Original Article

Controversies Related to Scientific Report Describing G-Forces from Studies on Platelet-Rich Fibrin: Necessity for Standardization of Relative Centrifugal Force Values

Richard Miron[1], Joseph Choukroun[2,3], Shahram Ghanaati[2]

[1]Department of Periodontology, University of Bern, Bern, Switzerland, [2]Department of Maxillofacial, Clinic for Maxillofacial and Plastic Surgery, Johann Wolfgang Goethe University, Frankfurt Am Main, Germany, [3]Pain Clinic, Nice, France

Abstract

Leukocyte and platelet-rich fibrin (PRF), a second-generation platelet concentrate has been the focus of intensive research endeavors over the last 2 decades. Over the years, numerous reports have however failed to accurately report g-force values which have caused considerable confusion in the field. These values have since been re-transcribed incorrectly in many studies moving forward, and this article aims to address this topic to avoid further confusion in the field. We address several reports in which PRF centrifugal g-forces have been calculated at the PRF clot (referred to as relative centrifugal force [RCF]-clot) as opposed to the international standard method described at the bottom of centrifugation tubes (RCF-max). We further highlight how RCF-clot is not only a deviation from the standard international method used to report g-force values, but one subject to significant error owing to centrifugation time, patient hematocrit levels, initial volume of blood collected, and other factors. For these reasons and those further reported throughout this article, we address this controversy in detail to avoid further confusion regarding the report of g-force values in future studies. Furthermore, we propose a standardization regarding the accurate report of g-force values in future studies investigating PRF at the RCF-max.

Keywords: L-PRF and A-PRF, L-platelet-rich fibrin, platelet-rich fibrin

Introduction

Leukocyte and platelet-rich fibrin (L-PRF), a second-generation platelet concentrate invented by Joseph Choukroun in 2001, has been the focus of intensive research endeavors over the past 2 decades.[1] Over the years, numerous reports, including those published initially by Choukroun *et al*. from 2001 to 2006 as well as others, have in fact misrepresented g-force values. These values have since been re-transcribed in a number of studies moving forward by many authors causing considerable confusion in the field.[2-5] Unfortunately, various research groups have continued to inaccurately misrepresent g-forces, and this article aims to clarify these miscalculations. One of the confusions that has been created in the field over the years is that various authors have reported centrifugal g-force at the PRF clot (referred to as relative centrifugal force (RCF)-clot – location at which the PRF clot is formed), whereas others have utilized the international standard method to report g-force calculated at the bottom of centrifugation tubes (RCF-max). Furthermore, initial studies published by Choukroun *et al*. and Dohan *et al*. in 2006 reported RCF-min values calculated at the upper portion of PRF tubes [Table 1]. This has caused considerable confusion for the readers since the majority of these studies have also not reported the location at which g-force values were actually calculated.

In this article, we highlight how "RCF-clot" is not only a deviation from the standard method used to report g-force

Address for correspondence: Dr. Richard Miron, Department of Periodontology, College of Dental Medicine, University of Bern, Bern 3010, Switzerland.
E-mail: richard.miron@zmk.unibe.ch

Access this article online

Quick Response Code:

Website: www.cellsindentistry.org

DOI: 10.4103/GFSC.GFSC_23_18

How to cite this article: Miron R, Choukroun J, Ghanaati S. Controversies related to scientific report describing g-forces from studies on platelet-rich fibrin: Necessity for standardization of relative centrifugal force values. Int J Growth Factors Stem Cells Dent 0;0:0.

at the bottom of centrifugation tubes [Figure 1][6] but also one subject to significant error and variation owing to centrifugation time, patient hematocrit levels, initial volume of blood collected, and other factors. For these reasons and those further reported throughout this article, we propose that a consensus be reached to standardize g-force values based on international centrifugation guidelines that have been referenced at the bottom of the centrifugation tubes (RCF-max) [Figure 1]. Although the g-forces applied throughout the centrifugation tube vary significantly as a result of tube angulation, rotor size, and/or bucket sizes/types, we demonstrate through several illustrations and photos the inability to accurately report g-force values at the RCF-clot. We further show how recent work by Cortellini *et al*. 2018 has in fact incorrectly reported g-force at the clot (RCF-clot).[3] This article aims to clarify these misunderstandings and proposes that a consensus be reached regarding more accurate means to report g-force values in future studies investigating PRF.

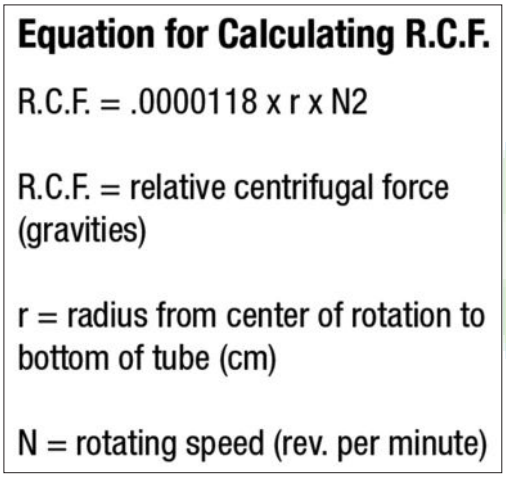

Figure 1: Reports on international guidelines to calculate g-force on centrifugation machines are standardized using the base of the centrifugation tubes were relative centrifugal force-max is located. Reprinted from https://druckerdiagnostics.com/g-force-calculator

Relative centrifugal force: Definition and calculation

One of the areas that has led to great confusion over the years is that RCF values have been calculated at various regions along a centrifugation tube. For these reasons, it is important to have a basic understanding of RCF values including calculations to obtain RCF-min, RCF-max, and RCF-av. The formula for RCF is as follows: RCF = 11.18 x r x $(N/1000)^2$ where N is revolutions per minute and r is the radius in mm.[6] Therefore, the radius plays a multiplying role on the relative centrifugal g-force. As the radius is increased, g-force values are exponentially larger. As shown in Figure 2a, representing a centrifuge (which typically range in angulation from 30 to 45 degrees), RCF values can easily be doubled between the RCF-min and RCF-max based on this increased radius distance [Table 1]. RCF-av represents the average g-force throughout an entire centrifugation tube. One of the confusions that has been created and expressed later in this article is that many articles related to PRF have often utilized RCF-min or RCF-max values without reporting exactly where the RCF values were calculated. This has caused significant difficulty for researchers to further reproduce data, and a general lack of understanding has been created as a result. To further complicate these matters, RCF-clot calculations were also introduced in the late 2000s which further confused many readers since these g-force values were never transparently provided by authors [Figure 2b]. For reasons later expressed in this article, RCF-clot has several drawbacks and limitations with inaccuracies in RCF calculations reported in the literature since the exact location of the clot can never be standardized at the same location, especially when liquid PRF versus PRF clots are created. While internationally, the standard method to report g-forces are calculated at the RCF-max, below we demonstrate many deviations from these standard methods with respect to PRF articles over the years.

Initial studies investigating leukocyte and platelet-rich fibrin: A range of studies reporting RPM and time from 2500 to 3000 RPM for 10–12 min

A recent letter to the editor of the Journal of Periodontology

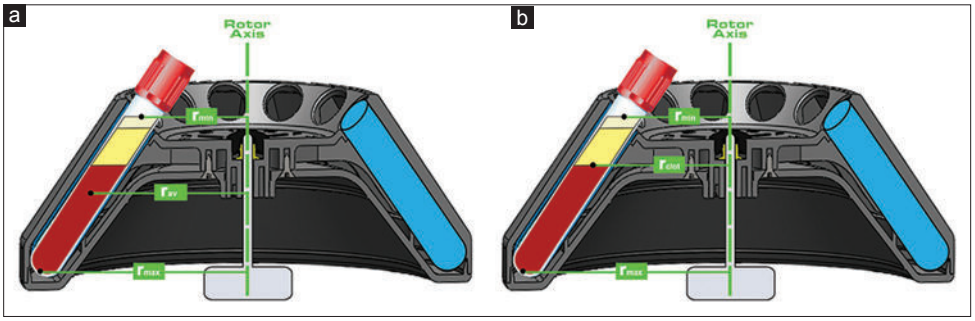

Figure 2: (a) the impact of the radius on relative centrifugal force values depending on the location at which relative centrifugal force values are calculated. The standard calculation for relative centrifugal force is: relative centrifugal force = 11.18 × r × (N/1000) 2 where N is revolutions per minute and r is the radius in mm. Significant differences are therefore reported between relative centrifugal force-min and relative centrifugal force-max. (b) Furthermore, within the PRF field, relative centrifugal force-clot values were introduced in the late 2000s further complicating the field since the majority of studies have not reported the location at which relative centrifugal force values were derived

Table 1: Representative table demonstrating inaccurately referenced g-force values to leukocyte and platelet-rich fibrin from original articles published in 2006

Device	RCF reported in study	RCFmin	RCFclot	RCFav	RCFmax
IntraSpin™		40 mm	50 mm	60 mm	80 mm
Rotor angulation: 33°					
Distance to rotor in mm					
Studies by Pinto and Quirynen					
2700 RPM	**408**	326	**408**	489	653
Choukroun *et al*. 2006					
2500 RPM	**280**	**280**	349	419	559
Dohan *et al*. 2006					
3000 RPM	**400**	**402**	503	604	805

While it has previously been reported that original articles describing g-forces of L-PRF at 400 g (in reference to the letter to the editor by Pinto and Quirynen),7 RCF-values in these original articles were actually calculated at RCF-min and not RCF-clot. Furthermore, the article referenced by Choukroun *et al*. did not report a g-force of ~400 g and instead reported a g-value of 280g. Notice that the g-forces referenced in these 2 original articles were both calculated at the RCF-min and not at the RCF-clot. These studies have an RCF-max value ranging from 559 g to 805 g. The L-PRF g-force utilized in our study performed by Fujioka-Kobayashi *et al*. utilized an RCF-max value of 708 g, well within this reference range. Confusion has been created since many studies have reported g-force values at different locations over the years either reported as RCF-min, RCF-max (standard in the field) or RCF-clot. L-PRF: Leukocyte and Platelet-Rich Fibrin, RCF: Relative centrifugal force

by Pinto and Quirynen[7] stated the following "In the materials and methods section of the above-mentioned article, L-PRF is processed at 708 RCF [2700 RPM in Duo Process centrifuge, Table 1 and Figure 2], which is far above the standard protocol of the original L-PRF (±408 RCF, Dohan *et al*. 2006, Choukroun *et al*. 2006)." In this statement, they utilize two studies published by Dohan *et al*. and Choukroun *et al*. in 2006 as having initially utilized g-forces of 408 RCF to produce PRF [Table 1].[8,9]

Actually, neither of these two studies actually reported an RCF-clot value of 408 RCF, and we wish to clarify the reported g-forces in these initial L-PRF articles. The article by Dohan *et al*. 2006, actually reported a centrifugation speed and time of 3000RPM for 10 min in a PC-O2 centrifuge (This centrifuge is dimensionally the same as the current centrifugation system offered today by Intralock, USA). In this study by Dohan *et al*. 2006, a difference in both centrifugation time and RPMs from the described protocols utilized in studies by Pinto and Quirynen (2700RPM and 12 min). The referenced 400 g force was at the RCF-min and not the RCF-clot [Table 1]. Furthermore, while attempts to report original L-PRF RCF values were described at 408 g and cite accordingly the work by Choukroun *et al*. in 2006, it is of significance to note that this study actually reported a g-force value of 280 g (not ~400 g). In the study by Choukroun *et al*. 2006, a centrifugation time and speed was reported at 2500RPM for 10 min (RCF-min 280 g, RCF-clot 349 g, and RCF-max at 559 g). In response to the comments made by these authors,[7] it is imperative that both these studies be carefully reviewed to avoid further confusion and kindly ask that future work citing these original L-PRF protocols be transcribed with a higher degree of scientific accuracy in the future. Furthermore, both these studies reported g-forces at the RCF-min and not at the RCF-clot or the RCF-max. In fact, numerous articles have miscalculated g-forces and/or time over the years, and it is imperative that this information be communicated to the reader to better characterize and report RCF values in future studies related to PRF.

Table 2 lists a number of studies reporting the g-forces and times over the years investigating L-PRF. These studies which are referenced since 2006 by Choukroun *et al*. are the focus of initial studies aimed at removing anticoagulants from blood concentrates. Unfortunately, a misrepresentation of the centrifugal g-forces is common in a number of these studies as pointed out in Table 2. Reports from the literature have shown that the majority of these initial studies which utilize the PC-O2 centrifuge (originally process for PRF, France, now IntraSpin by Intralock, USA) have commonly reported either inaccurate g-forces or RPM values over the years. Table 2 further demonstrates that numerous reports utilizing the same centrifugation system with the same settings (time and RPM) have also commonly reported different centrifugation g-forces (without mentioned if the g-force values are being calculated at the RCF-min, RCF-clot, or RCF-max), creating much confusion in the literature. Many studies have since re-transcribed these inaccurate values (with little knowledge on the accurate means to report g-force) which have not only led to confusion, but also difficulty in effectively advancing the field. Our understanding today regarding the effects of centrifugation g-force on PRF-based matrices has greatly been enhanced with improvements made utilizing the low-speed centrifugation concept (LSCC). Nevertheless, we believe that today a consensus must be reached to effectively report g-force values in future studies in an accurate and scientific manner and further advance the field.

Table 2: Scientific literature reporting leukocyte and platelet-rich fibrin at different spin cycles utilizing various centrifugations speeds and time with the according to reported g-forces

Author and year	Title	RPM and time	Reported g-force
Choukroun *et al*., 2006[8]	PRF: A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift	2500 RPM for 10 min	280 g
One of the first articles on PRF describing a centrifugation protocol of 2500 RPM for 10 min on a PCO2 machine (same rotor size utilized on the current Intra-Lok IntraSpin system). Today, it is known that the g-force reported in this study was, in fact, inaccurate since it was calculated at RCF-min. Based on the current rotor size of 80 mm, it is now known that this should have been represented at 559 g-force at RCFmax or 349 g at the RCFclot at 50 mm (both different from the proposed value of 400 g cited by Pinto and Quirynen)			
Dohan *et al*., 2006[9]	PRF: A second-generation platelet concentrate. Part III: Leukocyte activation: A new feature for platelet concentrates?	3000 RPM for 10 min	Approximately 400 g
Though this article was published in a group of five articles with Dohan and Choukroun as first authors in 2006, it is interesting to note that the reported centrifugation protocols and g-forces vary between the publications. Had this original protocol utilized the RCF-clot at 50 mm - this study would have utilized an RCF value of 503 g-force at the clot (reported in the study at~400 g however at the RCFmin). Had this report calculated the g-force based on the international guidelines at the bottom of the centrifugation tube, the reported g-force would be~805 g - higher than that reported in the study by Fujioka-Kobayashi *et al*. 2017 reported at 708 g			
Simonpieri *et al*. 2009[10]	The relevance of Choukroun's PRF and metronidazole during complex maxillary rehabilitations using bone allograft. Part II: implant surgery, prosthodontics, and survival	RPM not reported for 12 min	400 g
One of the difficulties with this study is the lack of reported RPMs with only the g-force reported. Though without question the reported g-force is a much more important reported value when compared to RPMs (owing to the potential differences in rotor sizes), it is impossible to know based on the info provided by these authors if the g-force was calculated at RCF-max or RCF-clot. This is precisely why a consensus regarding future reported g-forces needs to be standardized to avoid future confusion			
Dohan *et al*., 2009[11]	*In vitro* effects of Choukroun's PRF on human gingival fibroblasts, dermal prekeratinocytes, preadipocytes, and maxillofacial osteoblasts in primary cultures	RPM not reported for 12 min	400 g
Three years' following his first publication, Dohan *et al*. changed his protocol to 12 min and 400 g in Chourkoun's PRF. These studies utilized RCF-clot to report g-force			
Su *et al*., 2009[12]	*In vitro* release of growth factors from PRF: A proposal to optimize the clinical applications of PRF	2700 RPM for 12 min	700 g
One of the first studies where the g-force was in fact accurately calculated at the bottom of centrifugation tubes on an IntraLok centrifuge machine spinning at the most reported L-PRF protocols of 2700 RPM for 12 min. This study currently utilized RCF-max at the bottom of the centrifugation tubes			
Mazor *et al*., 2009[13]	Sinus floor augmentation with simultaneous implant placement using Choukroun's PRF as the sole grafting material: A radiologic and histologic study at 6 months	RPM not reported for 12 min	400 g
Dohan *et al*., 2010[14]	Three-dimensional architecture and Cell composition of a Choukroun's PRF Clot and membrane	3000 RPM for 10 min	Not reported
Once again, although many years had passed since the L-PRF was introduced, studies by leaders in the field including by Dohan Ehrenfest *et al*. continued to report Choukroun's PRF at various centrifugation speeds and times as highlighted in the above study			
Even 10 years' following the discovery of L-PRF by Choukroun *et al*., reports in the literature still vary regarding the "standard" L-PRF protocol. Here, the protocols were reported at 3000 RPM for 10 min - similar to the reports published by Dohan *et al*. in 2006			
Simonpieri *et al*., 2011[15]	Simultaneous Sinus-Lift and implantation using microthreaded implants and leukocyte- and PRF as Sole Grafting Material: A 6-year experience	RPM not reported for 12 min	400g
Simonpieri et al. 2012 reported an RCF value of 400g, however failed to report where this RCF value was calculated nor reported the RPM values or the centrifugation machine utilized			
Lekovic *et al*., 2012[16]	PRF and bovine porous bone mineral vs. PRF in the treatment of intrabony periodontal defects	RPM not reported for 10 min	1000 g
In this study, Lekovic *et al*. utilized a Labofuge 300; Heraus GmbH, Hanau, Germany centrifuge and did not report RPM but did report an RCF value of 1000g. It remains unknown if g-force was actually spun that high, was calculated at the bottom of centrifugation tubes or the RCF-clot; however, a lack of description further complicates the reproducibility of the study			
Kazemi and Fakhrjou 2015[17]	L-PRP Versus (L-PRF) For Articular Cartilage Repair of the Knee: A comparative evaluation in an animal model.	3000 RPM for 10 min	Not reported
Once again, reports in 2015 still utilize L-PRF at various centrifugation with no report on the RCF values produced in this study			
Pinto *et al*., 2017[18]	An Innovative Regenerative Endodontic Procedure Using Leukocyte and Platelet-rich Fibrin Associated with Apical Surgery: A Case Report	2700 RPM for 12 min	Approx. 400 g
This study utilized an approximate RCF value of 400 g in reference to the work by Dohan *et al*. in 2006, however, calculations of RCF-values are performed at the RCF-clot as opposed to the RCF-min (as was done in the Dohan *et al*. study from 2006) or by utilizing RCF-max which is the international standard			
Kobayashi *et al*., 2017[19]	Optimized Platelet-Rich Fibrin with the Low-Speed Concept: Growth factor release, biocompatibility, and cellular response	2700 RPM for 12 min	708 g

Contd...

Table 2: Contd...

Author and year	Title	RPM and time	Reported g-force
While the RCF-max value of 708 g falls within the range of original L-PRF values in studies by Choukroun *et al*. and Dohan *et al*. in 2006, the authors incorrectly transcribed an RPM value of 2700 - most commonly utilized on the IntraSpin system			
Afat *et al*., 2017[20]	Effects of leukocyte- and PRF alone and combined with hyaluronic acid on pain, edema, and trismus after surgical extraction of impacted mandibular third molars	3000 rpm for 10 min	Not reported
Even in 2017, several still utilize the initial published values by Dohan *et al*. at 3000 rpm for 10 min. No report on which centrifuge was utilized and no report on the g-forces are provided			
Nizam *et al*., 2018[21]	Maxillary sinus augmentation with leukocyte and platelet-rich fibrin and deproteinized bovine bone mineral: A split-mouth histological and histomorphometric study	Nüve Laboratory Equipment, NF200, Ankara, Turkey) for 12 min (RPM not reported)	400 g
Since another PRF centrifuge was utilized with no reported RPM, it is unknown where g-force was calculated in such a study - whether RCF-clot or RCF-max			
Tabrizi *et al*., 2018[22]	Does PRF increase the stability of implants in the posterior of the maxilla? A split-mouth randomized clinical trial	Intra-Lock system centrifuged at 28,000 rpm for 12 min	Not reported
Although g-force was not reported, the authors state using an Intra-Lock system whereby spun at 28,000 RPM. While these authors are sure one extra 0 was accidentally added, this highlights the lack of attention paid during the peer-review process warranting better standardization regarding the reported RPM/g-force for all studies			
Dohan *et al*., 2018[4]	The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of an L-PRF clot and membrane	2700 RPM for 12 min	400 g
While Dohan *et al*. reported in his original studies 3000 RPM for 10 min, here a common value of 2700 RPM is reported for 12 min with a PRF-clot value of 400 g. Note this g-force is equivalent to ~650 g at the bottom of this same centrifugation tube			
Meschi *et al*., 2018[23]	Root-end surgery with leukocyte- and PRF and an occlusive membrane: A randomized controlled clinical trial on patient' quality of life	Centrifuged at 702 RCF in an Intra-Lock® International (Florida, USA) centrifuge	702 RCF
Work by Meschi *et al*. utilize the Intra-Lock L-PRF system but accurately report RCF values at~700 g force at the base of the centrifugation tube			
Cortellini *et al*., 2018[3]	Leukocyte- and PRF block for bone augmentation procedure: A proof-of-concept study	2700 RPM for 3 min	408 g
In this study by Cortellini *et al*. in which both Pinto and Quirynen are co-authors, the authors introduce a very short centrifugation time; however, fail to recalculate the new RCF-clot owing to the new location of the PRF-layer separation. Inaccuracies such as these are subject to major confusion in the field. These miscalculations will be later better addressed in this article.			

*Comments are made certain studies to highlight their relevance. PRF: Platelet-rich fibrin, RCF: Relative centrifugal force, L-PRF: Leukocyte and Platelet-Rich Fibrin, L-PRP: Leukocyte and Platelet-Rich Plasma

The conclusions from Table 2 illustrates that

- Original g-forces for L-PRF were not reported correctly – neither at the RCF-clot nor at the RCF-max
- Much variation in reported g-forces are found in the literature – even for studies utilizing the exact same centrifuge with the same centrifugation settings (speed and time)
- Both Choukroun *et al*. as well as Dohan *et al*. have modified their centrifugation protocols over the years to improve their formulations. Both have changed from reporting RCF-min values to either RCF-clot/RCF-max values
- L-PRF protocols have varied in the literature from 2500 to 3000 RPM at a spin cycle ranging from 10 to 12 min
- The articles cited by Pinto and Quirynen describing the initial g-forces being valued at 400 g (Dohan *et al*. 2006, Choukroun *et al*. 2006) were in fact not utilizing and/or reporting either of these values neither at the RCF-clot nor the RCF-max.

Internationally g-force RCF values are calculated at the bottom of centrifugation tubes which corresponds with the maximal force applied to tubes

Several authors are correct in their calculations describing the effects of g-force based on rotor radius and effectively report RCF-min, RCFave, RCF-max, and RCF-clot. However, these authors deviate from reporting g-forces in their studies based on international centrifugation standards that effectively report g-force at the bottom of centrifugation tubes**.** While we certainly agree that the g-force applied to the PRF clot are different from RCF-max, below we highlight how reports from from these methods not only deviates from standard reports in the field but is subject to inaccuracies leading to future confusion in the field.

The problem with reporting only the g-force applied to either g-force max (end of tube) versus at g-force clot (platelet-rich fibrin-clot)

As example, to illustrate the drawback with reporting g-force at RCF-clot as presented by Pinto and Quirynen,[7] it is important

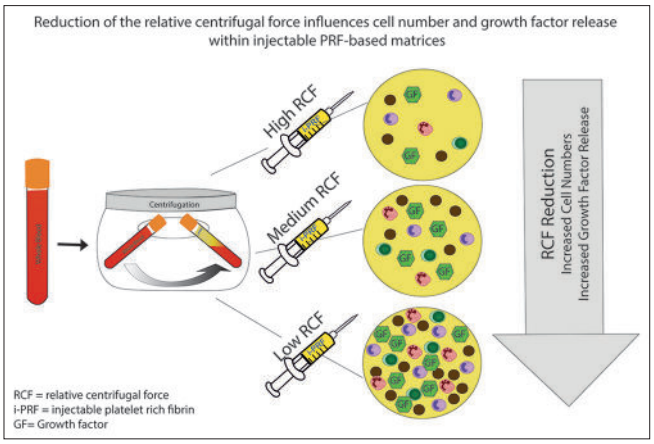

Figure 3: The effects of centrifugation time and speed on PRF-based matrices. As has now been shown in many studies, as g-force is increased, PRF clots are typically larger with less cell content/growth factors owing to the higher g-forces. Owing to recent modification to centrifugation speed and time, numerous studies have now shown that the low-speed centrifugation concept leads to smaller clots that contain more cells and growth factors. Reprinted with permission from Weng *et al*. 2018

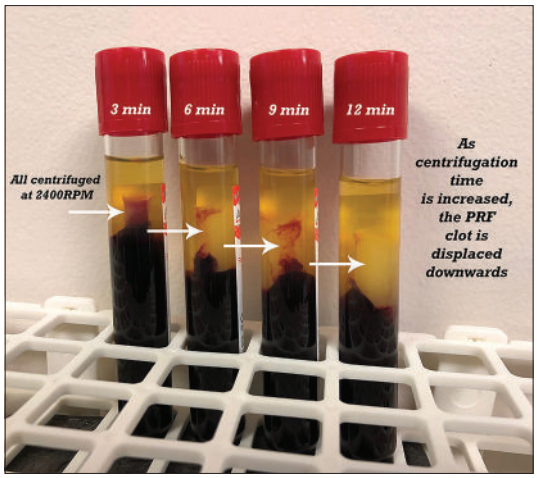

Figure 4: The effects of centrifugation time on the displacement of the PRF clot. Though all tubes were centrifuged at 2400 RPM on a Duo Centrifuge (relative centrifugal force-max = 708 g), notice how following 3, 6, 9, and 12 min, the clot is displaced downward. In such a situation, relative centrifugal force-clot cannot be standardized owing to the different location of the PRF clot, affecting the radius at which relative centrifugal force-clot is calculated

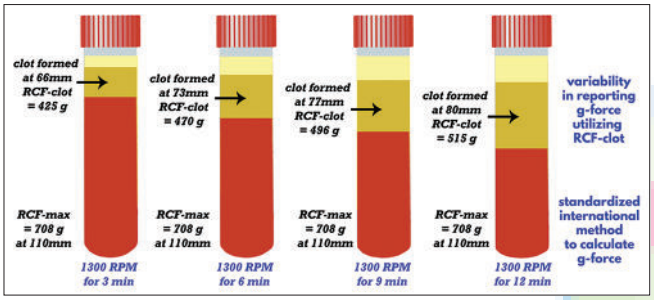

Figure 5: Illustration demonstrating once again the inability to accurately report relative centrifugal force-clot to report g-forces. While each of these tubes is centrifuged once again at 2400 RPM on a Duo Centrifuge for 3, 6, 9, and 12 minutes, notice how the relative centrifugal force-clot values change owing to the increased radius of the PRF clot. Therefore, though each of these tubes is centrifuged at the exact same speed (2400 RPM), relative centrifugal force-clot values range from 425–515 g. International guidelines recommend displaying g-force values at relative centrifugal force-max (708 g). Utilizing the relative centrifugal force-max values accurately reports g-forces in each of these scenarios

to first understand the effect of centrifugation force (speed and time) on the size separation between blood layers. As centrifugation speed increases (RCF/g-force is increased), the layer separation is increased. As previously reported, an increase in centrifugation time and speed has the effect of increasing the size of the PRF clot and reducing the content of cells and growth factors [Figure 3]. This concept was introduced in 2014 as the LSCC and has since been the basis of an array of studies since then demonstrating the positive effect of lower centrifugation speeds on PRF-based scaffolds.[24-31]

These articles all report the g-force at the bottom of centrifugation tubes based on international centrifugation guidelines and not at the clot since there are inherited errors with reporting RCF-clot. To illustrate the issue with reporting g-force at the radius where the RCF-clot is located, Figure 4

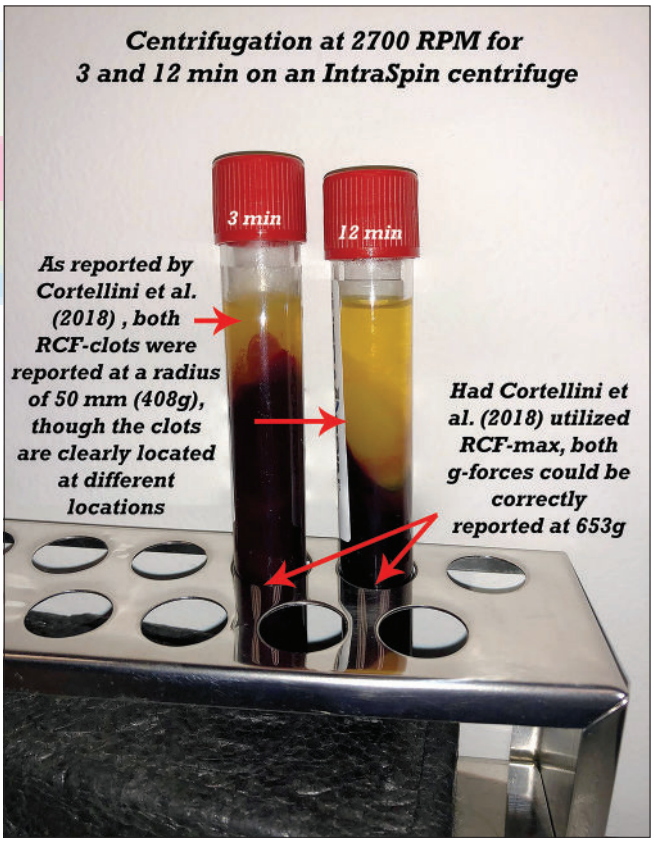

Figure 6: The effects of centrifugation time on location of PRF clots as reported by Cortellini *et al*. utilizing an IntraSpin centrifuge spun at 2700 RPM for 3 and 12 minutes. The authors report the exact same g-force at the relative centrifugal force-clot which assumed a 50 mm radius. Notice however that owing to the different clot locations, the error was created in their report owing to having calculated the clot to report relative centrifugal force values. Instead, had the authors reported g-force at the bottom of centrifugation tubes based on international guidelines, both values would accurately be reported at 653 g

demonstrates the effect of time on the position of PRF clots formed following spin cycles of 3 min, 6 min, 9 min, and 12 min at 2400 RPM (708 g-at the RCF-max-on a Duo Centrifuge, Process). Notice how though centrifugation was carried out at exactly the same speed, the position at which the clot is located is pushed downwards owing to the longer centrifugation cycle. Since RCF-clot utilizes the radius where the clot is located, g-force must then be reported differently. When g-force is calculated based on international guidelines at the bottom of the centrifugation tubes (110 mm), 708 g is utilized in all cases irrespective of the centrifugation time. This is most representative granted that all clots were created at the same centrifugation speed using the same machine and tubes.

Figure 5 illustrates this concept further. Based on the method of calculating RCF at the clot (RCF-clot[7]), even though RPMs remains unchanged, owing to an increase in centrifugation time, the PRF clot is displayed downward within the centrifugation tube affecting the radius at which the centrifugation g-force is calculated. This highlights the fact that though centrifugation is carried out at the exact same speed in RPMs (2400 RPM), the RCF-clot, in fact, varies based on centrifugation time (from 425–515 g). Owing to this discrepancy, international guidelines to report g-forces on a centrifugation system have been reported at the bottom of the centrifugation tubes to best report g-forces more accurately.

The major limitation with reporting the g-force at the relative centrifugal force-clot and its associated scientific inaccuracies

We would also like to further point to two recent articles published by Pinto and Quirynen and Cortellini *et al*. that has further created additional confusion utilizing RCF-clot as a means to represent g-force.[3,7] On a standard L-PRF clot produced with an IntraSpin device, the authors report that the clot is located at approximately a 50 mm radius (subject to variation). Nevertheless, based on their calculations, this reported g-force equates to a 408 g-force at the clot (reported as RCF-clot). Figure 6 illustrates the separation of layers at 2700 RPM when a 3 min protocol centrifuged is utilized on the IntraSpin centrifuge versus one that is centrifuged for 12 min. Notice the drastic difference in locations of the clots/separation layers based on this reported shorter centrifugation period.

The study by Cortellini *et al*. inaccurately reported a g-force of 408 g at the RCF-clot with the assumption that after 3 min, the location of the clot would be precisely the same (50 mm from the radius) as the location of the clot produced after a 12 min centrifugation period as the authors have reported in previous studies.[3] As can clearly be observed in Figure 6, these clots are nowhere near located at the same placement within the tubes owing to the reduction in centrifugation time (not speed). Had the authors used the RCF-max to report their data based on international centrifugation standards/guidelines, whether they centrifuged for 2700 for 3 min or 12 min, RCF-max would always be precisely reported at 653 g in either scenario. Owing to the different locations of the RCF-clot, the authors should have reported a different g-force based on their clot no longer being located at a 50 mm radius. Instead, the authors assumed/utilized the same g-force equivalent to the clot location after a 12-minute spin cycle. This is not simply a miscalculation in their study but more importantly the limitation with reporting RCF values at the clot. For these reasons, it is imperative that the scientific community be well informed of these limitations of reporting g-force values at RCF-clot to avoid future error in studies investigating platelet concentrations. At the very least, reporting exactly where the RCF values are being derived is a must.

Furthermore, it should also be noted that the location of the PRF clot may vary rather significantly from patient to patient depending on individual hematocrit levels of that patient. For these reasons, it is most logical that the standard method to report g-force in a consistent manner is to report g-force at the bottom of the centrifugation tubes. Although we do agree that the g-force which is applied to the clot may vary depending on the angulation of tubes and rotor size, reporting g-force at the bottom of the tubes represents a more standardized and effective way to report g-force and one in which is subject to NO variation. Nevertheless, at the bare minimum, we insist that g-force calculations be provided when authors wish to deviate from these standard methods.

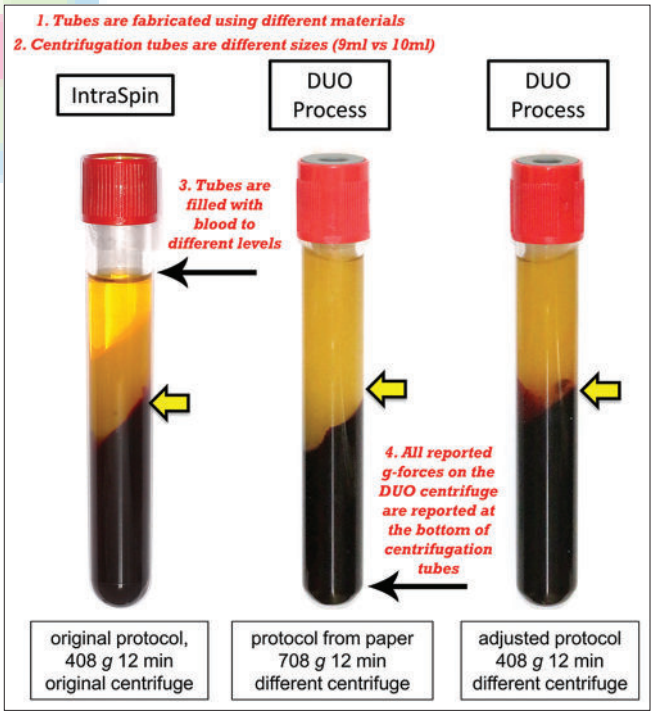

Figure 7: Although recent attempts have been made comparing L-PRF protocols, a direct comparison cannot be made owing to the number of variables introduces between systems. The tubes are (1) fabricated using different materials (plastic glass-coated tubes versus glass tubes), (2) are of different sizes (9 mL vs. 10 mL), (3) are filled to different initial blood levels, (4) are centrifuged and different machines with different settings, radiuses, and angulation of tubes

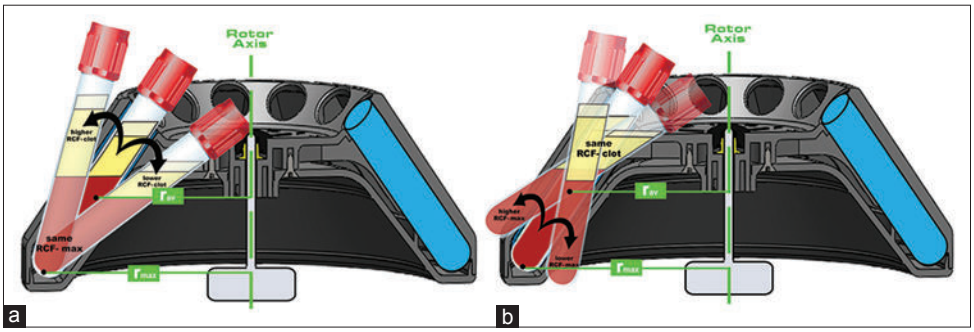

Figure 8: Illustration further demonstrating how no 2 centrifuges can report the same relative centrifugal force-clot/relative centrifugal force-max values if the angulation of tubes is different. Notice in (a), even if the relative centrifugal force-max is identical, a change of tube angulation will result in a different relative centrifugal force-clot. (b) demonstrates how even if relative centrifugal force-clot is the same, relative centrifugal force max is different and the applied g-forces throughout that tube will be also subject to variation. Therefore, when performing scientific studies investing the effects of centrifugation speed and time, it is most scientifically logical to utilized the same centrifugation device to minimize additional factors that may affect the results

It is not scientifically accurate to compare the effects of g-force or time on the quality of platelet-rich fibrin clots by utilizing different centrifugation systems – instead, the same centrifugation system should be utilized for such comparative studies

In the letter by Pinto and Quirynen,[7] several remarks regarding the effects of centrifugation speeds (RCF-values) and reference comparative studies between 2 centrifugation systems was also made. Though we can appreciate the effort placed into such comparative studies, the goal of the work by Fujioka-Kobayashi *et al*.[19] was to scientifically study the effect of centrifugation speed and time on growth factor release and PRF-based matrices. To do so, the authors had no intention of comparing two different centrifugation systems as the study would be deemed scientifically less relevant owing to the increased variability. As depicted by Pinto and Quirynen in their letter,[7] the authors display a comparison between clots formed from 2 entirely different systems [Figure 7]. By comparing results obtained from two different systems, too many variables are introduced including:

- Centrifugation tubes are fabricated from different materials (glass-coated plastic versus glass)
- Centrifugation tubes are different sizes (9 ml vs. 10 ml)
- Centrifugation tubes are filled with blood to different levels
- Centrifuges are fabricated using different materials
- Centrifugation rotors are fabricated with different sizes and rotor radiuses
- Centrifugation rotors are angled differently.

To specifically investigate the effects of centrifugation time and speed (g-force) on PRF-based matrices, it is logical and most scientifically accurate to minimize these number of variables. For these reasons, the study by Fujioka-Kobayashi *et al*. utilized one centrifugation machine with one sized rotor at the same angulation utilizing the same tubes filled to the same levels for all procedures.[19] Naturally, the entire clot is harvested and utilized. As the entire PRF clot was utilized for each experimental group – with the L-PRF clots being slightly larger owing to the higher g-forces utilized (708 g as reported at the base of the PRF centrifuge and not at the clot). As shown in Table 1, the g-force utilized to report L-PRF has ranged quite significantly even in original studies first published by Choukroun *et al*. in 2006 (RCF-max = 559 g, 2500 RPM for 10 min) to Dohan *et al*. 2006 (RCF-max 805 g, 3000 RPM for 10 min). The RCF-max, we have chosen in our study, corresponds to a g-force within this range of original studies (centrifuged at 2400 RPM-not 2700 RPM-which corresponds to an RCF-max value of 708 g).[19] The use of L-PRF in our study represents a centrifugation protocol that is based on high centrifugation speeds and times that were based on original protocols developed by Choukroun *et al*. which fit perfectly within the ranges of these original studies. The issue raised from the letter by Pinto and Quirynen is that these authors assumed that these original articles calculated RCF values at the RCF-clot (which is not the case) or that the study by Fujioka-Kobayashi *et al*. calculated g-force at RCF-clot (which is also not the case). This is why today we believe that all g-forces and RCF-values should be clearly reported at the RCF-max in future studies to avoid future confusion in the field and to improve scientific accuracy in future publications.

As the science has drastically evolved over the years and our understanding concerning the effects of PRF-based protocols have been optimized more recently, the effects of lowering centrifugation speed and time have more favorably shown that a reduction in g-force (RCF values can be calculated at RCF-min, RCF-clot or RCF-max) has led to clots that contain higher cellular content and growth factor release owing to the LSCC [Figure 3].[24-31] The article published in the Journal of Periodontology by Fujioka-Kobayashi *et al*. highlights how a reduction in not only centrifugation speed but also in time led to increased growth factor release and also improved cellular behavior further adding to the available literature supporting the LSCC.[19]

Biological differences of platelet-rich fibrin clots based on centrifugation parameters

It is also important to note that each centrifuge, owing to the

differences in rotor size, angulation of tubes, composition of tubes, and vibration of the centrifuge will result in a different biological PRF clots. Several authors are entirely correct in stating that "even a centrifugation at the same RPM will exert different centrifugal forces if the centrifuge rotors have different radius sizes, bucket types, or bucket sizes." We highlight in Figure 8 of this article, how even at the same g-force at RCF-clot or RCF-max, different g-forces will inevitably be reported at either the clot/end of tube if the tubes are angled differently. Therefore, while the original article by Fujioka-Kobayashi was designed to compare the effects of centrifugation speed and time on PRF-based matrices and their effect on cellular responses, it must also be reported that a PRF clot centrifuged on one machine at a similar g-force when compared to another may behave entirely differently. Therefore, a concept of biological differences of each PRF clot fabricated on various machines is certainly subject to differences. While variability between centrifuges may exist, these authors again wish to address strictly scientific facts:

1. PRF clots fabricated at lower centrifugation speeds and times improve growth factor release and cellular behavior owing to higher cellular content and growth factor accumulation. This has now been shown in many studies on PRF published since 2014, but also more recently with PRP
2. PRF clots fabricated at lower centrifugation speeds and times are smaller in size, however, contain more platelets and leukocytes, as well as with more growth factor release
3. The g-force calculated at the fibrin clot (RCF-clot) is subject to change owing to the centrifugation time – even when centrifuged at the exact same speed. For these reasons, this method of reporting g-force is inferior in accuracy and not commonly reported internationally
4. The g-force calculated at the end of the centrifugation tubes (RCF-max) is not subject to these differences – hence, why it is internationally utilized to report g-forces
5. The most recent report published by Cortellini *et al*. incorrectly assumed that the RCF clot was measured at a radius of 50 mm whether the PRF-clot was centrifuged for 3 min or 12 min. Evidence from Figure 5 clearly demonstrates that these clots are not located at all in the same location, and the authors introduce new error into their calculations of g-force. This should be avoided in future studies
6. To investigate the effects of centrifugation speed or time on PRF-based matrices, researchers in the field are encouraged to design experimental protocols that utilize precisely the same machine, with the same rotor sizes, utilizing the same tubes, to minimize unnecessary additional parameters into their study. Comparative studies whereby centrifugation machines are compared, with different centrifugation speeds, radiuses, angulations, with different tubes and even different spin cycles and times are not ideal to report scientifically the effects of relative g-force or time since too many variables are introduced into these studies.

Concluding statements

When evaluating and definitively when comparing medical devices and protocols in this arena, factual accuracy is of utmost importance. We, therefore, ask kindly that authors working in the field re-evaluate the articles published by Choukroun *et al*. and Dohan *et al*. in 2006 having reported initial g-forces of ~400 g. We show within this article that neither of these original studies have utilized the proposed centrifugation g-force reported in recent papers – neither at RCF-clot or RCF-max (one reported at 280–2500 rpm for 10 min, the other at 3000RPM for 10 min). We also ask kindly that all authors working in the PRF or PRP field reconsider reporting their g-forces at the bottom of centrifugation tubes since the use of RCF-clot for reporting g-forces both deviates from international standards but more importantly introduces new variability and error in reported values [Figure 5]. Authors that wish to investigate the effects of centrifugation speed or time in future publications should also utilize an appropriate experimental protocol. Such studies should ideally be designed using the same centrifugation machine, at the same centrifugation radius, with the same tube angulations, fabricated of the same composition, filled with blood to the same levels and utilizing the same centrifugation tubes. In the future, we sincerely hope this article provides the basis for more accurate scientific documentation/publication of PRF studies moving forward, and we sincerely hope a consensus be reached, and a stricter peer-review process be warranted regarding the report of g-force values in future scientific publications related to PRF.

Financial support and sponsorship

Nil.

Conflicts of interest

Disclosure: Richard Miron and Shahram Ghanaati report no conflict of interest in relation to any centrifuge or related devices. Joseph Choukroun is the owner of PRF for Process and a research fellow of the FORM-lab, in which objective and independent research and non-product-name-based research such as the invention of LSCC (low speed centrifugation concept) has been performed. Richard Miron is an independent researcher. All PRF-related work from Miron´s lab and FORM-lab and their associates have been performed aiming to use reproducible equipment for data acquisition, which enable to generate objective and transparent research, in order to better understand the regenerative potential of PRF.

References

1. Choukroun J, Adda F, Schoeffler C, Vervelle A. Platelet Rich Fibrin: An opportunity in perio-implantology. Implantodontie 2001.
2. Castro AB, Meschi N, Temmerman A, Pinto N, Lambrechts P, Teughels W, *et al.* Regenerative potential of leucocyte- and platelet-rich fibrin. Part A: Intra-bony defects, furcation defects and periodontal plastic surgery. A systematic review and meta-analysis. J Clin Periodontol 2017;44:67-82.
3. Cortellini S, Castro AB, Temmerman A, Van Dessel J, Pinto N, Jacobs R, *et al.* Leucocyte- and platelet-rich fibrin block for bone augmentation procedure: A proof-of-concept study. J Clin Periodontol

2018;45:624-34.
4. 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.
5. Pinto NR, Ubilla M, Zamora Y, Del Rio V, Dohan Ehrenfest DM, Quirynen M, *et al.* Leucocyte- and platelet-rich fibrin (L-PRF) as a regenerative medicine strategy for the treatment of refractory leg ulcers: A prospective cohort study. Platelets 2018;29:468-75.
6. Beck DJ, Bibby BG. A centrifugal technique of measuring food retention. J Dent Res 1961;40:148-60.
7. Pinto N, Quirynen M. Letter to the editor regarding fujioka-kobayashi *et al*. 2017 (JOP-16-0443.R1). J Periodontol 2018. Doi: 10.1002/JPER.18-0175. [Epub ahead of print].
8. 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.
9. 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.
10. Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM. The relevance of choukroun's platelet-rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part II: Implant surgery, prosthodontics, and survival. Implant Dent 2009;18:220-9.
11. Dohan Ehrenfest DM, Diss A, Odin G, Doglioli P, Hippolyte MP, Charrier JB, *et al. In vitro* effects of choukroun's PRF (platelet-rich fibrin) on human gingival fibroblasts, dermal prekeratinocytes, preadipocytes, and maxillofacial osteoblasts in primary cultures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:341-52.
12. Su CY, Kuo YP, Tseng YH, Su CH, Burnouf T. *In vitro* release of growth factors from platelet-rich fibrin (PRF): A proposal to optimize the clinical applications of PRF. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:56-61.
13. Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD, Dohan Ehrenfest DM, *et al.* Sinus floor augmentation with simultaneous implant placement using Choukroun's platelet-rich fibrin as the sole grafting material: A radiologic and histologic study at 6 months. J Periodontol 2009;80:2056-64.
14. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three-dimensional architecture and cell composition of a choukroun's platelet-rich fibrin clot and membrane. J Periodontol 2010;81:546-55.
15. Simonpieri A, Choukroun J, Del Corso M, Sammartino G, Dohan Ehrenfest DM. Simultaneous sinus-lift and implantation using microthreaded implants and leukocyte- and platelet-rich fibrin as sole grafting material: A six-year experience. Implant Dent 2011;20:2-12.
16. Lekovic V, Milinkovic I, Aleksic Z, Jankovic S, Stankovic P, Kenney EB, *et al.* Platelet-rich fibrin and bovine porous bone mineral vs. Platelet-rich fibrin in the treatment of intrabony periodontal defects. J Periodontal Res 2012;47:409-17.
17. Kazemi, D. and A. Fakhrjou, Leukocyte and Platelet Rich Plasma (L-PRP) Versus Leukocyte and Platelet Rich Fibrin (L-PRF) For Articular Cartilage Repair of the Knee: A Comparative Evaluation in an Animal Model. Iran Red Crescent Med J 2015;17:e19594.
18. Pinto N, Harnish A, Cabrera C, Andrade C, Druttman T, Brizuela C. An Innovative Regenerative Endodontic Procedure Using Leukocyte and Platelet-rich Fibrin Associated with Apical Surgery: A Case Report. J Endod 2017;43:1828-34. Doi: 10.1016/j.joen.2017.07.002.
19. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J, *et al.* Optimized platelet-rich fibrin with the low-speed concept: Growth factor release, biocompatibility, and cellular response. J Periodontol 2017;88:112-21.
20. Afat İM, Akdoğan ET, Gönül O. Effects of leukocyte- and platelet-rich fibrin alone and combined with hyaluronic acid on pain, edema, and trismus after surgical extraction of impacted mandibular third molars. J Oral Maxillofac Surg 2018;76:926-32.
21. Nizam N, Eren G, Akcalı A, Donos N. Maxillary sinus augmentation with leukocyte and platelet-rich fibrin and deproteinized bovine bone mineral: A split-mouth histological and histomorphometric study. Clin Oral Implants Res 2018;29:67-75.
22. Tabrizi R, Arabion H, Karagah T. Does platelet-rich fibrin increase the stability of implants in the posterior of the maxilla? A split-mouth randomized clinical trial. Int J Oral Maxillofac Surg 2018;47:672-5.
23. Meschi N, Fieuws S, Vanhoenacker A, Strijbos O, Van der Veken D, Politis C, *et al.* Root-end surgery with leucocyte- and platelet-rich fibrin and an occlusive membrane: A randomized controlled clinical trial on patients' quality of life. Clin Oral Investig 2018;22:2401-11.
24. 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.
25. Dohle E, El Bagdadi K, Sader R, Choukroun J, James Kirkpatrick C, Ghanaati S, *et al.* Platelet-rich fibrin-based matrices to improve angiogenesis in an *in vitro* co-culture model for bone tissue engineering. J Tissue Eng Regen Med 2018;12:598-610.
26. El Bagdadi K, Kubesch A, Yu X, Al-Maawi S, Orlowska A, Dias A, *et al.* Reduction of relative centrifugal forces increases growth factor release within solid platelet-rich-fibrin (PRF)-based matrices: A proof of concept of LSCC (low speed centrifugation concept). Eur J Trauma Eur J Trauma Emerg Surg. 2017. Doi: 10.1007/s00068-017-0785-7. [Epub ahead of print].
27. 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.
28. Kubesch A, Barbeck M, Al-Maawi S, Orlowska A, Booms PF, Sader RA, *et al.* A low-speed centrifugation concept leads to cell accumulation and vascularization of solid platelet-rich fibrin: An experimental study *in vivo*. Platelets. 2018:1-12. Doi: 10.1080/09537104.2018.1445835. [Epub ahead of print].
29. Wend S, Kubesch A, Orlowska A, Al-Maawi S, Zender N, Dias A, *et al.* Reduction of the relative centrifugal force influences cell number and growth factor release within injectable PRF-based matrices. J Mater Sci Mater Med 2017;28:188.
30. Cabaro S, D'Esposito V, Gasparro R, Borriello F, Granata F, Mosca G, *et al.* White cell and platelet content affects the release of bioactive factors in different blood-derived scaffolds. Platelets 2018;29:463-7.
31. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, *et al.* Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Investig 2016;20:2353-60.