OPEN

PRS GLOBAL OPEN

SPECIAL TOPIC

How Fat Grafting Works

Brogan G. A. Evans, MD*
Edward M. Gronet, MD†
Michel H. Saint-Cyr, MD†

Background: Fat grafting has been shown to improve diseased soft issue. Although the mechanism behind fat grafting's regenerative properties is currently debated, published studies agree that there is an associated vasculogenic effect. A systematic literature review was conducted to elucidate the biochemical pathways responsible for establishing neo-vasculature to grafted fat.

Methods: A systematic literature review was conducted by searching PubMed for current basic science and clinical research relating to fat grafting. In total, 144 of 269 (54%) articles met the inclusion criteria for our literature review. These 144 articles were summarized, with 86 of them (60%) used to construct this article at the authors' discretion.

Results: Fat grafting–induced neovascularization can be divided into 3 parts. First, tissue trauma induced via fat injection activates a host inflammatory response necessary for cellular recruitment. Recruited cells promote the formation of connective tissue and neo-vasculature at the graft site. Second, cellular elements within the lipoaspirate contribute to neovascularization through a cytokine burst. Third, a synergistic relationship is established between recruited inflammatory cells and the cytokine burst of grafted fat. The end product of these processes is the differentiation of progenitor cells and the creation of neo-vasculature at the graft site.

Conclusions: Establishing neovasculature is paramount for the survival of grafted fat. Fat graft take can be divided into 2 steps: imbibition and neovascularization. We believe this process occurs through 3 distinct concepts: host inflammation via graft injection, hypoxic response of lipoaspirate-derived cellular elements, and a synergistic relationship between host inflammation and grafted fat. *(Plast Reconstr Surg Glob Open 2020;8:e2705; doi: 10.1097/GOX.0000000000002705; Published online 14 July 2020.)*

INTRODUCTION

Chronic wounds, burn scars, and scleroderma all have one thing in common: poorly vascularized tissue. Fat grafting has been shown to accelerate wound healing in hostile environments in both animal models[1] and human subjects.[2–7] It is believed that fat grafting helps initiate a biochemical process responsible for soft-tissue repair,[8–15] and the current consensus agrees that fat grafting induces a vasculogenic effect.[6,14–35]

Once grafted, the adipocytes must establish a blood supply.[36,37] Until neovascularization can occur, the grafted tissue is limited to oxygenation via diffusion from surrounding vessels.[38] Current literature theorizes that cells on the periphery of the graft survive via adequate plasma-based oxygenation, whereas cells at the center of the graft undergo necrosis. An intermediate zone exists between the periphery and center zones, where graft precursor cells replace necrotic cells.[13,39,40] In order for this host-replacement zone to regenerate, a new blood supply must be established through neovascularization.[6,13,39,40] Via a paracrine effect exerted by the graft, and differentiation of host and graft progenitor cells, a molecular cascade is induced that promotes graft survival.[11,16,21,41–44]

Establishing neovascularization is an essential step in graft survival and improved clinical outcomes. This occurs via 2 distinct processes: vasculogenesis and angiogenesis.[45] Vasculogenesis is defined as the differentiation of mesodermal precursor cells into endothelial cells (ECs), and angiogenesis is defined as the proliferation of matured ECs from existing vasculature.[45] Through these processes, ECs adhere to one another, forming tubules that provide the architecture for neovascularization.[28,45] Insults such as hypoxia or inflammation encourage the sprouting of new vessels from existing vasculature.[8,23,24,28,33,41] Once

*From the *Texas A&M Health Science Center, College of Medicine, Bryan, Tex.; and †Division of Plastic Surgery, Baylor Scott & White Health, Temple, Tex.*

Received for publication June 3, 2019; accepted January 24, 2020.

DOI: 10.1097/GOX.0000000000002705

Disclosure: *The authors have no financial interest to declare in relation to the content of this article.*

neovascularization occurs, these new vessels deliver supplemental oxygen to heal damaged tissue.

Of clinical importance is that fat is grafted in a way that maximizes surface area contact with the recipient site.[46,47] Current technique recommends that fat be grafted in thin aliquots no >3 mm in diameter.[48] This is intended to maximize neovascularization because the center of aliquots >3 mm are outside the host-regenerative zone (1.5–2.0 mm from graft periphery), and become necrotic before neovascularization can occur.[36,37,39,48,49]

Because fat grafting continues to prove its usefulness in the treatment of soft-tissue pathology, filling of deflated areas, and wound healing, there is a need to identify the molecular pathways involved. We classify this process into 3 essential parts: induced inflammation from fat graft injection, the hypoxic response from harvesting lipoaspirate, and a synergistic interaction between inflammation and grafted fat. The purpose of this review is to better understand fat grafting at the cellular and molecular level and to present our believed mechanism of how fat grafting works.

METHODS

Our review searched PubMed for current clinical and basic science literature relating to fat grafting. Our search keywords included: "Fat Grafting," "Adipose Tissue Graft," "Lipoaspirate," "Fat Graft Wound Healing," "Adipose-Derived Stem Cells," "Adipose-Tissue Derived Stem Cells," and "Stromal Vascular Fraction." Queries for articles and abstracts that had titles containing one of these keywords yielded 6780 results. Our results were filtered further by selecting articles and abstracts that used the words "Plastic Surgery" 2087. Abstracts were screened for relevance at the discretion of the authors, and 246 articles were left for a full-text review. To this, we added 23 additional articles that commented on the molecular mechanisms of inflammation and neovascularization. These articles helped fill in gaps and clarify pathways found in our fat grafting literature review.

In total, 144 of 269 (54%) articles met the inclusion criteria for our literature review. These 144 articles were summarized, 86 of them (60%) were congruent with our overall thesis and were thus used to construct this article. These 86 articles chosen involved the following keyword: vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), erythropoietin, angiopoietin 1, insulin-like growth factor-1 (IGF-1), peroxisome proliferator-activated receptor-γ, hypoxia-inducing factor-1α (HIF-1α), transforming growth factor-β (TGF-β), macrophages, adipocytes, adipose-derived stem cells (ASCs), ECs, platelets, pericytes, bone marrow–derived mesenchymal stem cells (BM-MSCs), extracellular matrix (ECM), graft success and wound healing, scar alleviation and softness, immunosuppression, pain, and blood flow.

Articles were organized by theme, with published data extrapolated for comparison. Similarities were noted between publications, which helped establish our clinical and basic science outlook on fat grafting. In total, 86 of 144 (60%) of these articles were used in this review to outline our proposed molecular mechanism of fat grafting–induced neovascularization (Fig. 1). The authors' discretion was used to determine if an article met inclusion criteria. This systematic review uses currently published literature to support the authors' discussion on how fat grafting works.

RESULTS

Inflammatory Role in Neovascularization

Tissue trauma induces an inflammatory response to occur, which initiates an inflammatory cascade necessary for the formation of healthy granulation tissue (Fig. 2).[1,12] Tissue trauma in fat grafting is a result of surgical preparation of the recipient bed through scar release and the passage of injection cannulas.

With this in mind, tissue trauma induces a host inflammatory response, which activates the essential machinery to promote neovascularization. The trauma induced at the injection site recruits a variety of bone marrow–derived cells,[50] with platelets being the first to arrive.[12] Although newly migrated platelets help control bleeding through primary hemostasis, they also provide structural and biochemical support for local tissue. Platelets induce remodeling of local ECM through fibrin deposition, which enhances local ECM to support neovascularization.[51] This process is closely regulated by macrophages, which are recruited to the recipient site as monocytes within hours of tissue trauma.[51,52] Once present, macrophages contribute to tissue stability by increasing ECM remodeling and enhancing cell-to-cell interaction through collagen secretion.[53] Local platelets and ECs at the recipient site release PDGF,[8,24] which recruits bone marrow–derived pericytes to the recipient site.[27,28] The newly remodeled ECM binds PDGF with high affinity[54] and helps stabilize pericytes on newly formed vessels.[28,31]

BM-MSCs are next to arrive to the site of inflammation.[4,8,15,19,55–57] The BM-MSCs secrete VEGF, angiopoietin-1, and erythropoietin, which serve as powerful chemoattractants for additional monocytes.[8] Although multipotent BM-MSCs serve as a potent source for vascular and connective tissue cells in vasculogenesis,[15,28] monocytes also express a capacity to differentiate into ECs,[58,59] smooth muscle cells,[53] and adipocytes.[58]

The capacity of BM-MSCs to differentiate into ECs[60] provides necessary cellular building blocks for vasculogenesis.[45,60] Although ASCs help regenerate cells in a manner similar to that of BM-MSCs,[18,61,62] it is believed that BM-MSCs contribute to neovascularization through differentiation,[45,60] whereas ASCs contribute more through paracrine effects on local tissue.[15,21,41,42]

As the host inflammatory process continues to unfold, the adaptive immune system sends naive peripheral T helper cells ($CD4^+$ T-cells) to the graft site.[63] These T-cells help inhibit local tissue inflammation by releasing interleukin-4 (IL-4). IL-4 acts on recruited pro-inflammatory M1 macrophages, polarizing them to alternative M2 macrophages. IL-4's ability to limit the number of M1

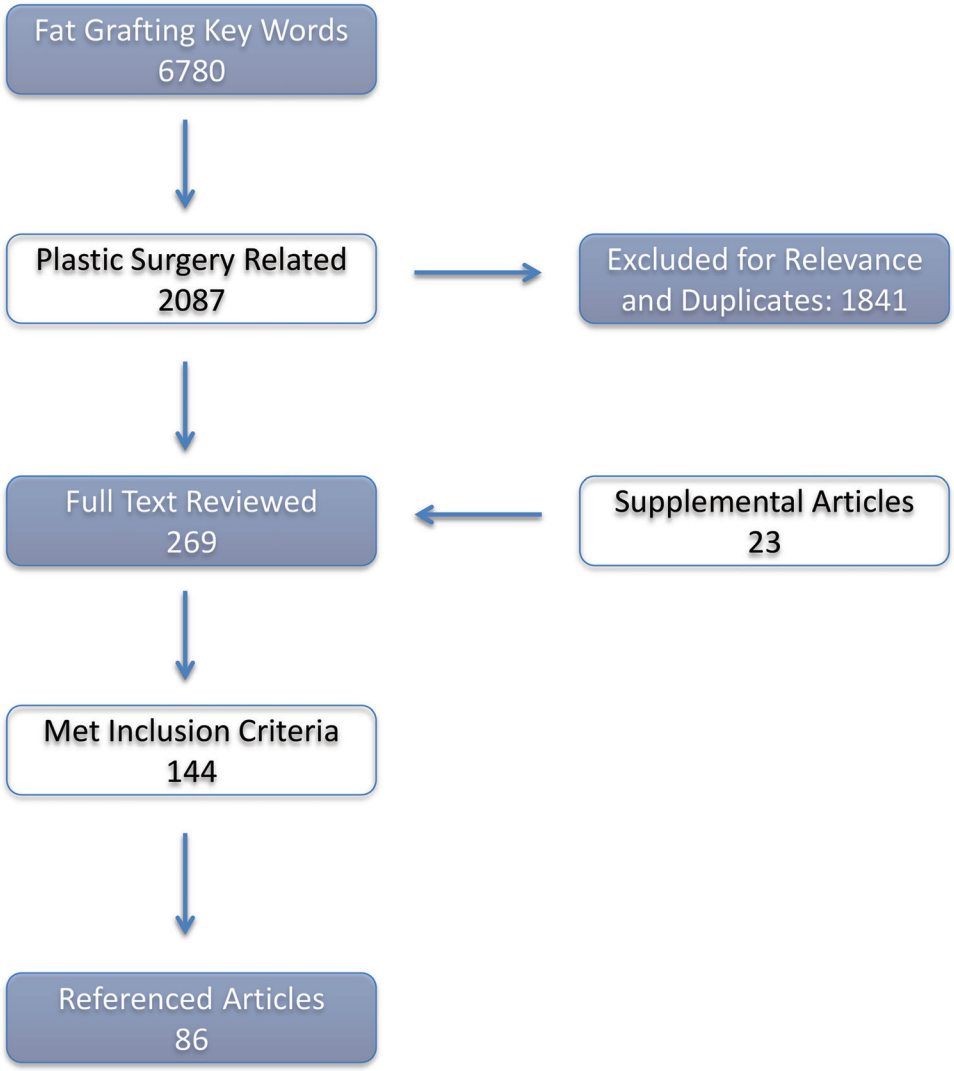

Fig. 1. A literature search was made to identify fat grafting articles relating to plastic surgery. Additional screening at the authors' discretion was used to identify the 86 articles referenced to construct this article.

macrophages at the graft site reduces M1-released cytokines such as tumor necrosis factor-α (TNF-α), a known inhibitor of adipocyte differentiation.[64] By limiting the release of TNF-α at the graft site, preadipocytes are able to differentiate into adipocytes through the activation of the peroxisome proliferator-activated receptor-γ, a transcription factor that upregulates gene expression necessary for preadipocyte differentiation.[64] The process of progenitor cell and preadipocyte differentiation into mature adipocytes largely occurs 7–14 days after grafting.[62,65]

Although TNF-α plays an inhibitory role in adipocyte differentiation, it conversely has been found to enhance ASCs' angiogenic abilities. Thus, although elevated levels of TNF-α before macrophage polarization inhibit preadipocytes differentiation, this ultimately contributes to graft neovascularization and microvascular ingrowth. In a study by Zubkova et al,[66] it was shown that TNF-α–enhanced ASCs showed increased transcription of the proangiogenic factors fibroblast growth factor 2 (FGF-2) and VEGF, as well as enhancing microvascular growth, accelerating blood flow recovery, and increasing arteriole density at the graft site. Overall, inflammation appears to play a key role in graft-induced neovascularization through its recruitment of essential cells and cytokines to the recipient site.

Lipoaspirate's Role in Neovascularization

Lipoaspirate contains multiple cell types necessary for neovascularization.[18,31,44,65,67–70] The graft contains cellular building blocks and precursor cells, both of which also produce cytokines (Fig. 3). Graft cells can be classified into 2 groups: adipocytes and the stromal vascular fraction (SVF).[44] The SVF is defined as all nonadipocyte cells. This includes numerous stromal cells, such as pericytes, fibroblasts, vascular ECs, leukocytes, and importantly, preadipocytes and ASCs.[19,26,31,55,71] The SVF has been shown to contain 28.1% ± 2.4% blood-derived cells, 28.9% ± 2.0% ASCs, 12.7% ± 2.9% ECs, and 10.7% ± 2.1% miscellaneous cells, such as fibroblasts and mural cells.[19] Adipose tissue

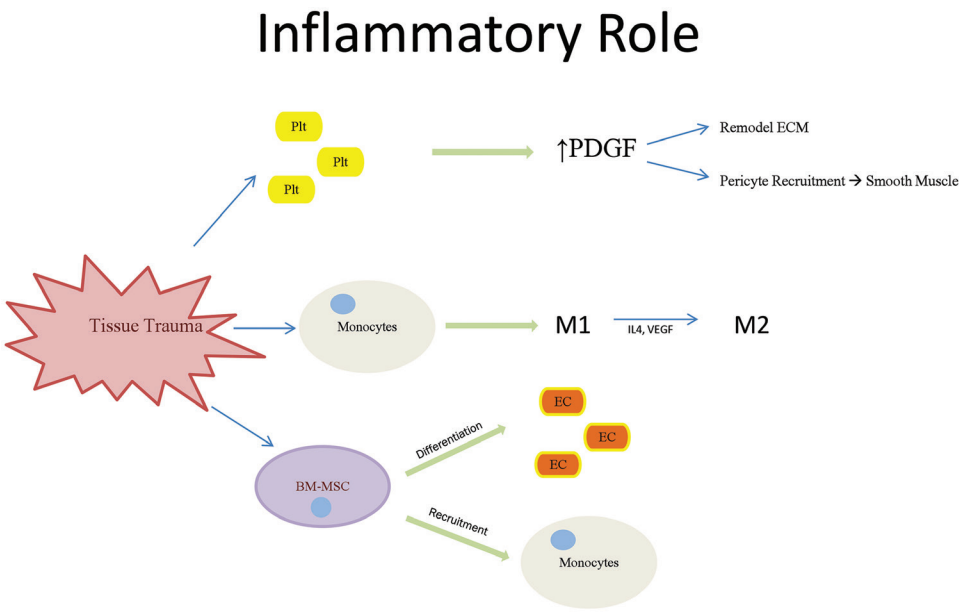

Fig. 2. Tissue trauma induces platelet, monocyte, and BM-MSC migration to the graft site. After hemostasis, platelets (Plt) help remodel the ECM and recruit pericytes to growing vessels. Recruited BM-MSCs differentiate into endothelial cells and recruit monocytes. Monocytes are polarized from M1 to M2 macrophages. Each process facilitates neovascularization.

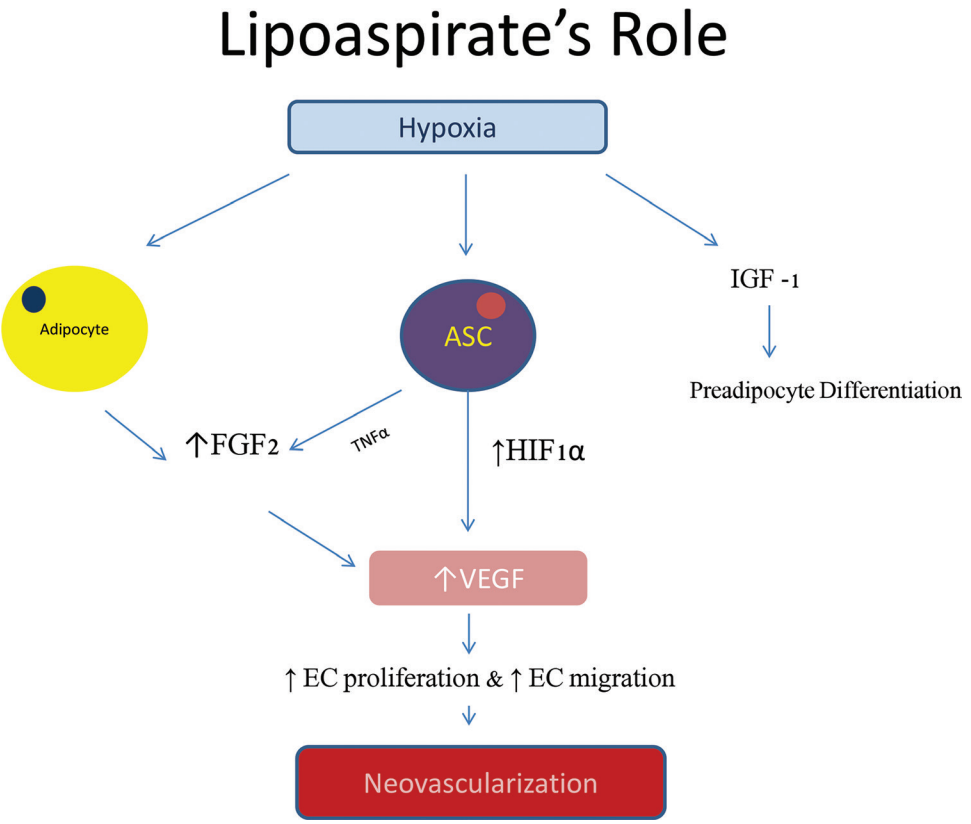

Fig. 3. Harvest forces hypoxia on grafted adipocytes and ASCs. As hypoxic adipocytes lyse, they release FGF-2 to the recipient site. ASCs secrete TNF-α and HIF-1α when hypoxic. TNF-α acts on ASCs and adipocytes to increase FGF-2 levels at the graft site. HIF-1α and FGF-2 upregulate VEGF levels, which promote EC proliferation and EC migration. IGF-1 is released to promote preadipocyte maturation (regenerative).

is an easily accessible source for multipotent stem cells[69]—possibly the highest percentage of adult stem cells within the human body.[72]

Preadipocytes and multipotent ASCs, found within the lipoaspirate, are able to promote graft success by replacing dying cells within the zone of regeneration via differentiation[27,68,73,74] and by promoting recipient site neovascularization.[16,74] Though preadipocytes do not retain multipotency like ASCs, they are found abundantly in adipose tissue (350,000 preadipocytes per 1 mL of adipose tissue) and retain the capacity to differentiate into mature adipocytes.[72]

Cell harvest creates acute ischemia. The most sensitive part of the lipoaspirate to acute ischemia is the mature adipocytes—a majority of which do not survive grafting.[13,19,39] Although adipocytes are sensitive to hypoxia, ASCs found within the SVF are more tolerant.[31,33,41] A laboratory study of fat cells by Suga et al[31] used flow cytometry to show that although the majority of adipocytes die within 24 hours of hypoxic exposure, nearly all ASCs remain viable after 72 hours. The less tolerant adipocytes lyse in hypoxic conditions and release large quantitates of FGF-2, whereas the more tolerant ASCs remain intact and upregulate their transcription of the cytokine HIF-1α.[28,31,33,41] Additional FGF-2 is released from ASCs secondary to stimulation by TNF-α.[66] Together, both FGF-2 and HIF-1α act on ECs and ASCs to increase transcription of VEGF at the graft site.[31,75]

Local paracrine signaling by ASCs serves a powerful role in fat grafting–induced vasculogenesis.[15,21,41,42] Elevated concentration of VEGF at the graft site increases vascular EC proliferation, inhibits EC apoptosis,[41] and mediates the mitosis and migration of vascular ECs into the graft.[23–25,76] Increased quantities of ECs present in the graft (and recruited from circulation) provide the necessary building blocks for neovascularization to occur. Vascular ingrowth can be visualized as early as 3 days after grafting,[41] with mesh-like dense capillary networks appreciable around 4 weeks.[21] Once vascular ingrowth begins, the host is able to directly oxygenate the grafted tissue and send host-derived ASC and BM-MSC into the graft to enhance tissue viability.[23,33,75] This migration of host progenitor cells into the graft occurs 4–14 days after grafting.[77] Additionally, ASCs within the graft retain their multipotency and contribute to graft survival through their differentiation into adipocytes, fibroblasts, pericytes, ECs, and even rudimentary nervous tissue.[15,16,26]

The cellular elements of lipoaspirate also contain a number of growth factors that contribute to its vasculogenic properties. Both bFGF and IGF-1 are found in large quantities in lipoaspirate,[25,31] with IGF-1 being adipose tissue's most abundant protein.[78] IGF-1 prolongs graft survival and promotes the differentiation of preadipocytes into mature adipocytes.[79,80] Ultimately, IGF-1's mitogenic properties increase graft exposure to essential proangiogenic growth factors, such as FGF-2, and VEGF through its ability to replace dying adipocytes.[75,79] bFGF contributes to neovascularization by recruiting additional ECs to the site of the graft and increasing local EC proliferation for ready use in growing vascular networks.[9,24,50,75,81] Pericytes within the graft contribute to its success by wrapping around the graft's newly developed vascular EC networks and providing additional structural support by differentiating into smooth muscle cells.[15,25,41,50,82] In addition to their ability to stabilize new blood vessels, it has also been shown that pericytes retain a progenitor capacity.[83,84] Thus, pericytes may further contribute to graft success by differentiating into adipocytes[83,84] and enhancing paracrine effects at the recipient site.

Neovascularization: A Product of Inflammation and Grafted Fat

Graft exposure to acute ischemia is an expected result of fat harvest.[19,23,43] Adipocytes and ASCs within the lipoaspirate respond to their newly hypoxic environment by releasing FGF-2, HIF-1α, and IGF-1 (Fig. 4). These cytokines promote neovascularization by establishing high levels of VEGF at the graft site.[23–26,57] VEGF promotes proliferation of local ECs[25,29,42,76,85] and increases the expression of key surface integrins necessary for cell migration.[27,29,76,85] Newly upregulated EC surface integrins help guide tip cell filopodial extensions from sprouting vessels through the remodeled ECM and into the graft.[22] The proangiogenic cytokine VEGF plays a critical role in this and has been shown to upregulate EC surface integrins 2- to 7-fold.[76]

Following initial inflammation and platelet migration, the host inflammatory response recruits bone marrow–derived monocytes and BM-MSCs to the site of tissue trauma.[8,15,20,86] These BM-MSCs act in combination with ASCs to recruit additional monocytes and ECs for increased neovascularization.[8,56,57] Together, the progenitor capacity of BM-MSCs,[60] ASCs,[18,61,62] and monocytes[58] provides an additional source of ECs and adipocytes at the graft site. Local paracrine effects enhance cellular differentiation and contribute to graft success by providing cellular elements for angiogenesis and vasculogenesis.

The high quantities of VEGF found at the grafting site[23–26,57] promote monocyte differentiation into M2 macrophages.[14,19,56] In addition to their anti-inflammatory properties,[14,50] newly matured M2 macrophages express a high affinity for tip cell filopodia and act in compliment with EC's surface integrins to enhance new vessel migration.[20]

The process of M2 macrophage–facilitated migration is explained in 2 parts. First, M2 macrophages act directly on endothelial tip cells—forming a physical linkage that allows them to propel new vessels through newly remodeled ECM.[20] Second, M2 macrophages work indirectly by upregulating EC expression of the surface ligand osteopontin (OPN).[76] OPN expresses a high affinity for EC surface integrins, which helps drive vessel migration and endothelial tip cell linkage to form vascular networks. VEGF helps facilitate this process further by increasing vascular permeability and allowing thrombin to cleave OPN ligands. This cleavage significantly increases OPN's affinity for neighboring EC surface integrins, which ultimately helps tip cell linkage.[76] This process acts in synergy with M2 macrophages to promote new vessel migration through the ECM. By experimentally inhibiting these integrins, new vasculature cross-sectional area has been shown to decrease

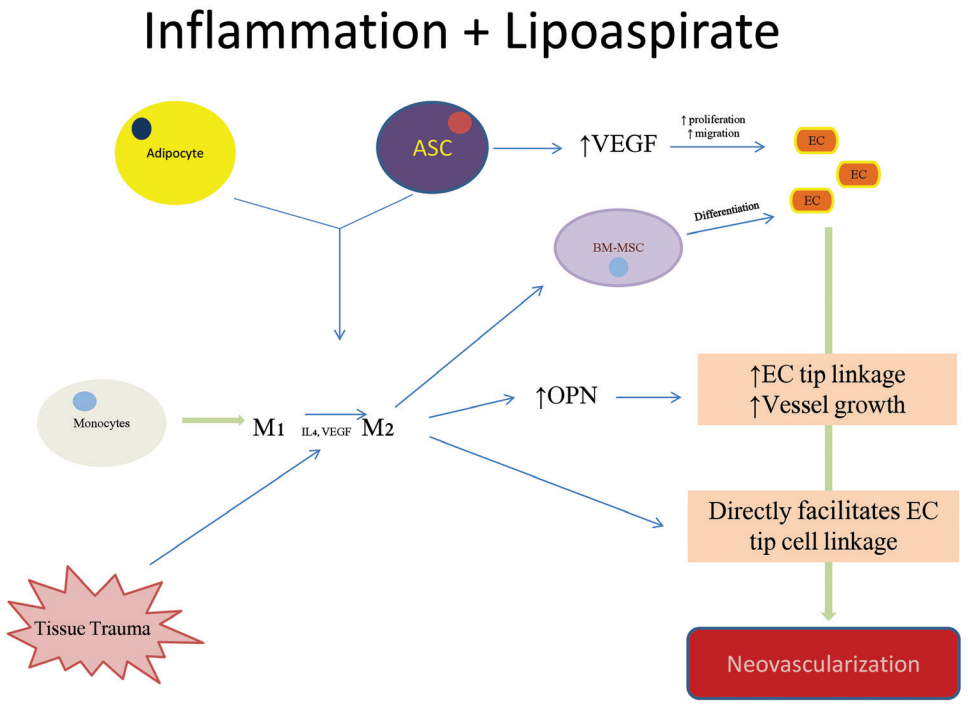

Fig. 4. Cellular elements within the lipoaspirate act in synergy with the tissue's response to injection to drive neovascularization at the graft site.

by 90%, whereas the total new vasculature area decreases by 82%.[29] Thus, fat grafting's ability to upregulate VEGF production and consequently integrin surface expression plays a key part in angiogenesis and vasculogenesis.

M2 macrophages are also a powerful recruiter for BM-MSCs.[14,50] After grafting, there is a gradual polarization of M1 macrophages to M2 macrophages at the graft site, coinciding with an increase in TGF-β. These M2 macrophages increase BM-MSC recruitment by producing C-X-C chemokine receptor type 4 (CXCR4), a protein that attracts hematopoietic stem cells. Further, they stimulate vessel growth in a VEGF-dependent manner and increase adipogenesis from the host.[50] This further supports that M2 macrophages not only play a critical role in the recruitment of BM-MSCs, but also that BM-MSCs serve as a potent source for cellular differentiation. BM-MSCs differentiation into vascular ECs contributes to graft success by enhancing vasculogenesis.[60] Additionally, BM-MSCs capacity to differentiate into adipocytes further augments paracrine signaling, but increasing proangiogenic growth factors and cytokines necessary for neovascularization. The differentiation of progenitor cells into adipocytes readily occurs throughout the first few weeks of grafting, with the majority of adipocyte differentiation complete 28 days after grafting.[21]

In the final steps of neovascularization, pericytes found within the graft, and those recruited by platelets and ECs, differentiate into smooth muscle cells to become part of the vessel walls.[25,28,31] This process is in parallel to ASCs and macrophages at the graft site, which release TGF-β1, which induces the differentiation of pericytes into smooth muscle cells[15,25,41,50,82] and stabilizes the vessel walls.[25,28]

DISCUSSION

The delivery or maintenance of well-vascularized tissue is a foundation of plastic surgery. Such tissue is either meticulously maintained or artfully transferred into a damaged area. Fat grafting is unique in that a nonvascularized graft appears to carry the ability to create a new vasculature where it is placed. This review is intended to explain how the body's response to surgical trauma initiates a healing cascade that complements the grafted cells' desire to survive and in doing so, creates a new vascular network. We have compartmentalized these biochemical processes into 3 essential parts: surgically induced inflammation from graft injection, the cellular components of the harvested lipoaspirate and their response to hypoxia, and a synergistic reaction between inflammation (recipient site) and grafted fat (donor).

It appears that inflammation plays an essential role in fat grafting–induced neovascularization.[6] The harvest and injection of processed lipoaspirate introduces cells subjected to hypoxia, their response is a cytokine burst. This burst is complemented—perhaps even augmented—by the grafted cells inducing a complementary cellular and cytokine response from the recipient site. This event leads to a cellular response through migration and cell division that forms local granulation tissue, beginning with its vasculature. The inflammation from injection mobilizes BM-MSCs and monocytes, which delivers precursor cells, helps remodel the ECM, and nurtures connections between growing blood vessels. The end result is increased levels of VEGF, as well as cellular building blocks that differentiate and directly foster neovascularization.

It is during the imbibition phase that grafted lipoaspirate is most hypoxic, explaining the necrosis of cells out of diffusion distance from recipient plasma. Graft hypoxia leads to a cytokine response as well as a loss of graft cells, especially mature adipocytes.[13,19,39] Once rudimentary vascular ingrowth occurs, host ASCs and BM-MSCs are able to migrate into the graft site and augment tissue viability through local paracrine signaling and cellular differentiation.

The cytokine burst increases VEGF concentrations at the graft site. The increased transcription of VEGF from ASCs and ECs and indirectly through adipocytes and macrophages allows for enhanced proliferation of EC, the recruitment of additional ECs to the graft site, as well as the upregulation of EC surface integrins necessary for migration and cell linkage. M2 macrophages directly facilitate EC linkage while signaling recruitment of BM-MSCs to the graft site to provide an additional source of vascular and connective tissue cells for vasculogenesis. Further, TGF-β released by ASCs induces recruited pericytes to become vascular smooth muscles cells, thus stabilizing the newly formed vascular networks. These new networks will increase blood flow and tissue oxygenation, regenerating, or at least revitalizing their recipient site.

Skin grafting is thought of in 3 distinct phases: imbibition, inosculation, and neovascularization. Unlike this well-described process, we postulate that fat grafting has only 2 phases for survival: imbibition and neovascularization. Imbibition is a plasmic phase reliant upon diffusion. Inosculation is the lining up of existing graft and recipient vessels. And neovascularization being creating and connecting these vessels. The initial survival of grafted fat is similar to skin grafts in the sense that it is based on access to plasmic imbibition. However, unlike skin grafting, there is not an inosculation phase in which capillary networks must align between graft and recipient—in fat grafting, these must all be created anew. Fat grafting must induce neovascularization to sustain sprouts from and connections to existing vascular networks.

We believe that graft-induced neovascularization and increased vascular density at the graft site is a key to fat grafting's regenerative ability. The idea of skin softness improving after fat grafting by Klinger et al[2–4] is, at its most basic level, the result of replacing an absent subcutaneous layer. The release of this adhesion creates a space, which the grafted fat fills, restoring a more normal tissue architecture. Grafting to such an area not only introduces soft tissue, but also cellular elements necessary to support vascular growth, increase tissue perfusion, and long-term repair, or as the authors measured it, softness.

However, our review is not without limitations. Articles used in this study were selected through author's discretion. This is largely due to the many different study designs used in this review, as well as the volume of published studies relating to fat grafting. Additionally, this review does not evaluate the use of fat grafting in irradiated tissue, rather it describes the biochemistry seen under ideal laboratory conditions. Irradiated tissue expresses a different microenvironment, which may alter the molecular pathways in which we believe fat grafting works. As fat grafting research continues to emerge, this article's thesis will surely be tested and refined.

CONCLUSIONS

Fat grafting mobilizes inflammatory cells and BM-MSCs to the site of injection, which supplies necessary cytokines and building material for new vessels. The lipoaspirate itself contains key elements and cellular machinery such as adipocytes, ASCs, and ECs, which act in synergy with recruited cells to promote a vasculogenic effect.

Through this literature review, we hypothesize that grafted ischemic adipose tissue induces local tissue neovascularization, which helps rejuvenate damaged soft tissue. Overall, although more research is needed to elucidate the exact mechanism by which fat grafting heals soft-tissue defects, it appears concerted that neovascularization plays a key role in its success. As ASCs and fat grafting are becoming increasingly popular areas for research in plastic surgery, understanding the mechanism behind fat grafting offers an exciting clinical potential for cosmetic and reconstructive surgery.

Edward M. Gronet, MD
Division of Plastic Surgery
Baylor Scott & White Health
2401 S 31st St
Temple, TX 76508
E-mail: doctorgronet@gmail.com

REFERENCES

1. Kao HK, Hsu HH, Chuang WY, et al. Experimental study of fat grafting under negative pressure for wounds with exposed bone. *Br J Surg.* 2015;102:998–1005.
2. Klinger FM, Caviggioli F, Forcellini D, et al. Breast fistula repair after autologous fat graft: a case report. *Case Rep Med.* 2011;2011:547387.
3. Klinger M, Caviggioli F, Klinger FM, et al. Autologous fat graft in scar treatment. *J Craniofac Surg.* 2013;24:1610–1615.
4. Klinger M, Lisa A, Caviggioli F, et al. Autologous fat grafting improves facial nerve function. *Case Rep Surg.* 2015;2015:520746.
5. Klinger M, Marazzi M, Vigo D, et al. Fat injection for cases of severe burn outcomes: a new perspective of scar remodeling and reduction. *Aesthetic Plast Surg.* 2008;32:465–469.
6. Raposio E, Bertozzi N, Grignaffini E, et al. Adipose-derived stem cells for treatment of chronic cutaneous ulcers in patients with critical limb ischemia: a pilot study. *Eur J Plast Surg.* 2018;41:459–464.
7. Stasch T, Hoehne J, Huynh T, et al. Débridement and autologous lipotransfer for chronic ulceration of the diabetic foot and lower limb improves wound healing. *Plast Reconstr Surg.* 2015;136:1357–1366.
8. Chen L, Tredget EE, Wu PY, et al. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One.* 2008;3:e1886.
9. Koellensperger E, von Heimburg D, Markowicz M, et al. Human serum from platelet-poor plasma for the culture of primary human preadipocytes. *Stem Cells.* 2006;24:1218–1225.
10. Lauritano D, Palmieri A, Vinci R, et al. Adipose derived stem cells: basic science fundaments and clinical application. An update. *Minerva Stomatol.* 2014;63:273–281.
11. Le Blanc K. Mesenchymal stromal cells: tissue repair and immune modulation. *Cytotherapy.* 2006;8:559–561.

12. Li J, Zhang YP, Kirsner RS. Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. *Microsc Res Tech*. 2003;60:107–114.
13. Mashiko T, Yoshimura K. How does fat survive and remodel after grafting? *Clin Plast Surg*. 2015;42:181–190.
14. Phipps KD, Gebremeskel S, Gillis J, et al. Alternatively activated M2 macrophages improve autologous fat graft survival in a mouse model through induction of angiogenesis. *Plast Reconstr Surg*. 2015;135:140–149.
15. Weyand B, Vogt PM. Potential of mesenchymal stem cell applications in plastic and reconstructive surgery. *Adv Biochem Eng Biotechnol*. 2013;130:55–67.
16. Bourne DA, James IB, Wang SS, et al. The architecture of fat grafting: what lies beneath the surface. *Plast Reconstr Surg*. 2016;137:1072–1079.
17. Del Vecchio D, Rohrich RJ. A classification of clinical fat grafting: different problems, different solutions. *Plast Reconstr Surg*. 2012;130:511–522.
18. Derby BM, Dai H, Reichensperger J, et al. Adipose-derived stem cell to epithelial stem cell transdifferentiation: a mechanism to potentially improve understanding of fat grafting's impact on skin rejuvenation. *Aesthet Surg J*. 2014;34:142–153.
19. Dong Z, Peng Z, Chang Q, et al. The survival condition and immunoregulatory function of adipose stromal vascular fraction (SVF) in the early stage of nonvascularized adipose transplantation. *PLoS One*. 2013;8:e80364.
20. Fantin A, Vieira JM, Gestri G, et al. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. *Blood*. 2010;116:829–840.
21. Fukumura D, Ushiyama A, Duda DG, et al. Paracrine regulation of angiogenesis and adipocyte differentiation during in vivo adipogenesis. *Circ Res*. 2003;93:e88–e97.
22. Herbert SP, Stainier DY. Molecular control of endothelial cell behaviour during blood vessel morphogenesis. *Nat Rev Mol Cell Biol*. 2011;12:551–564.
23. Hollenbeck ST, Senghaas A, Komatsu I, et al. Tissue engraftment of hypoxic-preconditioned adipose-derived stem cells improves flap viability. *Wound Repair Regen*. 2012;20:872–878.
24. Kemaloğlu CA. Nanofat grafting under a split-thickness skin graft for problematic wound management. *Springerplus*. 2016;5:138.
25. Nakagami H, Maeda K, Morishita R, et al. Novel autologous cell therapy in ischemic limb disease through growth factor secretion by cultured adipose tissue-derived stromal cells. *Arterioscler Thromb Vasc Biol*. 2005;25:2542–2547.
26. Planat-Benard V, Silvestre JS, Cousin B, et al. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. *Circulation*. 2004;109:656–663.
27. Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol*. 1995;11:73–91.
28. Semenza GL. Angiogenesis in ischemic and neoplastic disorders. *Annu Rev Med*. 2003;54:17–28.
29. Senger DR, Claffey KP, Benes JE, et al. Angiogenesis promoted by vascular endothelial growth factor: regulation through alpha1beta1 and alpha2beta1 integrins. *Proc Natl Acad Sci U S A*. 1997;94:13612–13617.
30. Sinno S, Wilson S, Brownstone N, et al. Current thoughts on fat grafting: using the evidence to determine fact or fiction. *Plast Reconstr Surg*. 2016;137:818–824.
31. Suga H, Eto H, Aoi N, et al. Adipose tissue remodeling under ischemia: death of adipocytes and activation of stem/progenitor cells. *Plast Reconstr Surg*. 2010;126:1911–1923.
32. Tevlin R, Atashroo D, Duscher D, et al. Impact of surgical innovation on tissue repair in the surgical patient. *Br J Surg*. 2015;102:e41–e55.
33. Thangarajah H, Vial IN, Chang E, et al. IFATS collection: adipose stromal cells adopt a proangiogenic phenotype under the influence of hypoxia. *Stem Cells*. 2009;27:266–274.
34. Wu Y, Chen L, Scott PG, et al. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells*. 2007;25:2648–2659.
35. Zielins ER, Brett EA, Blackshear CP, et al. Purified adipose-derived stromal cells provide superior fat graft retention compared with unenriched stromal vascular fraction. *Plast Reconstr Surg*. 2017;139:911–914.
36. Cook T, Nakra T, Shorr N, et al. Facial recontouring with autogenous fat. *Facial Plast Surg*. 2004;20:145–147.
37. Hong KY, Yim S, Kim HJ, et al. The fate of the adipose-derived stromal cells during angiogenesis and adipogenesis after cell-assisted lipotransfer. *Plast Reconstr Surg*. 2018;141:365–375.
38. Khouri RK Jr, Khouri RE, Lujan-Hernandez JR, et al. Diffusion and perfusion: the keys to fat grafting. *Plast Reconstr Surg Glob Open*. 2014;2:e220.
39. Eto H, Kato H, Suga H, et al. The fate of adipocytes after nonvascularized fat grafting: evidence of early death and replacement of adipocytes. *Plast Reconstr Surg*. 2012;129:1081–1092.
40. Pu LL. Mechanisms of fat graft survival. *Ann Plast Surg*. 2016;77(suppl 1):S84–S86.
41. Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation*. 2004;109:1292–1298.
42. Suga H, Glotzbach JP, Sorkin M, et al. Paracrine mechanism of angiogenesis in adipose-derived stem cell transplantation. *Ann Plast Surg*. 2014;72:234–241.
43. Yoshimura K, Shigeura T, Matsumoto D, et al. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *J Cell Physiol*. 2006;208:64–76.
44. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002;13:4279–4295.
45. Glotzbach JP, Levi B, Wong VW, et al. The basic science of vascular biology: implications for the practicing surgeon. *Plast Reconstr Surg*. 2010;126:1528–1538.
46. Fisher C, Grahovac TL, Schafer ME, et al. Comparison of harvest and processing techniques for fat grafting and adipose stem cell isolation. *Plast Reconstr Surg*. 2013;132:351–361.
47. Jurgens WJ, Oedayrajsingh-Varma MJ, Helder MN, et al. Effect of tissue-harvesting site on yield of stem cells derived from adipose tissue: implications for cell-based therapies. *Cell Tissue Res*. 2008;332:415–426.
48. Khouri RK Jr, Khouri RK. Current clinical applications of fat grafting. *Plast Reconstr Surg*. 2017;140:466e–486e.
49. Kato H, Mineda K, Eto H, et al. Degeneration, regeneration, and cicatrization after fat grafting: dynamic total tissue remodeling during the first 3 months. *Plast Reconstr Surg*. 2014;133:303e–313e.
50. Cai J, Feng J, Liu K, et al. Early macrophage infiltration improves fat graft survival by inducing angiogenesis and hematopoietic stem cell recruitment. *Plast Reconstr Surg*. 2018;141:376–386.
51. Cai J, Li B, Liu K, et al. Macrophage infiltration regulates the adipose ECM reconstruction and the fibrosis process after fat grafting. *Biochem Biophys Res Commun*. 2017;490:560–566.
52. Minutti CM, Knipper JA, Allen JE, et al. Tissue-specific contribution of macrophages to wound healing. *Semin Cell Dev Biol*. 2017;61:3–11.
53. Schnoor M, Cullen P, Lorkowski J, et al. Production of type VI collagen by human macrophages: a new dimension in macrophage functional heterogeneity. *J Immunol*. 2008;180:5707–5719.
54. Martino MM, Brkic S, Bovo E, et al. Extracellular matrix and growth factor engineering for controlled angiogenesis in regenerative medicine. *Front Bioeng Biotechnol*. 2015;3:45.
55. Ichim TE, Harman RJ, Min WP, et al. Autologous stromal vascular fraction cells: a tool for facilitating tolerance in rheumatic disease. *Cell Immunol*. 2010;264:7–17.

56. Seaman SA, Cao Y, Campbell CA, et al. Macrophage recruitment and polarization during collateral vessel remodeling in murine adipose tissue. *Microcirculation*. 2016;23:75–87.
57. Shweiki D, Itin A, Neufeld G, et al. Patterns of expression of vascular endothelial growth factor (VEGF) and VEGF receptors in mice suggest a role in hormonally regulated angiogenesis. *J Clin Invest*. 1993;91:2235–2243.
58. Kuwana M, Okazaki Y, Kodama H, et al. Human circulating $CD14^+$ monocytes as a source of progenitors that exhibit mesenchymal cell differentiation. *J Leukoc Biol*. 2003;74:833–845.
59. Kuwana M, Okazaki Y, Kodama H, et al. Endothelial differentiation potential of human monocyte-derived multipotential cells. *Stem Cells*. 2006;24:2733–2743.
60. Tepper OM, Capla JM, Galiano RD, et al. Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. *Blood*. 2005;105:1068–1077.
61. Levi B, James AW, Glotzbach JP, et al. Depot-specific variation in the osteogenic and adipogenic potential of human adipose-derived stromal cells. *Plast Reconstr Surg*. 2010;126:822–834.
62. Strong AL, Bowles AC, MacCrimmon CP, et al. Adipose stromal cells repair pressure ulcers in both young and elderly mice: potential role of adipogenesis in skin repair. *Stem Cells Transl Med*. 2015;4:632–642.
63. Choi P, Reiser H. IL-4: role in disease and regulation of production. *Clin Exp Immunol*. 1998;113:317–319.
64. Luzina IG, Keegan AD, Heller NM, et al. Regulation of inflammation by interleukin-4: a review of "alternatives." *J Leukoc Biol*. 2012;92:753–764.
65. Tonnard P, Verpaele A, Peeters G, et al. Nanofat grafting: basic research and clinical applications. *Plast Reconstr Surg*. 2013;132:1017–1026.
66. Zubkova ES, Beloglazova IB, Makarevich PI, et al. Regulation of adipose tissue stem cells angiogenic potential by tumor necrosis factor-alpha. *J Cell Biochem*. 2016;117:180–196.
67. Kølle SF, Fischer-Nielsen A, Mathiasen AB, et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. *Lancet*. 2013;382:1113–1120.
68. Philips BJ, Grahovac TL, Valentin JE, et al. Prevalence of endogenous $CD34^+$ adipose stem cells predicts human fat graft retention in a xenograft model. *Plast Reconstr Surg*. 2013;132:845–858.
69. Raposio E, Caruana G, Bonomini S, et al. A novel and effective strategy for the isolation of adipose-derived stem cells: minimally manipulated adipose-derived stem cells for more rapid and safe stem cell therapy. *Plast Reconstr Surg*. 2014;133:1406–1409.
70. Rigotti G, Marchi A, Galiè M, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. *Plast Reconstr Surg*. 2007;119:1409–1422; discussion 1423.
71. Yoshimura K, Sato K, Aoi N, et al. Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. *Aesthetic Plast Surg*. 2008;32:48–55; discussion 56.
72. Coleman SR. Structural fat grafting: more than a permanent filler. *Plast Reconstr Surg*. 2006;118(3 suppl):108S–120S.
73. Beane OS, Fonseca VC, Cooper LL, et al. Impact of aging on the regenerative properties of bone marrow-, muscle-, and adipose-derived mesenchymal stem/stromal cells. *PLoS One*. 2014;9:e115963.
74. Fu S, Luan J, Xin M, et al. Fate of adipose-derived stromal vascular fraction cells after co-implantation with fat grafts: evidence of cell survival and differentiation in ischemic adipose tissue. *Plast Reconstr Surg*. 2013;132:363–373.
75. Rophael JA, Craft RO, Palmer JA, et al. Angiogenic growth factor synergism in a murine tissue engineering model of angiogenesis and adipogenesis. *Am J Pathol*. 2007;171:2048–2057.
76. Senger DR, Ledbetter SR, Claffey KP, et al. Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the alphavbeta3 integrin, osteopontin, and thrombin. *Am J Pathol*. 1996;149:293–305.
77. Dong Z, Peng Z, Chang Q, et al. The angiogenic and adipogenic modes of adipose tissue after free fat grafting. *Plast Reconstr Surg*. 2015;135:556e–567e.
78. Pallua N, Pulsfort AK, Suschek C, et al. Content of the growth factors bFGF, IGF-1, VEGF, and PDGF-BB in freshly harvested lipoaspirate after centrifugation and incubation. *Plast Reconstr Surg*. 2009;123:826–833.
79. Blüher S, Kratzsch J, Kiess W. Insulin-like growth factor I, growth hormone and insulin in white adipose tissue. *Best Pract Res Clin Endocrinol Metab*. 2005;19:577–587.
80. Yuksel E, Weinfeld AB, Cleek R, et al. Increased free fat-graft survival with the long-term, local delivery of insulin, insulin-like growth factor-I, and basic fibroblast growth factor by PLGA/PEG microspheres. *Plast Reconstr Surg*. 2000;105:1712–1720.
81. Arnold F, West D, Kumar S. Wound healing: the effect of macrophage and tumour derived angiogenesis factors on skin graft vascularization. *Br J Exp Pathol*. 1987;68:569–574.
82. Marx M, Perlmutter RA, Madri JA. Modulation of platelet-derived growth factor receptor expression in microvascular endothelial cells during in vitro angiogenesis. *J Clin Invest*. 1994;93:131–139.
83. Farrington-Rock C, Crofts NJ, Doherty MJ, et al. Chondrogenic and adipogenic potential of microvascular pericytes. *Circulation*. 2004;110:2226–2232.
84. Mills SJ, Cowin AJ, Kaur P. Pericytes, mesenchymal stem cells and the wound healing process. *Cells*. 2013;2:621–634.
85. Jakeman LB, Winer J, Bennett GL, et al. Binding sites for vascular endothelial growth factor are localized on endothelial cells in adult rat tissues. *J Clin Invest*. 1992;89:244–253.
86. Chappell AG, Lujan-Hernandez J, Perry DJ, et al. Alternatively activated M2 macrophages improve autologous fat graft survival in a mouse model through induction of angiogenesis. *Plast Reconstr Surg*. 2015;136:277e.