Original Article

A basic analysis of platelet-rich fibrin: distribution and release of platelet-derived growth factor-BB

Warunee Pluemsakunthai[1)], Shinji Kuroda[2,*)], Hitoyata Shimokawa[3)] and Shohei Kasugai[1)]

[1)]Oral Implantology and Regenerative Dental Medicine, Global Center of Excellence (G-COE) Program, Tokyo Medical and Dental University, Tokyo, Japan
[2)]Oral Implantology and Regenerative Dental Medicine, Tokyo Medical and Dental University, Tokyo, Japan
[3)]Pediatric dentistry, Tokyo Medical and Dental University, Tokyo, Japan

Platelet-rich fibrin (PRF) is a natural reservoir for growth factors because it forms of a strong fibrin matrix. PRF is harvested by centrifuging blood collected without anticoagulants and functions by allowing slow release of several growth factors, which is useful for tissue regeneration. However, despite the multitude of clinical applications for PRF, there is little valid evidence for its performance based upon dynamic or molecular analyses. Clinical data on PRF clot, membrane, exudate, and supernatant forms, especially in the context of platelet-derived growth factor-BB (PDGF-BB) distribution, have not been sufficiently analyzed. In this study, PRF was separated into portions (upper and lower halves) containing different numbers of platelets and leukocytes. PDGF-BB levels were measured after guanidine hydrochloride (GndHCl) treatment or incubation in culture media. Our results demonstrated that a higher concentration of PDGF-BB accumulated in clots and membranes from the lower half of PRF. In addition, PDGF-BB could be efficiently extracted from PRF using GndHCl. The lower membrane portion, which dissolved in culture medium within 3 days, released PDGF-BB at a higher rate than the upper membrane portion, which was more stable in the medium. This study provides novel information about growth factor dynamics in PRF and will help clinicians develop strategies for tissue engineering by selectively utilizing the upper or lower portion of PRF.

Rec.1/3/2013, Acc.4/11/2013, pp164-172

*Correspondence should be addressed to:
Shinji Kuroda, Oral Implantology and Regenerative Dental Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan. Phone: +81-3-5803-4656, Fax: +81-3-5803-5934, E-mail: skuroda.mfc@tmd.ac.jp

Key words guanidine hydrochloride, platelet-derived growth factor-BB, extraction, platelet-rich fibrin, degradation

Introduction

Various techniques for fractionating and concentrating human blood have yielded a number of autologous platelet-derived materials that may improve regenerative grafting outcomes. Platelet α-granules contain a variety of growth factors such as transforming growth factor (TGFβ1, TGFβ2), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF-I, IGF-II), epidermal growth factor (EGF), platelet-derived epidermal growth factor (PDEGF), vascular endothelial growth factor (VEGF-A, VEGF-C), and hepatocyte growth factor (HGF)[1-5]. Concentrated platelets can accelerate tissue regeneration and increase the quality and quantity of newly formed tissues by functioning as an ideal reservoir for autologous growth factors. Local application of platelet concentrate has been used for several decades as an adjunct to wound healing[6-9]. The platelet concentrate releases abundant platelet-derived growth factors, which are essential regulators for proliferation, migration, and survival of mesenchymal cell lineages[10]. PDGF is a disulfide-linked dimeric protein composed of 2 polypeptide chains that may exist as a homodimer (PDGF-AA, PDGF-BB, PDGF-CC, PDGF-DD) or as a heterodimer (PDGF-AB)[11]. PDGF-AA and PDGF-BB regulate cell growth and chemotaxis. PDGF-BB is the most potent PDGF isoform, due to its ability to increase the number of collagen-synthesizing cells, promote angiogenesis, and stimulate mitogenesis of osteoblast precursors[12-16].

Platelet-rich plasma (PRP)[17] is the first generation of plasma rich in platelets (1 million/ μL)[3], has been used in various surgical fields[18-27], and is the family of human blood concentrates currently available for clinicians to enhance tissue healing. To prepare PRP, blood is collected with anticoagulant and immediately centrifuged twice. The resulting platelet concentrate is applied to the surgical site with a syringe, together with thrombin and/or calcium chloride to trigger platelet activation and fibrin polymerization[5]. The advantage of PRP is the release of significant growth factors in response to massive platelet activation by thrombin[28]. However, bone formation requires a slow release of growth factors to enable osteoblast immigration and periodic bone remodeling over a period of approximately 2 weeks[29]. PRP has rather low osteoneogenic performance[30] and has a short osteoconductive effect after grafting[31]. For this reason, other methods have been developed for long-term release of growth factors.

Choukroun's platelet-rich fibrin (PRF)[32] is the latest development in reliable platelet concentrates. To generate PRF, blood is collected without anticoagulants. It is immediately centrifuged for 12 min at 400 x g to generate PRF in a layer sandwiched between plasma and red blood cells (RBCs). A natural coagulation process then occurs and allows for easy collection of a leukocyte- and platelet-rich fibrin clot, without the need for biochemical modification of the blood[5]. The resulting PRF thus becomes a biostimulating fibrin-rich dense gel, resistant to traction and tearing, which can be compressed to act as a membrane. There is limited information on the clinical utility of the fibrin clot and supernatant, including the length and extent of their efficacy. Few studies have conducted dynamic analyses of PRF, focusing instead on the release of growth factors into the culture medium. This may be due to the difficulty in extracting growth factors trapped in the PRF matrix.

The aim of this study was to develop an optimal PRF utilization strategy by examining the characteristics of PRF supernatant, exudates, clots, and membranes. To this end, we performed a dynamic analysis of one of the growth factors released by PRF, PDGF-BB, focusing on its distribution in various parts of PRF and the distinct degradation rate of PRF membranes. To accurately measure PDGF-BB trapped within the fibrin matrix of PRF, we propose an extraction method for isolating growth factors from strong fibrin mesh by mechanical and chemical digestion with guanidine hydrochloride (GndHCl), followed by dialysis. The amount of PDGF-BB was compared to that obtained by the conventional method, demonstrating the effectiveness of the GndHCl extraction method. These results may help optimize clinical applications of PRF for a variety of purposes.

Materials and Methods

1)Isolation of PRF from blood

Blood was collected from 6 male donors (n = 6), who were non-smokers, with no history of anticoagulation drug intake, and aged between 25 and 65 years. The experimental procedures were approved by the Institutional Ethics Committee of Tokyo Medical and Dental University. PRF clots were prepared according to the method of Choukroun[32]. Venous blood (12 mL) was drawn and distributed evenly into 4 glass tubes and centrifuged at 400 x g at room temperature for 12 min (Heraeus Labofuge

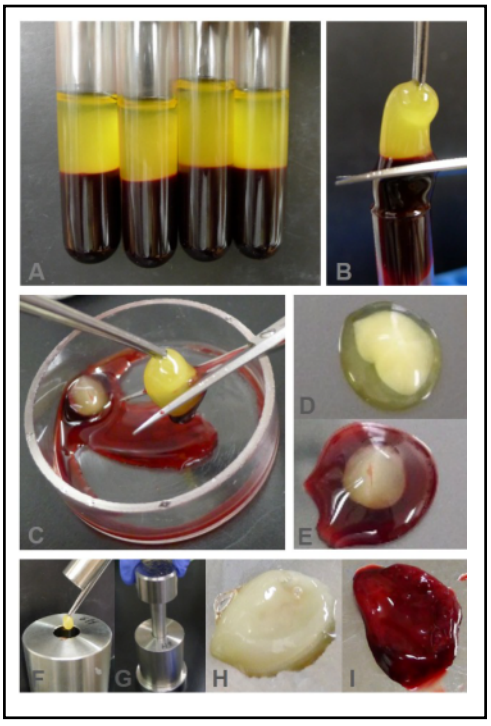

Fig.1 Isolation of PRF from blood

(A) Centrifugation of blood. Three layers were formed: the supernatant, middle gel layer, and bottom layer of RBCs. (B) Isolation of PRF by cutting at the upper 1 mm of the red blood cell layer. (C) PRF was divided into halves by length. (D)-(E) The upper and lower clots. (F)-(G) The upper and lower membranes were made by compressing the upper and lower clots using a homogenizer f to control membrane thickness. (H)-(I) The upper and lower membranes after processing.

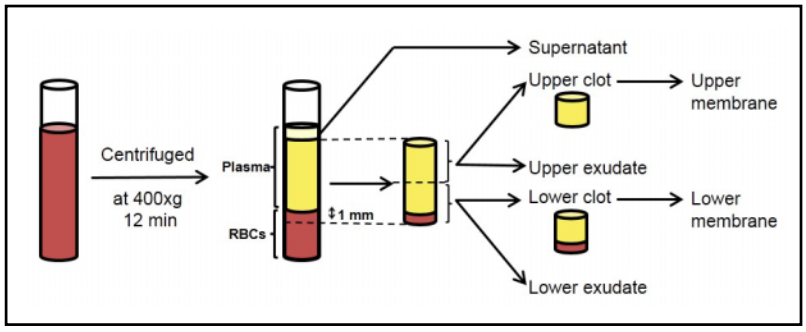

Fig.2 The method of sampling preparations

Whole blood samples (3 mL per tube) were centrifuged to separate into an upper liquid layer (supernatant), middle gel-layer, and lower RBC layer. The gel layer and top 1-mm of the RBC layer below comprised PRF. PRF was divided into an upper clot and lower clot, from which exudates could be extracted. Half of the clot samples were compressed to form PRF membranes. Consequently, 7 different samples were prepared: supernatant, upper clot, lower clot, upper membrane, lower membrane, upper exudate, and lower exudate.

300, Buckinghamshire, UK). After centrifugation, 3 layers are formed: the supernatant at the top, the middle layer, and the RBC layer at the bottom (Fig.1). The supernatant was removed and PRF was isolated by collecting the upper layer after cutting 1 mm below the junction between the middle and RBC layers.

2)Fractionation of PRF into upper and lower clots/membranes/exudates

As shown in Figure 1, PRF was divided into 2 parts by measuring macroscopically and cutting in half by length. PRF was separated into upper and lower layer clots, the exudates from these clots were collected (Fig.2) by syringe and stored at -80℃ for measurement of PDGF-BB. Half of the samples were used to prepare PRF membranes and the other half were used as PRF clots. The membranes were made by compressing either the upper or the lower clot to produce a strong, tear-resistant fibrin matrix membrane. The exudates released from the membranes were not included in the exudate samples because of their small amounts and inapplicability in clinical situations. Thus, 7 different groups (n = 6) were prepared: upper clot, lower clot, upper membrane, lower membrane, upper exudate, lower exudate, and supernatant from the top phase of the centrifuged blood. Each sample was used for direct PDGF-BB extraction analysis immediately after PRF preparation. Another set of PRF membranes was incubated in Dulbecco's Modified Eagle's Medium (DMEM) to profile PDGF-BB release during the experimental period; this was compared to the direct extraction method using GndHCL.

3)Direct extraction method by GndHCl for measurement of PDGF-BB concentration in PRF

All samples were placed in tubes containing 3-fold volume of sample weight of 4 M GndHCl in 50 mM Tris-EDTA (TE) buffer [50 mM Tris-HCl, 1 mM EDTA, pH 7.4]. However, the homogenized membranes were viscous and difficult to transfer the material into dialysis bags. We used 30-fold volume of sample weight of 4 M GndHCl to extract PDGF-BB from the membranes. The samples were homogenized using a Dounce homogenizer and centrifuged at 7,300 x g at 4℃ for 10 min. The supernatants were dialyzed for 1 week against distilled water to remove GndHCl and for 2 days against PBS. Distilled water and PBS were changed daily throughout dialysis. Guanidine hydrochloride extract was collected from all samples and stored at -80 ℃ until PDGF-BB measurement.

4)Incubation method to measure PDGF release and examine the stability of PRF membrane

The upper and the lower membranes were prepared and incubated in DMEM. At each experimental time point (20 min, 1 h, 4 h, 24 h, and 72 h), the conditioned medium was collected and stored at -80℃ for PDGF-BB measurement and the medium was replaced with fresh medium. The characteristics and rate of degradation of the PRF membranes were observed during the 1-week incubation period.

5)Enzyme-linked immunosorbent assay

PDGF concentration was determined by enzyme-linked immunosorbent assay (ELISA) using an ELISA kit (RayBiotech, Inc., Norcross, GA, USA) following the manufacturer's protocol. Absorbance was measured at 450 nm with a spectrofluorometer (ARVO SX, PerkinElmer, Waltham, MA, USA).

6)Statistical analysis

The amount of PDGF-BB per sample weight was shown in pg/mg for comparison between samples. This amount was calculated as shown below;

$$\text{PDGF-BB per sample weight (pg/mg)} = \frac{\text{PDGF-BB (ng/mL blood)} \times 1000}{\text{Sample weight (mg/mL blood)}}$$

All data are presented as mean ± standard error (S.E.). Intergroup comparisons were performed using Student's *t*-test for incubation method and Tamhane's T2 test for post hoc multiple comparisons among PDGF-BB values for GndHCl extraction method.

Results

1)Sample weight

Following routine procedures for managing PRF, PRF clots were removed from the glass tubes with forceps and held upright when cut (Fig.1). This action stretched the fibrin matrix and resulted in a loose fibrin matrix and light weight in the upper part (Table 1). On the other hand, the lower part had less stretched but more exudate than the upper part, resulting in greater weight and larger amount of exudate. Although there is a difference between the number of platelets and leukocytes[33], this difference may not affect the weight of the upper and the lower parts of PRF.

2)PDGF-BB distribution

To determine the distribution of PDGF-BB in each sample, we measured the PDGF-BB concentration in the 7 samples described above. We calculated the amount of PDGF-BB relative to the original sample weight and the results are expressed in pg/mg (Table 1, PDGF-BB per sample weight). In the direct extraction method, all samples were treated with GndHCl and dialyzed to extract PDGF-BB. The lower clot and membrane contained the highest concentrations of PDGF-BB and there was no significant difference between these samples. Conversely, the upper and the lower exudates contained the smallest concentrations of PDGF-BB and there was no significance between these samples (Tamhane's T2, $p < 0.05$).

Table 1 Amount and concentration of PDGF-BB from direct extraction method by GndHCl denote mean ± S.E., n = 6

Samples	Sample weight (mg/ml blood)	PDGF-BB (ng/ml blood, percentage of total PDGF-BB)	PDGF-BB per sample weight (pg/mg)
Supernatant	525.13 ±89.65	28.65 ±4.46 (41.29%)	55.19 ±3.69
Upper clot	36.89 ±4.93	1.66 ±0.28 (2.39%)	43.59 ±2.41
Lower clot	80.99 ±9.93	17.70 ±2.95 (25.51%)	213.17 ±12.5
Upper exudate	182.73 ±17.21	6.77 ±1.01 (9.76%)	35.98 ±2.7
Lower exudate	590.67 ±54.85	14.61 ±1.34 (21.05%)	24.92 ±1.27
Upper membrane	4.66 ±0.35	0.53 ±0.04	113.95 ±6.51
Lower membrane	18.69 ±2.02	3.71 ±0.26	208.75 ±26.73

3)PRF membrane degradation

To evaluate the degradation rate of PRF membranes and correlate it with PDGF-BB release from the membranes, PRF was incubated in cell culture medium and PDGF-BB concentrations were measured by ELISA after 20 min, 1 h, 4 h, 24 h, and 72 h. The lower membrane started to degrade from the RBC side and the proceeded to the other side over 3 d, whereas the upper membrane did not degrade until day 7 (Fig.3). The amount of PDGF-BB released from the upper and lower membranes was significantly different at each time point during the incubation period (student's *t*-test, $p < 0.05$). Both membranes rapidly released PDGF-BB from the start of the incubation until 4 h, followed by dramatically slower release for the remainder of the study (Fig.4A).

The amount of PDGF-BB reached 0.48 ± 0.05 ng in the

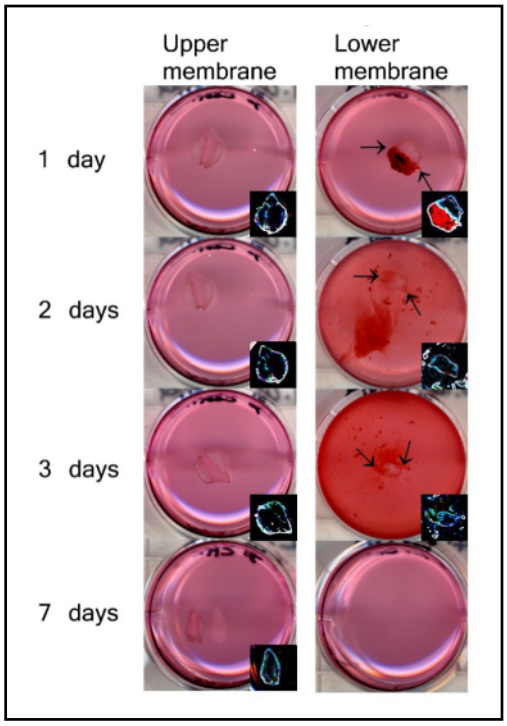

Fig.3 Degradation of PRF membranes incubated in culture medium

The upper and lower membranes were incubated in DMEM and the medium was collected at 20 min, 1 h, 4 h, 24 h, and 72 h for ELISA. The lower membrane degraded by more than 80% within 3 d, while the upper membrane maintained its original shape and started to degrade at day 7. The lower right panels show the outlines of the membranes which were highlighted in size and shape during the incubation period. Arrows indicate the red blood layer side where degradation began.

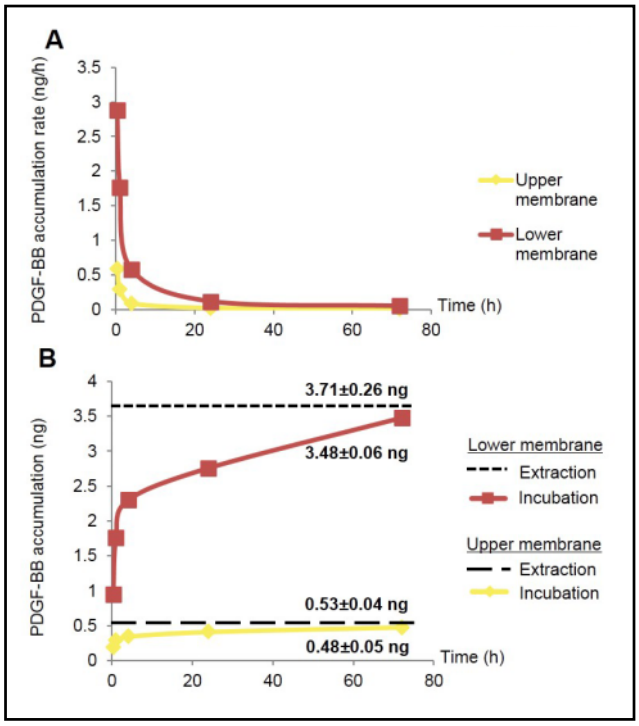

Fig.4 Release and accumulation of PDGF-BB from the upper and the lower membranes

(A) PDGF-BB release rate at 20 min, 1 h, 4 h, 24 h, and 72 h. (B) PDGF-BB accumulation. Dashed lines show the total amount of PDGF-BB extracted directly from both membranes; solid lines represent the amount of PDGF-BB released during incubation in culture medium. Both membranes showed rapid release of PDGF-BB in the first 4 h of incubation, followed by a slower release afterward. The total amount of extracted PDGF-BB is expressed as mean ± S.E., n = 6.

upper membrane and 3.48 ± 0.06 ng in the lower membrane of PRF prepared from 1-mL blood samples (Fig.4B). In comparison, the direct extraction method yielded 0.53 ± 0.04 ng PDGF-BB in the upper membrane and 3.71 ± 0.26 ng in the lower membrane. This result indicated that the direct extraction method using GndHCl is more efficient than the incubation method for PDGF-BB extraction from the PRF fibrin matrix.

Discussion

PRF is a pure natural matrix, full of leukocytes and platelets, produced from a patient's blood by one-time centrifugation without anticoagulants or chemical agents. Thus, PRF is likely to be easy to handle for clinical use.

In this study, we identified the lower clots and membranes to be the most abundant source of PDGF-BB among PRF fractions extracted by GndHCl. Furthermore, the upper and lower membranes exhibited different PDGF-BB-release properties, which can be partly explained by their distinct degradation rates.

To prove our hypothesis that PDGF-BB is trapped in the PRF fibrin matrix and can be extracted by GndHCl, we compared the concentration of PDGF-BB in the membranes, clots, and exudates. Notably, the lower clot and membrane showed higher concentrations of PDGF-BB, while the exudates contained PDGF-BB at very low concentrations (Fig.5), indicating that PDGF-BB in the PRF membranes was rarely released once the PRF clots were forcibly compressed. The upper clot, consisting of immature fibrin matrix[33], trapped only small concentrated

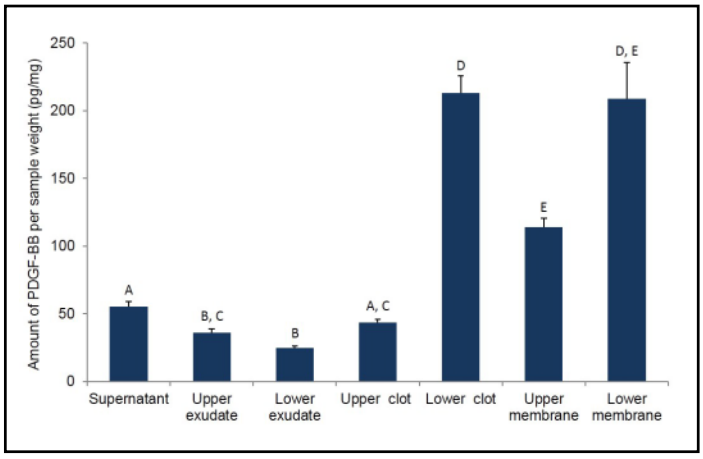

Fig.5 PDGF-BB yield from direct extraction
PDGF-BB was extracted with 4 M GndHCl immediately after PRF harvesting and then dialyzed. The amounts of PDGF-BB per sample weight (pg/mg) were calculated from ELISA results. Letters indicate significant difference according to Table 1 (Tamhane's T2, error bars denote mean ± S.E. $*p < 0.05$, n = 6). The lower clot and membrane had the highest concentration of PDGF-BB, while the upper and lower exudates had the lowest concentration of PDGF-BB.

amounts of PDGF-BB, which is reflective of the lower concentration in the upper membrane.

Support for our hypothesis was strengthened by comparing release of PDGF-BB after direct extraction to release after incubation in culture medium (Fig.4B). The amount of PDGF-BB released after 72 h incubation in culture medium, when the lower membrane had completely degraded, was similar to the total amount of PDGF-BB extracted by GndHCl treatment. In addition, the lower membrane contained approximately 7 times more PDGF-BB than the upper membrane, whether measured after extraction or incubation in culture medium. These results suggest that PDGF-BB strongly binds to the mature fibrin matrix that comprised the lower membrane and that GndHCl is effective for extraction of growth factors, at least PDGF-BB, from the PRF membrane.

Clinicians are often ambivalent about the supernatant and exudate because the importance or utility of these portions has not been well explained. We found that the supernatant contained the highest amount of PDGF-BB per 1 mL of blood (28.65 ± 13.39 ng, 41.29% of the total PDGF-BB) (Table 1), although the actual concentration in pg/mg of PDGF-BB was higher in the lower clot. Similarly, we found a large percentage of PDGF-BB (21.05%) in the lower exudate, although the concentration was quite low (24.92 ± 1.27 pg/mg). Thus, the supernatant and lower exudate should not be discarded; these samples provide a large volume and good source of active complex for accelerating wound healing and tissue regeneration by local application to surgical sites.

PRF is a popular natural scaffold, but its degradation rate is not well understood. We demonstrated that PDGF-BB release correlated with the degradation of PRF membranes. While the upper and the lower membranes released large amounts of PDGF-BB from α-granules of activated platelets for up to 4 h after blood collection, slow release of PDGF-BB from the membranes was observed even after 4 h (Fig.4B). We did note, however, that the lower membrane degraded faster than the upper membrane (Fig.3), which could be due to the concentrated platelets, leukocytes, red blood cells, and proteolytic enzymes present in the bottom phase of the PRF clots. Gassling et al. incubated the entire PRF clot in cell culture medium to detect PDGF-BB and found that the PDGF-BB concentration in the medium after 10 d was 0.018 ng/mL[34]. In our incubation method, although the total volume of the culture medium might be different, the lower membrane released 3.48 ± 0.06 ng/mL PDGF-BB within only 72 h, approximately 7 times more than that released by the upper membrane (Fig.4B). Consistent with this result, direct extraction method also yielded 7 times more PDGF-BB from the lower membrane than from the upper membrane.

There are 3 major phases of bone healing: inflammatory, reparative, and remodeling[35]. The inflammatory phase begins immediately after the initial disruption of bone and persists until bone formation is initiated. This phase lasts 3.4 days, longer in cases of a large defect[36]. In the incubation method, PRF membranes released a large amount of PDGF-BB within 4 h and continuously released until day 7 of experimental period. The use of PRF during this first week facilitates the inflammatory response and accelerates bone healing. During the initial phase of bone healing, PDGF from PRF induces mesenchymal cell migration, activation, and proliferation, angiogenesis, and chemotaxis

of acute inflammatory cells[2, 37]. Zumstein et al. reported that PRF clots cultured in medium showed a continuous slow release of TGF-β1, VEGF, and MPO (Myeloperoxidase) in the first 7 days, and IGF1, PDGF, and platelet activity in the first 8 h, followed by a decrease to near-zero at 28 days[38]. There are a number of potential growth factors in PRF for bone regeneration that play a role in replacing lost tissue and restoring vascular integrity during early bone healing[39] by stimulating the growth of osteoblasts, periodontal ligament cells, and gingival cells[33, 40-42]. Therefore, PRF is a good candidate for optimizing early bone and soft tissue healing processes.

The aim of this study is to give ideas of clinically applicable regions of PRF, where all samples have different properties. The upper and the lower membranes have both an advantage and a disadvantage. The upper membrane degraded slowly but contained a low amount of PDGF-BB. On the other hand, the lower membrane degraded fast but contained a large amount of PDGF-BB. Moreover, the supernatant and exudates also contained a lot of PDGF-BB due to their large volume. These may provide us of more appropriate ideas of utilization of each part of PRF or their combinations dependent upon purposes.

In conclusion, the results from the direct extraction by GndHCl and incubation methods gave rise to 3 main findings: (1) PDGF-BB can be extracted efficiently from PRF with GndHCl; (2) PRF releases PDGF-BB rapidly after preparation, and its slow but constant release continued over the experimental period; and (3) the lower membrane of PRF had a higher concentration of PDGF-BB and showed greater degradation compared to the upper membrane. We believe that, with future studies of bone regenerative effect of PRF, especially the commonly overlooked supernatant and exudates will be quite useful for guided bone and tissue regeneration such as tooth-extraction socket filling and wound sealing and that PRF delivery of its different portions into animal models can be necessary to classify their utilization optimal for different aspects of tissue recoveries.

Acknowledgments

The authors wish to express their gratitude to Dr. Jia Hao, Dr. Takayuki Miyahara, Dr. Kang Chen, Dr. Yu Yamashita, Dr. Shang Gao, Dr. Masaki Fujii, and Dr. Kanako Noritake who were abundantly helpful and offered invaluable assistance, support, and guidance.

Source of funding

This study was supported by Ministry of Education, Cultural, Sports, Science and Technology (MEXT) Scholarship (to W. Pluemsakunthai) and the Global Center of Excellence (G-COE) Program for Molecular Science in Tooth and Bone Diseases, Tokyo Medical and Dental University.

Conflict of interest

The authors have no conflicts of interest to declare.

References

1) Marx RE: Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg. 2004; 62: 489-496.
2) Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT: Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost. 2004; 91: 4-15.
3) Anitua E, Andia I, Sanchez M, Azofra J, del Mar Zalduendo M, de la Fuente M, Nurden P, Nurden AT: Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. J Orthop Res. 2005; 23: 281-286.
4) McNicol A, Israels SJ: Beyond hemostasis: the role of platelets in inflammation, malignancy and infection. Cardiovasc Hematol Disord Drug Targets. 2008; 8: 99-117.
5) Dohan Ehrenfest DM, Rasmusson L, Albrektsson T: Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol. 2009; 27: 158-167.
6) Knighton DR, Ciresi KF, Fiegel VD, Austin LL, Butler EL: Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). Ann Surg. 1986; 204: 322-330.
7) Knighton DR, Ciresi K, Fiegel VD, Schumerth S, Butler E, Cerra F: Stimulation of repair in chronic, nonhealing, cutaneous ulcers using platelet-derived wound healing formula. Surg Gynecol Obstet. 1990; 170: 56-60.
8) McAleer JP, Sharma S, Kaplan EM, Persich G: Use of autologous platelet concentrate in a nonhealing lower extremity wound. Adv Skin Wound Care. 2006; 19: 354-363.
9) Driver VR, Hanft J, Fylling CP, Beriou JM: A prospective, randomized, controlled trial of autologous plate-

let-rich plasma gel for the treatment of diabetic foot ulcers. Ostomy Wound Manage. 2006; 52: 68-70, 72, 74 passim.
10) Andrae J, Gallini R, Betsholtz C: Role of platelet-derived growth factors in physiology and medicine. Genes Dev. 2008; 22: 1276-1312.
11) Fredriksson L, Li H, Eriksson U: The PDGF family: four gene products form five dimeric isoforms. Cytokine Growth Factor Rev. 2004; 15: 197-204.
12) Pfeilschifter J, Krempien R, Naumann A, Gronwald RG, Hoppe J, Ziegler R: Differential effects of platelet-derived growth factor isoforms on plasminogen activator activity in fetal rat osteoblasts due to isoform-specific receptor functions. Endocrinology. 1992; 130: 2059-2066.
13) Battegay EJ, Rupp J, Iruela-Arispe L, Sage EH, Pech M: PDGF-BB modulates endothelial proliferation and angiogenesis in vitro via PDGF beta-receptors. J Cell Biol. 1994; 125: 917-928.
14) Rosenkranz S, Kazlauskas A: Evidence for distinct signaling properties and biological responses induced by the PDGF receptor alpha and beta subtypes. Growth Factors. 1999; 16: 201-216.
15) Heldin CH, Westermark B: Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev. 1999; 79: 1283-1316.
16) Lucarelli E, Beccheroni A, Donati D, Sangiorgi L, Cenacchi A, Del Vento AM, Meotti C, Bertoja AZ, Giardino R, Fornasari PM, et al: Platelet-derived growth factors enhance proliferation of human stromal stem cells. Biomaterials. 2003; 24: 3095-3100.
17) Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR: Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998; 85: 638-646.
18) Stuart JD, Kenney JG, Lettieri J, Spotnitz W, Baker J: Application of single-donor fibrin glue to burns. J Burn Care Rehabil. 1988; 9: 619-622.
19) Man D, Plosker H, Winland-Brown JE: The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery. Plast Reconstr Surg. 2001; 107: 229-237; discussion 238-229.
20) Duchesne B, Tahi H, Galand A: Use of human fibrin glue and amniotic membrane transplant in corneal perforation. Cornea. 2001; 20: 230-232.
21) Oyama T, Nishimoto S, Tsugawa T, Shimizu F: Efficacy of platelet-rich plasma in alveolar bone grafting. J Oral Maxillofac Surg. 2004; 62: 555-558.
22) Steigmann M, Garg AK: A comparative study of bilateral sinus lifts performed with platelet-rich plasma alone versus alloplastic graft material reconstituted with blood. Implant Dent. 2005; 14: 261-266.
23) Sammartino G, Tia M, Marenzi G, di Lauro AE, D1 Agostino E, Claudio PP: Use of autologous platelet-rich plasma (PRP) in periodontal defect treatment after extraction of impacted mandibular third molars. J Oral Maxillofac Surg. 2005; 63: 766-770.
24) Luengo Gimeno F, Gatto S, Ferro J, Croxatto JO, Gallo JE: Preparation of platelet-rich plasma as a tissue adhesive for experimental transplantation in rabbits. Thromb J. 2006; 4: 18.
25) Anitua E, Sanchez M, Nurden AT, Nurden P, Orive G, Andia I: New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol. 2006; 24: 227-234.
26) Hall MP, Band PA, Meislin RJ, Jazrawi LM, Cardone DA: Platelet-rich plasma: current concepts and application in sports medicine. J Am Acad Orthop Surg. 2009; 17: 602-608.
27) Marukawa E, Oshina H, Iino G, Morita K, Omura K: Reduction of bone resorption by the application of platelet-rich plasma (PRP) in bone grafting of the alveolar cleft. J Craniomaxillofac Surg. 2011; 39: 278-283.
28) Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B: 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.
29) Boyne PJ: Osseous repair of the postextraction alveolus in man. Oral Surg Oral Med Oral Pathol. 1966; 21: 805-813.
30) Casati MZ, de Vasconcelos Gurgel BC, Goncalves PF, Pimentel SP, da Rocha Nogueira Filho G, Nociti FH, Jr, Sallum EA: Platelet-rich plasma does not improve bone regeneration around peri-implant bone defects--a pilot study in dogs. Int J Oral Maxillofac Surg. 2007; 36: 132-136.
31) Gerard D, Carlson ER, Gotcher JE, Jacobs M: Effects of platelet-rich plasma at the cellular level on healing of autologous bone-grafted mandibular defects in dogs.

J Oral Maxillofac Surg. 2007; 65: 721-727.
32) Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, Dohan AJ, Mouhyi J, Dohan DM: Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101: 299-303.
33) Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB: Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol. 2010; 81: 546-555.
34) Gassling VL, Acil Y, Springer IN, Hubert N, Wiltfang J: Platelet-rich plasma and platelet-rich fibrin in human cell culture. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009; 108: 48-55.
35) Burchardt H, Enneking WF: Transplantation of bone. Surg Clin North Am. 1978; 58: 403-427.
36) Kalfas IH: Principles of bone healing. Neurosurg Focus. 2001; 10: E1.
37) Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, Dohan AJ, Mouhyi J, Dohan DM: Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101: e56-e60.
38) Zumstein MA, Berger S, Schober M, Boileau P, Nyffeler RW, Horn M, Dahinden CA: Leukocyte- and platelet-rich fibrin (L-PRF) for long-term delivery of growth factor in rotator cuff repair: review, preliminary results and future directions. Curr Pharm Biotechnol. 2012; 13: 1196-1206.
39) Peck MT, Marnewick J, Stephen L: Alveolar ridge preservation using leukocyte and platelet-rich fibrin: a report of a case. Case Rep Dent. 2011; 2011: 345048.
40) Clark RA, Folkvord JM, Hart CE, Murray MJ, McPherson JM: Platelet isoforms of platelet-derived growth factor stimulate fibroblasts to contract collagen matrices. J Clin Invest. 1989; 84: 1036-1040.
41) Blair P, Flaumenhaft R: Platelet alpha-granules: basic biology and clinical correlates. Blood Rev. 2009; 23: 177-189.
42) Sharma A, Pradeep AR: Autologous platelet-rich fibrin in the treatment of mandibular degree II furcation defects: a randomized clinical trial. J Periodontol. 2011; 82: 1396-1403.