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Original research

# Comparison study between plasma rich in growth factors and platelet-rich plasma for osteoconduction in rat calvaria

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ABSTRACT

*Objective:* Plasma rich in growth factors (PRGF) and platelet-rich plasma (PRP) can be rapidly obtained from patient blood. They are a new and potentially useful adjunct in oral and maxillofacial bone repair or regenerative surgery. The aim of this study was to compare the possibility of new bone formation using PRGF and PRP.
*Methods:* The osteogenic potential with transplantation of PRGF or PRP onto rat calvaria was evaluated by histologic examination and microCT. PRGF or PRP was prepared by centrifugation of rat whole blood (WB). First, the cells in the blood product were counted; there were no leukocytes in PRGF, and PRP included leukocytes. PRGF contained higher levels of TGF-β1 and PDGF-BB than PRP. Furthermore, PRGF or PRP was transplanted onto calvarial bone of rats.
*Results:* MicroCT showed that PRGF promoted an increase in bone volume when compared to PRP. Histological observation demonstrated that the PRGF group showed newly formed bone in a wide range. In addition, the PRP group showed numerous inflammatory cells compared to the PRGF group in HE-stained specimens. This suggests that PRP might delay bone regeneration due to the inflammatory response.
*Conclusions:* PRGF has more availability for bone regeneration than PRP, and PRGF may be useful in bone regeneration treatment.


## 1. Introduction

Platelet concentration products, which are autologous constituents of inductive factors obtained from blood, have high concentrations of platelets containing various growth factors [1]. The concentration of autologous platelets in plasma [platelet-rich plasma (PRP)] and the growth factors contained within platelets have been studied since 1998 [2]. The previous study reported that the combined use of autogenous bone with PRP increased radiographic and histomorphometric bone densities [2]. In addition, PRP has been used in bone augmentation for dental implants or fracture healing of jaw bone [2–5]. However, it has been shown that PRP formulations have different biological activities, depending on their preparation and administration [6–8].

The preparation of plasma rich in growth factors (PRGF) is one way to concentrate platelets [9,10], and in 1999, it was shown to have several advantages, including the enhancement of bone regeneration and rapid soft tissue healing [11]. This system is advantageous as it requires only one step of centrifugation and is leukocyte-free, thus avoiding higher levels of pro-inflammatory cytokines [3]. In addition, PRGF contains high levels of growth factors such as transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF), which are associated with bone regeneration [12]. However, there has been little basic research into the efficacy of PRGF in bone regeneration [9].

The aim of this study was to compare between PRGF and PRP to the availability of bone formation in transplantation of using histologic findings and microCT analysis.

## 2. Materials and methods

### 2.1. Preparations of PRGF and PRP

A total of 27 Sprague-Dawley male rats (age: 15 weeks) weighing 405–415 g were purchased from Japan SLC (Shizuoka, Japan). Rat whole blood (5 ml) was collected from the external jugular vein by syringe aspiration via direct venipuncture with a 21-gauge needle. Blood was immediately placed into 5-ml sterile extraction

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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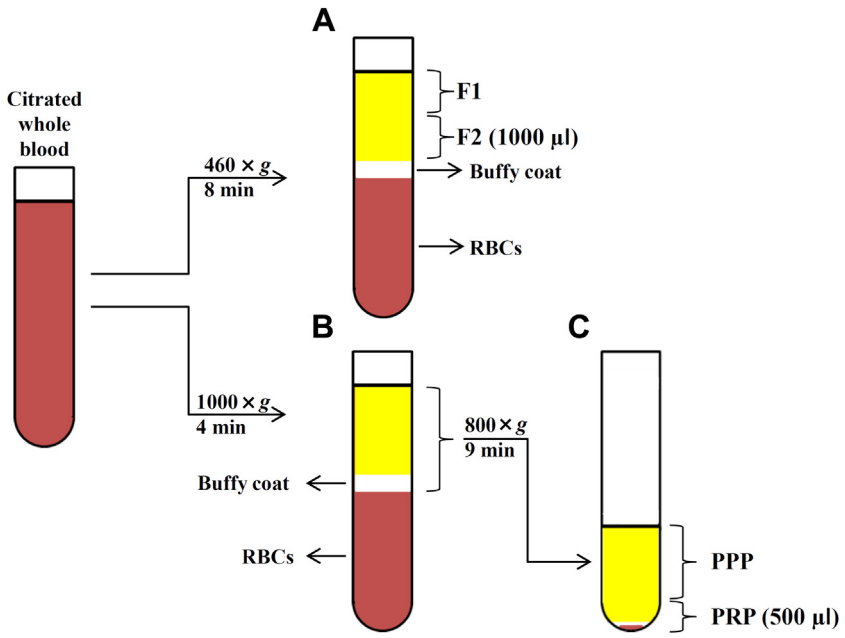


**Fig. 1.** Blood plasma fractions were obtained from citrate-anticoagulated whole blood.
(A) Tubes were centrifuged at 460*g* for 8 min to give PRGF. The bottom fraction contains erythrocytes, and the top fraction contains platelets. Both fractions are separated by the whitish buffy coat, which contains leukocytes. The plasma fraction (1 ml over the buffy coat) was collected to prepare Fraction 2 (F2). Fraction 1 (F1) was prepared from the upper layer of F2. (B, C) PRP was prepared at a separation spin of 1000 × *g* for 4 min, followed by a concentration spin of 800 × *g* for 9 min. (B) The layer of plasma containing the buffy coat was concentrated. (C) Plasma (500 µl) containing WBCs and RBCs were collected to prepare PRP. PPP was prepared from the upper layer of PRP in plasma.

tubes containing 0.5 ml of 3.8% sodium citrate as an anticoagulant (BTI Biotechnology Institute, Vitoria, Spain).

PRGF was prepared from whole blood (WB) centrifuged in accordance with Anitua's protocol [9,10,13] (Fig. 1A). Briefly, tubes were centrifuged at 460*g* for 8 min. The bottom fraction containing erythrocytes and the top fraction containing platelets are separated by the whitish buffy coat, which contains leukocytes. The plasma fraction (1 ml over the buffy coat) was collected as Fraction 2 (F2), while Fraction 1 (F1) was the layer above F2.

PRP was prepared from WB using the double-spin method described by Marx [5] (Fig. 1B and C). Briefly, WB was centrifuged at 1000 × *g* for 4 min, and the plasma layer containing the buffy coat was collected (Fig. 1B), followed by centrifugation at 800*g* for 9 min. The lower plasma layer (500 µl) containing white blood cells (WBCs) and red blood cells (RBCs) was collected to prepare PRP. The upper layer was platelet-poor plasma (PPP) (Fig. 1C). All animals were maintained and used in accordance with the Nihon University Intramural Animal Use guidelines (Ethics Committee Registration No: AP12MD018).

### 2.2. Characteristics of autologous blood product

Platelets, WBCs, and RBCs in PRGF and PRP were counted using Celltac α (MEK-6358; NIHON KOHDEN, Tokyo, Japan). Samples of serum, PPP, PRP, PRGF F1, and PRGF F2 for evaluating growth factor concentrations were stored at −80 °C before use. Levels of TGF-β1, PDGF-BB, and insulin-like growth factor (IGF)-1 were measured using ELISA (enzyme-linked immunosorbent assay) kits (Quantikine colorimetric ELISA kits; R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions.

### 2.3. Transplantation procedure

The transplantation procedure was designed to evaluate newly formed bone in the presence of PRP or PRGF F2 with artificial dam preparation using PTFE tubes (inner diameter, 3 mm; outer diameter, 4 mm; height, 2 mm) *in vivo*. Rats were randomly divided into three groups (n = 3 per group): the Control group (PTFE tube only), the PRP group (PRP in the PTFE tube), and the PRGF group (PRGF F2 in the PTFE tube).

All animals were anesthetized by intraperitoneal injection of 25 mg/kg pentobarbital sodium (Somnopentyl®; Kyoritsu Seiyaku, Tokyo, Japan), and the surgical site was prepared aseptically. A previous study reported that PRGF F2 had a higher platelet concentration and growth factors compared with PRGF F1 [9,13]. From this kind of reason, we used F2 in transplantation. Platelets in PRP or PRGF F2 were activated using 10% calcium chloride solution just prior to use [9,14,15]. Transplantation protocol into rats was performed as in previous reports [16–18]. Briefly, a linear skin incision was made along the edge of the skull with a #15 surgical blade. The periosteum of the calvaria was carefully removed with a raspatorium after elevation of the skin-periosteum flap. PRP or PRGF F2 was inserted into PTFE tubes, which were then placed on the calvarial surface. Overlying tissue was closed with sutures.

### 2.4. MicroCT analysis of transplants

Quantitative image analysis of new bone formation was performed using an *in vivo* microCT system (Rigaku-mCT, Tokyo, Japan). Rats were deeply anesthetized with intraperitoneal injection of sodium pentobarbital (25 mg/kg) and individually placed on the object stage. Calvarial bone at the PTFE tube site was scanned using a microCT system with an X-ray source of 90 kV/150 µA at 2, 4, and 8 weeks after transplantation. Imaging of newly formed bone in the area containing the inner cavity of the PTFE tube was then performed over a full 360°, with an exposure time of 2 min. An isotropic resolution of 20 × 20 × 20 $\mu m^3$ voxels was selected, which displayed the microstructure of new bone formation in rats. The original 3D images were displayed and analyzed using I-view software (J. Morita, Kyoto, Japan). Analysis of new bone formation was performed in the area containing the inner cavity of the PTFE tube. Linearity of the micro-CT scanner was established by scanning

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