Received: 10 May 2019 | Accepted: 4 August 2019

DOI: 10.1111/jicd.12458

ORIGINAL ARTICLE

WILEY

Distribution of platelets, transforming growth factor-β1, platelet-derived growth factor-BB, vascular endothelial growth factor and matrix metalloprotease-9 in advanced platelet-rich fibrin and concentrated growth factor matrices

Akira Takahashi[1] | Tetsuhiro Tsujino[2] | Sadahiro Yamaguchi[3] | Kazushige Isobe[4] | Taisuke Watanabe[5] | Yutaka Kitamura[6] | Kazuhiro Okuda[7] | Koh Nakata[8] | Tomoyuki Kawase[9]

[1]Private Practice, Kawasaki, Japan

[2]Private Practice, Hiroshima, Japan

[3]Private Practice, Ota, Japan

[4]Tokyo Plastic Dental Society, Tokyo, Japan

[5]Division of Anatomy and Cell Biology of the Hard Tissue, Institute of Medicine and Dentistry, Niigata University, Niigata, Japan

[6]Department of Oral and Maxillofacial Surgery, Matsumoto Dental University, Shiojiri, Japan

[7]Division of Periodontology, Institute of Medicine and Dentistry, Niigata University, Niigata, Japan

[8]Bioscience Medical Research Center, Niigata University Medical and Dental Hospital, Niigata, Japan

[9]Division of Oral Bioengineering, Institute of Medicine and Dentistry, Niigata University, Niigata, Japan

Correspondence
Dr Tomoyuki Kawase, Division of Oral Bioengineering, Institute of Medicine and Dentistry, Niigata University, Niigata, Japan.
Email: kawase@dent.niigata-u.ac.jp

Funding information
This research was funded in part by JSPS KAKENHI (grant no. 18K09595).

Abstract

Aim: Platelet-rich fibrin (PRF) matrices are compared with regard to their ability to retain and release growth factors. Although this ability is thought to influence regenerative outcomes, it remains unclear how it is regulated. To address this question, we compared advanced PRF (A-PRF) and concentrated growth factor (CGF) matrices in terms of distribution of platelets, transforming growth factor-β1, platelet-derived growth factor-BB, vascular endothelial growth factor and matrix metalloprotease-9 (MMP9).

Methods: Blood samples were obtained in glass tubes and immediately centrifuged to prepare A-PRF or CGF matrix according to their specific protocols. Both matrices were compressed, embedded in paraffin and subjected to immunohistochemical examination.

Results: Leukocytes and plasma proteins were localized on the proximal surface including the interface corresponding to buffy coat. In A-PRF, platelets were distributed homogenously, while growth factors and fibronectin were localized on the distal surface and MMP9 was mainly colocalized with leukocytes. In CGF, in contrast, platelets were localized on and below the proximal surface like leukocytes, growth factors were diffused homogenously and MMP9 was found in the plasma protein layers.

Conclusion: Although these preparations do not allow accurate quantification, platelet counts and growth factor levels seemed higher and leukocytes were less activated in A-PRF. This may explain A-PRF's higher ability to release growth factors.

KEYWORDS

advanced platelet-rich fibrin, concentrated growth factors, fibronectin, leukocytes, matrix metalloprotease-9, platelet-derived growth factor-BB, platelets, transforming growth factor-β1

1 | INTRODUCTION

Regenerative therapy using platelet-rich plasma (PRP) and its advanced derivatives, such as platelet-rich fibrin (PRF), is increasingly being applied in the dental field.[1-3] In fact, PRP and its derivatives (PRP/PRF) applied alone have been observed to produce convincing clinical outcomes for soft tissue wound healing.[3] PRP/PRF has been frequently and increasingly applied in hard tissue regenerative therapy, such as infrabony detect regeneration, furcation defect regeneration, guided bone regeneration, extraction socket management and sinus elevation, in the last decade.[4] However, these effects remain questionable and require further validation.[4,5] To compensate for such a possible drawback, PRP/PRF has been also applied in combination with autologous crushed bone, natural and synthetic bone substitutes, mesenchymal stem cells and osteogenic periosteal cell to obtain better results.[4,6-8] In any case, the clinical use of PRP/PRF is based on the evidence that PRP provides both signaling molecules (eg growth factors) and scaffolds in the well-known tissue engineering triad.[1,9-12] Among various regenerative therapies using natural and synthetic materials, it should be noted that the primary and biggest advantages of the PRP/PRF therapy are low cost and high safety rather than the predictable effectiveness. An additional advantage is their on-site preparation, which eliminates the need for storage and problems associated with storage, including stock space and dead stock.

Development of the leukocyte-rich PRF (L-PRF) preparation protocol by Dohan et al further reduced the time for preparation and the necessity of a highly technically competent operator,[13] and thereby made PRF therapy widely applied in regenerative dentistry. However, this progress has not necessarily led to substantial improvement in its efficacy. To improve its clinical efficacy and predictability, guidelines on PRP/PRF quality should be first established. Therefore, simple and quick methods should be developed to evaluate individual preparations prior to use.[14,15] However, especially in the case of insoluble PRF preparation, because several factors attesting to the quality of the preparation, such as platelet counts, cannot easily be quantified,[15] we accept standardization of preparation protocol as an alternative to minimize individual variations and thereby assure the minimal quality of individual PRF preparations.

Many research investigations have recently been performed to compare the performances and properties of major PRF derivatives.[16-20] We have compared the mechanical and degradation properties of advanced PRF (A-PRF) and concentrated growth factors (CGF) and found no statistically significant differences.[18] The current main topic in the comparison of PRF is their abilities to retain and release growth factors.[16,19,21] In some studies,[19,21] an A-PRF matrix prepared by low-speed centrifugation has been demonstrated to be superior to a CGF matrix prepared by high-speed centrifugation. However, contradictory findings have also been reported[16] and no additional evidence to support such differences has been presented to date. Thus, this functional ability remains to be analyzed more carefully.

In general, it is thought that the ability of growth factor retention depends on the mechanism of growth factor adhesion and/or storage, while the release ability depends mainly on the degradability of cross-linked fibrin fibers. However, assuming that the degradability is almost equal between A-PRF and CGF matrices, as was demonstrated in our previous study,[18] their release ability can be considered largely influenced by the retention ability. Thus, it is the manner of growth factor distribution. The aim of this work was to demonstrate possible differences in growth factor distribution. Thus, we compared the distribution of transforming growth factor (TGF)-β1, platelet-derived growth factor-BB (PDGF-BB), vascular endothelial growth factor (VEGF), matrix metalloprotease-9 (MMP9), platelets and leukocytes in A-PRF with CGF matrices. In general, blood cells and soluble factors were localized on the proximal surface due to inclusion of the interface between red thrombus and PRF matrix and the distal surface, respectively; however, these distributions were modified as centrifugal force was reduced.

2 | MATERIALS AND METHODS

2.1 | Preparation of PRF matrices

Blood samples were collected, without anticoagulants, from 10 non-smoking healthy male volunteers aged 30-63 years. The study design and consent forms for all procedures (project identification code: 2297) were approved by the ethics committee for human participants at the Niigata University School of Medicine (Niigata, Japan) and complied with the Declaration of Helsinki of 1964, as revised in 2013.

Fresh blood samples (~9.0 mL) from each donor were collected into vacuum plain glass tubes, A-PRF+ (Jiangxi Fenglin Medical Technology, Fengcheng, China) or BD Vacutainer (Becton Dickinson, Franklin Lakes, NJ, USA). Blood was immediately centrifuged at 200 g for 14 minutes (A-PRF protocol) using a Duo centrifuge (Process for PRF, Nice, France) or by the CGF protocol using a program that automatically changes the centrifugal speed as follows: 30 seconds, acceleration; 2 minutes, 692 g; 4 minutes, 547 g; 4 minutes, 592 g; 3 minutes, 855 g; and 36 seconds, deceleration. This CGF protocol was carried out using a Medifuge centrifugation (Silfradent, Santa Sofia, Italy). All centrifugations were performed at ambient temperature.

Quality checks were carried out on individual blood samples by performing platelet and other blood cell counts using a pocH 100iV automated hematology analyzer (Sysmex, Kobe, Japan).

2.2 | Immunohistochemical examination

Freshly prepared A-PRF and CGF clots were gently, but not fully, compressed with a stainless-steel PRF compression device (PRF stamper; JMR, Niigata, Japan),[22] washed 3 times with phosphate-buffered saline (PBS) and fixed in 10% neutralized formalin. After the fixed A-PRF and CGF membranes were divided into 7 pieces, the

3 pieces shown in Figure 1A were dehydrated in a series of ethanol washes, embedded in paraffin and sectioned at a thickness of 6 μm.

Distribution of platelets in PRF matrices was determined using a previously described immunohistochemical method,[22] outlined as follows: deparaffinized sections were antigen-retrieved using Liberate Antibody Binding Solution (Polysciences, Warrington, PA, USA) for 15 minutes and blocked with 0.1% Block ACE (Sumitomo Dainippon Pharma, Osaka, Japan) in 0.1% Tween-20-containing PBS (T-PBS) for 1 hour. The specimens were then probed with a rabbit polyclonal anti-CD41 antibody (GeneTex, Hsinchu City, Taiwan), diluted 1:400 in ImmunoShot Mild (CosmoBio, Tokyo, Japan) overnight at 4°C. This was followed by incubation with horseradish peroxidase-conjugated goat antirabbit immunoglobulin (Ig)G antibody (Cell Signaling Technology, Danvers, MA, USA) (1:100 diluted in T-PBS) for 1 hour at ambient temperature. Immunoreactive proteins were visualized following the addition of 3,3′-diaminobenzidine (DAB) substrate solution (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA).[23]

Likewise, distributions of TGF-β1, PDGF-BB, VEGF and MMP9 were visualized using a rabbit polyclonal anti-TGF-β1 (1:400 in dilution) (ProteinTech, Rosemont, IL, USA), a rabbit polyclonal anti-PDGF-BB (1:200 in dilution) (GeneTex), a rabbit polyclonal anti-VEGF (1:200 in dilution) (GeneTex) and a rabbit polyclonal anti-MMP9 (1:400 in dilution) (ProteinTech).

3 | RESULTS

The distribution of $CD41^+$ platelets in A-PRF and CGF matrices is shown in Figure 2. In the A-PRF matrix, platelets were distributed homogenously from the surface to deep layers, while in the CGF matrix, platelets were localized on and below the proximal surface region including the interface between red thrombus and fibrin matrix (the upper direction in each photomicrograph), which is illustrated in Figure 1B. However, platelets and leukocytes were not always colocalized there: most leukocytes were placed on the plasma protein layer. Although quantitative analysis was technically limited, platelet counts seemed higher in the A-PRF than CGF matrix. In negative controls, the CGF matrix was stained with normal rabbit IgG and horseradish peroxidase-conjugated antirabbit IgG. No positive-staining was found in either the low- or the high-magnification image. Because the primary antibodies used in the study were derived from rabbits, these images can be used as negative controls for the other staining.

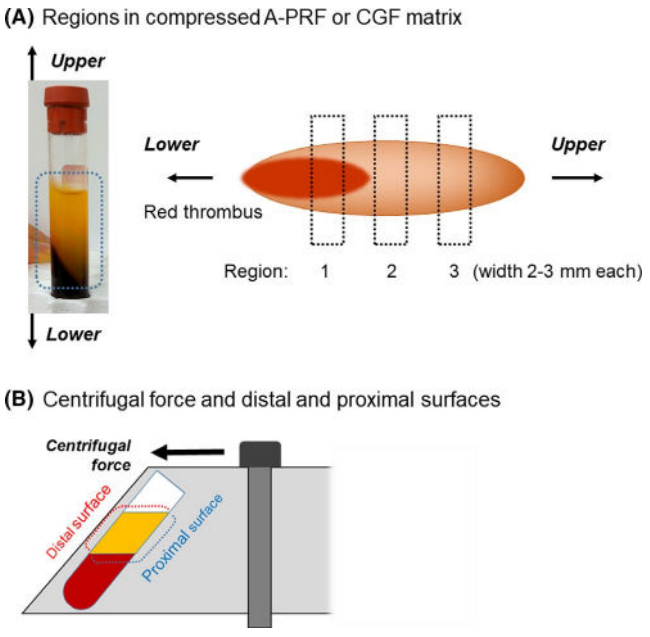

FIGURE 1 (A) Regions in compressed A-PRF or CGF matrix. This image is the proximal surface. (B) Centrifugal force and distal and proximal surface of A-PRF or CGF matrix. A-PRF, advanced platelet-rich fibrin; CGF, concentrated growth factors

The distribution of TGF-β1 in A-PRF and CGF matrices is shown in Figure 3. In the A-PRF matrix, TGF-β1 was homogenously distributed in region 1, while in both regions 2 and 3, TGF-β1 was concentrated on the distal surface of the regions, the other surface of the plasma protein layer. In contrast, in the CGF matrix, although a similar finding was observed in region 2, generally the levels of positivity (ie area and intensity) were apparently lower in the CGF than in the A-PRF matrix.

The distribution of PDGF-BB in A-PRF and CGF matrices is shown in Figure 4. As observed in TGF-β1, PDGF-BB was widely and diffusibly distributed in region 1 of the A-PRF matrix and localized mainly in the distal surface regions. In contrast, PDGF-BB was diffusibly but weakly distributed in the CGF matrix. Condensation in the distal surface was not observed.

The distribution of VEGF in A-PRF and CGF matrices is shown in Figure 5. Unlike TGF-β1 or PDGF-BB, in this experiment, both matrices were weakly positive for VEGF or immunologically VEGF-like compounds.

The distribution of MMP9 in A-PRF and CGF matrices is shown in Figure 6. This distribution pattern was distinguishable from those of growth factors. The positive compounds could be recognized as dot-like objects. In the A-PRF matrix, MMP9 was colocalized in leukocytes and their surrounding region, whereas in the CGF matrix, MMP9 was released from leukocytes and localized in the plasma protein layer (indicated by arrows). Thus, MMP9 was found to not be colocalized with leukocytes in the CGF matrix.

4 | DISCUSSION

To our knowledge, there have been a limited number of publications reporting the blood cell distribution in these kinds of fibrin clots.[21,24] However, they have not examined growth factor distribution. This study, for the first time, demonstrated clear differences in the distribution of growth factors, proteases and adhesion molecules, along with that of platelets and leukocytes, in A-PRF and CGF matrices.

The essential difference between A-PRF and CGF preparation protocols is centrifugal force, namely speed. Unfortunately, there are few reports comparing A-PRF with CGF. However, there is some

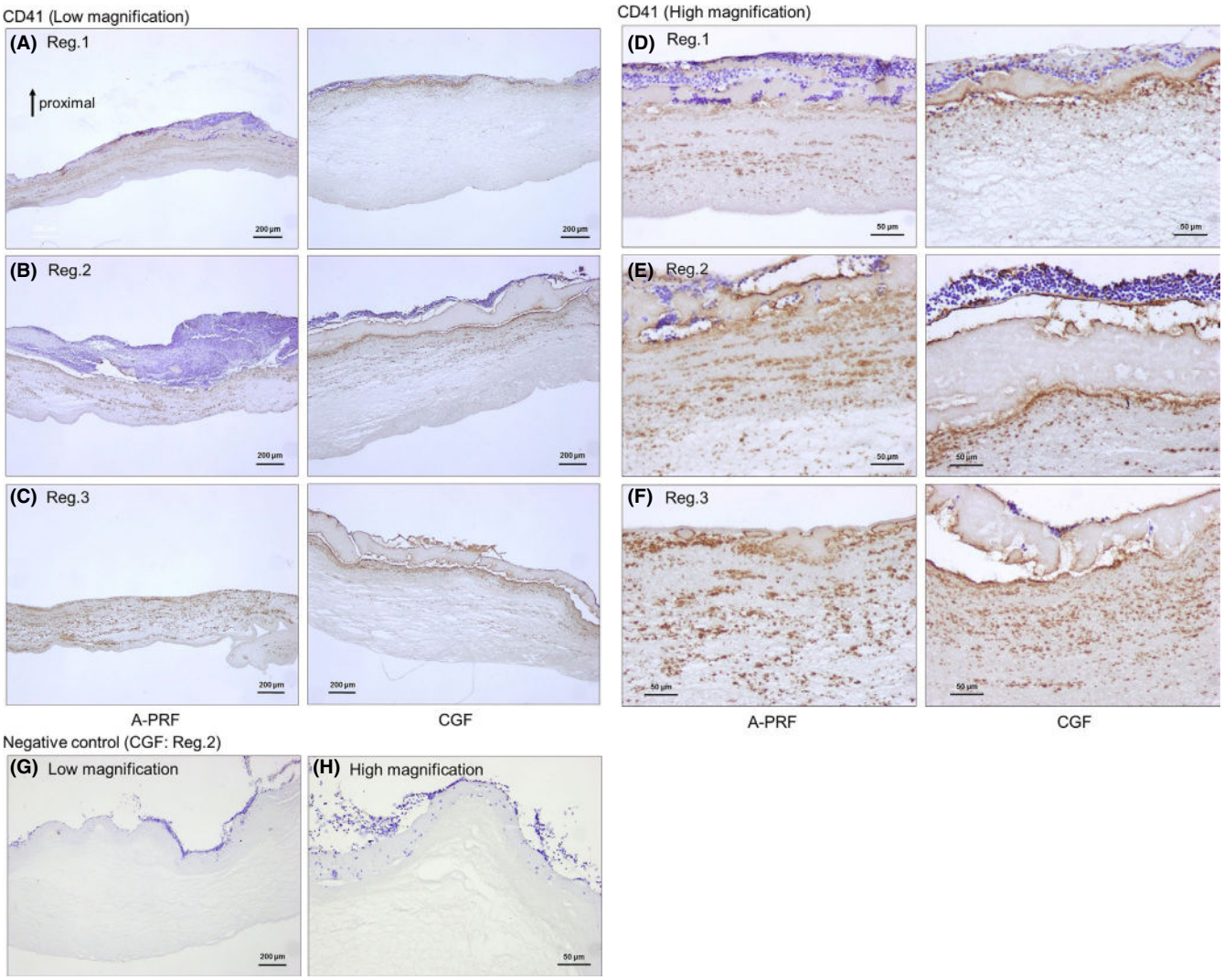

FIGURE 2 Distribution of CD41$^+$ platelets in A-PRF and CGF matrices. (A,D) Region 1, (B,E) region 2, (C,F) region 3. (A-C) Low magnification, (D-F) high magnification, (G, H) negative control staining using normal rabbit IgG. A-PRF, advanced platelet-rich fibrin; CGF, concentrated growth factors; IgG, immunoglobulin G

literature available that explores the differences between A-PRF and L-PRF. In terms of preparation protocol, CGF and L-PRF are prepared through high-speed centrifugation. In this aspect, the comparison between A-PRF with L-PRF seems similar to that between A-PRF and CGF. In several reports,[19,21,25] it was concluded that the low-speed centrifugation concept, which is applied in A-PRF preparation, produces PRF clots with a greater ability to retain more growth factors and to release them for a longer period of time. Dohan Ehrenfest et al reported contradictory findings that L-PRF has higher capacity as a carrier of growth factors and is resistant to degradation.[16] Thus, they concluded that L-PRF is longer lasting than A-PRF, although a shaker was not always used and striking differences were not evident.

The clear difference in growth factor release between A-PRF and L-PRF remains to be clarified. In this study, we found that certain growth factors were condensed in the distal surface in regions 2 and 3, but not region 1, in the A-PRF matrix. In contrast, growth factors were not condensed in any regions. The possible explanation of these phenomena is that growth factors may be washed away from the A-PRF matrix by centrifugation-dependent fluid flow and consequently condensed in the distal surface at the end of centrifugation. In contrast, in CGF preparation, growth factors may be completely washed out from the CGF matrix before the end of centrifugation, probably due to higher fluid flow caused by higher-speed centrifugation. Because the immunohistochemical method using polyclonal antibodies is limited in quantification and specificity, other more quantitative and specific approaches should be performed to confirm those findings and terminate the debate.

Prior to this study, we raised a working hypothesis that due to fibrin matrix formation, platelets and leukocytes may not be fractionated mainly by centrifugal force. Thus, the differences in centrifugal force may not reflect clear differences in platelet and leukocyte distribution. As expected, leukocytes were localized on the proximal surface of the fibrin matrix regardless of centrifugal forces, whereas platelet distribution largely depended on

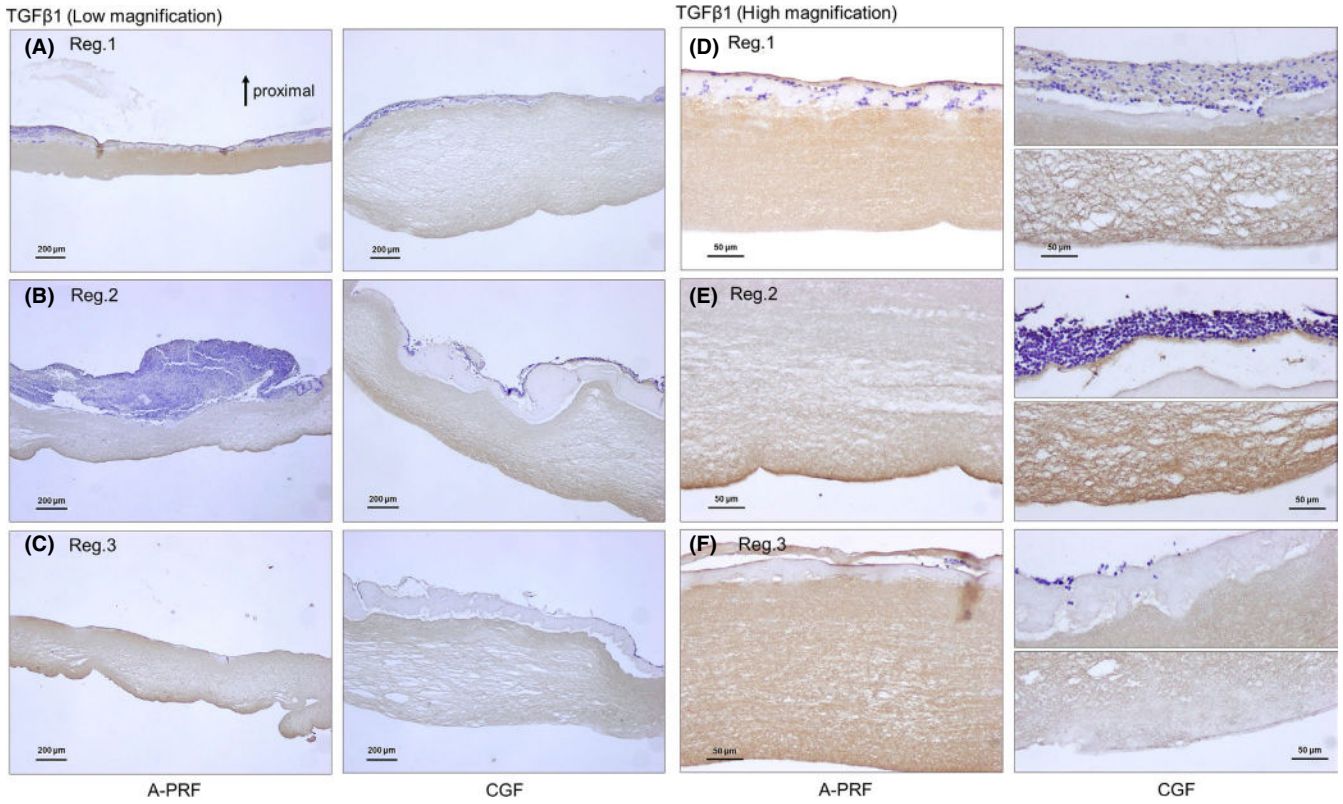

FIGURE 3 Distribution of TGF-β1 in A-PRF and CGF matrices. (A,D) Region 1, (B,E) region 2, (C,F) region 3. (A-C) Low magnification, (D-F) high magnification. A-PRF, advanced platelet-rich fibrin; CGF, concentrated growth factors; TGF-β1, transforming growth factor-β1

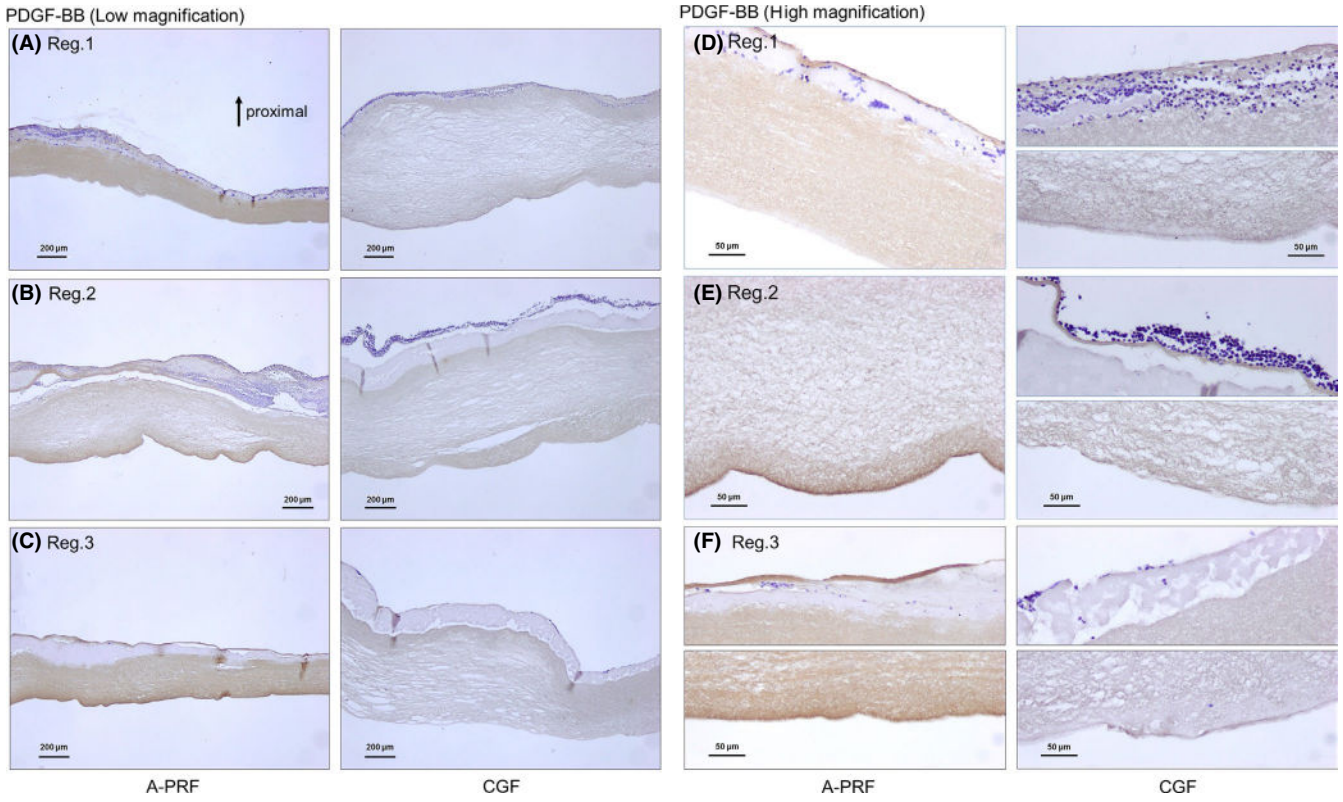

FIGURE 4 Distribution of PDGF-BB in A-PRF and CGF matrices. (A,D) Region 1, (B,E) region 2, (C,F) region 3. (A-C) Low magnification, (D-F) high magnification. A-PRF, advanced platelet-rich fibrin; CGF, concentrated growth factors; PDGF-BB, platelet-derived growth factor-BB

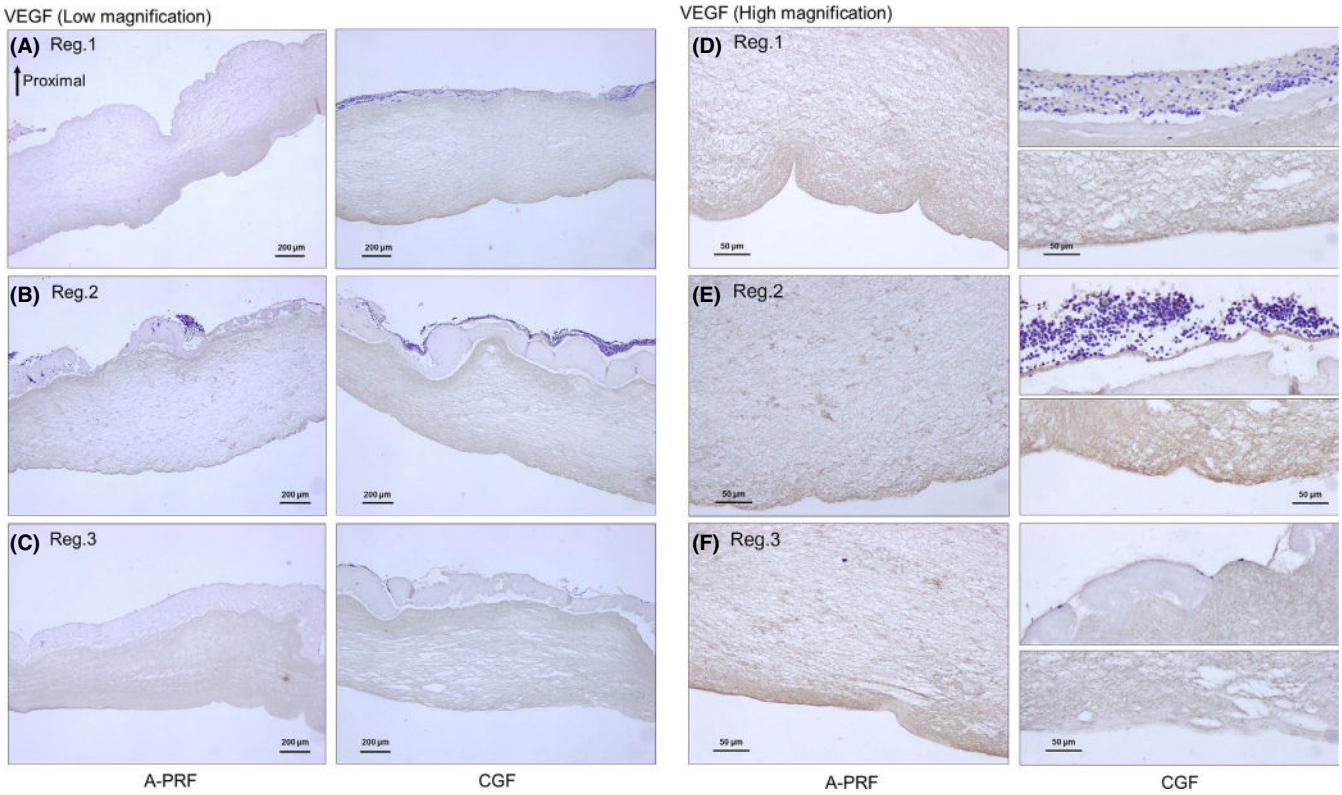

FIGURE 5 Distribution of VEGF in A-PRF and CGF matrices. (A,D) Region 1, (B,E) region 2, (C,F) region 3. (A-C) Low magnification, (D-F) high magnification. A-PRF, advanced platelet-rich fibrin; CGF, concentrated growth factors; VEGF, vascular endothelial growth factor

centrifugal force. At high-speed centrifugation, platelets were localized on and below the proximal surface, while at low-speed centrifugation, were homogenously distributed from surface to deep layers. At low-speed centrifugation, interestingly, MMP9 was co-localized with some leukocytes, indicating that those leukocytes are not activated.

In this study, we obtained no direct evidence regarding the activation status of platelets in fibrin matrices; however, the relative resting status of leukocytes indicates that platelets may be less activated than leukocytes.[26-29] Taken together with basic diffusability under low-speed centrifugation, it is plausible that growth factors do not diffuse away to extra-matrix spaces but stay longer within the matrix. To sustain the soluble growth factors inside the matrix, those factors should be anchored with insoluble material (structures), such as fibrin fibers and the plasma membrane of blood cells. It is theoretically possible that growth factors adsorbed to fibrin fibers or those caged in resting platelets can be retained and released as the fibrin matrix is degraded. In addition, localization of growth factors, namely to the surface or to deep layers of the fibrin matrix, may significantly influence their release to the extra-matrix spaces. In the A-PRF matrix, growth factors were concentrated in the distal surface regions but also detected in deep layers at higher levels than in the CGF matrix. Taken together, our data support the conclusion that A-PRF functions as a long-lasting carrier of growth factors although its superiority for clinical applications, relative to other matrices, is still controversial.

Based on a number of preclinical and clinical studies and distributors' sales, we speculate that the A-PRF protocol is the most commonly used for the preparation of generic PRF matrices. However, all A-PRF users do not necessarily use genuine centrifuge and blood-collection tubes: some clinicians use standard centrifuges equipped with an angle rotor and glass tubes provided by a 3rd party.

Debate exists in Europe as to whether genuine devices and expendables should be used for genuine A-PRF or L-PRF (or CGF),[30] but current focus on the debate would not lead to further progress in PRF therapy. In fact, we confirmed no substantial differences between a Duo centrifuge and a 3rd party's centrifuge or between A-PRF + glass tubes and BD's glass tubes (Vacutainer) at the immunohistological level. However, it is important to pay attention to the quality of the devices (eg registration or approval as a medical device by regulatory authorities). For example, silica-coated plastic tubes, which are increasingly used as alternatives to glass tubes, produce a distinguishable type of fibrin matrix in terms of platelet distribution and contamination of silica particles (Kawase et al, manuscript in submission).

On the other hand, in Japan, where the related market is actually dominated by distributors of CGF devices,[2] most Japanese fibrin-matrix users have no opportunity to consider which protocols are suitable for preparation of the best PRF matrix.[2] In other words, these users are least aware of the safest devices and the protocols that produce higher quality fibrin matrices. In our newly published

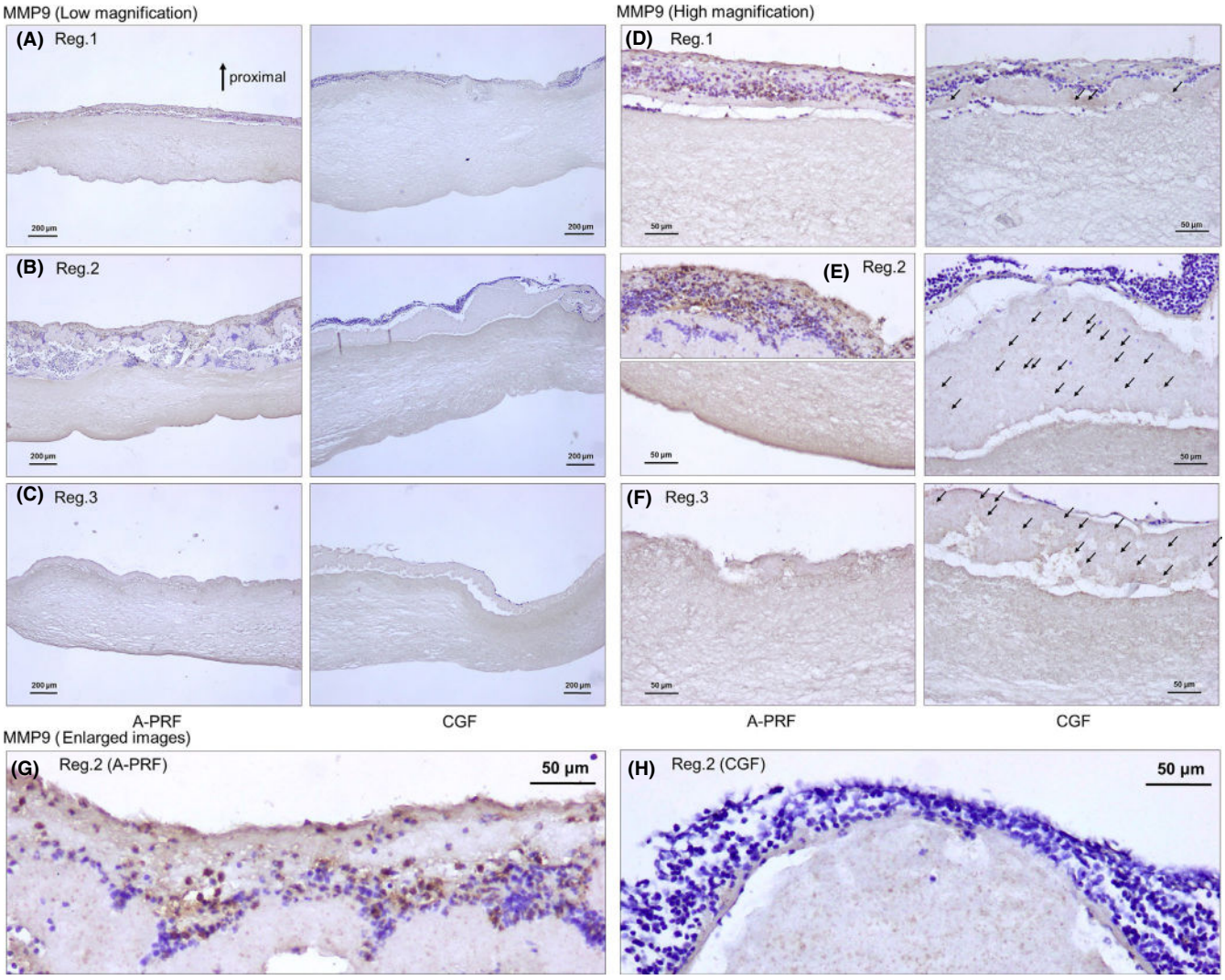

FIGURE 6 Distribution of MMP9 in A-PRF and CGF matrices. (A,D) Region 1, (B,E) region 2, (C,F) region 3. (A-C) Low magnification, (D-F) high magnification, (G, H) enlarged images of region 2. The arrows indicate MMP9 in the plasma protein layer. Note: MMP9 was not colocalized with leukocytes in the CGF matrix (H vs G). A-PRF, advanced platelet-rich fibrin; CGF, concentrated growth factors; MMP9, matrix metalloprotease-9

article,[31] to alert those users, we provided evidence that an "A-PRF-like matrix" prepared with silica-coated plastic tubes has potential risks for tissue regeneration at implantation sites. Although the overheated debate in Europe sometimes produces substantial bias, even among academic investigators, the oligopolistic market in Japan unfortunately deprives opportunities for debate. This is a "silent" bias, which may be worse than the commercial bias in Europe. We, research investigators, need to evaluate individual types of fibrin matrix from a neutral standpoint for substantial progress of PRF therapy.

4.1 | Conclusions

It has often been reported that the A-PRF matrix is superior to the CGF matrix in terms of growth factor retention and release. In this study, we demonstrated that TGF-β1 and PDGF-BB are widely distributed at higher levels in the A-PRF matrix than CGF matrix and that these factors are concentrated in the distal surface region, a phenomenon which was not observed in the CGF matrix. Thus, the differences in growth factor distribution may be a key factor regulating growth factor release.

AUTHOR CONTRIBUTIONS

Conceptualization, AT and TK; methodology, TK; validation, TT; formal analysis, TT and TK; investigation, AT, TT, SY, KI, TW, YK and KO; resources, AT and TT; data curation, KO and KN; original draft preparation, TK; review and editing, AT, TT and TK; visualization, TK; supervision, KN; project administration, TW and TK; and funding acquisition, TK

ORCID

Tomoyuki Kawase https://orcid.org/0000-0003-4160-396X

REFERENCES

1. Kawase T. Platelet-rich plasma and its derivatives as promising bioactive materials for regenerative medicine: basic principles and concepts underlying recent advances. *Odontology*. 2015;103:126-135.
2. Kawase T, Tanaka T. An updated proposal for terminology and classification of platelet-rich fibrin. *Renerative Therapy*. 2017;7:80-81.
3. Miron RJ, Fujioka-Kobayashi M, Bishara M, Zhang Y, Hernandez M, Choukroun J. Platelet-rich fibrin and soft tissue wound healing: a systematic review. *Tissue Eng Part B Rev*. 2017;23(1):83-99.
4. Miron RJ, Zucchelli G, Pikos MA, et al. Use of platelet-rich fibrin in regenerative dentistry: a systematic review. *Clin Oral Investig*. 2017;21(6):1913-1927.
5. Albanese A, Licata ME, Polizzi B, Campisi G. Platelet-rich plasma (PRP) in dental and oral surgery: from the wound healing to bone regeneration. *Immun Ageing*. 2013;10(1):23.
6. Nagata M, Hoshina H, Li M, et al. Ogose A and others. A clinical study of alveolar bone tissue engineering with cultured autogenous periosteal cells: coordinated activation of bone formation and resorption. *Bone*. 2012;50(5):1123-1129.
7. Nowotny J, Farack J, Vater C, et al. Translation of cell therapy into clinical practice: validation of an application procedure for bone marrow progenitor cells and platelet rich plasma. *J Appl Biomater Funct Mater*. 2016;14(1):e1-e8.
8. Okuda K, Kawase T, Nagata M, et al. Tissue-engineered cultured periosteum sheet application to treat infrabony defects: case series and 5-year results. *Int J Periodontics Restorative Dent*. 2013;33(3):281-287.
9. Chieruzzi M, Pagano S, Moretti S, et al. Nanomaterials for tissue engineering in dentistry. *Nanomaterials (Basel)*. 2016;6(7):134.
10. Sadeghi-Ataabadi M, Mostafavi-Pour Z, Vojdani Z, Sani M, Latifi M, Talaei-Khozani T. Fabrication and characterization of platelet-rich plasma scaffolds for tissue engineering applications. *Mater Sci Eng C Mater Biol Appl*. 2017;71:372-380.
11. Sanchez M, Anitua E, Delgado D, et al. Platelet-rich plasma, a source of autologous growth factors and biomimetic scaffold for peripheral nerve regeneration. *Expert Opin Biol Ther*. 2017;17(2):197-212.
12. Textor JA, Murphy KC, Leach JK, Tablin F. Ultrastructure and growth factor content of equine platelet-rich fibrin gels. *Am J Vet Res*. 2014;75(4):392-401.
13. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101(3):e37-e44.
14. Kawase T, Takahashi A, Watanabe T, Tsujino T. Proposal for point-of-care testing of platelet-rich plasma quality. *Int J Growth Factors Stem Cells Dent*. 2019;2(1):13-17.
15. Kitamura Y, Watanabe T, Nakamura M, et al. Platelet counts in insoluble platelet-rich fibrin clots: a direct method for accurate determination. *Front Bioeng Biotechnol*. 2018;6:4.
16. Dohan Ehrenfest DM, Pinto NR, Pereda A, et al. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane. *Platelets*. 2018;29(2):171-184.
17. Giannini S, Cielo A, Bonanome L, et al. Comparison between PRP, PRGF and PRF: lights and shadows in three similar but different protocols. *Eur Rev Med Pharmacol Sci*. 2015;19(6):927-930.
18. Isobe K, Watanebe T, Kawabata H, et al. T and others. Mechanical and degradation properties of advanced platelet-rich fibrin (A-PRF), concentrated growth factors (CGF), and platelet-poor plasma-derived fibrin (PPTF). Int. *J Implant Dent*. 2017;3(1);17.
19. Kobayashi E, Fluckiger L, Fujioka-Kobayashi M, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. *Clin Oral Investig*. 2016;20(9):2353-2360.
20. Masuki H, Okudera T, Watanabe T, et al. Growth factor and pro-inflammatory cytokine contents in PRP, plasma rich in growth factors (PRGF), advanced-platelet-rich fibrin (A-PRF) and concentrated growth factors (CGF). *Int J Implant Dent*. 2016;2:19.
21. El Bagdadi K, Kubesch A, Yu X, et al. Reduction of relative centrifugal forces increases growth factor release within solid platelet-rich-fibrin (PRF)-based matrices: a proof of concept of LSCC (low speed centrifugation concept). *Eur J Trauma Emerg Surg*. 2017;45(3):467-479.
22. Kobayashi M, Kawase T, Horimizu M, Okuda K, Wolff LF, Yoshie H. A proposed protocol for the standardized preparation of PRF membranes for clinical use. *Biologicals*. 2012;40(5):323-329.
23. Kawase T, Nagata M, Okuda K, et al. Platelet-rich fibrin extract: a promising fetal bovine serum alternative in explant cultures of human periosteal sheets for regenerative therapy. *Int J Mol Sci*. 2019;20(5):1053.
24. Ghanaati S, Booms P, Orlowska A, et al. Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol*. 2014;40(6):679-689.
25. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low-speed concept: growth factor release, biocompatibility, and cellular response. *J Periodontol*. 2017;88(1):112-121.
26. Allegrezza-Giulietti A, Serretti R, Beccerica E, Muti S, Ferretti G, Cervini C. Platelet release products modulate some aspects of polymorphonuclear leukocyte activation. *J Cell Biochem*. 1991;47(3):242-250.
27. Libby P. Fanning the flames: inflammation in cardiovascular diseases. *Cardiovasc Res*. 2015;107(3):307-309.
28. Lindsey M, Wedin K, Brown MD, et al. Matrix-dependent mechanism of neutrophil-mediated release and activation of matrix metalloproteinase 9 in myocardial ischemia/reperfusion. *Circulation*. 2001;103(17):2181-2187.
29. Rhee BG, Hall ER, McIntire LV. Platelet modulation of polymorphonuclear leukocyte shear induced aggregation. *Blood*. 1986;67(1):240-246.
30. 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(3):158-167.
31. Tsujino T, Takahashi A, Yamaguchi S, et al. Evidence for contamination of silica microparticles in advanced platelet-rich fibrin matrices prepared using silica-coated plastic tubes. *Biomedicines*. 2019;7(2):45.

How to cite this article: Takahashi A, Tsujino T, Yamaguchi S, et al. Distribution of platelets, transforming growth factor-β1, platelet-derived growth factor-BB, vascular endothelial growth factor and matrix metalloprotease-9 in advanced platelet-rich fibrin and concentrated growth factor matrices. *J Invest Clin Dent*. 2019;e12458. https://doi.org/10.1111/jicd.12458