

# Enhanced Lipofilling Outcomes Using Platelet-Rich Plasma: An Innovative Approach

# Munir Abdulmoula

munir\_plastic@yahoo.com

Asso. Prof. of plastic surgery in Misrata university\medical collage Department of Plastic Surgery, National Cancer Institute of Misrata, Misrata, Libya

Article information	Abstract
Key words	Introduction:
Autologous fat transplantation, Platelet- rich plasma (PRP), Fat graft survivability, Soft tissue augmentation, Graft retention, Donor site selection, Healing mechanisms, Minimally traumatic techniques, Secondary calcifications,	Adipose tissue for autotransplantation is easily accessible. Due to comparatively variable and unexpected survival, the popularity of fat transfer surgery has fluctuated throughout the years. The outcome of autologous fat tissue grafts is influenced by numerous factors, some of which the surgeon can manage, like Aseptic procedures, low pressure suction, cautious handling of graft tissues, and the use of minimally traumatic cannulae. There is growing evidence that careful donor site selection and manipulation of specific healing mechanisms that regulate cellular recruitment, migration, and differentiation at the recipient site may improve the consistency and dependability of fat grafts.
Microcyst formation.	The purpose of this paper to investigate the improving the
Received: <b>08-12-2024</b>	survivability and outcome of using transplanted fat with PRP as an alternative to soft tissue augmentation using only fat tissue transfer as
Accepted: <b>06-01-2025</b>	the ordinary procedure
Available: 29-01-2025	<ul> <li>Materials and Methods:</li> <li>The study here is a preliminary clinical investigation combined with a description of a novel technique. It introduces a new method of enhancing autologous fat transplantation with platelet-rich plasma (PRP) and reports initial clinical observations.</li> <li>Results: A higher percentage of graft volume retention (about 91% of the volume of the transferred tissue after 6months post-surgery) appears to be produced following the use of these procedures with all the patients, and regarding to the complication, I observe that The percentage of secondary calcifications occurred in 4% of the patients, and microcyst formation was observed in 3% of them.</li> <li>Discussion: This technique aims to promote or accelerate the healing phase following grafting, increase the intended augmentation retention volume, potentially reduce secondary calcifications and microcyst formation, and maximize transplant unit volume by reducing extracellular fluids transferred with the grafts.</li> </ul>

# I) Introduction

Most tissue augmentation and transplantation surgeries continue to use autologous grafts. It is widely established that autologous tissue grafts survive transfer techniques and subsequently live in the recipient site using the concepts of induction and conduction.1-7 The ideal transplant tissue should be readily available, with low antigenicity and donor site morbidity, consistent retention and

munir\_plastic@yahoo.com

outcomes, and no disease transmission (Figure 1). Autologous fat grafting fits all of these characteristics and hence is a readily available resource for tissue enhancement.

Fat graft survival has been described as uncertain, possibly due to inconsistent collection, handling, and transfer techniques (4,5,8,11). As a result, unless the exact same methodology is used, it is sometimes impossible to replicate or compare the findings of one study to other experiences in fat tissue transplantation. Standardization of such critical components of autologous fat grafting is under underway. Consistent approaches will help to compare ongoing clinical trials to establish the safety and efficacy of such grafts. Overall patient health, genetic predisposition for fat storage in donor sites, pre-transplantation and post-transplantation diet, basic metabolic rate, a traumatic harvest and handling, recipient bed preparation, and graft immobilization in recipient sites are some of the factors that seem to have a significant impact on the success of autologous fat transplantation (Figure 2).

The goal of this project is to investigate the possibility of improving the survivability and clinical effectiveness of using transplanted fat with PRP as an alternative to soft tissue augmentation, which is usually performed by only fat tissue transfer. This project will outline a method for efficiently isolating and combining autologous PRP with platelet-derived growth factors (PDGF) to the transplanted fat.

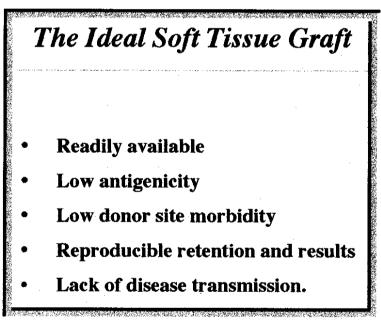


Figure 1. Characteristics of the ideal soft tissue graft.

Host Consideration	Surgical Consideration
<ul> <li>Overall patient health</li> <li>Genetic predisposition for fat storage in donor sites</li> <li>Pre- and Post-transplantation diet</li> <li>Basic metabolic rate</li> <li>Atraumatic harvest and handling</li> <li>Proper preparation of recipient bed</li> <li>Graft immobilization in the recipient sites</li> </ul>	<ul> <li>Selection of appropriate Donor sites (predisposed to storage, hereditary deposits)</li> <li>Selection of appropriate recipient sites</li> <li>Low pressure harvest of autologous fat</li> <li>Handling and preparation of harvested fat</li> <li>Transfer techniques into recipient sites</li> </ul>

Figure 2. Host and perioperative considerations that will influence the success of an autologous fat graft.

# **II) Materials and Methods**

This study is preliminary clinical investigation with a description of a novel technique. It introduces a new method of Enhanced Lipofilling Outcomes Using Platelet-Rich Plasma and reports initial clinical observations.

The research includes:

1. protocol for PRP isolation and incorporation into fat grafting.

2. Observations of PRP-enhanced fat grafts, including their impact on graft retention, tissue healing and complications rates.

## A) Sequestration of Platelet Rich Plasma:

The cell separator machine, which is employed concurrently in the operating room when autologous fat is harvested for transplantation, is used to isolate PRP.

400 450–mL of autologous whole blood is extracted using a specialized large-bore peripheral intravenous catheter. Citrate phosphate dextrose, an anticoagulant, are added.

After that, the blood is centrifuged at 5600 rpm to separate the material into its three main components: platelet poor plasma layer (PPP), PRP, and red blood cells. Three distinct layers are produced by the varying densities of these constituents: the lowest density PPP on the surface, the PRP layer in the center, and the blood cell layer at the bottom.

About 200 mL of the least dense PPP layer are removed. In order to precisely separate the PRP layer (about 70 mL) from the denser cellular components, the sample is then re-centrifuged at a speed of 2400 rpm. the straw-colored PRP will turn red. The majority of newly produced platelets with the highest potential for activity are known to be found near the top of the blood cell layer.<sup>3, 15</sup>

The isolated PRP can then be brought into the operating room to be added to the autologous fat cells that have been extracted and cleaned. Usually completed during the fat harvesting and rinsing phase, the process takes 20 to 30 minutes.

#### **B)** Closed Syringe Harvest of Donor Adipocytes:

Adipocytes are extracted using a minimally traumatic approach and the standard tumescent fluid volume infiltration (fluid to graft ratio of 2:1) made of normal saline, Klein solution, or lactated Ringer solution. I think that using blunt cannulae with a wider diameter, eliminating air from the system, and using low vacuum pressures all work together to reduce damage to the fat tissues.

By using a superpolished titanium cannula system specifically designed for tumescent infiltration and adipocyte withdrawal, will eliminates a significant portion of the potential damage caused by fat-cell membranes coming into contact with the rough stainless-steel surfaces of conventional cannulae.

It is not appropriate to compare fat obtained with a syringe to fat obtained with a machine vacuum pump and trap system. Air is totally removed from the system by using sterile saline to displace all of the air from the syringe and harvesting cannula. The significance of this observation may be better understood in light of the fact that cavitation and cellular vaporization are caused by the fast rise and fall of pressure gradients connected to machine vacuum systems, which damages cells close to the harvesting cannula tip.

The quick variations in pressure that occur when a household vacuum cleaner is transferred from a linoleum floor to dense pile carpeting provide a straightforward comparison for this. Increased cellular trauma may arise from the cavitational effect caused by the pressure differential that forms in thetissues.<sup>31</sup>

To reduce intracellular lidocaine and debris (such as cellular remains, blood products, free lipids, etc.), the collected fat is meticulously and repeatedly rinsed in sterile saline solution. To create the cleanest graft material feasible, at least three rinses are often performed.32 After this phase of grafting preparation is finished, PRP and other additional materials may be added to improve the grafts' longevity and tissue acceptance.

## **C)** Fat Graft Preparation With PRP-Platelet Gel:

After the fat graft has been washed, PRP is added at a 1:10 ratio. It is then gently shaken and left undisturbed for a minimum of 10 minutes. There's evidence that growth factors can bind to certain receptor sites on human adipocytes for up to four hours.12

A discernible rise in extracellular fluids from the cellular components occurs when the treated PRP is introduced to the prepared graft. The measured fat volume is typically reduced by about 60% as a result of this increase in extracellular fluids. When compared to traditional techniques of handling and transferring autologous fat in a suspension form, this visible concentration of the fat for transplantation is thought to be valuable since it indicates that the graft contains roughly 60% more cellular components. For the actual transfer into the recipient bed that has been pretunneled, the condensed graft material can then be put into injection syringes of different sizes that include large bore cannulae or needles.

#### **III)** Results:

Total This technique is currently being investigated and Initial clinical findings are very encouraging from the standpoint of reduction of loss of fullness associated with the expected gradual removal of extracellular fluids used to transport the grafts into the recipient beds. The apparent concentration and greater quantity of cellular grafts observed after utilization of these techniques seem to yield a higher proportion of graft volume retention where about 91% of the



volume of the transferred fat tissue after 6months post-surgery (photos 1a&b,2a&b).

Photos1a&b: facial lipofilling pre and post procedure, photos taken with patient permission for publication



Photos2a&b: temporal area lipofilling pre and post procedure, photos taken with patient permission for publication.

To reduce the pressures of distribution into the recipient locations, we have chosen larger bore introduction instruments due to the denser consistency(because of PRP gel). Blunt cannulae measuring 2.1 to 3.0 mm have been shown to be effective and efficient for big volume transfers.

In order to inject the small aliquots of graft materials through smaller bore devices, cells are exposed to increased pressures that are expected to be damaging to cellular integrity. In addition, we believe that effective but limited pretunneling with blunt cannulae will likewise create a small corridor of potential space to receive the graft without forcing it against closed resistance.

## A) Risks and Complications:

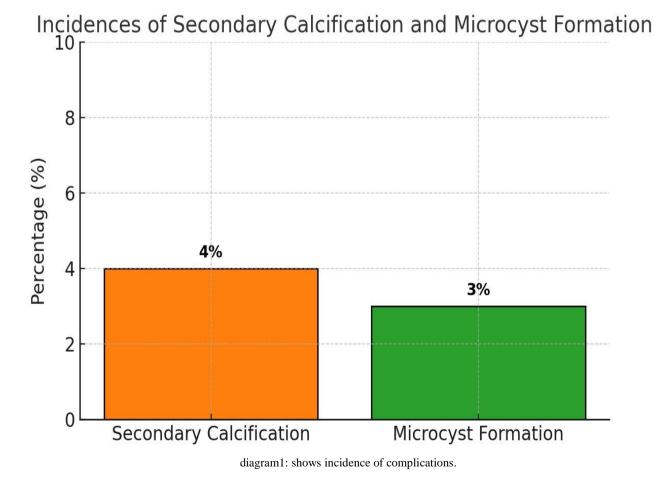
Complications related to autologous fat transplantation are usually rare and are like the type, frequency, and severity of typical liposuction treatments and are related to donor site morbidity.

There have been reports of palpable nodules in recipient site following autologous fat grafting.<sup>4, 5, 8, 11, 34, 35</sup> Palpable nodules following fat transplantation, in my opinion, are because of delayed healing.

liponecrosis that leads to calcification and microcyst development can occur in both minor and large volume transfers<sup>.9, 34, 35</sup>

here in this project the complications were as following:

The percentage of secondary calcifications occurred in 4% of the patients, and microcyst formation was observed in 3% of them (diagram1).



Because PRP-enhanced fat grafts may speed up the revascularization process and graft acceptance, i think they may decrees the likelihood of developing such findings5.

# **IV) Discussion:**

Numerous biochemical additives and substances that are thought to affect adipocyte development, differentiation, or both in vitro and in vivo are widely documented in the literature. These investigations are predicated on the theory that these substances might have the ability to modify metabolic processes or trigger the growth or differentiation of precursor forms. Heparin, calcium, insulin, thyroid hormone, bezafibrate, and vitamin E are some of these substances.16, 17, 39–42 Several of these substances were said to be lipogenic or to impede lipolysis without showing any discernible modifications in the process of differentiation.

There isn't any strong proof that any one additive has had a significant impact on the adipocyte population's developmental or metabolic processes or graft acceptance, but The presence of excessive extracellular fluid and cellular debris is one known limiting factor42-48.

Usually, the Surgeons have overcorrected the transplanted volume by as much as 30 to 50 percent due to the long-term effects of fat transplantation.5, 11 This degree of overcorrection might not be as significant given our findings of the significant decrease in graft mass linked to the injection of PRP, calcium chloride, and thrombin .

It is easiest to categorize the variables that the surgeon can control and that seem to affect the outcome of fat grafting into the several processes that are included in the procedure. The following categories can be used to practically divide the processes (Figure 2).

## A) Selection of Appropriate Donor Sites:

It seems that autologous fat has donor-site memory, meaning that it might preserve the storage and metabolic traits of adipocytes that are typical of the locations chosen for harvest. This has major clinical implications for identifying the best donor site locations, which means that each patient's shown capacity for fat storage and levels of fat metabolic activity is taken into consideration when choosing a donor site rather than the surgeon's convenience.

Cells that are genetically marked as active in storage are thought to be ideal donor sites. These are what are known as primary deposit sites because they are metabolically resistant (i.e., to diet and exercise). With tendencies of specific diet and exercise-resistant deposits that seem to connect with female-to-female or male-to-male phenotypic expression, these donor areas exhibit a strong familial pattern.

# **B)** Selection of Appropriate Recipient Sites

Researchers frequently used recipient sites that were not adipocytes' natural home in early experimental models, such as those developed by Eppley<sup>23</sup>. The results could have been affected because these regions might not have used the potential of a naturally occurring source of native precursor cells to serve as an extra substrate for the resident or recruited growth factors that affect graft acceptance .

Grafts put in fat-bearing beds may have shown success in recent animal trials of fat grafting into sites containing other native fat and connective tissue elements. <sup>47</sup>

# C) Low Pressure Harvest of Autologous Fat

Using low-pressure closed-syringe procedures to remove grafts seems to increase high cell viability in lipoharvesting.<sup>5, 30, 31</sup> The amount of fat available for transplantation, the location of main deposits (genetically predisposed deposit locations), and volume needs all have a direct impact on the harvest phase

The careful application and distribution of sufficient tumescent solution into the donor site before pretunneling and low-pressure vacuum application have an impact on the graft extraction phase. To aid in the mobilization of fat cells from the investing matrix and to provide a means of extracting the saline–cellular suspension, a sufficient fluid media (tumescent fluids) must be provided. With the use of closed syringe harvesting procedures for both large and small volume removal, larger bore, atraumatic cannulae are increasingly used. Niechajev and Sevcuk5 found that vaporization of fat cells and cellular injury were really caused by suction-assisted aspiration of fat cells under maximal negative pressure (machine suction).

According to reports, the ideal and least stressful aspiration pressure was 0.5 atm or lower.<sup>5</sup> Early reports of the use of a closed-syringe system showed a significant decrease in cellular and site trauma utilizing syringes and modest aspiration pressures in an air-free system, which supported these findings.<sup>31</sup> It is thought that less cavitation and cell injury during harvesting occur when there are less abrupt changes in pressure at the cannula tip.

## D) Handling and Preparation of Harvested Fat

Sterile saline or balanced salt solutions are still the most widely used medium for cell extraction because of the concern that solutions that are too hypertonic or hypotonic may harm the extracted cells.

Thoroughly washing the extracted graft tissues is thought to efficiently remove debris and free lipids from the graft in addition to lowering intracellular lidocaine concentrations.<sup>32</sup>

Grafts that are rinsed and maximally cellularized can cause the host bed to experience a less severe inflammatory response. During the initial healing stages linked to any graft acceptance, it would not be desirable to have excessive inflammatory cellular and vascular reactions.

By lowering intracellular lidocaine levels, which are known to have an undesired metabolic effect, serial rinse of collected fat cells may also have a significant metabolic impact.<sup>32</sup>

## E) Transfer Techniques Into Recipient Sites

Large diameter blunt needles or cannulae (2.1–3.0 mm diameter) are considered to be important in decreasing pressure trauma during both extraction and implantation, in accordance with the view that managing cellular grafts with least pressures is important. A healthy recipient site with sufficient vascular, nutrition delivery system, and supportive inflammatory potential is essential, just like in any graft.<sup>5</sup>. It is believed that the beginning and maintenance of appropriate wound healing mechanisms directly affect the outcome of transplantation. These subsequently encourage the transplanted fat cells' early integration and survival.

When fat cells are removed and transplanted, normal ischemia and hypoxic circumstances develop. During the centripetal revascularization process, plasmatic imbibition is necessary for the initial cellular viability.<sup>5, 7</sup> Currently, it is thought that in order to shield the fat cells that are most centrally placed from possible central necrosis and liquefaction, tiny aliquots of fat should be moved into tunnels that have been created.<sup>4,5,8–11,31</sup> To lessen this risk, care should be made to prevent excessive

volume clumping of cellular constituents during fat tissue grafting. It is thought that this effect plays a significant role in reports of erratic and fluctuating resorption rates.

The most common complications in the recipient sites are 2ndry fat calcification and micro cyst formation, here in the diagram (2) I am showing the complications rates in the enhanced lipofilling technique compared with traditional lipofilling procedure.

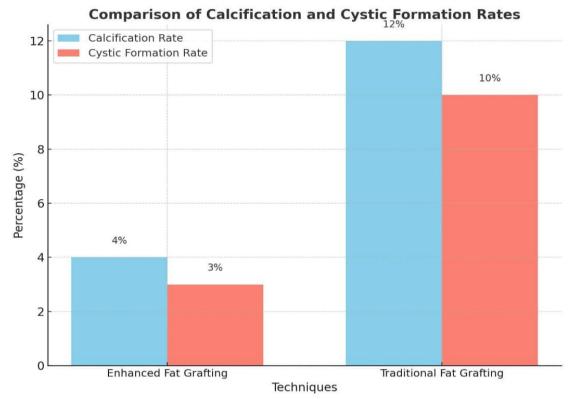


Diagram3: comparing the complications between enhanced fat grafting and traditional fat grafting.

According to this research, PRP-enhanced autologous fat grafts seem to be a better option (retention of 91% of the volume of the transplanted tissue) than traditional fat grafting methods for soft tissue augmentation (retention of only 60% of the volume of the transplanted tissue)17-21. Diagram3

As of right now, the process is intended to sequester a sizable amount of blood and seems to be most appropriate for larger volume transplants required for idiopathic subcutaneous fat atrophy, smoothing trochanteric depressions, posttraumatic defects, retroglandular breast enlargement, or other lipodystrophic disorders. In order to address nasolabial folds, smaller scar depressions, and lip, cheek, and chin augmentation, I am improving this procedure to allow for little volume transfers.

So, Initial clinical findings are very encouraging with utilization of more condensed graft placements. I have observed that the apparent concentration of grafts seems to yield a higher proportion of correction with less volume of graft required.

Despite the consistency of these results, there is currently no way to specifically quantify graft acceptance and survival. <sup>7,41</sup>

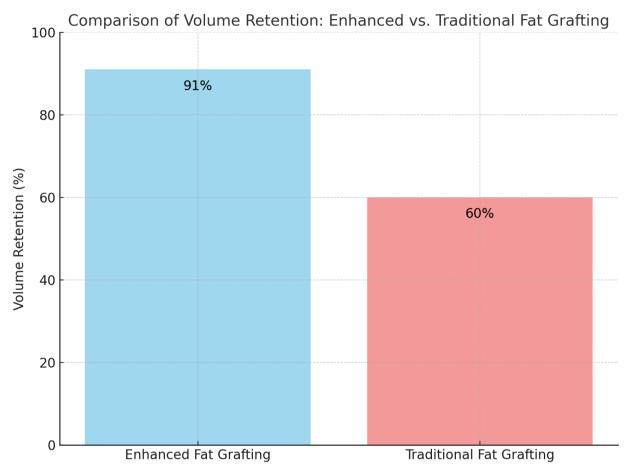


Diagram2: comparing the volume retention between enhanced fat grafting and traditional fat grafting

The effectiveness of soft tissue augmentation may be greatly increased by enriching grafts with PRP and growth factors during autologous fat transplantation surgery. It may be able to achieve greater understanding and clinical predictability through such improvement. The most likely keys to success are thought to be following stringent basic biological and surgical guidelines throughout the entire adipocyte harvesting and transfer procedure. Stated differently, fat transplants require careful collection, handling, and transplantation. The vast range of reported experiences and claims may be explained by the prevalence of abuses during the extraction and preparation process.

Platelets appear to bind more when the calcium chloride or thrombin to PRP ratio is changed to 1.5:10, which lowers the number of platelets in the liquid media. The quantity and method of applying bovine thrombin may have a direct impact on the possible activation of specific antibodies.24 For instance, excessive use of thrombin and CaCl has been linked to coagulopathy in neurosurgery and cardiovascular procedures.<sup>24, 25, 36, 38</sup>

To determine the best dose response curves for these substances and to look for alternate ways to activate PRP for fat grafting, it is crucial to build more clinical experience with modest volume exposures. The components of sequestered blood are being used to create autologous thrombin. The use of autologous thrombin will alleviate the concern of using a bovine source.

This study offers a straightforward and efficient technique for separating platelet gel and PRP for possible use in autologous fat grafting. The importance of growth factors in the healing process of wounds is undeniable. I propose that adding these elements to autologous fat grafts could improve adipocyte survival and promote preadipocyte development. To ascertain the optimal therapeutic use

of such procedures, long-term clinical documentation and quantification of such grafts are required. According to early clinical research, there may be a greater percentage of transplant volume retention than with the traditional fat grafting techniques we have used in the past.

# V) Conclusion:

The availability of autologous fat cells and the technological ability to carefully harvest and transport them to tissue that requires augmentation make it crucial to design standards that will expand augmentation capabilities, improve safety and efficacy, and improve predictability. Concentrated autogenic growth factors have a great deal of promise to improve each of these characteristics, just like in bone transplant technology.

It is thought that the angiogenic potential provided by growth factor availability further improves the recipient bed's integrity and healing capability. PRP deserves extensive laboratory and supervised clinical research since it might be the perfect supplement to autologous fat grafts. This notion and concept raises a number of issues that merit consideration. Additional research is desperately needed in the areas of measuring the life of implanted fat cells, investigating metabolic factors to improve graft survival, and standardizing methods for fat transplantation research.

## **VI) References**

- [1]. Kim WS, Park BS, Sung JH. Protective role of adipose-derived stem cells and their soluble factors in photoaging. Arch Dermatol Res 2009;301:329-36.
- [2]. Miller J, Jeffery C. Fat hypertrophy after autologous fat transfer. Ophthal Plast Reconstr Surg 2002;18:228-31.
- [3]. Coleman SR. Facial recontouring with lipostructure .Clin Plast Surg 1997;24:347-67.
- [4]. Coleman SR. Hand rejuvenation with structural fat grafting. Plast Reconstr Surg 2002.44-110:1731:
- [5]. Cohen G, Treherne A. Treatment of facial lipoatrophy via autologous fat transfer. J Drugs Dermatol 2009;8:486-9.
- [6]. Renom S, Maria J, Joan F. Treatment of facial fat atrophy related to treatment with protease inhibitors by autologous fat injection in patients with human immunodeficiency virus infection. Plast Reconstr Surg 2004.5-114:551:
- [7]. Nishimura T, Hashimoto H, Nakanishi I (Furukawa M. Microvascular angiogenesis and apoptosis in the survival of free fat grafts. Laryngoscope 2000;110:1333-8.
- [8]. Gutiérrez Santamarça J, Masi JGridilla J, Pamias Romero J, et al .Fat grafting is a feasible technique for the sequelae of head and neck cancer treatment. J Craniomaxillofac Surg. 2017;45:93–98.
- [9]. Sinna R, Delay E, Garson S, et al. Breast fat grafting (lipomodelling) after extended latissimus dorsi flap breast reconstruction: a preliminary report of 200 consecutive cases. J Plast Reconstr Aesthet Surg. 2010;63:1769–1777.
- [10]. Missana MC, Laurent I, Barreau L, et al. Autologous fat transfer in reconstructive breast surgery: indications, technique and results .Eur J Surg Oncol. 2007;33:685–690.
- [11]. Delay E, Garson S, Tousson G, et al. Fat injection to the breast :technique, results, and indications based on 880 procedures over 10 years. Aesthet Surg J. 2009;29:360–376.
- [12]. Bertossi D, Zancanaro C, Trevisiol L, et al. Lipofilling of the lips :ultrastructural evaluation by transmission electron microscopy of injected adipose tissue. Arch Facial Plast Surg. 2003;5:392–398.
- [13]. Stallworth CL, Wang TD. Fat grafting of the midface. Facial Plast Surg. 2010; 26:369–375.
- [14]. Pu LLQ. Towards a more rationalized approach to autologous fat grafting. J Plast Reconstr Aesthetic Surg. 2012; 65:413–419.
- [15]. Condé-Green A, de Amorim NF, Pitanguy I. Influence of decantation washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: a comparative study. J Plast Reconstr Aesthet Surg. 2010; 63:1375–1381.
- [16]. Crandall DL, Hausman GJ, Kral JG. A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives. Microcirculation 1997; 4:211–232.

- [17]. 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.
- [18]. Carpaneda CA, Ribeiro MT. Percentage of graft viability versus injected volume in adipose autotransplants. Aesthetic Plast Surg .1994.19–18:17 :
- [19]. Baran CN, Celebioğlu S, Sensoz O, et al. The behavior of fat grafts in recipient areas with enhanced vascularity. Plast Reconstr Surg. 2002; 109:1646–1651; 1652.
- [20]. Martinez-Zapata MJ, Mart-ÇCarvajal AJ, Solà I, et al. Autologous platelet-rich plasma for treating chronic wounds. Cochrane Database Syst Rev. 2016;2016:CD006899.
- [21]. Foster TE, Puskas BL, Mandelbaum BR, et al. Platelet-rich plasma :from basic science to clinical applications. Am J Sports Med .2009.2272–37:2259 \$
- [22]. Cohn CS, Lockhart E. Autologous platelet-rich plasma: evidence for clinical use. Curr Opin Hematol. 2015;22:527–532.
- [23]. Frautschi RS, Hashem AM, Halasa B, et al. Current evidence for clinical efficacy of platelet rich plasma in aesthetic surgery: a systematic review. Aesthetic Surg J. 2016;37: sjw178.
- [24]. Sommeling CE, Heyneman A, Hoeksema H, et al. The use of platelet-rich plasma in plastic surgery: a systematic review. J Plast Reconstr Aesthet Surg. 2013;66:301–311.
- [25]. Amable PR, Carias RB, Teixeira MV, et al. Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. Stem Cell Res Ther . 2013.4:67:
- [26]. Marx RE. Clinical application of bone biology to mandibular and maxillary reconstruction. Clin Plast Surg. 1994;21:377–392.
- [27]. Knighton DR, Hunt TK, Scheuenstuhl H, et al .Oxygen tension regulates the expression of angiogenesis factor by macrophages. Science. 1983;221:1283–1285.
- [28]. Marx RE, Ehler WJ, Tayapongsak P, Pierce LW .Relationship of oxygen dose to angiogenesis induction in irradiated tissue. Am J Surg. 1990;160:519–524.
- [29]. Carpaneda CA, Ribiero MT. Study of histologic alterations and viability of the adipose graft in humans .Aesth Plast Surg. 1993;17:43–47.
- [30]. Jones JK, Lyles ME. The viability of human adipocytes after closed-syringe liposuction harvest. Am J Cosm Surg. 1997;14:275–279.
- [31]. Alexander RW. Liposculpture in the superficial plane: closed syringe system for improvement in fat removal and free fat transfer. Am J Cosm Surg. 1994 *11:127.134–*
- [32]. Alexander RW, Maring T, Aghabo T. Autologous fat grafting: a study of residual intracellular adipocyte lidocaine concentrations after serial rinsing with normal saline. Am J Cosm Surg. 1999;16:123–126.
- [33]. Hood AG. Perioperative autologous sequestration III: a new physiologic glue with wound healing properties. Proc Am Acad Cardiovasc Perf. 1993;14 :126.129–
- [34]. Bircoll M. Autologous fat tissue augmentation .Am J Cosm Surg. 1987;4:141–149.
- [35]. Bircoll M. Autologous fat transplantation: an evaluation of microcalcification and fat cell survivability following (AFT) cosmetic breast augmentation .Am J Cosm Surg. 1988;5:283–288.
- [36]. Rapaport SI, Zivelin A, Minow RA, et al. Clinical significance of antibodies to bovine and human thrombin and factor V after surgical use of bovine thrombin. Am J Clin Pathol. 1992;97:84–91.
- [37]. Berruyer M, Amiral J, Ffrench P, et al. Immunization by bovine thrombin used with fibrin glue during cardiovascular operations. Development of thrombin and factor V inhibitors. J Thorac Cardiovasc Surg Vol. 1993;105:892–897.
- [38]. Israels SJ, Israels ED. Development of antibodies to bovine and human factor V in two children after exposure to topical bovine thrombin. Am J Pediatr Hematol Oncol. 1994;16:249–254.
- [39]. Menschik Z. Vitamin E and adipose tissue. Edinburgh Med J. 1944;51:486–489.
- [40]. Brownsey RW, Edgell NJ, Hopkirk TJ, Denton RM. Studies on insulin-stimulated phosphorylation of acetyl-CoA carboxylase, ATP citrate lyase and other proteins in rat epididymal adipose tissue. Biochem J .1984.743–218:733:
- [41]. Sidman RL. The direct effect of insulin on organ cultures of brown fat. Anat Rec. 1956;124:723–739.

- [42]. Renold AE, Marble A, Fawcett DW. Action of insulin on deposition of glycogen and storage of fat in adipose tissue. Endocrinology. 1950;46:55–66.
- [43]. Leslie ML, Antoniades HN, Geyer RP. Stimulation of phospholipid and cholesterol ester synthesis by platelet derived growth factor in normal and homozygous familial hypercholesterolemia human skin fibroblasts. Biochim Biophys Acta. 1982;711:290–304.
- [44]. Krawisz BR, Scott RE: Coupling of preadipocyte growth arrest and differentiation; I. Induction by heparinized medium containing human plasma. J Cell Biol. 1982;94:394–399.
- [45]. Brandes R, Hertz R, Arad R, Naishtat S, Weil S 'Bar-Tana J. Adipocyte conversion of cultured 3T3-L1 preadipocytes by bezafibrate. Life Sci. 1987;40:935–941.
- [46]. Flores-Delgado G, Marsch-Moreno M, Kuri -Harcuch W. Thyroid hormone stimulates adipocyte differentiation of 3T3 cells. Mol Cell Biochem. 1987;76:35.43–
- [47]. Ullmann Y, Hyanms M, Ramon Y, Beach D (Peled IJ, Lindenbaum E. Enhancing the survival of aspirated human fat injected into nude mice. Plast Reconstr Surg. 1998;101:1940–1944.
- [48]. Eppley BL, Sidner RA, Platis JM, Sadove MA .Bioactivation of free-fat transfer; a potentially new approach to improving graft survival. Plast Reconstr Surg. 1992;90:1022–1030.