability.[106] Additional studies have shown that ASCs seeded onto biomaterials, such as a silk fibroin-chitosan scaffold, transformed into fibrovascular, endothelial, and epithelial elements of repaired tissue.[104]

However, the primary mechanism for the therapeutic effect of ASCs is possibly the secretion of a wide range of paracrine molecules. ASCs provide angiogenic support by secreting molecules essential for angiogenesis, such as stromal cell–derived factor 1 (SDF-1), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-BB, basic FGF, Ang-1, IGF-1, MMPs, IL-6, and IL-8.[107] VEGF stimulates the mobilization, recruitment, and migration of progenitor endothelial cells, SDF-1 promotes endothelial cell survival, vascular branching, and pericyte recruitment, and Ang-1 inhibits pericyte apoptosis, recruiting them for the maturation of forming blood vessels.[108,109] FGF helps to maintain vascular integrity. When ASCs are exposed to hypoxic environments, the levels of secreted VEGF and/or HGF, as well other growth and differentiation factors, such as TNF-α, FGF, EGF and ascorbate, increase.[109] Zografou *et al.* also reported increased collagen density due to the paracrine effects of ASCs on collagen production and secretion of fibroblasts.[110]

Finally, ASCs demonstrate immunosuppressive capacities by down-regulating the inflammatory response. ASCs can modulate monocytes/macrophages, dendritic cells, T-cells, and B-cells by direct cell-to-cell interaction and paracrine effects. However, the underlying mechanisms remain unclear.[104]

Isolation Process

ASCs share several potentially therapeutic properties with other MSCs. BMSCs were the first adult stem cells to be studied in relation to regenerative and cell-based therapy. Both cell types could modulate the immune response, secrete paracrine factors, and promote therapeutic angiogenesis, thereby providing the basis for regeneration processes.[111] However, since Zuk and colleagues first isolated ASCs in 2001 from adipose tissue obtained after liposuction, these cells have been clinically more attractive than BMSCs, due to their higher isolation yield, ease of harvest after a minimally invasive procedure, and abundance.[15, 104]

ASCs can be harvested from subcutaneous adipose tissue by standard tumescent liposuction procedures under local-assisted anesthesia. Several independent groups have developed procedures to isolate and characterize ASCs, although the procedure described by Zuk *et al.* remains the most widely used.[112] This method includes enzymatic digestion of the lipoaspirate adipose tissue (0.075% collagenase type I) followed by a series of centrifugation and washing steps with Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS). The isolation protocol proposed by Zuk was bench-derived, like those described by other groups, and therefore, not appropriate for clinical purposes because of its complex, time-consuming (the entire process takes an average of 2 hours), and expensive nature.[113-116] In 2016, Raposio *et al*. described a method that was specifically designed for clinical application, which appeared easy, safe, and fast (80 minutes), allowing the collection of a ready-to-use ASC pellet.[117] Once the lipoaspirate was harvested, ASCs were isolated by both mechanical (centrifuge) and enzymatic (collagenase) means. The entire process was performed under a closed automated system in order to maximize sterility and safety. Flow cytometric assay revealed that 9.06×10^5 (SD ± 6.6×10^5) ASCs were isolated from 100 ml of adipose tissue and ASCs accounted for 25.9% of the pellet cells, which was a considerably higher yield than those reported in literature.[94] The same group also reported the therapeutic effectiveness of the isolated ASC pellet in articles currently at press. The method proposed by Raposio *et al.* required the use of an automated device. Many such devices are currently available because of the related advantages. An automated device allows for closed-system fat processing, protocol standardization, and reduced operative time.[95] However, there is a drawback: most of these devices require the use of animal-derived reagents and collagenase.[118] ASCs have a high yield upon isolation and, therefore, do not require extensive manipulation or *ex vivo* expansion before application. Thus, therapies based on ASC application do not require compliance with "cell manufacturing" in accordance with current good manufacturing practice (cGMP) guidelines.[119] These restrictions are not applied in cases of minimal manipulation [Regulation (EC) No 1394/2007 of the European Parliament and of the Council], and ASCs are considered advanced therapy medicinal products.[112]

In the United States, however, when ASCs are enzymatically isolated, the cells are regarded as more than minimally manipulated and therefore a drug. Thus, an investigational new drug application is required prior to using enzymatically isolated ASCs in clinical settings.[120] This regulation does not apply in cases of ASCs isolated by mechanical means during the same operative session. Therefore, high-yield ASC isolation processes with minimal handling were conceived following the findings of Yoshimura *et al*.,[97, 113,121-123] who reported that a significant amount of ASCs (enough to be used clinically without cell expansion) could be isolated from what they called the liposuction aspirate fluid (LAF).

In the light of these findings, Francis *et al.* isolated cells capable of tri-lineage differentiation by mechanically separating the SVF directly from lipoaspirate.[113] Soon after, Bianchi *et al.* described a non-expanded, ready-to-use fat product obtained by pushing aspirated fat through size reduction filters while allowing waste products to exit in a closed system.[124]

Raposio *et al*. also described an effective alternative procedure performed only by mechanical means.[118] ASCs were isolated with the help of a vibrating shaker and a centrifuge under a laminar airflow bench. The entire isolation process lasted approximately 15 minutes and yielded a mean of 5×10^5 (SD: $\pm 1 \times 10^5$) ASCs with a 97% viable cell rate. The clinical effectiveness of the isolated ASC pellet was also reported.[101]

Clinical Experience with ASCs Combined with Autologous Fat Grafts

ASCs or SVF, in combination with autologous fat grafts, were used in an attempt to reduce the rapid loss of graft volume that usually occurs within 3-6 months.[95] The hypothesis was that ASCs might be able to enhance tissue revascularization, thereby improving graft survival. As a result, reabsorption rate may be reduced and steady, making the cosmetic outcome more predictable.

Pre-clinical trials have highlighted the possible mechanism of action of ASCs in enhancing the volume retention of fat grafts.[125-127] Due to their multipotency, ASCs may differentiate not only into mature adipocytes contributing to direct tissue regeneration, but also into endothelial and vascular mural cells.[125,128,129] ASCs may also promote angiogenesis and graft survival by releasing angiogenic growth factors in response to the hypoxia typical of the central portion of fat grafts. Finally, ASCs persist in the engrafted adipose tissue as ASCs.[126]

The first report of ASCs added to autologous fat transfer was by Yoshimura *et al.*, who referred to it as "cell-assisted lipotransfer" (CAL).[130, 131] In their work, patients underwent CAL for breast augmentation. Patients were highly satisfied, no adverse events were reported, and the authors stated that fat transfers enriched with ASCs apparently increased the augmentation effects. However, no comparative arm was used. The same group applied CAL to patients with facial lipoatrophy.[132] All of the faces of each of the patients were treated with fat graft combined with ASCs, while the contralateral portion served as control and was treated with standard lipoinjections. CAL was reported to ensure better cosmetic outcome and graft retention.

Peltoniemi *et al.* reported a contradictory result from their study in which breast enhancement with CAL showed no significant improvement over traditional fat transfer. The retention rates of the fat grafts were objectively analyzed by MRI.[133]

In 2011, Kim *et al*. published the results of a clinical trial wherein 31 patients were treated for facial depressed scars.[133] There was no control group, and fat grafts were enriched with ASCs that were expanded *ex vivo*. They observed 74.6% volume retention with stable outcome at 1 year.[134] Kolle *et al.* also performed a single-center, triple-blind, randomized, placebo-controlled trial wherein ASCs were expanded *ex vivo*. Patients that underwent CAL injections showed a 64.6% improvement in the outcome compared to the control group.[135]

Other groups have investigated the potential of CAL vs. standard lipoinjections in face reconstructive surgery or breast augmentation and have reported that the addition of ASCs to fat grafts promoted statistically superior volume retention.[136-140] However, there are few reports of CAL in facial cosmetic surgery.

Mailey *et al.* reported that even when CAL and control groups showed similar satisfaction in terms of symmetry, scarring, and deformity, patients that underwent CAL observed a significant increase in skin improvement.[141]

Finally, Charles-de-Sá *et al.* recently published an outstanding paper in which they analyzed the histological and ultrastructural changes that occur in aged facial skin after CAL.[11] They also compared the results with those obtained after only injecting expanded ASCs. The ASCs injected were 16.204 ± 5516 cells within the CAL, and 2×10^6 cells when injected alone. Both treatments produced overlapping results. Skin appeared more hydrated. Newly formed oxytalan elastic fibers were dispersed in the papillary dermis, and the area of elastosis decreased. The reticular dermis modified its tridimensional architecture at ultrastructural level, indicating the presence of a richer microvascular bed. However, larger amounts of adipose tissue at the dermal-subcutaneous interface were observed only in the group treated with ASCs alone. The quality of the results was not influenced by the number of ASCs injected. Thus, even patients with poor subcutaneous adipose tissue may benefit from such treatment. This study was the first to demonstrate the histological and ultrastructural changes in the skin after either CAL or ASC-only treatment, thereby validating their skin rejuvenating effects.

Bioengineered Fillers

Currently available soft-tissue fillers may act as injectable materials for engineered tissue replacement.[26] ASCs vehicled by soft-tissue fillers could provide them with the biological properties of CAL or even traditional lipoinjection by promoting rejuvenation and regenerative effects in aged facial skin. By identifying the appropriate combination of injectable materials and ASCs, the potential of the stem cells for angiogenesis and vasculogenesis may be utilized to correct the vascular disconnection between the dermis and subcutaneous tissue.[11] Moreover, injectable materials may act as predefined-shape 3D scaffolds with the advantage of adapting to voids, allowing clinicians to tailor the treatment for individual patients. Injectable materials were studied for minimally invasive delivery of cell-based therapy in engineered tissue replacement. It is essential to find suitable vehicles that are easily injectable and safe for both patients and vehicled cells.[25] Such vehicles could prevent excessive ASC diffusion, allowing sustained release at the injection site. Synthetic polymers such as PLA are popular bioengineering materials, and many have been approved by the FDA for *in vivo* trials.[142] However, they lack innate bioactivity, and *in situ* gelation produces toxic byproducts. Natural biopolymers have been studied in preclinical settings.[138-142] Bioactivity, cell adhesion, and limited immune response are their main advantages. Collagen was the first to be studied as a stiff sponge.[148,149] However, it undergoes reabsorption too fast, and showed limited vascularization in injectable form.[150,151] Alginate and fibrin were also tested, but showed limited adjunctive advantages.[146,152-153] HA is the most commonly used soft-tissue filler in tissue engineering.[26] Due to its carboxylic acid side chain and disaccharide backbone that allow for chemical modification, the degradation, strength, and interactions of HA can be specifically designed. Its biocompatibility, bioavailability, and tolerance as an injectable vehicle

for ASCs have been demonstrated by Lequeux *et al*. *in vitro* and in an animal model.[25] To date, bioengineered fillers have not been tested in any human trial because further pre-clinical research is required.

5. Conclusions

Facial aging is a complex process that involves all of the tissues of the face. It, therefore, cannot be addressed completely by only one technique. Traditional lipoinjection and other soft-tissue fillers are required to treat the deflation of face volume that occurs over time.[155-158] CAL seems to improve one of the main drawbacks of fat grafting, namely the unpredictable reabsorption rate of the grafted adipose tissue; however, a real consensus has not been reached. The rejuvenation and regenerative effect of autologous fat transfer could be explained by the presence of ASCs; however, optimization for cell dose efficacy and standardization of isolation protocol may improve future applications.[159-162] Safety and effectivity should also be clearly outlined. Finally, bioengineered fillers are in a very early stage of development, and could be further developed as semi-permanent fillers with characteristic biological properties of engrafted adipose tissue, resulting in an even less minimally invasive procedure. In conclusion, ASCs could emerge as an effective and novel anti-aging therapeutic agent in cosmetic surgery. However, extensive analysis of their actual effectiveness and mechanism of action are required.

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