International Journal of Medical Science and Clinical Research Studies

ISSN(print): 2767-8326, ISSN(online): 2767-8342

Volume 04 Issue 08 August 2024

Page No: 1441-1446

DOI: https://doi.org/10.47191/ijmscrs/v4-i08-03, Impact Factor: 7.949

Fat Graft. The Science behind It.

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ABSTRACT

Fat grafting is a method used to speed up the healing of wounds in challenging conditions, both in animals and humans. It triggers a biochemical process that promotes the repair of soft tissues and has a positive effect on blood vessel formation. The process involves two main processes: vasculogenesis and angiogenesis. Grafting fat in small portions, no larger than 3mm in diameter, enhances the growth of new blood vessels. The inflammatory role in neovascularization is essential for the development of healthy granulation tissue. In fat grafting, tissue trauma triggers an inflammatory response, which activates the growth of new blood vessels. Platelets, macrophages, and bone marrow-derived mesenchymal stem cells (BM-MSCs) help stabilize pericytes on newly formed blood vessels. This review aims to provide a deeper understanding of fat grafting at the cellular and molecular level.

The inflammatory process in grafts involves the adaptive immune system recruiting naïve peripheral T helper cells to suppress local tissue inflammation, releasing cytokines like TNF-α. This inhibits the differentiation of adipocytes, allowing preadipocytes to differentiate into adipocytes. TNF-α improves the angiogenic capabilities of adipose-derived stem cells (ASCs), promoting the formation of new blood vessels and small blood vessels in the graft. Lipoaspirate, a key component of neovascularization, comprises many cell types essential for neovascularization, including cellular building blocks and precursor cells. Adipose tissue is a readily accessible source of multipotent stem cells, potentially having the highest percentage of adult stem cells in the human body. ASCs play a significant role in promoting the growth of new blood vessels in fat grafting through local signaling, increasing the concentration of VEGF at the graft site.

Fat grafting is a unique method of plastic surgery that involves injecting a graft without blood vessels into an injured area. This process triggers a healing cascade that supports the survival of transplanted cells and facilitates the formation of a new vascular network. The process involves inflammation, cellular elements of the harvested lipoaspirate, and the combined effect of inflammation at the recipient site and the transplanted fat from the donor. Inflammation activates bone marrow-derived mesenchymal stem cells (BM-MSCs) and monocytes, facilitating the movement of cells and division, resulting in the formation of blood vessels. The grafted lipoaspirate experiences hypoxia, triggering a cytokine response and the depletion of graft cells. This process enhances tissue viability and blood supply, promoting regeneration.

KEYWORDS: fat grafting, stem cells, TCD4

ARTICLE DETAILS

Published On:
06 August 2024

Available on:
https://ijmscr.org/

INTRODUCTION

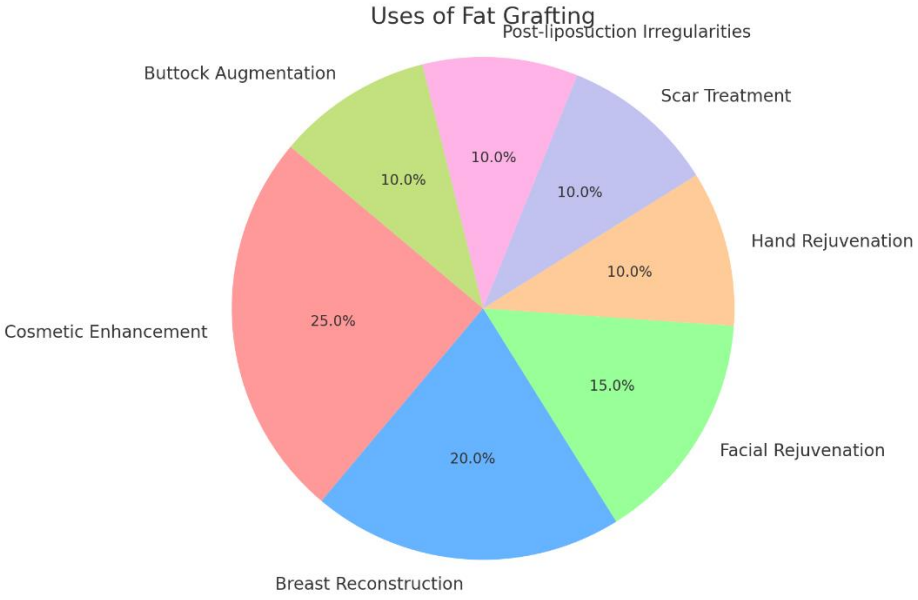
Chronic wounds, burn scars, and scleroderma have a common characteristic: they all have inadequately vascularized tissue. Studies have demonstrated that fat grafting can speed up the healing of wounds in challenging conditions, both in animals and humans. It is believed that fat grafting triggers a biochemical process that promotes the repair of soft tissues, and there is a general agreement that it also has a positive effect on blood vessel formation (1).

After being transplanted, the adipocytes need to develop a blood supply. Until new blood vessels can form, the transplanted tissue can only receive oxygen through diffusion from nearby vessels. According to recent studies, it is believed that cells at the edges of the graft survive because they receive enough oxygen from the plasma, while cells in the center of the graft die due to lack of oxygen, resulting in necrosis. A transitional area exists between the outer and inner areas, where cells that will become grafts replace dead cells. In order for this area to regenerate, a new blood supply must be created through the formation of new blood vessels. This is achieved through the graft releasing certain substances and the development of new cells from both the host and the graft. This process triggers a series of molecular events that support the survival of the graft (2).

Establishing the growth of new blood vessels is a crucial step in ensuring the success of a transplant and achieving better clinical results. There are two main processes involved in the formation of new blood vessels: vasculogenesis and angiogenesis. Vasculogenesis refers to the transformation of precursor cells into endothelial cells, while angiogenesis involves the growth and proliferation of mature endothelial cells from existing blood vessels. During these processes, endothelial cells adhere to each other and form tubules, which serve as the framework for the development of new blood vessels. Factors like hypoxia or inflammation can stimulate the sprouting of new vessels from existing ones. Once new blood vessels are formed, they provide additional oxygen to help heal damaged tissue (3).

It is clinically important to graft fat in a manner that maximizes contact with the recipient site. The current technique suggests grafting fat in small portions no larger than 3mm in diameter. This is done to enhance the growth of new blood vessels because portions larger than 3mm are located outside the area where the host can regenerate tissue (1.5-2.0mm from the edge of the graft) and will die before new blood vessels can form (1).

Due to the ongoing demonstration of the effectiveness of fat grafting in treating soft-tissue disease, restoring deflated regions, and promoting wound healing, it is necessary to determine the specific biochemical pathways that are involved. The process may be divided into three crucial components: the inflammation caused by injecting fat grafts, the hypoxic response resulting from extracting lipoaspirate, and the synergistic interplay between inflammation and grafted fat. The objective of this review is to get a deeper comprehension of fat grafting at the cellular and molecular level and to provide our hypothesized mechanism of action for fat grafting (2).



The Inflammatory Role in Neovascularization

Tissue trauma triggers an inflammatory response, which is essential for the development of healthy granulation tissue. In fat grafting, tissue trauma occurs during the surgical preparation of the recipient bed, involving scar release and the use of injection cannulas (4).

Considering this, tissue damage triggers an inflammatory reaction in the body, which activates the necessary mechanisms to stimulate the growth of new blood vessels. The damage caused at the injection site attracts a range of cells produced from the bone marrow, with platelets being the first to arrive. While the newly migrating platelets assist in controlling bleeding through primary hemostasis, they also

offer structural and biochemical support to the surrounding tissue. Platelets initiate the modification of the surrounding extracellular matrix (ECM) by depositing fibrin, which in turn enhances the local ECM to support the formation of new blood vessels. This process is carefully controlled by macrophages, which are attracted to the injured tissue site as monocytes shortly after the trauma occurs. Once present, macrophages contribute to tissue stability by promoting ECM modification and improving cell-to-cell communication through the secretion of collagen. At the recipient site, platelets and endothelial cells release platelet-derived growth factor (PDGF), which attracts pericytes derived from the bone marrow. The newly modified ECM has a strong affinity for PDGF and helps to stabilize pericytes on the newly formed blood vessels (4, 5).

Bone marrow-derived mesenchymal stem cells (BM-MSCs) migrate to the site of inflammation. They release substances such as VEGF, angiopoietin-1, and erythropoietin, which attract more monocytes. BM-MSCs have the ability to develop into various types of cells in blood vessel formation, while monocytes can also differentiate into endothelial cells, smooth muscle cells, and adipocytes (6).

BM-MSCs possess the ability to transform into ECs, which is crucial for the formation of blood vessels. ASCs also aid in cell regeneration, but it is thought that BM-MSCs primarily contribute to the development of new blood vessels through differentiation, while ASCs have a greater impact on local tissue through paracrine effects (7).

As the inflammatory process progresses, the adaptive immune system recruits naïve peripheral T helper cells (CD4+ T-cells) to the graft site. These T-cells release interleukin-4 (IL-4) to suppress local tissue inflammation. IL-4 stimulates the transformation of pro-inflammatory M1 macrophages into alternative M2 macrophages. IL-4 restricts the number of M1 macrophages at the graft site, which in turn reduces the release of M1-released cytokines like tumor necrosis factor-α (TNF-α). TNF-α is known to inhibit the differentiation of adipocytes. 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 increases the expression of genes necessary for preadipocyte differentiation. The process of progenitor cell and preadipocyte differentiation into mature adipocytes mainly occurs 7-14 days after grafting (8).

While TNF-α inhibits adipocyte development, it has been discovered to improve the angiogenic capabilities of ASCs. Therefore, while increased levels of TNF-α prior to macrophage polarization hinder the differentiation of preadipocytes, this eventually promotes the formation of new blood vessels and the proliferation of small blood vessels in the graft. Zubkova et al conducted a study which demonstrated that TNF-α–enhanced ASCs exhibited elevated transcription of the proangiogenic factors fibroblast growth factor 2 (FGF-2) and VEGF. Additionally, these cells enhanced microvascular growth, expedited blood flow recovery, and increased arteriole density at the graft site. In general, inflammation seems to have a significant impact on graft-induced neovascularization by attracting necessary cells and cytokines to the recipient site (9).

Aspect	Molecular Basis
Adipocyte Survival	Involves angiogenesis and revascularization for oxygen and nutrient supply.
Stem Cells	Mesenchymal stem cells (MSCs) in adipose tissue contribute to tissue regeneration and differentiation.
Growth Factors	Various growth factors like VEGF (vascular endothelial growth factor), PDGF (platelet-derived growth factor), and FGF (fibroblast growth factor) promote cell survival, angiogenesis, and tissue integration.
Extracellular Matrix	Provides structural support and regulates cell behavior through signaling pathways involving collagen, elastin, and proteoglycans.
Cytokines and Chemokines	These signaling molecules mediate inflammation and immune response, crucial for graft acceptance and integration.
Hypoxia Response	Hypoxia-inducible factors (HIFs) regulate the cellular response to low oxygen levels, promoting angiogenesis and adipogenesis.
Oxidative Stress	Reactive oxygen species (ROS) are managed by antioxidant mechanisms to prevent cellular damage.
Adipokines	Adipose tissue-derived hormones like leptin and adiponectin influence metabolic and inflammatory processes.
Immune Response	Macrophages and other immune cells modulate inflammation, tissue remodeling, and healing.
Lipolysis and Lipogenesis	Balance of fat breakdown and synthesis regulated by hormones and enzymes like insulin and lipases.

The role of lipoaspirate in neovascularization

Lipoaspirate comprises many cell types essential for neovascularization, including cellular building blocks and precursor cells that also secrete cytokines. Graft cells may be categorized into two distinct groups: adipocytes and the stromal vascular fraction (SVF). The SVF refers to any cells that are not adipocytes. The stromal vascular fraction (SVF) consists of various types of cells, including pericytes, fibroblasts, vascular endothelial cells (ECs), leukocytes, preadipocytes, and adipose-derived stem cells (ASCs). Studies have shown that the SVF contains approximately 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. Adipose tissue is a readily

accessible source of multipotent stem cells, potentially having the highest percentage of adult stem cells in the human body (10).

Preadipocytes and multipotent ASCs, which are present in the lipoaspirate, contribute to the success of grafts by replacing dying cells in the regeneration area through differentiation and by promoting the growth of new blood vessels at the recipient site. While preadipocytes do not possess the same ability as ASCs to differentiate into multiple cell types, they are abundant in adipose tissue (with 350,000 preadipocytes per 1 mL of adipose tissue) and can still mature into adipocytes (11).

Cell harvest results in the development of acute ischemia. The mature adipocytes in the lipoaspirate are highly sensitive to acute ischemia and are unlikely to survive after grafting. On the other hand, the adipose-derived stem cells (ASCs) found within the stromal vascular fraction (SVF) are more resistant to hypoxia. A study conducted by Suga et al. used flow cytometry to demonstrate that while most adipocytes die within 24 hours of hypoxic exposure, almost all ASCs remain viable even after 72 hours. Adipocytes that have less tolerance break down in low oxygen conditions and release a significant amount of FGF-2. On the other hand, more tolerant ASCs remain intact and increase their production of the cytokine HIF-1α. Additional FGF-2 is released from ASCs due to stimulation by TNF-α. Both FGF-2 and HIF-1α work together to stimulate ECs and ASCs to increase the production of VEGF at the graft site (12).

ASCs play a significant role in promoting the growth of new blood vessels in fat grafting through local signaling. This is achieved by increasing the concentration of VEGF at the graft site, which leads to the proliferation of endothelial cells (ECs) and prevents their death. VEGF also stimulates the division and movement of ECs into the graft. As a result, there is an increased number of ECs in the graft, both from the circulation and recruited locally, which is essential for the formation of new blood vessels. Vascular ingrowth can be observed within 3 days after grafting, with dense capillary networks becoming visible around 4 weeks. Once vascular ingrowth starts, the host is able to supply oxygen directly to the grafted tissue and send host-derived ASC and BM-MSC cells into the graft to improve tissue viability. This migration of host progenitor cells into the graft occurs between 4 and 14 days after grafting. Furthermore, ASCs within the graft maintain their ability to differentiate into various cell types, including adipocytes, fibroblasts, pericytes, endothelial cells, and even rudimentary nervous tissue, contributing to the survival of the graft (13).

The lipoaspirate's cellular components also possess several growth factors that enhance its ability to form blood vessels. Both bFGF and IGF-1 are found in large quantities in lipoaspirate, with IGF-1 being adipose tissue's most abundant protein. IGF-1 prolongs graft survival and promotes the differentiation of preadipocytes into mature adipocytes. 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. 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. 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. In addition to their ability to stabilize new blood vessels, it has also been shown that pericytes retain a progenitor capacity. Thus, pericytes may further contribute to graft success by differentiating into adipocytes and enhancing paracrine effects at the recipient site (14).

DISCUSSION

Ensuring the presence of well-vascularized tissue is a fundamental aspect of plastic surgery, whether it is for delivery or maintenance purposes. This tissue is either carefully preserved or skillfully transplanted into an injured location. Fat grafting is distinctive in that a graft without blood vessels seems to possess the capability to generate a new network of blood vessels in the area where it is implanted. This study aims to elucidate the mechanism by which the body's response to surgical damage triggers a healing cascade that synergistically supports the survival of transplanted cells and facilitates the formation of a new vascular network. We have divided these biochemical processes into three crucial components: the inflammation caused by graft injection, the cellular elements of the harvested lipoaspirate and their reaction to hypoxia, and the combined effect of inflammation at the recipient site and the transplanted fat from the donor (1, 4, 7-10).

Inflammation is a crucial factor in the development of new blood vessels caused by fat grafting. The process of collecting and injecting processed lipoaspirate introduces cells that experience low oxygen levels, leading to a burst of cytokines as their reaction. The burst is enhanced by the transplanted cells, which in turn stimulate a corresponding cellular and cytokine response in the recipient location. The occurrence of this event triggers a biological reaction characterized by the movement of cells and their division, resulting in the formation of granulation tissue in the immediate vicinity. This process starts with the development of blood vessels. The injection-induced inflammation activates bone marrow-derived mesenchymal stem cells (BM-MSCs) and monocytes, facilitating the transportation of precursor cells, promoting the restructuring of the extracellular matrix (ECM), and supporting the formation of connections among developing blood vessels. The final outcome is elevated amounts of VEGF, together with cellular components that specialize and directly promote the formation of new blood vessels (10).

The grafted lipoaspirate experiences hypoxia to the greatest extent during the imbibition phase, which accounts for the

cell necrosis occurring outside the diffusion range of recipient plasma. Graft hypoxia triggers a cytokine response and the depletion of graft cells, particularly mature adipocytes. Once there is initial vascular growth, host ASCs and BM-MSCs can migrate to the graft site and enhance tissue viability through local paracrine signaling and cellular differentiation (14).

The release of cytokines leads to an elevation in the levels of vascular endothelial growth factor (VEGF) at the location of the graft. The upregulated production of VEGF from ASCs and ECs, as well as indirectly through adipocytes and macrophages, facilitates the increased proliferation of ECs, the recruitment of more ECs to the graft site, and the upregulation of EC surface integrins required for motility and cell adhesion. M2 macrophages directly promote the connection of endothelial cells (EC) and signal the recruitment of bone marrow-derived mesenchymal stem cells (BM-MSCs) to the graft site. This recruitment provides an extra supply of cells for the formation of blood vessels (vasculogenesis) and connective tissue. In addition, the release of TGF-β by ASCs prompts pericytes that have been recruited to transform into vascular smooth muscle cells, therefore enhancing the stability of the recently developed vascular networks. These newly formed networks will enhance the circulation of blood and the supply of oxygen to the tissues, so promoting the regeneration or revitalization of the area they are located in (10-14).

Skin grafting is seen as a process consisting of three separate phases: imbibition, inosculation, and neovascularization. In contrast to this well-explained procedure, we propose that fat grafting simply consists of two stages for its survival: imbibition and neovascularization. Imbibition is a process that depends on the diffusion of substances through the plasma phase. Inosculation refers to the alignment of preexisting graft and recipient vessels. Neovascularization involves the formation and connection of these blood vessels. The initial viability of transplanted fat is comparable to that of skin grafts, as it relies on the absorption of plasma. However, unlike in skin grafting, there is no phase of inosculation where capillary networks need to align between the graft and recipient. In fat grafting, all these networks must be established from scratch. Neovascularization is necessary for fat transplantation to establish and maintain linkages with pre-existing vascular networks (12).

FINAL REMARKS

Fat grafting recruits inflammatory cells and bone marrow-derived mesenchymal stem cells (BM-MSCs) to the injection site, providing essential cytokines and materials for the formation of new blood vessels. The lipoaspirate consists of essential components and cellular machinery, including adipocytes, ASCs, and ECs, which work along with recruited cells to stimulate a vasculogenic response.

Based on our analysis of existing research, we propose that the transplantation of ischemic adipose tissue stimulates the growth of new blood vessels in the surrounding tissue, hence promoting the regeneration of injured soft tissue. In summary, while further study is required to fully understand the precise process by which fat grafting repairs soft-tissue lesions, it is evident that neovascularization is a crucial factor in its effectiveness. With the rising popularity of ASCs and fat grafting in plastic surgery research, it is crucial to comprehend the underlying process of fat grafting. This understanding holds great promise for cosmetic and reconstructive surgery.

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