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Research Article

SEM Evaluation of Surface Morphology and Cell Structure in PRF Membrane Prepared in Clockwise and Counter Clockwise Centrifugation Method

P Rajababu, G Jagadish Reddy, Atchuta Abhinav*, Ch. Tejaswini Reddy, Palle Ajay Reddy and G Pradeep Raj

Department of Periodontics, Army College of Dental Sciences, India

***Corresponding Author:** Atchuta Abhinav, Department of Periodontics, Army College of Dental Sciences, India.

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Abstract

Background: Platelet-rich fibrin is an autologous blood concentrate which is a rich source of various growth factors which help in periodontal regeneration. Variation in the centrifugation method may enhance or improve the fibrin network arrangement in the Platelet-rich fibrin. The aim of the present study is to compare the surface morphology of fibrin network and cell structure in PRF membrane prepared in clockwise, counter clockwise and combination of both clockwise and counterclockwise centrifugation method through SEM analysis.

Material and Method: 5 Five healthy dental students (male and female) were recruited from Department of Periodontics, Kamineni institute of Dental Sciences. 15 ml of blood was collected from each participant and prepared platelet-rich fibrin through various centrifugation methods and the obtained PRF's were sent to SEM analysis.

Results: PRF prepared in clockwise direction showed 20% each of highly organized homogenized fibrin network, organized fibrin network and woven fibrin network and 40% of organized and mature fibrin network. PRF prepared in counter clockwise direction showed 20% each of highly organized homogenized fibrin network, organized and mature fibrin network and woven fibrin network and 40% of organized fibrin network. PRF prepared in combination of two directions showed 20% of highly organized homogenized fibrin network and 80% of organized and mature fibrin network.

Conclusion: Within the limitations of this study, the PRF prepared in both clockwise and counterclockwise centrifugation technique showed highly irregular surface and more organized and mature fibrin network with numerous platelets and leucocytes entrapped within it when compared with other techniques. However, further long term clinical trials are required to know the effects of centrifugation direction on fibrin formation and its therapeutic benefits in periodontal regeneration.

Keywords: *Blood; Centrifugation, Platelet-Rich Fibrin; Growth Factors; Wound Healing; Scanning Electron Microscopy*

Introduction

Blood is a mixture of plasma, various types of cells and platelets (biologically-active cellular fragments). Thrombocytes have an important role in coagulation and prevent excessive blood loss in venous injuries. They contain numerous cytokines and growth factors that influence bony regeneration and maturation of soft tissue [1]. In the past two decades, increased understanding of the physiological roles of platelets in wound healing and after tissue injury has led to the idea of using platelets as therapeutic tools [1]. Platelet-rich fibrin (PRF) is an autologous concentration of human platelets, was found to be more favourable for periodontal regeneration. PRF is a slowly and

naturally polymerizing fibrin matrix in which growth factors are present [2]. Platelet-rich fibrin (PRF) was first developed in France by Choukroun., *et al.* in 2001 as an autologous biomaterial that contains leucocytes and platelet-rich fibrin (PRF) [3]. Unlike other platelet-rich products, the technique requires neither anticoagulant nor bovine thrombin (or any other gelling agent) [4-7]. Platelets contain high quantities of key growth factors, such as PDGF-AB (platelet- derived growth factor AB), TGFb-1 (transforming growth factor b-1) and VEGF (vascular endothelial growth factor), which are able to stimulate cell proliferation, matrix remodelling and angiogenesis. The use of these growth factors to enhance healing is an interesting option [8]. The PRF membrane being autologous in nature and cost effective compared to any other membrane offers significant advantages over commercially available membranes.

A new centrifugation method for the improvement of platelet rich fibrin products stated that the new prf formed with both clockwise and counter clockwise directions show a superior fibrin network than normal prf which was prepared in counter clockwise rotation [9].

The scanning electron microscope (SEM) is a research instrument and it's effective development was begun in 1953 [10,11]. Scanning Electron Microscopy (SEM) is a powerful method for the investigation of surface structures. This technique provides a large depth of field, which means, the area of the sample that can be viewed in focus at the same time is actually quite large. SEM also has the advantage that the range of magnification is relatively wide allowing the investigator to easily focus-in on an area of interest, on a specimen that was initially scanned at a lower magnification [12].

The basic steps involved in SEM sample preparation include surface cleaning, stabilizing the sample with a fixative, rinsing, dehydrating, drying, mounting the specimen on a metal holder and coating the sample with a layer of a material that is electrically conductive [13].

Aim of the Study

The aim of the present study is to compare the surface morphology and cell structure in PRF membrane prepared in clockwise, counter clockwise and combination of both clockwise and counterclockwise centrifugation method through SEM analysis.

Materials and Methods

The study was conducted in Department of Periodontics, Kamineni Institute of Dental Sciences, Narketpally, Nalgonda and a written informed consent was obtained from each study participant and the study protocol was approved by Institutional Ethical Committee with allotment number KIDS/IEC/PERIO/2019/001. SEM analysis was carried out in Department of Physics, Osmania university, Hyderabad. The study has been registered in Clinical trials Registry, India with CTRI No: CTRI/2019/02/017624.

Inclusion criteria

Five healthy dental students (male and female) were recruited from Department of Periodontics, Kamineni institute of Dental Sciences.

Exclusion criteria

1. Any systemic illness
2. Periodontitis
3. Smokers
4. Intake of any medication which interfere coagulation.

The participants who were selected for the study were informed about the study schedule with all details and informed consent was obtained.

Sample collection

15 ml of blood was collected from each study participant by venipuncture from the antecubital vein. The collected blood was divided into 5 ml each into three glass tubes and were processed for PRF preparation using three different protocols:

1. 5 ml blood within the glass tube was placed in the clockwise centrifuge with 3000 rpm for 10 minutes.
2. 5 ml blood within the glass tube was placed in the anti-clockwise centrifuge with 3000 rpm for 10 minutes.
3. 5 ml blood within the glass tube was placed initially in clockwise centrifuge with 3000 rpm for 5 minutes and later placed in anti-clockwise centrifuge with 3000 rpm for 5 minutes.

The obtained PRF's from all three different protocols from all the study participants were collected in sample collection containers with 10% formalin as a fixative and preservative. All the samples obtained were sent to the Department of Physics, Osmania University on the same day for SEM processing.

SEM processing

Initially a small size of sample necessary for processing and imaging was cut and is subjected to air drying as PRF is a wet sample. Later the sample is loaded on to an aluminium sample stub with a double sided carbon adhesive tape (Figure 1a). These sample loaded aluminium stubs were placed in the sputter coater chamber for Gold sputtering for 10 minutes (Figure 1b). Later these Gold sputter coated samples which were loaded on the aluminium stubs were placed inside the SEM chamber for imaging (Figure 1c and 1d).

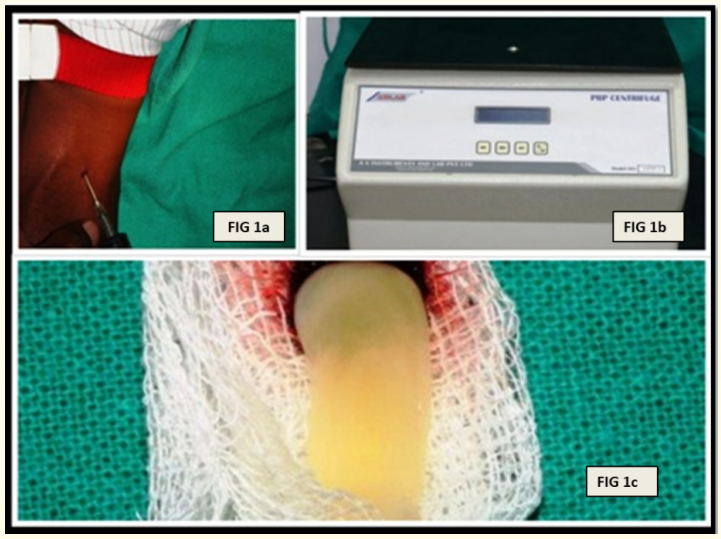

Figure 1: *1a: Venipuncture (Collection of blood). 1b: Clockwise centrifugation machine. 1c: Platelet rich fibrin.*

Statistical analysis

Data were analyzed using statistical software. The association between the variables of the data was tested using Pearson chi square test with mean and p value ($p \leq 0.05$ is statistically significant).

Results

Platelet-rich fibrin was evaluated under SEM to visualize its surface morphology, fibrin network arrangement and the cell types that were trapped within it. Under different magnifications of SEM, the surface morphology of PRFs prepared in three different techniques appeared to be highly irregular. The fibrin network arrangement varied in different PRF preparation techniques. PRF prepared in combination of both clockwise and anticlockwise centrifugation technique showed mostly Organized and mature fibrin network (Figure 2a and 2b) whereas PRF prepared in anticlockwise centrifugation technique mostly presented an Organized Fibrin Network (Figure 2c and 2d) and PRF prepared in clockwise centrifugation technique presented combination of Organized and mature fibrin network and Highly organized (homogenized) fibrin network (Figure 2e and 2f). PRF on SEM evaluation showed some spherical structures with an irregular surface which can possibly be identified as leucocytes. There are also presence of dense clusters of activated platelets resting on a mature fibrin background can be identified. The number of platelets and leucocytes identified varies according to the technique, field of vision and the surface of PRF which is being examined. Some PRF samples may present with numerous platelets and leucocytes while other may have scanty or few platelets and leucocytes.

Figure 2: *2a: PRF samples loaded on to aluminium stubs. 2b: Gold sputter coating machine. 2c: Scanning electron microscope. 2d: PRF samples after gold sputtering placed into the SEM chamber.*

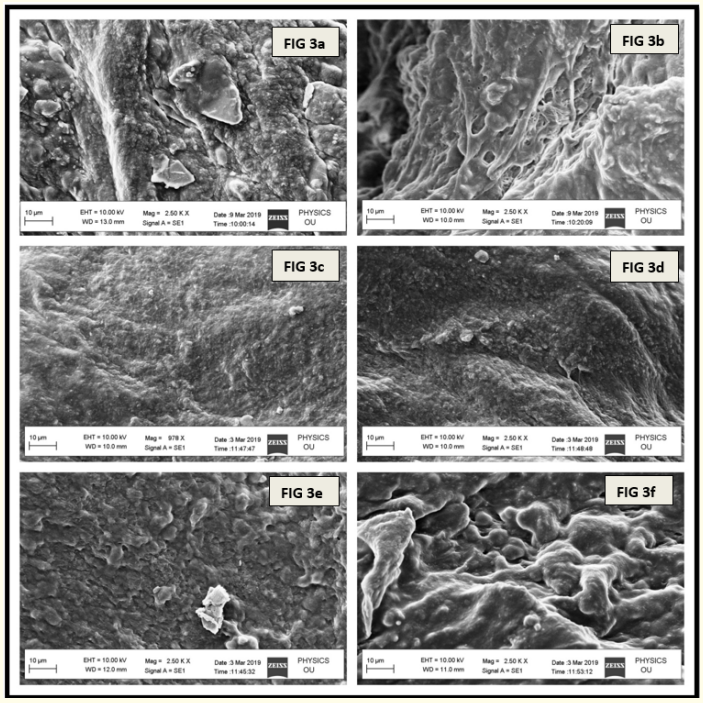

Figure 3: *3a and 3b: PRF prepared in clockwise and counterclockwise centrifugation methods showing irregular surface with organized and mature fibrin network and numerous platelets and leucocytes. 3c and 3d: PRF prepared in counterclockwise centrifugation method showing irregular surface with organized fibrin network with few platelets and leucocytes. 3e and 3f: PRF prepared in clockwise centrifugation method showing irregular surface with organized and mature fibrin network with few platelets and leucocytes.*

This result showed that, for the fibrin matrix maturation, the centrifugal method was also important, like centrifugation time. Fibrin formation was made more organized and denser with 2-way direction centrifugation.

PRF biology

PRF has the characteristic of polymerizing naturally and slowly during centrifugation. The thrombin concentrations are almost physiologic which is crucial to determine the 3-dimensional organization of a fibrin network. During gelling, based on different centrifugation methods, the fibrin fibrillae can be assembled between them in different biochemical architectures: condensed tetramolecular or bilateral junctions and connected trimolecular or equilateral junctions [14]. Bilateral junctions are constituted with strong thrombin concentrations and allow the thickening of fibrin polymers; this leads to the constitution of a rigid network, not very favorable to cytokine enmeshment and cellular migration. On the other hand, weak thrombin concentrations imply a very significant percentage of equilateral junctions. These connected junctions allow the establishment of a fine and flexible fibrin network able to support cytokines enmeshment and cellular migration. Moreover, this 3-dimensional organization will give great elasticity to the fibrin matrix. The density of fibrin matrix is therefore greatly influenced by centrifugation method.

The complex structure of the fibrin matrix and its density are key parameters of any platelet concentrate [15,16]. 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. By the two way centrifugation method the density of the fibrin network can be improved.

Centrifugation Method	Fibrin Network							
	Highly Organized Homogenized Fibrin Networ		Organized and Mature Fibrin Network		Organized Fibrin Network		Woven Fibrin Network	
	Count	% within centrifugation Method	Count	% within centrifugation Method	Count	% within centrifugation Method	Count	% within centrifugation Method
Anticlockwise	1	20%	1	20%	2	40%	1	20%
Clockwise	1	20%	2	40%	1	20%	1	20%
Anticlockwise + Clockwise	1	20%	4	80%	0	0%	0	0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.750[a]	6	.452
Likelihood Ratio	6.959	6	.325
Linear-by-Linear Association	2.301	1	.129
N of Valid Cases	15		
p ≤ 0.05 is statistically significant			

Table 1: *Centrifugation method and fibrin network cross tabulation.*

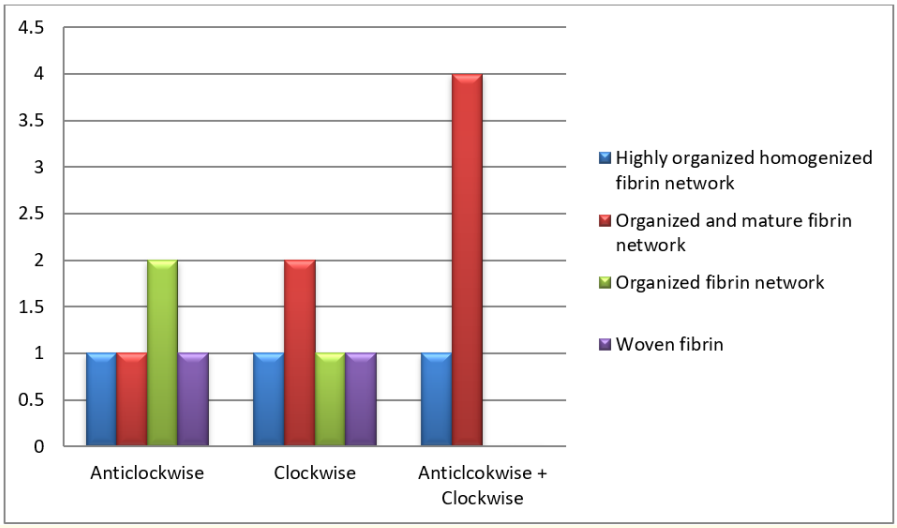

Figure 1: *Centrifugation method and fibrin network cross tabulation.*

Discussion

The rationale of using the PRF membrane lies in the fact that the platelets have granules that act as reservoirs of many Growth Factors that play a crucial role in hard and soft tissue repair mechanism. These include platelet derived GFs, transforming Growth Factor -β, vascular endothelial Growth Factor and epidermal Growth Factor [17]. PRF is considered as a fibrin biomaterial. Its molecular structure with low thrombin concentration is an optimal matrix for migration of endothelial cells and fibroblasts. It permits a rapid angiogenesis and an easier remodeling of fibrin in a more resistant connective tissue [7].

Choukroun and his associates were amongst the pioneers for using PRF protocol in oral and maxillofacial surgery to improve bone healing in implant dentistry. Several studies have examined the effectiveness of PRF in intrabony defects and Grade II furcation defects and have found positive clinical and radiographic outcomes [18-21].

In this study, Platelet rich fibrin was evaluated under SEM to visualize its surface morphology, fibrin network arrangement and the cell types that are trapped within it in order to better understand its biologic properties. The PRF under SEM evaluation showed a highly irregular surface with numerous to scanty platelets and leucocytes entrapped within it. The type of centrifugation technique showed little difference in fibrin network arrangement of PRF. A comparison of SEM images of PRF's prepared in different centrifugation techniques revealed that PRF prepared in both clockwise and anticlockwise technique showed more organized and mature fibrin network and also numerous platelets when compared to other techniques whereas leucocytes found were scanty. The arrangement of fibrin network can be correlated to the centrifugation speed and direction but the platelet and leukocyte entrapment within the fibrin background cannot be justified according to the centrifugation technique. The complete blood picture may vary from one individual to another and hence it may show variations in the cellular arrangement in the PRF. As presence of platelets and leukocytes in PRF are beneficial for wound healing process, as they can be a rich source of Growth Factors, it is always suggested to preserve a small RBC layer at the PRF clot end to collect as many platelets and leukocytes as possible [16].

Conclusion

The ease of preparation and cost effectiveness of PRF membrane offers a huge advantage over other commercially available membranes. The PRF prepared in both clockwise and counterclockwise centrifugation technique showed highly irregular and more organized and mature fibrin network with numerous platelets entrapped within it when compared with other techniques. As PRF is considered to be a rich source of Growth factors, it can widely be used in periodontal regeneration. However furthermore *in-vitro* studies are to be conducted to support the properties of PRF. We believe with this study that the innovations of platelet rich products which previously focused on the duration and speed of centrifugation may be directed to the direction of centrifugation.

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