# Fat Graft Retention: Adipose Tissue, Adipose-Derived Stem Cells, and Aging

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**Summary:** Over the past 30 years, there has been a dramatic increase in the use of autologous fat grafting for soft-tissue augmentation and to improve facial skin quality. Several studies have highlighted the impact of aging on adipose tissue, leading to a decrease of adipose tissue volume and preadipocyte proliferation and increase of fibrosis. Recently, there has been a rising interest in adipose tissue components, including adipose-derived stem/stromal cells (ASCs) because of their regenerative potential, including inflammation, fibrosis, and vascularization modulation. Because of their differentiation potential and paracrine function, ASCs have been largely used for fat grafting procedures, as they are described to be a key component in fat graft survival. However, many parameters as surgical procedures or adipose tissue biology could change clinical outcomes. Variation on fat grafting methods have led to numerous inconsistent clinical outcomes. Donor-to-donor variation could also be imputed to ASCs, tissue inflammatory state, or tissue origin. In this review, the authors aim to analyze (1) the parameters involved in graft survival, and (2) the effect of aging on adipose tissue components, especially ASCs, that could lead to a decrease of skin regeneration and fat graft retention. *(Plast. Reconstr. Surg.* 151: 420e, 2023.)

**Clinical Relevance Statement:** This review aims to enlighten surgeons about known parameters that could play a role in fat graft survival. ASCs and their potential mechanism of action in regenerative medicine are more specifically described.

Adipose tissue (AT) is present in large quantity and represents the ideal filler for correcting and remodeling purposes. For two decades, extensive work has been done on adipose-derived stem/stromal cells (ASCs) and their use in regenerative medicine.[1,2] Those cells would be the key factor for fat graft survival because of their ability to differentiate and synthesize growth factors.[3,4] However, ASCs are not the only cellular component involved in graft survival. This review aims to describe other parameters that can have an effect on graft survival, with a focus on macrophage polarization, vascularization promotion, and extracellular matrix (ECM) remodeling. We also depict the effect of aging on those phenomena.

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*Received for publication November 18, 2020; accepted March 1, 2022.*

DOI: 10.1097/PRS.0000000000009918

## ADIPOSE TISSUE AGING

The main function of subcutaneous AT is energy storage through lipids. It is a major endocrine organ[5] with strong immunomodulatory properties,[6–8] and also undergoes changes with age.[9] Age induces facial subcutaneous AT deflation.[10,11] AT volume decrease leads to a loss of projection, inducing excessive traction on the lower eyelid.[12] Using magnetic resonance imaging, Wysong et al. observed an age-related decrease in AT thickness in infraorbital and temporal zones and on the medial cheek.[13] In contrast to these findings, Gosain et al. showed an increase in fat volume in the medial cheek on aged people.[14] Age is negatively correlated with preadipocyte proliferation on subcutaneous but not omental depots. Furthermore, preadipocyte proliferation and differentiation capacities are down-regulated with age.[15] There are also differences between fat depots, as preadipocyte properties vary according to their localization.[16] Facial adipocytes have different morphology

**Disclosure:** *The authors have no financial interest to declare in relation to the content of this article. No funding was received for this article.*

according to their fat depot; for instance, the average adipocyte size of nasolabial fat is larger than deep medial cheek fat.[17] In addition, several reports have shown that age reduces vascularity, angiogenic capacity, and vascular endothelial growth factor (VEGF) expression, and increases fibrosis in aged AT mice.[18] Ultraviolet irradiation could also impairs AT by inhibition of preadipocyte differentiation, mediated by inflammatory cytokines such as interleukin (IL)-1α, IL-6, and tumor necrosis factor-α.[19,20]

## ADIPOSE-DERIVED STEM/STROMAL CELLS

Since the mid-1990s, autologous fat grafting has become a standard technique in plastic surgery. Lipofilling is now admitted as an alternative to synthetic polymer fillers.[21–23] AT is not only a simple filler for volumetric effects, but also combines regenerative effects in skin on grafting.[24,25] AT is composed of adipocytes and stromal vascular fraction (SVF) cells, including immune cells (eg, macrophages and lymphocytes), endothelial cells and their progenitors, smooth muscle cells, pericytes, and mesenchymal stem/stromal cells called ASCs[26–31] (Fig. 1). ASCs were described by Zuk et al. in early 2000s.[32,33] They are multipotent cells with high differentiation capacity[27,34] that have gained attention for therapeutic and cosmetic applications.[3,35] They are used to treat and improve wound healing,[36–39] scars,[24,40–43] hair regeneration,[44,45] and facial aging.[46,47] Fat grafting improves skin quality, leading to a reduction of dermal epidermal junction flattening, with noticeable reconstruction of normal ridge pattern and dermal papillae.[48,49] However, two systematic reviews focusing on therapeutic and aesthetic use of lipofilling for skin quality improvement, wound healing, and hair growth demonstrate that these findings may be somewhat limited because of no significant effect on healthy skin.[50,51] The authors conclude that even if the clinical outcomes show improvement, there is no robust clinical study (with high level of evidence) that shows a significant effect on skin quality.

### ECM Modulation

As ASCs can modify surrounding cell behavior, they can remodel dermal ECM.[42,46] ASCs promote dermal fibroblasts and epidermal keratinocyte proliferation and migration, not only by cell-to-cell direct contact, but also by paracrine activation through secretory factors. ASCs can also enhance the secretion of ECM proteins such as collagens or fibronectin[52–55] and act as modulators of ECM by collagen and matrix metalloproteinase (MMP)/tissue inhibitors of MMP synthesis regulation.[56–61] Indeed, ASCs can modulate homeostasis of MMPs and their endogenous inhibitors (tissue inhibitors of MMPs).[62–64] Those cells are known to induce a better collagen organization and a decrease of α-smooth muscle actin expression, markers of dermal fibrosis improvement.[65–67] ASC can inhibit profibrotic factors such as transforming growth

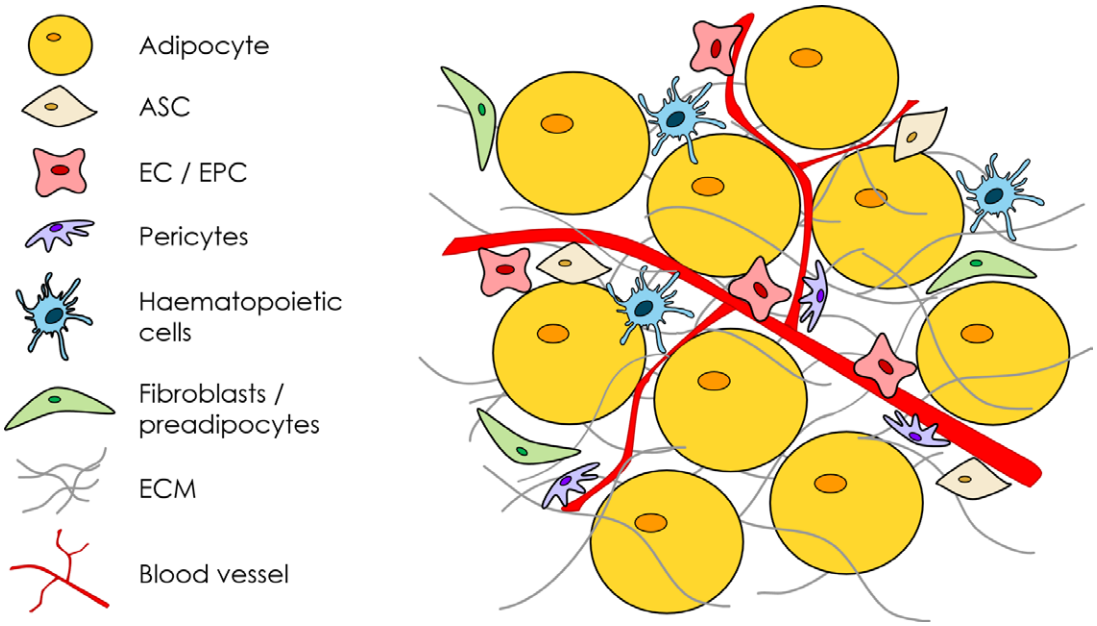


**Fig. 1.** Adipose tissue composition. *EC*, endothelial cells; *EPC*, endothelial progenitor cells.

factor (TGF)-β1 or IL-6[68–70] and increase the antifibrotic factor TGF-β3.[43] The antifibrotic effect of ASCs is also mediated by their paracrine activity. Indeed, ASCs secrete basic fibroblast growth factor (b-FGF), also called FGF-2,[71,72] hepatocyte growth factor (HGF),[71–74] and IL-10,[75] which are known to decrease TGF-β1 expression,[76] stop fibroblast-to-myofibroblast differentiation,[77,78] and induce myofibroblast apoptosis.[61,79]

## Immunomodulatory Properties

### Effect on Immune Cells

ASCs have strong immunomodulatory effects on both innate and adaptive immune systems.[80] These cells are able to partially suppress lymphocyte proliferation and inhibit B-lymphocyte proliferation and differentiation into plasmocytic cells.[81] Treatment with SVF cells or ASCs greatly attenuated the activities of T-helper 1 and T-helper 17 cells and their associated proinflammatory cytokines.[82] Some studies have shown that ASC secretome is a pivotal player in immunomodulatory or angiogenic properties.[83–85] However, cell characteristics may vary between patients according to age, sex, body mass index (BMI), or metabolic state. As an example, ASCs derived from patients affected by type 2 diabetes showed increased expression of inflammatory markers and high reduction of their immunosuppressive activities.[86]

### Focus on Macrophages

ASCs can modulate monocyte and macrophage behavior through soluble factors.[87] Some authors suggested that ASCs could modulate inflammation through regulation of macrophage polarization. Indeed, ASC-conditioned media (CM) significantly reduced the production of TNFα, nitric oxide, and prostaglandin $E_2$ and the activation of nuclear factor-κB by macrophages.[87] Co-culture of M0 or M1 macrophages with ASCs or ASC-CM increases alternative M2 macrophage marker expression such as CD206, CD163, or IL-10.[82,88–92] This alternative activation is paired with a decrease of proinflammatory M1 macrophage markers such as CD80, IL-1β, IL-6, or TNF-α. Some immunomodulatory roles of ASCs remain unclear, such as expression of toll-like receptors,[93,94] hematopoiesis support,[95] and cytokine release at a basal or stimulated state.[73] New therapeutic strategies are considering use of ASC-CM or extracellular vesicles to modulate inflammation,[92,96] as cells secrete inflammatory cytokines such as granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, IL-6, IL-8, or TNF-α.[55,72,73,97] Macrophage polarization switches from M0 or M1 to M2 could be mediated by those soluble factors. Indeed, exosomes from ASCs polarize macrophages toward M2 phenotypes through the transactivation of arginase-1 by exosome-carried active STAT3.[98]

Because of their ubiquitous presence and ability to secrete numerous cytokines, macrophages can interfere at each angiogenic step. Macrophages can modulate ECM by MMP synthesis,[99] synthesized factors that can modulate endothelial cell proliferation and migration either in a proangiogenic (Ang2, b-FGF, TNF-α, VEGFC) or antiangiogenic (IL-10, TSP-1, $VEGFA_{xxx}b$) pathway.[100] M2 macrophages are known to promote angiogenesis and tissue regeneration, whereas M1 macrophages are considered antiangiogenic, although these classifications are controversial. Jetten et al. indicated that M2 macrophages have higher potential to increase the number of endothelial cells and tubular structures when compared to M1 macrophages.[101] In contrast, Spiller et al. have demonstrated that M1 macrophages secrete high levels of angiogenic stimulators, including VEGF, and M2 macrophages secrete high levels of PDGF-BB (a chemoattractant-stabilizing pericytes), promote anastomosis of sprouting endothelial cells, and secrete the highest levels of MMP9, an important protease involved in vascular remodeling.[102]

These data suggest that ASCs could affect immune cells, especially macrophages, leading to a proresolutive phenotype. This immunomodulation could improve tissue regeneration, as alternatively activated macrophages increase angiogenesis.

## Role in Vascularization

Beneficial effects of ASCs on wound healing involved promotion of vascular regeneration. ASCs can differentiate into endothelial vascular cells[103–105] and promote the vascular network when co-cultured with endothelial cells.[106] Furthermore, endothelial cells co-cultured with either ASCs or bone marrow–derived stromal cells induce stable vascular structures.[107] However, ASC co-cultures developed more junctions and higher network density within the same time frame.[107] ASCs bear many hallmarks of pericytes and provide vascular stability through functional interaction with endothelial cells.[108] Fat grafts supplemented with ASCs have a higher capillary

density, indicating that ASCs could promote neovascularization through expression of various growth factors, including VEGFA and insulin-like growth factor-1.[109] Paracrine function is a key factor of ASC regenerative effects, and many authors have been interested in the ASC secretome.[73,110] Those cells are able to synthesize a high variety of factors such as leptin, VEGF, HGF, b-FGF, TGF-β, IL-8, platelet-derived growth factor (PDGF), PlGF, or SDF-1,[71–74,97,111–116] involved at different steps of angiogenesis. ASCs can form capillary-like tubes, which are dependent on PDGF and the b-FGF signaling pathway.[117] ASCs increased endothelial cells growth and reduced endothelial cell apoptosis through VEGF, HGF, and TGF-β secretion.[72] ASCs support endothelial tubulogenesis by VEGF-A and VEGF-D expression.[113] FGF and VEGF are promoters for ASC proliferation, migration, attachment, and endothelial differentiation and have a co-stimulatory effect on ASC endotheliogenesis.[118]

### Impact of Aging on ASCs

The regenerative potential of ASCs is based on their differentiation potential and paracrine effects.[3,4,119] However, mesenchymal stem/stromal cell functionality declines with age.[120] Aging decreases osteogenic differentiation[121,122] and ASC telomere length.[114,123–125] The effect of aging on proliferation and adipogenic potential is still controversial.[121,123] Some authors state that aging has no effect on ASC yield, viability, and proliferation,[126,127] whereas others show that cellular proliferation and migration decrease with age.[121,122,128] Another study shows no correlation between age and ASC yield, or with the capacity of preadipocytes to undergo differentiation.[129] Furthermore, ASC immunomodulatory potential is increased in infants compared to elderly, as they better suppress T-cell proliferation, down-regulate the secretion of interferon-γ, and increase the percentage of T-regulatory cells.[122] Another study shows that aged ASCs failed to induce $CD3^+CD4^+$ T-cell suppression compared to young ASCs.[130] Age also impairs angiogenic capacities of ASCs,[18,131] as it decreases the ability of ASCs to differentiate toward endothelial cells and secretion of proangiogenic factors.[131] Surprisingly, the literature is scarce concerning the effect of aging on ASC secretome. Aging reduces VEGF and b-FGF mRNA expression from white AT and isolated cells[18,123] and protein expression of TGF-β1 and fibronectin.[132] Angiogenic factors (VEGF, PlGF, HGF, angiopoetin-1, and angiogenin) protein and mRNA expression from ASC-CM decrease with patient age, whereas no changes were observed in the levels of antiangiogenic factors thrombospondin-1 and endostatin.[114,125] Although those studies have been performed on in vitro conditions, future research should explore their role in vivo.

## FAT GRAFT RETENTION

For more than a century, surgeons had used AT as a filling product. In 1893, Neuber was the first to use fat to correct facial scar.[133] In the 1980s, multiple surgeons described the use of fat grafts in the cosmetic field. Despite promising therapeutic applications of fat grafting, the long-term results are often disappointing because of variable and unpredictable partial absorption.[134–136] Several studies have reported resorption rates of 20% to 70% within 1 year, especially for large-volume fat grafting.[136–140] In the mid-1990s, Coleman introduced a new technique to decrease traumatic handling of fat during liposuction.[141,142] Even if his technique remains the standard for fat grafting, the numerous optimizations of each step of the procedure[143,144] (eg, harvesting, processing, and injection) make comparisons between studies very difficult. However, many studies focus on survival rate of graft volume injected without taking into account the recipient-site volume. Khouri and Khouri suggested replacing percentage graft retention by more clinically relevant percentage augmentation: final volume augmented/initial recipient-site volume.[145,146]

### Fat Graft Survival Theories

In 1923, Neuhof and Hirschfeld proposed the *host replacement theory*.[147] In this theory, grafted fat cells die after transplantation and are partly replaced by infiltration either by host cells, which become fat cells, or by fibrous tissue.

In 1950, Peer contradicted this theory and proposed the *cell survival theory*,[148,149] defined as follows: "Living human autogenous grafts tend to retain their specific structure, following free transplantation in unlike tissue, when the cells survive as living entities. When the cells fail to survive, the graft is replaced by fibrous tissue or mixed connective-tissue derivatives." In his studies, Peer also demonstrated survival of the graft vascular system and anastomosis between host blood vessels and the vascular system of the graft, previously excluded.

Both graft survival and host replacement theories can explain partly fat graft survival process. In 2012, Eto et al. challenged the cell survival theory and found that adipocytes die easily under ischemic conditions, whereas ASCs or progenitor

cells could survive and were activated and contributed to AT repair later.[150] The authors have proposed the *graft replacement theory*, which defined the injected AT particle into three main zones. The most superficial zone is called the "surviving zone," where both adipocytes and ASCs survive; the "regenerating zone," where adipocytes die but ASCs survive and provide new adipocytes to replace the dead ones; and the "necrotic zone," where adipocytes and ASCs die.

### Parcel Size and Injected Volume

After Eto et al. highlighted the impact of fat microdroplets graft size,[150] some teams described other essential parameters to improve fat graft survival (eg, oxygen diffusion). Khouri et al., have nicely modeled parameters involved in fat graft percentage augmentation.[151] The study predicts that fat particles thinner than 0.16 cm in radius do not have a region of central necrosis, because oxygen supply is sufficient for all cells included in the particle. Otherwise, several surgeons have suggested that injecting too much fat into a small recipient site can increase interstitial fluid pressure (IFP) enough to constrict capillaries, inducing ischemia in the grafted tissues.[152–154] The model described by Khouri et al. predicts that a given tissue compartment can accommodate approximately 60% of its weight in interstitial fluid before reaching a critical IFP (9 mmHg), beyond which any additional fluid causes a drastic IFP increase and capillary perfusion decrease.[151] The injection step is crucial, as fat grafts have to be distributed in small droplets at varying depths in the soft tissue to allow oxygen supply and avoid excessive IFP at the recipient site.

### ASCs and Vascularization

As seen above, fat graft survival depends on surgical techniques but also on the AT biology. Philips et al. demonstrated that there is a strong correlation between SVF percentage of $CD34^+$ progenitors and human graft retention in mice.[135] These $CD34^+$ progenitors could be ASCs, and their concentration within the SVF may be one of the factors used to predict human fat graft percentage augmentation. Other studies have also demonstrated that ASC-enriched grafts improved fat graft survival through angiogenesis stimulation,[103,109,155–158] and that fat graft enriched in proangiogenic factors improved the graft viability by means of increased vascularization.[159,160] As high-density fat contained more vasculogenic progenitor cells and vascularity cytokines, this fat induces a better fat graft survival compared to low-density fat.[161] These studies show that, through their proangiogenic capacities, ASCs could improve fat graft percentage augmentation.

### Match between Harvest and Recipient Site

Although the literature suggests that AT is of mesoderm origin, one study demonstrates that adipocytes around the salivary gland come from neural crest of neuroectoderm.[162] It has recently been reported that the individual fat depots exhibit distinct embryonic origins and express different HOX codes.[163–165] Kouidhi et al. have shown the existence of an opposite gradient from the upper to the lower body between expressions of HOXC10 and the neural crest marker PAX3, which highlights diverse embryonic origins.[166,167] Those data are completed by another study that show a different HOX code between abdominal and facial preadipocytes.[168] Another study demonstrates different mouse AT embryonic origins according to the fat depot.[169] Kouidhi et al. have highlighted the match between embryonic origin from AT donor and receptor sites as a critical parameter for clinical outcomes,[166,167] as a mismatch of embryonic origins between harvested and recipient AT could lead to an impairment of grafted ASCs for tissue regeneration.[170] Furthermore, some authors show that facial preadipocytes have a better adipogenic potential compared to abdominal ones.[171] However, Kouidhi et al. have shown that ASCs extracted from either chin or knee have the same triglyceride concentration and lipolytic activity but that chin ASCs have the potential to differentiate into brown-like adipocytes, whereas knee ASCs can only differentiate into white adipocytes.[166]

Considered together, those data highlight the difference in regenerative potential according to the harvest site. In contrast, studies on donor-site influence on graft survival remain conflicting.[172] However, those studies have investigated differences between knee, thigh, abdomen, or breast, but did not take into account facial AT.

### Other Factors

Some authors mentioned that donor age could decrease fat graft survival in mice, according to the recipient site.[173,174] Interestingly, another study concludes that according to fat process, age has a negative or no effect on volume retention.[136] Donor sex could matter in fat

graft survival, as fat graft volume retention was higher and reaches a stable state earlier in men than in women.[175] Otherwise, even if low estrogen level induced favorable inflammation status and adipocyte hypertrophy (which improve fat graft retention), a continuing decreased estrogen level led to fat graft fibrosis.[176] Inflammatory status, including macrophage and cytokine release, could also play a key role in graft percentage augmentation, even if this phenomenon is yet to be described.[177] Phipps and colleagues have demonstrated that fat graft supplementation with M2 macrophages improve autologous fat graft volume retention with a higher vascular density, suggesting that M2 macrophages improve fat graft survival by promoting angiogenesis.[178] Intriguingly, the prevalence of M2 macrophages has been correlated with a higher BMI and prevalence of M1 macrophages has been correlated with a lower BMI.[179] The authors suggest that inflammatory response of lower BMI patients could inhibit angiogenesis and decrease blood flow of the graft, leading to a lower graft survival.

Fat graft survival is multiparametric and could rely on ASC number and potential, vascularization potential, age, BMI, sex, or embryonic origin. Several studies mentioned also that all processes of fat grafting, including the harvesting site or cannula, processing step, or surgeon gesture for injection, could influence graft survival.[143,172,180]

## CONCLUSIONS

Mesenchymal cells from hypodermis, ASCs, are key players in regeneration of surrounding tissues such as dermis or AT itself. Their regenerative potential is expressed through their multipotency or paracrine effects.[3,4] ASCs have numerous beneficial effects on ECM remodeling, immunity, and vascularization, summarized in Figure 2. As lipofilling is used for soft-tissue reconstruction, the oncologic safety of fat grafting is a hot topic. One study shows an increase of breast cancer cells when co-cultured with ASCs.[181] However, another study analyzed the effect of ASCs and lipoaspirate on proliferation of human breast cancer cell lines and revealed there is no proliferation increase

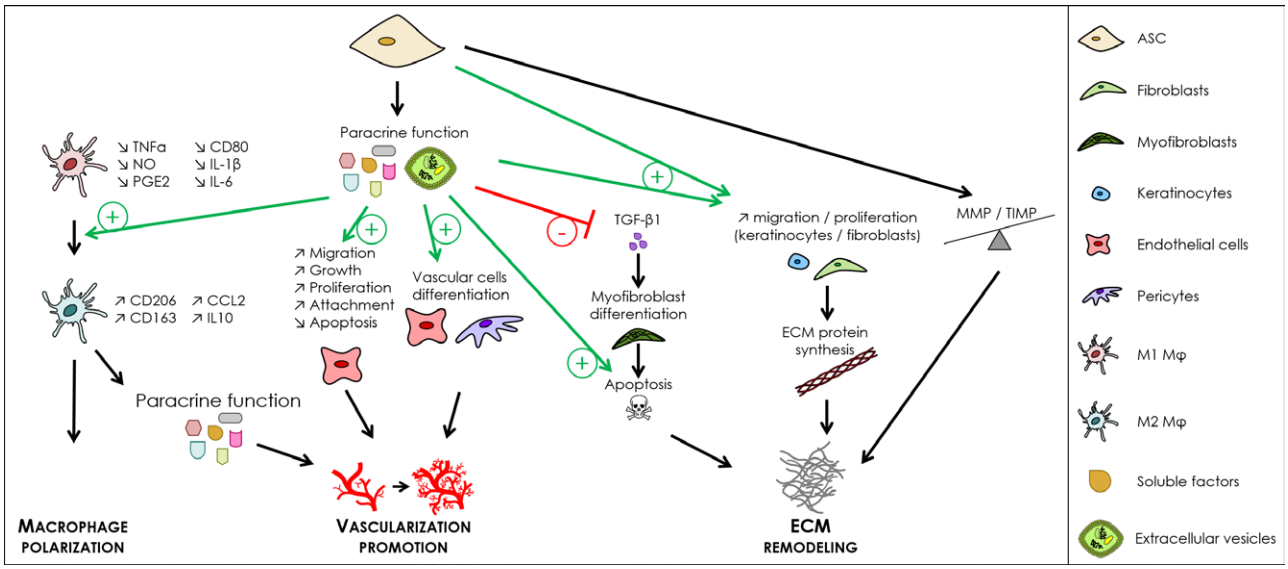


**Fig. 2.** Synthetic scheme of ASC mode of action on macrophage polarization, vascularization promotion, and ECM remodeling. Because of cell-to-cell contact and paracrine function, they are able to modulate macrophage switch, vascularization, and ECM remodeling. Co-culture of ASCs with macrophages increases alternative M2 macrophage marker expression and decreases proinflammatory M1 macrophage markers. ASC extracellular vesicles could also mediate this macrophage switch. Those macrophages are involved in vascularization through paracrine factors secreted. ASCs could differentiate into endothelial cells (*EC*) and bear pericyte hallmarks. They promote vascular network when co-cultured with endothelial cells and increase endothelial cell growth, proliferation, and migration; and decrease endothelial cell apoptosis by means of direct contact through soluble factors and extracellular vesicles. ASCs promote fibroblast and keratinocyte proliferation and migration, enhance ECM protein secretion, and induce a better collagen organization. They can also modulate MMP/tissue inhibitors of MMP (*TIMP*) balance and are able to inhibit profibrotic factors such as TGF-β1, inhibit fibroblast-to-myofibroblast differentiation, and induce myofibroblasts apoptosis. *NO*, nitric oxide; $PGE_2$, prostaglandin $E_2$; *EPC*, endothelial progenitor cells.

of the cells.[182] The authors even observed that lipoaspirate and ASCs inhibit the proliferation of breast cancer cells. Furthermore, a cohort study examining 300 affected breasts reconstructed with fat grafting (and 300 matched control patients) shows no significant differences in the locoregional recurrence rates between groups after 5-year follow-up, suggesting that there is no evidence that fat grafting is associated with increased rates for cancer relapse in patients with breast cancer.[183]

Aging is a phenomenon that impairs all tissues and is characterized by proliferative and differentiation capacities decrease of cell types. ASC regenerative potential has been demonstrated in many fields, including cutaneous aging.[46,47] Beneficial ASC effects on fibroblasts and adipocytes and their ECM could be impaired with age. However, despite these promising results with ASCs, several questions remain. The impact of age on ASC yield, proliferative capacity, or multipotent potential still needs to be elucidated,[123,126] as does the effect of aging on ASC paracrine function. Indeed, there is no study to date concerning aging impact on complete secretome quality and quantity. Some authors mentioned that age could act negatively on graft survival. However, other factors listed in Figure 3 need to be taken in account, such as embryonic origin or HOX code match between harvested and recipient site, particle size, or inflammatory state of AT. Vascularization speed of AT after injection is also a therapeutic path encountered. ASCs strongly interact with surrounding cells, especially with vascular cells such as endothelial cells or pericytes, and immune cells such as macrophages. Cell-to-cell communication is mediated by soluble factors. We still do not know how donor age influences ASC capacity to polarize macrophages. Moreover, the link between ASCs, macrophage polarization, and vascularization has not been well described yet. Further research should be undertaken to investigate the aging effect on fat depots from different anatomical sites, regarding regenerative potential and paracrine function. This work could lead to determination of factors

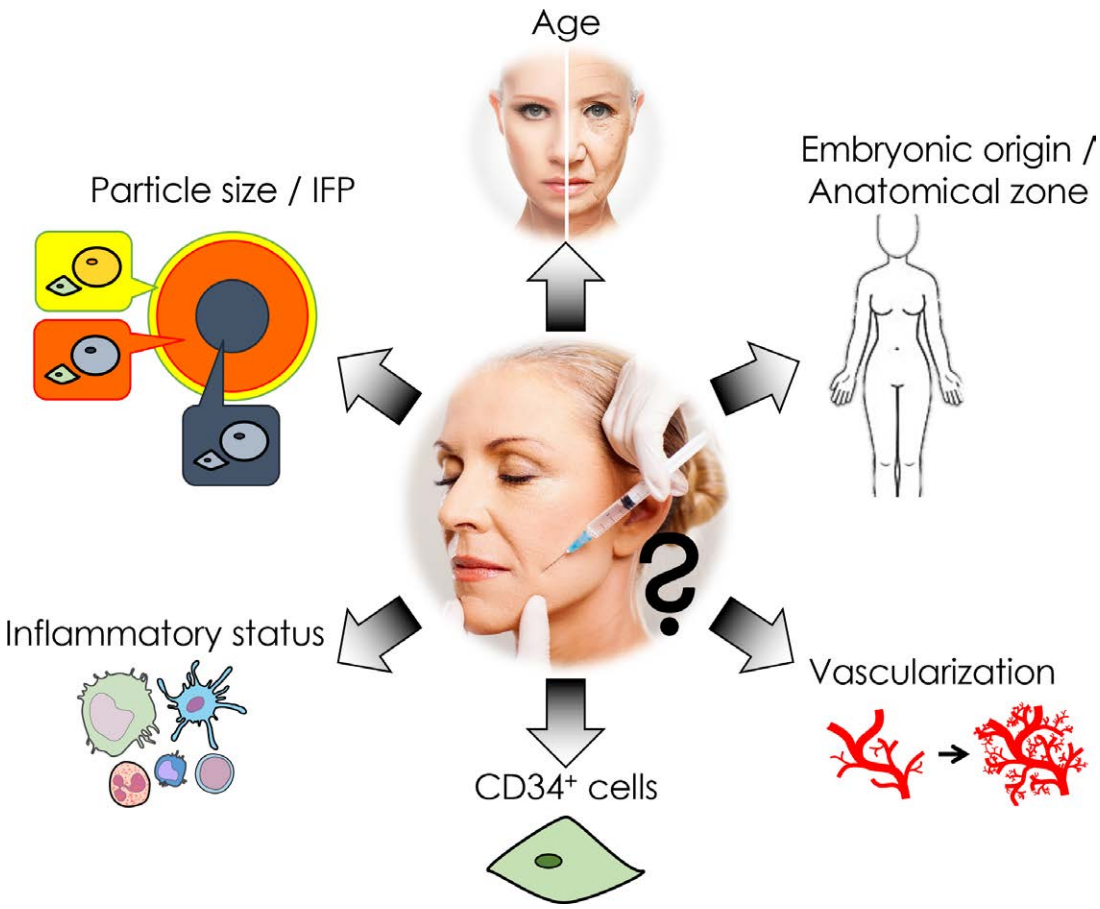


**Fig. 3.** Factors involved in fat graft percentage augmentation.

involved in graft percentage augmentation and open new therapeutic ways for fat grafting.

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