Platelet-Rich Plasma and Platelet-Rich Fibrin Enhance the Outcomes of Fat Grafting: A Comparative Study

Shaoheng Xiong, M.D.
Lihong Qiu, M.D.
Yinjun Su, M.D., Ph.D.
Hui Zheng, M.D.
Chenggang Yi, M.D., Ph.D.

Xi'an, Shaanxi, People's Republic of China

Background: Autologous fat grafting is an efficient procedure in plastic surgery. However, its long-term tissue absorption is variable and technique-dependent. Platelet-rich plasma positively affects fat-grafting outcomes but still has shortcomings, and platelet-rich fibrin has been reported to have efficacy in fat transplantation. Here, we compared the effects of platelet-rich fibrin and platelet-rich plasma in fat grafting using histologic analysis.

Methods: Twenty rabbits were divided randomly into two groups. In each group, the groin region fat pads were cut into 1-mm^3 granules. Platelet-rich fibrin–treated or platelet-rich plasma–treated fat granules were transplanted into one ear, whereas the contralateral ear was transplanted with normal saline–treated fat granules. Histologic characteristics and capillary density of grafted tissue were analyzed 12 weeks after fat grafting.

Results: The grafted fat in the platelet-rich fibrin–treated group showed higher tissue retention than that in the control group [weight retention, 19.57 percent (interquartile range, 13.87 to 29.93 percent) versus 9.04 percent (interquartile range, 6.16 to 16.80 percent), $p < 0.05$; and volume retention, 18.00 percent (interquartile range, 10.50 to 26.50 percent) versus 8.00 percent (interquartile range, 5.75 to 13.25 percent), $p < 0.05$] and higher neovascularized capillary density than that in the platelet-rich plasma–treated and control groups. The platelet-rich plasma–treated group showed higher vessel density without superior tissue retention compared with the control group.

Conclusion: Platelet-rich fibrin increased tissue retention, quality, and vascularization of grafted fat compared with the control group and showed effects similar to those of platelet-rich plasma on tissue retention and histologic graft improvement. (*Plast. Reconstr. Surg.* 143: 1201e, 2019.)

Autologous fat grafting can result in long-lasting effects on tissue augmentation; therefore, adipose tissues are considered an ideal type of soft-tissue filler by plastic surgeons. This evolutionary procedure is one of the most commonly used strategies for aesthetic and reconstructive operations, including soft-tissue defects, facial rejuvenation, and breast augmentation, because of its multiple advantages (e.g., abundance, ease of collection, formation of microlesions, and lack of allergic reactions).[1,2] However, the outcomes of fat grafting are variable and technique-dependent. Significant progress has been made with various techniques in fat grafting, with the advent of Brava (Brava LLC, Miami, Fla.). However, although the retention predictability of grafted fat has been improved, the quality of grafted tissue is still unsatisfactory.[3–6] Recent studies have shown that early neovascularization of grafted fat plays a critical role in improving the quality and retention of

From the Department of Plastic Surgery, Xijing Hospital, Fourth Military Medical University.
Received for publication May 5, 2018; accepted November 7, 2018.

DOI: 10.1097/PRS.0000000000005624

Disclosure: *None of the authors has a financial interest in any of the products or devices mentioned in this article.*

A "Hot Topic Video" by Editor-in-Chief Rod J. Rohrich, M.D., accompanies this article. Go to PRSJournal.com and click on "Plastic Surgery Hot Topics" in the "Digital Media" tab to watch.

A Video Discussion by Alexes Hazen, M.D., accompanies this article. Go to PRSJournal.com and click on "Video Discussions" in the "Digital Media" tab to watch.

transplanted tissue.[7–9] Platelet concentrates, concentrated solutions of autologous platelets prepared by collecting the patient's own blood and submitting it to centrifugation several times, may help to address this predicament in fat grafting.

Platelet-rich plasma, a first-generation platelet concentrate, contributes to the degranulation of platelets, resulting in the release of various growth factors—including vascular endothelial growth factor (VEGF), basic fibroblast growth factor, platelet-derived growth factor, and epithelial growth factor—and cytokines.[10] Platelet-rich plasma reportedly improves grafted fat retention[11–13] by means of increased neovascularization,[14] enhanced proliferation and differentiation of adipose-derived stem cells,[15] and direct nutrient infiltration to the grafts. However, using exogenous additives during platelet-rich plasma preparation may cause adverse effects. Moreover, applying activators can trigger the sudden release of growth factors in platelet-rich plasma within 1 day, which may reduce its efficacy.[16] Importantly, the efficacy of platelet-rich plasma for outcomes in fat grafting remains controversial.[17]

The second-generation platelet concentrate platelet-rich fibrin was recently reported[18]; it is superior to platelet-rich plasma in many aspects, with its preparation being simpler than that of platelet-rich plasma and requiring only one centrifugation step. Moreover, it does not require the addition of exogenous additives, which promote natural physiologic polymerization of fibrin. The three-dimensional fibrin structure of platelet-rich fibrin facilitates platelet capture and growth factor bonding, which enhances the gradual and long-tern release of growth factors and cytokines.[19,20] This fibrin mesh also provides a framework for cell proliferation and differentiation and new blood vessel formation. A clinical self-control study comparing the effects of platelet-rich fibrin or platelet-rich plasma combined with a fat graft in facial lipostructure indicated that a greater average resorption was observed on the platelet-rich plasma/fat side (0.9 ± 0.3 with platelet-rich fibrin versus 1.4 ± 0.5 with platelet-rich plasma). However, the results were subjective and inconclusive, as the study evaluated the tissue resorption by comparing presurgical and postsurgical photographic views rather than using a more objective measurement such as three-dimensional scanning or magnetic resonance imaging.[21] Thus, platelet-rich fibrin may perform better and replace platelet-rich plasma in fat-grafting applications, although further studies are needed to support this. This study compared the effects of platelet-rich plasma or platelet-rich fibrin associated with fat grafting by histologically evaluating their functions in a rabbit fat transplantation model.

MATERIALS AND METHODS

Platelet-Rich Fibrin and Platelet-Rich Plasma Preparation

Platelet-rich fibrin preparation was performed as reported previously. Briefly, 10 ml of blood was drawn from rabbits and transferred into a sterile tube without any anticoagulant, and the sample was centrifuged immediately for 12 minutes at 1000 *g* in a laboratory centrifuge (Centrifuge 5804 R; Eppendorf, Germany). This centrifugation condition and the naturally polymerized fibrinogen resulted in a three-layer product in the tube; the platelet-rich

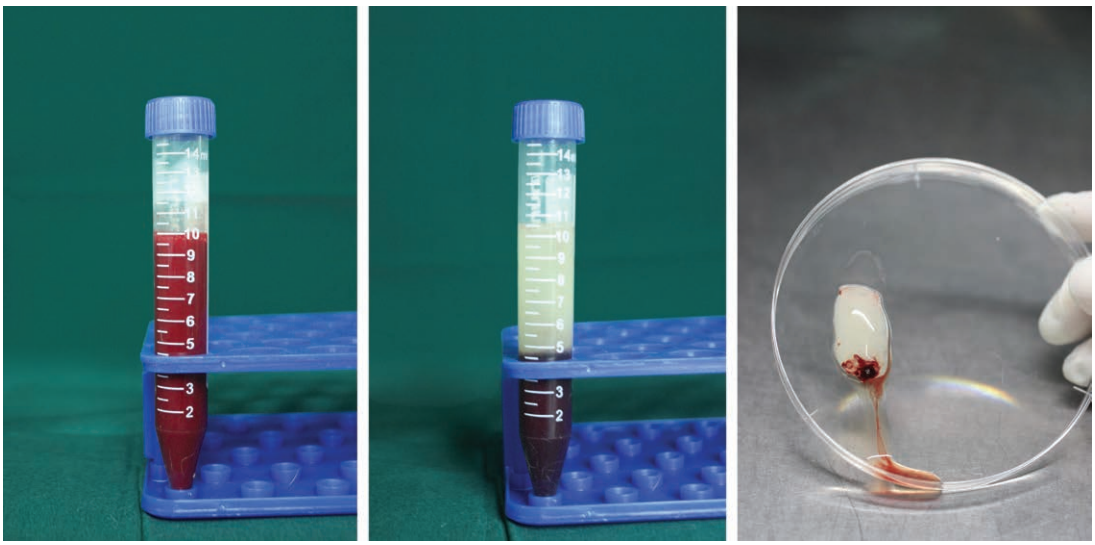

Fig. 1. Preparation of platelet-rich fibrin. (*Left*) Blood collection. (*Center*) After centrifugation (1000 *g* for 10 minutes). (*Right*) Platelet-rich fibrin was separated.

fibrin clot was in the middle layer (Fig. 1). Platelet-rich fibrin was then placed into a refrigerator at 4°C for less than 2 hours before fat grafting. During fat transplantation, the platelet-rich fibrin clot was cut into fragments (approximately 1 mm^3) and then mixed with prepared fat granules.

Platelet-rich plasma was prepared using a two-step centrifugation protocol. Briefly, 10 ml of blood was collected from the arteries of rabbits' ear and mixed with 1.5 ml of trisodium citrate. The blood samples were then divided into three layers (from top to bottom: platelet-poor plasma, platelets, and red blood cells) after the first centrifugation at 300 *g* for 10 minutes, and most of the bottom red blood cell layer was discarded. Next, the top and middle layers were transferred into new sterile syringes without anticoagulant to perform the second centrifugation at a speed of 800 *g* for 15 minutes. In this step, platelets were concentrated into the bottom, and the redundant superficial layer of platelet-poor plasma (approximately two-thirds) was discarded. Finally, the condensed platelets with few red blood cells were gently suspended with supernatant platelet-poor plasma to yield platelet-rich plasma (Fig. 2). Platelet-rich plasma was kept in the anticoagulated state and moved to the refrigerator

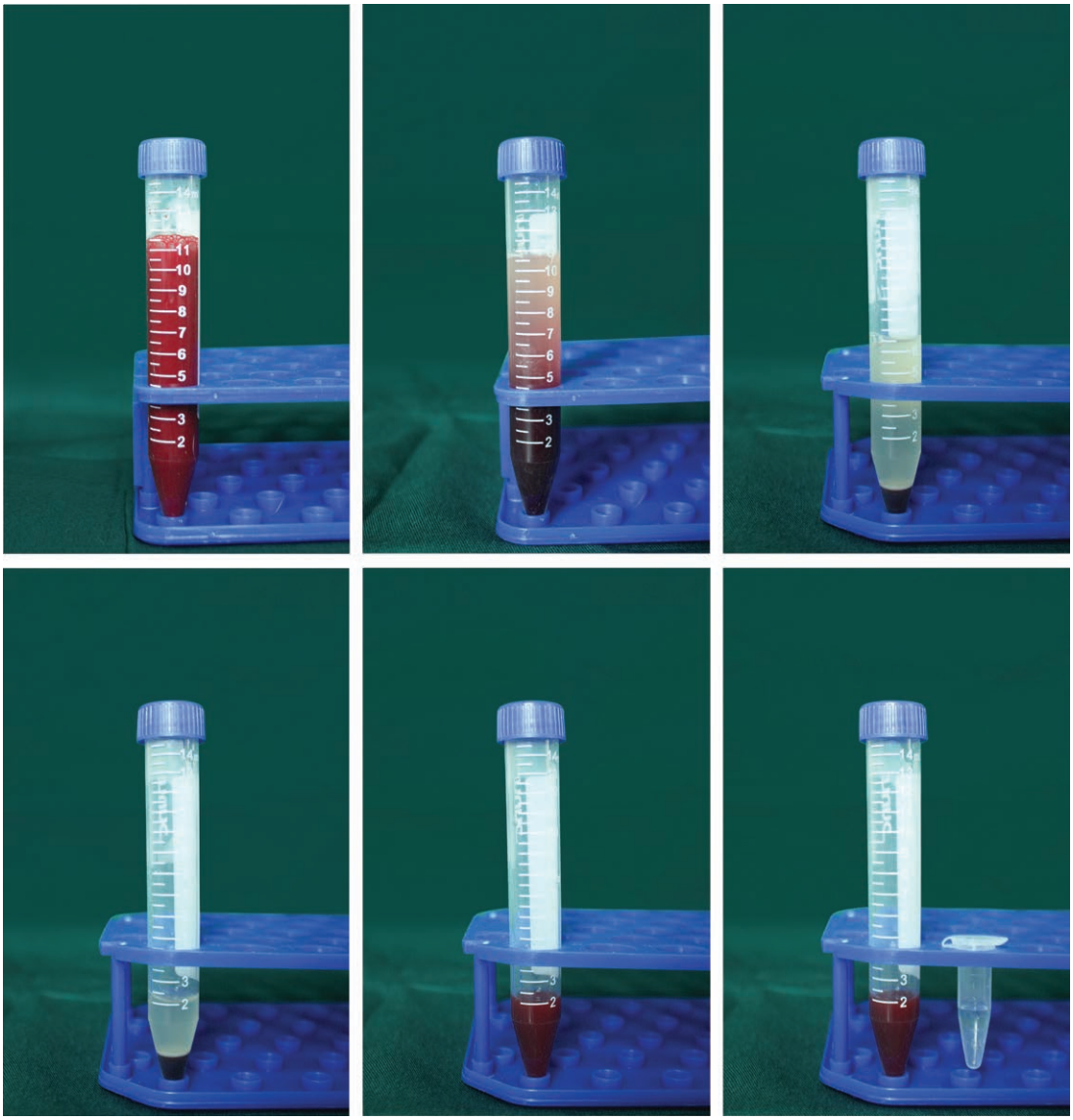

Fig. 2. The preparation of platelet-rich plasma. (*Above*, *left*) Blood collection. (*Above*, *center*) After the first centrifugation (300 *g* for 10 minutes), the bottom layer of red blood cells was discarded. (*Above*, *right*) After the second centrifugation (800 *g* for 15 minutes), (*below*, *left*) most of the serum was removed. (*Below*, *center*) The remaining serum was mixed with red blood cells to obtain platelet-rich plasma, and (*below*, *right*) platelet-rich plasma was activated by 10% calcium chloride before use.

at 4°C for less than 2 hours before use. Platelet-rich plasma was activated with calcium chloride within 5 minutes before being transplanted with fat.

Animals

The 20 female New Zealand rabbits (11 months old; average weight, 3.3 to 3.8 kg) (Experimental Animal Center, Fourth Military Medical University, Shaanxi, People's Republic of China) used in this study were divided randomly into two groups (platelet-rich fibrin and platelet-rich plasma; $n = 10$ per group). Platelet-rich fibrin–treated or platelet-rich plasma–treated fat granules were transplanted into one ear; the contralateral ear of each rabbit, which served as the control, was transplanted with normal saline–treated fat granules (platelet-rich fibrin group, normal saline 1; platelet-rich plasma group, normal saline 2) (Fig. 3). Antibiotic prophylaxis was given (intramuscular injection of 100,000 U/kg penicillin; North China Pharmaceutical Co. Ltd., Shijiazhuang, Hebei Province, People's Republic of China). Our experimental procedures and the use of animals in this study were approved by the Institutional Animal Care and Use Committee at the Fourth Military Medical University, Xi'an, People's Republic of China (approval no. LL-KY-20131226).

Surgical Procedure

Rabbits were anesthetized by pelltobarbitalum natricum (intravenous injection, 1.5%, 2 ml/kg; Merck, Darmstadt, Germany), and a sterile field was created around the inguinal region. Adipose tissue was then excised in a standard surgical procedure, with 4 g of clean adipose tissue harvested from each animal. Next, pure adipose tissue for transplantation was separated from harvested tissue, and blood vessels and fascia were discarded. The adipose tissues were then cut into small pieces (approximately 1 mm^3) to create adipose granules for transplantation.

Adipose granules were transplanted into the ears of rabbits because of the absence of autologous adipose tissue in this region. The prepared adipose granules were divided into two parts of 1 ml each, one of which was mixed with platelet-rich fibrin or platelet-rich plasma and grafted into one ear, and the other was mixed with normal saline and transplanted into the other ear. The grafted volume was measured using a sterile syringe. The mixing ratio between platelet-rich fibrin or platelet-rich plasma and fat was 1:2. All of the surgical procedures, including fat preparations and transplantations, and platelet-rich fibrin and platelet-rich plasma preparations, were performed by the same person.

Histologic Evaluation

The retention of grafted fat tissue was evaluated by macroscopic analysis of weight and volume retention. The rabbits were killed at the end of 12 weeks, the grafted fat pad was dissected, and the weight and volume of grafts were measured. Volume measurements were performed through the liquid overflow method.

The scoring systems for evaluating the histologic characteristics of grafted fat have been widely used in current studies.[22–24] The grafted fat pads were fixed in 10% formaldehyde and embedded in paraffin. The paraffin-embedded tissues were cut into 4-mm sections, deparaffinized, and rehydrated for hematoxylin and eosin staining. To evaluate the histologic characteristics, histologic sections were assessed using four parameters: the appearance of cysts or vacuoles, the degree of fibrosis, cell integrity, and the degree of tissue inflammation. The histologic parameters were graded by blinded evaluation as reported previously using a semiquantitative scale of 0 to 5 (0 = absence, 1 = minimal presence, 2 = minimal to moderate presence, 3 = moderate presence, 4 = moderate to extensive presence, and 5 = extensive presence).[25] All evaluations were performed blindly and independently by two experienced pathologists.

Assessment of Neovascularized Capillary Density

Neovascularized capillary density of grafted fat tissue was analyzed by the Weidner method with minor modifications. The paraffin-embedded tissues were stained immunohistochemically for CD31 at 12 weeks after transplantation. Briefly, the paraffin-embedded tissues were cut into 4-mm sections, dewaxed, and rehydrated. The sections were then subjected to microwave antigen retrieval in citric acid buffer (pH 6.0). Sections were incubated in 3% hydrogen peroxide in methanol solution for 10 minutes to block endogenous peroxidase activity in the tissue and with 5% normal bovine serum and then monoclonal mouse anti-rabbit primary antibodies targeting CD31 (ab187376; Abcam, Cambridge, United Kingdom) overnight at 4°C to block the nonspecific binding of immunoglobulins. Slides were then rewarmed for 1 hour at room temperature, washed with phosphate-buffered saline three times (10 minutes each), and then incubated with biotinylated mouse anti-rabbit secondary antibodies for 15 minutes at room temperature. Finally, the sections were reacted with diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis,

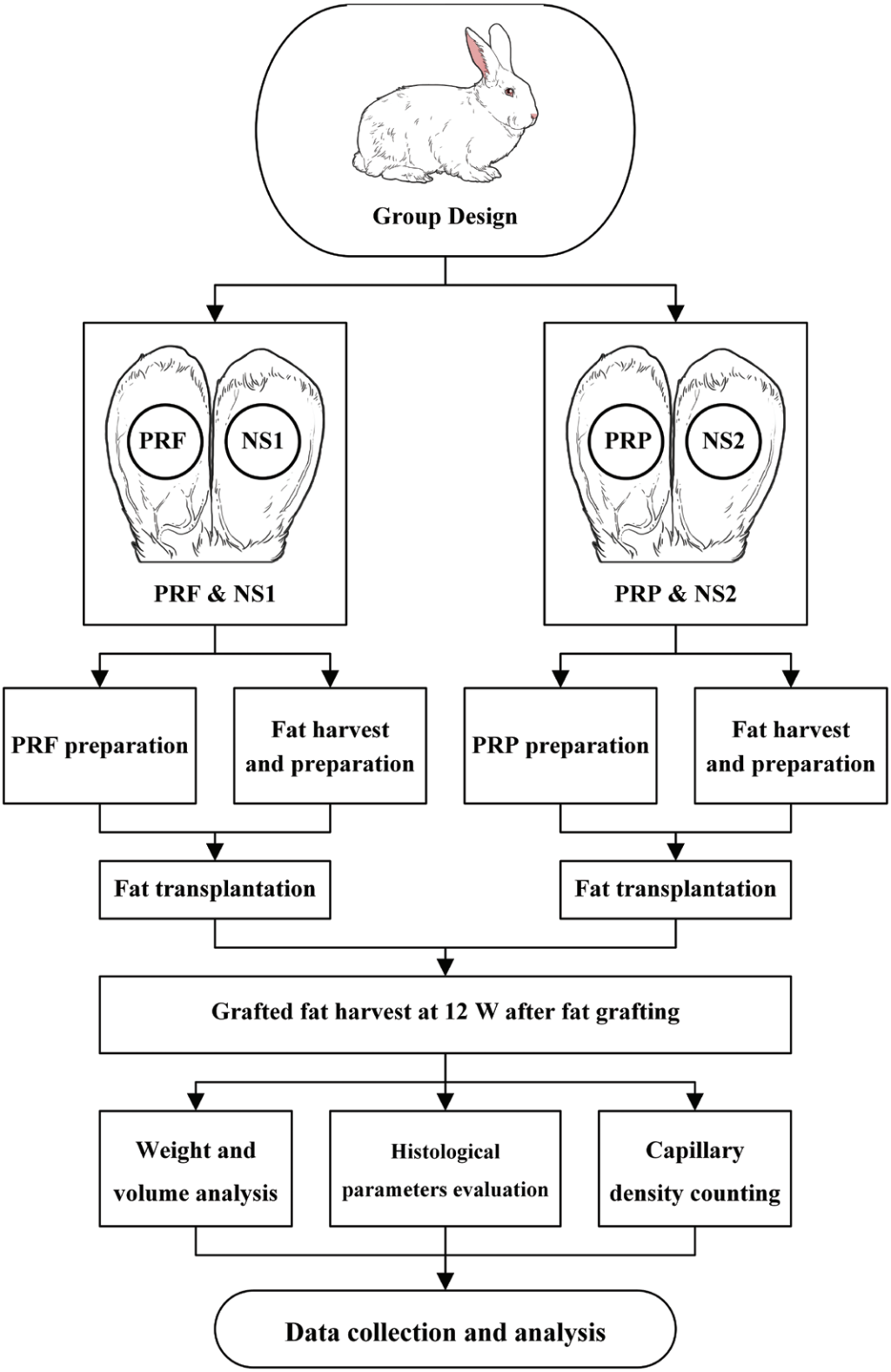

Fig. 3. Experimental design. *PRP*, platelet-rich plasma *PRF*, platelet-rich fibrin; *NS1*, normal saline 1; *NS2*, normal saline 2.

Mo.) for 4 minutes at room temperature and counterstained with hematoxylin. The slides were sealed by neutral balsam.

The neovascularized capillary densities were counted in 10 randomized fields on each section under a light microscope (Nikon 55i; Nikon

Corp., Tokyo, Japan) (original magnification, × 20). The randomization was performed as follows: each slide was pictured from the first complete countable field of view in the upper left corner of the tissue (under 20× magnification), horizontally from left to right, extracting an observation field from each field of view until the last countable field of view of the line. Going to the next line, the cycle was repeated from right to left. The fields of view were pictured alternately at intervals and each picture was numbered. Finally, 10 randomized fields on each section were chosen (from a random number table). All of the evaluations were performed blindly by two of the authors.

Statistical Analysis

Data are presented as medians (interquartile range) in tissue retention analysis and histologic evaluations. Means ± standard deviations were used to express the result of neovascularized capillary counting. SPSS Version 16.0 (SPSS, Inc., Chicago, Ill.) was used for analysis. In tissue retention analysis and histologic evaluation of grafted fat, comparisons of means in A (between platelet-rich fibrin and normal saline 1) and B (between platelet-rich plasma and normal saline 2) were performed with Wilcoxon signed rank tests, whereas comparisons of means in C (between platelet-rich fibrin and platelet-rich plasma) were performed with Mann-Whitney *U* tests. In neovascularized capillary density assessment, comparisons of means in A and B were performed with paired-samples *t* tests, whereas comparisons of means in C were performed with independent-samples *t* test. Differences with values of $p < 0.05$ were considered statistically significant.

RESULTS

Weight and Volume Retention of Grafted Fat

The weight and volume retention of grafted fat were observed at 12 weeks after fat grafting. The platelet-rich fibrin–treated group showed a higher weight and volume retention rate than the control group [weight retention, 19.57 percent (interquartile range, 13.87 to 29.93 percent) versus 9.04 percent (interquartile range, 6.16 to 16.80 percent), $p = 0.005$; and volume retention, 18.00 percent (interquartile range, 10.50 to 26.50 percent) versus 8.00 percent (interquartile range, 5.75 to 13.25 percent), $p = 0.005$] (Table 1). No statistically significant results were observed in comparisons between platelet-rich plasma and normal saline 2 [weight retention, 13.50 percent (interquartile range, 12.37 to 17.09 percent) versus 10.93 percent (interquartile range, 9.73 to 15.33 percent), $p = 0.059$; and volume retention 12.50 percent (interquartile range, 10.50 to 15.25) versus 8.00 percent (interquartile range, 7.00 to 12.00 percent), $p = 0.074$] (Table 1) and between platelet-rich fibrin and platelet-rich plasma (weight retention, 19.57 percent (interquartile range 13.87 to 29.93 percent) versus 13.50 percent (interquartile range, 12.37 to 17.09 percent), $p = 0.112$; and volume retention, 18.00 percent (interquartile range, 10.50 to 26.50 percent) versus 12.50 percent (interquartile range 10.50 to 15.25 percent), $p = 0.139$] (Table 1) (Fig. 4). Thus, platelet-rich fibrin increased the volume and weight retention of grafted fat compared with the control group.

Histologic Evaluation

The general observations of grafted fat are shown in Figure 5. Cysts/vacuoles and fibrosis were less frequent in the platelet rich fibrin–treated group than in the control group ($p < 0.05$). Fat integrity was higher in the platelet rich fibrin–treated group than in the control group ($p < 0.05$). Significantly less fibrosis and inflammation were observed in the platelet-rich plasma–treated group than in the control group (Table 2).

Capillary Density Assessment in Grafted Fat Tissue

To analyze the presence of neovascularized capillaries in grafted fat tissue, we measured the

Table 1. Comparisons of Weight and Volume Retention Rate

	A			B			C		
	PRF	NS1	*p*	PRP	NS2	*p*	PRF	PRP	*p*
Weight, %	19.57 (13.87–29.93)	9.04 (6.16–16.80)	<0.05	13.50 (12.37–17.09)	10.93 (9.73–15.33)	>0.05	19.57 (13.87–29.93)	13.50 (12.37–17.09)	>0.05
Volume, %	18.00 (10.50–26.50)	8.00 (5.75–13.25)	<0.05	12.50 (10.50–15.25)	8.00 (7.00–12.00)	>0.05	18.00 (10.50–26.50)	12.50 (10.50–15.25)	>0.05

PRF, platelet-rich fibrin; PRP, platelet-rich plasma; NS, normal saline.
*Data are presented as medians (interquartile range). Comparisons of means in A (between PRF and NS1) and B (between PRP and NS2) were performed with Wilcoxon signed rank tests; comparisons of means in C (between PRF and PRP) were performed with Mann-Whitney *U* tests. Results with values of $p < 0.05$ were considered statistically significant.

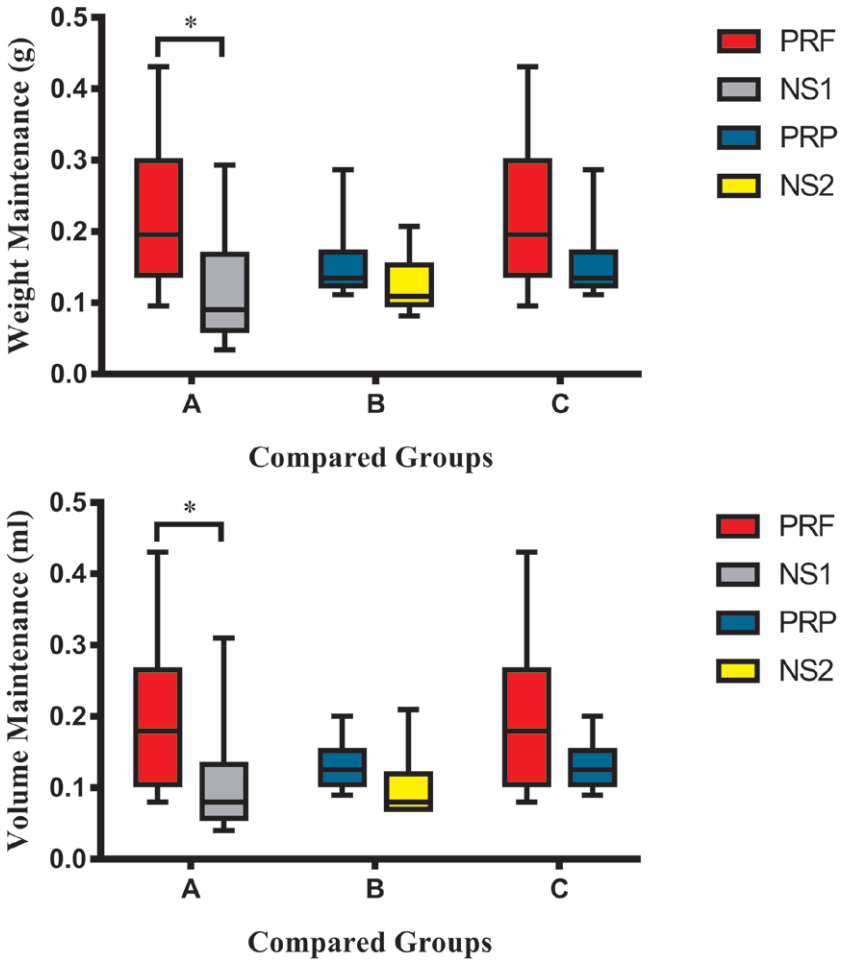

Fig. 4. Box plot showing the weight (*above*) and volume (*below*) retention of grafted fat at 12 weeks after transplantation. *PRF*, platelet-rich fibrin; *PRP*, platelet-rich plasma; *NS*, normal saline; *A*, comparison between platelet-rich fibrin and normal saline 1; *B*, comparison between platelet-rich plasma and normal saline 2; *C*, comparison between platelet-rich fibrin and platelet-rich plasma.

capillary density by immunohistochemical staining of CD31 (Fig. 6). The vascular densities per optical field in platelet-rich fibrin–treated, normal saline 1–treated, platelet-rich plasma–treated, and normal saline 2–treated groups were 17.38 ± 2.35, 5.66 ± 2.01, 10.40 ± 2.54, and 5.56 ± 1.45, respectively, at 12 weeks after transplantation (Table 3). Significant differences were found between multiple comparisons at 12 weeks after fat grafting (Fig. 6, *below*), suggesting that both platelet-rich fibrin and platelet-rich plasma enhanced the neovascularization of grafted fat tissue and that platelet-rich fibrin increased the vascular density to a greater extent than platelet-rich plasma ($p < 0.05$).

DISCUSSION

In this study, we compared the effects of platelet-rich fibrin and platelet-rich plasma on fat grafting and observed whether platelet-rich fibrin was more effective than platelet-rich plasma during fat transplantation using a rabbit autologous fat grafting model. Our results showed that the grafted tissue was better maintained in the platelet-rich fibrin group than in the control group. However, there were no significant differences between the platelet-rich plasma and control groups or between the platelet-rich fibrin and platelet-rich plasma groups in tissue retention. In addition, the platelet-rich fibrin group displayed higher cell integrity, fewer cysts/vacuoles, and lower fibrosis than the control group. The platelet-rich plasma group displayed lower tissue inflammation and lower fibrosis than the control group. However, no differences between the histologic characteristics of the platelet-rich fibrin and platelet-rich plasma groups were observed. Moreover, the

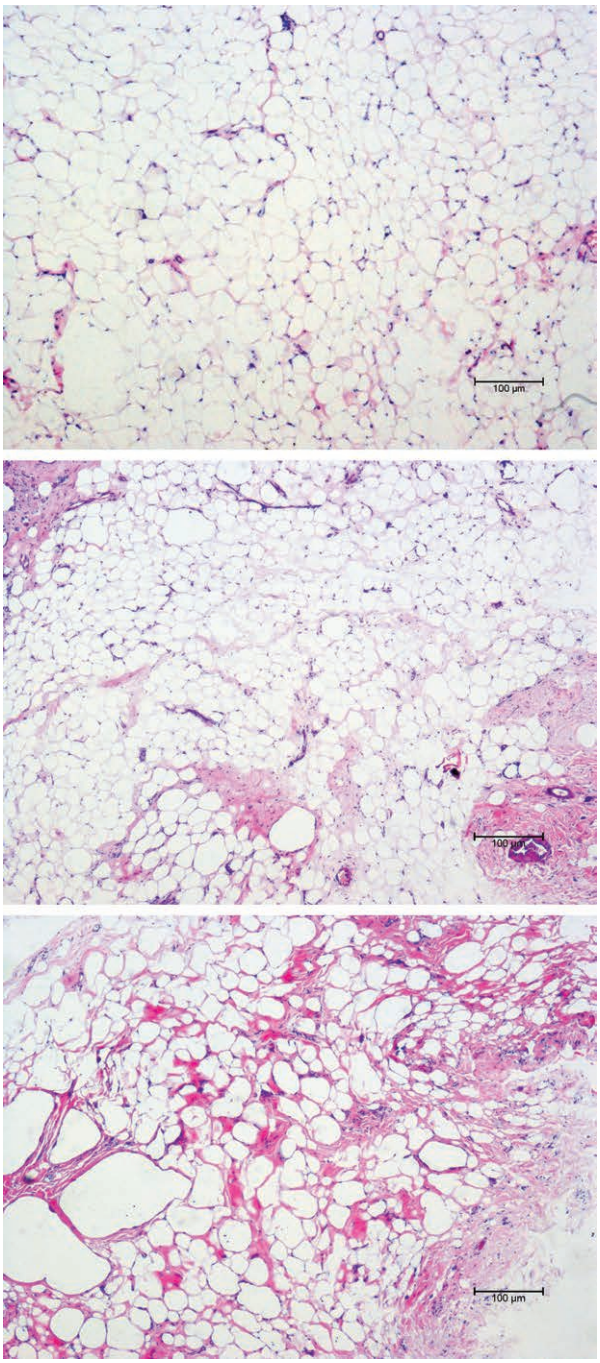

Fig. 5. Histologic analysis of grafted fat at 12 weeks after transplantation. Tissues were cut into 4-μm sections, and each section was stained with hematoxylin and eosin. Representative photographs of the grafted fat of platelet rich fibrin–treated (*above*), platelet-rich plasma–treated (*center*), and normal saline–treated (*below*) groups are shown (original magnification, × 10).

neovascular density of grafts in the platelet-rich fibrin group was higher than those in the platelet-rich plasma and control groups at 12 weeks after fat transplantation. Thus, our findings suggested that platelet-rich fibrin improved outcomes in fat grafting compared with those in the control group by increasing tissue retention, enhancing graft quality, and stimulating graft fat neovascularization.

Platelet-rich fibrin increased tissue retention and enhanced graft quality compared with the control group, contributing to improvement of neovascularization and increased neovascular density. Similarly, current studies have shown that platelet-rich fibrin increases vascularization by stimulating the production of angiogenesis-related factors (e.g., VEGF, basic fibroblast growth factor, platelet-derived growth factor, and epidermal growth factor), which leads to new blood vessel formation by modulating the activation, proliferation, and migration of endothelial cells.[26,27] Mammoto et al. reported that platelet-rich fibrin induces vascular network formation in mice.[14] In another in vivo study, mRNA and protein expression of hypoxia-inducible factor-1α and VEGF and tissue neovascularization were increased in the platelet-rich fibrin treatment group.[28] Moreover, our results showed that platelet-rich fibrin induced a higher capillary density than platelet-rich plasma, potentially because of the unique three-dimensional fibrin structure of platelet-rich fibrin, which plays an important role in tissue repair and regeneration. Unlike platelet-rich plasma, platelet-rich fibrin does not contain anticoagulants, permitting natural and progressive polymerization during centrifugation. This progressive polymerization resulted in a high-density, highly coherent, homogeneous three-dimensional fibrin structure framework; this structure significantly facilitated the incorporation of platelets and circulating growth factors/cytokines into the fibrin mesh.[29] Thus, platelet-rich fibrin significantly prolongs the release time of growth factors/cytokines, which could have long-term effects on fat grafting by gradually releasing these growth factors, in contrast to the rapid release observed for platelet-rich plasma. Indeed, platelet-rich fibrin has been shown to release more growth factors for a longer period and can strongly induce cell migration compared with platelet-rich plasma.[30] Moreover, this three-dimensional fibrin framework provided a perfect scaffold for neovessel formation[31] and was particularly favorable for the migration and proliferation of certain cells, such as adipose-derived stem cells. Recent studies[32–35] have shown that abundant angiogenesis is crucial for maintaining and improving the quality of grafted tissue. Therefore, platelet-rich fibrin increased the neovascularization of grafts and further improved the tissue

Table 2. Comparisons of Histologic Evaluations

	A			B			C		
	PRF	NS1	*p*	PRP	NS2	*p*	PRF	PRP	*p*
Cysts/vacuoles	1.00 (0.75–2.00)	2.00 (2.00–3.00)	<0.05	2.00 (1.00–2.25)	1.5 (1.00–3.00)	>0.05	1.00 (0.75–2.00)	2.00 (1.00–2.25)	>0.05
Fibrosis	2.00 (1.00–3.00)	4.00 (2.00–4.00)	<0.05	3.00 (2.75–3.00)	4.00 (3.00–4.00)	<0.05	2.00 (1.00–3.00)	3.00 (2.75–3.25)	>0.05
Integrity	4.00 (2.75–4.25)	3.00 (2.00–3.00)	<0.05	3.00 (3.00–4.00)	3.00 (2.75–3.00)	>0.05	4.00 (2.75–4.25)	3.00 (3.00–4.00)	>0.05
Inflammation	2.00 (1.75–3.25)	3.00 (2.00–3.25)	>0.05	2.00 (1.75–3.00)	3.00 (2.75–4.00)	<0.05	2.00 (1.75–3.25)	2.00 (1.75–3.00)	>0.05

PRF, platelet-rich fibrin; PRP, platelet-rich plasma; NS, normal saline.
*Data are presented as medians (interquartile range). The scores were made independently by two of the authors blinded to the groups. Comparisons of means in A (between PRF and NS1) and B (between PRP and NS2) were performed with Wilcoxon signed rank tests; comparisons of means in C (between PRF and PRP) were performed with Mann-Whitney *U* tests. Results with values of $p < 0.05$ were considered statistically significant.

retention of grafted fat. Notably, no significant difference was found in tissue retention between platelet-rich fibrin and platelet-rich plasma, which may be related to the limited number of animals or the limited improvement between platelet-rich fibrin and platelet-rich plasma.

Furthermore, our results supported that platelet-rich plasma could increase the angiogenesis of

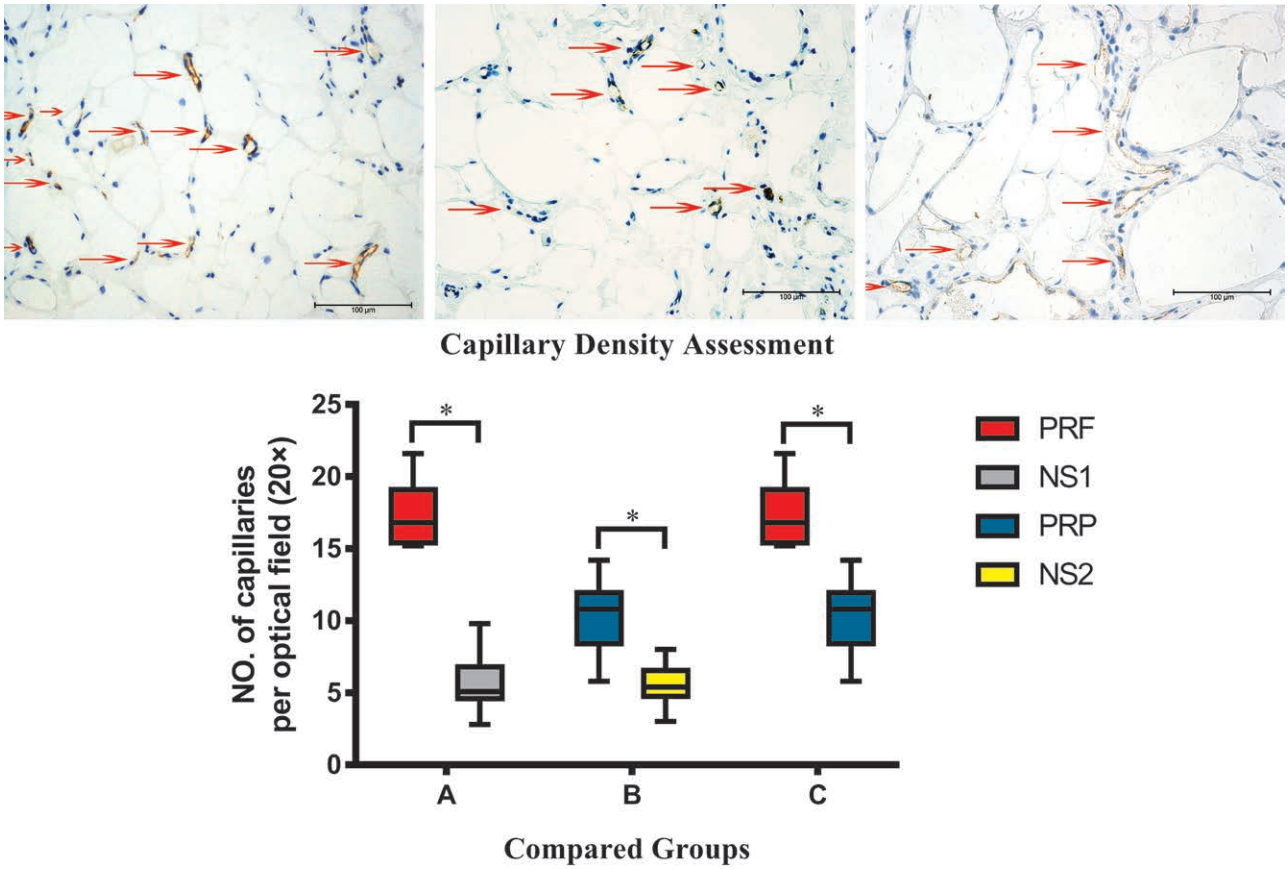

Fig. 6. Immunohistologic staining and capillary counts in the grafted fat at 12 weeks after transplantation. Tissues were cut into 4-μm sections, and paraffin-embedded sections were incubated with monoclonal anti-CD31 antibodies. Representative micrographs of platelet-rich fibrin–treated (*above*, *left*), platelet-rich plasma–treated (*above*, *center*), and normal saline–treated (*above*, *right*) grafts are shown (original magnification, × 20). The *red arrows* indicate vessels. The box plot (*below*) shows the numbers of $CD31^+$ capillaries per optical field (original magnification, × 20; $n = 10$). *PRF*, platelet-rich fibrin; *PRP*, platelet-rich plasma; *NS*, normal saline; *A*, comparison between platelet-rich fibrin and normal saline 1; *B*, comparison between platelet-rich plasma and normal saline 2; *C*, comparison between platelet-rich fibrin and platelet-rich plasma.

Table 3. Comparisons of Capillary Count

	A			B			C		
	PRF	NS1	*p*	PRP	NS2	*p*	PRF	PRP	*p*
MVD	17.38 ± 2.35	5.66 ± 2.01	<0.05	10.40 ± 2.54	5.56 ± 1.45	<0.05	17.38 ± 2.35	10.40 ± 2.54	<0.05

MVD, microvessel density; PRF, platelet-rich fibrin; PRP, platelet-rich plasma; NS, normal saline.
*Data are presented as means ± standard deviations. Comparisons of means in A (between PRF and NS1) and B (between PRP and NS2) were performed with paired-samples *t* tests; comparisons of means in C (between PRF and PRP) were performed with independent-samples *t* tests. Results with values of $p < 0.05$ were considered statistically significant.

grafts compared with that in the control group; however, this effect was inferior to that of platelet-rich fibrin, which may explain the positive effects on grafted tissue quality (increasing the newly formed vessels) and the negative effects of platelet-rich plasma on tissue retention (insufficient vascularization). The effects of platelet-rich plasma are mainly associated with degranulation of platelets. Several fundamental studies have shown the positive effects of platelet-rich plasma on inducing neoangiogenesis, improving adipose-derived stem cell proliferation, and blocking apoptosis.[36–38] In addition, a recent systematic review of the effects of platelet-rich plasma and fat cotransplantation showed that platelet-rich plasma improved the results of fat grafting in most published in vivo studies.[17] However, the clinical applications of platelet-rich plasma are still controversial, and it is difficult to evaluate the efficiency of platelet-rich plasma in fat grafting without consistent and ample methodologic details. Some other studies also demonstrated that the beneficial in vitro effects of adding platelet-rich plasma may only be partially reflected in fat transplantation in the clinical setting.[39–42] The controversies of applying platelet-rich plasma in the clinic can be explained by the following three concepts. First, there is large variability in the obtained platelet-rich plasma (because of preparation methodologies and activation) among various studies, leading to discrepant results. In addition, the progressive use of commercial devices can further worsen this issue because of their wide applications and varying preparation protocols. Second, the suitable mixing ratio of platelet-rich plasma to fat is still unclear. In our experiment, we used a 1:2 ratio of platelet-rich plasma to fat, which is the most commonly used mixing ratio in most in vivo studies reporting positive results.[17,43] However, we did not observe positive effects of platelet-rich plasma combined with fat grafting. There are large variations in the mixing ratio in platelet-rich plasma applications among current in vitro and in vivo studies. Some studies have even shown opposite outcomes using different ratios; for example, some studies have reported that 5 percent platelet-rich plasma is the optimal ratio to promote the proliferation of adipose-derived stem cells, and increased concentrations showed inhibitory effects.[44,45] In contrast, another study demonstrated concentration-dependent effects of platelet-rich plasma using this ratio. This discrepancy has limited the applications of platelet-rich plasma for fat grafts.[38] Third, the use of an activator would reduce the efficiency of platelet-rich plasma. The process of neovascularization and tissue remodeling of grafted fat is protracted, lasting for a long period and requiring a stable and continuous level of growth factors/cytokines. However, the use of an activator in platelet-rich plasma led to a burst of released growth factors/cytokines, decreasing the lifespan of these biomolecules and ultimately resulting in levels too low to support tissue neovascularization. In addition, time-consuming preparation and high cost of platelet-rich plasma have also hindered its development.

There are some limitations to this study. First, the dose-response relationship between platelet-rich plasma/platelet-rich fibrin and fat is still unknown. We used various concentrations, and there is still no consensus on the optimal concentration for application of platelet-rich plasma/platelet-rich fibrin. Moreover, because we prepared platelet-rich plasma or platelet-rich fibrin using equal amounts of blood and applied the same ratio to fat, the dose-response relationships between platelet-rich plasma and platelet-rich fibrin may even be different for the same amounts of platelet-rich plasma or platelet-rich fibrin. The dose-response relationships of platelet-rich plasma/platelet-rich fibrin should be examined in further studies. Second, the tissue evaluations were limited by the animals and reagents used in this study. The rabbit seems the most suitable animal for this study. Small animals such as Sprague-Dawley rats were unsuitable for this study, as adequate fat for fat grafting or enough blood for platelet-rich fibrin or platelet-rich plasma preparation could not have been obtained. The same could be said for nude mice. Moreover, experimental results from large animals are more informative than those from small animals. However,

the currently available experimental reagents such as antibodies are more specific for research in Muridae animals (such as Sprague-Dawley rats and nude mice) than for research in rabbits. This imposed a limitation in terms of the availability of appropriate reagents for tissue evaluation. Finally, the underlying mechanisms of platelet-rich plasma/platelet-rich fibrin in fat grafting remain unclear.

In future research, we intend to explore the detailed molecular mechanisms of platelet-rich plasma/platelet-rich fibrin in fat transplantation. Furthermore, more high-quality, placebo-controlled, randomized, double-blind clinical trials, and more objective measurement methods, are necessary to evaluate the effects of platelet-rich plasma/platelet-rich fibrin in fat grafting.

CONCLUSIONS

To the best of our knowledge, this is the first comparative preclinical study of the effects of platelet-rich plasma or platelet-rich fibrin on autologous fat grafting. In this rabbit autologous fat transplantation model, platelet-rich fibrin enhanced the weight and volume retention of grafted fat, increased the neovascularization of grafts, and improved the quality of transplanted tissue by reducing cyst/vacuole numbers, decreasing fibrosis, and improving cell integrity at 12 weeks after grafting. Even though no differences in tissue retention were observed between the platelet-rich plasma and control groups, platelet-rich plasma significantly increased the angiogenesis of grafted tissue and improved the quality of grafts. Considered together, our results showed that platelet-rich fibrin could improve the outcomes of fat grafting. There was no significant difference in tissue retention and quality improvement of grafts between platelet-rich fibrin and platelet-rich plasma.

Chenggang Yi, M.D., Ph.D.
Department of Plastic Surgery
Xijing Hospital
Fourth Military Medical University
No. 15, Changle West Road
Xi'an, Shaanxi 710032, People's Republic of China
yichg@163.com

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (grant nos. 81471884, 81671932, and 81701919). The authors thank their colleagues at the Institute of Plastic Surgery, Xijing Hospital, Fourth Military Medical University, for cooperation and support; and Chen Xiong for assistance in drawing the schematic diagram.

REFERENCES

1. Coleman SR. Structural fat grafting: More than a permanent filler. *Plast Reconstr Surg.* 2006;118(Suppl):108S–120S.
2. Pu LLQ. Fat grafting for facial rejuvenation and contouring: A rationalized approach. *Ann Plast Surg.* 2018;81(Suppl 1):S102–S108.
3. Khouri RK, Khouri RK Jr. Current clinical applications of fat grafting. *Plast Reconstr Surg.* 2017;140:466e–486e.
4. Khouri RK, Rigotti G, Khouri RK Jr, et al. Tissue-engineered breast reconstruction with Brava-assisted fat grafting: A 7-year, 488-patient, multicenter experience. *Plast Reconstr Surg.* 2015;135:643–658.
5. Khouri RK, Khouri RK Jr, Rigotti G, et al. Aesthetic applications of Brava-assisted megavolume fat grafting to the breasts: A 9-year, 476-patient, multicenter experience. *Plast Reconstr Surg.* 2014;133:796–807; discussion 808–809.
6. Khouri RK Jr. Discussion: Enhancement of progenitor cells by two-step centrifugation of emulsified lipoaspirates. *Plast Reconstr Surg.* 2018;142:110–111.
7. Pu LL. Mechanisms of fat graft survival. *Ann Plast Surg.* 2016;77(Suppl 1):S84–S86.
8. Khouri RK Jr. Discussion: Improved long-term volume retention of stromal vascular fraction gel grafting with enhanced angiogenesis and adipogenesis. *Plast Reconstr Surg.* 2018;141:687e–688e.
9. Zhang Y, Cai J, Zhou T, et al. Improved long-term volume retention of stromal vascular fraction gel grafting with enhanced angiogenesis and adipogenesis. *Plast Reconstr Surg.* 2018;141:676e–686e.
10. Frautschi RS, Hashem AM, Halasa B, Cakmakoglu C, Zins JE. Current evidence for clinical efficacy of platelet rich plasma in aesthetic surgery: A systematic review. *Aesthet Surg J.* 2017;37:353–362.
11. Pires Fraga MF, Nishio RT, Ishikawa RS, Perin LF, Helene A Jr, Malheiros CA. Increased survival of free fat grafts with platelet-rich plasma in rabbits. *J Plast Reconstr Aesthet Surg.* 2010;63:e818–e822.
12. Cervelli V, Palla L, Pascali M, De Angelis B, Curcio BC, Gentile P. Autologous platelet-rich plasma mixed with purified fat graft in aesthetic plastic surgery. *Aesthetic Plast Surg.* 2009;33:716–721.
13. Sasaki GH. The safety and efficacy of cell-assisted fat grafting to traditional fat grafting in the anterior mid-face: An indirect assessment by 3D imaging. *Aesthetic Plast Surg.* 2015;39:833–846.
14. Mammoto T, Jiang A, Jiang E, Mammoto A. Platelet rich plasma extract promotes angiogenesis through the angiopoietin1-Tie2 pathway. *Microvasc Res.* 2013;89:15–24.
15. Felthaus O, Prantl L, Skaff-Schwarze M, et al. Effects of different concentrations of platelet-rich plasma and platelet-poor plasma on vitality and differentiation of autologous adipose tissue-derived stem cells. *Clin Hemorheol Microcirc.* 2017;66:47–55.
16. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009;108:707–713.
17. Luck J, Smith OJ, Mosahebi A. A systematic review of autologous platelet-rich plasma and fat graft preparation methods. *Plast Reconstr Surg Glob Open* 2017;5:e1596.

18. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101:e37–e44.
19. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101:e45–e50.
20. Jiménez-Aristizabal RF, López C, Álvarez ME, Giraldo C, Prades M, Carmona JU. Long-term cytokine and growth factor release from equine platelet-rich fibrin clots obtained with two different centrifugation protocols. *Cytokine* 2017; 97:149–155.
21. Keyhan SO, Hemmat S, Badri AA, Abdeshahzadeh A, Khiabani K. Use of platelet-rich fibrin and platelet-rich plasma in combination with fat graft: Which is more effective during facial lipostructure? *J Oral Maxillofac Surg*. 2013;71:610–621.
22. Xing W, Mu D, Wang Q, Fu S, Xin M, Luan J. Improvement of fat graft survival with autologous bone marrow aspirate and bone marrow concentrate: A one-step method. *Plast Reconstr Surg*. 2016;137:676e–686e.
23. Luan A, Zielins ER, Wearda T, et al. Dynamic rheology for the prediction of surgical outcomes in autologous fat grafting. *Plast Reconstr Surg*. 2017;140:517–524.
24. Kokai LE, Jones TL, Silowash R, et al. Optimization and standardization of the immunodeficient mouse model for assessing fat grafting outcomes. *Plast Reconstr Surg*. 2017;140:1185–1194.
25. Yi CG, Xia W, Zhang LX, et al. VEGF gene therapy for the survival of transplanted fat tissue in nude mice. *J Plast Reconstr Aesthet Surg*. 2007;60:272–278.
26. Martínez CE, Smith PC, Palma Alvarado VA. The influence of platelet-derived products on angiogenesis and tissue repair: A concise update. *Front Physiol*. 2015;6:290.
27. Roy S, Driggs J, Elgharably H, et al. Platelet-rich fibrin matrix improves wound angiogenesis via inducing endothelial cell proliferation. *Wound Repair Regen*. 2011;19:753–766.
28. Xu FT, Liang ZJ, Li HM, et al. Ginsenoside Rg1 and platelet-rich fibrin enhance human breast adipose-derived stem cell function for soft tissue regeneration. *Oncotarget* 2016;7:35390–35403.
29. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101:e45–e50.
30. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. *Clin Oral Investig*. 2016;20:2353–2360.
31. Schär MO, Diaz-Romero J, Kohl S, Zumstein MA, Nesic D. Platelet-rich concentrates differentially release growth factors and induce cell migration in vitro. *Clin Orthop Relat Res*. 2015;473:1635–1643.
32. Suga H, Eto H, Aoi N, et al. Adipose tissue remodeling under ischemia: Death of adipocytes and activation of stem/progenitor cells. *Plast Reconstr Surg*. 2010;126:1911–1923.
33. Sunaga A, Sugawara Y, Katsuragi-Tomioka Y, Kobayashi E. The fate of nonvascularized fat grafts: Histological and bioluminescent study. *Plast Reconstr Surg Glob Open* 2013;1:e40.
34. Eto H, Kato H, Suga H, et al. The fate of adipocytes after nonvascularized fat grafting: Evidence of early death and replacement of adipocytes. *Plast Reconstr Surg*. 2012;129: 1081–1092.
35. Garza RM, Rennert RC, Paik KJ, et al. Studies in fat grafting: Part IV. Adipose-derived stromal cell gene expression in cell-assisted lipotransfer. *Plast Reconstr Surg*. 2015;135: 1045–1055.
36. Kakudo N, Morimoto N, Kushida S, Ogawa T, Kusumoto K. Platelet-rich plasma releasate promotes angiogenesis in vitro and in vivo. *Med Mol Morphol*. 2014;47:83–89.
37. Fukaya Y, Kuroda M, Aoyagi Y, et al. Platelet-rich plasma inhibits the apoptosis of highly adipogenic homogeneous preadipocytes in an in vitro culture system. *Exp Mol Med*. 2012;44:330–339.
38. Cervelli V, Scioli MG, Gentile P, et al. Platelet-rich plasma greatly potentiates insulin-induced adipogenic differentiation of human adipose-derived stem cells through a serine/threonine kinase Akt-dependent mechanism and promotes clinical fat graft maintenance. *Stem Cells Transl Med*. 2012;1:206–220.
39. Willemsen JCN, Van Dongen J, Spiekman M, et al. The addition of PRP to facial lipofilling. *Plast Reconstr Surg*. 2018;142:796e–798e.
40. Rigotti G, Charles-de-Sá L, Gontijo-de-Amorim NF, et al. Expanded stem cells, stromal-vascular fraction, and platelet-rich plasma enriched fat: Comparing results of different facial rejuvenation approaches in a clinical trial. *Aesthet Surg J*. 2016;36:261–270.
41. Fontdevila J, Guisantes E, Martínez E, Prades E, Berenguer J. Double-blind clinical trial to compare autologous fat grafts versus autologous fat grafts with PDGF: No effect of PDGF. *Plast Reconstr Surg*. 2014;134:219e–230e.
42. Salgarello M, Visconti G, Rusciani A. Breast fat grafting with platelet-rich plasma: A comparative clinical study and current state of the art. *Plast Reconstr Surg*. 2011;127:2176–2185.
43. Xiong S, Qiu L, Zhao J, et al. The role of platelet concentrates in facial fat grafting. *Ann Plast Surg*. 2018;81(Suppl 1):S117–S123.
44. Kakudo N, Minakata T, Mitsui T, Kushida S, Notodihardjo FZ, Kusumoto K. Proliferation-promoting effect of platelet-rich plasma on human adipose-derived stem cells and human dermal fibroblasts. *Plast Reconstr Surg*. 2008;122:1352–1360.
45. Liao HT, James IB, Marra KG, Rubin JP. The effects of platelet-rich plasma on cell proliferation and adipogenic potential of adipose-derived stem cells. *Tissue Eng Part A* 2015;21:2714–2722.