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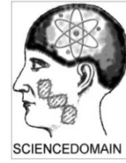

A New Centrifugation Method for the Improvement of Platelet-rich Fibrin Products: A Preliminary Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author MT designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author HÖ managed the literature searches. Author ZK helped with analyses of the study performed the microscopy analysis. Authors SA and EÖ managed the experimental process. Author MA helped with the statistical analyses. Authors MA and EF helped with the writing manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Nowadays, there are many articles about Platelet Rich Plasma/Platelet Rich Fibrin families. A novel platelet-rich product called titanium prepared platelet-rich fibrin (T-PRF) has stronger and thicker fibrin than that of the classic glass tube prepared platelet-rich fibrin. Strong fibrin structure is important to extend the time for resorption of fibrin *in-vivo*, and increase the

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release time of growth factors.
Objective: In this preliminary study of a new centrifugation method, we aimed to change the direction of fibrin formation during the platelet aggregation, and make T-PRF much denser and more resistant. According to our hypothesis, it can make it possible to use in guided bone, and guided tissue regeneration more successfully.
Methods: Blood samples of 10 healthy male volunteers were collected, and four 10ml blood samples, one for each of four groups, were transferred to a Ti tube from each volunteer. The first group was centrifuged for a 20-minute period clockwise (T-PRF group), and the other groups were centrifuged for a total of 20 minutes with two-minute (2min MT-PRF group), five-minute (5 min MT-PRF group), and ten-minute (10min MT-PRF group) periods clockwise and counter-clockwise.
Results: By hematoxylin and eosin stain, the 10min MT-PRF group showed a better-organized network with continuous integrity compared to the other groups. With the immunofluorescent staining, fibrin seemed thicker and better organized in the 10 min MT-PRF group. SEM examination showed more complex and denser fibrin clusters in the 10 min MT-PRF group than the other groups.
Conclusion: This pilot study defines 10 min MT-PRF as a new autogenous product with superior fibrin network. Our results showed that, fibrin formation was made more organised and denser with 2-way direction centrifugation.

Keywords: Tissue engineering; biomaterial(s); centrifugation; scanning electron microscopy (SEM); wound healing; blood; histomorphometry.

1. INTRODUCTION

When developed in 2001 by Chouckroun [1], the leukocyte and platelet-rich fibrin (L-PRF) moved itself to the forefront of other blood products, as it was obtained as a result of natural clotting, it was completely autogenous, and no foreign anticoagulant or clotting agent was used [2].

L-PRF is also a natural fibrin matrix that consists of leukocytes, proteins like vitronectin, fibronectin, bone morphogenetic proteins (BMP), cytokines, and growth factors, and it activates the vascular system and angiogenesis, releases growth factors like platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF) that are involved in tissue healing [3-8]. Due to L-PRF's natural fibrin framework properties, growth factors can keep their activity in fibrin mesh that allows for their progressive release over time (7–11 days) and stimulate tissue regeneration effectively [7].

In recent years, L-PRF has begun to be widely used for soft and hard tissue healing [9-16]. It has been reported in studies on healing of soft tissue that employing PRF alone as a soft tissue graft has been very successful, and it has considerable advantages compared to other non-autogenous materials or autologous subepithelial connective tissue grafting [9-16]. Nevertheless, many studies have shown that the L-PRF resorption period is 7–11 days in vivo in humans, which is successful in soft-tissue healing; however, its success with guided bone regeneration (GBR) and guided tissue regeneration (GTR) techniques in bone healing remains unclear. Moreover, silica in the glass tube is required for the formation of platelet activation and the formation of fibrin in L-PRF. Some physicians [17] worry about a possible health hazard with glass-evacuated blood collection tubes with silica activators. O'Connell [17] described the unavoidable silica contact.

New products (titanium prepared platelet-rich fibrin (T-PRF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF)) have been developed in recent years to improve L-PRF and to get rid of the limitations. The achievements of these blood concentrations that are fully autogenous have been evaluated in studies [18-23]. Despite this, there are still a number of question marks. The first basic question to be asked is what purpose we can use these second generation platelet-rich products for:

1. For soft tissue healing?
2. As a biological material in addition to the graft material used in the hard tissue healing, for providing to faster healing rates and enhanced tissue regeneration?
3. As a biological barrier membrane in the hard tissue healing?

4. As a scaffold to the stem cells, growth factors, or other biological materials in applications?
5. As a hard tissue graft material alone? Or for all of these goals?

We believe that the more advanced newly developed products are required for the improved L-PRF beside the need for further qualified clinical and experimental studies in order to answer these important problems.

Titanium has noncorrosive and hemocompatibility properties. These are key properties for biomaterials that come into contact with the blood [24-27]. In our study group's initial trials, we observed that titanium-induced platelet activation similar to glass tubes, and the clot produced in the titanium tubes was clinically identical compared to glass tubes. The fibrin structure of T-PRF seemed to have been woven more tightly and thicker than classic L-PRF. We also established that the fibrin carpet formed with titanium had a firmer network structure. Strong fibrin structure is important to extend the time for resorption of fibrin and increase the release time of growth factors [18,20,23]. T-PRF is also used to avoid any short- and/or long-term negative effects of dry glass or glass-coated plastic tubes and to eliminate the concerns regarding silica.

In this pilot study, we aimed to modify T-PRF and make this autogenous product much denser and more resistant and therefore to extend the resolved period of fibrin. We invented a centrifuge which can also reverse the tube filled with blood after a certain time to produce T-PRF. According to our hypothesis, when this is achieved, it can make it possible to use in GBR and GTR more successfully.

2. MATERIALS AND METHODS

Blood samples were collected from 10 healthy male volunteers (age range of 23 to 37 years) at the GATA Haydarpasa Hospital (Istanbul, Turkey). None of the participants had systemic diseases, and none were smokers. The participants had not taken medications within 2 weeks that could interfere with coagulation. The patients who were selected for the study were informed about the study schedule with all details and signed informed consent, and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. The study protocol was approved by the Institutional Committee of Ethics in Dental Research of the Faculty of Dentistry at Cumhuriyet University (Sivas, Turkey).

A blood sample of each volunteer was drawn from the antecubital vein of the subject's right or left arm in four attempts (a total of 40 ml blood, 10 ml for each of the four groups, was collected from patients with 10 ml injectors). 10 ml blood was transferred to grade IV titanium tube for each group. The blood was quickly collected, and the titanium tubes were immediately centrifuged with a specific table centrifuge at room temperature. The first group was centrifuged at 2700 RPM for a 20-minute period clockwise (T-PRF group), and the second, third, and fourth groups were centrifuged at 2700 RPM for two-minute (2 min MT-PRF group), five-minute (5min MT-PRF group), and ten-minute (10 min MT-PRF group) periods clockwise and counter-clockwise (MT-PRF groups were centrifuged rotating one clockwise and one counter-clockwise in 2-minute, 5-minute, and 10-minute periods for a total of 20 minutes). After centrifugation, the T-PRF clots were removed from the tubes using sterile tweezers, separated from the RBC base using scissors, and placed on sterile woven gauze. In each series, the clots were left on sterile woven gauze to release their serum slowly over 20 minutes. Half of each clot, after sectioning the clot into two parts along its long axis, was processed for SEM evaluation and fixed in 2.5% glutaraldehyde. The other half of each clot was sectioned for a second time into two parts along its long axis; one part was used for the fluorescence microscopy analysis, and the other part was used for the light microscopy analysis.

2.1 Histological Procedures for Light Microscopy

The T-PRF clots were dehydrated in increasing gradients of alcohol (70%, 95%, and 100%) and placed in toluene before including the paraffin. After complete dehydration, the clot was 0.5 mm thick. For each T-PRF membrane, a series of 20 successive 7-mm sections was created along the long axis of the clot (i.e., 140 mm of the clot thickness could be analysed in a longitudinal and reliable manner). These sections were stained with hematoxylin and eosin.

2.2 Histological Procedures for the Fluorescence Microscopy

The frozen sections of the T-PRF clot specimens were used for the direct immunofluorescent

method (DIF) (Fibrinogen, FITC (Ventana, Catalogue Number: 760-2685). An automatic immunohistochemical staining device, Ventana BenchMark XT (Ventana Medical Systems, Inc., Arizona, USA), was used, and the immunofluorescent staining was performed using a human fibrinogen antibody. The sections were evaluated with a fluorescent microscope (Laika DM 2500, Leica and Zeiss Co., Cambridge, England).

2.3 Histological Procedures for SEM Evaluation

The morphologic evaluation of the T-PRF clots was conducted with a scanning electron microscope (LEO 440, Leica and Zeiss Co., Cambridge, England). The clots were fixed in 2.5% glutaraldehyde for 1 hour and treated for desiccation. The specimens were sputter coated with 20 nm of gold/palladium and subsequently examined in a scanning electron microscope. Photographs were taken at 20 kV using a magnification from 51 to 5000.

2.4 Histomorphometric Analysis

Histometric analysis was performed by an examiner blinded with respect to the treatment rendered. Pictures from three different areas of each section (0,348 mm²) were taken with an attached camera photofluorescence microscope in groups with an original magnification ×400 (Laika DM 2500, Leica and Zeiss Co., Cambridge, England). The digital images were saved on a computer. The Clemex Vision-Lite 5.0 software (Clemex Technologies, Quebec, Canada) was used for the histomorphometric analysis.

2.5 Statistical Analysis

Statistical computations were carried out using software programme (SPSS 14.0, SPSS Inc., Chicago, IL, USA). After evaluation of normality with Kolmogorov-Smirnov test, data were analysed with Mann-Whitney U test for pairwise comparisons. P values less than 0.05 were considered statistically significant.

3. RESULTS

3.1 Light Microscopy Study

With the hematoxylin and eosin staining, it was observed that fibrin clot was formed in each of the four group samples.

3.1.1 2 min MT-PRF group

In terms of fibrin structure with the hematoxylin and eosin stain of the same samples, the 2 min MT-PRF samples showed a flabbier fibrin texture and erythrocyte islets in the fibrin were observed (Fig. 1).

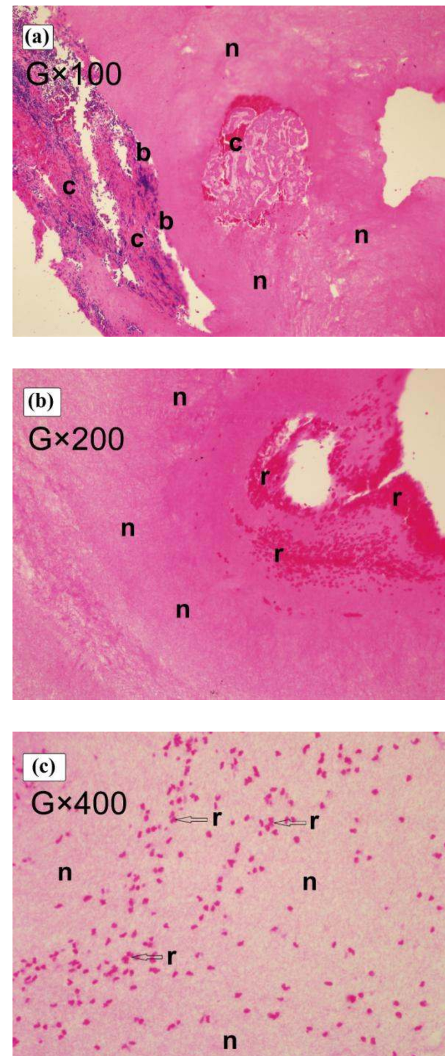

Fig. 1. The light microscopy analysis of the 2 min MT-PRF clot. Hematoxylin and eosin staining

(a) *Flabbier fibrin network (light pink (n)) and cellular components (c) were detectable. The border (b) between the cellular structures (c), and fibrin network (n).*

(b) *Fibrin texture (n) and erythrocyte islets (r) in the fibrin were observed.*

(c) *Fibrin network (n) with continuous integrity. Red blood cells (r) embedded in the entire fibrin network. The magnifications (G) are indicated in each panel.*

3.1.2 5 min MT-PRF group

The 5-minute group samples showed well-organised structure with hematoxylin and eosin stain. The leukocyte nuclei were stained dark blue with hematoxylin. Small erythrocyte islets were observed in the fibrin network (Fig. 2).

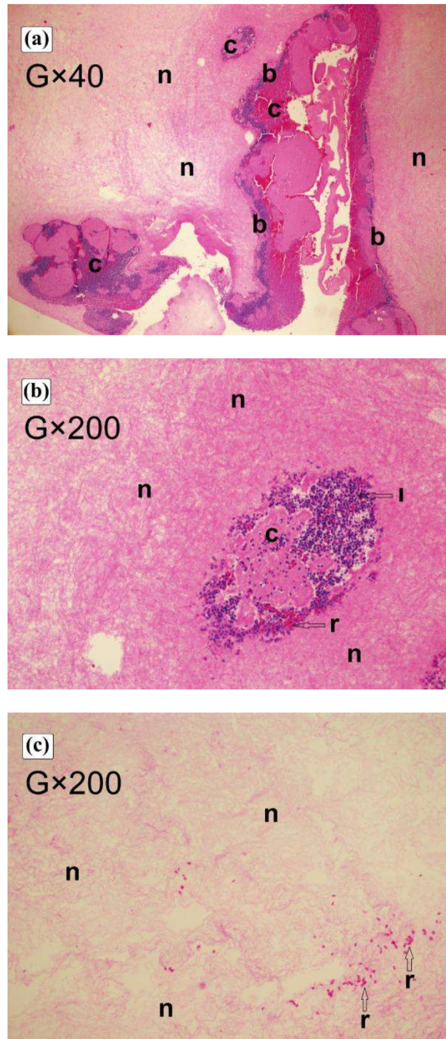

Fig. 2. The light microscopy analysis of the 5 min MT-PRF clot. Hematoxylin and eosin staining

(a) *The mature fibrin network (light pink (n)) and cellular components (c) were easy detectable. The border (b) between the cellular structures (c), and fibrin network (n).*
(b) *Cellular structures (c) embedded in the fibrin mesh (n). The leukocyte nuclei (l) were stained dark blue with hematoxylin. Erythrocyte islets (r) in the cellular area (c) were observed.*
(c) *Well-organised, wooly fibrin (n). Red blood cells (r) embedded in the fibrin network. The magnifications (G) are indicated in each panel.*

3.1.3 10 min MT-PRF group

The 10-minute MT-PRF group samples showed well-organised fibrin mesh structure by hematoxylin and eosin stain. The 10min MT-PRF group showed the best-organised network with continuous integrity compared to the other groups. The RBCs and cytoplasm of the leukocytes were easily detectable, as they were a darker pink. The leukocyte nuclei were stained dark blue with hematoxylin (Fig. 3).

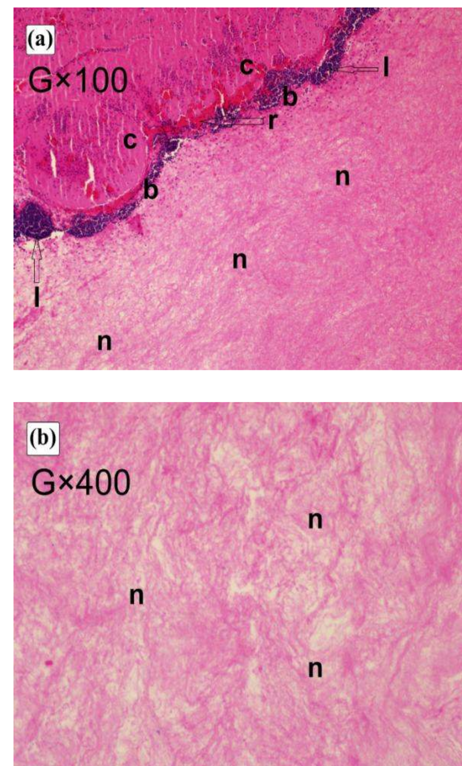

Fig. 3. The light microscopy analysis of the 10 min MT-PRF clot. Hematoxylin and eosin staining

(a) *Mature, and dense fibrin network (light pink (n)) and cellular components (c) were well separated from each other. Thick border (b) between the cellular structures (c), and fibrin network (n). Leukocyte (l), and erythrocyte (r) islets in the cellular area (c) were observed.*
(b) *Well-organised, mature, wooly, and often textured fibrin (n). The magnifications (G) are indicated in each panel.*

3.1.4 T-PRF group

The T-PRF group showed a well-organised fibrin mesh structure with continuous integrity by hematoxylin and eosin stain (Fig. 4).

3.2 Fluorescence Microscopy Study

3.2.1 2 min MT-PRF group

The fibrin networks were not observed to be mature and dense in this group. Wide dark areas were observed in a messy and fragmented-looking fibrin structure. The areas rich in collagen in the form of islands draw attention (Fig. 5a).

3.2.2 5 min MT-PRF group

With the immunofluorescent staining, the fibrin network appeared mature and dense in this group. A mature and dense T-PRF fibrin network was present with big gaps between the fibrin meshwork (Fig. 5b).

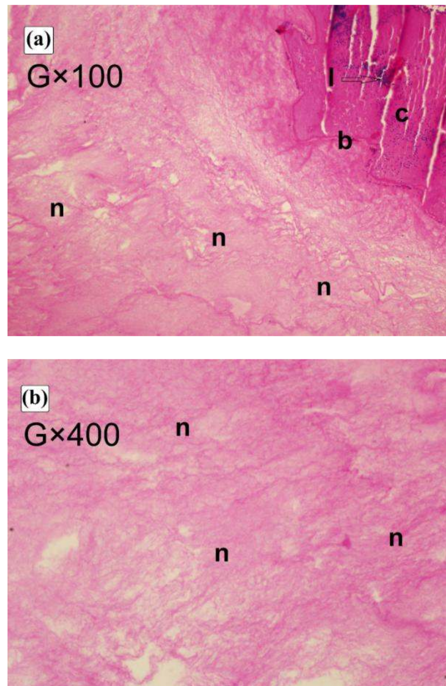

Fig. 4. The light microscopy analysis of the T-PRF clot. Hematoxylin and eosin staining
(a) *Mature fibrin network (light pink (n)) and cellular components (c) were well separated from each other. Border (b) between the cellular structures (c), and fibrin network (n) was easily detectable. Leukocytes (l), in the cellular area (c) were observed.*
(b) *Well-organised, and mature, fibrin (n). The magnifications (G) are indicated in each panel.*

3.2.3 10 min MT-PRF group

With the immunofluorescent staining, the 10 min MT-PRF group's fibrin seemed thicker and best organised, compared to the other groups. The fibrin network with small gaps appeared mature and dense (Fig. 5c).

3.2.4 T-PRF group

With immunofluorescent staining, the T-PRF group's fibrin network appeared mature and dense. However, it can be said that the fibrin network was not as dense as the 10 min MT-PRF group's fibrin and gave a more loosely woven carpet impression (Fig. 5d).

3.3 SEM Evaluation

In all groups, the RBCs were located either outside of the matrix or adhered to the matrix at the border of the red area and yellow clot. A comparison of the images between all groups revealed that fibrin seemed thick and best organised in the 10min MT-PRF group. The T-PRF and 5min MT-PRF groups had also a well-organised fibrin mesh structure. SEM examination showed that more complex and denser fibrin clusters occurred in the 10 min MT-PRF group than the other groups. In the 2 min MT-PRF group, cells were located in the fibrin matrix (Fig. 6).

3.4 Histomorphometric Evaluation

Histometric analysis from fluorescence microscopy images of 10min MT-PRF group and T-PRF group examples showed that 10min MT-PRF group fibrin network covers a statistically significant larger area than T-PRF groups network (Fig. 7).

4. DISCUSSION

In this study, we evaluated different centrifuge methods to create more powerful fibrin structure. We have shown that the structure of T-PRF fibrin, the second generation of a blood concentrate, can be made denser with the help of bidirectional rotatable centrifugation, without adding any substances and without destroying its autogenous structure. We have also shown that the densest fibrin network could be achieved by rotating 10 minutes clockwise and 10 minutes counter-clockwise (10 min MT-PRF group), and fibrin mesh separates from the cells well and platelets aggregate and also erythrocytes decompose in full.

Many studies have shown the positive regenerative effects of L-PRF in soft and bone tissues [7-16,28-31]. But it was still unknown which one was the most effective technique and how the releasing period of the cells increased in the fibrin scaffold depending on varying

centrifugation time and speed or type of tube. Recently, Ghanaati et al. [19] developed a new fibrin structure (A-PRF) with a decrease in centrifugation speed and an increase in centrifugation time. They stated that specific cell types were scattered differentially depending on the centrifugal force. Ghanaati et al. [19] used A-PRF as a biological material in addition to the graft materials used in hard tissue to promote faster healing.

We believe with this study that the innovations of platelet rich products which previously focussed on the duration and speed of centrifugation may be directed to the direction of centrifugation. Although A-PRF [19], and CGF [21-22] products associated with duration and speed of centrifugation are claimed to offer additional advantages due to excess growth factors, they have not been shown to be advantageous in terms of their natural fibrin matrix structure. This means that the use of these products alone is not predictable in terms of osteoconductive way. They have been mostly proposed to be employed in hard tissue healing as a biological material together with graft materials.

In spite of this, we observed in our previous studies that when we used T-PRF with non-autogenous graft materials, the magic was ruined, resorption time of the graft material was not shortened, and the inflammatory response in the system caused by the foreign substances resorption in the tissues was just as when we placed the graft [23]. Our previous studies have shown that T-PRF with a conventional centrifugation method can also be used alone successfully for hard tissue healing [18-23]. According to our hypothesis, modified T-PRF (10min MT-PRF) that offers a much denser natural matrix will be more successful than T-PRF in terms of the osteoconductive effect. We also believe that the healing agents and the growth factors it invites to the region due to its osteoinductive properties are as important as the growth factors in the contents of the natural matrix that remains longer in the body.

In this pilot study, we have tried to modify T-PRF and make this autogenous product much denser and more resistant. The aim of this study was to increase the effectiveness of T-PRF by means of extending the period of resorption. In this study, it was observed that fibrin clot was formed in each of the four group samples. The T-PRF, 5 min MT-PRF, and 10 min MT-PRF groups showed well-organised structure, but when compared between groups, the best results were seen in the 10 min MT-PRF group.

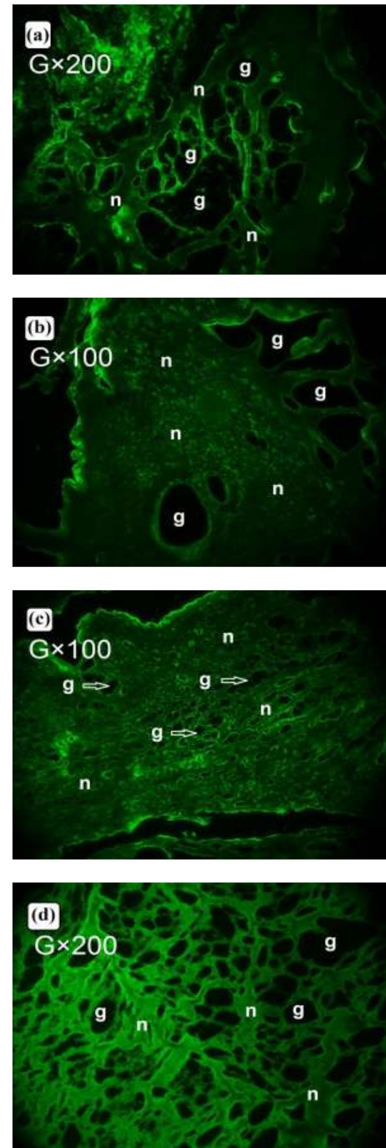

Fig. 5. The immunofluorescent microscopy analysis of the 2min MT-PRF group, 5min MT-PRF group, 10min MT-PRF group, and T-PRF group fibrin network structure

(a) *The 2min MT-PRF group. Non-uniform fibrin network (n) with big gaps (g).*
(b) *The 5min MT-PRF group. Mature and dense fibrin network with large gaps (g) between the fibrin meshwork.* ***(c)*** *The 10min MT-PRF group. Well-organised, often textured fibrin carpet (n) with small gaps (g)*
(d) *The T-PRF group. Mature, and uniform fibrin network (n) with small gaps (g). The magnifications (G) are indicated in each panel.*

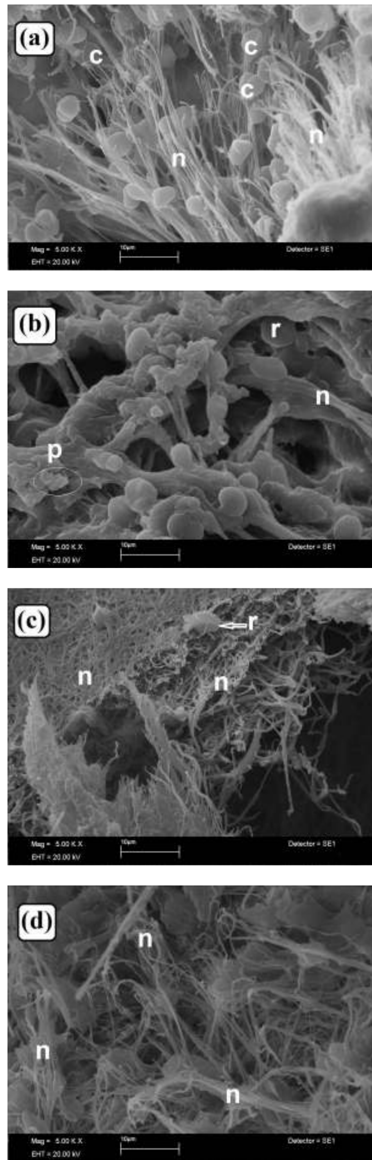

Fig. 6. Scanning electron micrographs of the 2min MT-PRF group, 5min MT-PRF group, 10min MT-PRF group, and T-PRF group fibrin network structures

(a) *The 2min MT-PRF group. Loosely woven fibrin network (n). Cellular components (c) were enmeshed in the fibrin matrix. (SEM; original magnification × 5000).*
(b) *The 5min MT-PRF group. Organized and mature fibrin structure tended to form a network (n). The red blood cells (r) trapped within the fibrin matrix. Platelets were often enmeshed in the fibrin network but sometimes appeared as aggregates (p (white circle)) (SEM; original magnification × 5000).*
(c) *The 10min MT-PRF group. Well-organised, mature, wooly, and often textured fibrin carpet (n). The red blood cell (r) in the outer surface of the thick fibrin carpet. (SEM; original magnification × 5000).*
(d) *The T-PRF group. Well-organised fibrin mesh structure. (SEM; original magnification × 5000).*

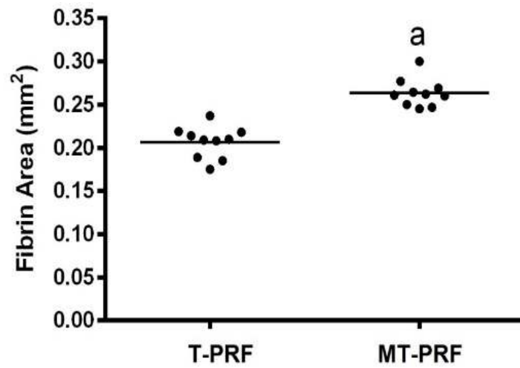

Fig. 7. Results of the histometry measurements (mm²)
[a]$p < 0.05$ vs. T-PRF group

The complex structure of the fibrin matrix and its density are key parameters of any platelet concentrate [3,32]. Growth factors and cytokines can be released by a suitable biologically carrier such as a fibrin matrix that supports their release in the wound environment. The greater the density of the fibrin clot, the more it can be a stronger biological healing matrix by supporting cell migration and growth factor release [3,32]. When the centrifuge runs, most platelets are activated in a few minutes after contacting the tube walls, which initiates the coagulation cascade, and as a result, the clot is formed. This clot consists of a three-dimensional fibrin scaffold that contains activated platelets, growth factors, cytokines, and other blood cells [32]. In this study, we evaluated different centrifuge methods to create more powerful fibrin structure. The 10 min MT-PRF group showed a better-organised network with continuous integrity compared to the other groups. This result showed that, for the fibrin matrix maturation, the centrifugal method was also important, like centrifugation time. Fibrin formation was made more organised and denser with 2-way direction centrifugation.

5. CONCLUSION

This pilot study defines modified T-PRF (10 min MT-PRF group) as an autogenous platelet and leukocyte-rich fibrin product with a superior fibrin network to T-PRF. More research on clinical parameters such as resorption time, clinical success, and many other aspects of this new product are required.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Choukroun J, Adda F, Schoeffler C, Vervelle A. An opportunity in perio-implantology: The PRF (in French). Implantodontie. 2001;42:55-62.
2. 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 in Biotechnology. 2009;27:158-167.
3. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Sammartino G, Charrier JB. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in human cell cultures: Growth factor release and contradictory results. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics. 2010;110:418-21.
4. Dohan Ehrenfest DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics. 2006;101:37-44.
5. Dohan Ehrenfest DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics. 2006;101:45-50.
6. Dohan Ehrenfest DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics. 2006;101:51-55.
7. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, Dohan AJJ, Mouhyi J, Dohan Ehrenfest DM. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing, Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics. 2006;101:56-60.
8. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, Dohan AJJ, Mouhyi J, Dohan Ehrenfest DM. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics. 2006;101:299-303.
9. Del Corso M, Sammartino G, Dohan Ehrenfest DM. Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: A 6-month study. Journal of Periodontology. 2009; 80:1694-7.
10. Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD, Dohan Ehrenfest DM. 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. Journal of Periodontology. 2009;80:2056-64.
11. Lee EH, Kim JY, Kweon HY, Jo YY, Min SK, Park YW, Choi JY, Kim SG. A combination graft of low- molecular-weight silk fibroin with Choukroun platelet-rich fibrin for rabbit calvarial defect. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics. 2010;109:33-8.
12. Pradeep AR, Sharma A. Autologous platelet rich fibrin in the treatment of mandibular degree II furcation defects: A Randomized Clinical Trial. Journal of Periodontology. 2011;82:1396-403.
13. Simonpieri A, Choukroun J, Del Corso M, Sammartino G, Dohan Ehrenfest DM. Simultaneous sinus-lift and implantation using microthreaded implants and leukocyte- and platelet-rich fibrin as sole grafting material: A six-year experience. Implant Dentistry. 2011;20:2-12.
14. Ezirganli S, Polat S, Barıs E, Tatar I, Celik HH. Comparative investigation of the effects of different materials used with a titanium barrier on new bone formation. Clinical Oral Implants Research. 2013;24:312-9.
15. Ozdemir H, Ezirganli S, Kara MI, Mihmanli A, Baris E. Effects of platelet rich fibrin alone used with rigid titanium barrier. Arch Oral Biol. 2013;58:537-44.
16. Tunali M, Ozdemir H, Arabaci T, Gurbuzer B, Pikdoken ML, Firatli E. Clinical evaluation of autologous platelet-rich fibrin

(L-PRF) in the treatment of multiple adjacent gingival recession defects: A 12-month study. Int J Periodontics Restorative Dent. 2015;35:105-14.
17. O'Connell SM. Safety issues associated with platelet-rich fibrin method. Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics. 2007;103: 587(author reply)587-93.
18. Tunali M, Ozdemir H, Kucukodacı Z, Akman S, Yaprak E, Firatli E. *In vivo* evaluation of titanium-prepared platelet-rich fibrin (T-PRF): A new platelet concentrate. British Journal of Oral and Maxillofacial Surgery. 2013;51:438-43.
19. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, Landes C, Sader R, Kirkpatrick C, Choukroun J. Advanced platelet-rich fibrin: A new concept for cell-based tissue engineering by means of inflammatory cells. J Oral Implantol. 2014;40:679-89.
20. Tunali M, Ozdemir H, Kucukodacı Z, Akman S, Yaprak E, Toker H, Firatli E. A novel platelet concentrate: Titanium-prepared platelet-rich fibrin. BioMed Research International; 2014. Article ID 209548.
21. Kim TH, Kim SH, Sándor GK, Kim YD. Comparison of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factor (CGF) in rabbit-skull defect healing Arch Oral Biol. 2014;59:550-8.
22. Kim JM, Sohn DS, Bae MS, Moon JW, Lee JH, Park IS. Flapless transcrestal sinus augmentation using hydrodynamic piezoelectric internal sinus elevation with autologous concentrated growth factors alone, Implant Dent. 2014;23:168-74.
23. Tunali M, Ozdemir H, Kucukodacı Z, Ezirganli S, Baris E, Akman S, Atay A, Firatli E. A novel platelet concentrate for guided bone regeneration: Titanium Prepared Platelet-Rich Fibrin (T-PRF). Gulhane Med J. 2015;57:102-6.
24. Breme HJ, Biehl V, Helsen JA. Metals and implants, In Helsen JA, Breme HJ, (eds.), Metals as Biomaterials. Chichester: Wiley 1998;37-72.
25. Park JB. Metallic biomaterials, In Bronzino JD, (ed.). The biomedical engineering handbook. Boca Raton: CRC Press. 1995;537–51.
26. Cranin AN, Lemons JE. Dental implantation, In Ratner BD, Hoffman AS, Schoen FJ, et al. (eds.) Biomaterials science-an introduction to materials in medicine. San Diego: Elsevier Academic Press. 2004;555-72.
27. Takemoto S, Yamamoto T, Tsuru K, Hayakawa S, Osaka A, Takashima S. Platelet adhesion on titanium oxide gels: Effect of surface oxidation. Biomaterials 2004;25:3485-92.
28. Marenzi G, Riccitiello F, Tia M, di Lauro A, Sammartino G. Influence of leukocyte- and platelet-rich fibrin (L-PRF) in the healing of simple postextraction sockets: A split-mouth study. BioMed Research International; 2014. Article ID 369273.
29. Kazemi D, Fakhrjou A, Dizaji VM, Alishahi MK. Effect of autologous platelet rich fibrin on the healing of experimental articular cartilage defects of the knee in an animal model. BioMed Research International; 2014. Article ID 486436.
30. Troedhan A, Wainwright M, Kurrek A, Schlichting I. Biomechanical stability of dental implants in augmented maxillary sites: results of a randomized clinical study with four different biomaterials and PRF and a biological view on guided bone regeneration. BioMed Research International; 2015. Article ID 850340.
31. Oncu E, Alaaddinoglu EE. The effect of platelet-rich fibrin on implant stability. Int J Oral Maxillofac Implants. 2015;30:578–82.
32. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Sammartino G, Charrier JB. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. Journal of Periodontolology. 2010;81:546-55.

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