COMPARISON OF TENSILE STRENGTH OF L-PRF AND A-PRF USING LSCC TECHNIQUE WITH COMMERCIALLY AVAILABLE GTR MEMBRANE

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ABSTRACT

Platelet Rich Fibrin (PRF) has gained tremendous awareness in recent years to be used in wide variety of medical field for soft tissue regeneration including regenerative periodontal therapy. PRF also termed as L-PRF is an autologous autogenous biomaterial consisting of growth factors and cytokines which provide the optimal environment for tissue regeneration and wound healing. A fairly new concept to dentistry is the low-speed centrifugation concept (LSCC). According to this theory, leukocyte infiltration into the red blood cell fraction can be reduced by lowering the relevant centrifugation force (RCF) or g force and increasing spin time. Hence the PRF clot produced will have a looser fibrin structure gradually enhancing the release of leukocytes and growth factors. This PRF type is termed as advanced PRF (A-PRF). The impact of tensile strength of A-PRF in comparison with L-PRF and commercially available GTR membrane is evaluated in this study. Blood samples were collected from a total of 40 volunteers in 10 cc plain vacuum glass tubes without anticoagulant. Blood samples were immediately centrifuged in GYROZEN 406 Centrifuge machine (Gyrozen Co. Ltd. Korea) following the centrifugal protocol for A-PRF (n=20) and L-PRF (n=20). Within the limitation of the study we concluded that Lyoplant® showed superior tensile strength than L-PRF which in turn showed superior tensile strength than A-PRF. Based on the available information, it can be said that PRF is a healing biomaterial that can be used in regenerative surgical operations to speed healing, however its suitability as a barrier membrane is questionable given its inferior mechanical qualities.

Keywords: *L-PRF, A-PRF, LSCC, Tensile Strength and Mechanical Properties.*

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INTRODUCTION

Platelet Rich Fibrin (PRF) is a fibrin matrix consisting of platelet, leukocytes and cytokines, growth factors and other cells which are slowly released gradually over time from its membrane.[1] As a result of the presence of leukocytes, the PRF is also termed as L-PRF (leukocyte Platelet Rich Fibrin).[2, 3] The WBC (White Blood Cells) present in PRF have important part in healing of wound and prevention of infection due to the presence of macrophages and neutrophils which phagocytize the debris, necrotic tissue and microbes.[4] Furthermore the fibrin matrix of PRF is rich in growth factors.[5] This slow and sustained release of leukocytes and growth factors in PRF help boost up immune system and the platelets help achieve hemostasis.[6]Being very economical and autologous in nature in comparison with other commercial membranes, PRF is highly beneficial.

A fairly new concept to dentistry is the low-speed centrifugation concept (LSCC).[7] This idea suggests that by lowering the relevant centrifugation force (RCF) or g force and increasing the spin time the infiltration of leukocyte into the red blood cell fraction is minimized.[8-12] Early vascularization, accelerated soft tissue growth, in-

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creased cytokines, and the release of bone morphogenic proteins (BMPs) [13] are the outcomes, which cause the production of new bone.[14] The resultant advance platelet rich fibrin (A- PRF) using LSCC concept, revolutionized the use of PRF in periodontal regeneration by inducing bone repair by stimulating the cell multiplication in periosteum during normal healing of wound.[15] This foundation of growth factors (GF) entrapped within the A-PRF membrane makes it the third generation. Many tissue engineered GTR membranes are available and successfully rendering good services as scaffold for tissue regeneration. However, the most important culture comes from the patient himself, when tissue engineering techniques are generally measured with platelet concentrates rich with leukocyte.[16]

In addition to scientific proof of an acceptable membrane's effectiveness, clinical handling and mechanical qualities are also crucial considerations. The eventual outcome of GTR may be impacted by the membrane's mechanical properties. The clinical outcome after healing may depend on the material's tensile strength.[17] Additionally, a scaffold's robust mechanical properties offer a better support for regeneration. [18] Hence mechanical properties should be considered while developing a GTR membrane for its clinical use. To the best of our knowledge, there hasn't been a comparison of mechanical properties in various PRF types made with LSCC in Pakistan. Additionally, not enough evidence based research has been done on their mechanical characteristics as a barrier membrane as to which membrane is more mechanically strong. In addition, because the fibrin membrane degrades gradually at the implanted site in vivo, it is poorly understood how mechanical properties change during degradation process. In the present investigation, we expected that the mechanical characteristics of the fibrin membrane are comparable to those of the GTR membrane that is commercially available. We compared these characteristics among L-PRF, A-PRF, and a biodegradable bovine membrane control "Lyoplant®" To the best of our knowledge, this pioneering study reports the tensile strength and strain of the membranes originated by two different PRF derivate (L-PRF, and A-PRF) in Pakistan. The hypothesis being that LSCC has an impact on mechanical properties of PRF.

MATERIAL AND METHODS

This Experimental study was conducted in the department of Science of Dental Material Department, Hamdard University from 4th February 2019 to 4th November 2019 after approval of the institutional review board. The study lasted for 9 months after receiving the approval from Ethical committee. The total sample size was 19 which rounded up to 20. The sampling was done by purposive sampling method. The sample size was calculated by Openepi software version 3. Mean of group 1 was 0.01923(±0.00676) and group 2 was 0.02885(±0.01297) with confidence interval 95% and power of the study was 80%. Blood samples were taken from 20 healthy, disease-free, and non-smoking male's volunteers aged between 32 and 50. The volunteers had no known history of any systemic disease or medication dependency. Any patient taking medication of any sort was excluded from the study. Blood sample were collected in 10 cc plain vacuum glass tubes without anticoagulant. Blood samples were immediately centrifuged in GYROZEN 406 Centrifuge machine (Gyrozen Co. Ltd. Korea).

The L-PRF was fabricated by centrifuging the freshly drawn blood at 708 RCF equivalents to 2330 RPM for 10 minutes Table 1. The RCF was values were adjusted on the automated screen selector and the corresponding RPM values were predefined. Upon completion of centrifuge process, three distinct phases were visible in the test tube Fig 2. The bottom most phase was RBC which was not required for the study and hence was discarded. The middle portion was the yellow colored PRF which was separated from RBC and was our study material Fig 3. The topmost plasma poor platelet liquid was removed.

The A-PRF was fabricated by centrifuging the freshly drawn blood at 208 RCF equivalents to 1260 RPM for 13 minutes Table 1. Upon completion of centrifugation process the sample were handled with the same methodology as was done for L-PRF sample group.

Upon separation, the samples were squeezed to make a membrane in a specially designed squeezing instrument Fig 4. Digital caliper was used to measure the thickness of each membrane at three different locations which are start, middle and end. The membranes hence produced were ensured to have a uniform 0.6 mm thickness, 3.2 mm width, and 10 mm length. Any sample lacking uniformity of thickness was excluded from the study. The membrane hence formed was subjected for micro-tensile test using Universal Testing Machine; (MODEL 4301, INSTRON Lloyd Instrument Ltd. Fareham, UK) Fig 5. The UTS utilizes the speed of 1mm per minute with 1000 N load at room temperature 27 +/- 3. The results of tensile strength and strain were recorded.

The GTR membrane used as control was LYOPLANT® (Lot # 220194, Aesculap® Aesculap AG 78532 Tuttlingen, Germany). It is a bovine collagen resorbable duramater substitute made from lyophilized bovine pericardium. This membrane has a uniform thickness of 0.6 mm. However for the purpose of this study it was cut into strips of 10mm length and 3.2 mm width. After obtaining the desired size the GTR membranes were hydrated in PBS for 30 minutes and were subjected to

UTM micro tensile testing. The results of all three test groups were compared using SPSS Version 23 to analyze the data. To determine the mean difference between the experimental groups, one sample t-test and post-hoc Tukey's test were employed. A p value of ≤ 0.05 was considered significant.

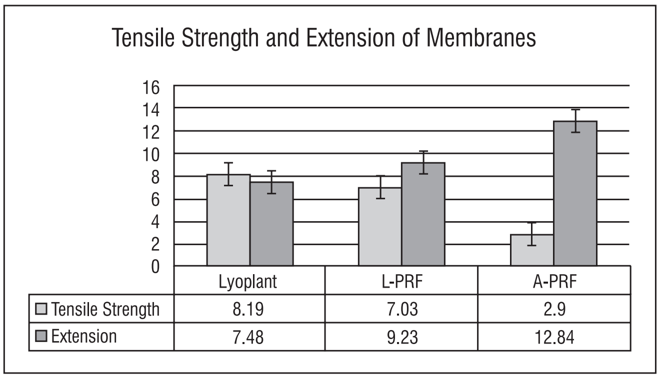
The tensile strength of the membrane was calculated using following formula:

$$\textit{Tensile strength (MPa)} = \frac{\textit{Load X 10}}{\textit{Thickness (mm) X Width (mm)}}$$

Where the thickness of the membrane is 0.60 mm and the width of the membrane is 3.2mm. 1.92

RESULTS

The Mean values of tensile strength on for Lyoplant® was 8.19 (± 1.55), L-PRF 7.03 (±1.38) and A-PRF 0.35(±0.04) and the extension percent for Lyoplant® was 7.48 (± 0.65), L-PRF 9.23 (± 0.79) and A-PRF 12.84 (± 1.22) as shown in Table 2.



*The tensile strength of L-PRF was significantly better than A-PRF

Fig 1: Tensile strength and Extension of the experimental groups

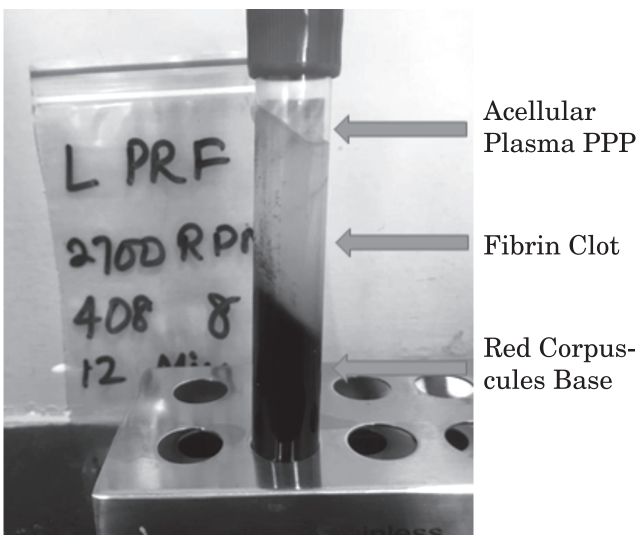


Fig 2: Centrifuge Tube of L-PRF with three distinct Phases

Fig 3: Fibrin Clot after removal of RBC and PPP.

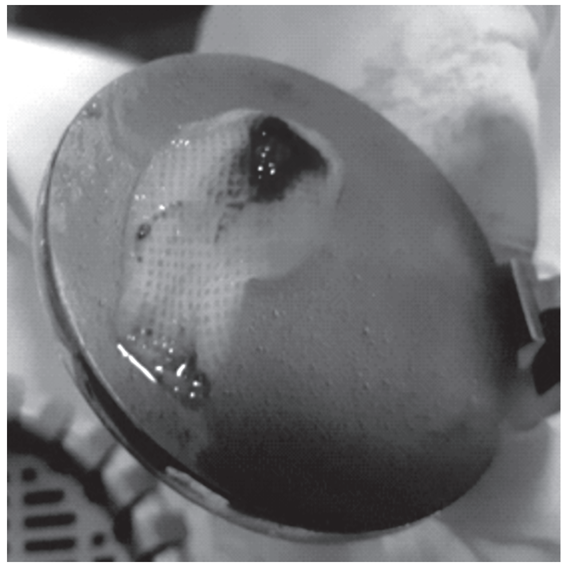


Fig 4: Squeezed membrane in a specially designed squeezing instrument

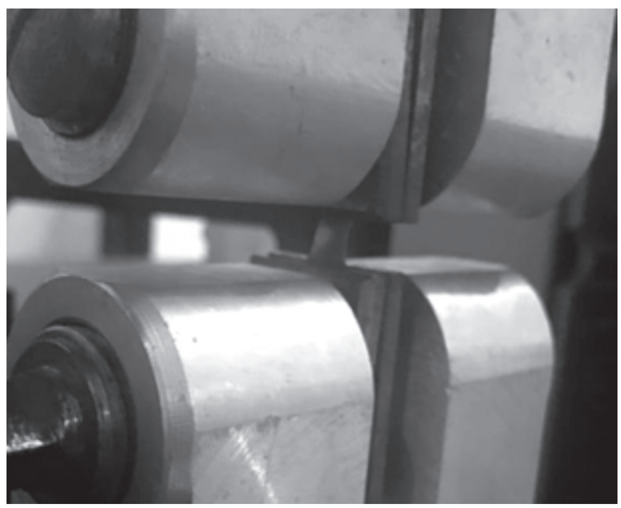


Fig 5: Tensile strength testing of PRF membrane

TABLE 1: CENTRIFUGAL PROCESS OF PRF PREPARATION

Sample (n=40)	RCF	RPM	Time in minutes
L-PRF (n=20)	708	2330	10
A-PRF (n=20)	208	1260	13

TABLE 2: TENSILE STRENGTH AND EXTENSION WITH RESPECT TO EXPERIMENTAL GROUPS AND CONTROL

Sample	Load (Kg)	Extension (%)	Tensile Strength (Mpa)	p Value
Lyoplant®	1.57	7.48	8.19	<0.001*
L PRF	1.35	9.23	7.03	<0.001*
A PRF	0.4	12.84	2.08	<0.001*

A one sample t-test with a ≤ 0.05 p-value was used.

DISCUSSION

The main reason for using PRF is that it harbors a reservoir of growth factors in its platelet alpha granules.[19] These growth factors are essential for the repair of both soft and hard tissues. Platelet-derived growth factors, transforming growth factor beta, vascular endothelial growth factor, and epidermal growth factor are the growth factors that are significant. They allow for quick angiogenesis and simpler fibrin rebuilding in more resilient connective tissue [20] and bone in order to improve healing in implant dentistry, Choukran[21] et al were among the first to use the PRF protocol in oral and maxillofacial surgery. The low speed centrifugation concepts (LSCC) have been created in an effort to enhance the therapeutic results.[22] The fundamental idea of LSCC is an all-encompassing method for obtaining blood concentrates from 710 x g to 44 x g under various physical circumstances, from solid to liquid, for various uses in medicine and dentistry.[21,22] In this study the two types of solid PRF membranes i.e. L-PRF and A-PRF were centrifuged based on newer LSCC concept with RCF 708 being high and RCF 208 being intermediate as described by Choukran et al. [1] and Ravi & Santhanakrishnan. [23] The scope of this study precluded us from comparing the mechanical characteristics of L-PRF and A-PRF with a control, commercially available GTR membrane, Lyoplant®. The results of this study show that L-PRF performed significantly better p<0.001when it comes to tensile strength as compared to A-PRF. The results of this study are in consistence with the findings of other researchers who narrate that as the RCF or g- force is decreased the tensile strength of PRF also decreases. Lee[18] *et al* concluded that varying speeds can modify tensile strength. It was discovered that by reducing the RCF and increasing spin time, the cell and the growth factors concentration in A-PRF (Advanced-PRF) increased threefold to eightfold showed enhanced presence of neutrophilic granulocytes in the distal part of the clot contributing the induction of new bone formation.[22]

The tensile strength of PRF was compare with bovine collagen Duramater, the Lyoplant® used as control. It was discovered that the tensile strength of Lyoplant® was significantly greater than that of L-PRF p <0.001. This result was expected; since the PRF is an autologous membrane without any additives to improve the mechanical properties other than the inherent resistance provided by the fibrin matrix[25,26].

It was observed that the tensile strength of all samples increased with increasing density, since the L-PRF was denser as compared to A-PRF. Lam et al[27] refers it to the influence of platelets on the clot and noted a favorable impact, which in addition to fibrin was source of adhesive characteristics. This finding was consistent with the finding of Karimi et al 2022.[9] The tensile strength values of this study closely match Lee[14] *et al* and Khorshidi[24] *et al*. but differ from other researchers; this is due to different centrifuge apparatus, variation in timing protocol and Instron apparatus used. The significant difference between L-PRF and A-PRF in our result suggests that as the RCF is increased, the tensile strength of PRF biomaterial improves. The results of our study however are in contradiction with that of Ravi and Santhanakrishnan[1], Simões-Pedro[26] *et al* and Pascoal[28] *et al*. In our study the average age of volunteers was 42 years whereas Simões-Pedro[26] had average age of 23 years, which could have been one of the contributing factors.

PRF has a higher tendency to thrust the growth factors in initial healing phase thereby decreasing post-surgical inflammation. It was interesting to find a notable increase in the elasticity as the RCF is decreased. The results of this study show that A PRF strained much more than L-PRF which in turn strained more than Lyoplant®. Although A PRF had significantly low tensile strength but it strained the most among all the test groups. The membranes hence produced with LSCC are more resilient to fracture when applied in multiple situations.

CONCLUSION

The PRF membrane has a significant advantage over other commercially available membranes due to its cost-effectiveness, simplicity of manufacture, and ability to be both autogenous and autologous. The initial results from the evaluation of its mechanical characteristics and its comparison to other collagen membranes do not indicate favorable outcomes. Its applicability in GTR operations might be constrained by its lack of stiffness and quicker deterioration. Although PRF can be used in regenerative surgical techniques to speed up healing, its suitability as a barrier membrane is uncertain given its subpar mechanical qualities.

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