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Clinical Study

Platelet-rich plasma enhances bone union in posterolateral lumbar fusion: A prospective randomized controlled trial

Go Kubota, MD, PhD[a], Hiroto Kamoda, MD, PhD[b], Sumihisa Orita, MD, PhD[c], Kazuyo Yamauchi, MD, PhD[c], Yoshihiro Sakuma, MD, PhD[d], Yasuhiro Oikawa, MD, PhD[e], Kazuhide Inage, MD, PhD[c], Takeshi Sainoh, MD, PhD[f], Jun Sato, MD, PhD[g], Michihiro Ito, PhD[h], Masaomi Yamashita, MD, PhD[i], Junichi Nakamura, MD, PhD[c], Takane Suzuki, MD, PhD[j], Kazuhisa Takahashi, MD, PhD[c], Seiji Ohtori, MD, PhD[c,*]

[a]*Department of Orthopedic Surgery, Eastern Chiba Medical Center, Chiba, Japan*
[b]*Department of Orthopaedic Surgery, Chiba Cancer Center, Chiba, Japan*
[c]*Department of Orthopedic Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan*
[d]*Department of Orthopaedic Surgery, National Hospital Organization Chiba Medical Center, Chiba, Japan*
[e]*Department of Orthopaedic Surgery, Chiba Children's Hospital, Chiba, Japan*
[f]*Department of Orthopaedic Surgery, Sainou Hospital, Toyama, Japan*
[g]*Department of Orthopaedic Surgery, Chiba Aoba Municipal Hospital, Chiba, Japan*
[h]*Department of Clinical Laboratory, Chiba University Medical Hospital, Chiba, Japan*
[i]*Department of Orthopaedic Surgery, Social Insurance Funabashi Central Hospital, Chiba, Japan*
[j]*Department of Environmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan*

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Abstract

BACKGROUND CONTEXT: Platelet-rich plasma (PRP) accelerates bone union in vivo in a rodent model of spinal fusion surgery. However, PRP's effect on bone union after spinal surgery remains unclear.
PURPOSE: The objective of this study was to evaluate the efficacy of PRP after posterolateral lumbar fusion (PLF) surgery.
STUDY DESIGN/SETTING: Single-center prospective randomized controlled clinical trial with 2-year follow-up.
PATIENT SAMPLE: The patient sample included a total 62 patients (31 patients in the PRP group or 31 patients in the control group).
OUTCOME MEASURES: The outcome measures included the bone fusion rate, the area of bone fusion mass, the duration of bone fusion, and the clinical score using the visual analog scale (VAS).
MATERIALS AND METHODS: We randomized 62 patients who underwent one- or two-level instrumented PLF for lumbar degenerative spondylosis with instability to either the PRP (31 patients) or the control (31 patients) groups. Platelet-rich plasma-treated patients underwent surgery using an autograft bone chip (local bone), and PRP was prepared from patient blood samples immediately before surgery; patients from the control group underwent PLF without PRP treatment. We assessed platelet counts and growth factor concentrations in PRP prepared immediately before surgery. The duration of bone union, the postoperative bone fusion rate, and the area of fusion mass were assessed using plain radiography every 3 months after surgery and by computed tomography at 12 or 24 months. The duration of bone fusion and the clinical scores for low back pain, leg pain, and leg numbness before and 3, 6, 12, and 24 months after surgery were evaluated using VAS.

FDA device/drug status: Not applicable.

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The authors declare that there are no conflicts of interest.

Go Kubota wrote and prepared the manuscript, and all of the authors participated in the study design. All authors have read, reviewed, and approved the article.

Approval code: 805 Graduate School of Medicine, Chiba University.

* Corresponding author. Department of Orthopedic Surgery, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8677, Japan. Tel: (81) 43-226-2117; fax: (81) 43-226-2116.

E-mail address: sohtori@faculty.chiba-u.jp (S. Ohtori)

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RESULTS: Data from 50 patients with complete data were included. The bone union rate at the final follow-up was significantly higher in the PRP group (94%) than in the control group (74%) (p=.002). The area of fusion mass was significantly higher in the PRP group (572 mm^2) than in the control group (367 mm^2) (p=.02). The mean period necessary for union was 7.8 months in the PRP group and 9.8 months in the control group (p=.013). In the PRP, the platelet count was 7.7 times higher and the growth factor concentrations were 50 times higher than those found in plasma (p<.05). There was no significant difference in low back pain, leg pain, and leg numbness in either group at any time evaluated (p>.05).
CONCLUSIONS: Patients treated with PRP showed a higher fusion rate, greater fusion mass, and more rapid bone union after spinal fusion surgery than patients not treated with PRP.

Keywords: Bone union; Platelet-rich plasma; Posterolateral lumbar fusion; Randomized; Spine; Surgery

Introduction

Autologous bone grafting is the most accepted procedure for achieving bone union in spinal fusion surgery. However, a significant number of cases of non-union, ranging between 5% and 43%, have been reported [1]. Non-union results in poor clinical outcomes and increased medical expenditure. Therefore, new surgical strategies using biological and alternative substitutes to autologous bone grafts to accelerate spinal union have been developed.

Platelet-rich plasma (PRP) has recently gained attention in the field of bone and soft-tissue regeneration [2–4]. Platelet-rich plasma contains a high concentration of platelets and osteoinductive autologous growth factors in a small amount of plasma, such as platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) [5–7]. Platelet-rich plasma appears to promote bone formation and to shorten the period required for spinal fusion in posterolateral lumbar fusion (PLF) and lumbar interbody fusion in a rat model of spine surgery [8,9]. However, the advantages of PRP in clinical situations remain controversial [10–16]. A major shortcoming has been the lack of controlled clinical trials to evaluate how and to what extent PRP increases the rate of spinal fusion [17].

The aim of the present prospective randomized controlled study was to assess the efficacy of PRP when added to autograft bone (local bone) in PLF surgery, and to evaluate the quality of human-derived PRP.

Materials and methods

Study design, selection of participants, and randomization

After approval by our institutional review board, this single-center prospective randomized controlled clinical trial was conducted between July 2009 and November 2015. The trial was registered in the University Hospital Medical Information Network clinical trials registry in Japan. All patients gave their written informed consent to participate in the trial before their enrollment.

The present participants were patients from our hospital diagnosed with lumbar spinal stenosis with spondylolisthesis plus instability. Spinal instability was defined as an anterior translation of >5% of the vertebra and a translocation of >5° between the flexion and extension positions on the lumbar radiographic examination. Patients with more than two-level pathologic lesions, past lumbar surgery, or other infectious or inflammatory diseases, such as spinal tumors, infection, and trauma, were excluded. After obtaining written informed consent to participate in this trial, the patients were examined by the case-reviewing committee, and the accepted patients were randomly divided into the control and the PRP groups in a 1:1 ratio using a computer-generated random number sequence. The patients were randomized to each group using a minimization method. Age and sex were additional stratification factors.

PRP preparation

Immediately before the surgery, 400 mL of peripheral venous blood was withdrawn from each patient to prepare the PRP. The blood was processed using a two-stage centrifugation method (CR7B3; Hitachi Koki, Tokyo, Japan). The first centrifugation was performed at 1,660 rpm for 5 minutes. Consequently, the plasma was separated from the red blood cells. The second centrifugation was performed at 1,450 rpm for 15 minutes to pellet the platelets. The pelleted platelets were temporarily isolated from the supernatant platelet-poor plasma. To adjust the PRP volume, a portion of the platelet-poor plasma was mixed with the pelleted platelets. Finally, 22 mL of PRP was generated, of which 2 mL was used to count the number of platelets with a hematology analyzer and to assess the concentration of growth factors (TGF-β and PDGF) using a sandwich enzyme-linked immunosorbent assay.

During the surgery, 0.5 mL of 1,000 U/mL liquid thrombin solution (Mochida Pharmaceutical Co., Tokyo, Japan) and 1 mL of 2% calcium chloride solution (Otsuka Pharmaceutical Co., Tokyo, Japan) were added to the 20 mL of PRP to activate the platelets and to prepare a PRP gel that was mixed with the autogenous local bone graft for insertion into the graft site. Red blood cells were returned to patients during surgery.

Surgical technique

Decompression and posterolateral fusion surgery at the level of spondylolisthesis were performed in all patients. One- or two-level posterolateral fusion was performed using pedicle screws and a local bone graft. Bilateral facet fusion was performed in all patients. Local bone was generated from the decompression site lamina. No additional osteoconductive products were used for spinal fusion. In the PRP group, 20 mL of activated PRP was divided equally and placed on both sides over the adjacent lumbar transverse processes together with the local bone graft. In the control group, PRP was not used. The same team of surgeons performed all surgical procedures in a similar manner using either CD Horizon Legacy (Medtronic, Memphis, TN, USA) or CD Horizon Solera (Medtronic) fixation systems.

Outcome measures

Quantification of platelet count and growth factor concentrations

After the preparation of PRP, the platelets in whole blood and in the PRP were counted using a hematology analyzer (K-4500; Sysmex, Kobe, Japan). The concentrations of the main isoenzymes of TGF-β and PDGF (PDGF-AA, PDGF-AB, and PDGF-BB) in the blood and in the activated PRP were measured using Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's instructions.

Assessment of final bone union rate and measurement of area of bone union

The final bone union rate and the area of bone union across the transverse processes was assessed at 12 or 24 months after surgery using a coronal plane from a three-dimensional image reconstructed from computed tomography (CT). Bone union revealed on CT was defined as bridging bone remodeling across the transverse processes between the adjacent vertebrae. If bone union was confirmed, we also evaluated the area of bony fusion on a coronal plane from a three-dimensional image reconstructed from CT. The area of bone union was measured using a computer-linked digitizer. The images were evaluated by three surgeons blinded to the condition. Fusion was defined as when at least two observers simultaneously detected bone fusion. To measure the area of fusion, the average measurement by two surgeons was used. We evaluated the fusion and the area of fusion at each of the two sites in the one-level PLF and at each of the four sites in the two-level PLF.

Assessment of duration of bone union

The duration of bone union was assessed over 24 months after surgery using anteroposterior radiographic images and lateral flexion-extension radiographic images. Radiological findings were obtained every 3 months after surgery. Radiographic spinal union was defined as including both (1) bridging bone formation across the adjacent transverse processes and (2) less than 1.5° instability between the flexion and the extension positions.

Assessment of the duration of bone union was performed by three different surgeons blinded to the treatments. The time of bone union was the period between the surgery and the time when at least two observers simultaneously detected bone fusion.

Clinical evaluation

We evaluated the change in low back, leg pain, and leg numbness before and 3, 6, 12, and 24 months after surgery. To evaluate pain, the visual analog scale (VAS) scores (0, no pain; 10, worst pain) for low back pain, leg pain, and leg numbness were recorded and compared.

Adverse events

All adverse events, their severity (mild, moderate, or severe), and the investigator's decision regarding their relationship with the PRP treatment (none, unlikely, possible, or probable) were reported.

Statistical analysis

A Student *t* test with standard error of the mean (SEM) was used to assess the statistical significance of any differences. SEM was calculated using the standard error of proportions. All data were evaluated statistically using SPSS software (version 19.0; SPSS, Chicago, IL, USA). $p<.05$ was considered significant.

Results

Patients' demographic data

A Consolidated Standards of Reporting Trials (CONSORT) flow diagram for the trial participants is shown in Fig. 1. We enrolled 63 patients. After exclusion (n=1), 62 patients were assigned to the PRP (n=31) or the control (n=31) group and underwent surgery. Data from 12 patients were excluded because these were incomplete. Ultimately, 50 patients were followed up and evaluated. Table 1 shows the preoperative demographic characteristics of the patients who were followed up. No significant difference was found between the

Table 1
Demographic characteristics of patients

	Control	PRP	p
Number of patients	25	25	
Sex	M 14, F 11	M 15, F 10	>.99
Age, mean year±SEM (range)	65.3±1.99 (33–78)	65.1±2.14 (30–83)	.474
Number of fixation levels: number of patients	1 level: 15 2 levels: 10	One level: 18 Two levels: 7	.288

M, male; F, female; SEM, standard error of the mean.

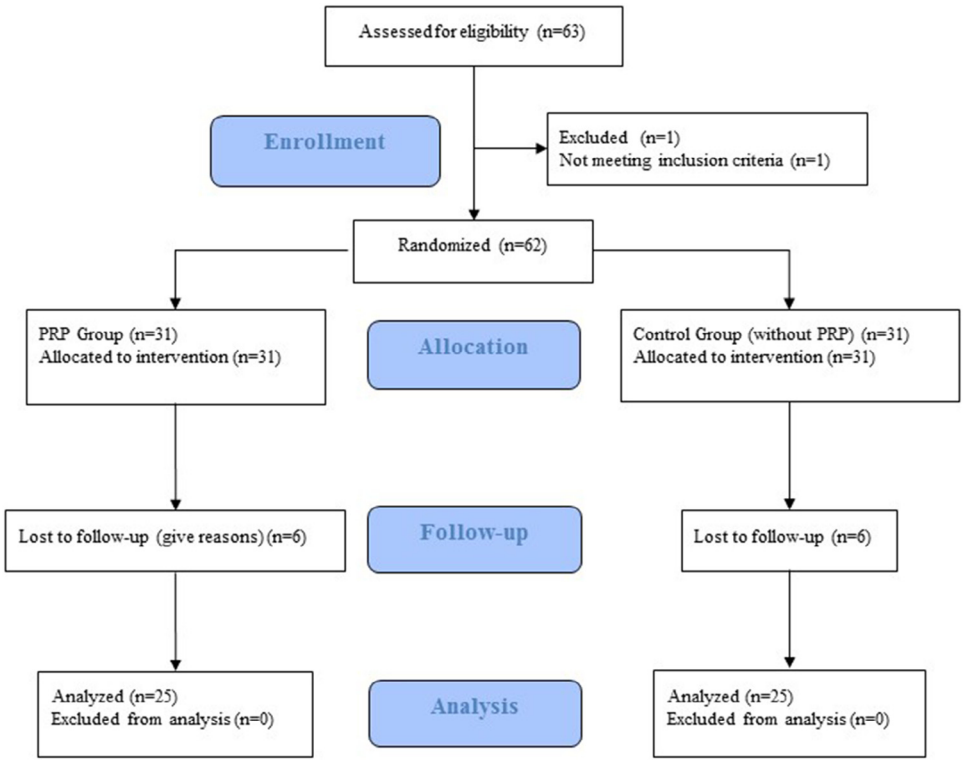

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram. PRP, platelet-rich plasma.

groups for sex (p>.99) or age (p=.47). In the PRP group, one fixation level was used in 18 patients and two fixation levels were used in seven patients, whereas in the control group, one fixation level was used in 15 patients and two fixation levels were used in 10 patients (Table 1). No significant differences were found in the number of fixation levels between the groups (p=.288).

Blood serum and PRP platelet counts

The mean blood serum platelet counts were $26.7\pm2.11\times10^4/\mu L$ (mean±SEM) and the PRP platelet counts were $205\pm27.0\times10^4/\mu L$ (Table 2). The platelet count in PRP was 7.7 times higher than that in the blood (p<.05).

Growth factor concentrations

The mean blood serum and the PRP TGF-β concentration and the PDGF-AA, PDGF-AB, and PDGF-BB concentrations are shown in Table 2. The concentrations of all growth factors in PRP were significantly higher than those measured in the blood serum (p<.05).

Assessment of final bone union rate and measurement of the area of bone union

Posterolateral lumbar fusion was performed bilaterally, so the total numbers of segments evaluated by CT were 70 sites in the control group and 64 sites in the PRP group.

By CT, 74.2% of segments exhibited fusion across the transverse processes in the control group; 93.7% of segments exhibited fusion in the PRP group. The rate of bone fusion in the PRP group was significantly faster than that in the control group (p=.012, Table 3). The area of fusion mass was significantly greater in the PRP group (572 mm^2) compared with the control group (367 mm^2) (p=.02). The mean times

Table 2
Platelet counts and concentrations of PDGF isoenzymes and TGF-β

	Blood serum	PRP	p
Platelets ($\times10^4/\mu L$)	26.7±2.11	205±27.0	<.05
PDGF-AA (ng/mL)	0.24±0.04	13.5±1.15	<.05
PDGF-AB (ng/mL)	0.37±0.02	47.7±4.12	<.05
PDGF-BB (ng/mL)	0.27±0.03	24.5±3.12	<.05
TGF-β (ng/mL)	1.65±0.12	117.9±5.15	<.05

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

Table 3
Evaluation of spinal fusion

	Control	PRP	p
Number of fixation levels: number of patients	One level: 15 Two levels: 10	One level: 18 Two levels: 7	.288
Number of evaluated sites (all sites across the transverse processes)	70	64	.135
Bone union rate (CT)	52/70 (74.2%)	60/64 (93.7%)	.012
Area of bone union (mm^2)	366.9±41	572.2±62	.02
Period until bone union (mo)	9.4±0.42	7.8±0.43	.013

CT, computed tomography; PRP, platelet-rich plasma.

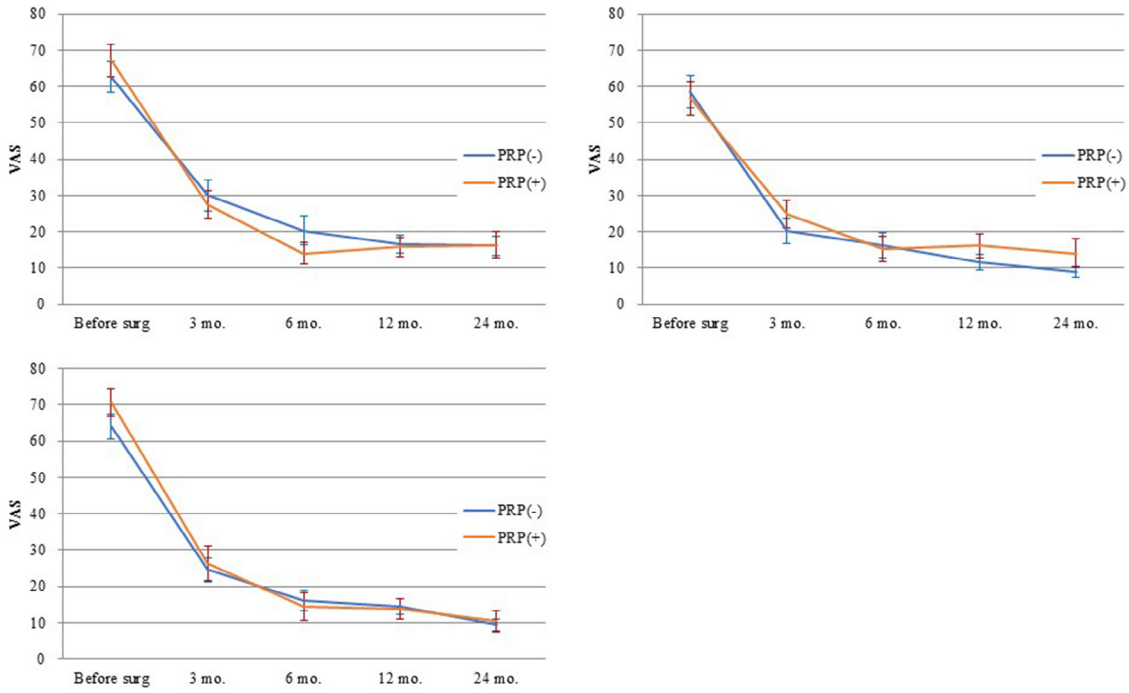

Fig. 2. Three scores significantly improved after surgery compared with before surgery in the two groups (p<.05). Pain score: VAS scores for low back pain (Top Left), leg pain (Bottom), and leg numbness (Top Right) were not significantly different between the two groups before surgery or at any time after surgery. Data shown as mean±standard error of the mean. VAS, visual analog scale; PRP, platelet-rich plasma.

required for bone union were 9.8±0.6 months in the control group and 7.8±0.6 months in the PRP group (Table 3). There was a significant difference in the fusion period between the groups (p<.05).

Pain score

The VAS scores for low back pain, leg pain, and leg numbness were not significantly different between the two groups before surgery (p>.05, Fig. 2). The three scores significantly improved after surgery in both groups (p<.05, Fig. 2). However, VAS scores at each point during follow-up (24 months) after surgery were not significantly different between the two groups (p>.05, Fig. 2).

Adverse events

There were no adverse events in either group.

Discussion

In the present study, patients treated with PRP showed a higher fusion rate and greater fusion mass after spinal fusion surgery compared with those patients not treated with PRP. The use of PRP in the PLF surgery shortened the time of bone union by approximately 2 months. In addition, the average platelet count was 7.7 times higher and the growth factor concentrations in PRP were 50 times higher than those measured in the blood plasma. These results suggest that the use of PRP in PLF surgery can significantly promote bone fusion and can reduce the time required for bone union.

Platelet-rich plasma is a type of plasma that is prepared from the patient's own blood by simple centrifugation and has a high platelet concentration. Platelet-rich plasma contains many growth factors, such as PDGF or TGF-β, which produce therapeutic effects by enhancing chemotaxis and proliferation of mesenchymal stem cells [18–20].

The first clinical report of the use of PRP was by Marx et al. in 1998 for the treatment of patients with mandibular bone defects [21]. Marx et al. recommended a minimally effective platelet concentration in PRP of at least $100\times10^4/\mu L$, targeting a four- to fivefold increase over the usual blood levels [22]. In the present study, the average platelet count in PRP was 7.7 times higher than that measured in the blood. Therefore, we consider that the PRP used in the present study was effective to promote bone union.

Lowery et al. first reported lumbar spinal fixation using platelet concentrates in 19 patients. Bone union was confirmed at the time of the second surgery in 5 patients, whereas bone union was confirmed using radiographic assessment in the remaining 14 patients [12]. Hee et al. reported higher bone union rates at 4 and 6 months postoperatively in 23 patients treated with autograft and platelet gel concentrates than in the traditional autograft control group [10]. However, other researchers have reported decreased autograft fusion rates despite the addition of PRP [13,14]. Therefore, the utility of

PRP in promoting spinal fixation has been controversial. However, previous studies were not prospective controlled clinical trials.

In the present prospective randomized controlled trial, the use of PRP may have resulted in a significant promotion of bone fusion and a shorter period of bone union because we used an appropriate PRP concentration that promotes bone formation. We also speculate that in the studies that reported the ineffectiveness of PRP, the recommended dose may not have been achieved because most of these studies failed to contain data regarding the platelet count or the growth factor concentration in the PRP [10–14].

Direct application of growth factors to a bone defect can cause a significant increase in callus density and volume [23,24], but their high cost limits their clinical applicability. In the present study, we confirmed that the average growth factor concentrations in PRP were more than 50 times greater than those measured in the blood. Therefore, we suggest that PRP can be a low-cost alternative for the direct application of growth factors.

In the present study, although there was a significant difference in the fusion rate, there was no significant difference in the VAS score between the two groups during follow-up. Some authors have reported a discrepancy between bone union and clinical results [25,26]. Successful arthrodesis occurred in 83% of the patients with instrumented posterolateral fusion surgery; however, successful fusion was not related to patient outcomes [25]. By contrast, pseudoarthrosis of the fusion mass was seen in 36% of the patients, and the clinical results were excellent for these patients because of the development of a fibrous union that appeared to provide sufficient structural support to prevent progressive olisthesis [26]. Therefore, we concluded that the present findings support these earlier findings.

The present study has several limitations. First, PRP may induce heterotopic bone formation. Care should be taken to keep PRP separate from the soft tissues of the spinal canal to prevent this adverse effect [27]. Second, the amount and quality of the local autograft was not uniform between patients, although this limitation existed in both treatment groups. Finally, platelet handling can cause premature degranulation and growth factor release. Concentrate preparation was performed as carefully as possible before the gel application into a graft composite.

In conclusion, the present prospective randomized controlled study demonstrated that PRP can improve bone union rate and enlarge bone mass after PLF surgery, and can significantly shorten the period of union by approximately 2 months. The average platelet count was approximately 7.7 higher and the growth factor concentrations in the PRP were approximately 50 times higher than those measured in the blood plasma. These results suggest that the use of PRP in PLF surgery can promote bone union and can shorten the time of fusion if sufficiently high concentrations of platelets and bone union-related growth factors are used.

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