Subcutaneous Injections of Platelet-Rich Plasma into Skin Flaps Modulate Proangiogenic Gene Expression and Improve Survival Rates

Weiwei Li, M.D., Ph.D.
Mitsuhiro Enomoto, M.D., Ph.D.
Madoka Ukegawa, M.D.
Takashi Hirai, M.D.
Shinichi Sotome, M.D., Ph.D.
Yoshiaki Wakabayashi, M.D., Ph.D.
Kenichi Shinomiya, M.D., Ph.D.
Atsushi Okawa, M.D., Ph.D.

Tokyo, Japan

Background: Flap necrosis remains a major complication of reconstructive surgery. To improve skin flap survival, various treatments with vasodilators, antiplatelet drugs, or the local administration of growth factors have been performed. However, the sufficient prevention of skin necrosis is not well established. Platelet-rich plasma has been used as an autologous factor and includes various growth factors. The authors evaluated whether or not platelet-rich plasma can improve skin flap survival in an experimental rat model.
Methods: Cranially based dorsal cutaneous flaps were elevated in 48 rats. The animals received subcutaneous injections of either platelet-rich plasma (100 μl) or platelet-poor plasma (100 μl). The rats were divided into three groups: the platelet-rich plasma group ($n = 16$), the platelet-poor plasma group ($n = 16$), and the nontreatment group ($n = 16$). Flap survival was measured and histologic specimens were collected on day 7. Real-time polymerase chain reaction specimens were collected after 8 hours, 24 hours, 3 days, and 7 days.
Results: Platelet-rich plasma significantly improved flap survival rates (61.2 percent) compared with the platelet-poor plasma treatment (35.8 percent) and nontreatment groups (28.0 percent). A histologic analysis showed that significantly fewer inflammatory cells and an increased blood vessel density were observed in the platelet-rich plasma rats versus the platelet-poor plasma or nontreatment rats. In addition, platelet-rich plasma treatment significantly increased the mRNA levels of vascular endothelial growth factor and platelet-derived growth factor.
Conclusion: Platelet-rich plasma modulates the genes involved in angiogenesis and improves skin flap survival. (*Plast. Reconstr. Surg.* 129: 858, 2012.)

Random-pattern skin flaps are widely used in reconstructive plastic surgery. However, ischemic necrosis in the distal skin flap remains a major complication with high morbidity during surgical reconstructive procedures. The pathogenesis of skin flap ischemic necrosis remains unclear. The consensus is that the cellular activation of proinflammatory mediators, insufficient vascularity, and thrombosis are the principal factors in the pathogenesis of flap ischemic necrosis.[1,2] Pharmacologic efforts to preserve the existing microcirculation have included the use of vasodilators, antiplatelet agents, and antibodies against the adhesion molecules and cytokines involved in leukocyte trafficking and microthrombus formation.[3,4] More recently, the administration of exogenous angiogenic factors has been shown to augment the blood supply and improve flap survival.[5,6] Of the angiogenic agents that have been examined, vascular endothelial growth factor (VEGF) has emerged as a key factor that is induced by ischemia.[7] The clinical treatment of skin flap ischemic necrosis remains controversial. Numerous approaches, such as hyperbaric oxygen, ischemic preconditioning, pharmacologic agents, and growth factor delivery to ischemic tissues, have been used to reduce ischemic necrosis in cases of impending skin flap failure.[8,9]

From the Department of Orthopaedic Surgery and the Developmental Division of Advanced Orthopaedic Therapeutics, Graduate School, Tokyo Medical and Dental University.
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Platelet-rich plasma is a reserve of various growth factors.[10,11] Platelet-rich plasma can be collected autologously and costs less than medication such as alprostadil. Platelet-rich plasma is 100 percent biocompatible and safe. Thus, for clinical use, no special considerations concerning antibody formation and the possible risk of infection are needed. Clinical devices are currently available to automatically prepare platelet-rich plasma. For a long time, autologous platelet-rich plasma has been used intraoperatively to clinically enhance bone regeneration, reduce inflammation, and decrease blood loss in the fields of orthopedics and plastic surgery. However, the evaluation of platelet-rich plasma quality remains controversial, and treatment with poor-quality platelet-rich plasma results in nonbeneficial effects.[12] This is likely because of the faster degradation of the growth factors in platelet-rich plasma, because some authors suggest using the sustained-release form of platelet-rich plasma to deliver the optimal effects of platelet-rich plasma. Platelets are a rich source of the complex group of growth factors that are essential for natural wound repair.[13] These growth factors consist of VEGF, transforming growth factor (TGF)-α1 and TGF-α2, TGF-α, epidermal growth factor (EGF), fibroblast growth factor (FGF-2), the three isomers of platelet-derived growth factor (PDGF-AA, PDGF-BB, and PDGF-AB), platelet thromboplastin, thrombospondin, coagulation factors, endostatin, calcium, serotonin, histamine, and hydrolytic enzymes.[14,15] Platelet-rich plasma contains these various growth factors, and cytokines play important roles in regenerating damaged tissue.[12,16,17] However, little is known about the mechanism of platelet-rich plasma–induced regeneration of damaged skin tissue.

We hypothesized that skin flaps treated with platelet-rich plasma gels would exhibit enhanced flap survival rates because these gels release various growth factors and cytokines. The objective of this study was to assess whether the application of a platelet-rich plasma gel would increase skin flap survival in an experimental rat model and change the gene expression of cytokines and growth factors in the platelet-rich plasma–treated flaps.

MATERIALS AND METHODS

Preparation of Platelet-Rich Plasma and Platelet-Poor Plasma

All of the procedures were approved by the Ethics Committee of the Tokyo Medical and Dental University. Under general anesthesia, adult male Wistar rats (250 to 300 g) were given an intraperitoneal injection of chloral hydrate (400 mg/kg), and whole blood (8 to 10 ml) was collected from each rat by means of cardiac puncture and into sterile syringes containing 10% acid citrate dextrose (Terumo, Tokyo, Japan), an anticoagulant. The whole blood was centrifuged at 200 *g* for 15 minutes at room temperature, and the upper layers (including the buffy coat) were transferred to a new centrifuge tube to remove the bottom layer that contained most of the red blood cells and platelets. After the transferred layers were further centrifuged at 500 *g* for 10 minutes, 250 μl of platelet-rich plasma from 10 ml of whole blood was collected from the bottom layer. The upper layer consisted of platelet-poor plasma. The platelet concentrations in the platelet-rich plasma, platelet-poor plasma, and whole blood were automatically counted using a hematology analyzer. The platelet-rich plasma (200 μl) was activated with a 2% calcium chloride solution (100 μl). The platelet-rich plasma contained various cytokines and growth factors that were immobilized in the gel. We measured the TGF-β1 and PDGF-BB concentrations in the platelet-rich plasma and platelet-poor plasma, and the serum was quantified in each experiment using an enzyme-linked immunosorbent assay kit (R&D Systems, Inc., Minneapolis, Minn.). The analysis was performed according to the manufacturer's instructions.

Rat Ischemic Skin Flap Model and Treatment

Twelve-week-old male Wistar rats weighing 250 to 300 g (n = 48) were assigned randomly to the nontreatment control group (n = 16), the platelet-poor plasma group (n = 16), or the platelet-rich plasma group (n = 16). Anesthesia was induced by intraperitoneal injection of chloral hydrate (280 mg/kg). After shaving the dorsal hair, a cranially based dorsal random-pattern flap (11 × 3 cm) was outlined with water-fast markers using anatomical landmarks extending from the scapular tip to the hip joint; the same vessels were included in each flap.[18] The flap, including the skin, subcutaneous tissue, and superficial fascia (panniculus carnosus), was then raised to ensure uniformity. The flap tissue, including the skin and superficial fascia, was dissected using a scalpel, with no effort to achieve hemostasis. A platelet-rich plasma or platelet-poor plasma gel was prepared, and a total of 100 μl of gel was injected subcutaneously at four points on the skin flap (25 μl of platelet-poor plasma or platelet-rich plasma per point) located at one-third and two-thirds of the flap length through a Hamilton syringe. The

injected flaps were sutured back into their original positions after a silicone sheet was placed underneath to avoid vascular ingrowth from the wound bed. Sutures were placed at 5-mm intervals using 5-0 monofilament nylon to facilitate the measurement of the tissue survival duration. The sample collections were performed postoperatively after 8 hours, 24 hours, 3 days, and 7 days ($n = 3$ at each time point) for polymerase chain reaction. The collection spots were the proximal 1-cm interval region on the middle line of the flap pedicle (Fig. 1). After the macroscopic observation ($n = 4$ in each group), a skin specimen was collected for histologic analysis 7 days after surgery.

Macroscopic Measurement of the Flap Survival Rate

The flap survival rate was observed on postoperative day 7, when the border of the necrotic area on the flap was defined. The rats were killed with intraperitoneal chloral hydrate (400 mg/kg), and standardized digital photographs of the flaps were collected with a digital camera (Cybershot T900; Sony, Tokyo, Japan) and transferred to a computer. Dark zones and zones covered with scabs were defined to be necrotic, and the remaining areas were defined to be viable. To assess the survival rates, the digital image was processed using image analysis software (Photoshop CS; Adobe Systems, Inc., San Jose, Calif.). Pixels were then counted after evaluating the necrotic and viable areas. The total and viable areas of each flap were measured, and the survival rate was expressed as a percentage of the total flap area (flap survival rate = viable area/total area × 100 percent). The calculated ratios were used for statistical analysis to examine the significance of the differences between the groups.

Histology

A full-thickness skin specimen (1 × 0.5 cm) was harvested from the flap's midline, 3 cm proximal to the cranial end of the flap, 7 days after surgery. The samples were fixed overnight in 4% paraformaldehyde at 4°C, washed with phosphate-buffered saline, and immersed in 70% ethanol for 24 hours at 4°C. The samples were subsequently embedded into paraffin blocks, cut into 5-μm-thick sequential sections, dewaxed (xylene, three times, for 5 minutes), rehydrated by passage through a decreasing graded alcohol series, and rinsed in water. Routine hematoxylin and eosin staining was performed. The specimens were examined under a light microscope by a pathologist blinded to the treatment group. The polymorphonuclear cells in the panniculus carnosus were quantified in three sections under 40× magnification, as described previously.[5,19]

Immunohistochemistry to Measure Microvessel Density

The sections were immunostained with a rabbit polyclonal antibody raised against rat von Willebrand factor (1:100; Abcam, Cambridge, Mass.) to evaluate the microvessel density. A secondary biotinylated goat anti-rabbit antibody was applied for 30 minutes. The slides were then exposed to ABC reagent (Vector Laboratories, Burlingame, Calif.) for 30 minutes and 3,3′-diaminobenzidine–peroxidase substrate solution (Vector) for 3 minutes to visualize the antibody binding. After nuclear counterstaining with hematoxylin and washing in tap water for 15 minutes and distilled water for 10 minutes, the preparations were mounted with UltraMount (LabVision Corp., Thermo Fisher Scientific, Fremont, Calif.).

The average number of von Willebrand factor–immunopositive vessels in the hypodermis was

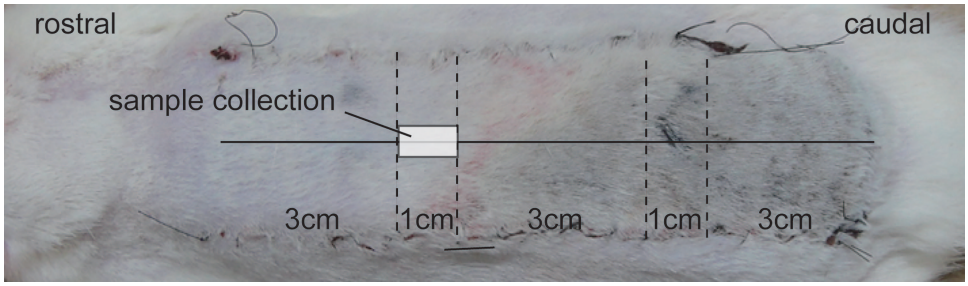

Fig. 1. Representative skin flap 7 days after platelet-rich plasma treatment. A flap 11 cm long was divided into proximal, middle, and distal regions, 3 cm in length, with 1-cm intervals in between them. Specimens for either polymerase chain reaction or histology were collected from the proximal 1-cm interval region (sample size, 1 × 0.5 cm).

used for the analysis of angiogenesis. To ensure that the observed differences in the number of blood vessels were not an indirect effect of an overall increase in flap survival, this value was compared across the platelet-rich plasma, platelet-poor plasma, and nontreatment group to minimize the effect of existing vessels in the skin tissues before surgery. To eliminate bias and misinterpretation, the microvessel density (40× magnification) was assessed in five fields for each specimen by an independent researcher blinded to the treatment group. The images were obtained on an Olympus microscope (Olympus, Tokyo, Japan).

Real-Time Polymerase Chain Reaction of Skin Tissue

Full-thickness biopsies (1 ×0.5 cm) at proximal 1-cm intervals from the flap's midline were performed 8 hours, 24 hours, 3 days, and 7 days after flap elevation. The skin tissues were frozen in liquid nitrogen and stored at −80°C. The biopsy specimens were placed in 1 ml of ice-cold QIAzol Lysis reagent (Qiagen, Valencia, Calif.), homogenized with a TissueRuptor (Qiagen), and isolated using an RNeasy Lipid Tissue Mini Kit (Qiagen). First-strand cDNA was prepared using the QuantiTect Reverse Transcription Kit, according to the manufacturer's instructions. To investigate growth factor expression, we selected *VEGF*, *EGF*, *PDGF-AA*, and *PDGF-BB*. The expression of these genes was quantified by real-time polymerase chain reaction using the Mx3000P QPCR System (Stratagene Corp., La Jolla, Calif.). The genes of interest and their primer sequences were synthesized by Takara Bio, Inc. (Shiga, Japan) and are listed in Table 1. These specific genes were selected on the basis of their known or putative roles in platelet-rich plasma's mechanism of action. The reactions (total volume, 25 μl) used 10 ng of cDNA, 12.5 μl of SYBR Premix Ex TagII (Takara Bio), and 1 μM of each gene-specific primer. The cycling conditions were as follows: 10 minutes at 50°C for reverse transcription, 10 minutes at 95°C for initial activation, 40 cycles of 15 seconds at 95°C for denaturation, and 1 minute at 60°C for annealing/elongation. The product specificity of the polymerase chain reaction was confirmed by a dissociation curve. Each run included a nontemplate control. The fluorescence was acquired at 74°C. The results were evaluated using Mx3000p analysis software and the 2^{DDCt} relative quantification technique.[20] Gluteraldehyde-3-phosphate dehydrogenase (*GAPDH*) served as a reference housekeeping gene, and the samples from untreated rat skin served as baseline calibrators. All of the reactions were performed in duplicate.

Table 1. Primers Used for Real-Time Polymerase Chain Reaction Analysis

Gene	Primer
VEGF	
Forward	GTTCGAGGAAAGGGAAAGGGTC
Reverse	GCGAGTCTGTGTTTTTGCAGGA
EGF	
Forward	TGCACTGGGTCCGAAACAG
Reverse	GTAGCGGTCCACGGATTCA
PDGF-AA	
Forward	TGGGTCCCACACTGTTAAGCA
Reverse	CCGTCCTGGTCTTGCAAACT
PDGF-BB	
Forward	AACATGACCCGAGCACATTCTG
Reverse	CGTCTTGCACTCGGCGATTA
GAPDH	
Forward	GGCATCCTGACCCTGAAGTA
Reverse	GGGGTGTTGAAGGTCTAAA

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, Calif.). The results of all of the experiments were expressed as mean ± SD. For the flap survival rate comparisons, a two-tailed unpaired *t* test (assuming equal variances) was used. Two-way analysis of variance was used for the statistical analysis of the real-time polymerase chain reaction results, and one-way analysis of variance was applied to the histologic data. Statistical significance was set at $p < 0.05$.

RESULTS

Platelet Number and TGF-β1 and PDGF-BB Concentrations in Platelet-Rich Plasma

The number of platelets was higher in platelet-rich plasma than in platelet-poor plasma or whole blood. A 6-fold increase in platelet concentration was found in platelet-rich plasma compared with platelet-poor plasma and whole blood (Table 2). We measured TGF-β1 and PDGF-BB as functional markers in platelet-rich plasma. Enzyme-linked immunosorbent assay showed that more TGF-β1 and PDGF-BB were released from platelet-rich plasma than from platelet-poor plasma (Table 3).

Table 2. Comparison of Platelet Concentration in Plasma

	No. of Platelets (×10⁴)
PRP	477.2 ± 145.9*
PPP	12.9 ± 2.4
Whole blood	84.3 ± 22.6

PRP, platelet-rich plasma; PPP, platelet-poor plasma.
*$p < 0.05$.

Table 3. Comparison of TGF-β1 and PDGF-BB Concentration in Plasma

	TGF-β1 (pg/ml)	PDGF-BB (pg/ml)
PRP	251.1 ± 42.9*	38.8 ± 2.3*
PPP	10.9 ± 8.3	1.1 ± 0.6
Serum	49.1 ± 8.4	3.6 ± 1.9

PRP, platelet-rich plasma; PPP, platelet-poor plasma.
*$p < 0.05$.

Flap Survival after Platelet-Rich Plasma Treatment

Postoperatively, no increased bleeding or hemorrhage was noted during flap elevation or in the subsequent week. Seven days later, skin flaps were collected on the platelet-rich plasma, platelet-poor plasma, and nontreatment rats. Their necrotic and viable regions were clearly demarcated (Fig. 2, *above* and *second* and *third rows*). The flap survival rate was calculated for each treatment. The platelet-rich plasma treatment (61.2 ± 9.1 percent) resulted in a significantly improved flap survival rate compared with the platelet-poor plasma treatment (35.8 ± 11.3 percent) and the nontreatment group (28.0 ± 5.4 percent) (Fig. 2, *below*).

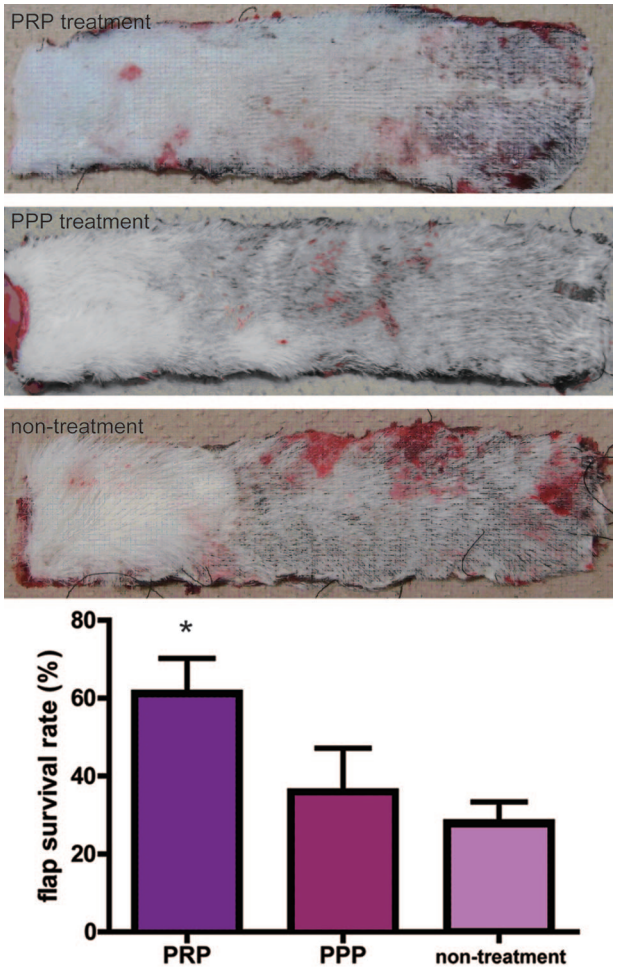

Fig. 2. Appearance of skin flaps in the platelet-rich plasma (*PRP*), platelet-poor plasma (*PPP*), and control group rats 7 days after surgery. The necrotic and viable regions are clearly demarcated (*above* and *second* and *third rows*). (*Below*) Platelet-rich plasma injection significantly improved flap survival compared with platelet-poor plasma injection and nontreatment.

Histologic Evaluation of Inflammation and Microvessels

Infiltrating polymorphonuclear cells were observed in each flap (Fig. 3, *above* and *second row*). Quantification revealed significantly fewer inflammatory cells in the platelet-rich plasma group (0.9 ± 0.3 cells per high-power field) than in the platelet-poor plasma (3.9 ± 0.3 cells per high-power field) and control groups (2.3 ± 0.5 cells per high-power field; $p < 0.05$) (Fig. 3, *below*, *left*).

Von Willebrand factor–positive blood vessels were observed in the platelet-rich plasma– and platelet-poor plasma–treated flaps (Fig. 3, *third row*). Quantification revealed significantly more capillaries in the platelet-rich plasma group (3.6 ± 0.2 vessels per high-power field) than in the platelet-poor plasma (1.0 ± 0.2 vessels per high-power field) and control groups (0.7 ± 0.1 vessels per high-power field) (Fig. 3, *below*, *right*). *PRP*, platelet-rich plasma; *PPP*, platelet-poor plasma.

mRNA Expression of Growth Factors after Platelet-Rich Plasma Treatment

To investigate which growth factors were related to skin flap survival, the expression of VEGF, EGF, PDGF-AA, and PDGF-BB mRNA in platelet-rich plasma, platelet-poor plasma, or untreated skin tissues was analyzed after 8 hours, 24 hours, 3 days, and 7 days. VEGF was significantly up-regulated in the platelet-rich plasma treatment group after 24 hours and 3 days (Fig. 4, *above*, *left*). PDGF-AA was significantly up-regulated after 3 and 7 days (Fig. 4, *below*, *left*). In contrast, EGF and PDGF-BB were significantly up-regulated in the platelet-rich plasma treatment group after 24 and 8 hours, respectively (Fig. 4, *right*). EGF and PDGF-BB were both strongly expressed in the acute phase after the platelet-rich plasma treatment, whereas VEGF and PDGF-AA were strongly expressed in the chronic phase. These growth factors and cytokines that are released from platelet-rich plasma might improve the ischemic flap environment and enhance the flap survival rate.

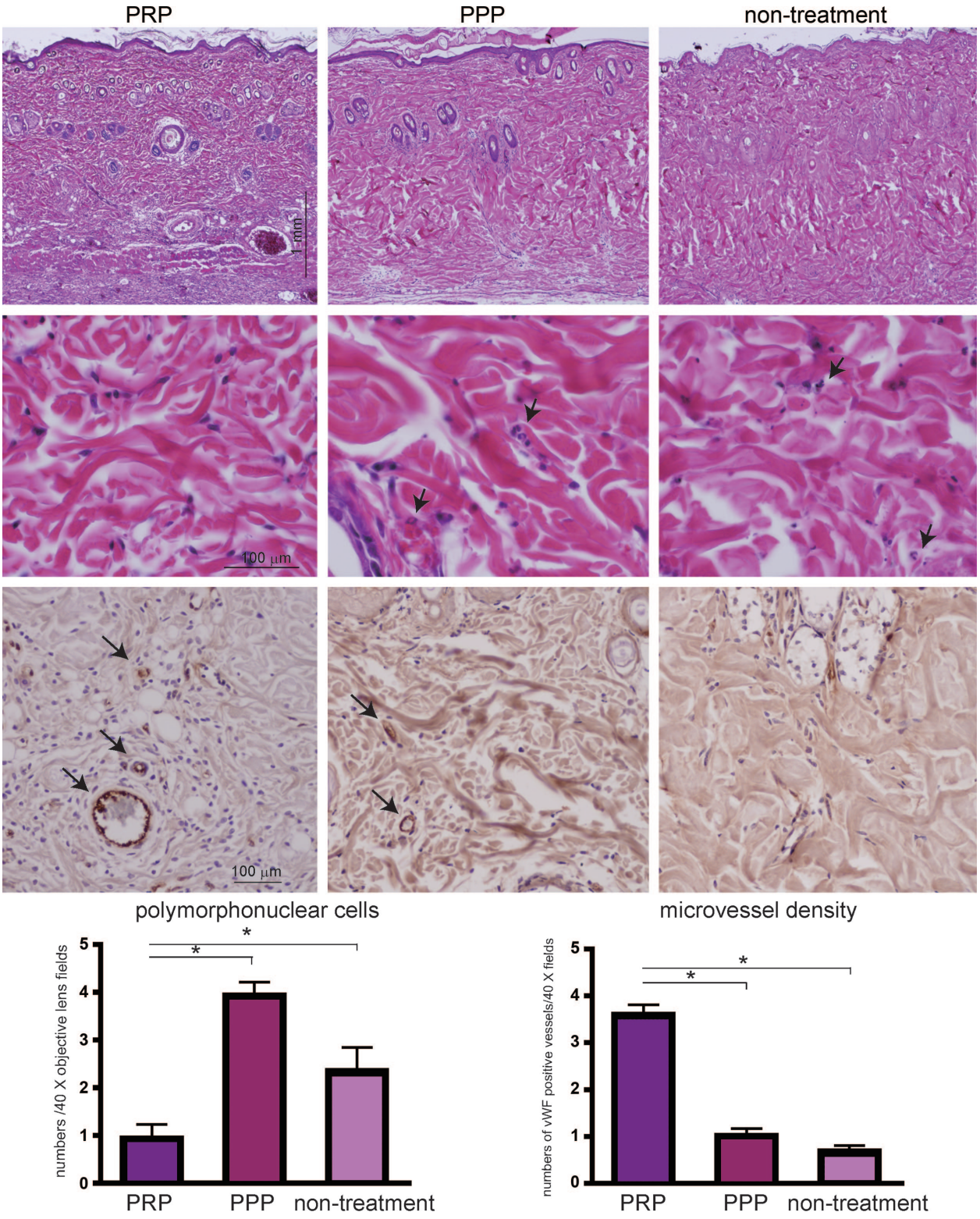

Fig. 3. Hematoxylin and eosin staining (*above* and *second row*) and von Willebrand factor immunostaining (*third row*) of skin-flap dermis in each treatment group on postoperative day 7. The number of polymorphonuclear cells (*arrows* in *second row*) is increased in the platelet-poor plasma–treated and untreated rat dermis. A significant decrease in the number of polymorphonuclear cells was observed in the platelet-rich plasma treatment group (*below*, *left*) (*$p < 0.05$). Von Willebrand factor–positive cells (*arrows* in *third row*) were observed after surgery. There was a significant increase in the microvessel density in the platelet-rich plasma–treated group (*below*, *right*) (*$p < 0.05$). *PRP*, platelet-rich plasma; *PPP*, platelet-poor plasma.

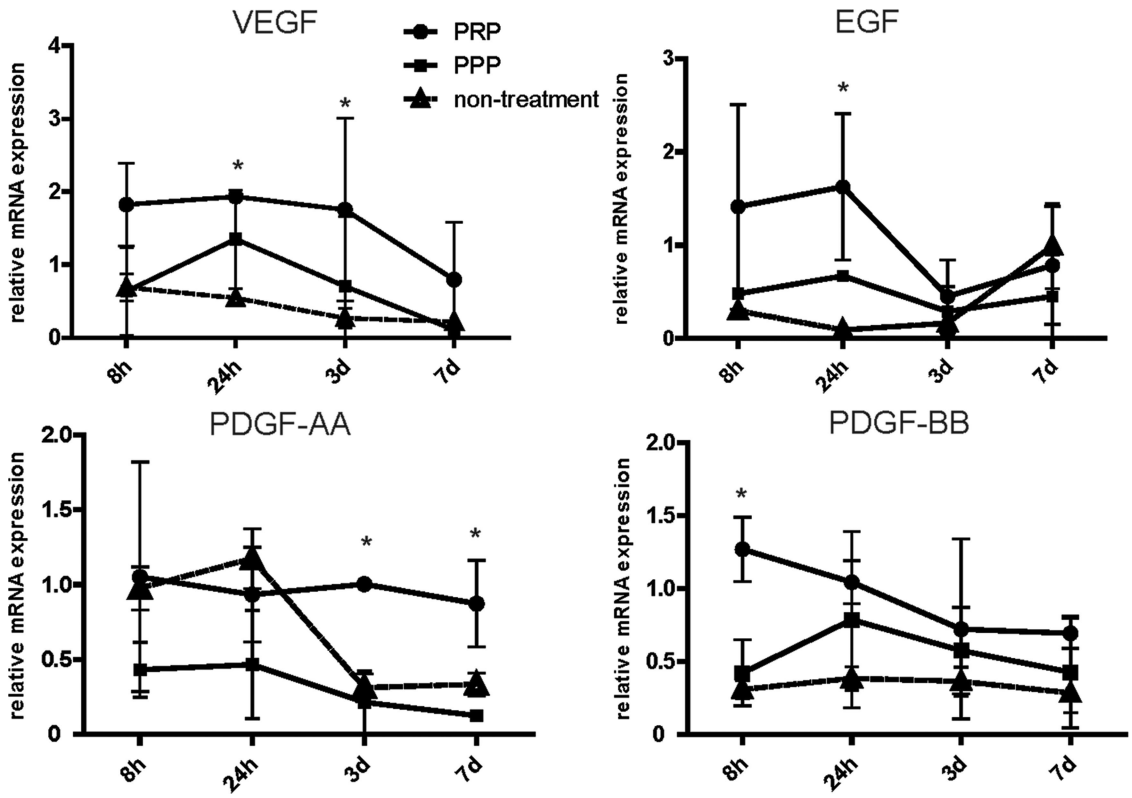

Fig. 4. VEGF, EGF, PDGF-AA, and PDGF-BB mRNA expression in platelet-rich plasma (*PRP*), platelet-poor plasma (*PPP*), or untreated skin tissues was analyzed after 8 hours, 24 hours, 3 days, and 7 days. Significant changes in each factor were observed at different time points (*$p < 0.05$). Two-way analysis of variance was performed to test for any significant differences. The transcript levels were expressed relative to the levels in intact specimens (baseline = 1).

DISCUSSION

We have proposed an autologous rat platelet-rich plasma gel preparation with a simpler technique and rapid platelet-rich plasma extraction. With this protocol, we were able to reach a concentration at least six times greater than that in an average blood sample. These results were superior to those of other studies, which have been unable to obtain a two-fold increase in the patient's platelet concentration.[12,21,22]

Platelet-rich plasma contains various cytokines and growth factors, including TGF-β and PDGF-BB, which were detected by enzyme-linked immunosorbent assay. TGF-β is particularly important because, once it is activated, it affects most aspects of tissue repair, including initiation and termination of the treatment of wounds that are over a decade old.[23] It can activate fibroblasts for procollagen formation that is induced by collagen deposition and wound healing. PDGF associated with TGF-β increases tissue vascularization, promotes fibroblast proliferation, increases collagen formation, stimulates granulation tissue production, and increases the wound's tensile strength when applied locally in animal models.[24,25] In the very early phases after platelet-rich plasma injection, TGF-β might play an important role in promoting tissue healing in our ischemic flap. In contrast, TGF-β negatively influence angiogenesis.[26] According to our results, most proteins secreted from platelet-rich plasma could react positively on angiogenesis.

This study demonstrated that VEGF mRNA expression was enhanced in the platelet-rich plasma group after 8 hours and was maintained until 3 days after surgery. Platelet activation induces the release of VEGF, a mediator of angiogenesis that stimulates endothelial cell proliferation.[27] VEGF, a vascular permeability factor released from the wound epithelium and extracellular matrix by endothelial cell–derived proteases, stimulates endothelial cell proliferation and increases vascular permeability. These influence the extravasation of plasma proteins and create a temporary support structure on which activated endothelial cells, leukocytes, and epithelial cells can subsequently migrate.[28,29] In

fact, we analyzed von Willebrand factor expression in the flaps 24 hours after surgery. Expression in the group treated with platelet-rich plasma was higher than in the other groups (data not shown). The platelet-rich plasma gel contains more VEGF and induces VEGF expression from endothelial cells, increasing the microvessel density and preventing tissue necrosis from the early phase. To fully understand how angiogenesis occurs in a skin flap with platelet-rich plasma treatment, further analysis of blood flow will be needed using laser Doppler or speckle flowgraphy in the early phase.

In this study, expression of PDGF-BB mRNA was significantly enhanced after 8 hours, and expression of PDGF-AA mRNA was maintained from 3 to 7 days. The release of PDGF into a wound bed can have a chemotactic effect on monocytes, neutrophils, fibroblasts, mesenchymal stem cells, and osteoblasts. PDGF is also a powerful mitogen for fibroblasts and smooth muscle cells and is involved in all three phases of wound healing (i.e., angiogenesis, the formation of fibrous tissue, and reepithelialization).[30] However, different PDGF isoforms have different biological functions. In an in vitro study conducted by Marx,[12] PDGF-BB had a stronger mitogenic effect than PDGF-AA or PDGF-AB on microvascular endothelial cells, and PDGF-AA failed to induce any endothelial proliferative response despite inducing receptor autophosphorylation. However, PDGF-AA affects smooth muscle cells and might affect angiogenesis indirectly.[31] VEGF also promotes mural cell accumulation, presumably through the release of PDGF-BB. Platelet-rich plasma treatment may induce PDGF-AA into skin flaps in cooperation with other growth factors, such as PDGF-BB and VEGF.

EGF is released by platelets and is chemotactic for fibroblasts, and its topical application accelerates epidermal regeneration and increases a wound's tensile strength.[32] However, the EGF mRNA in the platelet-rich plasma–treated tissues was only temporarily expressed after 24 hours. At a minimum, high expression of EGF plays an important role in promoting reepithelialization in the early phase after surgery.

FGF-2 plays a role in granulation tissue formation, reepithelialization, and tissue remodeling in acute wounds.[33] In fact, we performed semiquantitative real-time polymerase chain reaction for FGF-2 before performing the real-time polymerase chain reaction analyses. According to this result, the expression of FGF-2 mRNA did not change at any time point (data not shown). In our skin flap model, growth factors other than FGF-2, which plays an important role during chronic wound healing, may be more important instead.[33]

Platelet-rich plasma essentially replicates the wound-healing process, but the lack of erythrocytes may assist in bypassing the inflammatory stage. This may lead to an earlier initiation of the proliferative phase, which may explain the relatively early improvements. As we continue to refine our understanding of the biological processes that occur with use of platelet-rich plasma, we will be more able to tailor platelet-rich plasma treatment to each particular application. In the future, besides use in flaps, the most common use will be for treating deep diabetic foot ulcers and orthopedic applications.

CONCLUSIONS

This study shows that a subcutaneous platelet-rich plasma injection significantly improves the survival of cutaneous flaps and is strongly correlated with angiogenesis. Platelet-rich plasma administration could be an important technique for starting angiogenesis by recruiting the endothelial cells that line the blood vessels. To achieve wide clinical application of platelet-rich plasma, basic research is necessary to verify the related clinical reports. It is an active area of investigation and plastic surgery research.

Weiwei Li, M.D., Ph.D.
Department of Plastic Surgery
Beijing Tsinghua Hospital
Tsinghua University
Haidian District
Beijing 100084, People's Republic of China
weiweili711@gmail.com

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