Journal of Oral Biosciences xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Original Article

Advanced platelet-rich fibrin (A-PRF) has an impact on the initial healing of gingival regeneration after tooth extraction

Yu-Hao Liu [a, 1], Masahiro To [b, 1], Toshimitsu Okudera [a], Satoko Wada-Takahashi [b], Shun-Suke Takahashi [c], Chen-yao Su [a, d], Masato Matsuo [a, *]

[a] Department of Clinical Oral Anatomy, Kanagawa Dental University, 82, Inaoka, Yokosuka, Kanagawa, 238-8580, Japan
[b] Department of Physiology, Kanagawa Dental University, 82, Inaoka, Yokosuka, Kanagawa, 238-8580, Japan
[c] Department of Pharmacology, Kanagawa Dental University, 82, Inaoka, Yokosuka, Kanagawa, 238-8580, Japan
[d] International Society of Blood Biomaterials (ISBB), Sec.2, Linong Street, Taipei, 112 Taiwan

ARTICLE INFO

Article history:
Received 12 October 2021
Received in revised form
11 November 2021
Accepted 15 November 2021
Available online xxx

Keywords:
PRF
Gingival tissue
Regeneration
VEGF
Micro vasculature

ABSTRACT

Objectives: Platelet-rich fibrin (PRF) is widely used in wound healing because it contains several growth factors, including vascular endothelial growth factor (VEGF). In this study, we investigated the effects of advanced PRF (A-PRF) in early-stage gingival regeneration after tooth extraction.
Methods: Blood sample was collected from females beagle dogs (age: 12 months) before tooth extraction for A-PRF preparation. All animals were sacrificed by perfusion-fixation on postoperative days 1, 3, and 7. The upper jaws were prepared for hematoxylin and eosin staining and immunostaining (for CD34 and VEGF). The lower jaw samples were prepared for scanning electron microscope observations. Blood flow in the gingiva before and after surgery was measured using laser Doppler flowmetry.
Results: In the A-PRF group, a large number of microvessels were observed in the gingival tissue on postoperative day 1. The microvessels in the control group were fewer and sparse. Regarding the vascular resin cast, a large number of new blood vessels were observed on postoperative day 1 in the A-PRF group. A stronger CD34-positive signal was obtained around the blood vessels in the A-PRF group than in the control group. Further, a strong VEGF-positive signal was observed in the perivascular tissue in the A-PRF group. Gingival blood flow was significantly higher in the A-PRF group after surgery.
Conclusion: A-PRF had a positive impact on angiogenesis in the gingiva through the induction of VEGF expression. Thus, A-PRF may be beneficial for gingival tissue regeneration.

1. Introduction

Regenerative medicine is emerging in recent years. Several techniques have been developed for modern dentistry to accelerate the regeneration of both hard and soft tissues [1,2]. In general, regenerative materials can be divided into two, namely, autologous and non-autologous materials. Ideally, a non-autologous graft for soft tissue augmentation should promote hemostasis, provide infection resistance, and favor the formation of granulation tissue, with low postoperative morbidity and a fast-healing time [2]. Self-derived regenerative materials are easy to obtain and are thought to possess no risk of allergies, rejection, and infection [1–3]. Therefore, they are ideal materials for regeneration.

Furthermore, angiogenesis and the formation of newly formed blood vessels are mandatory to provide the required nutrition, oxygen, immunological cells, mesenchymal stem cells, and growth factors, especially in the initial phase of healing [2,4,5]. A growing body of literature points towards angiogenesis as an essential physiological process that occurs during the repair and regeneration of damaged bone and soft tissues. The newly formed blood vessels not only provide nutrients and growth factors but also function as a delivery route of stem cells and progenitors to the site of the defect [2,4,6]. However, to date, most studies focus on time intervals in weeks or months [6,7] and seldom conduct comparisons of tissue regeneration within a week.

Platelet-rich fibrin (PRF) is an example of platelet-concentrating material used in self-regenerative medicine. PRF was developed in

Abbreviations: PRF, Platelet-rich fibrin; VEGF, vascular endothelial growth factor; A-PRF, advanced-PRF; CGF, concentrated growth factors; H&E, Hematoxylin and eosin.

* Corresponding author. Fax: +81 46 822 9646.
E-mail address: m.matsuo@kdu.ac.jp (M. Matsuo).
[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.job.2021.11.001
1349-0079/

2006 [8]. PRF fibrin matrix possesses flexibility, elasticity, and strength. It is composed of thrombin concentrates with equilateral bonds forming fine and flexible fibrin networks that support cytokines and cell migration [7–9]. Owing to the widespread use of PRF, platelet concentrates based on different production processes, such as advanced-PRF (A-PRF) and concentrated growth factors (CGF), have been developed [10]. These extensions have their characteristics and are used in tissue regeneration studies and have clinical applicability in different fields [11].

In this study, we aimed to investigate the recovery of the gingival tissue using A-PRF. Dogs were used as experimental animals. H&E staining and immunostaining were performed to observe the recovery of the gingival tissue on the first-, third-, and seventh-day post-surgery. In addition, we used laser Doppler flowmetry to measure changes in the blood flow rate to the gingival tissue before and after surgery.

2. Materials and methods

2.1. Animals

A total of six, one-year-old, weighing approximately 10 kg, female beagle dogs (Oriental Kobo, Tokyo, Japan) were used in this study. The oral cavity of each dog was checked before surgery [12].

All animal experiments were conducted in compliance with the protocol, reviewed, and approved by the Institutional Animal Ethics Committee of Kanagawa Dental University (Permit Number 19-007).

2.2. Preparation of A-PRF

Under general anesthesia administration, blood samples were collected in 10 mL tubes without anticoagulants. Samples were centrifuged at 200 ×g for 8 min [13]. After discarding the red blood cell fraction, the remaining A-PRF clots were placed on a dry gauze to eliminate excess serum and incubated at room temperature for 10 min till clotting.

2.3. Surgery

Two premolars (2nd and 3rd) were extracted bilaterally from the upper and lower jaws at experimental sites. A-PRF derived from autologous blood of the animals was placed at the right side of the dentition in the upper and lower jaws. The contralateral side without A-PRF (left side) was used as the control. Under general anesthesia administration, the premolar teeth were extracted along the gingival margin. Atropine (0.04 mg/kg subcutaneous injection) was administered as a pre-anesthetic before general anesthesia administration. Anesthesia induced by the administration of propofol (6 mg/kg intravenous injection) was maintained using isoflurane. After confirming the sufficient efficacy of anesthesia, the experimental procedure was performed ensuring no pain or discomfort to the animals. After filling the socket with A-PRF, the gingival flap was sutured; a similar procedure was performed for the control.

2.4. Morphological examinations

2.4.1. Hematoxylin and eosin (H&E) staining

Histological observations were performed at one-, three-, and seven-days post-surgery. All animals were sacrificed by perfusion-fixation in the upper jaws were resected after reflux fixation (10% formalin) and immersion-fixed overnight. The samples were decalcified in 5% hydrochloric acid and embedded in paraffin. They were cut into 4 μm disto–mesial plane sections and stained with Hematoxylin and Eosin (H&E) according to the standard procedure and observed under a light microscope (Olympus Optical, Tokyo, Japan).

2.4.2. Calculation of blood vessel density

We followed the method described previously by Aung PP et al. with slight modifications [14]. After observing the surgical area of gingival tissue under an optical microscope (Olympus Optical, Tokyo, Japan) at 100×, five areas with dense blood vessels were selected. We observed these five areas at 400× magnification, then counted the number of blood vessels in each field of view and calculated this mean value; blood vessels with a diameter <40 μm were recorded [15]. Each group were observed four times (n = 4).

2.4.3. Electron microscopy examination

After perfusion fixation, the synthetic resin (Mercox; Ladd Research, Williston, VT, USA) was injected from the inferior alveolar arteries into the lower jaw. The soft tissues were dissolved in 5% hypochlorous acid; all specimens were then washed thoroughly with water and freeze-dried. After ion-coating with platinum-palladium, the specimens were examined using a scanning electron microscope (JSM6301F; JEOL, Tokyo, Japan).

2.4.4. Immunohistochemistry

The paraffin-embedded implanted upper jaws of 4 μm sections were used for immunohistochemistry (IHC) analysis. The protocol as follows: 1) sections were dewaxed, and rehydrated; 2) they were autoclaved at 120 °C for 5 min in 10 Mm citrate buffer (pH 6); 3) after washing with PBS, endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in methanol; 4) slides were washed again with PBS and incubated in blocking buffer for 30 min at room temperature; 5) the primary antibodies used were anti-CD-34 (EP373Y, abcam, Tokyo, Japan) and anti-VEGF (VEGF; SC-7269, COSMO BIO CO., Tokyo, Japan) at 1: 200 dilution and incubated overnight; 6) the slides were washed thrice with PBS for 5 min each time; 7) polyclonal secondary antibody (Dako, USA) was added to tissue sections and co-incubated for 60 min; 8) the slides were washed thrice with wash buffer for 5 min each and the staining signal was developed by incubating the tissue with Metal Enhanced DAB Substrate Working Solution (Dako, USA) for 30 min; 9) the slides were washed for 1 min under tap water, and 10) hematoxylin staining was performed for 10 s, slides were washed for 5 min with tap water, and the dye was fixed. Image analysis after IHC staining was performed by observed using light microscopy (Olympus Optical, Tokyo, Japan).

2.5. Laser-Doppler-blood-flow examination

After administering anesthesia, the gingival blood flow in all animals was estimated using an LDF meter (TBF-LN1; Unique Medical Co., Ltd., Tokyo, Japan) with a laser Doppler probe of 2.0 mm of diameter at the roots of the first to fourth molars on either side of the upper and lower jaws. The recorded gingival blood flow was analyzed using data analysis software (Chart v 4.2; AD Instruments, Inc., Colorado Springs, CO, USA). The animals were re-tested and values were compared at the same location after one-, three-, and seven days post-surgery. Blood flow measurements were recorded using a non-contact probe, 2 mm in diameter. Six peri-gingival buccal and lingual points measurements were taken and the average values were recorded. Total blood flow in the marginal and attached gingivae was determined in a cylindrical area with a diameter and depth of 2 mm.

2.6. Statistical analysis

All statistical analyses were performed using the GraphPad Prism software (La Jolla, CA, USA). The data were expressed as mean ± standard deviation and were analyzed using a two-way analysis of variance followed by Tukey's test for significance. Statistical significance was considered as $P < 0.05$.

3. Results

3.1. Morphological observations

3.1.1. Hematoxylin and eosin (H&E) staining

No new blood vessels were observed in the control group on the first-day post-operation (Fig. 1B), however, in the A-PRF group, new and multiple blood vessels between the epithelial processes of the gingivae were observed (Fig. 1C). On the third day post-operation, capillaries were observed in the gingivae of the control group animals, but these were less in number as compared to the A-PRF group (Fig. 1D). In the A-PRF group, not only were there many new blood vessels, but the diameter of the blood vessels also showed an increase (Fig. 1E). On the seventh day, similar to the findings on the third day, new blood vessels were observed in the gingivae of the control group animals (Fig. 1F). In the A-PRF group, as a whole, the structure of gingival tissue was normal, and not only new blood vessels but also many large-diameter blood vessels were observed in the gingivae (Fig. 1G). There was no apparent difference in the tissue repair process between the two groups after the seventh day.

3.1.2. Density of blood vessels

The statistical results for each area of each group's slices are shown in Fig. 2. The density in the A-PRF group on the first day post-surgery was significantly different from that of the control group; the density in the A-PRF group on the third-day post-surgery was also significantly different from the seventh day in the control group. The density of blood vessels tended to decrease over time.

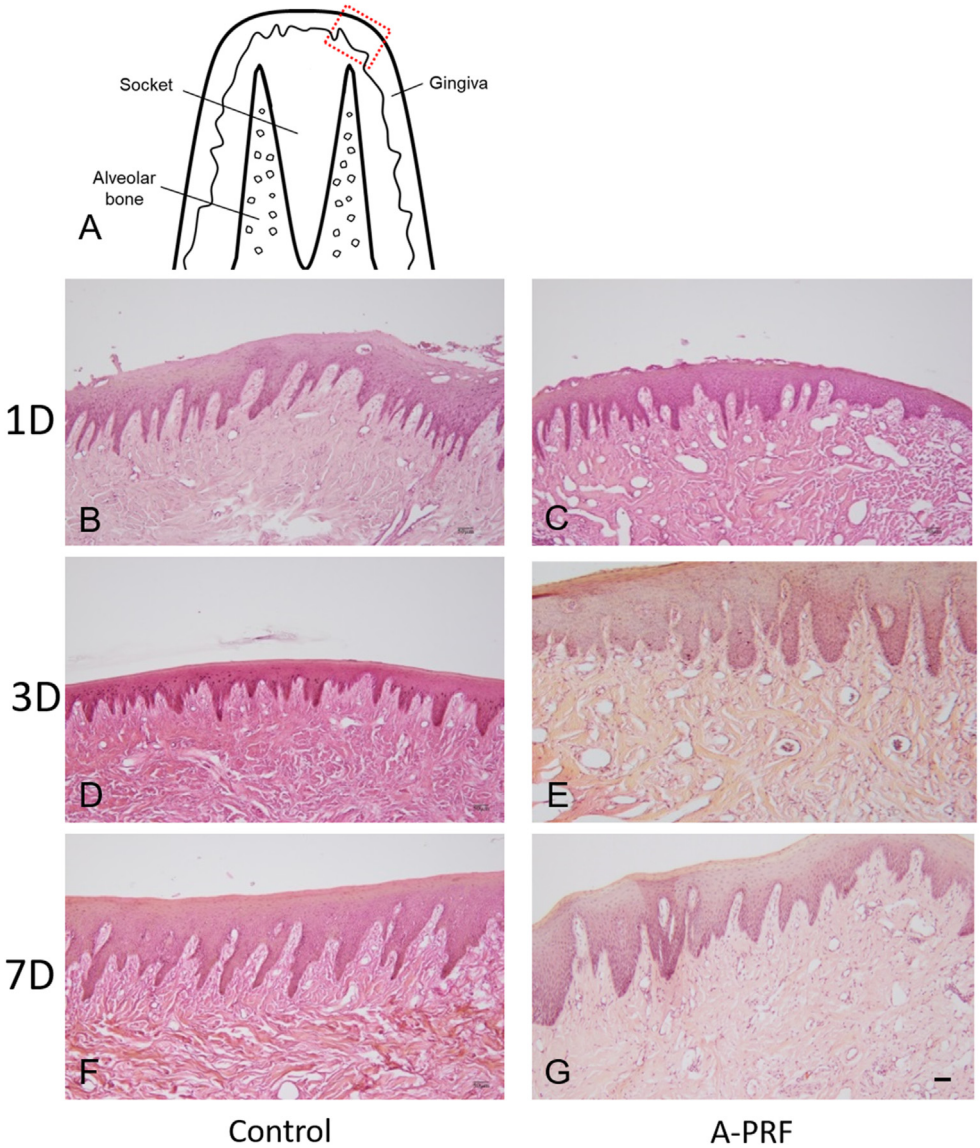

Fig. 1. Hematoxylin-eosin staining in gingival tissue for each group. The observation area shows in A. Histological changes at first, third, and seventh-day post-operation in the control (B, D, F) and A-PRF groups (C, E, G). Scale bar = 50 μm.

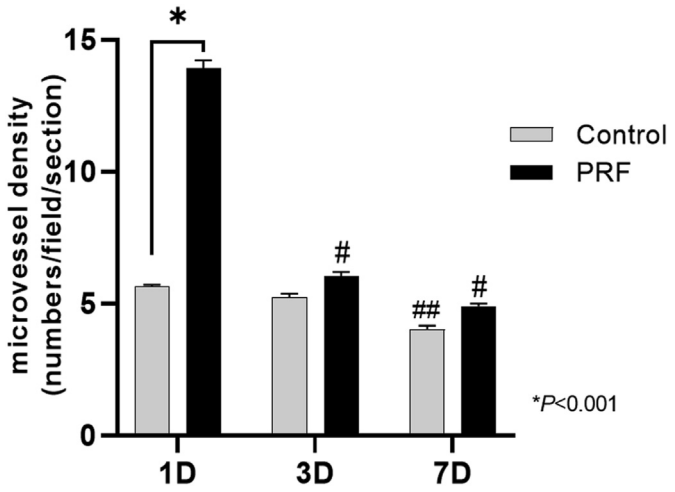

Fig. 2. The density of blood microvessels in each group. The graph shows the comparison of the density of blood microvessels in each group at each time point (n = 4). Two-way (PRF × days) analysis of variance followed by Tukey's test. *$P < 0.001$. #$P < 0.001$, versus the first day in the A-PRF group. ##$P < 0.001$, versus first day in the control group.

3.1.3. Resin cast model observations

The alveolar fossa was filled with thrombus in the control group on the first day. Capillaries with small diameters were observed (Fig. 3A). In the A-PRF group, abundant new capillaries were observed around the epithelium. Thin blood vessels were present in the superficial layer and flat and large diameter blood vessels were present in the deeper layers (Fig. 3B). On the third day, in the control group, a few thick blood vessels and capillary networks were observed (Fig. 3C). In the A-PRF group, a dense capillary network and thick blood vessels were observed along with a clear two-layered vascular structure (Fig. 3D). On the seventh day, the gingivae of the control group animals had low blood vessel density (Fig. 3E), and thick blood vessels were observed in the capillary network. In the A-PRF group, thick blood vessels were observed in the superficial capillary network (Fig. 3F).

3.2. Immunohistochemistry

On the first day, less CD34 expression was detected in the control group (Fig. 4A), while, in the A-PRF group, a strong CD34-positive signal was detected in the vascular endothelium (Fig. 4B). On the third day, the control group also showed some CD34 expression, however, it was still scarce as compared to that in the A-PRF group (Fig. 4C). A-PRF group showed marked expression of CD34 (Fig. 4D). On the seventh day, less CD34 expression was detected in the gingival tissue of the control animals (Fig. 4E). In the A-PRF group, the CD34 expression appeared strong around the blood vessel wall and in many large blood vessels (Fig. 4F).

On the first day in the control group, VEGF positive signal was low (Fig. 5A). On the first day in the A-PRF group, the VEGF-positive signal was strongly evident and almost distributed throughout the blood vessels (Fig. 5B). On the third day in the control group, VEGF expression was detected around the blood vessels but was weak as compared to the A-PRF group (Fig. 5C). In the A-PRF group, similar to the first day, VEGF expression was observed around the blood vessels (Fig. 5D). On the seventh day in the control group, the VEGF

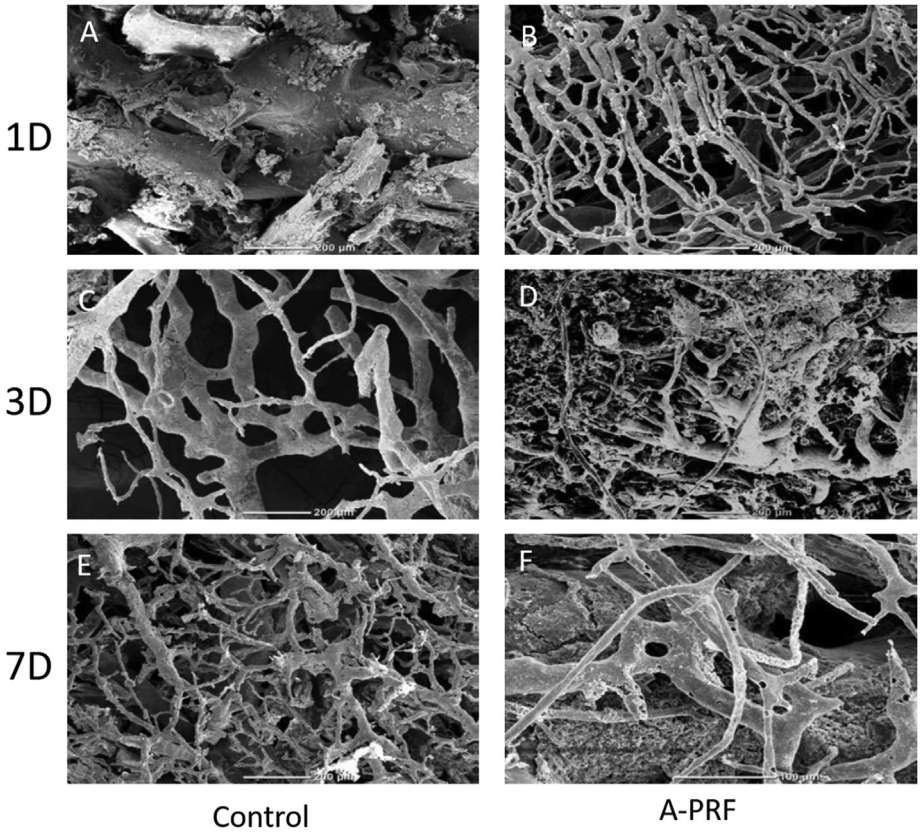

Fig. 3. Resin casts of the gingival vascular network in each group. Observation of gingival blood vessels at first, third, and seventh-day post-operation in the control (A, C, E) and A-PRF groups (B, D, F). Scale bar = 200 μm.

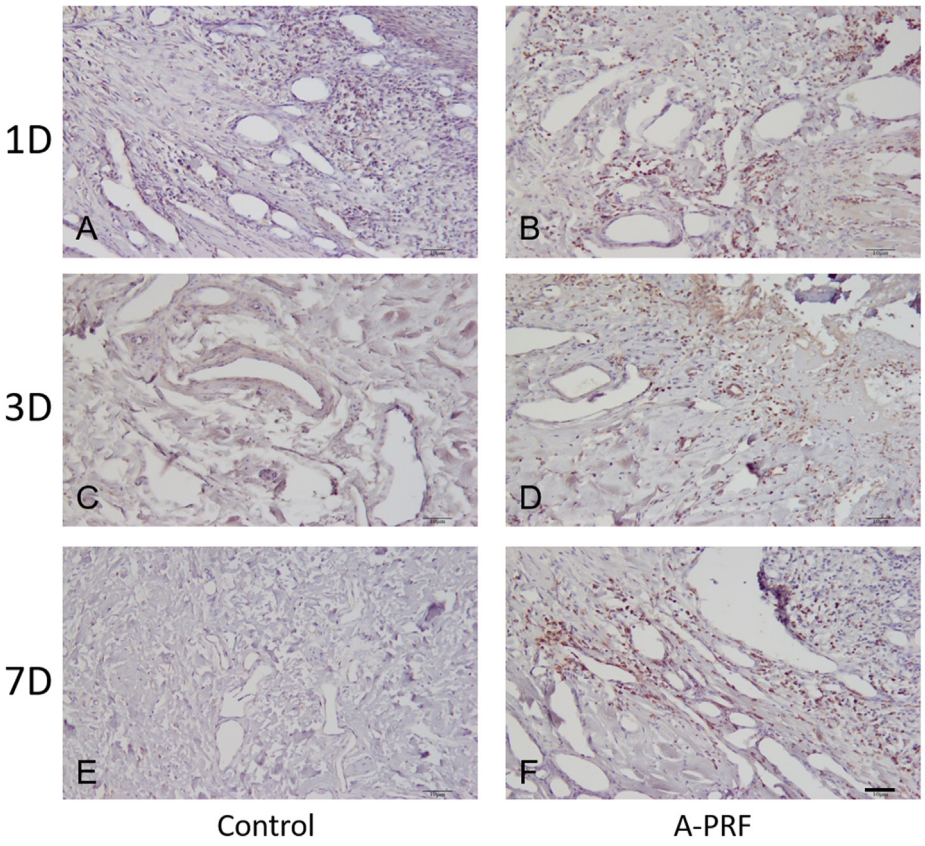

Fig. 4. Representative images of immunofluorescence analysis for CD34. Immunohistological reactions for CD34 in gingival tissue at first, third, and seventh-day post-operation in the control (A, C, E) and A-PRF groups (B, D, F). CD34 antigen is calibrated around the blood vessels from the first day and an increase on third- and seventh-day post-surgery was observed. Scale bar = 10 μm.

signal was absent (Fig. 5E), however, in the A-PRF group, marked expression of VEGF was seen around the blood vessels (Fig. 5F).

3.3. The laser Doppler flowmetry test

The measurements of laser Doppler flowmetry are shown in Fig. 6. Blood flow on the first day in the A-PRF group was significantly higher than that in the normal gingivae (36.68 ± 6.35 mV/min/100 g vs. 29.22 ± 4.46 mV/min/100 g). However, there were no significant differences between the other groups.

4. Discussion

The findings of this study indicated that A-PRF had an impact on the regeneration of gingival tissue through the acceleration of angiogenesis. PRF is widely used for the promotion of the regeneration of both soft and hard tissues. Based on modified protocols, several methods for enriching growth factors have been developed [2,10,11,16]. These previous studies show that the materials can accelerate tissue repair in damaged areas [16,17]. A-PRF was created by slight modifications in the production method of PRF [13,17]. A previous study has reported that A-PRF can release more growth factors, especially VEGF, relative to PRF or other blood biomaterials [16]. As A-PRF has a dense fibrous structure [7,17], it has a harder texture than PRF which makes it more suitable for fixing it at a specific position during surgery. Our previous study shows that A-PRF is effective in alveolar bone regeneration [13]. The present study clearly indicated that A-PRF had an impact on the regeneration of gingival tissue by accelerating the angiogenesis in the early stage of wound healing.

Combining the histological examination and scanning electron microscopy results, many microvessels were detected in the gingival tissue in the A-PRF group, relative to the control group. Additionally, the results also showed that the density of blood vessels in the gingival tissue of the A-PRF group was higher than that in the control group. For the comparison of blood vessel density, the method used in this study was previously reported in the determination of the benign and malignant tumors and prognostic evaluation [14,18–20]. The examination of tissue activity using the density of blood vessels is quite similar to wound healing as the high density of blood vessels is required in the newly forming tissue [2,21,22]. Our findings suggested that A-PRF could promote wound healing in the gingival tissue by inducing its vascularization.

CD34, commonly found in vascular endothelial cells and mesenchymal stem cells, is an antigen used in the evaluation of angiogenesis or vascular distribution [18,23]. VEGF increases endothelial cell proliferation and promotes angiogenesis [24,25]. The amount and distribution of VEGF in the tissues indicate tissue repair activity [25]. A marked increase in expressions of VEGF and CD34 proteins was detected in the gingival tissue of the A-PRF group relative to the control group. Moreover, there was a tendency for higher expression on the first day as compared to the seventh day. These findings were in line with our microvessel density observations using scanning electron microscopy. The wounds are generated in the operation area post-surgery and the tissues subsequently undergo self-repair [2,7,22]. To carry these series of steps, the proper functioning of blood vessels, especially the capillaries, is particularly important [21]. The tissues stimulate some blood vessels through cytokines to increase their permeability, thereby, completing the movement and function of leukocytes through

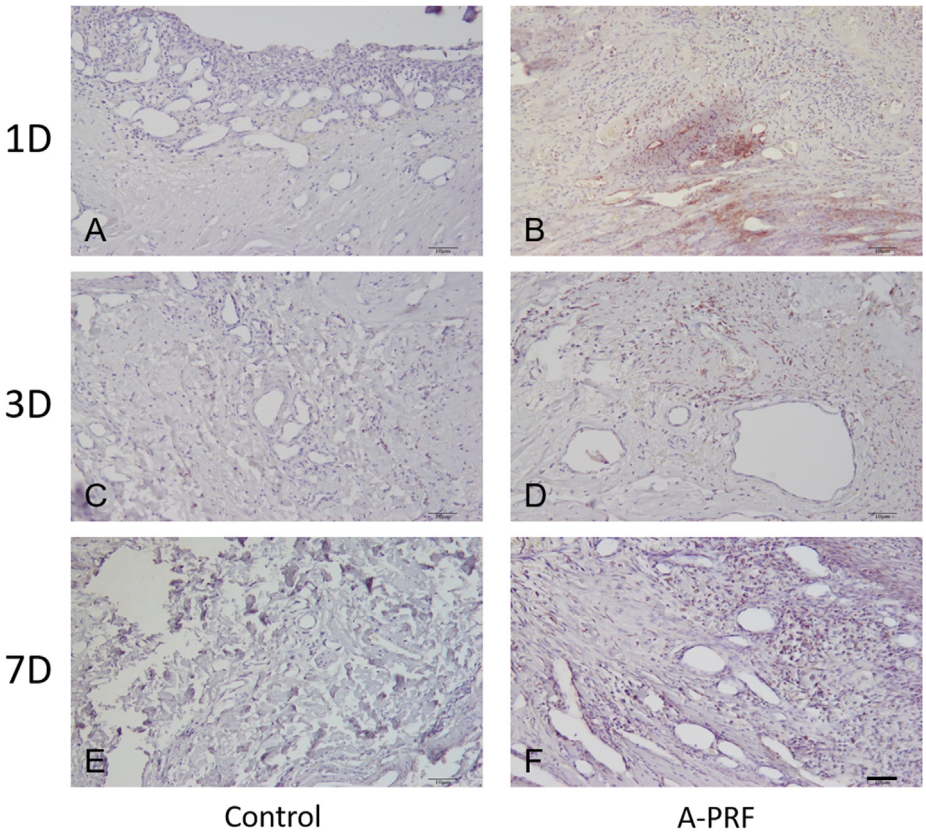

Fig. 5. Representative images of immunofluorescence analysis for VEGF. Immunohistological reactions for VEGF in gingival tissue at first, third, and seventh-day post-operation in the control (A, C, E) and A-PRF groups (B, D, F). VEGF antigen is calibrated from the first day, and on the third and seventh days post-surgery, the positive signal strength decreases relative to that on the first day. Scale bar = 10 μm.

diffusion; simultaneously, there is an exchange of oxygen and other substances [26]. However, at a higher density of microvessels, the diffusion efficiency is much higher than in the normal state [27]. Our results showed an increase in the density of microvessels on the first day in the A-PRF group. As A-PRF is rich in VEGF and other growth factors related to angiogenesis, after A-PRF filling, we speculated that a large number of new blood vessels were induced in the gingival tissue which could promote blood supply to the sockets.

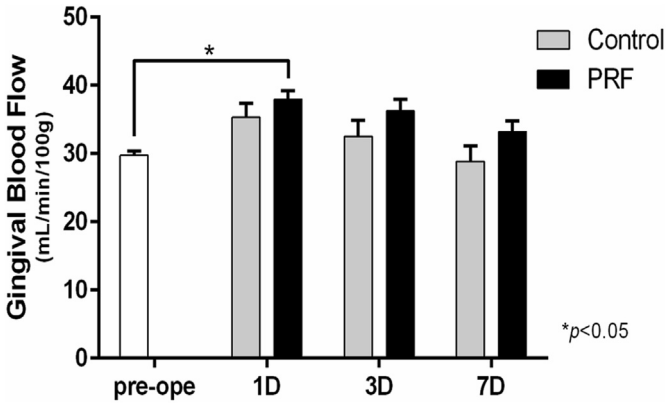

Fig. 6. Gingival blood flow estimate by laser Doppler flowmetry. The graph shows a comparison of the gingival blood flow in each group for each time point (N = 4). Gingival blood flow was significantly different between the A-PRF group on the first day (36.68 ± 6.35 mV/min/100 g) and before surgery (pre-op; 29.22 ± 4.46 mV/min/100 g). Two-way (PRF × days) analysis of variance followed by Tukey's test. *$P < 0.05$.

In addition to evaluating tissue regeneration by morphological examinations, observing the blood flow to the tissue is also an objective evaluation method [12]. The damaged tissue must be removed quickly and the new tissue must grow back [2,27]. In addition, for tissue regeneration, a high blood supply is necessary [27]. Therefore, the number and density of blood vessels in the tissue are closely related to the blood flow. Our results showed that the blood flow in the two groups was higher on the first-day post-surgery, and the blood flow decreased gradually on the third day and seventh day, thereafter. Only on the first day in the experimental group, the blood flow was significantly higher than that before the operation. Collectively, these findings suggested that A-PRF promoted blood flow by inducing angiogenesis in the gingival tissue at an early stage of wound healing.

5. Conclusion

In conclusion, the findings indicated that A-PRF could induce the formation of blood vessels for regeneration of the gingival tissue. However, from the view point of tissue regeneration, tissue regeneration cannot be accomplished by the regeneration of blood vessels alone and requires other cells and growth factors. Future studies should investigate the involvement of other factors in tissue regeneration.

Ethical statement

This study was approved by the Ethics Committee of the Kanagawa Dental University Animal Experiment (approval number,

19-007). All experiments were conducted following the Kanagawa Dental University Animal Experiment Guidelines.

Funding

This research was partially supported by the Japan Society for the Promotion of Science and the Grant-in-Aid for Scientific Research (C), grant number 17K11632.

CRediT authorship contribution statement

Yu-Hao Liu: Animal Surgery, Data curation, Investigation, Writing – original draft. **Masahiro To:** Data curation, Investigation, Writing – original draft. **Toshimitsu Okudera:** Animal Surgery, Data curation. **Satoko Wada-Takahashi:** Data curation, Investigation, Supervision. **Shun-Suke Takahashi:** Data curation, Investigation, Supervision. **Chen-yao Su:** Writing – original draft, Data curation, Investigation, Supervision. **Masato Matsuo:** Conceptualization, Animal Surgery, Data curation, Investigation, Writing – original draft, Supervision.

Conflicts of interest

The authors declare no conflicts of interest.

References

[1] Chisini LA, Conde MCM, Grazioli G, Martin ASS, Carvalho RV, Sartori LRM, et al. Bone, periodontal and dental pulp regeneration in dentistry: a systematic scoping review. Braz Dent J 2019;30:77–95.
[2] 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 B Rev 2017;23:83–99.
[3] Chuang MH, Ho LH, Kuo TF, Sheu SY, Liu YH, Lin PC, et al. Regenerative potential of platelet-rich fibrin releasate combined with adipose tissue-derived stem cells in a rat sciatic nerve injury model. Cell Transplant 2020;29:963689720919438.
[4] Saghiri MA, Asatourian A, Garcia-Godoy F, Sheibani N. The role of angiogenesis in implant dentistry part ii: the effect of bone-grafting and barrier membrane materials on angiogenesis. Med Oral Patol Oral Cir Bucal 2016;21:e526–37.
[5] Shaikh MS, Zafar MS, Pisani F, Lone MA, Malik YR. Critical features of periodontal flaps with regard to blood clot stability: a review. J Oral Biosci 2021;63:111–9.
[6] Kurobane T, Shiwaku Y, Anada T, Hamai R, Tsuchiya K, Baba K, et al. Angiogenesis involvement by octacalcium phosphate-gelatin composite-driven bone regeneration in rat calvaria critical-sized defect. Acta Biomater 2019;88:514–26.
[7] Hatakeyama I, Marukawa E, Takahashi Y, Omura K. Effects of platelet-poor plasma, platelet-rich plasma, and platelet-rich fibrin on healing of extraction sockets with buccal dehiscence in dogs. Tissue Eng 2014;20:874–82.
[8] Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, 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:e37–44.
[9] Granéli C, Thorfve A, Ruetschi U, Brisby H, Thomsen P, Lindahl A, et al. Novel markers of osteogenic and adipogenic differentiation of human bone marrow stromal cells identified using a quantitative proteomics approach. Stem Cell Res 2014;12:153–65.
[10] Masuki H, Okudera T, Watanebe T, Suzuki M, Nishiyama K, Okudera H, et al. Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (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.
[11] Rochira A, Siculella L, Damiano F, Palermo A, Ferrante F, Carluccio MA, et al. Concentrated growth factors (cgf) induce osteogenic differentiation in human bone marrow stem cells. Biology (Basel) 2020;9.
[12] Yamamoto R, Amano K, Takahashi SW, To M, Takahashi S, Matsuo M. Changes in the microcirculation in periodontal tissue due to experimental peri-implantitis. J Oral Biosci 2021;63:153–60.
[13] To M, Su CY, Hidaka K, Okudera T, Matsuo M. Effect of advanced platelet-rich fibrin on accelerating alveolar bone formation in dogs: a histological and immunofluorescence evaluation. Anat Sci Int 2019;94:238–44.
[14] Aung PP, Leone D, Feller JK, Yang S, Hernandez M, Yaar R, et al. Microvessel density, lymphovascular density, and lymphovascular invasion in primary cutaneous melanoma-correlation with histopathologic prognosticators and braf status. Hum Pathol 2015;46:304–12.
[15] Tucker WD, Arora Y, Mahajan K. Anatomy, blood vessels. In: Statpearls. Treasure Island (FL). StatPearls Publishing; 2021.
[16] Lei L, Yu Y, Han J, Shi D, Sun W, Zhang D, et al. Quantification of growth factors in advanced platelet-rich fibrin and concentrated growth factors and their clinical efficacy as adjunctive to the gtr procedure in periodontal intrabony defects. J Periodontol 2020;91:462–72.
[17] Dohan Ehrenfest DM, Pinto NR, Pereda A, Jiménez P, Corso MD, Kang BS, 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;29:171–84.
[18] Kukreja I, Kapoor P, Deshmukh R, Kulkarni V. Vegf and cd 34: a correlation between tumor angiogenesis and microvessel density-an immunohistochemical study. J Oral Maxillofac Pathol 2013;17:367–73.
[19] Basnaker M, Sr S, Bnvs S. Expression of endoglin (cd-105) and microvessel density in oral dysplasia and squamous cell carcinoma. J Clin Diagn Res 2014;8:Zc91–4.
[20] Yi F, Yang L, Wang S, Guo L, Huang C, Xie Y, et al. Microvessel prediction in h&e stained pathology images using fully convolutional neural networks. BMC Bioinf 2018;19:64.
[21] Filipowska J, Tomaszewski KA, Niedźwiedzki Ł, Walocha JA, Niedźwiedzki T. The role of vasculature in bone development, regeneration and proper systemic functioning. Angiogenesis 2017;20:291–302.
[22] Blatt S, Burkhardt V, Kämmerer PW, Pabst AM, Sagheb K, Heller M, et al. Biofunctionalization of porcine-derived collagen matrices with platelet rich fibrin: influence on angiogenesis in vitro and in vivo. Clin Oral Invest 2020;24:3425–36.
[23] Nagasaki M, Sakaguchi W, Fuchida S, Kubota N, Saruta J, Suzuki K, et al. Comparison of cd34 expression in fibrous reactive hyperplasia and healthy oral mucosa. J Oral Biosci 2020;62:88–92.
[24] Adini A, Adini I, Chi ZL, Derda R, Birsner AE, Matthews BD, et al. A novel strategy to enhance angiogenesis in vivo using the small vegf-binding peptide pr1p. Angiogenesis 2017;20:399–408.
[25] Friedlander LT, Hussani H, Cullinan MP, Seymour GJ, De Silva RK, De Silva H, et al. Vegf and vegfr2 in dentigerous cysts associated with impacted third molars. Pathology 2015;47:446–51.
[26] Keles GC, Cetinkaya BO, Ayas B, Isildak I, Diraman E, Koprulu H, et al. Levels of gingival tissue platelet activating factor after conventional and regenerative periodontal surgery. Clin Oral Invest 2007;11:369–76.
[27] Morss Clyne A, Swaminathan S, Díaz Lantada A. Biofabrication strategies for creating microvascular complexity. Biofabrication 2019;11:032001.