

Platelet and growth factor concentrations in activated platelet-rich plasma: a comparison of seven commercial separation systems

Satoshi Kushida · Natsuko Kakudo ·
Naoki Morimoto · Tomoya Hara · Takeshi Ogawa ·
Toshihito Mitsui · Kenji Kusumoto

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Abstract Platelet-rich plasma (PRP) is blood plasma that has been enriched with platelets. It holds promise for clinical use in areas such as wound healing and regenerative medicine, including bone regeneration. This study characterized the composition of PRP produced by seven commercially available separation systems (JP200, GLO PRP, Magellan Autologous Platelet Separator System, KYOCERA Medical PRP Kit, SELPHYL, MyCells, and Dr. Shin's System THROMBO KIT) to evaluate the platelet, white blood cell, red blood cell, and growth factor concentrations, as well as platelet-derived growth factor-AB (PDGF-AB), transforming growth factor beta-1 (TGF- β 1), and vascular endothelial growth factor (VEGF) concentrations. PRP prepared using the Magellan Autologous Platelet Separator System and the KYOCERA Medical PRP Kit contained the highest platelet concentrations. The mean PDGF-AB concentration of activated PRP was the highest from JP200, followed by the KYOCERA Medical PRP Kit, Magellan Autologous Platelet Separator System, MyCells, and GLO PRP. TGF- β 1 and VEGF concentrations varied greatly among individual samples, and there was almost no significant difference among the different systems, unlike for PDGF. The SELPHYL system produced PRP with low concentrations of both platelets and growth factors. Commercial PRP separation systems vary widely, and familiarity with their individual advantages is

important to extend their clinical application to a wide variety of conditions.

Keywords Platelet-rich plasma · Growth factor · Comparison

Introduction

Platelet-rich plasma (PRP) is blood plasma that has been enriched with platelets. As a concentrated source of autologous platelets, PRP contains several different growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF- β), and vascular endothelial growth factor (VEGF), as well as other cytokines that stimulate healing of bone and soft tissue. It has been shown that PRP has the potential to promote cell proliferation [1], chemotaxis [2], cell differentiation, and angiogenesis [2]. Therefore, PRP has become increasingly popular as a clinical treatment in a wide variety of medical applications in almost all fields of surgery [3], particularly for chronic wounds [4], maxillofacial bone defects [5], tendon healing in orthopedics [6], and the fields of cosmetic surgery [7].

However, even though there are many methods of preparing PRP, few comparisons of PRP produced by various platelet separation models have been reported. Differences in the purification method used to produce PRP may give rise to differences in the amount of whole blood required, final yield, liquid components, and growth factor concentrations, and knowledge of these differences may enable PRP kits to be used in ways suitable for clinical applications.

The purpose of this study was to evaluate the platelet, white blood cell (WBC), red blood cell (RBC), and growth

S. Kushida (✉) · N. Kakudo (✉) · N. Morimoto · T. Hara ·
T. Ogawa · T. Mitsui · K. Kusumoto
Department of Plastic and Reconstructive Surgery,
Kansai Medical University, 2-5-1 Shin-machi, Hirakata,
Osaka 573-1010, Japan
e-mail: kushidas@takii.kmu.ac.jp

N. Kakudo
e-mail: kakudon@hirakata.kmu.ac.jp

factor concentrations, as well as platelet-derived growth factor-AB (PDGF-AB), TGF- β , and VEGF concentrations in the PRP produced by seven commercial PRP separation systems.

Materials and methods

Platelet Separation Systems

The study was conducted in accordance with the principles of the Declaration of Helsinki 1996 and Good Clinical Practice standards. The study protocol, informed consent form, and other study-related documents were reviewed and approved by our institutional ethics committee, who found the protocol acceptable.

The whole blood was collected from five healthy subjects (four men, one woman, 26–35 years old) who were not taking medication. A single technician collected 150 mL of blood from each participant using a 19-gauge arteriovenous fistula needle (TERUMO Corporation, Tokyo, Japan).

Whole blood samples were collected with the appropriate ratio of anticoagulant according to the manufacturer's instructions, and each sample was simultaneously processed using each PRP kit: JP200 (BS Medical Co., Ltd., Tokyo, Japan), GLO PRP (Glofinn Oy, Salo, Finland), Magellan Autologous Platelet Separator System (Medtronic Inc., Minneapolis, MN), KYOCERA Medical PRP Kit (KYOCERA Medical Corporation, Osaka, Japan), SELPHYL (Cascade Medical Enterprises, LLC, Wayne, NJ), MyCells (Kaylight Technologies Ltd, Holon, Israel), and Dr. Shin's System THROMBO KIT (GRAND AESPIO IMC., Seoul, Korea).

Centrifugation was performed two times in four systems and once in three for the preparation of PRP, following to each original protocol. PRP was separated by tube centrifugation in 4, gel separation in 2, and fully automated centrifugation in one system. The preparation process was semi-closed in 4, closed in 2, and open in 1. The necessary whole blood volume was 8–60 mL, and the final volume of PRP was 0.6–3 mL. All PRP samples were collected and activated according to each manufacturer's protocol. A 1:1 (v/v) mixture of 0.5 M CaCl₂ and autologous thrombin was prepared in advance as an activator [1]. A 10:1 (v/v) mixture of PRP and the activator was incubated for 5 min at room temperature for the JP200, GLO PRP, Magellan Autologous Platelet Separator System, SELPHYL, and Dr. Shin's System THROMBO KIT. On the other hand, PRP produced by the KYOCERA Medical PRP Kit and SELPHYL was activated with 0.5 M CaCl₂ at one-tenth of the amount of the total PRP according to the manufacturer's instructions. The supernatants of the activated samples

were stored at -80°C for future analyses to determine the concentrations of PDGF-AB, active TGF- β 1, and VEGF.

Quantification of platelet, WBC, and RBC concentrations

All whole blood and PRP samples were sent to the University Hospital Clinical Laboratory immediately after PRP collection. Platelet, WBC, and RBC counts were performed using an XE-2100 Automated Hematology System (SYS-MEX CORPORATION, Kobe, Japan).

Quantification of growth factors

The PDGF-AB, TGF- β 1, and VEGF concentrations in the preparation of activated PRP were determined by a commercially available sandwich enzyme-linked immunosorbent assay technique kit (Quantikine, R&D Systems, Inc., Minneapolis, MN, USA). Growth factor concentrations were measured according to the manufacturer's instructions. All assays were performed in triplicate.

Statistical analysis

All data are presented as mean \pm SD. The Tukey–Kramer test for multiple comparisons among the seven groups was used. For all statistical analyses, the significance was set at $p < 0.05$.

Results

Each system produced 0.6–3 mL of PRP per whole blood sample, even though the starting whole blood volume was different for each system.

The price of the disposable kit used for the preparation of PRP varied from US\$50 to US\$500. The Magellan Autologous Platelet Separator System was the most expensive (US\$500/kit). JP200 and GLO were the least expensive (US\$50/kit) (Table 1).

Platelet concentration of PRP

The average whole blood platelet concentration was $16.8 \times 10^4/\mu\text{L}$. The mean platelet concentration of PRP was the highest from the Magellan Autologous Platelet Separator System ($152.1 \times 10^4/\mu\text{L}$), followed by the KYOCERA Medical PRP Kit ($131.2 \times 10^4/\text{mL}$) and GLO PRP ($89.1 \times 10^4/\text{mL}$). There was no significant difference between the Magellan Autologous Platelet Separator System and the KYOCERA Medical PRP Kit. The mean PRP platelet concentration was the lowest from SELPHYL ($8.8 \times 10^4/\text{mL}$) (Fig. 1a).

Table 1 Protocols for the platelet-rich plasma separation systems tested

System	Number of centrifuge times	Centrifuge force and time	Features	Preparation process	Whole blood volume (mL)	Final volume of PRP (mL)	Cost/1 kit
JP200	2	1,000×g 6 min 800×g 8 min	Tube centrifugation	Open system	20	1	\$50
GLO PRP	2	1,800×g 3 min 1,800×g 6 min	Tube centrifugation	Semi-closed system	8.5	0.6	\$95
MAGELLAN	2	610×g 4 min 1,240×g 6 min	Fully automated centrifugation	Closed system	60	3	\$500
KYOCERA	2	600×g 7 min 2,000×g 5 min	Tube centrifugation	Semi-closed system	20	2	\$100
Selphyl	1	525×g 15 min	Gel separation	Semi-closed system	8	2	\$215
MyCells	1	2,054×g 7 min	Gel separation	Closed system	10	1	\$120
Dr. Shin's System	1	1,720×g 8 min	Tube centrifugation	Semi-closed system	8.5	1	\$190

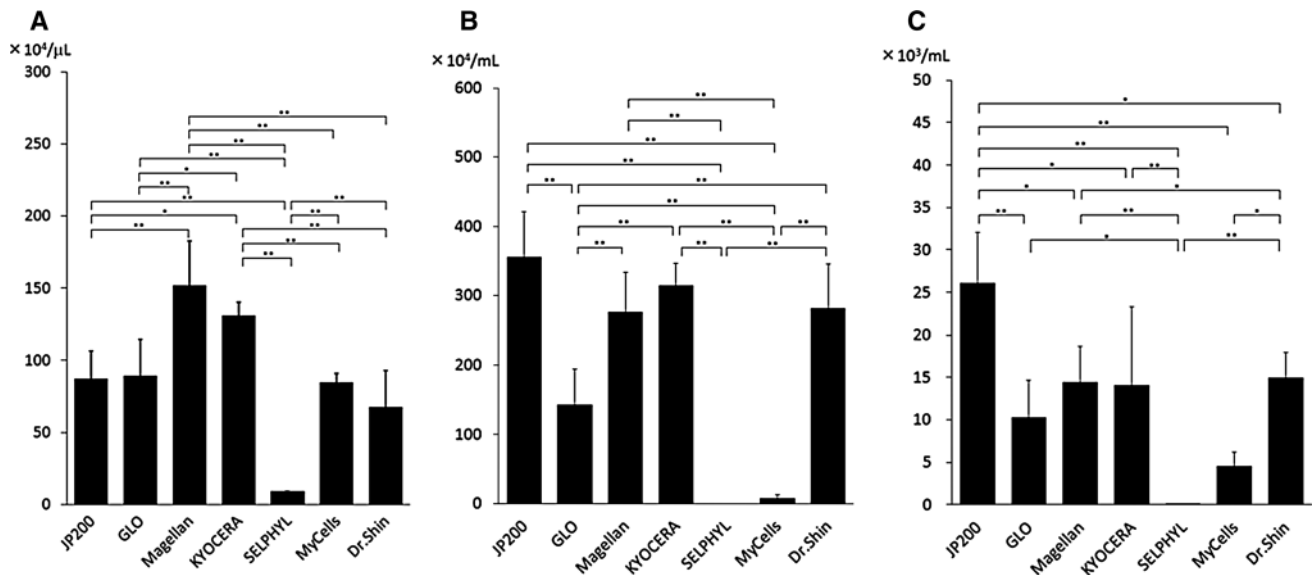


Fig. 1 a Platelet concentrations, b red blood cell concentrations, c white blood cell concentrations of PRP with JP200, GLO PRP, Magellan Autologous Platelet Separator System, KYOCERA Medical

PRP Kit, SELPHYL, MyCells, and Dr. Shin's System THROMBO KIT. Data are presented as mean ± SD (**p < 0.01; *p < 0.05)

RBC concentration of PRP

The mean RBC concentration of PRP was the highest from JP200 ($355.8 \times 10^4/\text{mL}$), followed by the KYOCERA Medical PRP Kit ($314.4 \times 10^4/\text{mL}$) and Dr. Shin's System THROMBO KIT ($282.2 \times 10^4/\text{mL}$) (Fig. 1b).

WBC concentration of PRP

The mean WBC concentration of PRP was the highest from JP200 ($26.1 \times 10^3/\text{mL}$), followed by Dr. Shin's System

THROMBO KIT ($14.9 \times 10^3/\text{mL}$), the Magellan Autologous Platelet Separator System ($14.4 \times 10^3/\text{mL}$), and the KYOCERA Medical PRP Kit. The mean RBC concentration of PRP was the lowest from SELPHYL ($0.3 \times 10^3/\text{mL}$) (Fig. 1c).

PDGF-AB concentration of PRP

The mean PDGF-AB concentration of activated PRP was the highest from JP200 (93.5 ng/mL), followed by the KYOCERA Medical PRP Kit (76.2 ng/mL), the Magellan

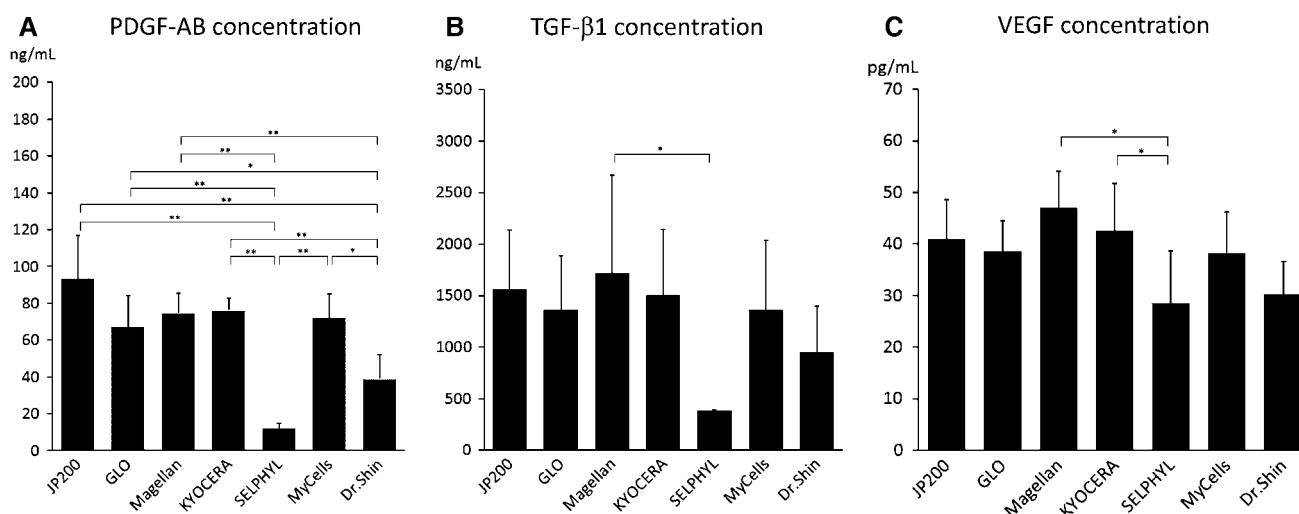


Fig. 2 **a** The platelet-derived growth factor-AB concentrations, **b** the transforming growth factor beta-1 concentrations, **c** the vascular endothelial growth factor concentrations of activated PRP with JP200, GLO PRP, Magellan Autologous Platelet Separator System,

KYOCERA Medical PRP Kit, SELPHYL, MyCells, and Dr. Shin's System THROMBO KIT. Data are presented as mean \pm SD (** $p < 0.01$; * $p < 0.05$)

Autologous Platelet Separator System (74.8 ng/mL), MyCells (72.2 ng/mL), and GLO PRP (67.3 ng/mL). There was no significant difference among the five groups with the highest concentrations. The mean RBC concentration of PRP was the lowest from SELPHYL (12.2 ng/mL) (Fig. 2a).

TGF-β1 concentration of PRP

The mean TGF-β1 concentration of activated PRP was the highest from the Magellan® Autologous Platelet Separator System (1,719.0 pg/mL), followed by JP200 (1,563.0 pg/mL) and the KYOCERA Medical PRP Kit (1,508.2 pg/mL). The mean VEGF concentration of activated PRP was the lowest from SELPHYL (384.0 pg/mL). The difference was significant only between the Magellan® Autologous Platelet Separator System and SELPHYL (Fig. 2b).

VEGF concentration of PRP

The mean VEGF concentration of activated PRP was the highest from the Magellan® Autologous Platelet Separator System (47.0 ng/mL), followed by KYOCERA Medical PRP Kit and JP200 (Fig. 2c).

Discussion

In this study, seven different PRP preparation processes were compared with respect to platelet, RBC, and WBC yields of the procedure and growth factor release. Although there have been a few previous studies comparing two or

three types of commercial PRP kits [7–9], this is the first comparative investigation to address seven types at the same time.

Measurements of platelet and growth factor concentrations are widely used to evaluate PRP [1, 8]. Furthermore, Wibrich et al. [9] suggested that different individuals may require different platelet concentration ratios to achieve a comparable biological effect. Castelio et al. [8] compared different PRP kits by producing PRP from blood samples from a single individual, and they emphasized that a comparison of platelet and growth factor concentrations was required. In the present experiment, their comments were taken into account, and single-donor PRP produced by seven commercially available PRP separation systems was compared. Platelet concentration is important in the production of PRP. Platelet α -granules contain growth factors such as PDGF, TGF- β , and VEGF. Some researchers have suggested that PRP should achieve a three- to fivefold increase in platelet concentration over baseline [10, 11]. In this study, all kits other than the SELPHYL achieved a platelet concentration increase of at least threefold.

In general, PDGF is a powerful mitogen for fibroblasts and smooth muscle cells and is involved in all three phases of wound healing, including angiogenesis, the formation of fibrous tissue, and re-epithelialization [12]. The primary effect of PDGF seems to be its mitogenic activity to fibroblasts and vascular muscle cells [13]. The most important specific activities of PDGF include angiogenesis and chemotaxis for fibroblasts and collagen synthesis.

Transforming growth factor stimulates the proliferation of undifferentiated mesenchymal stem cells and stimulates

chemotaxis of endothelial cells and angiogenesis [13]. VEGF increases angiogenesis and vascular permeability [14].

The concentrations of platelets and growth factors have been reported to exhibit a directly proportional relationship [15]. In the present study, although PRP containing more than a certain concentration of platelets tended to show higher PDGF concentrations, the relationship was not always directly proportional. Although the PDGF concentration in the PRP produced by the JP200 had the highest mean value, there were no significant differences with the KYOCERA Medical PRP Kit, the Magellan Autologous Platelet Separator System, MyCells, and GLO. TGF- β and VEGF concentrations varied greatly among individual samples, meaning that there was almost no significant difference among the different systems. One reason for this may have been the small number of samples. The present results suggest that PDGF should be used as an indicator when comparing methods for producing PRP in terms of growth factor concentrations because of its low individual variability.

Kakudo et al. [1] previously reported the concentration of platelets, PDGF-AB, and TGF- β from JP200; the median whole blood platelet count was $16.74 \times 10^4/\mu\text{L}$, and the median platelet count in the PRP from JP200 was $132.26 \times 10^4/\mu\text{L}$. In the present study, the mean whole blood platelet count was $16.8 \times 10^4/\text{mL}$, and that in the PRP from JP200 was $87.1 \times 10^4/\text{mL}$, a value that was somewhat low. This may have been because the JP200 is a manual blood cell layer removal system for extracting PRP. The transfer from the blood cell layer to the centrifuge tube for platelet concentration is performed manually, making the task more cumbersome, and this means that practice is required to produce PRP of consistent quality.

An automated blood cell layer isolation system has therefore been developed with the aim of compensating for the disadvantage of this manually operated system. This automates the transfer operation from the blood cell layer to the centrifuge tube for platelet concentration, there is almost no scope for the introduction of technical errors, and PRP can be produced simply by almost anyone. The Magellan Autologous Platelet Separator System consists of a microprocessor-controlled centrifuge, syringe pumps, and necessary single-use processing components. With the platelet separator instrument, PRP is automatically and quickly separated from anticoagulated blood and dispensed into a separate sterile syringe [16]. Kakudo et al. [16] previously reported the efficiency of PRP produced by the Magellan Autologous Platelet Separator System for intractable skin ulcers, such as diabetic and venous ulcers. The mean platelet concentration in PRP prepared using the Magellan system

was increased 7.1 ± 0.79 times, and epithelialization of the ulcer took an average of 6.6 weeks. In this study, the average whole blood platelet concentration was $16.8 \times 10^4/\mu\text{L}$, and the mean platelet concentration of PRP from the Magellan Autologous Platelet Separator System was $152.1 \times 10^4/\mu\text{L}$. Therefore, the mean platelet concentration increase was about 9.0 times. Such use of an automated blood cell layer separation system enables the production of high-concentration PRP irrespective of the proficiency of the operator. In addition, it has been reported that PRP prepared using this Magellan Autologous Platelet Separator System was effective for the following conditions: postoperative surgical incision sites [17], punch biopsy wounds [18], postoperative pain after total shoulder arthroplasty [19], and bariatric surgery [20].

Castillo et al. [8] reported that the platelet concentration of the Magellan was $780.2 \times 10^3/\mu\text{L}$. They also reported that concentrations of the growth factors PDGF-AB, VEGF, and TGF- β 1 were 34.4 ± 10.7 , 1.2 ± 0.8 , and 0.2 ± 0.1 ng/mL, respectively. In the present study, higher values were obtained from the Magellan system for both the platelet count and growth factor levels than those found by Castillo et al. This was probably because, while they harvested a final volume of 6 mL of PRP from 26 mL of whole blood, 3 mL of PRP was harvested from 56 mL of whole blood in the present study. The advantage of the Magellan is that the amounts of whole blood and PRP can be set as desired using the panel. In the field of oral surgery, for example, PRP may need to be used in narrow tooth extraction cavities, whereas in plastic surgery, a greater volume will be required for ulcers or pressure sore pockets, making the fact that it is possible to set the final volume of PRP a major advantage of the Magellan. On the other hand, it does entail the disadvantage that the fully automated Separator System must be purchased separately, meaning that a larger initial investment is required.

Platelet-rich plasma produced by SELPHYL has usually been used for improvement of deep nasolabial folds in aesthetic and cosmetic surgery [21, 22]. Sclafani et al. [23] reported that when PRP produced by SELPHYL was injected into the deep dermis, activated fibroblasts and new collagen deposition and development of new blood vessels occurred. In the present study, the platelet count in the PRP produced by the SELPHYL system was the lowest of any of the seven types of kit surveyed, and this was reflected in the concentrations of growth factors except for VEGF. Notwithstanding the low platelet concentration, however, its low RBC count means that it does not leave any bruising when injected subcutaneously, which is a major advantage from the cosmetic perspective. In the present study, PRP prepared using

MyCells had a similarly low RBC count to that produced by SELPHYL, but it had both a high platelet concentration and high levels of PDGF and VEGF. There have as yet been no reports of the clinical use of MyCells, but on the basis of the present results, it may hold promise for use in cosmetic surgery.

It has been proposed that the role of WBCs in PRP may be related to the concentrated presence of leukocytes, which can provide a local environment at the site of PRP injection with increased immunomodulatory capability that may aid in preventing or controlling infection at the identified site of injury [8]. However, there are currently no controlled animal studies and clinical studies to compare the effect between leukocyte-rich PRP and leukocyte-poor PRP. In the present study, PRP from the JP200 showed the highest WBC count. Future studies are required to investigate whether WBCs in PRP exert any action *in vivo*.

There have been no previous reports of the recently developed GLO PRP, KYOCERA Medical PRP Kit, and Dr. Shin's System THROMBO KIT, and the present study is the first to demonstrate the features and efficacies of these kits. All of these kits produced PRP with platelet concentrations and growth factor contents above a certain level, and all are semi-closed kits using syringe centrifugation, enabling their use with general-purpose centrifuges, which means that they offer the advantage of enabling PRP to be prepared simply and inexpensively.

The price of the disposable kit used for the preparation of PRP varied from US\$50 to US\$500. JP200 was the least expensive (US\$50), but the PRP prepared with these kits showed high PDGF concentrations, and they are considered to be clinically cost-effective.

Further larger studies are necessary to characterize the system variability in platelet, WBC, RBC, and growth factor concentrations in the PRP from commercial PRP separation systems. It will also be necessary to carry out comparative investigations of the effects of these various kits *in vivo*. A wide variety of commercial PRP separation systems is available, and it will be important to proceed with their clinical application in ways that make the best use of their individual advantages.

Various PRP kits are expected to be developed in the future, but the PDGF concentration in PRP is considered to serve as a reasonable index for comparing their performance. Presently, JP200 and GLO appear to be clinically recommendable from the viewpoint of cost-performance. Lastly here we added that these data are based on each protocol at the experimental timing of 2012.

Conflict of interest None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this manuscript.

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