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OUR EXPERIENCE IN PROCUREMENT OF VARIOUS TYPES OF PLATELET RICH FIBRIN WITH RESPECT TO THEIR CENTRIFUGATION TIME

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Abstract

Background: A second-generation platelet concentrate called platelet-rich fibrin (PRF) has been the subject of extensive research for the past two decades. The debate over relative centrifugal force (RCF), revolutions per minute (RPM), and relative time in the production process of various platelet concentrates has significantly complicated the topic during the last few years. The following factors need to be taken into account in order to produce the precise sub-type of PRF: Rotor angulation for the tube holder, Revolutions per minute (RPM), Relative centrifugal force (RCF), Rotor dimensions, Centrifugation model employed, Composition, size & form of used tubes. **Objective:** The study's objective was to assess how these variables interacted to produce the three different subtypes of PRF (S-PRF, A-PRF, and I-PRF) solely by adjusting the RPM and Duration without altering the rotor's angulation or size. **Materials and Methods:** Fifteen healthy volunteered patients for the study underwent surgical removal of impacted mandibular 3rd molars. Their peripheral blood was drawn and three subtypes of PRF were produced using a tabletop centrifuge (REMI C-852). Conical end glass tube with rubber stopper & counterweight balance were used. S-PRF, A-PRF, and I-PRF were obtained at 2700 rpm for 12 minutes, 1500 rpm for 14 minutes, and 700 rpm for 3 minutes, respectively. Statistical analysis was done using independent t-test in IBM SPSS 26.0 software. **Results and Conclusions:** The three subtypes of PRF have their own indications in different fields of oral and maxillofacial surgeries. They have enriched growth factors for excellent wound healing & tissue regeneration.

Keywords: Advanced platelet-rich fibrin, Standard platelet-rich fibrin, Injectable platelet-rich fibrin, Centrifugation.

INTRODUCTION

Several ideas for therapeutically relevant tissue production using minimally invasive techniques have been developed in the modern era. Even though different biomaterials are available in this field autogenous biomaterials are the gold standard forever. In this non-invasive autologous blood, products are preferred both by the patients & experienced surgeons one such non-invasive autologous blood product that can be used as a good biomaterial is platelet concentrates. Megakaryocytes produce platelets as a-nucleated cells. Coagulation factors, growth factors, Cytokines, Chemokines, Adhesion molecules

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and Integrins are only a few of the components found in platelets. Marx et al.[1] and Anitua et al.[2] established the use of platelet concentrates in medicine in the late 1990s. For a very long time, several disciplines of medicine, especially dentistry, have used platelet concentrates to treat a range of ailments.[3]

The first generation of platelet concentrates is represented by platelet-rich plasma (PRP). Platelet rich plasma is a subset of plasma that contains more platelets than peripheral blood does. PRP has three to four times as many growth factors as in peripheral blood. PRP has an advantageous impact on tissue regeneration and wound healing.[4] PRP is made up of a variety of plasma proteins, which are thought to be essential for the connective tissue stroma to undergo repair. PRP was shown to have a remarkable capacity for growth and to include a supra-physiological amount of growth factors that promote quicker healing.[5] But the inclusion of anticoagulants, bovine serum, and a multi-step centrifugation procedure restricts the therapeutic use of PRP and necessitates the use of alternative, clinically viable methods.[6]

In order to overcome the disadvantages, the 2nd generation platelet concentrates where introduced. Original research conducted by Drs. Joseph Choukroun and David Dohan that resulted in the creation of a platelet concentrate using blood taken without the use of an anticoagulant.[7]

Platelet Rich Fibrin(PRF) belongs to the 2nd generation platelet concentrates which do not require additional anticoagulation agents andare obtained by a simple centrifugation process. Leucocytes and platelets from peripheral blood are abundant in the PRF clot. By serving as the primary protagonists to influence the various phases of wound healing, leucocytes play a critical role. Leucocytes have an active role in lymphogenesis and angiogenesis. Platelets & leucocytes have synergistic effects &act as a reservoir of cytokines. The ability of platelet-rich fibrin (PRF) to promote the regeneration of both hard and soft tissues for a variety of clinical indications has been thoroughly investigated.[8] PRF-based membranes are used in a number of in vivo studies to hide the alveolar ridge on the augmentation side. They are also used as a sub sinus filling material in sinus lift procedures. They are also useful for small otologic surgeries. Further studies have demonstrated that the biological characteristics of PRF obtained from a certain individual may vary depending on the centrifugation speed.[6]

Injectable PRF is a totally autologous biomaterial with almost minimal hypersensitive reaction since it does not require the use of an anticoagulant. Differential centrifugation was initially explained by Drs. Ghannati and Choukran, who additionally introduced the world with I-PRF, a patented product created by Dr. Choukran.[9] They came to the conclusion, along with a few other authors, that slow-speed centrifugation yields more cells and their growth factors, that injectable PRF is a byproduct of this particular technique.[7,9]

The benefits of PRF include its one-step, streamlined procedure, use of an autologous blood sample, natural polarisation with less immunological reactivity, and ability to be used with bone grafts. PRF that contains a greater number of white blood cells is called

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as A-PRF or advanced platelet-rich fibrin. They are used in tissue regeneration studies and have clinical applicability in different fields.[10]

In this regard, the current study shows that different sub-types of PRF can be produced using a single centrifuge, provided that the revolution speed and time are modified.

MATERIALS AND METHOD

The Institutional Ethics Committee gave permission for this study to be conducted in the Department of Oral & Maxillofacial Surgery at Madha Dental College & Hospital, Kundrathur, Chennai, Republic of India (Ref No 013/MDC/IEC/2019). Fifteen adult patients between the ages of 18 and 35 who had their impacted mandibular third molars surgically removed made up the study sample. Following a thorough case history documentation, patients underwent a clinical examination, and the third molar was assessed radiographically.

1. Rotor angulation for the tube holder

- Fixed angulation - 33°

2. Revolutions per minute (RPM)

- 2700RPM for S-PRF
- 1500RPM for A-PRF
- 700RPM for I-PRF

3. Relative centrifugal force (RCF)

- Fixed RCF- 1750g

4. Dimensions of the rotor

- Fixed dimension – 295x295x25mm

5. Centrifugation model used

- REMI C-852, REMIELEKTROTECHNIK LTD, VASAI, INDIA

6. Composition, size & shape of tubes used

- To create fluid blood concentrates for our study, sterile conical end glass test tubes (Borosil, India) with a volume of 10 ml were utilised. The glass test tubes were sterilized in the formalin chambers for 48 hrs and washed with normal saline prior to use. These glass test tubes were closed using sterile rubber stoppers. The blood was withdrawn using the clinically approved butterfly blood collection method. The success of obtaining the desired subtype of PRF is purely dependent on the time of transfer of collected blood to the glass test tubes. Tincture of time is crucial.

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METHODS OF PREPARATION OF ADVANCED PRF

A total of five milliliters of venous blood was drawn in sterile conditions from the patient's left ante-cubital fossa and put in a glass test tube without the addition of any anti-coagulants. This glass test tube was instantly spun at 1300 rpm for 8 minutes in a centrifuge (REMI C-852, REMIELEKTROTECHNIK LTD, VASAI, INDIA). The centrifuged blood was separated into three fractions: red blood cells were found in the lowest fraction, advanced fibrin clot was found in the middle, and the upper fraction held cellular plasma. The A-PRF clot was initially separated from the red blood cells and the straw-colored cellular plasma was removed. Ample precautions were made during dissection to remove as little red blood cells as feasible. The consistency of the obtained A-PRF was a thick gel. It was then taken from the test tube with Adson’s non-toothed forceps and was inserted into the surgical areas.

METHODS OF PREPARATION OF INJECTABLE PRF

A total of five milliliters of venous blood was drawn in sterile conditions from the patient's left ante-cubital fossa and put in a glass test tube without the addition of any anti-coagulants. This glass test tube was instantly spun at 700 rpm for three minutes in a centrifuge (REMI C-852, REMIELEKTROTECHNIK LTD, VASAI, INDIA). The centrifuged blood was separated into three fractions: red blood cells were found in the lower portion, fibrin liquid was found in the middle, and yellow cellular plasma was found in the upper fraction. A sterile syringe was then used to aspirate the yellow-colored liquid platelet-rich fibrin, leaving the remaining red blood cells at the bottom of the test tube. Adequate care was used during aspiration to avoid aspirating the red blood cells. The obtained I-PRF was liquid in consistency. It was then taken from the test tube with a sterile syringe with a wide bore needle and was inserted into the surgical areas.

METHODS OF PREPARATION OF STANDARD PRF

A total of five millilitres of venous blood was drawn in sterile conditions from the patient's left ante-cubital fossa and put in a glass test tube without the addition of any anti-coagulants. This glass test tube was instantly spun at 2700 rpm for 12 minutes in a centrifuge (REMI C-852, REMIELEKTROTECHNIK LTD, VASAI, INDIA). The centrifuged blood was separated into three fractions: red blood cells were found in the lower fraction, fibrin clot was found in the middle, and cellular plasma was found in the upper fraction. Initially, the straw-colored cellular plasma was removed, and the red blood cells and PRF clot were separated. An adequate amount of attention was taken during dissection to remove as little red blood cells as feasible. The consistency of the obtained S-PRF was a clot. It was then taken from the test tube with Adson’s toothed forceps and was inserted into the surgical areas.

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PREPARATION OF A-PRF, I-PRF AND S-PRF

Fig 1, Fig 2, Fig 3, Fig 4 and Fig 5 shows the methodology of collecting blood, centrifugation process and procured A-PRF, I-PRF and S-PRF respectively.

Figure 1: * Method of Obtaining Blood

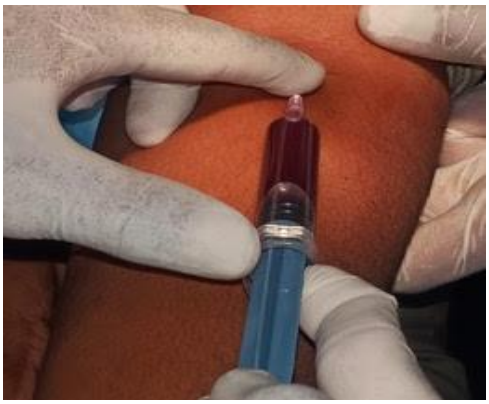

Figure 2: † Centrifugation Process

Fig 3: ‡ Procured Advanced Platelet Rich Fibrin

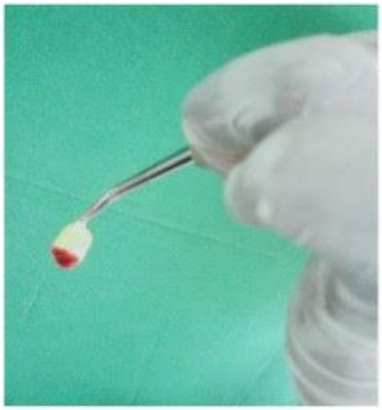

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Fig 4: § Procured Injectable Platelet Rich Fibrin

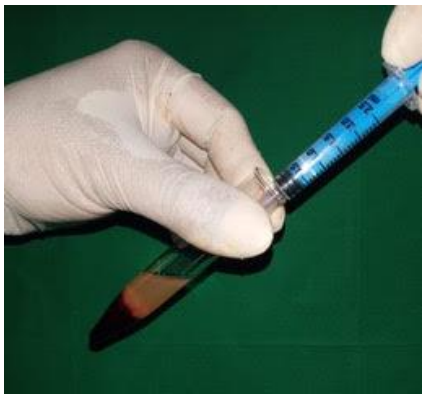
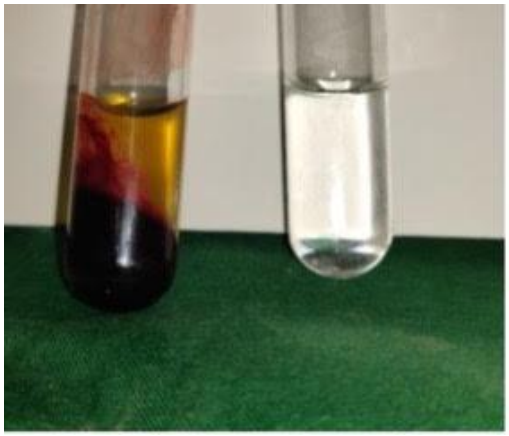


Fig 5: || Procured Injectable Platelet Rich Fibrin



Statistical Analysis

Independent t-test used in IBM SPSS 26.0 software.

RESULTS

We obtained S-PRF at 2700 rpm for 12 minutes, A-PRF at 1500 rpm for 14 minutes & I-PRF at 700 rpm for 3 minutes.

DISCUSSION

Choukroun et al. in France were the first to use PRF, a brand-new platelet concentrate. It is preferable to PRP since the streamlined processing method does not involve biochemical blood handling. PRF can also be utilised to encourage bone growth, haemostasis, graft stabilisation, and wound healing. Direct stem cell migration and the healing process are made simple by the better organisation of the fibrin matrix.[11] Studies have demonstrated that PRF releases growth factors. In vitro studies have shown much better results for PRF when compared to PRP.[12] Dohan et al. demonstrated that PRF has stronger healing effects than PRP and that growth factors are released from it more

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slowly.[13] As a result of the findings, it has been established that it is feasible to obtain three subtypes of PRF using the same centrifuge but altering the revolution speed and time within the clinical routine. In our study, we have obtained S- PRF at 2700 rpm for 12 minutes. In a study done by Kobayashi E et al, they used ten millilitres of whole blood without anticoagulant and centrifuged at 2,700 rpm (325g) for 12 min.[14] S-PRF was produced using a sterile glass coated plastic tube (9 mL; 2700 rpm for 12 minutes) in a study done by Ghanaati et al.[6]

According to a study by Fujioka-Kobayashi et al.[15], the PRF was recovered from the upper portion of the tube while the WBC and platelets were forced to the bottom of the tubes by the strong centrifugal forces. They demonstrated that increasing spin duration and decreasing centrifugal speed to 200 g at 1300 RPM can enhance the quantity of leukocytes and platelets in the PRF. This was termed as Advanced PRF or A-PRF. In our study, we have obtained A-PRF at 1500 rpm for 14 minutes. This is in accordance with the study done by Ghanaati et.al [6]. Using a PRF box, which is utilised in dentistry for making a PRF membrane, a-PRF clot created by the aforementioned process can be compressed. The clot is covered by a flat metal plate, which flattens it into a membrane. Additionally, the apparatus contains a cylindrical hole where the clot can be inserted. The clot can be put to a light weight, which forces the water out and transforms it into a tiny plug-like structure. These PRF membranes and plugs are both very beneficial in dentistry. PRF is used to treat wounds or ulcers, and the clot can be applied directly to the wound base and squeezed to fit there, promoting healing.[16] The distribution pattern of neutrophilic granulocytes can be altered by altering the centrifugation protocols in terms of centrifugation time and speed.[6] As a result of their phagocytotic ability, degranulation, and neutrophilic extracellular traps, neutrophilic granulocytes are regarded as early inflammatory cells.[17]

Other than platelets these advanced fibrin clots contain T- Lymphocytes, B- Lymphocytes, monocytes, macrophages and stem cells. In our study,I- PRF was obtained at 700 rpm for 3 minutes. According to a study, the injectable form of PRF is created by centrifuging blood at 700 rpm (60 g) for three minutes in clear plastic tubes with no coatings.[18] A liquid PRF can be created, as demonstrated by Miron et al[19], by lowering the centrifugal force and the spin duration. This was termed as I-PRF or Injectable-PRF. For three minutes, the centrifugal speed was maintained at 60 g. Due to the brief centrifugation period, the preparation stays liquid and the separation takes place before the clot has a chance to form. In most cases, a 10 mL tube can only yield 1–1.5 ml of the total volume of I-PRF. It has been discovered to have more WBC and platelets than L-PRF and A-PRF. Before it starts to coagulate and form a clot, it remains in liquid state for 15 to 20 minutes. The acquired I-PRF can be injected into the scalp and face skin at this time, or it can be combined with bone grafting materials, shaped into the desired shape, and allowed to clot. Choukroun and Ghanaati[9] showed in their clinical research that centrifugation done at a low speed and just fast enough for the platelets to get separated from red blood cells, produces the most remarkable outcomes and much more leukocytes, platelets, and growth factors than the conventional PRF methodology. Another success of this unique

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technology was the development of injectable liquid PRF (i-PRF) without using any anticoagulants. According to the authors, i-PRF prepared using the centrifugation at low speed is significantly enriched in leukocytes, platelets and growth factors like transforming growth factor-1 and vascular endothelial growth factor (VEGF), both of which are crucial for angiogenesis and neovascularization. Monocytes, which are present in A-PRF and i-PRF, are crucial for vascularization, VEGF production, and bone development. The monocytes generate BMP-2 and have BMP receptors.[20]

CONCLUSION

We postulate that these three sub-types of PRF have their own indications in different fields of oral and maxillofacial surgeries. These sub-types of PRF have enriched growth factors for excellent wound healing & tissue regeneration. Further studies are needed in near future in large numbers to obtain more efficient platelet concentrates.

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