Application of Platelet-Rich Plasma and Platelet-Rich Fibrin in Fat Grafting: Basic Science and Literature Review

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Due to the natural properties of fat, fat grafting remains a popular procedure for soft tissue volume augmentation and reconstruction. However, clinical outcome varies and is technique dependent. Platelet-rich plasma (PRP) contains α -granules, from which multiple growth factors such as platelet-derived growth factor, transforming growth factor- β , vascular endothelial growth factor, and epidermal growth factor can be released after activation. In recent years, the scope of PRP therapies has extended from bone regeneration, wound healing, and healing of musculoskeletal injuries, to enhancement of fat graft survival. In this review, we focus on the definition of PRP, the different PRP preparation and activation methods, and growth factor concentrations. In addition, we discuss possible mechanisms for the role of PRP in fat grafting by reviewing *in vitro* studies with adipose-derived stem cells, preadipocytes, and adipocytes, and preclinical and clinical research. We also review platelet-rich fibrin, a so-called second generation PRP, and its slow-releasing biology and effects on fat grafts compared to PRP in both animal and clinical research. Finally, we provide a general foundation on which to critically evaluate earlier studies, discuss the limitations of previous research, and direct plans for future experiments to improve the optimal effects of PRP in fat grafting.

Introduction

AUTOLOGOUS FAT GRAFTING is an important treatment option for small to medium soft tissue defects derived from tumor ablation, congenital deformity, and traumatic injury. The advantages are that autologous fat is easy to obtain in large quantities and the procedure is less uncomfortable and risky to patients; the operation is of short duration, can sometimes be performed under local anesthesia, and simultaneously achieves an aesthetic result in both the donor and recipient sites. However, the disadvantages of fat grafting are an unpredictable and variable reabsorption rate of around 40%–60%, resulting in the need for repeated procedures, and microcalcifications and cyst formation due to fat necrosis.^{1,2} Reabsorption and fat necrosis are believed to be caused by insufficient neoangiogenesis around the fat graft, thus resulting in adipocyte apoptosis due to lack of nutrient supply and accumulation of metabolic waste.

Several strategies have been reported to enhance fat graft survival, such as adjunct therapy by adding the stromal vascular fraction (SVF), enhancing angiogenesis by addition of growth factors, or use of chemical cell-stimulating factors, such as insulin or erythropoietin.^{3–8} Among these, platelet-

rich plasma (PRP) has recently emerged as a new matrix to enhance fat graft survival. PRP, which is derived from whole blood through double-spin centrifugation, contains multiple growth factors and adhesion molecules in α -granules. PRP is believed to be safer and more practical in clinical adjunctive therapy than other recombinant growth factors or stem cell therapies. In addition, PRP is an economic way to obtain multiple growth factors at one time that meet the requirements for highly complex processes during tissue repair or regeneration. PRP has been demonstrated to be effective in bone regeneration, wound healing, and improvement of musculoskeletal sports injuries.^{9–15} Recently, clinicians extended the scope of PRP therapy to soft tissue augmentation by combining PRP with fat grafting. Although some successful clinical results were reported,^{16,17} evidence supporting the application of PRP combined with fat grafts in soft tissue augmentation remains limited, as only a few basic research and preclinical studies in small animals have been conducted.^{15,18–23} Furthermore, no details on molecular mechanisms are addressed in the literature. In this review article, we discuss the possible molecular mechanisms of PRP in fat graft survival based on a review of the current literature. This review discusses published *in vitro*, animal,

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and human studies and provides guidance for future research and clinical application.

The Definition of PRP

According to Marx *et al.*, PRP is the autologous platelet concentration above baseline normal platelet count in a small volume of plasma.¹¹ Usually, the normal adult human platelet count ranges between 150,000 and 350,000/ μL , with an average of $200,000/\mu\text{L} \pm 75,000/\mu\text{L}$.^{9,11} It has been shown that a concentration of ~ 1 million platelets per μL , or approximately four to seven times more than the usual baseline platelet count, produces clinical benefits.^{9,11} Platelets contain two basic granules: α -granules and dense granules. There are ~ 50 – 80 α -granules per platelet. The α -granules are ~ 200 – 500 nm in diameter. At least seven fundamental protein growth factors have been proven to exist within α -granules for initiating wound healing. These growth factors include the three isomers of platelet-derived growth factor (PDGF-AA, PDGF-BB, and PDGF-AB), transforming growth factor- β (TGF- $\beta 1$ and TGF- $\beta 2$), vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF).¹¹ The α -granules also contain three proteins known to act as cell adhesion molecules: fibrinogen, fibronectin, and vitronectin.¹⁴ In addition to the seven basic growth factors, scientists have also found other growth factors such as the insulin-like growth factor (IGF-I, IGF-II), fibroblast growth factor (FGF), endothelial cell growth factor (ECGF), and platelet-derived angiogenesis factor (PDAF).^{9,14}

Bioactive factors are also contained in dense granules, including serotonin, histamine, dopamine, calcium, and adenosine, which are also involved in wound healing.¹⁴ These bioactive factors are involved in inflammation, the first stage of wound healing. Serotonin and histamine secreted by aggregated platelets increase the permeability of capillaries, allowing inflammatory cells to migrate from the capillary lumen into the wound site and activate macrophages.

Preparation of PRP

Ideally, blood used to generate PRP should be collected before initiation of surgery because platelets will aggregate in the surgical site to initiate the clotting cascade and reduce circulating platelet counts.²⁴ PRP has traditionally been prepared by double-spin centrifugation of anticoagulated blood. The first centrifugation (soft spin) separates the platelet layer from the plasma and red blood cells. The lower red blood cell layer (specific gravity = 1.09) is discarded. The upper plasma (specific gravity = 1.03) and middle layers (specific gravity = 1.06) contain platelets that are collected and further centrifuged again during the hard spin, and precipitated platelets are collected with part of the plasma as PRP. No consistent centrifugal force or time has been reported in the literature. A higher centrifugal force for the second spin was recommended to shorten the preparation time and increase platelet numbers.^{25,26} However, high centrifugation will cause platelet fragmentation, which will result in the release of some growth factors during preparation and compromise bioactivity.⁹ Dugrillon *et al.* studied the influence of centrifugal force on growth factor release and found that platelet counts increased gradually as the centrifugal force increased from 400 to 1200 g .²⁷ However, the concentration of TGF- β showed a biphasic response with a significant increase from 400 to 800 g , but without further

increase at 1000 and 1200 g .²⁷; 800 g seems to be the optimal centrifugal force for the second spin.

Although centrifugation is an easy method to prepare PRP, it is best suited for laboratory research because it is labor intensive when a large volume is required and sterility is not easy to maintain. As such, there are two kinds of commercial devices available for making PRP. One type is the standard cell separator and salvage devices that can separate PRP from one unit of whole blood, which is suitable to generate large volumes of PRP required for procedures such as fat grafting in breast reconstruction. Usually, the standard cell separator yields platelet concentrations from two to four times the baseline.⁹ The advantages of these devices are that they can automatically produce large volumes of PRP and residual red blood cells and plasma can be reinfused back to the patient to avoid blood volume depletion. The other type of device is designed to generate small volumes of PRP, which are required in clinical procedures such as in bone grafting, fat grafting, or treatment of knee cartilage sports injuries. Hence, some point-of-care systems, such as Curasan, PCCS, Anitus, SmartPreP, GPS, and the Symphony II system, are designed to produce ~ 6 mL of PRP from 45 to 60 mL of blood.^{9,24,28} However, the range of concentrated platelets is wide, from a less than two- to eightfold increase over baseline.⁹

Activation of PRP

Activation is a process of degranulation that results in α -granules fusing to the platelet membrane, with the secretory proteins becoming bioactive by the addition of histones and carbohydrate side chains.^{9,11} Marx *et al.* described the activation by mixing 6 mL of PRP, 1 mL of calcium chloride/thrombin mixture (10 mL of 10% calcium chloride mixed with 10,000 units of bovine thrombin), and 1 mL of air to act as a mixing bubble.¹² However, activation of PRP by thrombin usually results in a burst of growth factors, releasing within 10 min of clotting, and more than 95% will be released in 1 h.^{9,11} Hence, Marx *et al.* recommended that thrombin should be applied to the reconstruction site within 10 min after PRP activation.¹¹ This method is usually used to prepare and collect total growth factors from PRP. Since this first report, additional multiple activation methods have been reported.

The addition of CaCl_2 alone rather than thrombin is an alternative way to activate PRP. The addition of CaCl_2 results in the formation of autologous thrombin from prothrombin within PRP and the eventual formation of a loose fibrin matrix, which will entrap the growth factors, resulting in the slow secretion of growth factors over 7 days. This method is most used in the clinical application of PRP for fat grafting for soft tissue augmentation.

Another method to collect growth factors from PRP is the freeze/thaw cycle.^{29,30} Tubes containing PRP are placed in a -80°C freezer for 24 h, followed by a 37°C water bath for 1 h, and then centrifugation at 2000 g for 10 min. The supernatant is then filtered with a $0.22\text{-}\mu\text{m}$ sterile filter and stored in aliquots of 5 mL at -80°C .

Variable Concentrations of Growth Factors in PRP

Method of PRP activation

The PRP activation method influences the concentration of growth factors being released from PRP. The concentration

of growth factors in PRP varies in published reports due to different preparation methods, different centrifugal forces, and different activation methods (Table 1). Kim *et al.* compared growth factor release between four different activation methods: (1) 10% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; (2) 0.1% Triton-X; (3) 142.8 U/mL of thrombin and 14.3 mg/mL $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; (4) 10 U/mL of thrombin and 2 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ after pre-activation with shear stress and 20 $\mu\text{g}/\text{mL}$ collagen. All four methods can adequately activate platelets.¹⁰ No conclusion was made as to which method was best for activation. PDGF and TGF- β were better released by methods 2 and 3. VEGF was better released by method 4. FGF was better released by method 1. Eppley *et al.* activated PRP using thrombin/ CaCl_2 methods; PDGF-BB (120 ng/mL), TGF- β 1 (120 ng/mL), VEGF (995 ng/mL), and EGF (129 ng/mL) were found.³¹ Weibrich *et al.* evaluated growth factor release by the freeze/thaw cycle from 115 patient samples. A large amount of growth factor release was found in PDGF-AB (117 ng/mL), TGF- β 1 (169 ng/mL), and IGF-I (84 ng/mL), while PDGF-BB (10 ng/mL) and TGF- β 2 (0.4 ng/mL) were found in small amounts only.²⁹ No correlation was found between the

growth factor content and platelet count in whole blood or with PRP.

Method of PRP preparation and preservation

The method of PRP preservation can affect PRP efficacy. For example, Pietramaggiori *et al.* studied the growth factor concentration among three different preservation methods: fresh frozen, freeze-dried with a stabilization solution, and freeze-dried without a stabilization solution. The results showed all three methods can effectively release growth factors with TGF- β (334.4 ng/mL), PDGF (8672 pg/mL), EGF (2185.2 pg/mL), and VEGF (330.8 pg/mL) in fresh frozen PRP; TGF- β (314.8 ng/mL), PDGF (7304 pg/mL), EGF (2016.4 pg/mL), and VEGF (346.4 pg/mL) in fresh-dried without a stabilization solution; and TGF- β (245.2 ng/mL), PDGF (7784 pg/mL), EGF (2064.8 pg/mL), and VEGF (268 pg/mL) in freeze-dried PRP with a stabilization solution.¹³ The possibility of delivering growth factors using platelets by freeze-drying and frozen methods could extend the shelf-life of platelet products. Eppley *et al.* activated PRP

TABLE 1. OVERVIEW OF GROWTH FACTOR CONCENTRATION RELEASE FROM HUMAN PRP

References	Centrifugation method	Activation method	Mean platelet count in PRP/whole blood	Mean PDGF-AB/TGF- β 1
Weibrich <i>et al.</i> ²⁹	Hemonetics gradient density cell separator	Freeze-thaw cycle	1,407,640/266,040/ μL	PDGF-AB: 117 ng/mL TGF- β 1: 169 ng/mL
Eppley <i>et al.</i> ³¹	3200 rpm 12 min	Thrombin + CaCl_2	1,603,000/197,000/ μL	PDGF-BB: 17 ng/mL TGF- β 1: 120 ng/mL
Tsay <i>et al.</i> ⁵⁶	1. 200 g 15 min 2. 200 g 10 min	Thrombin	Nil	PDGF-AB: 32.5 ng/mL ^a TGF- β 1: 11.4 ng/mL ^a
Kakudo <i>et al.</i> ³⁶	1. 1700 rpm 7 min 2. 3200 rpm 5 min	Thrombin + CaCl_2	1,322,600/167,400/ μL	PDGF-AB: 144.46 pg/mL TGF- β 1: 96.38 pg/mL
Huang <i>et al.</i> ⁵⁷	Obtained from blood bank	Thrombin + CaCl_2	1,240,010/188,750/ μL	PDGF-AB: 86.45 ng/mL TGF- β 1: 8.27 ng/mL
Pietramaggiori <i>et al.</i> ¹³	Platelets purchased from blood bank	Sonication	1,200,000/ μL	Fresh frozen PRP PDGF-AB: 8.67 ng/mL TGF- β 1: 334.4 ng/mL Freeze-dried PRP without stabilization solution PDGF-AB: 7.3 ng/mL TGF- β 1: 314.8 ng/mL Freeze-dried PRP with stabilization solution PDGF-AB: 7.78 ng/mL TGF- β 1: 245.2 ng/mL
Weibrich <i>et al.</i> ²⁸	1. Blood bank 2. Crurasan 3. PCCS2000 4. PCCS 2001 5. Anitua 6. SMARTPreP 7. Friadent-Schutze	Not mentioned	1. 1,434,300/260,370/ μL 2. 1,072,290/289,200/ μL 3. 2,205,890/289,200/ μL 4. 1,641,800/274,200/ μL 5. 513,630/274,200/ μL 6. 1,227,890/276,810/ μL 7. 1,440,500/276,810/ μL	1. PDGF-AB: 133.6 ng/mL TGF- β 1: 268.7 ng/mL 2. PDGF-AB: 321.1 ng/mL TGF- β 1: 83.9 ng/mL 3. PDGF-AB: 267.7 ng/mL TGF- β 1: 560.2 ng/mL 4. PDGF-AB: 156.7 ng/mL TGF- β 1: 289.5 ng/mL 5. PDGF-AB: 47 ng/mL TGF- β 1: 73.3 ng/mL 6. PDGF-AB: 208.3 ng/mL TGF- β 1: 77.2 ng/mL 7. PDGF-AB: 251.6 ng/mL TGF- β : 196.8 ng/mL

^aGrowth factor concentration release at day 1.

PRP, platelet-rich plasma; PDGF, platelet-derived growth factor; TGF, transforming growth factor.

using thrombin/CaCl₂ methods; PDGF-BB (120 ng/mL), TGF- β 1 (120 ng/mL); VEGF (995 ng/mL); and EGF (129 ng/mL) were found.

The method of PRP preparation also affects the growth factor concentrations in PRP. Weibrich *et al.* compared PRP obtained from the blood bank to five point-of-care methods; the growth factor concentrations are listed in Table 1.^{28,32} Increased platelet concentrations are believed to elevate released secretory proteins.³¹ However, Eppley *et al.* and Weibrich *et al.* found that the correlation between the platelet count and secretory growth factor concentration is not high and that it is hard to predict the growth factor level by platelet concentrations.^{9,29,31} Possible reasons are high variability in cellular production or storage of biologically active substances, variable releases with different activation methods, and contribution of growth factors from other cellular (leukocytes) or plasmatic sources.²⁸

The Effects of PRP on Fat Graft Survival

Fat graft implantation

Fat grafts contain at least two cell groups: mature adipocytes and the SVF. The SVF is a heterogeneous cell population, including endothelial cells, smooth muscle cells, pericytes, leukocytes, mast cells, preadipocytes, and multipotent adipose-derived stem cells (ASCs). Mature adipocytes are sensitive to ischemic environments; they may die or dedifferentiate. The dedifferentiated adipocytes may redifferentiate into mature adipocytes if adequate vascular supply is established.^{32–34} Proliferation and differentiation of preadipocytes and ASCs are also responsible for fat graft survival.³²

After surgical implantation, fat grafts initially survive through nutrient diffusion from the plasma. Thus, smaller grafts have better survival rates than larger grafts because the higher surface to volume ratio of smaller grafts results in a larger area in contact with the vascular bed. Subsequently, neovascularization, which often occurs as early as 48 h post-transplantation, will begin supplying nutrients to the fat grafts. Large grafts may exhibit higher liquefaction, necrosis, and cyst formation, especially in the central part, due to poorer nutrient diffusion from the plasma and inadequate neovascularization to the central part.³²

Fat graft survival

The retention of fat grafting is known to be affected by size. Eto *et al.* described a three-zone theory of fat graft fate established using a mouse model.³⁵ The most superficial zone, which is less than 300 μ m thick, is the surviving zone. In the surviving zone, both adipocytes and ASCs survive. The second zone is the regenerating zone, in which adipocytes die as early as day 1, but ASCs survive and provide new adipocytes to replace the dead ones. The most central zone is the necrotic zone, where both adipocytes and adipose-derived stromal cells die, no regeneration is expected, and the dead space will be absorbed or filled with scar tissue.

Role of PRP in fat graft survival

PRP may increase fat graft survival by (1) providing nutrient support from its plasma component; (2) increasing angiogenesis from multiple angiogenic growth factors, such

as PDGF, PDAF, and VEGF; and (3) enhancing the proliferation and adipogenic differentiation of preadipocytes and ASCs in the regeneration zone. Many preclinical studies have confirmed increased angiogenesis after adding activated PRP to the fat graft. However, the effects of PRP on mature adipocytes and ASCs have not been extensively examined. Many articles describe that PRP either promoted the proliferation of ASCs or that fetal bovine serum can be substituted as the expansion medium *in vitro*. The optimal concentration of active PRP (aPRP) for prompting ASC proliferation is still controversial. Kakudo *et al.*³⁶ determined that 1% and 5% are the suitable aPRP concentrations for proliferation of human ASCs; in contrast, a greater than 5% aPRP concentration will inhibit ASC proliferation. However, in Cervelli's study,¹⁶ they found a dose-dependent effect of aPRP on the proliferation of human ASCs. ASC proliferation increased as the concentration of aPRP was elevated from 1% to 50%. Some articles concluded that autogenous PRP was able to replace the role of fetal bovine serum in the culture medium. The advantages are that fetal bovine serum can enhance the proliferation of ASCs and carries no risk of transmitting viruses, bacterial diseases, or Creutzfeldt-Jakob disease.

Mechanistic role of PRP on fat graft survival

The molecular influence of PRP on ASCs and mature adipocytes has been even less addressed in the literature. Liu *et al.*³⁷ studied PRP in osteoporosis and found that PRP can upregulate the osteogenesis potential and downregulate the adipogenesis potential of preadipocytes (3T3-L1 cell line). It was also determined that PRP can transdifferentiate mature adipocytes into osteoblasts by increasing the expression of osteogenic-specific genes such as RunxII, OPN, and OCN and mineralizing and decreasing the expression of adipogenic-specific genes such as PPAR- α and Leptin in a PRP-treated group. Fukaya *et al.*³⁸ identified proliferative preadipocytes, so-called ceiling culture-derived proliferative adipocytes (ccdPAs), from adipose tissue. They demonstrated that PRP can inhibit the apoptosis of highly adipogenic homogeneous preadipocytes (ccdPAs) by reducing the levels of DAPK1 and BIM mRNA expression; they further concluded that PRP may improve the outcome of adipose tissue transplantation by enhancing the antiapoptotic activities of the implanted preadipocytes. Cervelli *et al.*¹⁶ studied the effect of PRP on ASCs and found that PRP alone did not increase the adipogenesis of ASCs. However, with insulin it greatly potentiates adipogenesis in human ASCs through a FGFR-1- and Erb2-regulated Akt mechanism.

Specific role of growth factors in PRP

PRP is a natural cocktail of growth factors. Adipogenic differentiation is influenced by a complex process involving multiple hormones and growth factors. IGF induces the adipogenic differentiation of 3T3-L1 cell lines by enhancing the ability of the PPAR ligand.^{39,40} TGF- β 1 has been demonstrated to inhibit adipogenesis in bone marrow mesenchymal progenitor cells through its target gene (connective tissue growth factor).⁴¹ EGF and PDGF were reported to inhibit the adipocyte conversion due to decreasing PPAR α transcriptional activity after the activation of EGF or PDGF receptors with subsequent phosphorylation of PPAR by the

MAP kinase signaling pathway.³⁹ However, PDGF is also found to stimulate adipose conversion of 3T3-L1 preadipocytes dramatically when it is added to the adipogenic medium (insulin + corticosterone + 3-isobutyl-1-methylxanthine) compared to the adipogenic medium or PDGF alone.⁴² The process is believed to be mediated through the expression of CCAAT/enhancer-binding proteins.⁴² In addition, Stagner *et al.* described that the withdrawal of PDGF from the adipogenic medium not only decreased capacity for differentiation, but also induced the apoptosis of 3T3-L1 preadipocytes.⁴³ Craft *et al.* also showed the increased fat graft survival in nude mice by the effect of long-term delivery of PDGF by microspheres.⁴⁴ In summary, the growth factors alone seem to inhibit adipogenesis, except for IGF. In addition, the role of PDGF on adipogenesis is controversial.

Although most of the growth factors do not favor adipogenesis, most of them indeed stimulate angiogenesis. Rophael *et al.*, found the cocktail of angiogenic growth factors (VEGF, FGF, PDGF-BB) does not only enhance early angiogenesis, but also late *de novo* adipogenesis compared to each single growth factor in a murine tissue engineering model.⁴⁵ Hence, the angiogenic effect of PRP might not only maintain the survival of mature fat cells, but also induce *de novo* adipogenesis.

Preclinical Studies

Several animal studies have been conducted to demonstrate the efficacy of PRP on fat graft survival (Table 2). The results are controversial and difficult to compare due to the variability in fat graft source, fat graft harvest methods, ratio of PRP to fat graft, method of PRP activation, and recipient site. Por *et al.* mixed human lipoaspirates with PRP at a ratio of 4:1 in an experimental group and with saline in a control group,²⁰ and then implanted on the scalps of nude mice. After 4 months, there were no significant differences between the PRP group and the saline group in fat graft survival, vasculogenesis, cyst formation, fibrosis, necrosis, or inflammation. However, their poor results were criticized because no activation agent (thrombin or CaCl_2) was added in their study to release growth factors from platelets. Pires Fraga *et al.* used a rabbit model to study the effect of PRP on fat graft survival.¹⁹ Fat grafts were harvested from the dorsal scapular region and mixed with a near identical volume of autologous PRP, which was activated by CaCl_2 and thrombin. The mixtures were implanted in the subcutaneous ear of the rabbit model, and the results showed a significant increase in viable adipocytes and angiogenesis in the PRP group and an increased necrotic area and inflammation area in the saline group. Rodriguez-Flores *et al.* harvested rabbit groin fat through liposuction and mixed it with the same volume of PRP activated by CaCl_2 to augment the lip.²¹ The outcome was no different in angiogenesis and viable adipocytes between the PRP and control groups, but a lower inflammatory reaction and cyst formation in the PRP group than in the control group 4 months after implantation. Nakamura *et al.* combined rat inguinal fat with PRP activated by CaCl_2 at a ratio of 4:1. The mixture was implanted on a dorsal subcutaneous pocket.²² The results showed similar histology between the PRP and control groups 10 days postoperatively; however, the control group expectedly had fewer normal adipocytes at 20 days postoperatively and the

PRP group had more granulation tissue and capillary formation and good maintenance of normal adipocytes for at least 120 days. Oh *et al.* mixed human lipoaspirated fat graft and activated PRP (by thrombin and CaCl_2) at a ratio of 7:2 and implanted it on the scalps of nude mice.¹⁸ They found higher PRP volume and weight in the control group after 10 weeks. The histology was analyzed, but only with a semiquantitative method with a grading scale. The results showed reduced cyst and fibrosis formation in the PRP group and no difference in integral fat and inflammation between the PRP and control groups. In summary, studies suggest that maintenance of viable adipocytes and increased angiogenesis can be achieved by activated PRP; PRP also can reduce inflammation and cyst formation.

Clinical Studies

Few clinical studies have been reported in the literature. Salgarello *et al.* presented their early experience with autologous fat graft combined with PRP at a ratio of 1:9 for breast reconstruction.⁴⁶ PRP was obtained using the Regenkit Extracell Adipocyte with one spin centrifuge at 3500 rpm for 5 min. PRP was activated by CaCl_2 . Plastic surgeons analyzed clinical outcomes for breast surgery using a grading scale. Breast ultrasound and mammography were used to detect fat necrosis. No difference in the grading score was found between the PRP and saline groups. The percentage of patients who experienced fat necrosis in the two groups did not differ significantly. Gentile *et al.* addressed the positive effect of PRP on the maintenance of clinical fat graft in breast reconstruction.¹⁷ One hundred patients were divided into two groups: one was treated with PRP/fat graft and the other group was treated by PRP only. The etiologies were breast soft tissue defect by unilateral breast hypoplasia, which is an outcome of breast cancer reconstruction and prostheses removal. Platelets were produced using a cascade system with 1100 g centrifugation for 10 min. PRP was activated by Ca^{2+} . The patients treated with PRP added to autologous fat grafts showed 69% maintenance of the contour restoration after 1 year, while the fat graft only group showed 39% maintenance. Gentile *et al.* also demonstrated the PRP could yield similar volume maintenance of fat graft as SVF by 69% and 63%, respectively.⁴⁷ Cervelli *et al.* further showed that 40% is the optimal PRP ratio for fat graft maintenance up to 50 weeks.¹⁶ They also found that local injection of insulin after 7 and 15 days in the 40% PRP/fat group further increased soft tissue restoration after 12 weeks compared with the 40% PRP/fat only group. However, these two studies mainly used a subjective evaluation to score the maintenance of defect restoration by (1) presence of asymmetry, deformity, and irregularity; (2) results of treatment area; (3) reabsorption of fat in one or more regions; (4) time of stabilization of the transplanted fat; and (5) need for retreatment. Although they claimed they also used an objective method by comparing the preoperative and postoperative photos at the same brightness, contrast, and size, they still used a subjective scoring system, which were not truly objective measurements. Currently, many quantitative tools or software can differentiate changes in three-dimensional volume, such as CT, MRI, and 3d-MD, which are more objective, reproducible, and examiner independent for explaining clinical results than a subjective scoring system.⁴⁸

TABLE 2. OVERVIEW OF ANIMAL STUDIES

	Animal model	Preparation of PRP	Mean platelets in PRP/in whole blood	Activation method	Growth factor concentration	PRP source	Fat graft source	Fat graft: PRP	Injection site	Follow-up period	Comment
Por <i>et al.</i> ²⁰	Nude mice	Processed by Medtronic Magellan system	280,000/nil/ μ l	Nil	Nil	Human	Human fat graft	4:1	Scalp	4 months	No increase in angiogenesis and viable adipocytes
Nakamura <i>et al.</i> ²²	Rat	Sugimori's method ⁵¹	1,400,000/440,000/ μ l	CaCl ₂	Nil	Rat	Rat inguinal fat	4:1	Subcutaneous dorsal pocket	4 months	Increased angiogenesis and viable adipocytes
Pires Fraga <i>et al.</i> ¹⁹	Rabbit	1. 1450 rpm 10 min 2. 2100 rpm 10 min	Nil	CaCl ₂ and thrombin	Nil	Rabbit	Rabbit dorsal scapular fat	Around 1:1	Ear	6 months	Increased angiogenesis and viable adipocytes
Rodriguez-Flores <i>et al.</i> ²¹	Rabbit	Anitua's method ⁵²	Nil	CaCl ₂	Nil	Rabbit	Rabbit groin fat pad	1:1	Lip	3 months	Less inflammation reaction Less oil cyst formation No increase in angiogenesis and viable adipocytes
Oh <i>et al.</i> ¹⁸	Nude mice	160 g 10 min 400 g 10 min	82,200/nil/ μ l	CaCl ₂ and thrombin	Nil	Human	Human	7:2	Scalp	10 weeks	Less fibrosis, less cyst formation, increased angiogenesis, similar integral adipocytes and inflammation

Platelet-Rich Fibrin and its Effect on Fat Grafts

Platelet-rich fibrin (PRF), so-called second-generation PRP, was first described by Choukroun *et al.* in France, mainly for use in oral and maxillary surgery.⁴⁹ The advantage of PRF is that, there is no need for anticoagulants or thrombin additives. PRF is very simple to obtain: one draws 10 mL of blood from the patient into a tube without adding anticoagulants and immediately centrifuges the blood at 3000 rpm for 10 min. Due to the absence of anticoagulant in the blood, the coagulation cascade is initiated immediately after the blood contacts the glass wall. The fibrinogen is transformed into a fibrin clot by the circulating thrombin, which is transformed from prothrombin after initiation of the coagulation cascade. The fibrin clot is obtained at the middle part of the tube after centrifugation, with the red blood cells at the bottom and the acellular plasma in the top. Concentrated platelets are believed to be trapped in the fibrin clot. The platelets are activated and growth factors are released and trapped in the fibrin polymer. The short duration between blood aspiration and centrifugation is the key factor in producing consistently clinical useful PRF. Several studies confirmed the gradual release of PDGF and TGF for 28 days from PRF, comparing the burst release of PRP within 1 day.^{50,51} A possible explanation is that PRF polymerizes with 3D architecture progressively, slowly, and naturally during centrifugation, which helps to entrap cytokines released from platelets with the fibrin polymer. In contrast, PRP is activated with a high concentration of thrombin, which makes the polymerization rapid, followed by strong contraction of the clots, from which fluids will expel. This will result in difficulties in entrapping cytokines released from platelets.

Due to the retention and slow release of growth factors from the platelets by PRF, some have begun to study whether PRF provides better fat graft survival than PRP. Liu *et al.* studied the effect of autologous PRF and/or SVF on fat graft survival.⁵⁰ They implanted a mixture of fat graft with PRF alone, SVF alone, or PRF with SVF on the rabbit ear. After 4 weeks, there was higher microvessel density and remaining adipose tissue area in the PRF+SVF+fat graft group compared to the other groups. The PRF and SVF only groups had similar microvessel density and remaining adipose tissue, but were still significantly higher than in the control group with fat graft only. After 24 weeks, the fat graft absorption rate was highest in the fat graft only group, followed by the fat graft with PRF group and the fat graft with SVF group, and the least in the fat graft with PRF and SVF group. The study addressed the effect of both PRF and SVF on increasing fat graft survival. In addition, PRF combined with SVF had a synergic effect on further fat graft survival. Keyhan *et al.* studied the effect of facial liposuction by comparing the combination of fat graft with either activated PRP or PRF.⁵² The outcome was evaluated by the amount of reabsorption, which was estimated by comparing pre- and postsurgical photographic views, pain, edema, and bruising. The results suggest that the combination of fat and PRF is more effective than fat and PRP in facial liposuction surgery.

Future Directions

In summary, activated PRP appears to increase fat graft survival in most small animal studies and some clinical

studies. Preclinical studies showed that the increased maintenance of mature adipocytes may be due to increased angiogenesis. However, the *in vitro* studies raised some concerns regarding osteogenic differentiation or transdifferentiation effect of PRP on preadipocytes and mature adipocytes. Furthermore, multipotent ASCs are a component of fat grafts, which are capable of differentiating directly to osteoblasts when exposed to PRP. Liu *et al.* showed that human PRP could induce the proliferation and osteogenic differentiation of human adipose-derived stromal cells; 10%–12.5% of human PRP seemed to be the optimal concentration.⁵³ It was also found that PRP could be combined with ASCs as injectable tissue-engineered bone to generate ectopic bone in the inguinal area of nude mice.⁵³ More detailed molecular experiments should be conducted to determine if the addition of PRP would induce bone formation in fat grafts and if PRP can induce adipogenic differentiation of ASCs.

Although most small animal studies report the success of PRP in fat grafting, to translate the results to clinical application, studies in large animal models should be performed, as larger animals simulate the anatomic, physiological, and biomechanical environments of humans far better than rodents. Nude mice and rabbits are not ideal for fat grafting experiments because these animals have very thin subcutaneous tissue. Obtaining lipoaspirates by liposuction to mimicking clinical situations cannot be achieved in mice or rabbit subcutaneous tissue. In contrast, lipoaspirates can be obtained with the Coleman procedure by harvesting abdominal subcutaneous fat from large animals to augment the recipient area, adequately mimicking clinical conditions.

The ratio of fat graft to PRP should be feasible to apply in clinical situations. The 1:1 ratio applied in two of the animal studies seemed unreasonable to apply in a clinical situation. For example, if a 100-mL fat graft is used to reconstruct a soft tissue defect in the breast, then 100 mL PRP is required at a ratio of 1:1, which means 1000 mL blood would need to be aspirated from the patient, because only 1 mL PRP is obtained from 10 mL of whole blood. In breast reconstruction, a 200–300 mL of fat graft is routinely needed, making the use of PRP impossible due to large loss of blood. Although a cell separator machine may be used to reinfuse the platelet-depleted blood back to the patient to compensate for the blood loss, the large platelet deprivation could cause abnormal coagulation. Hence, the minimally effective ratio of PRP to fat graft should be defined in animal studies.

PRF, the second generation of PRP, may be more effective than PRP due to the slow and long-term release of growth factors from the fibrin matrix. Kurita *et al.* demonstrated that PRP impregnated in biodegradable gelatin hydrogel can more effectively induce angiogenesis for critical ischemia treatment than PRP only.⁵⁴ Sell *et al.* incorporated PRP into an electrospun scaffold of silk fibroin, polyglycolic acid, or polycaprolactone.³⁰ They found sustained release of growth factor proteins up to 35 days in culture. The bioactivity of the PRP-electrospun scaffolds was demonstrated by enhancing the proliferation of ASCs and increasing chemotaxis of macrophages. Hence, strategies to incorporate slow releasing mechanisms of PRP components with fat grafts are a promising direction for future research. Since fat graft maintenance is achieved partly by proliferation and adipogenic-differentiation of ASCs and SVF has been demonstrated to be effective in fat graft survival,^{3,4,55} the addition of

PRP to SVF or ASCs to explore synergistic effects warrants further study.

Finally, controlled clinical studies are lacking. Available clinical studies are truly case-control studies. Double-blinded randomized controlled clinical studies are required to provide powerful evidence-based support in the future.

Conclusions

Most small animal studies and clinical outcomes studies appear to demonstrate the increased maintenance of fat graft volume by PRP, although there were a few studies with negative results. PRF, with its slow-release of growth factors, seemed to have a better effect on fat grafts than PRP. However, there is much variability in study design, methodology, and evaluation. Standard PRP preparation and activation methods should be established for fat grafting. We recommend that platelet numbers or growth factor concentrations should be recorded in every animal or clinical study despite different preparation methods, which can help researchers recognize the true effects of PRP. Activation methods should also be described precisely in published research. Quantitative measurements of volume change by 3d-MD, CT, or MRI should be used instead of a subjective scoring system. Furthermore, molecular mechanisms of PRP on fat grafting should be studied in more detail to support clinical use. Large animal studies and more randomized controlled clinical studies will be required to obtain consistent outcomes and establish guidelines.

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