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ORIGINAL ARTICLE

The effect of age, gender, and time between blood draw and start of centrifugation on the size outcomes of platelet-rich fibrin (PRF) membranes

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Abstract

Objectives Platelet-rich fibrin (PRF) has been utilized in regenerative dentistry as a supra-physiological concentrate of autologous growth factors capable of stimulating tissue regeneration. Due to the variability in the macroscopic morphology/size of PRF membranes observed between patients, we were interested in studying the effects of patient age, gender, and time between blood draw and the start of centrifugation on the size outcomes of PRF membranes. Despite PRF therapy being increasingly more popular in private practice, to date, no study has investigated the effects of the delay between blood draw and the start of centrifugation in a clinical setting.

Materials and methods A total of 60 patients enrolled in this study were divided into 6 groups of 10 patients each, including male and female patients categorized into age groups 21–40, 41–60, and 61–80 years. From each patient, a total of five PRF membranes were fabricated from 10-mL tubes following centrifugation starting after 0, 30, 60, 90, and 120 s. In total, 300 PRF membranes were produced in this study to investigate the effects of patient age, gender, and time on the size outcomes of PRF membranes.

Results A longer delay by the clinician before starting centrifugation following blood draw led to a smaller final size of PRF membranes. At 90 s following blood draw, a significant (13%) reduction in PRF membrane size was observed. After 120 s, a significant (23%) reduction was observed. Additionally, female patients had on average 17% larger membranes compared to men ($p < 0.05$, 300 samples). Lastly, the size outcomes of the PRF membranes was largest in patients aged 61–80, followed by those aged 41–60 and 21–40. However, no statistically significant differences in PRF membrane sizes were reported between age groups.

Conclusions The time at which a centrifugation procedure begins following blood draw is critical to optimize the size outcomes of PRF membranes. In general, approximately 15 s is required per tube to harvest 9–10 cc of blood. Therefore, a 60- to 90-s interval between blood draw and the start of centrifugation should be a parameter that is respected by clinicians to avoid significant changes in the macroscopic morphology/size of fabricated PRF membranes. Furthermore, females and older patients produced larger membranes, likely due to lower red blood cell counts derived from their peripheral blood.

Clinical relevance The findings from the present study demonstrate that on average, a clinician has approximately 60–90 s between blood draw and the start of the centrifugation cycle to produce standard-sized PRF membranes. Shortly thereafter, a significant reduction in size is observed. Additionally, females and older patients were found to produce larger PRF membranes. Centrifugation protocols may therefore be adapted accordingly.

Keywords Fibrin · Blood platelets · Centrifugation · Wound healing · Platelet-rich fibrin

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Introduction

The use of platelet-rich fibrin (PRF) in dentistry has seen a wide and steady increase in regenerative therapy over the past decade [1]. Due to its ability to collect peripheral blood and concentrate blood-derived growth factors following centrifugation, PRF has been utilized for tissue regeneration in a therapy that is derived entirely from autologous sources [2, 3]. Platelet-rich plasma (PRP) was initially developed in the mid to late 1990s with widespread use not only in dentistry, but also in many areas of medicine including maxillofacial surgery, orthopedic surgery, and esthetic medicine [4–8]. Despite its initial use as a platelet concentrate capable of stimulating tissue regeneration, concerns were raised regarding the use of bovine thrombin and various other anti-coagulants [4, 9, 10].

When anti-coagulants were removed from the centrifugation process, PRF (a second-generation platelet concentrate following the introduction of PRP) forms a three-dimensional fibrin matrix that has since been highlighted as being advantageous because it allows for a more slow and gradual release of growth factors over time [11]. Furthermore, modifications to centrifugation speed and time, known as the low-speed centrifugation concept, have been further shown to release higher concentrations of growth factors [12–14].

Over the past few years, several clinicians have reported great variability in the macroscopic morphology/size of PRF membranes between patients. The rationale of this study was therefore to address this question by studying the effects of patient age, gender, and time between blood draw and the start of centrifugation on the size outcomes of PRF membranes. Furthermore, the extent to which the time between blood draw and the start of centrifugation affects the size outcomes of PRF membranes is unknown. Because roughly 15 s is required to collect one vial of peripheral blood (9–10 cc) with a standard butterfly needle, the second aim of the study was to investigate the maximum number of seconds required between blood draw and the start of centrifugation before alterations occur in the final size outcomes of PRF clots. We therefore hypothesized that both patient age and gender would affect the final size outcomes of PRF clots and that a delay between blood harvest and the start of centrifugation would negatively impact the size of the final PRF clots.

Materials and methods

Preparation of PRF

Blood samples were collected with the informed consent of 60 volunteer donors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No ethical approval was required for this study because human samples were not identified, as previously described [15]. The donors were separated equally into 30 female patients and 30 male patients. The factors that affect fibrin clot formation and structure include genetic factors, acquired factors (such as abnormal concentration of thrombin and factor XIII in plasma, blood flow, platelet activation, oxidative stress, hyperglycemia, hyper-homocysteinemia, medications, and cigarette smoking), and other parameters (such as microgravity, pH, temperature, reducing agents, and concentration of chloride and calcium ions) [16]. All patients with any of the above conditions were excluded. All patients were included if systemically healthy, non-smoking, and not taking any medications. The patients were then equally divided into groups of 20 with age ranges of 21–40, 41–60, and 61–80 years. The six groups of ten patients each were therefore divided as follows: (1) ten female patients aged 21–40, (2) ten male patients aged 21–40, (3) ten female patients aged 41–60, (4) ten male patients aged 41–60, (5) ten female patients aged 61–80, and (6) ten male patients aged 61–80. Five tubes of 10 mL of whole blood was collected from each individual patient, and centrifugation began after 0, 30, 60, 90, and 120 s. Centrifugation was carried out at 1300 RPM (200 g-force as calculated at RCF max) for 8 min at room temperature on a Duo Centrifuge and 10 mL glass tubes (Process for PRF, Nice, France). Following centrifugation, the caps of the centrifugation tubes were removed and exposed to air for 5 min to induce further membrane clotting (standard protocol by Process for PRF). Thereafter, the membranes were removed, the red clot attached to the yellow PRF membrane was separated as previously described [17–19], and the final PRF clot was measured both in width and height. A total surface area of each of the 300 membranes was calculated by multiplying the width and height measurements of each individual PRF membrane with a Vernier caliper. Thereafter, membrane sizes were compared between males and females, between age groups, and between the various times between blood draw and the start of centrifugation.

Statistical analysis

All experiments were performed with a minimum of 20 patients per group. Means and standard errors were calculated, and data were analyzed for statistical significance using one-way analysis and *t* test for membrane size differences with GraphPad Prism 6.0 software (GraphPad Software, Inc., La Jolla, CA, USA; $*p$ values < 0.05 were considered significant).

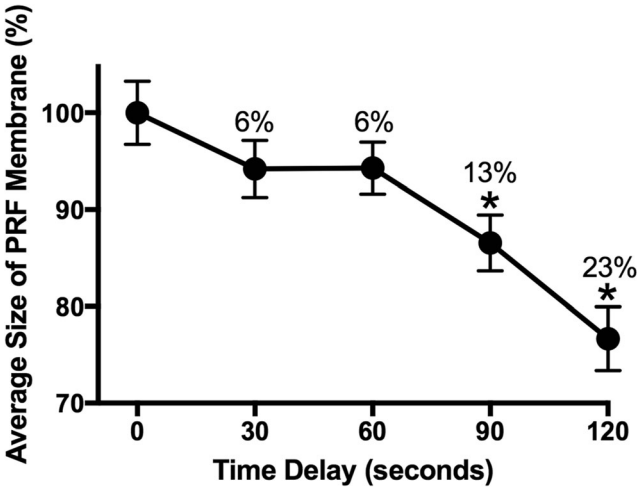

Fig. 1 Average size of PRF membranes from 60 patients following blood draw after an initial wait period of 0, 30, 60, 90, and 120 s prior to centrifugation. Notice that after 90 s, the PRF membranes were significantly reduced in size (by 13%). Following a 120 s wait period, these membranes were further significantly reduced in size (by 23%) compared to the control (0-s wait period). (*$p < 0.05$ indicates a significant difference between 0 s from centrifugation and the investigated time period of 90 and 120 s)

Results

Effect of time between blood draw and centrifugation

In a first series of experiments, the effect of time between blood draw and the start of centrifugation on the size outcomes of PRF membranes was investigated in 60 patients from all age groups (Fig. 1). Interestingly, while little change in the sizes of PRF membranes was observed within the first 60 s, by 90 s, a significant (13%) reduction in the size of PRF membranes was observed. Furthermore, after 120 s, a significant (23%) reduction was observed (Fig. 1). These findings suggest that a clinician has up to 90 s to draw all PRF tubes; otherwise, the first tubes (drawn over a 90-s interval) will result in a PRF membrane displaying a significant reduction in size.

PRF membrane size differences between males and females

In a second experiment, the size outcomes of PRF membranes were compared between 30 male versus 30 female patients. Significantly larger membranes were produced in females at all investigated time points with significant discrepancies observed at the earlier time periods (Fig. 2). When the data were grouped together from all 300 samples, female membranes were on average 17% larger than those of male patients (Fig. 3). These findings highlight the fact that clinicians may expect larger membranes in females compared to males, irrespective of the time at which centrifugation is carried out (though larger membranes were routinely produced when centrifugation began as immediately as possible following blood draw).

PRF membrane size differences between patient ages

In a final set of experiments, the size of PRF membranes was compared between 60 patients equally grouped into age categories of 21–40, 41–60, and 61–80 years (Fig. 4). In general, older people (male and female) showed larger membranes, though no significant difference was observed between the age groups (Fig. 4, Table 1). While only a 4% difference in PRF membrane size was observed between patients aged 41–60 compared to those aged 21–40, a 15% increase in membrane size was observed in patients between the ages of 61–80 compared to those aged 21–40 (Fig. 5). This was particularly relevant for females aged 61–80 (Table 1). These findings highlight the fact that older patients may also produce larger membranes and not solely females.

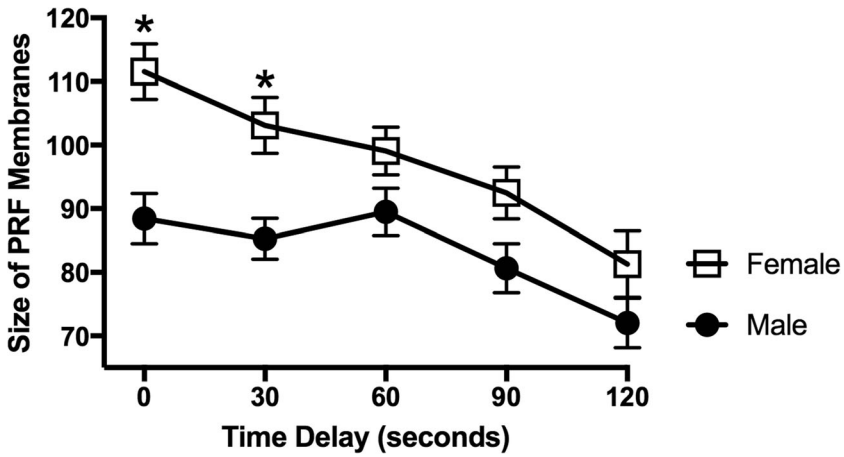

Fig. 2 Comparison of the average size of PRF membranes between males and females in 60 patients. Notice that at earlier time points, the female PRF membranes were significantly larger compared to male PRF membranes. (*$p < 0.05$ indicates a significant difference between male and female PRF membrane sizes (%))

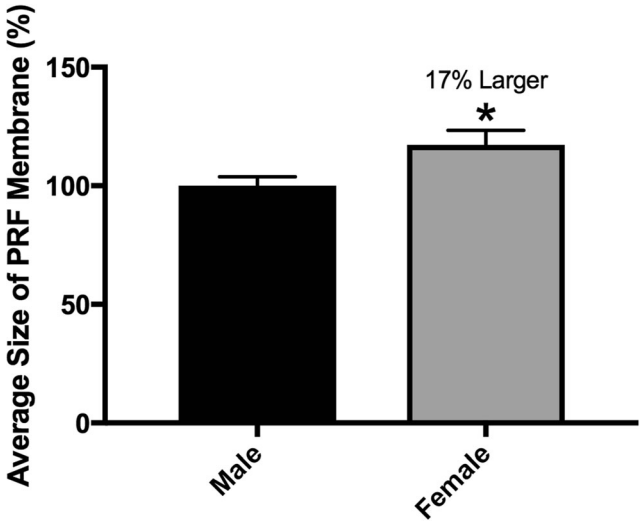

Fig. 3 Bar graph representing the average size of PRF membranes from males and females in 60 patients. On average, the female PRF membranes were 17% larger (*$p < 0.05$ indicates a significant difference between male and female PRF membrane sizes (%))

Discussion

PRF has gained tremendous momentum for a variety of regenerative procedures in dentistry, including for the management of extraction sockets [20–23], gingival recessions [24–26], palatal wound closure [27–29], and the regeneration of periodontal defects [30], amongst others [31]. In other medical fields, PRF has also been utilized for the management of hard-to-heal leg ulcers including diabetic foot ulcers, venous and chronic leg ulcers, facial soft tissue defects, laparoscopic cholecystectomy, deep nasolabial folds, superficial rhytids, acne scars, chronic rotator cuff tears, and acute traumatic ear drum perforations [32].

Despite the widespread use of PRF, it remains interesting that through numerous blood draws within our clinics, it became apparent that there were significant discrepancies between the macroscopic morphology/size of PRF membranes following centrifugation between patients. We were therefore interested to investigate the size outcomes of PRF membranes in a patient population that compared males versus females, patient age groups, and the time between blood draw and the initiation of centrifugation.

In the present study, one noticeable trend was the size of the membranes produced between male and female patients. On average, the size of PRF membranes produced from females was 17% larger than those produced in males (Fig. 3). As the role of centrifugation is to separate blood layers transitionally over time, these differences were thought to be observed due to females generally containing lower hematocrit levels within their peripheral blood compared to males [33, 34]. As a result, the layer separation between plasma layers is increasingly more difficult in patients (males) with higher hematocrit levels. The same was also observed in the present study in elderly patients. As a patient ages, their concentration of RBCs also tends to decrease. As a result, the separation of plasma layers was also shown to be more easily separated, producing larger PRF membranes. Based on these findings, it may be anticipated that as a general rule, females and older patients typically produce larger PRF membranes compared to males and younger patients. Furthermore, a previous report by Yajamanya et al. found that the fibrin network of PRF-based membranes was less dense as patient age increased [18]. This factor may have also played a role in the separation of layers. Further studies are needed to investigate all factors that may affect the size outcomes of PRF clots.

Another important parameter investigated in the present study was the duration between blood draw and the start of centrifugation. In general, we found that on average, it took roughly 15 s to fill each individual centrifugation tube of 9–10 cc. The present study found that at 90 s, a significant (13%) reduction in the size of the PRF membrane was found, and by

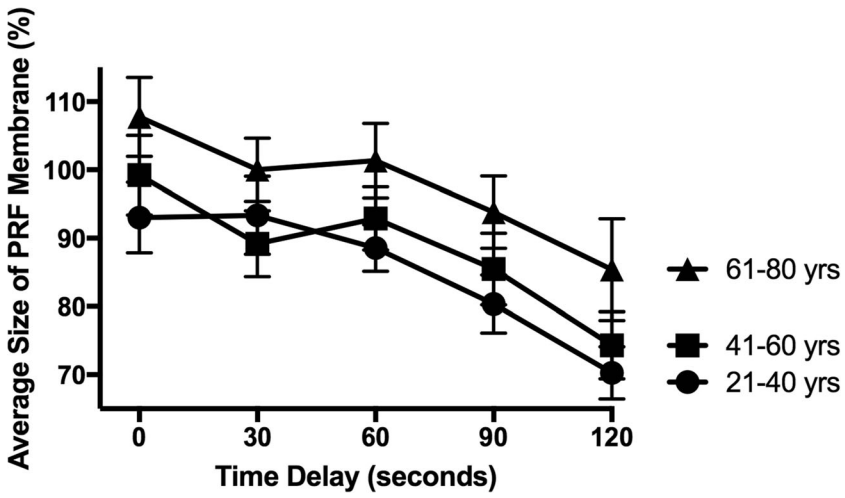

Fig. 4 Comparison of the average size of PRF membranes between various age groups including (a) 21–40, (b) 41–60, and (c) 61–80 years. While no significant differences were noticed between the groups, in general, older patients produced larger membranes

Table 1 Analysis in the groups demonstrates trends between the various age groups

Tukey's multiple comparisons test	Mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted *p* value
Male 21–40 vs. female 21–40	− 8.491	− 29.06 to 12.08	No	ns	0.7945
Male 21–40 vs. male 41–60	− 4.931	− 25.5 to 15.64	No	ns	0.9745
Male 21–40 vs. female 41–60	− 9.764	− 30.33 to 10.8	No	ns	0.6868
Male 21–40 vs. male 61–80	− 1.965	− 22.53 to 18.6	No	ns	0.9997
Male 21–40 vs. female 61–80	− 31.63	− 52.19 to − 11.06	Yes	***	0.0010
Female 21–40 vs. male 41–60	3.559	− 17.01 to 24.13	No	ns	0.9941
Female 21–40 vs. female 41–60	− 1.274	− 21.84 to 19.29	No	ns	> 0.9999
Female 21–40 vs. male 61–80	6.526	− 14.04 to 27.09	No	ns	0.9195
Female 21–40 vs. female 61–80	− 23.14	− 43.7 to − 2.572	Yes	*	0.0211
Male 41–60 vs. female 41–60	− 4.833	− 25.4 to 15.73	No	ns	0.9766
Male 41–60 vs. male 61–80	2.966	− 17.6 to 23.53	No	ns	0.9975
Male 41–60 vs. female 61–80	− 26.7	− 47.26 to − 6.131	Yes	**	0.0060
Female 41–60 vs. male 61–80	7.799	− 12.77 to 28.37	No	ns	0.8453
Female 41–60 vs. female 61–80	− 21.86	− 42.43 to − 1.298	Yes	*	0.0326
Male 61–80 vs. female 61–80	− 29.66	− 50.23 to − 9.097	Yes	**	0.0020

120 s, this increased significantly to 23% (Fig. 1). These results clearly indicate that if each PRF tube draw lasts approximately 15 s, by the seventh tube draw (> 90 s), the clinician should expect that the resulting PRF membrane begins to decrease in size significantly.

The present study revealed significant changes in the macroscopic morphology and sizes of PRF membranes; however, it remains unstudied how changes in PRF membrane size may affect the cell and growth factor content within PRF membranes. Over time, it is theoretically more difficult to transiently separate layers (resulting in smaller PRF membranes). Thus, it may also be hypothesized that these membranes may also contain fewer cells and less growth factors. This assumption requires further study but potentially carries significant clinical relevance. Furthermore, the centrifugation protocol in this study utilized lower centrifugation speeds. These advancements were founded by work conducted by Ghanaati et al. whereby lower centrifugation speeds were shown to contain a higher number of cells including leukocytes prior to the formation of a fibrin clot [35]. Nevertheless, these lower centrifugation speeds produce smaller membranes caused by a reduction in the overall g-force during the centrifugation process. Future research remains necessary to investigate if such significant differences would also be observed utilizing various centrifugation protocols/settings. In summary, despite PRF therapy being increasingly more popular in regenerative dentistry, to date, this study is the first to investigate the effect of the duration between the blood draw and the start of centrifugation. We therefore report an optimal blood draw of up to 90 s to produce PRF membranes.

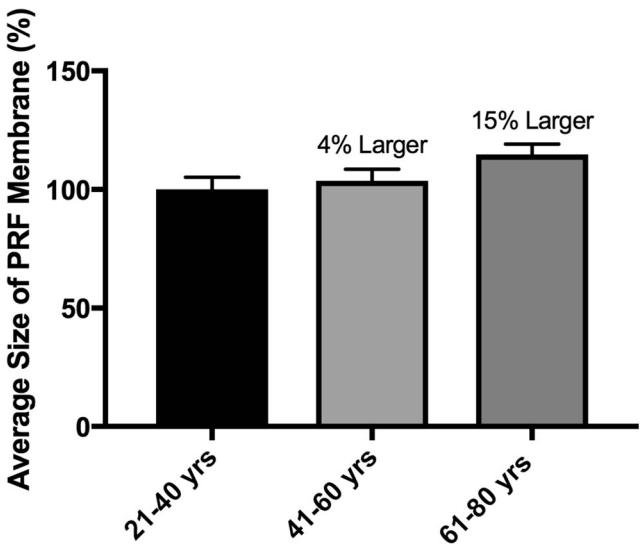

Fig. 5 Bar graph representing the average size of PRF membranes between various age groups including (a) 21–40, (b) 41–60, and (c) 61–80 years. While no significant differences were noticed between the groups, in general, older patients produced larger membranes

Conclusion

A 60- to 90-s interval between blood draw and the start of centrifugation should be a stringently respected parameter by clinicians to avoid significant changes in the macroscopic morphology of fabricated PRF membranes. By 120 s, a significant (23%) reduction in PRF membrane size was observed. Furthermore, both females and older patients produced larger membranes compared to those of males and younger patients. It may be possible to further optimize centrifugation protocols based on differences in patient age and gender. However, future research is required to validate these findings.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval No ethical approval was required for this study, as human samples were not identified.

Informed consent For this type of study, informed consent was provided prior to blood draw to conduct the outlined experiments.

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