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PREPARATION, CHARACTERIZATION, AND COMPARISON OF RHEOLOGICAL PROPERTIES OF L-PRF, A-PRF AND ALB-PRF - AN IN-VITRO PILOT STUDY

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ABSTRACT Platelet-rich fibrin (PRF) has gained clinical significance in recent years for regenerative procedures. Here, we compared the preparation, characterization and rheological properties of Platelet Rich Fibrin (PRF), Advanced-Platelet Rich Fibrin (A- PRF) and Albumin-Platelet Rich Fibrin (Alb-PRF). Blood samples from 10 volunteers were taken. For PRF preparation, 10mL of whole blood will be centrifuged as per standard protocol. For A-PRF preparation, blood samples will be centrifuged at 1,500rpm for 14 minutes. For Alb-PRF preparation, blood samples will be centrifuged at 700g for 8 minutes and then heated at 75°C for 10 minutes to create denatured albumin. Following heating, the albumin gel will be allowed to cool to room temperature for 10 minutes. Then, liquid PRF will be mixed with the cooled albumin gel to form Alb-PRF. The different generations of PRF are assessed for the various properties. Highest degradability rate and the least swelling ratio was seen in L-PRF while A-PRF and Alb-PRF has shown no significant differences. There was no significant difference between A-PRF and Alb-PRF compared to L-PRF in regenerative periodontal therapy and both have sustained release of growth factors.

KEYWORDS : Platelet rich fibrin, albumin gel, periodontal regeneration, rheological properties

INTRODUCTION

An autologous platelet derivative known as platelet concentrate has advanced significantly in recent years for use in several regeneration therapies.[1] Built on the concept that a blood supply is a requirement for tissue regeneration, the idea of using platelet concentrates was created with the goal of using human blood proteins as a reservoir of growth factors capable of maintaining angiogenesis and tissue ingrowth. The regenerative property of platelets was introduced in 1970's.[2] In 1997, Whitmann et al, introduced the use of platelet-rich plasma, which is the first platelet derivative in the field of oral surgical procedures as it has various advantages in regenerative procedures.[3] Various studies showed PRP has limited potential to stimulate bone regeneration as it releases growth factors quickly, just before the cell outgrowth from the surrounding tissue.[4,5] It has also been demonstrated that bovine thrombin which is used for PRP preparation may have toxic effects on the body cells.[6]

Later in 2001, Choukroun et al introduced platelet rich fibrin (PRF) which has brought a great revolution in the field of regenerative surgical procedures. The PRF introduced by Choukroun et al was rich in leukocytes, and hence it was also known as Leukocyte rich PRF (L-PRF). In last few years, the protocol for preparation has undergone various modifications which led to modified variations in PRF with different properties.[7]

The various modified PRF are Advanced PRF (A-PRF), Advanced plus PRF (A+PRF), Injectable PRF (I-PRF), Titanium PRF (T-PRF), Horizontal PRF (H-PRF) and Albumin PRF (Alb-PRF). These PRFs differs in the process of preparation such as the difference in centrifugation speed, centrifugation time, the angle of centrifugation or by the tube material.[6] A-PRF is a second-generation platelet concentrate in which the fibrin matrix is produced by lowering the centrifugation force which causes higher concentrates of growth factors. According to recent studies, unlike A-PRF, heating a liquid platelet-poor plasma (PPP) layer can extend heated albumin's resorption properties from 2 weeks to more than 4 months which lead to modification in the protocol and was known as Alb-PRF. PRF in the form of a membrane and platelet gel can be used in combination with bone grafts, as it promotes wound healing, bone growth and maturation, wound sealing, and hemostasis.[7] The present article aims to compare the process of preparation, characterization and the rheological properties such as degradability and swelling property of L-PRF, A-PRF and Albumin PRF (Alb-PRF).

MATERIALS AND METHODS

The study was designed to be an in-vitro observational study. This study has been approved by the Institutional Ethical Committee, Meenakshi Ammal Dental College, MAHER, Chennai (MADC/IEC-I/56/2022). This study was conducted in White Lab of Saveetha Dental College and Hospital, Chennai, over a period of 1 month from September to October of 2022.

The sample size was not estimated as it is a pilot study. The study sample consists of 9 volunteers who were systemically healthy, who were 20 and 25 years of age were included in the present study based on the inclusion and exclusion criteria. The inclusion criteria include systemically healthy person with 20–25 years of age, who are not on any drugs and non-smokers. The exclusion criteria include person with systemic diseases, smokers, pregnant and lactating women and those who have consumed antibiotics within the last 3 months.

PREPARATION OF L-PRF, A-PRF AND ALB-PRF

The blood samples from 9 volunteers were collected and the blood was processed for PRF clot preparation. For L-PRF preparation, 10 ml of whole blood were centrifuged as per standard protocol. (Fig.1a) For A-PRF preparation, blood samples were centrifuged at 1,500 rpm(230g) for 14 minutes. (Fig.1b) For Alb-PRF preparation, blood samples were centrifuged at 700g for 8 minutes and 2 ml of sample were collected in

syringe separately and heated at 75°C for 10 minutes to create denatured albumin. Later, heated albumin gel were allowed to cool to room temperature for 10 minutes; following which the remaining centrifuged PRF were mixed with the cooled albumin gel to form Alb-PRF. (Fig.1c)

Evaluation Of Swelling Ratio

The swelling ratio is defined as the fractional increase in the weight of the hydrogel due to water absorption. Firstly, the weight of the Eppendorf tubes were weighed in electronic micro-weighing scale without loading the membranes and the tubes were labelled. Later, the membranes were loaded to labelled tubes along with phosphate buffer solution and the Eppendorf tubes were then weighed. The samples were taken out of the phosphate buffer solution at different times and individually weighed. The weight of the tubes with the membrane were measured for alternative seven days to evaluate the swelling ratio. The swelling ratio percentage was evaluated with the following formula,

$$\text{Swelling ratio} = \frac{Ws - Wd}{Wd}$$

Where, Ws is weight of membrane after swelling
Wd is weight of dried membrane

Evaluation Of Degradation Test

The degradation tests of the prepared L-PRF, A-PRF and Alb-PRF were conducted by placing the PRF membranes in 10 ml of pH 7.4 phosphate buffer solution on an orbital shaker set at 50 rpm. All the three PRF were weighed on an electronic micro weighing scaling and then they were weighed separately at the start of the experiment followed by weighing the membranes separately for alternative seven days to evaluate the degradation profile. the degradation profiles were expressed as the accumulated weight loss of the membrane.

The degradation percentage of the PRF membranes was calculated using the following formula,

$$\%\ \text{degradation} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

Characterization Of Prf

The prepared samples of L-PRF, A-PRF and Alb-PRF were stored in phosphate buffer solution under refrigeration for five days. Later, it was dried at room temperature and analyzed. The characteristic analysis of the different generations of PRF were done by using Fourier Transform Infrared (FTIR) spectroscopy.

RESULTS

Evaulation Of Swelling Ratio

After seven days, the swelling ratio of L-PRF, A-PRF and Alb-PRF were compared. (Fig.2) It was found that the swelling ratio of PRF was the lowest followed by the swelling ratio of A- PRF and Alb-PRF. There were no significant changes observed in A-PRF and Alb-PRF.

Evaluation Of Degradation Test

The degradability of L-PRF, A-PRF and Alb-PRF were observed for seven days (Fig.3) . On evaluation, L-PRF was completely degraded by the fifth day while A-PRF and Alb-PRF remained stable without getting degraded till the seventh day. While comparing the A-PRF and Alb-PRF, there were no significant change in the degradation percentage.

Fourier Transforming Infrared (ftir) Spectrometric Analysis

The FTIR analysis was obtained from the three different PRF membranes to identify the changed bands among the three different membranes. L-PRF showed vibrations at 1639.16 and 1545.61 characteristic of amide I and amide II groups respectively. (Fig.4a) On analysis of A-PRF, slight change in the vibrations which was at 1636.26 and 1540.93 for amide I and amide II groups was observed respectively. (Fig.4b) While Alb-PRF showed vibrations which was at 1635.24 and 1465.63 for amide I and amide II groups respectively. (Fig.4c) There was a significant difference in the peak of the amide II group in Alb-PRF compared to L-PRF and A-PRF indicating the crosslinking of N-H bonds.

DISCUSSION

PRF has brought a revolution in periodontal tissue regeneration since its discovery due to its acceleration in hard tissue and soft tissue healing. It has a molecular structure with low thrombin concentration which is an optimal matrix for migration of endothelial cells and fibroblasts.[7] It permits rapid angiogenesis and an easier remodeling of fibrin in a more resistant connective tissue.[8] There are various studies that have evaluated the efficacy of PRF in regenerative procedures such as root coverage, intrabony defects, etc. As there are various protocol modifications that have been made with several advantages, it also important to know the characteristics and properties of different types of PRF. Here, this study is aimed at comparing the L-PRF, A-PRF and Alb-PRF.[6]

On examining the swelling ratio of all three PRF, it was found that the L-PRF had the least swelling ratio compared to other two PRF membranes. As swelling ratio is important to evaluate the property of controlled release of the growth factors, here we can find that swelling property is increased in A-PRF and Alb-PRF, hence there is controlled release of growth factors from the above-mentioned PRF. Similarly, by the degradation test, the slower the membrane resorbs there is increased time for the release of growth factors. The variation in centrifugation protocols which are used can influence the degradation properties and increase the rate of resorption.

Hence in our study, we can observe that the L-PRF loses it structural integrity and resorbs in five days which is shorter duration than the A-PRF and Alb-PRF showing that there is longer duration release of growth factors by these PRF. A study done by Sam et al in 2014[9] on properties of PRF showed that PRF membrane was comparable to other membranes in terms of maintain its physical property upto 6 days and then gets degraded to about 36% of its initial weight. Similarly, S Ravi et al in 2020 [10] compared the properties of L-PRF, A-PRF and T-PRF and stated that maximum amount of degradation was found in L-PRF which was about 85.75% followed by A-PRF about 84.18%.

A study done by Kawase et al in 2015 [11], applied heat treatment to standard PRF membranes through membrane compression to investigate the degradation properties of PRF which has shown improvement in the resorption properties of PRF membranes. One of its limitations was that the growth factor activity was greatly reduced during the denaturation process, as the heating process causes the cells to undergo apoptosis.[11]

In a study done by Mourao et al in 2015 [12], he proposed the utilization of the PPP layer as the heat-treated component with extended PRF-resorption properties, along with advantages such as membrane stability over time. Later, retention of cells from the buffy coat layer must be done, so that growth factors help to maintain the regenerative properties of PRF within the heated PPP layer.[12]

In a study done by Fujioka et al in 2021, they combined the liquid PRF along with albumin gel. By combining these, the authors had hypothesized that Alb- PRF would demonstrate viable cells capable of releasing growth factors over time while simultaneously improving the cellular activity of gingival fibroblasts. [13]

Using the various modified PRF membranes such as Albumin -PRF, Horizontal-PRF and Leukocyte-PRF, Gheno et al in 2021 experimented in vivo comparison of biocompatibility and stability in rodents in which they observed that the L-PRF and H-PRF had reduction in membrane site between 7 to 14 days and near complete resorption was seen by 21 days while Alb-PRF was still stable after 21 days, by which they had concluded that the membrane stability can be extended by heat treatment process.[14]

Based on the properties we assessed, our study revealed that A-PRF and Alb-PRF maintain the structural integrity compared to L-PRF which loses its integrity with faster degradation rate and low swelling ratio which was suggestive of shorter duration of release of growth factors. While the A-PRF and Alb-PRF had a longer release of growth factors with better resorption properties, Alb-PRF had peak in the amide group which shows the cross-linking properties which was observed through FTIR spectroscopy which also suggest that the crosslinking bond can decrease the rate of degradation and increase the time of membrane action in the site.

CONCLUSION

Platelet rich fibrin is a natural fibrin framework containing growth factors that may maintain their action for a comparatively longer time and efficiently promote tissue regeneration. For efficient tissue regeneration, sustained and long term release of growth factors plays

an important role. Among the various properties observed, it was evident that the structural integrity of the fibrin framework was maintained for the study period in A-PRF and Alb-PRF which is considered better than L-PRF. As there was no significant difference between A-PRF and Alb-PRF, both were considered more favourable compared to L-PRF in regenerative periodontal therapy. Future studies and clinical testing with longer duration and with increased sample size are required to further validate the properties of the L-PRF, A-PRF and Alb-PRF.

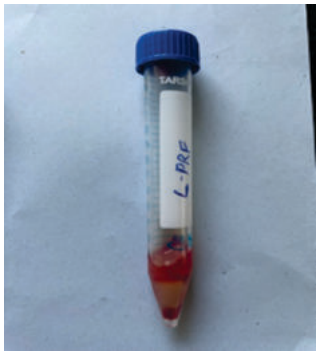

Fig.1a. L-PRF

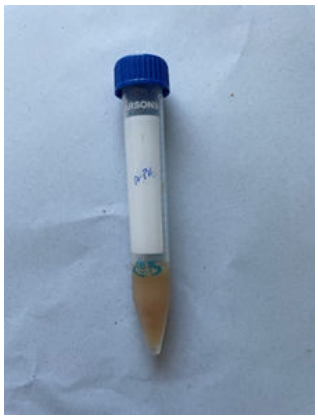

Fig.1b. A-PRF

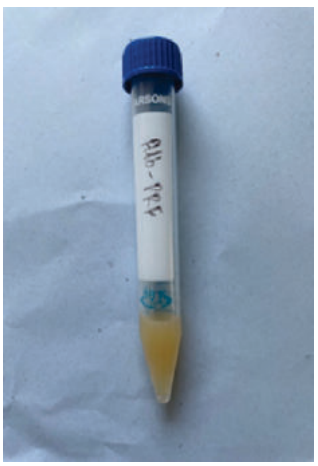

Fig.1c. Alb-PRF

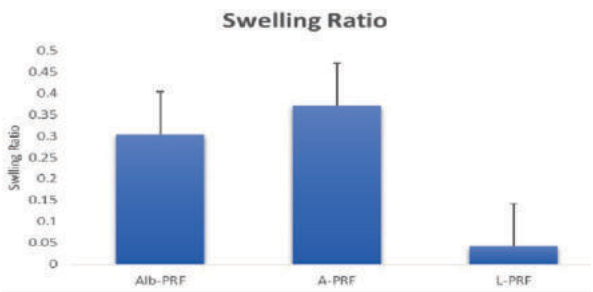

Fig.2

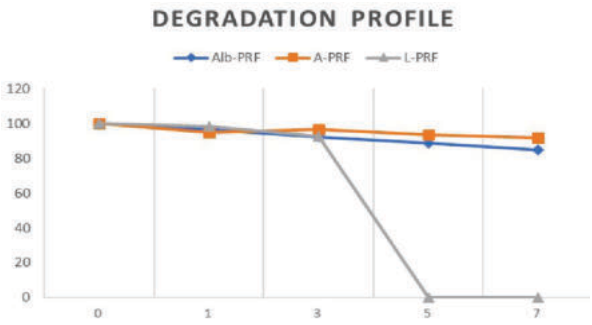

Fig.3

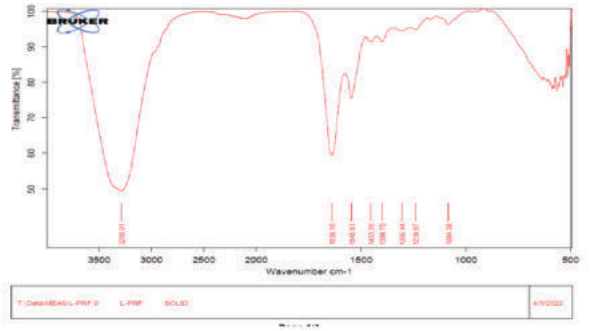

Fig.4a. L-PRF FTIR

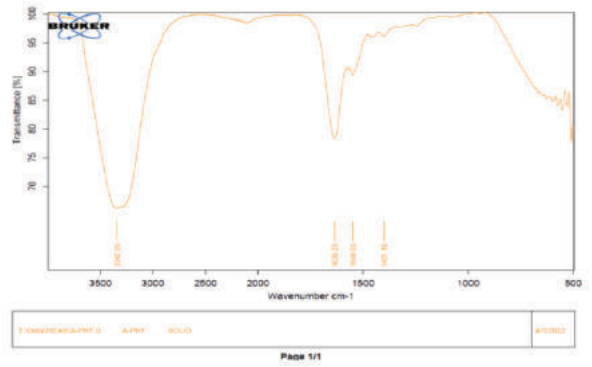

Fig.4b. A-PRF FTIR

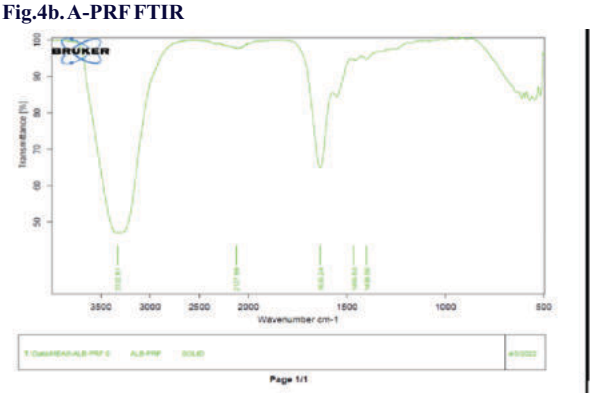

Fig.4c. Alb-PRF FTIR

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