

# CCD

CONTEMPORARY CLINICAL DENTISTRY

[www.contempclindent.org](http://www.contempclindent.org)

Volume 10 / Issue 3 / July - September 2019

# Regenerative Capacity of Leukocyte-rich and Platelet-rich Fibrin in Indirect Sinus Elevation Procedure May be Dependent on Model-Specific Modification of the Centrifugation Cycle

## Abstract:

**Context:** To compare optical density (OD) and fibrinogen content of leukocyte-rich and platelet-rich fibrin (L-PRF) generated by standard protocol (2700 rotations per minute [RPM] for 12 min) versus relative centrifugal force (RCF)-adjusted protocol across two widely used laboratory centrifuges with swing-out rotors. **Aims:** Centrifuges for PRF production generate forces in excess of 800 g. The study aimed to evaluate OD, fibrinogen content and effectiveness in bone-added osteotome sinus floor elevation (BAOSFE) of leukocyte-rich and platelet-rich fibrin (L-PRF) generated by the standard protocol (2700 RPM for 12 min) versus a RCF-adjusted protocol to generate precisely 400 g of force across two centrifuges with swing-out rotors. The outcomes were compared to a standard centrifuge configured to generate L-PRF as per the original Choukroun guidelines. **Settings and Design:** Sample size for the present study was calculated using proportional power calculation. A minimum sample size of 8 per group was needed to detect a bone height difference of 2 mm when the power of the test is 0.80 at a significance level of 0.05. **Subjects and Methods:** Based on the centrifuge and protocol used to generate L-PRF, 10 participants were assigned to each of the following groups as follows: D group, fixed angle centrifuge (DUO Quattro®) at default setting. R-O group: Swing-out centrifuge (Remi 8C®) + standard protocol. R-A group: Remi 8C® centrifuge + RCF-adjusted protocol. C-O group: Swing-out centrifuge (Remi C854®) + standard protocol. and C-A group: Remi C854® + RCF-adjusted protocol. OD, fibrinogen content, and gain in bone fill and bone height after BAOSFE were the evaluated outcomes. **Statistical Analysis Used:** Data were analyzed using GraphPad Prism® Software version 6.0 (GraphPad Software Inc., La Jolla, USA) and SAS Software® version 9.3 versions (SAS, New Delhi, India). Data were summarized by mean  $\pm$  standard deviation for continuous data and median  $\pm$  inter-quartile range for the score data. The comparison between different time points was done by analysis of one-way repeated measures test, followed by *post hoc* test for score data. The comparison between two groups for repeated data was made by analysis of two-way repeated measures test and followed by *post hoc* test. Spearman's Rho correlation test was used to test the correlation between prognosis and the other variables. **Results:** L-PRF from the Remi C854® centrifuge with RCF-adjusted protocol showed OD ( $P = 0.152$ ) and fibrinogen content ( $P = 0.232$ ) identical to those from the DUO Quattro® centrifuge. L-PRF from Remi 8C® centrifuge with the RCF-adjusted protocol resulted in maximum postoperative bone height gain ( $7.01 \pm 1.44$  mm) and bone fill ( $13.50 \pm 4.51$  mm<sup>2</sup>) which was higher than that of the outcomes from the DUO Quattro® centrifuge ( $6.82 \pm 2.92$  mm and  $12.32 \pm 5.31$  mm<sup>2</sup>). **Conclusions:** A reduction in RCF resulted in a less dense clot and had a positive influence on the regenerative potential of L-PRF in BAOSFE procedure.

**Keywords:** Alveolar ridge augmentations, centrifugation, maxillary ridge augmentations, platelet-rich fibrin

## Introduction

PRF is an autologous, second-generation platelet concentrate and is a healing biomaterial with great potential for the bone- and soft-tissue regeneration without initiating local inflammatory reactions.<sup>[1]</sup> The generation of PRF requires

a centrifugation cycle<sup>[1,2]</sup> which is dependent on the time, rotations per minute (RPM), angulation of the tubes to the axis of the rotor, and the g-force generated by the centrifuge (relative centrifugal force [RCF]).<sup>[1,3]</sup> In these terms, the standard protocol to generate leukocyte-rich and platelet-rich fibrin (L-PRF) is to run a centrifuge at 2700 RPM for 12 min at

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

**Rampalli Viswa Chandra, Varanasi Vaishnavi, Y. S. H. S. Chakravarthy**

Department of Periodontics,  
SVS Institute of Dental Sciences,  
Mahbubnagar, Telangana, India

**Address for correspondence:**  
Dr. Y. S. H. S. Chakravarthy,  
Department of Periodontics,  
SVS Institute of Dental Sciences,  
Mahbubnagar - 509 002,  
Telangana, India.  
E-mail: yshschakri@gmail.com

## Access this article online

### Website:

www.contempclindent.org

DOI: 10.4103/ccd.ccd\_715\_18

### Quick Response Code:



**How to cite this article:** Chandra RV, Vaishnavi V, S. Chakravarthy YS. Regenerative capacity of leukocyte-rich and platelet-rich fibrin in indirect sinus elevation procedure may be dependent on model-specific modification of the centrifugation cycle. Contemp Clin Dent 2019;10:433-9.

around 400 g in tubes having a fixed angulation of 33° to the central axis of rotation.<sup>[1,2]</sup>

Considerable ambiguity in the protocol and armamentaria for PRF exists, and these are frequently not clear or vary across the individual studies.<sup>[1-4]</sup> With respect to the tube angulation, centrifuges are of two types; fixed angle and swing-out rotors.<sup>[4]</sup> PRF generation requires the tubes to be at a 33° angle to the axis of rotation and hence, a fixed angle centrifuge is preferred.<sup>[1-3]</sup> Interestingly, studies on the effects of platelet concentrates on bone regeneration have utilized both swing-out<sup>[3,5]</sup> and fixed angle centrifuges<sup>[1,2]</sup> for PRF generation. Laboratory centrifuges have swing-out rotors with tube angulation >33°; these centrifuges show increased tube vibration and parasitic acceleration which may cause possible cell death and slow release of mediators in the obtained platelet aggregate.<sup>[1,2]</sup> PRF generation also requires precise centrifugation environment, which is specified in terms of RCF and expressed as “g.”<sup>[6]</sup> The G-force and RPM of a centrifuge are related by the formula  $RCF = 1.12 \times r \times (RPM/1000)^2$  where, r is the center of the centrifuge to tube end distance in millimeters.<sup>[6]</sup> Contrary to popular perception, it is the RCF that affects the preparation, constituents, and clinical efficacy of PRF and not the RPM.<sup>[7]</sup> Peck *et al.*<sup>[7]</sup> have stated that RCF is critical to the production of L-PRF and must be calculated for each centrifuge used; however, most centrifuges do not have a provision for setting up the RCF generated by them.<sup>[3,6,7]</sup> The center of centrifuge to tube end distance is as critical to the process of producing PRF as the RPM<sup>[6,7]</sup> and it is difficult to assume that there will be a uniform rotor radii across the wide array of centrifuges available.<sup>[3,4,6,7]</sup> Assuming that the RPM required is 2700 and the tube length is 100 mm, the RCF as calculated by the formula  $1.12 \times r \times (RPM/1000)^2$  will be 816 g which is clearly in excess of the 400 g recommended in the literature.<sup>[1,2]</sup> Using the same formula, a precise 400 g of force can be generated in a 100-mm tube if the centrifuge is run around 1900 rpm. Therefore, it may necessary to adjust the RPM across different centrifuge models to generate precisely 400 g of force for the preparation of PRF which is biologically and clinically acceptable.<sup>[3,6,7]</sup>

Platelet concentrates have been used with osteotome sinus floor elevation (OSFE) for increasing the residual bone height (RBH) in the atrophic posterior maxillae.<sup>[8-12]</sup> Studies on the use of PRF in sinus elevation have either used a fixed angle centrifuge<sup>[8,12]</sup> or have not described the nature of centrifuge clearly.<sup>[9-12]</sup> The addition of a bone grafts such as hydroxyapatites also results in faster *de novo* bone formation in the sinus radiographically and histologically.<sup>[13,14]</sup> Novel Hydroxyapatites such as nanostructured Zn-substituted monetite are replaced completely by the natural bone, thereby avoiding the disadvantages of nonresorbable materials interfering with normal bone remodeling process.<sup>[15]</sup>

The study has two objectives. The first objective of this study was to compare optical density (OD) and fibrinogen content of L-PRF generated by standard protocol (2700 RPM for 12 min)<sup>[1,2]</sup> versus RCF-adjusted protocol across two widely used laboratory centrifuges with swing-out rotors. The adjusted protocol is derived by measuring the “r” for both the centrifuges and applying this in the formula  $g = 1.12 \times r \times (RPM/1000)^2$  to measure the RPM required when the  $g = 400$ ; the optimum g-force required to generate L-PRF with optimum qualities.<sup>[1,2]</sup> The values obtained will be compared to L-PRF obtained from a centrifuge configured to generate L-PRF as per the original Choukroun guidelines.<sup>[1,2,13]</sup> The second objective of this study is to compare the effectiveness of L-PRF obtained across these three centrifuges in sinus augmentation using the bone-added osteotome sinus floor elevation (BAOSFE) technique.

## Subjects and Methods

The purpose of this trial is to study, in BAOSFE technique, the effect of L-PRF generated by model-specific modification of the centrifugation cycle across two swing-out laboratory centrifuges and a fixed angle centrifuge designed as per the original Choukroun guidelines. Approval from the Institution Ethical Committee (SVSIDS/PERIO/1/2015) was obtained, and informed consent was taken from all the participants.

### Sample size

Sample size for the present study was calculated using proportional power calculation. A minimum sample size of 8 per group was needed to detect a bone height difference of 2 mm when the power of the test is 0.80 at a significance level of 0.05.

### Source of data and participant flow

From a subject pool of 88 participants, systematically healthy participants between 25 and 50 years with edentulous area in the posterior maxillary region having a favorable interarch distance, RBH  $\geq 4$  mm and  $\leq 8$  mm and willing for delayed implant placement were included in the study. Medically compromised patients, participants having uncontrolled periodontal disease or an active sinus infection and smokers were excluded from the study. Nearly 50 participants (mean age =  $38.22 \pm 9.08$  years/29 males) satisfied the inclusion criteria and 10 participants each were assigned to the following groups. (1) D group: participants from whom L-PRF was obtained from a centrifuge designed as per original Choukroun guidelines (DUO Quattro®, Nice, France). (2) R-O group: L-PRF was obtained from a laboratory swing-out centrifuge (Remi 8C®, Mumbai, India) conforming to the original centrifugation cycle (2700 RPM for 12 min). (3) R-A group: L-PRF was obtained from a laboratory swing-out centrifuge (Remi 8C®, Mumbai, India) as per a RCF-adjusted protocol. The protocol was revised as follows: two operators (Rampalli

Viswa Chandra and Varanasi Vaishnavi) calculated the “*r*” value for the centrifuge [Figure 1] and an average was taken (*r* = 125 mm). The RPM required to generate 400 g of RCF in a 125-mm tube was calculated (RPM = 1690). The centrifuge was run at 1700 RPM for 12 min. (4) C-O group: L-PRF was obtained from a laboratory swing-out centrifuge (Remi C854®, Mumbai, India) conforming to the original centrifugation cycle (2700 RPM for 12 min). (5) C-A group: L-PRF was obtained from a laboratory swing-out centrifuge (Remi C854®, Mumbai, India) as per a RCF-adjusted protocol. The “*r*” value was 130 mm and the revised RPM to generate 400 g of force was 1650. Unlike other centrifuges, this centrifuge lacks a digital display and hence the centrifuge was run at setting “2” (corresponding to 1400RPM) for 14 min. A nonrandomized, open-label protocol was followed and participants were treated sequentially in the order of recruitment into the study.

### Investigations and interventions

From each participant (*n* = 50), 30 mL of blood was drawn into three silicone coated 10 mL tubes (Becton Dickinson, Gurgaon, India) devoid of an anticoagulant from the antecubital vein rapidly for the production of L-PRF. Corresponding to the group, the participant was in, either the preset button for L-PRF was selected (D group; “PRF(L)”) or the RPM was set (R-O and C-O groups) or adjusted accordingly (R-A and C-A groups) for each centrifuge. Three clots were obtained per participant after the centrifuge cycle was completed.

### Optical density and fibrinogen content in leukocyte-rich and platelet-rich fibrin clots

One clot per participant was sliced vertically into two broad segments for OD and fibrinogen estimation. Estimation of OD is an acceptable method to estimate platelet behavior and to analyze the kinetics of fibrin clot formation. OD of L-PRF clots was estimated as follows:<sup>[3,16,17,18]</sup> the clots were centrifuged, washed, and dried in a manner described by Saifer and Newhouse.<sup>[16]</sup> The OD of this standard clot was read in a spectrophotometer (Systronics 1203®, Ahmedabad,

India) at 570 nm after preparing the clots as per standard protocols.<sup>[16,17]</sup> The fibrinogen content in the L-PRF clot was assayed by the immunoturbidimetric method by using a commercially available kit (Fibrinogen Assay®, Diazyme Europe, Dresden, Germany) as per standard protocols.<sup>[17]</sup>

### Bone-added osteotome sinus floor elevation procedure

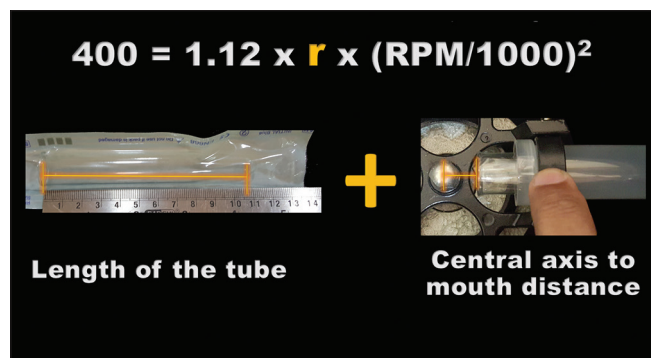
Two clots from each participant were used for the BAOSFE procedure. Briefly, the procedure was done as follows:<sup>[11]</sup> a crestal incision was given on the palatal aspect of the anticipated osteotomy site and a full-thickness flap was elevated for adequate visualization. The site was marked with a small round carbide bur and a 2-mm twist drill to the depth of 1-mm short of sinus floor was made which was then widened to 3 mm. The L-PRF obtained was initially cut into small pieces with a surgical scissor. These were placed inside the osteotomy as a cushion during sinus elevation. A suitable-sized osteotome (<3 mm) was used to advance the sinus floor by 1 mm with each mallet stroke, until the desired bone height is obtained. To check for the maxillary sinus membrane integrity, the patient was asked to perform the Valsalva maneuver. The remainder of the L-PRF was mixed with a Zn-substituted monetite scaffold (Siloss®, Azure Bio, Madrid, Spain) and was packed into the osteotomy site by tapping it with osteotome and mallet. The mucoperiosteal flap was then repositioned and sutured.

### Radiographic assessment

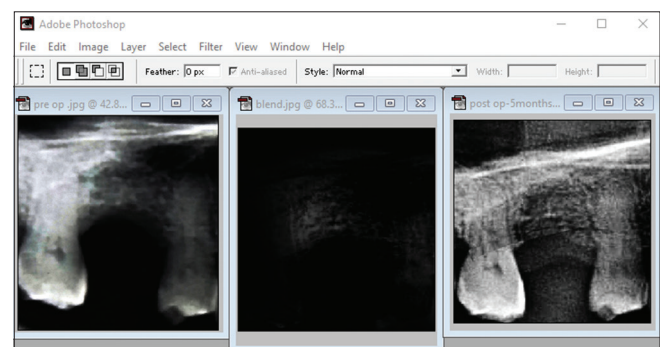
Radiographs were taken with digitalized RVG machine (Carestream Dental RVG 5200®, Kodak, New Delhi, India) at 60 kVp/2 mA with inactive interface at baseline and 6 months. The gain in bone height was calculated by measuring the distance between the sinus floor and the most occlusal aspect of the alveolar ridge at the baseline and at 6 months just before the implant placement. The evaluation of bone fill was performed by using digital-subtraction technique and morphometric area analysis by using specific tools in two image processing software [Figures 2 and 3].<sup>[19]</sup>

### Digital-subtraction technique

The radiograph obtained at 6 months was subtracted from the one taken at the baseline by using commercially available



**Figure 1:** The *r* value in the formula  $1.12 \times r \times (\text{rotations per minute}/1000)^2$  is calculated from the central axis to the end of the centrifuge tube connected to the rotor. We have calculated this distance by adding the length of the inner tube placed in the centrifuge and the central axis to mouth distance



**Figure 2:** Pre- and post-treatment radiographs were subtracted in adobe Photoshop® to identify the areas of bone fill



image processing software (Adobe Photoshop® 6.0, Adobe Systems, San Jose, USA). To reduce the brightness and contrast variations, both images were adjusted based on the levels and the curves in the software. Before digital subtraction, both scans were moved in appropriate directions as needed, to reduce the geometric distortion. These images were then superimposed and subtracted by selecting the image>calculation>exclusion>new channel tools. The excluded residual bone layer was outlined by using the polygonal lasso tool and the layer was copied and saved as a separate joint photographic expert group document at low compression.

### Morphometric area analysis

After digital subtraction, the digitized and excluded residual bone layer was transferred to open source software for the area calculation (ImageJ®, Research Services Branch, NIH, and Bethesda, Maryland, USA). The layer was converted into a grayscale image, and the measurement scale was set to account for any magnification/reduction of the radiograph because of the RVG. The area of the layer was calculated (in mm<sup>2</sup>) by initially enclosing the entire area with the rectangular selection tool and then by using analyze > analyze particles tool.

### Statistical analysis

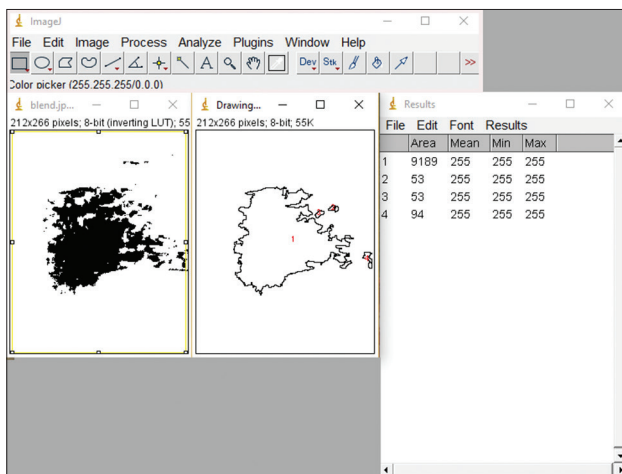
Data were analyzed by GraphPad Prism® software version 6.0 (GraphPad Software Inc., La Jolla, USA) and SAS software® version 9.3 versions (SAS, New Delhi, India). Data were summarized by mean ± standard deviation for continuous data and median ± inter-quartile range for score data. The comparison between different time points was made by analysis of one way repeated measures test and followed by *post hoc* test for score data. The comparison between two groups for repeated data was made by analysis of two way repeated measures test and followed by *post hoc* test. Spearman's Rho correlation test

was used to test the correlation between prognosis and the other variables. All the values of  $P < 0.05$  were considered as statistically significant, and  $<0.001$  were considered highly statistically significant.

## Results

All enrolled participants ( $n = 50$ ) completed the treatment and one participant each from the R-O and R-A groups was lost during follow-up because of geographic relocation. Mild postsurgical pain ( $n = 12$ ) and swelling ( $n = 21$ ) were the most common postsurgical complaints. However, no untoward effects or complications were observed. Forty-eight participants were included in the final analysis.

Table 1 shows the results of intergroup comparison of OD and fibrinogen content between all the groups. There were a significant intergroup difference in ODs of the L-PRF generated by the centrifuges ( $P = 0.021$ ). The highest OD was observed in L-PRF clots from the R-O group ( $2.00 \pm 1.65$ ). The ODs of L-PRF from the R-O, R-A, and C-O groups showed a significant to highly significant difference when compared to that of the OD of L-PRF from the D group. L-PRF clots from C-A group showed identical OD values to those from the D group ( $P = 0.152$ ). Modifying the centrifugation cycle resulted in highly significant reduction ( $P \leq 0.001$ ) in the OD of L-PRF in R-A and C-A when compared to that of the R-O and C-O groups, respectively. Similar trends were observed in the fibrinogen analysis between all the groups as well. The highest fibrinogen content was observed in L-PRF clots from the R-O group ( $19.01 \pm 5.87$  mg/dL). The fibrinogen content in L-PRF from the R-O, R-A, and C-O groups showed a significant to highly significant difference when compared to those from the D group ( $P = 0.032$ ). L-PRF clots from C-A group showed identical fibrinogen values to those from the D group ( $P = 0.232$ ). Modifying the centrifugation cycle resulted in highly significant



**Figure 3:** Morphometric area analysis was performed after digital subtraction and was calculated (in mm<sup>2</sup>) after converting the layer into a gray scale image in ImageJ®

**Table 1: Intergroup comparison of optical density and fibrinogen content between all groups**

Parameter	Group	Mean±SD	F	P
OD	D	0.84±0.32	1.892	0.021 <sup>a</sup>
	R-O	2.00±1.65 <sup>†</sup>		
	R-A	0.96±0.35 <sup>‡,†</sup>		
	C-O	1.56±0.82 <sup>†</sup>		
	C-A	0.88±0.67 <sup>++</sup>		
Fibrinogen (mg/dL)	D	7.39±1.12	2.362	0.032 <sup>a</sup>
	R-O	19.01±5.87 <sup>†</sup>		
	R-A	8.44±1.67 <sup>‡,†</sup>		
	C-O	13.72±7.22 <sup>†</sup>		
	C-A	7.74±8.23 <sup>++</sup>		

<sup>a</sup>Significant; <sup>†</sup>Highly significant and <sup>‡</sup>Significant when compared to the D Group; <sup>++</sup>Highly significant when compared to R-O Group; <sup>+++</sup>Highly significant when compared to C-O Group. D: DUO Quattro®; R: Remi 8C®; C: Remi C854®; O: Original and A: Adjusted protocol; OD: Optical density; SD: Standard deviation

reduction ( $P \leq 0.001$ ) in the fibrinogen content of L-PRF in R-A and C-A when compared to that of the R-O and C-O groups, respectively.

Table 2 summarizes the intergroup comparison of bone height and bone fill between all groups. L-PRF application resulted in significant bone height gain in all the groups ( $P = 0.029$ ). R-A group showed the maximum postoperative bone height gain ( $7.01 \pm 1.44$  mm) which was similar to the bone height gain observed in the D group ( $6.82 \pm 2.92$  mm) with no statistical significant difference between them ( $P = 0.361$ ). Bone height gain R-O, C-O, and C-A groups showed a significant to highly significant difference when compared to those from the D group. Modifying the centrifugation cycle resulted in highly significant ( $P \leq 0.001$ ) gain in bone height in R-A and C-A when compared to that of the R-O and C-O groups, respectively. L-PRF application resulted in significant bone height gain in all the groups ( $P = 0.030$ ), and R-A group showed maximum bone fill among all the groups ( $13.50 \pm 4.51$  mm<sup>2</sup>). D group showed significant to highly significant differences in bone fill when compared to that of the other groups. Modifying the centrifugation cycle resulted in highly significant ( $P \leq 0.001$ ) bone fill in R-A group when compared to that of the R-O group.

When the OD and the bone height/bone fill were compared to each other [Table 3], the correlations were as follows; there was a positive and significant correlation between OD and BH in the D ( $r = 0.3923$ ;  $P = 0.0293$ ) and R-A ( $r = 0.4831$ ;  $P = 0.0490$ ) groups. There was also a positive and significant correlation between OD and SF in the D ( $r = 0.2217$ ;  $P = 0.0476$ ) and C-A ( $r = 0.18561$ ;  $P = 0.0389$ ) groups.

## Discussion

Pinto *et al.*<sup>[2]</sup> have stated that centrifuge characteristics can directly impact the architecture, cell content, and the biologic signature of an L-PRF clot. At 2700 RPM, the two laboratory swing-out centrifuges used in this study, Remi 8C<sup>®</sup> and Remi C854<sup>®</sup>, generate a g-force of 1020 g and 1060 g, respectively. The ratio between the cellular elements and the fibrin matrix in L-PRF can vary depending on the centrifugal force used<sup>[7,13,20]</sup> and a higher centrifugal force can result in a thicker clot with fewer cellular elements. Modifying the centrifugation cycle resulted in highly significant reduction in the OD of L-PRF in both the centrifuges. Adjusting the RCF to 400 g by reducing the RPM results in less dense fibrin clot containing more cells<sup>[1,2,20]</sup> and may improve the physical and regenerative properties of the L-PRF.<sup>[1-3,7]</sup> L-PRF clots from the Remi C854<sup>®</sup> laboratory centrifuge with a RCF-adjusted protocol showed identical OD values to those generated from the centrifuge customized for L-PRF generation (DUO Quattro<sup>®</sup>). In the absence of a digital display, the tubes were run at a slightly lower RPM corresponding to the manual controls than the Remi

**Table 2: Intergroup comparison bone height and bone fill between all groups**

Parameter	Group	Mean±SD	F	P
BH (mm)	D	6.82±2.92	1.859	0.029 <sup>a</sup>
	R-O	5.22±3.28 <sup>‡</sup>		
	R-A	7.01±1.44 <sup>+</sup>		
	C-O	4.89±1.19 <sup>+</sup>		
	C-A	5.82±3.56 <sup>‡,++</sup>		
Bone fill (SF) (mm <sup>2</sup> )	D	12.32±5.31	3.182	0.030 <sup>a</sup>
	R-O	10.67±6.89 <sup>‡</sup>		
	R-A	13.50±4.51 <sup>‡,+</sup>		
	C-O	9.62±4.22 <sup>+</sup>		
	C-A	10.01±5.20 <sup>‡</sup>		

<sup>a</sup>Significant; <sup>+</sup>Highly significant when compared to R-O Group;

<sup>++</sup>Highly significant when compared to C-O Group; <sup>‡</sup>P value represents significant. D: DUO Quattro<sup>®</sup>; R: Remi 8C<sup>®</sup>; C: Remi C854<sup>®</sup>; O: Original and A: Adjusted protocol; BH: Bone height; SD: Standard deviation; SF: Sinus floor

**Table 3: The correlation between optical density versus bone height and bone fill using Spearman correlation**

Correlation	Group	Spearman's Rho	P
OD versus BH	D	0.3923	0.0293 <sup>a</sup>
	R-O	-0.23573	0.1346
	R-A	0.4831	0.0490 <sup>a</sup>
	C-O	0.78962	0.1466
	C-A	0.16882	0.0513
OD versus SF	D	0.2217	0.0476 <sup>a</sup>
	R-O	0.17847	0.6328
	R-A	0.84357	0.0671
	C-O	-0.27659	0.2383
	C-A	0.18561	0.0389 <sup>a</sup>

<sup>a</sup>Significant. D: DUO Quattro<sup>®</sup>; R: Remi 8C<sup>®</sup>; C: Remi C854<sup>®</sup>; O: Original and A: Adjusted protocol; OD: Optical density; BH: Bone height; SF: Sinus floor

8C<sup>®</sup> (1400 RPM vs. 1700 RPM) which may have resulted in a less dense L-PRF.

During L-PRF preparation, almost the entire soluble fibrinogen is transformed into insoluble fibrin that polymerizes into a stable three-dimensional matrix.<sup>[21]</sup> Rather than assaying only the zone of fibrin, sample preparation mimicked the actual procedure after centrifugation, that is., the lifting of clot from the collection tube and the separation of PRF from the red blood cell (RBC) base using scissors. In L-PRF, fibrinogen is undetectable or found in minute quantities,<sup>[22]</sup> but we were able to detect sub-optimal quantity of fibrinogen. The L-PRF clot used for fibrinogen assay had a part of the intermediate buffy-coat which can retain components such as fibrinogen even after centrifugation.<sup>[21,22]</sup> Contamination from the lower RBC-rich layer cannot be ruled out as well. Insoluble fibrinogen can lead to uncontrollable coagulation and unpredictable platelet activation.<sup>[22,23]</sup> Vibrations and excessive g-forces can also affect the subsequent fibrin polymerization<sup>[1,22,23]</sup>

and modifying the centrifugation cycle resulted in highly significant reduction ( $P \leq 0.001$ ) in the fibrinogen content of L-PRF in both the swing-out centrifuges, whereas L-PRF clots from Remi C854® centrifuge run with RCF-adjusted protocol showed identical fibrinogen content to those from the DUO Quattro® centrifuge ( $P = 0.232$ ). This reduction in fibrinogen content caused by reducing the RPM may improve the biological and physical signature of L-PRF.<sup>[21-23]</sup>

PRF is an acceptable regenerative material in sinus floor elevation<sup>[8-14]</sup> and L-PRF application resulted in significant gain in bone height in all the groups ( $P = 0.029$ ). L-PRF from Remi 8C® centrifuge with the RCF-adjusted protocol resulted in maximum postoperative bone height gain ( $7.01 \pm 1.44$  mm) which was higher than the outcome from the DUO Quattro® centrifuge ( $6.82 \pm 2.92$  mm). Centrifuges producing high G-forces cause vibrations which can result in the production of aberrant cell population and an inconsistent fibrin matrix and reduction in these forces can offset this effect.<sup>[1,2,13]</sup> The Remi 8C® centrifuge is the heaviest centrifuge of the three and is least at risk of vibrations during centrifugation.<sup>[1]</sup> Reducing the RCF to 400 g by following a RCF-adjusted protocol or by using a fixed-angle centrifuge (DUO Quattro® centrifuge) which is essentially free from parasitic vibrations<sup>[1,2]</sup> also results in optimum release of growth factors<sup>[2,13,21]</sup> contributing to the gain in bone height in this study. When the OD of the clot was correlated to the bone height obtained, there was a positive and significant correlation between OD of L-PRF and bone height when DUO Quattro® centrifuge ( $r = 0.3923$ ;  $P = 0.0293$ ) and Remi 8C® centrifuge with RCF-adjusted protocol ( $r = 0.4831$ ;  $P = 0.0490$ ) were used. Fibrin matrix density is an important determinant in the process of tissue regeneration,<sup>[24,25]</sup> and reparative processes occur more slowly in denser clots;<sup>[24,25]</sup> L-PRF produced at higher g-forces results in a denser clot.<sup>[7,24,25]</sup> The Remi 8C® centrifuge with RCF-adjusted protocol runs at lower RPM resulting in less dense clots and better reparative processes contributing to improved gain in bone height. Similar trends were observed for bone fill as well with L-PRF from Remi 8C® centrifuge with RCF-adjusted protocol showing maximum bone fill among all the groups ( $13.50 \pm 4.51$  mm<sup>2</sup>). When OD of the clot was correlated to the bone fill obtained, there was a positive and significant correlation between OD of L-PRF and bone height when DUO Quattro® centrifuge ( $r = 0.2217$ ;  $P = 0.0476$ ) and Remi C854® centrifuge with adjusted protocol ( $r = 0.18561$ ;  $P = 0.0389$ ) were used. This centrifuge was run at a lower RPM (~1400) resulting in clots which are less dense.

This study has some limitations worth noting. We have assumed 400 g of RCF as the standard for L-PRF based on the pioneering studies on PRF generation.<sup>[1,2,13,21]</sup> The PRF protocol is an open-access system<sup>[26]</sup> and the ideal RCF to generate PRF currently seems to vary from 400 g<sup>[26]</sup> to 800 g.<sup>[27]</sup> Regardless of the RCF considered as normal, the RPM must be lower than 2700 RPM to achieve a lower

G-force<sup>[28]</sup> which is the very essence of this study. The  $r$ -value in the formula  $1.12 \times r \times (\text{RPM}/1000)^2$  is generally calculated from the central axis to the end of the centrifuge tube (outer tube) connected to the rotor at 90° angulation to the same axis. We have calculated this distance by adding the length of the inner tube (the tube into which blood is drawn into and placed in the outer centrifuge tube) and the central axis to tube-mouth distance. This distance is shorter by 3–5 mm as there is a stopper which balances the outward force exerted on the inner tube during centrifuging.<sup>[6]</sup> This calculation, we feel is more specific as blood collects at the end of the inner tube during centrifugation and is easy to record. Remi C854® has no provision to display the RPM; we had to assume a value of 1400 RPM for 14 min by analyzing the graduations on the analog control.

PRF is a product from a centrifuge and understanding the principles of centrifugation is essential as the RCF can affect the quantity, quality, and the regenerative capacity of the PRF matrix. A reduction in RCF by adjusting the RPM had a positive influence on the regenerative potential of L-PRF in BAOSFE procedure. Critical issues behind using a centrifuge for PRF generation such as the choice of rotor, RCF-RPM relationship and even using terms such as “PRF” and “NonPRF centrifuge” to clearly identify centrifuges ideal for generating PRF must be addressed to generate further clarity on the effectiveness of these devices.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

### References

1. Dohan Ehrenfest DM, Pinto NR, Pereda A, Jiménez P, Corso MD, Kang BS, et al. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane. *Platelets* 2018;29:171-84.
2. Pinto N, Pereda A, Jiménez P, Corso M, Kang BS, Wang HL, et al. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors and fibrin architecture of a leukocyte – And platelet-rich fibrin (L-PRF) clot and membrane. Part 2: Macroscopic, photonic microscopy and scanning electron microscopy analysis of 4 kinds of L-PRF clots and membranes. *POSEIDO* 2014;2:141-54.
3. Chandra V, Varanasi V, Boya S. Effect of centrifuge type and rotation dynamics on PRF generation. *Int Poster J Dent Oral Med* 2017;19:1110.
4. Johnson C, Attridge T, Smith H. Advantages of the fixed-angle rotor for the separation of density-labelled from unlabelled proteins by isopycnic equilibrium centrifugation. *Biochim Biophys Acta* 1973;317:219-30.
5. Shivashankar VY, Johns DA, Vidyath S, Sam G. Combination of platelet rich fibrin, hydroxyapatite and PRF membrane in the management of large inflammatory periapical lesion. *J Conserv Dent* 2013;16:261-4.



6. Majekodunmi SO. A review on centrifugation in the pharmaceutical industry. *Am J Biomed Eng* 2015;5:67-78.
7. Peck MT, Hiss D, Stephen L. Factors affecting the preparation, constituents, and clinical efficacy of leukocyte and platelet – Rich fibrin (L-PRF). *S Afr Dent J* 2016;71:298-302.
8. Chen Y, Cai Z, Zheng D, Lin P, Cai Y, Hong S, *et al.* Inlay osteotome sinus floor elevation with concentrated growth factor application and simultaneous short implant placement in severely atrophic maxilla. *Sci Rep* 2016;6:27348.
9. Inchingolo F, Tatullo M, Marrelli M, Inchingolo AM, Scacco S, Inchingolo AD, *et al.* Trial with platelet-rich fibrin and bio-oss used as grafting materials in the treatment of the severe maxillary bone atrophy: Clinical and radiological evaluations. *Eur Rev Med Pharmacol Sci* 2010;14:1075-84.
10. Neamat AH, Ali SM, Boskani SW, Mahmud PK. An indirect sinus floor elevation by using piezoelectric surgery with platelet-rich fibrin for sinus augmentation: A short surgical practice. *Int J Case Rep Images* 2017;8:380-4.
11. Narang S, Parihar AS, Narang A, Arora S, Katoch V, Bhatia V, *et al.* Modified osteotome sinus floor elevation using combination platelet rich fibrin, bone graft materials, and immediate implant placement in the posterior maxilla. *J Indian Soc Periodontol* 2015;19:462-5.
12. Toffler M, Toscano N, Holtzclaw D. Osteotome-mediated sinus floor elevation using only platelet-rich fibrin: An early report on 110 patients. *Implant Dent* 2010;19:447-56.
13. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, *et al.* Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:299-303.
14. Belouka SM, Strietzel FP. Sinus floor elevation and augmentation using synthetic nanocrystalline and nanoporous hydroxyapatite bone substitute materials: Preliminary histologic results. *Int J Oral Maxillofac Implants* 2016;31:1281-91.
15. Padilla S, Garcia de Castro A, Garzon-Gutierrez A, Benito L, Enciso S. Novel nanostructured Zn-substituted monetite based biomaterial for bone regeneration. *J Nanomed Nanotechnol* 2015;6:325.
16. Saifer A, Newhouse A. Photometric microdetermination of plasma fibrinogen with a thrombin-ninhydrin procedure. *J Biol Chem* 1954;208:159-79.
17. Harmoinen A, Uppa H, Lehtinen M, Jokela H, Koivula T. Fibrinogen: A comparison of an immunoturbidimetric method with four conventional methods. *Clin Lab Haematol* 1983;5:101-7.
18. Gabbasov ZA, Popov EG, Gavrilov IYu, Pozin EYa. Platelet aggregation: The use of optical density fluctuations to study microaggregate formation in platelet suspension. *Thromb Res* 1989;54:215-23.
19. Yellarthi PK, Rampalli VC, Anumala N, Devaraju RR. Assessment of bone-fill following regenerative periodontal therapy by image subtraction using commercially available software. *J Indian Acad Oral Med Radiol* 2014;26:13-8.
20. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, *et al.* Advanced platelet-rich fibrin: A new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol* 2014;40:679-89.
21. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, *et al.* Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:e51-5.
22. Hatakeyama I, Marukawa E, Takahashi Y, Omura K. Effects of platelet-poor plasma, platelet-rich plasma, and platelet-rich fibrin on healing of extraction sockets with buccal dehiscence in dogs. *Tissue Eng Part A* 2014;20:874-82.
23. Araki J, Jona M, Eto H, Aoi N, Kato H, Suga H, *et al.* Optimized preparation method of platelet-concentrated plasma and noncoagulating platelet-derived factor concentrates: Maximization of platelet concentration and removal of fibrinogen. *Tissue Eng Part C Methods* 2012;18:176-85.
24. Weibrich G, Kleis WK, Hafner G, Hitzler WE. Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. *J Craniomaxillofac Surg* 2002;30:97-102.
25. Voss A, McCarthy MB, Allen D, Cote MP, Beitzel K, Imhoff AB, *et al.* Fibrin scaffold as a carrier for mesenchymal stem cells and growth factors in shoulder rotator cuff repair. *Arthrosc Tech* 2016;5:e447-51.
26. Dohan Ehrenfest DM, Andia I, Zumstein MA, Zhang CQ, Pinto NR, Bielecki T, *et al.* Classification of platelet concentrates (Platelet-rich plasma-PRP, platelet-rich fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: Current consensus, clinical implications and perspectives. *Muscles Ligaments Tendons J* 2014;4:3-9.
27. Kobayashi M, Kawase T, Horimizu M, Okuda K, Wolff LF, Yoshie H, *et al.* A proposed protocol for the standardized preparation of PRF membranes for clinical use. *Biologicals* 2012;40:323-9.
28. Choukroun J, Ghanaati S. Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: The first introduction to the low speed centrifugation concept. *Eur J Trauma Emerg Surg* 2018;44:87-95.