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EVALUATION OF STRUCTURE OF PLATELET-RICH FIBRIN MEMBRANES

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ABSTRACT

This study aimed to collect and evaluate fibrin membrane by centrifugation method. 3 mL of whole blood were collected into 10 mL glass tubes without anticoagulant and immediately centrifuged at 1500 rpm (group A), 3000 rpm (group B), and 4000 rpm (group C) for 10 min. The results showed that the ratio of fibrin membrane clot from group A less than group B and group C. The histological analysis with H-E staining demonstrated that fibrin membrane in the group B exposed homogeneous structure than the other groups. The results of analysis by scanning electron microscopy (SEM) revealed that fibrin membrane in the group B exposed homogeneous distribution of fibrin fibers than the other groups. The average diameter of fibrin

fibers of group A, group B, and group C were 0.143 ± 0.007 µm, 0.203 ± 0.007 µm, and 0.080 ± 0.005 µm, respectively.

Key word: hematoxylin-eosin staining, fibrin membrane, fibrin fibers.

INTRODUCTION

In hematology and blood transfusion, platelet level plays an important role in the treatment and prevention of bleeding in cases of tissue defects [9,10,11].Fibrin membrane formation involves thrombin-mediated proteolysis and removal of the N tail of fibrinopeptide from the Aα and Bβ chains. Another fibrin monomer allows staggered attachment with a single fibrin monomer into protofibrils. The combining subsequent protofibrils into fibers creating a fiber network for blood clot stability [2]. Furthermore, fibrin is able to bind more proteins and growth factors in healthy tissue or be released into the wound to heal wounds, including fibronectinand vitronectin, FGF, VEGF, insulin-like growth factors.This protein complexesassociate to the fibrin membranesand promote healing wounds through intermediate receptor interactions [11]. In the first blood drawing, the anticoagulant (such as EDTA, calcium chloride or thrombin) is not necessary to apply for the PRF membrane collection to stimulate polymer polymerization reaction [13]. The PRF membranes exposed a higher concentration of cytokines in their structures than basal levels, which can stimulate autologous recovery [4], cartilage repair [3], regeneration after oral surgery and maxilla [5], and wound healing [7].

With the potential for application in treatment, there have been many studies focusing on the methods of obtaining and proving the PRF membrane which has the potential to become a biological material.The PRF membrane was applied for performing autologous grafting on cartilage pieces of the injured knee [13]. The PRF membrane structure contains autologous platelets without external supplementation which store and release cytokines in a controlled manner [7]. There was three platelet cytokine (platelet-derived growth factor-BB, transforming growth factor-beta 1: TGF-b1,and insulin-like growth factor 1) have been studied [6]. These cytokines quantification were investigated in local PRF in rabbits [8]. In this study, we aimed to collect and evaluate some biological properties of platelet-rich fibrin.

MATERIAL AND METHODS

The PRF membrane collection

3 mL of whole blood from patients were drawn into 10 mL glass tubes without anticoagulant and immediately centrifuged at 1500 rpm (group A), 3000 rpm (group B), and 4000 rpm (group C) for 10 min. The PRF clotformation was observed in the middle of each tube.Each the PRF clot was collected from the tube by separating from the redblood cell base. The clots were gently pressed to obtain the fibrin membrane.

Scanning electron microscope (SEM) analysis

SEM was applied to examine the surface microstructure of membrane. The PRF membrane was dehydrated by passing the specimens through a graded series of ethanol-water mixtures, and then dried by the critical-point method. After drying the sample was sputter-coated with gold, and examined under the SEM. ImageJ software (National Institutes of Health, Bethesda, MD) was applied to estimatesurface microstructure of membrane and the diameter of fibrin fibers

Hematoxylin-Eosin (H-E) staining

The PRF membrane dehydration was carried out with 10% fomaldehyde before fixing in paraffin. The Rotary 3006 EM Prm cutting device is used to cut samples into 3–5 mm sections for histological analyses. Hematoxylin-Eosin (H-E) staining process will be carried out as follows: the glass slides with samples were incubated with xylene in tank for 5 minutes to remove paraffin, then samples were incubated in 100° Ethanol, 95° Ethanol, 80° Ethanol, and 70° Ethanol [15]. The slide samples were washed with distilled water. The samples were observed under microscope.

Statistical analysis

The data were analyzed for statistical significance by one-way ANOVA where $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The PRF membrane collection

After centrifugation, the blood samples were divided into two separate layers: the upper layer is yellow with platelet-rich fibrin, the lower layer is dark red blood. As seen in the Figure 1, the ratio of PRF clot from group B and group C was higher than group A. The was no statistically significant diffrence in the ratio of PRF clot between group B and group C. The ratio of PRF clot of group C was 1.16-fold higher than group A.

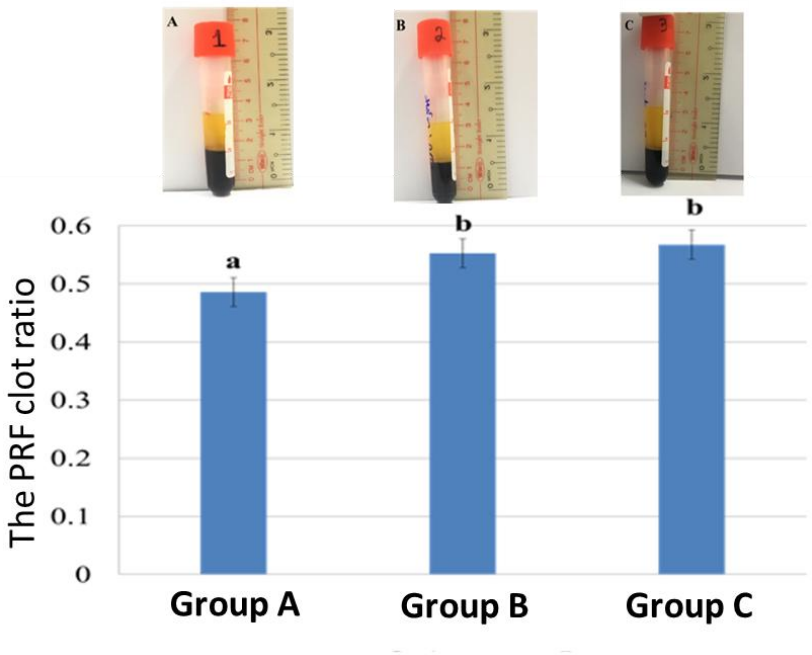

Figure 1.The PRF collection. A, B, C: group A, group B, group C. *a, b, c: statistically significant diffrence (P≤0.05).*

Hematoxylin-Eosin (H-E) staining

The histological structure of PRF membranes was estimated by Hematoxylin-Eosin staining. The PRF membranes from group A showed the heterogeneous structure, as indicated by the loose linkage between fibrin subunits in the membrane (Figure 2A1, 2A2, 2A3).The PRF membranes from group C exposed the more homogeneous structure than Group A. However, theerythrocytes and small cavities were observed in the PRF membranes from group C (Figure 2C1, 2C2, 2C3). No red blood cells are found on the outside and inside of the PRC membrane

from group B (Figure 2B1, 2B2, 2B3). The fibrin membrane obtained from group B showedmore homogeneous structure than group A and group C.

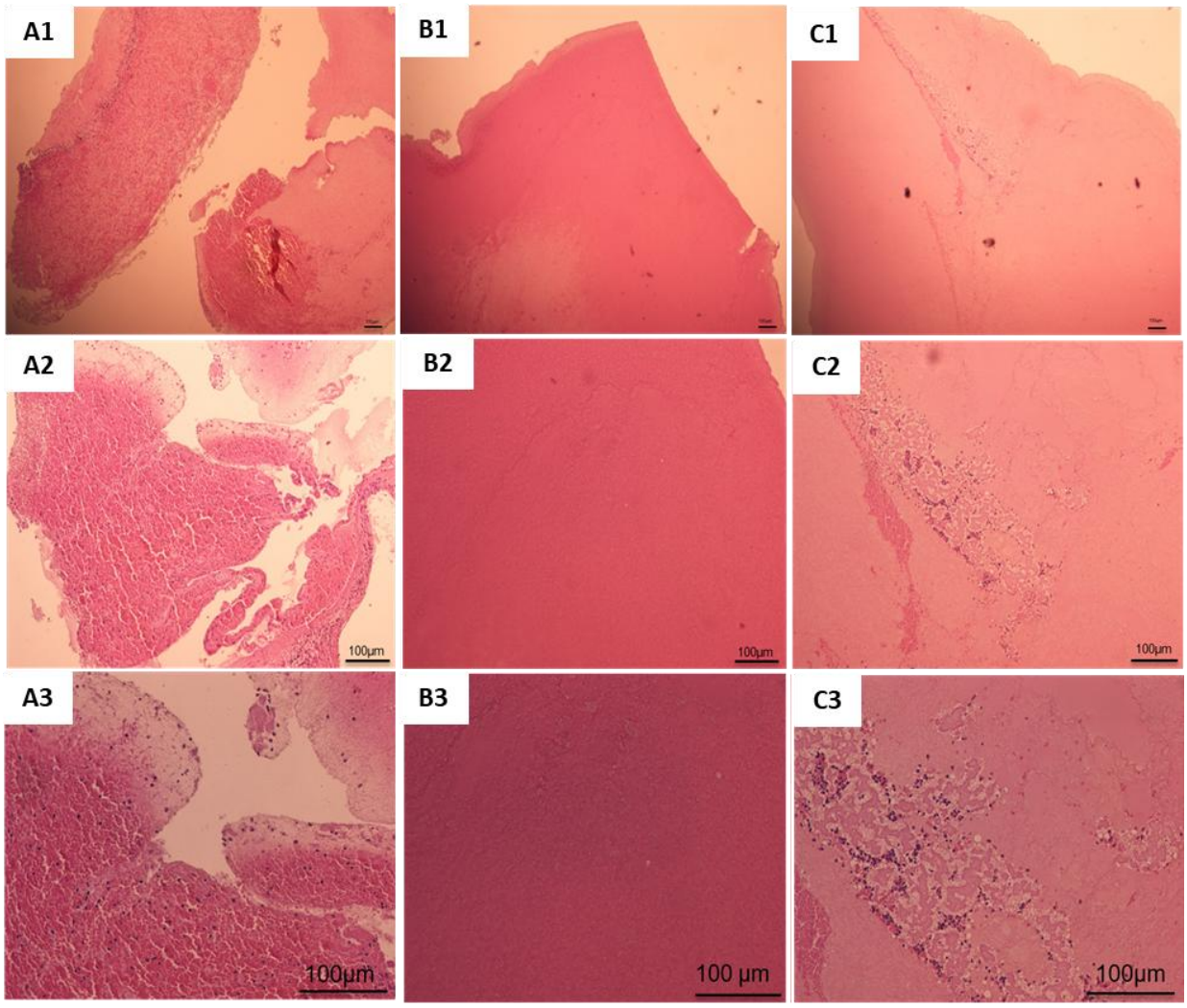

Figure 2.Hematoxylin-Eosin (H-E) staining of PRF membranes. A1, A2, A3: the PRF membranes from group A in the magnification of 100X, 200X, 400X; B1, B2, B3: the PRF membranes from group B in the magnification of 100X, 200X, 400X; C1, C2, C3: the PRF membranes from group C in the magnification of 100X, 200X, 400X.

The PRF membrane analysis by SEM

The observing the fibrin membrane under the scanning electron microscope (SEM) demonstrated that the surface structure is different between the experimental groups (Figure 3). The PRF membrane from group C showed the uneven and rough surface structure, many positions exposed theholes, the number of blood cells from group C is less than group A.In group

B, the PRF membrane structure showed the homogeneous surface.Thenumber of fibrin fibers of PRF membrane from group B is higher than the other two groups.

Thediameter measurement of fibrin fiber in group A, group B and group C wasconducted by software Image J (Figure 4). The average diameter of fibrin fibers in group A, group B and group C is 0.143 ± 0.007 µm, 0.203 ± 0.007 µm, 0.080 ± 0.005 µm, respectively. Our results were consistent to study from Bai and colleagues who reported that the diameter of fibrin fibers is about of 0.079- 0.179 µm [1].

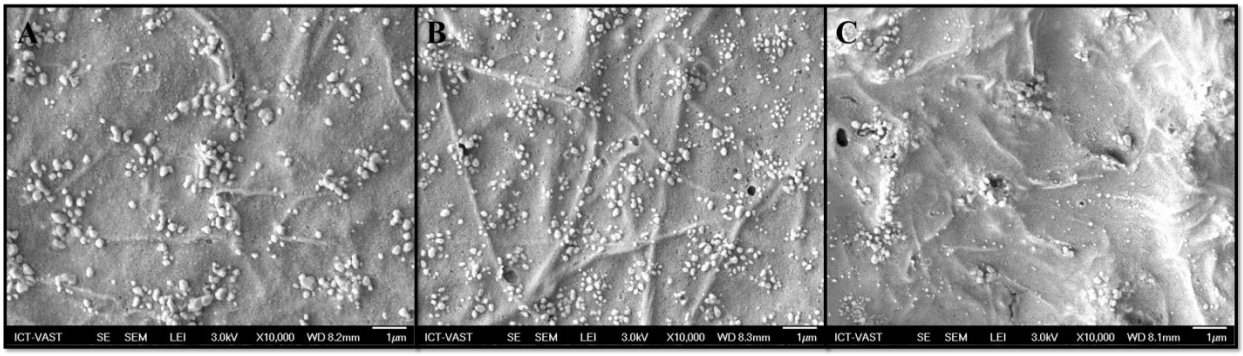

Figure 3.*Scanning electron microscopic image at high magnification (10000X). A,B,C: the PRF membranes from group A, group B, group C.*

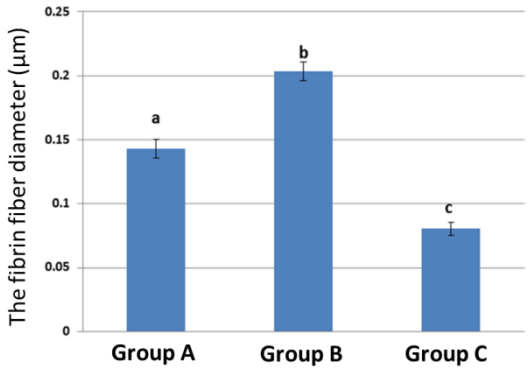

Figure4.*The changes of fibrin fiber diameter in the PRF membrane.a, b, c: statistically significant difference (P≤0.05).*

In recent studies, Takayama and colleagues have reported that platelet-rich fibrin membranes were not only used in the clinical tooth loss, but also showed a significant effect of reducing pain and swelling [12]. Many methods for isolating platelet-rich fibrin membranes such as chemical precipitation, chromatography, ultra filtration and other methods [11]. But in this

study, we applied the centrifugation method to collect the PRF membrane by directly inducing polymerization of fibrin fibers to form the fibrin network. In our study, the ratio of fibrin clot per total volume of the tube is about 50% higher than other studies [14].

CONCLUSION

The present study showed that centrifuge method could be efficiently applied to collect the PRF membrane which could be considered as the potential biomaterial for healing treatment in regenerative surgical procedures.

Acknowledgements

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

[1] M. Y. Bai, C. W. Wang, J. Y. Wang, M. F. Lin, W. P. Chan, ”Three-dimensional structure and cytokine distribution of platelet-rich fibrin,”*CLINICS*, vol. 72(2), pp. 116-124, Feb 2017.

[2] E. Borie, D. G. Oliví, I. A. Orsi, K. Garlet, B. Weber, V. Beltrán, ”Platelet-rich fibrin application in dentistry: a literature review,”*International journal of clinical and experimental medicine*, vol. 8(5), pp. 7922-7929, May 15 2015.

[3] Y. C. Chang, K. C. Wu, J. H. Zhao, “Clinical application of platelet-rich fibrin as the sole grafting material in periodontal intrabony defects,”*Journal of Dental Sciences*, vol. 6(3), pp. 181-188, Sep 2011.

[4] R. Civinini, A. Macera, L. Nistri, B. Redl, M. Innocenti, “The use of autologous blood-derived growth factors in bone regeneration,” *Clinical Cases in Mineral and Bone Metabolism*, vol. 8(1), pp. 25-31, Jan-Apr 2011.

[5] C. B. Desai, U. R. Mahindra, Y. K. Kini, M. K. Bakshi, "Use of platelet-rich fibrin over skin wounds: Modified secondary intention healing," *Journal of Cutaneous and aesthetic Surgery*, vol. 6(1), pp. 35-37, Jan 2013.

[6] E. D. M. Dohan, "How to optimize the preparation of leukocyte- and platelet-rich fibrin (L-PRF, Choukroun's technique) clots and membranes: introducing the PRF Box," *Oral Surgery, oral medicine, oral pathology, oral radiology, and endodontics*, vol. 110(3), pp. 275-278, Sep 2010.

[7] E. D. M. Dohan, T. Bielecki, R. Jimbo, G. Barbé, C. M. Del, F. Inchingolo, "Do the fibrin architecture and leukocyte content influence the growth factor release of platelet concentrates? An evidencebased answer comparing a pure platelet-rich plasma (P-PRP) gel and a leukocyte- and platelet-rich fibrin (L-PRF)," *Current Pharmaceutical Biotechnology*, vol. 13(7), pp. 1145-1152, Jun 2012.

[8] E. D. M. Dohan, T. Bielecki, A. Mishra, P. Borzini, F. Inchingolo, G. Sammartino, "In search of a consensus terminology in the field of platelet concentrates for surgical use: platelet-rich plasma (PRP), plateletrich fibrin (PRF), fibrin gel polymerization and leukocytes," *Current Pharmaceutical Biotechnology*, vol. 13(7), pp. 1131-1137, Jun 2012.

[9] E. D. M. Dohan, C. M. Del, A. Diss, J. Mouhyi, J. B. Charrier, "Three dimensional architecture and cell composition of a Choukroun'splateletrich fibrin clot and membrane," *Journal of Periodontol*, vol. 81(4), pp. 546-555, Apr 2010.

[10] K. Hotwani and K. Sharma, "Platelet rich fibrin - a novel acumen into regenerative endodontic therapy," *Restorative Dentistry Endodontics*, vol. 39(1), pp. 1-6, Jan 2014.

[11] A. E. Ismail, "Purification of fibrinogen from human plasma," Chemical &Biomolecular Engineering Theses, Dissertations, & Student Research. The Graduate College at the University of Nebraska, Lincoln, Nebraska.

[12] T. Kanayama, T. Sigetomi, H. Sato, M. Yokoi, "Crestal approach sinus floor elevation in atrophic posterior maxilla using only platelet rich fibrin as grafting material: A computed tomography evaluation of 2 cases," *Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology*," vol. 26(4), pp. 519-525, Oct 2014.

[13] R. Landesberg, M. Roy, R. S. Glickman, "Quantification of growth factor levels using a simplified method of platelet-rich plasma gel preparation," *Journal of Oral and Maxillofac Surgery*," vol. 58(3), pp. 297-300, Mar 2000.

[14] N. R. Pinto, A. Pereda, P. Jiménez, M. D. Corso, B. S. Kang, H. L. Wang, M. Quirynen, "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," Part 2: macroscopic, photonic microscopy and Scanning Electron Microscopy analysis of 4 kinds of L-PRF clots and membranes, vol. 2(2), pp. 141-154, 2014.

[15] S. R. Yajamanya, A. Chatterjee, C. N. Babu,and D. Karunanithi, "Fibrin network pattern changes of platelet-rich fibrin in young versus old age group of individuals: A cell block cytology study," J Indian SocPeriodontol, vol. 20(2), pp. 151-156, 2016.