



Research Paper

The evaluation of platelet-rich fibrin for preventing alveolar ridge atrophy in a rat tooth extraction socket model

Accepted

ABSTRACT

The aim of this present research was to evaluate if alveolar ridge resorption following tooth extraction could be prevented by application of platelet-rich fibrin (PRF) membrane. Platelet-rich fibrin (PRF) is generally used as a natural scaffold in various clinical treatments as a reservoir of a vast number of biologically active molecules which are associated to tissue regeneration. However, the osteogenic potential of PRF membrane in a tooth extraction model has not been well evaluated. The autologous PRF membrane was transplanted into the tooth extraction socket of Wistar rat immediately after incisor extraction. The mandibles were subjected to microcomputed tomography and histological analyses for the evaluation of bone and soft tissue healing. PRF showed the prominent improvement in bone formation, soft tissue healing and blood homeostasis. Three days after surgery, soft tissue healing occurred quickly without signs of inflammation. After 4 weeks, PRF also accelerated the growth of soft tissue and the proliferation of bone cells. The sockets filled with the PRF membrane retained their original shapes and were prevented from ridge atrophy. This study demonstrated a simple and effective alveolar ridge preservation method using PRF membrane as a bleeding stopper and a natural scaffold for assisting bone and soft tissues regeneration.

Warunee Pluemsakunthai, Shinji Kuroda,
Hidemi Nakata and Shohei Kasugai

Department of Oral Implantology and
Regenerative Dental Medicine, Graduate
School of Medical and Dental Sciences,
Tokyo Medical and Dental University,
Yushima 1-5-45, Bunkyo-ku, Tokyo 113-
8510, Japan.

*Corresponding author. E-mail:
skuroda.mfc@tmd.ac.jp. Tel/Fax: +81 3
5803 4656.

Key words: Platelet, plate-rich fibrin, regeneration, ridge atrophy, tooth extraction, scaffold, autologous, osteogenic.

INTRODUCTION

Tooth extraction induces severe alveolar bone resorption around the extraction socket, which may result in a continuous decline of residual bone volume and strength as well as, impaired masticatory function, speech disorder, dynamic changes of facial expression and enhancing the pathologic fracture possibility (Misch et al., 2004). The healing process is accompanied with substantial reduction of the alveolar bone height and width, which may also impair aesthetic reconstruction (Mecall et al., 1991, 1992; Schropp et al., 2003; Serino et al., 2003). Such bone loss would be followed by soft tissue recession resulting in reduction or loss of keratinized marginal gingiva and disappearance of interdental papillae (Sclar, 2003). Unattended for a long time, alveolar ridge would become severely diminished. Furthermore, restoration with dental implant is sometimes very difficult when the destruction

reaches maxillary sinus or blood vessel and nerve. Therefore, recovery of alveolar ridges with its sufficient tissue volume, contour and height by tissue management and simultaneous grafting into the extraction sockets may compensate for post-extraction bone loss and its associated esthetic decline.

Understanding of the growth factors involved in bone and soft tissue healing can lead to development of the method for alveolar recovery in the extraction sockets. Platelet rich fibrin (PRF) is a rich source of wound-healing promoting growth factors (Kang et al., 2011). It has been clinically applied for maxillofacial reconstruction techniques such as sinus floor augmentation and alveolar ridge augmentation for dental implant treatments (Choukroun et al., 2006; Mazor et al., 2009). These clinical effects might result from a significant amount of growth factors and cytokines released

from platelets and leukocytes trapped in the fibrin structure of PRF (Dohan et al., 2006; Pluemsakunthai et al., 2013).

Platelets and leukocytes are stimulated by centrifugation during the PRF production process (Dohan et al., 2006). Growth factors and cytokines released from PRF including platelet-derived growth factor (PDGF), transforming growth factor- β 1 (TGF- β 1), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), interleukin-1 β (IL-1 β) and interleukin-4 (IL-4) contribute to promoting soft and hard tissue healing by initiating bone formation and stimulating collagen formation (Bolander, 1992; Cromack et al., 1990; Dohan et al., 2009; Pierce et al., 1991).

Conclusive evidence for their beneficial effect on alveolar bone healing is still being explored despite the fact that PRF increases growth factor levels in bony defects (Dohan et al., 2006; Gassling et al., 2009; He et al., 2009; Su et al., 2009). In the present study, we delivered PRF into rat tooth extraction sockets to determine the effect of PRF on hemostasis, soft tissue healing, alveolar ridge resorption and osteoconduction property.

MATERIALS AND METHODS

Figure 1 shows the outline of this study.

Animals

Twenty-four (24) healthy male Wistar rats aged 15 weeks were used in this study. The experimental design and procedures were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University (#0120243A). Every two rats were kept and fed per cage at 22°C under a 12 h light/dark cycle, with free access to normal diet and tap water.

Preparation of PRF

Venous blood was drawn from the femoral vein of each rat without anticoagulant in a 3 ml glass tube and immediately centrifuged at 400x room temperature for 12 min (Heraeus Labofuge 300, Buckinghamshire, UK). Three layers were formed after centrifugation: the supernatant at the top and the middle layer, and the RBC layer at the bottom (**Figure 2**). PRF clot was isolated by collecting the upper layer after cutting 1 mm below the junction between the middle and RBC layer. Thereafter, PRF clot was pressed between 2 gauzes to produce a PRF membrane.

Incisor extraction and PRF delivery

The root apex of rat incisor is not going to be closed through life so that those teeth keep growing. The eruption rate of

the lower incisor is about 2.8 mm/week (0.4 mm/day) (Addison et al., 1915). Under diethyl ether anesthesia, the lower right incisor was trimmed horizontally every three days at the level of the marginal gingiva with a diamond disk using a high-speed micromotor handpiece and normal saline sprayed through the handpiece for irrigation and cooling. Three days after the third treatment, all of the animals were anesthetized with an intraperitoneal injection of the combination of ketamine-xylazine (40 mg/kg; 5 mg/kg) and the lower right incisors carefully pulled out along the long axis to prevent damage to the gingival tissue and the alveolar bone by a minimally traumatic technique. The entire incisor was successfully extracted without root fracture in all rats. Immediately after extraction, the alveolar socket was filled with the autologous PRF membrane using a root canal plugger in the experimental group and the control group was filled with a natural blood clot. PRF membrane was well controlled of thickness and approximately positioned 1 mm deep from the edge of the socket.

Total bleeding time measurement

According to Duke's method, the total bleeding time (TBT) was measured immediately after extraction (Loscalzo et al., 2003). Round shape filter paper, 10 cm in diameter was used to draw off the blood at the edge of the socket. Cessation of bleeding was determined by blotting away the blood every 30 s until the blood stopped staining the paper filter (Bowie et al., 1980; Lind, 1984). The TBT connotes the operating time (OT) and the bleeding time (BT), where the OT is the period for PRF delivery into the socket, and the BT is the hour between the beginning of bleeding and the cessation. The formula is given as:

Total Bleeding Time (TBT) = Operating Time (OT) + Bleeding Time (BT)

Anatomical and histological examination

Three rats from each group were sacrificed at 3 days, 1, 2, and 4 weeks after the incisor extraction. The mandible was dissected out, a photograph taken and placed in 10% neutral buffered formalin for 2 weeks, decalcified in 10% EDTA at pH 7.4 for 4 weeks and then embedded in paraffin. Coronal sections, about 5 μ m thick, were cut, stained with haematoxylin-eosin and observed.

Micro-computed tomography (MicroCT) analysis

Biopsies were also sampled and scanned to calculate new bone volume (BV, mm³) and bone mineral density (BMD, mg/cm³) within the socket by micro-computed tomography (μ CT, SMX-90CT, Shimadzu, Kyoto, Japan). Thereafter, three-

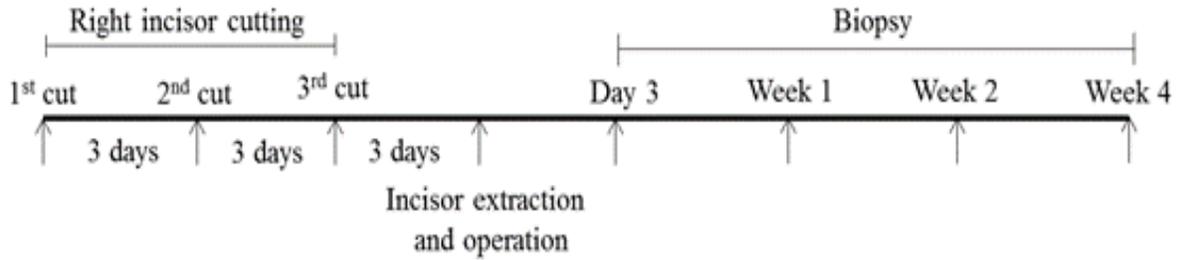


Figure 1: Scheme shows a study design and an experimental time line. Every third day lower right incisor was cut at the level of marginal gingiva. Three days after the third cut, all of the animals were anesthetized and only right incisor was carefully pulled out by a traumatic technique. Immediately after incisor extraction, the autologous PRF was used to fill the alveolar socket in the experimental group. In the control group, the sockets were filled with natural blood clot. Total bleeding time (TBT) was measured immediately after extraction in accordance with Duke’s method. Three rats from each group were sacrificed at 3 days, 1, 2, and 4 weeks after incisor extraction. The mandible was dissected out and all of the specimens analyzed by histological and micro-computed tomography methods.



Figure 2: Histological images at fourth week after tooth extraction. Control group showed many spaces in the extraction socket and newly formed trabecular bone was small and not strong enough to retain the height and width of alveolar ridge. In PRF group, PRF acts as space maintainer to support alveolar shape. PRF membrane forms strong bridge for the gingiva to grow on. Bone cell migrated completely into the fibrin of PRF and formed a new bone to support alveolar bone height and width for over 4 weeks.

dimensional microCT images were reconstructed and the structural indices calculated using a three-dimensional image analysis system (Tri 3D-BON, Ratoc System Engineering, Tokyo, Japan).

Statistical analysis

All data are presented as mean \pm standard deviation (S.D.). The changes in hemostasis and microCT variables between PRF-treated and the control groups were evaluated specifically at each time points (3 days, 1, 2, and 4 weeks) using the unpaired Student’s *t*-test. *P*-value less than 0.05

were considered to be statistically significant.

RESULTS

The effect of PRF on hemostasis

The bleeding attenuated and stopped within a few minutes in the PRF group. On the other hand, the bleeding continued for a couple of hours until it coagulated in the control group. The PRF group took 0.54 ± 0.40 min to acquire complete hemostasis, which was significantly shorter than that in the control group (45.36 ± 4.38 min).

Control group

3 days

1 week

4 week



PRF group

3 days

1 week

4 week



Figure 3: Photographic of the tooth extraction healing of control and PRF group at 3 days, 1 and 4 weeks post-operation. PRF accelerated wound healing and no sign of inflammation was observed whereas the control group showed red, swelling and bleeding soft tissues covered the extraction socket. Figure 3b shows a cross-section of the incisor socket, while C and D shows the longitudinal sections of incisor socket at the center of the defect.

Clinical and histological evaluation of PRF on soft tissue and bone healing

By the end of the 4 weeks of the study all of the 24 extraction sockets healed uneventfully. **Figure 3** shows that clinically, soft tissue healing appeared to be more rapid in

the PRF-treated group. During the healing process, the control sites showed signs of inflammation including redness and swelling. The PRF group quickly healed and was filled with granulation tissue within 3 days. PRF also prevented soft tissue invasion, bacteria, and worked as a space maintainer for protecting collapse of the alveolar

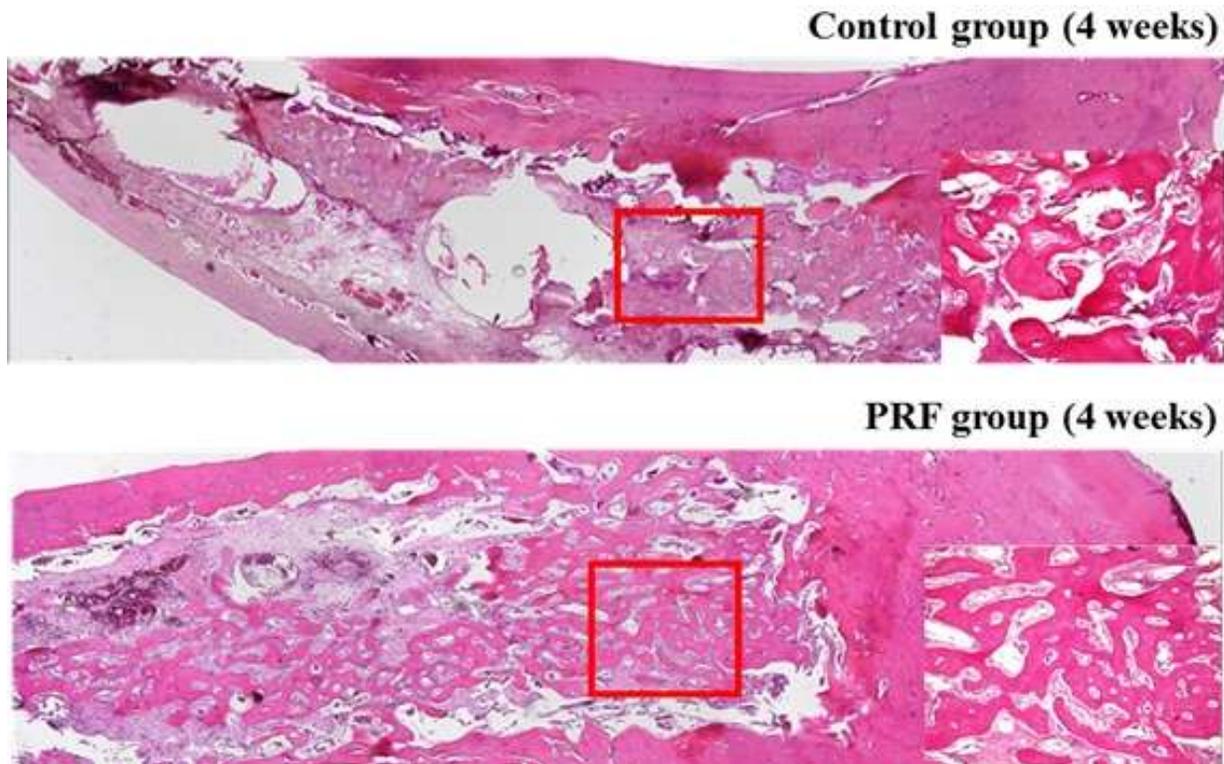


Figure 4: Micro-computed tomography (MicroCT) analysis images of control and PRF groups at 4 weeks post-operation. The images represent cross-section, mesio-distal longitudinal section, and bucco-lingual longitudinal section of incisor socket at the center of the defect. PRF group showed highly significant different new bone formation after applying PRF immediately in a tooth extraction. White area in the socket shows new bone formation which had prominent new bone formation in PRF groups compared with the control group.

process. The immature epithelium extended from the surrounding gingiva to cover the socket within the first 3 days in the PRF group.

The PRF membrane functioned as a space maintainer in the alveolar bone and a plug for hemostasis in the socket, where the osteoblasts were observed to generate new woven bone in the socket adjacent to the cortical wall without obstruction by gingival in growth at 1 week. Bone formation at 2 weeks continued on the surface of the socket wall and the space was filled with trabecular. Until 4 weeks, osteoblasts had migrated into and completely replaced the fibrin of PRF, forming a new bone to support and maintain the alveolar bone height and width. New bone formation was also found in control, particularly on the wall of the extraction socket at 1 week. After 4 weeks, trabecular bone started to occupy the tooth extraction space of the control group, which was less in volume and was not enough to maintain the original shape of the alveolar ridge.

Micro-computed tomography (MicroCT) analysis of PRF on new bone formation

Figure 4 shows the new bone formation of the control group was compromised, compared with that in the PRF-filled

defects at 4 weeks. The newly formed bone was compared by limiting the region of interest (ROI) only inside the tooth socket. PRF group gained higher BV and BMD as compared to the control group. There were no significant difference in the BV and BMD of the immature bone in this ROI during 3 days to 2 weeks post-operation (Figure 5).

DISCUSSION

This present study clinically examined hemostasis and new bone formation in the tooth-extraction socket histologically and by microCT. The anatomical observation of quick cessation of bleeding within a few minutes by plugging the extraction socket with the PRF membrane clarified that the membrane acted as not only a plug against bleeding but also a space maintainer for osteogenesis. From the histological analysis, the incisor sockets which had been covered with the PRF membrane were replaced with a new bone preventing ridge atrophy (Figure 6). In contrast, the control group left residual space in the alveolar socket which would gradually but incompletely healed, led to collapse of the alveolar ridge. Furthermore, the microCT evaluation showed significantly more bony regeneration in which the PRF membrane was also replaced with new bone by osteoblast

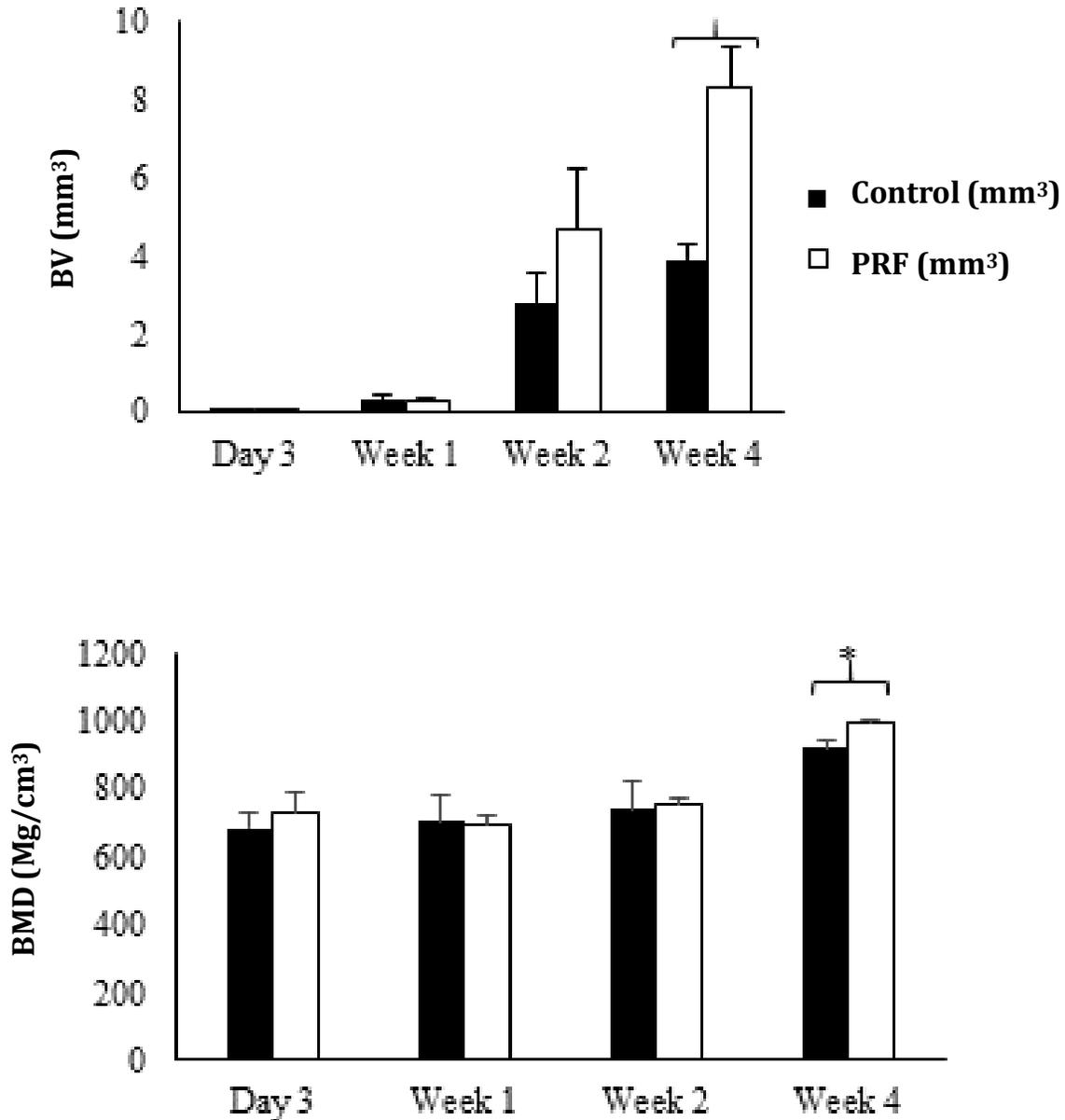


Figure 5: Graphs demonstrate a newly formed bone volume (BV, mm³) and a bone mineral density (BMD, mg/cm³) of control and PRF groups at 3 days, 1, 2, and 4 weeks after operation by microCT analysis. PRF group gradually increased newly formed bone more than control group from and had highly significant different new BV and BMD at week 4.

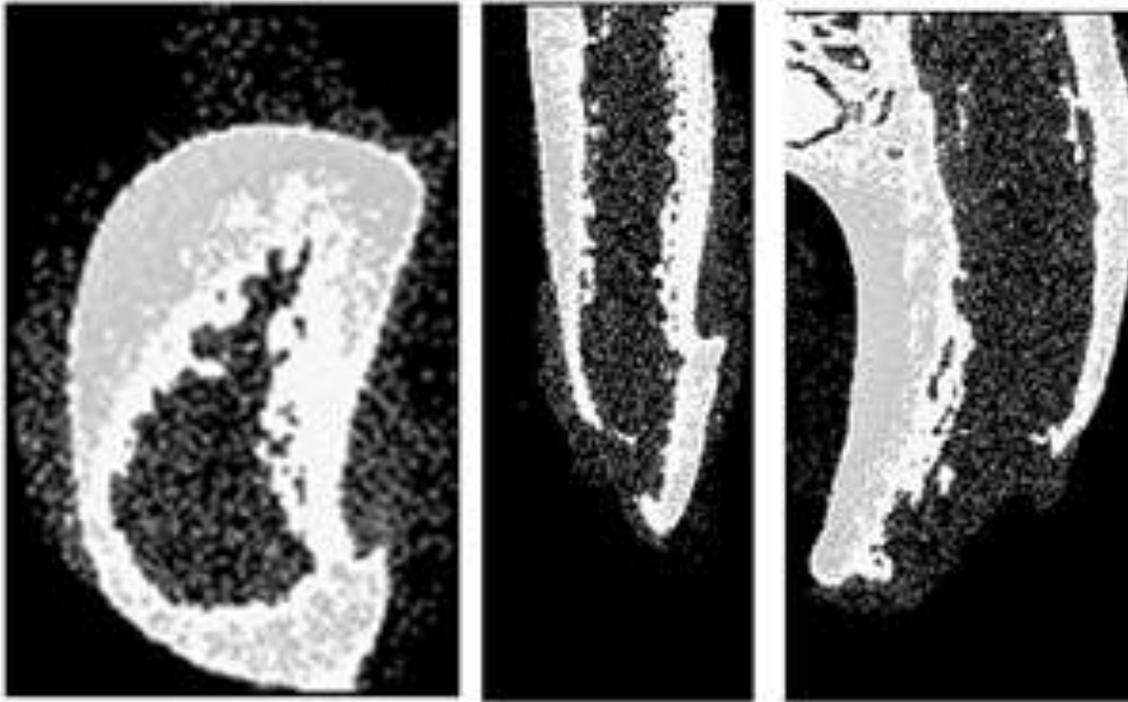
invasion into the membrane within 4 weeks (Figure 4).

Tooth extraction is often necessary because of caries, periodontitis, endodontitis or fracture in the dental field. However, atrophy of the alveolar bone after the extraction would be disadvantageous and followed by prosthetic treatments in many cases. Therefore, effective healing of bone and soft tissues for bone preservation in the extraction sockets could be beneficial for dental restoration. There are several studies examining alveolar bone preservation to be quantitatively analyzed.

The present prospective study of PRF in rat tooth-

extraction sockets concluded that PRF provided the efficacious and desirable bone regeneration. PRF can provide a three-dimensional scaffold in order to generate and maintain the proper bone volume in the extraction socket for preparation of optimal esthetics and function with prostheses. Fibrin can generally play an essential role to support bony space and release vital cytokines and cells to accelerate wound healing (Dohan et al., 2006). Previous studies reported that bFGF, VEGF, PDGF and angiopoietin, which are main angiogenic soluble factors, scatter and bind to fibrin networks (Sahni et al., 1998). PRF also allows early

Control group (4 weeks)



PRF group (4 weeks)



Figure 6: Histological images of control and PRF group at 4 weeks post-operation. PRF group was filled with trabecular bone and acted as a space maintainer whereas the control group was filled with spaces and less trabecular bone when compared to the PRF group.

angiogenesis to infiltrate into the interconnected fibrin network in the whole bone lack area (Choukroun et al, 2006). Therefore, the strong fibrin of PRF may as well support new bone regeneration for further constitutive prosthetic treatment.

Conclusion

This study demonstrated a simple and effective alveolar ridge preservation method using PRF membrane which acted as a space maintainer, a bleeding stopper and a natural scaffold for assisting bone and soft tissues regeneration. In summary, PRF would be useful for developing a method to prevent the severe alveolar ridge atrophy after tooth extraction.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Drs. Daisuke Sato, Masaki Fujii, Kang Chen and Yu Yamashita who offered invaluable guidance, support and assistance. This study was supported by the Global Center of Excellence (G-COE) Program for International Research Center for Molecular Science in Tooth and Bone Disease, Tokyo Medical and Dental University and the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

- Addison WHF, Appleton JL (1915). The structure and growth of the incisor teeth of the albino rat. *J Morphol* 26(42-96).
- Bolander ME (1992). Regulation of fracture repair by growth factors. *Proc Soc Exp Biol Med* 200(2): 165-70.
- Bowie EJ, Owen CA, Jr. (1980). Standardization of the bleeding time. *Scand J Haematol Suppl* 37(87-94).
- Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, Dohan AJ, Mouhyi J, Dohan DM (2006). 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* 101(3): e56-60. 10.1016/j.tripleo.2005.07.011.
- Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, Dohan AJ, Mouhyi J, Dohan DM (2006). 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* 101(3): 299-303. 10.1016/j.tripleo.2005.07.012.
- Cromack DT, Porras-Reyes B, Mustoe TA (1990). Current concepts in wound healing: growth factor and macrophage interaction. *J Trauma* 30(12 Suppl): S129-33.
- Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B (2006). Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101(3): e45-50. 10.1016/j.tripleo.2005.07.009.
- Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B (2006). Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 101(3): e51-5. 10.1016/j.tripleo.2005.07.010.
- Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammartino G (2009). Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): a gold standard to achieve for all surgical platelet concentrates technologies. *Growth Factors* 27(1): 63-9. 10.1080/08977190802636713.
- Gassling VL, Acil Y, Springer IN, Hubert N, Wiltfang J (2009). Platelet-rich plasma and platelet-rich fibrin in human cell culture. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 108(1): 48-55. 10.1016/j.tripleo.2009.02.007.
- He L, Lin Y, Hu X, Zhang Y, Wu H (2009). A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 108(5): 707-13. 10.1016/j.tripleo.2009.06.044.
- Kang YH, Jeon SH, Park JY, Chung JH, Choung YH, Choung HW, Kim ES, Choung PH (2011). Platelet-rich fibrin is a Bioscaffold and reservoir of growth factors for tissue regeneration. *Tissue Eng Part A* 17(3-4): 349-59. 10.1089/ten.TEA.2010.0327.
- Lind SE (1984). Prolonged bleeding time. *Am J Med* 77(2): 305-12.
- Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD, Dohan Ehrenfest DM (2009). Sinus floor augmentation with simultaneous implant placement using Choukroun's platelet-rich fibrin as the sole grafting material: a radiologic and histologic study at 6 months. *J Periodontol* 80(12): 2056-64. 10.1902/jop.2009.090252.
- Mecall RA, Rosenfeld AL (1991). Influence of residual ridge resorption patterns on implant fixture placement and tooth position. 1. *Int J Periodontics Restorative Dent* 11(1): 8-23.
- Mecall RA, Rosenfeld AL (1992). The influence of residual ridge resorption patterns on implant fixture placement and tooth position. 2. Presurgical determination of prosthesis type and design. *Int J Periodontics Restorative Dent* 12(1): 32-51.
- Misch CE, Wang HL, Misch CM, Sharawy M, Lemons J, Judy KW (2004). Rationale for the application of immediate load in implant dentistry: Part I. *Implant Dent* 13(3): 207-17.
- Misch CE, Wang HL, Misch CM, Sharawy M, Lemons J, Judy KW (2004). Rationale for the application of immediate load in implant dentistry: part II. *Implant Dent* 13(4): 310-21.
- Pierce GF, Mustoe TA, Altrock BW, Deuel TF, Thomason A (1991). Role of platelet-derived growth factor in wound healing. *J Cell Biochem* 45(4): 319-26. 10.1002/jcb.240450403.
- Pluemsakunthai W, Kuroda S, Shimokawa H, Kasugai S (2013). A basic analysis of platelet-rich fibrin: distribution and release of platelet-derived growth factor-BB. *Inflammation and Regeneration* 33(3): 164-172.
- Sahni A, Odrliji T, Francis CW (1998). Binding of basic fibroblast growth factor to fibrinogen and fibrin. *J Biol Chem* 273(13): 7554-9.
- Schropp L, Wenzel A, Kostopoulos L, Karring T (2003). Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *Int J Periodontics Restorative Dent* 23(4): 313-23.
- Serino G, Biancu S, Iezzi G, Piattelli A (2003). Ridge preservation following tooth extraction using a polylactide and polyglycolide sponge as space filler: a clinical and histological study in humans. *Clin Oral Implants Res* 14(5): 651-8.
- Su CY, Kuo YP, Tseng YH, Su CH, Burnouf T (2009). In vitro release of growth factors from platelet-rich fibrin (PRF): a proposal to optimize the clinical applications of PRF. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 108(1): 56-61. 10.1016/j.tripleo.2009.02.004.