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REVIEW ARTICLE

Optimization of platelet-rich fibrin

Richard J. Miron[1] | Masako Fujioka-Kobayashi[2] | Anton Sculean[1] | Yufeng Zhang[3]

[1]Department of Periodontology, University of Bern, Bern, Switzerland

[2]Department of Oral and Maxillofacial Surgery, School of Life Dentistry at Tokyo, The Nippon Dental University, Tokyo, Japan

[3]Department of Oral Implantology, University of Wuhan, Wuhan, China

Correspondence
Richard J. Miron, Department of Periodontology, University of Bern, Bern, Switzerland.
Email: richard.miron@zmk.unibe.ch and rick@themironlab.com

1 | INTRODUCTION

Platelet concentrates, including platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), have been utilized in regenerative medicine for nearly three decades owing to their ability to rapidly secrete autologous growth factors and ultimately speed wound healing.[1,2] Many years ago, Marx and colleagues[3] proposed that by concentrating platelets utilizing a centrifugation device, growth factors derived from blood could be collected from a platelet-rich plasma layer and later utilized in surgical sites to promote local wound healing.[1,2] Today, it has been well established that platelet concentrates act as a potent mitogen capable of the following (Figure 1):

- Accelerating the revascularization of tissues (angiogenesis)
- Acting as a potent recruitment agent of various cells, including stem cells
- Inducing the prompt multiplication of various cell types found in the human body (proliferation) (QR Code 1 SCAN ME)

In fact, the field has become so widespread that several textbooks have been dedicated to the technology, and an entire issue in Periodontology 2000 has been devoted precisely to this topic.[4] Over the years, many improvements have been made to optimize PRF production (QR Code 2 SCAN ME). However, to date, a number of clinicians produce PRF with little knowledge of basic principles, using various laboratory centrifuges and test tubes without a proper understanding of their optimization. Therefore, to improve clinical outcomes, this overview article aims to fill this void and address the recent common errors made by clinicians.

First, a general understanding regarding differences between rotations per minute (RPM) and relative centrifugal force (RCF; g-force) will be thoroughly explained. Then, a discussion of the low-speed centrifugation concept, fixed angle vs. horizontal centrifugation, and protocols dedicated to maximizing platelet concentrations will be described in detail. Thereafter, the importance of chemically modified PRF tubes without the addition of chemical additives, as well as the regulation of temperature to induce/delay clotting, will be thoroughly explained. This article is the first of its kind summarizing all recent literature on PRF, and it is designed to help clinicians optimize PRF generation.

2 | PROBLEMS IN THE LITERATURE BETWEEN REPORTS OF RPM VERSUS RCF (G-FORCE)

One of the most common errors made by clinicians in clinical practice is the lack of general understanding of the differences between RPM and RCF. A very clear understanding of the calculations needed to convert rotations per minute (RPM) into relative centrifugal force (RCF, aka g-force) is provided here utilizing simple and straightforward formulas.

Unfortunately, with the desire to create commercial profitably within companies, much debate and confusion has also been created, leading to not only confusion in the field but also, more importantly, thousands of clinicians not utilizing appropriate centrifugation protocols to produce PRF. Even worse, numerous scientific publications

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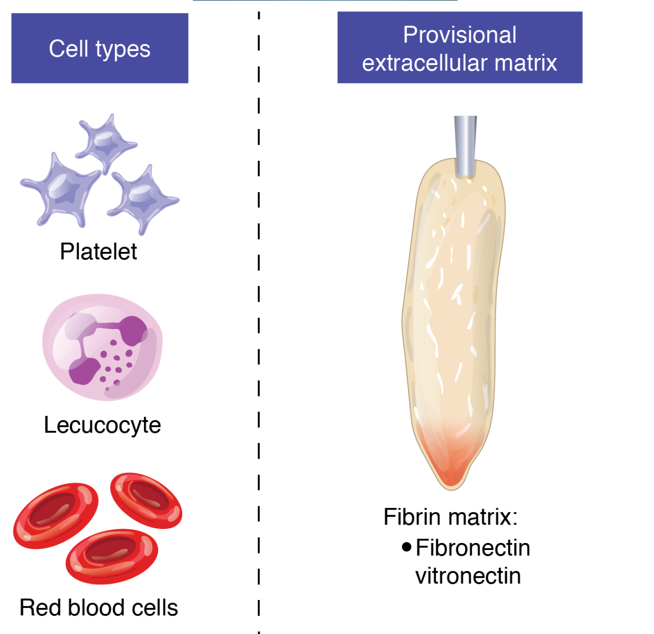

FIGURE 1 Three main components of PRF all derived naturally from the human body. These include (1) cells (platelets, leukocytes, and red blood cells), (2) a provisional three-dimensional extracellular matrix scaffold fabricated from autologous fibrin (including fibronectin and vitronectin), and (3) a wide array of over 100 bioactive molecules, including most notably PDGF, TGF-beta, VEGF, IGF, and EGF. Adapted with permission from Miron et al.[38]

that are well cited in the literature from nearly two decades ago have actually failed to accurately report g-force values in their studies, which has further led to considerable confusion in the field.[5] If company X reports that the optimized PRF protocol is produced at a 2700 RPM protocol for 12 min, this can only be utilized on a system with exactly that rotor diameter and angulation. Any deviation from this sizing will change RCF/g-force values applied to those cells, leading to unevenly distributed platelets and growth factors; ultimately, both platelets and growth factors will be completely lost until the protocol is corrected.

3 | UNDERSTANDING RCF

The goal of centrifugation as a whole is simply to apply relative force in a circular fashion to separate layers based on their density. Centrifugation systems have been utilized for decades successfully and are considered standard equipment in the majority of medical hospitals and research laboratories. In basic laboratory sciences, RCF values (and not RPM values) are always reported in scientific literature.

The goal of reporting RCF values in the literature is very simple: an RCF/g-force value allows for the application of centrifugation conditions irrespective of laboratory, country, or rotor size of the centrifugation device utilized. This is an internationally recognized way to transmit working conditions from one colleague to another for reproducibility purposes. Simply put, during the spin cycle, the further away an object is from its spinning axis/rotor, the more g-force it receives during rotation (Figure 2). One illustration that addresses this point fully is one commonly used in education. For example, imagine holding a child close to you and spinning them in circles near your body at one rotation per second. That child would receive a certain g-force. Imagine now holding that child on a long rope and spinning/lassoing him or her around a large room with the

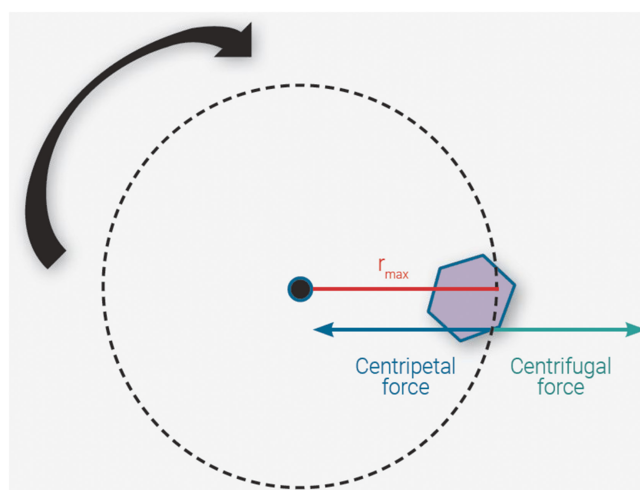

FIGURE 2 Schematic illustration of the centrifugation process during the rotation of an object (red symbol) and the resulting centrifugal force (green arrow) and centripetal force (blue arrow) in relation to the maximum radius (orange line).

same speed of one rotation per second. Naturally, that child would be traveling at a much greater velocity and subject to much higher g-forces. Therefore, the report of rotations per minute (RPM) commonly utilized in PRF centrifugation parameters is entirely useless, much like informing a parent that a child will enjoy spinning at one rotation per second. The precise radius is absolutely needed to better understand the speed and gravitational force exerted on a sample, and any change to that radius will certainly affect its experienced gravitational force.

The entire purpose of reporting actual RCF values is to be able to relate the g-force received on that object to another operator in another part of the world that may have a centrifugation device with a larger or smaller radius. With adequate reports of RCF values,

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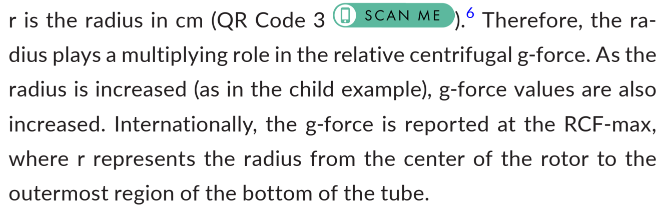
any colleague could adapt appropriate centrifugation conditions to reproduce the experiment. Thus, reporting RPM values for the production of PRF is entirely useless, as it can only be utilized specifically for one device utilized in those experiments. Any deviation from that device produces errors unless the RCF is reported, and the conditions are appropriately corrected.

The formula for RCF is a relatively simple one as follows: $RCF = 11.18 \times r \times (N/1000)^2$ where N is revolutions per minute and



r is the radius in cm (QR Code 3 SCAN ME).[6] Therefore, the radius plays a multiplying role in the relative centrifugal g-force. As the radius is increased (as in the child example), g-force values are also increased. Internationally, the g-force is reported at the RCF-max, where r represents the radius from the center of the rotor to the outermost region of the bottom of the tube.

Unfortunately, over the years, there have been many errors in the report of RPM and/or RCF values in scientific publications.[5] Furthermore, RCF/RPM errors have been incorrectly retranscribed in many newer publications in recent years. Entire companies have attempted to mask these parameters for profitability of their company, which has created further confusion in the field, ultimately leading to many clinicians being unsure how to optimize their own centrifugation devices. Sadly, many patients have received substandard treatment care with PRF as a result.

4 | CONSENSUS REPORT FROM 2019 ON STANDARDIZATION OF THE REPORT OF RCF VALUES: RECAP OF THE WORK BY MIRON, PINTO, QUIRYNEN, AND GHANAATI[7]

Owing to the reporting issues of centrifugation parameters, in 2019, a consensus report was written among several international colleagues to address past issues. The summary from this consensus article briefly proposed that in the future, all articles on PRF report the following parameters[7]:

- Dimensions of the rotor (radius at the clot and end of the tube).
- Rotor angulation for the tube holder.
- Revolutions per minute (RPM) and time.
- RCF value calculated at either the RCF-min, RCF-clot, or RCF-max.
- Composition and size of tubes utilized to produce PRF.
- Centrifuge model utilized.

By simply stating each of these parameters, further research in the field could be better standardized and reproduced. Effectively, should proper parameters not be followed accordingly, the production process of PRF will be different, and the quality of the fibrin mesh, as well as the concentration of cells and growth factors, will not be accurate or comparable. Consequently, much gained scientific knowledge has been lost over the years.

5 | THE LOW-SPEED CENTRIFUGATION CONCEPT

In the early 2000s, work began on producing PRF with the main aim of removing anticoagulants from PRP since it is well known that clotting is one of the first and most important steps in healing.[4] Thus, the first version of PRF, also known as leukocyte and platelet-rich fibrin (L-PRF), aimed at rapidly separating cell layers (platelets/leukocytes from red blood cells) prior to clotting. Following the standard 10–12 min spin cycle, a resulting PRF clot was formed.

Following over a decade of extensive clinical and basic in vitro research with the original L-PRF protocol, it was discovered in 2014 that centrifugation carried out at these initially relatively high centrifugation speeds (~700 g – utilized in L-PRF protocols) led to the great majority of leukocytes located either at the buffy coat zone (between the red blood cell layer and the upper plasma layer) or more commonly at the bottom of centrifugation tubes (Figure 3).[8] It was discovered that longer and higher centrifugation speeds led to more cells being pushed further down the centrifugation tube (a major disadvantage, since PRF is collected from the upper portion of PRF tubes).

Pioneering research led to the development of an advanced platelet-rich fibrin (A-PRF) protocol whereby lower centrifugation speeds (~200 g) led to a higher accumulation of platelets and leukocytes more evenly distributed throughout the upper PRF layers (Figure 4).[8] These newer protocols more favorably led to a higher

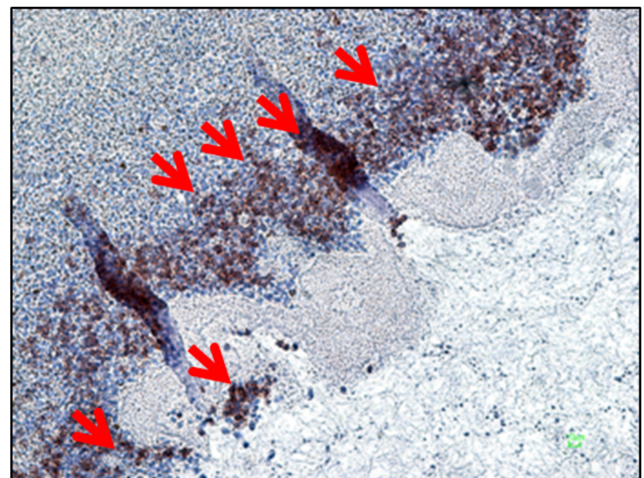


FIGURE 3 Histologic observation of leukocytes following centrifugation. The resulting white blood cells have been shown to be contained basically in the layers between the plasma PRF layer and the red blood cell clot. This finding demonstrated quite clearly that the g-force was excessive, necessitating the development of newer protocols aimed at improving the retention of leukocytes within the PRF matrix. Reprinted with permission from Ghanaati et al.[8]

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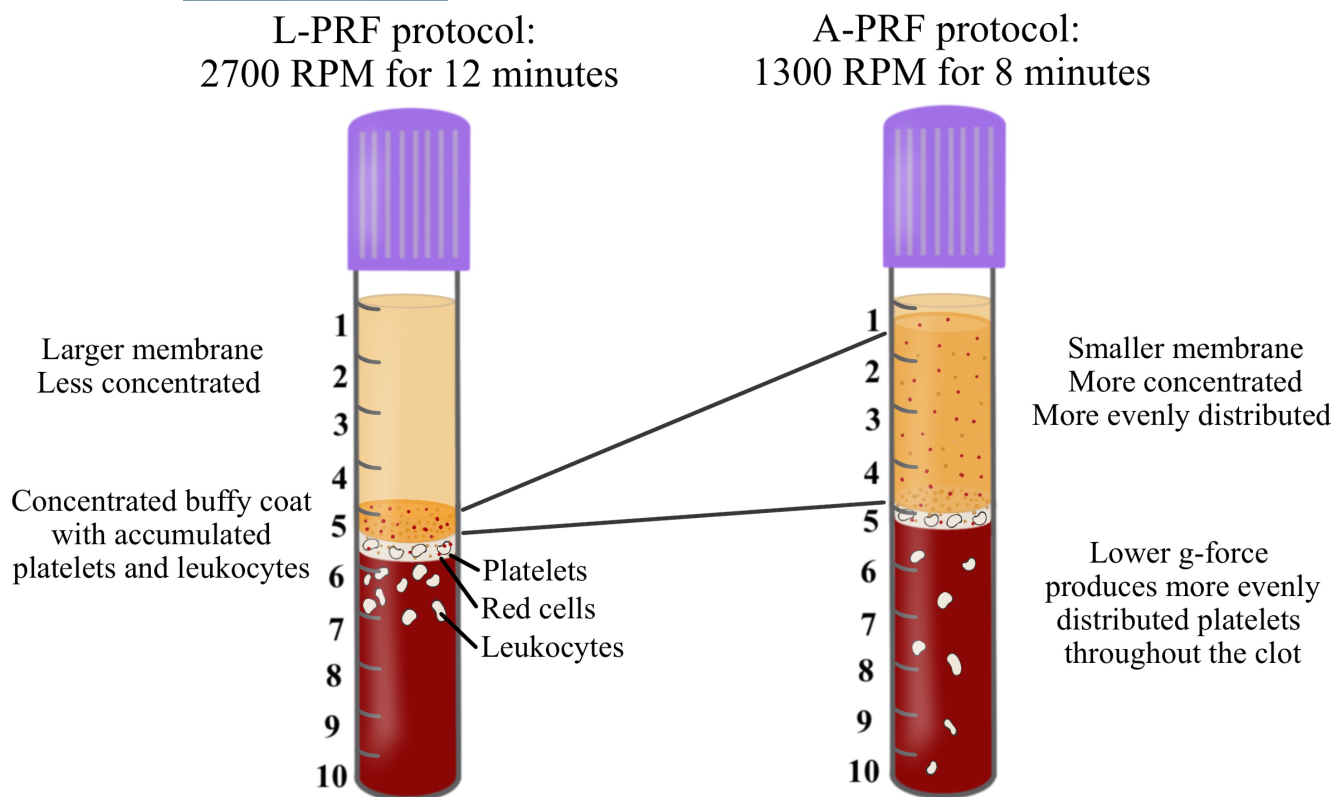

FIGURE 4 Summary of the findings comparing L-PRF and A-PRF protocols. While neither was typically able to collect leukocytes (reviewed later in the chapter), the lower centrifugation speeds using the A-PRF protocol allowed for the more even distribution of platelets in the upper layers. Reprinted with permission from Miron et al.[14]

release and concentration of growth factors over a 10-day period when compared to PRP or L-PRF.[9] In 2015–2017, our research team further demonstrated that optimization of platelet-rich fibrin could be achieved by not only reducing centrifugation speed but also time. The A-PRF protocol was therefore modified from 14 min at 200 g as originally described in 2014 to an 8-min protocol.[9]

Following an array of basic research studies on this topic, it was additionally observed that by further reducing g-force and time, it was possible to obtain a plasma layer that had not yet turned solid and converted into fibrin (i.e., scientifically liquid fibrinogen but often referred to as liquid PRF for simplicity). In a study titled "Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry?",[10] it was demonstrated that at lower centrifugation speeds and times (~60 g for 3 min), a liquid platelet-rich fibrin (termed injectable-PRF or i-PRF) could be obtained following centrifugation. While these protocols typically produced minimal volumes (~1–1.5 mL), it was shown that both platelets and leukocytes were even more highly concentrated when compared to L-PRF or A-PRF. This liquid PRF layer could be utilized clinically for approximately 15–20 min, during which time fibrinogen and thrombin had not yet converted to a fibrin matrix (i.e., remained liquid). However, this has since been utilized for injection into various joints/spaces similar to PRP, with the reported advantages of a longer growth factor release time owing to its natural ability to clot following injection (no anticoagulants utilized). Furthermore, the concept of "sticky" bone was also developed. Importantly, improvements in this space occurred a few years later when a chemically modified plastic PET tube as well as cooling temperatures could keep liquid PRF liquid for up to 4 h, as addressed later in this article.

6 | FIXED ANGLE VERSUS HORIZONTAL CENTRIFUGATION

PRF has been optimized in another key way over the past decade with research investigating horizontal centrifugation. Simply, horizontal centrifugation is routinely utilized in high-end research labs as well as in medical hospitals owing to its better ability to separate

layers and cell types based on density (QR Code 4). A simple Google search comparing both devices highlights the main roles of either centrifugation device (fixed angle devices are generally used for pelleting).

Following several basic research projects, our research group discovered that horizontal centrifugation actually led to up to four times greater concentrations of cells when compared to most currently available and commercial fixed angle devices commonly used to produce L-PRF and A-PRF (especially leukocytes).[11] During this study, a

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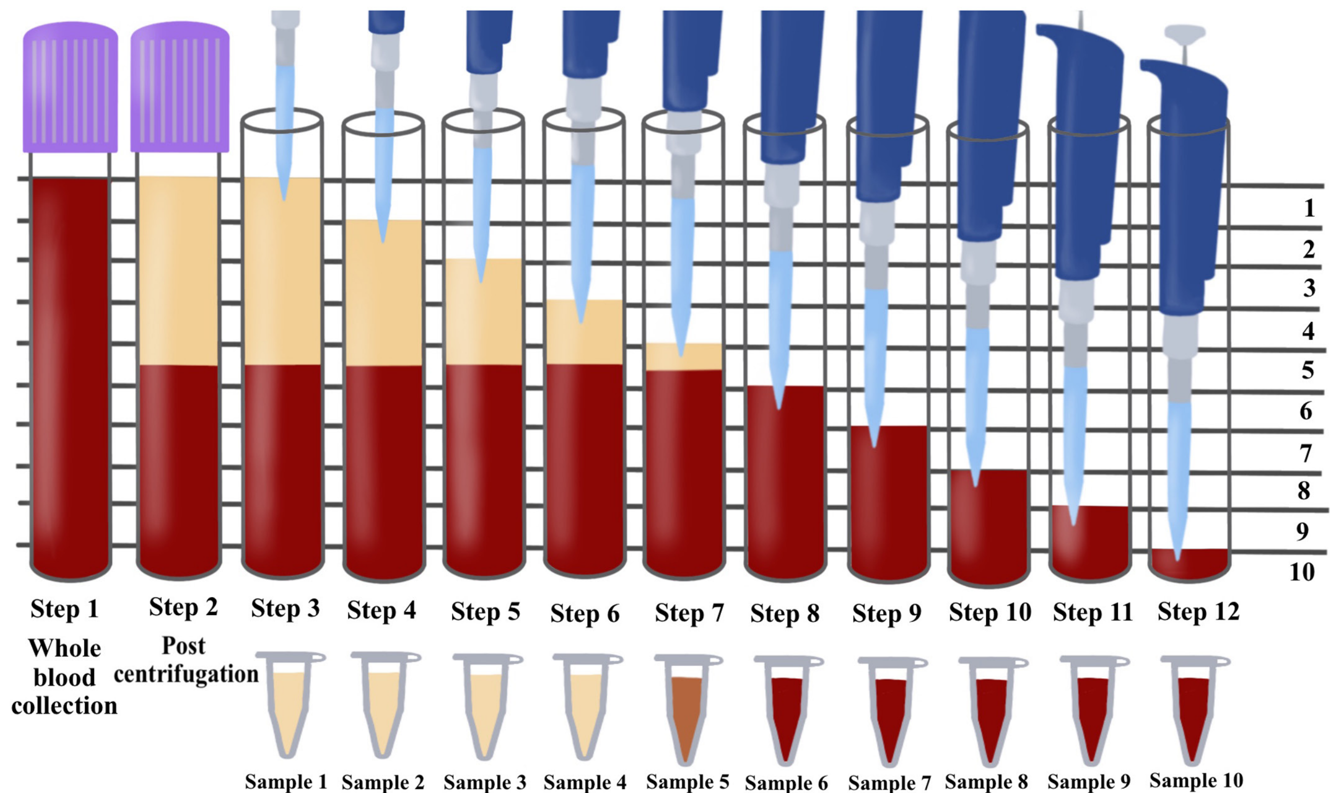

FIGURE 5 Illustration demonstrating the proposed novel method to quantify cell types following centrifugation of PRF. Currently, one of the limitations is that whole blood is compared to the total plasma concentration following centrifugation. This, however, does not give a proper representation regarding the location of cells following centrifugation. By utilizing the proposed technique in this study of sequentially pipetting 1 mL of volume from the top layer downward, it is possible to send each of the 10 samples for CBC analysis and accurately determine the precise location of each cell type following centrifugation with various protocols. Note that one layer (in this case, layer 5) will contain some yellow plasma and red blood cells. This is typically the location of the buffy coat, where a higher concentration of platelets is typically located. Reprinted with permission from Miron et al.[11]

novel method for quantifying platelet concentrates was developed whereby instead of quantifying the cell types in whole blood or from the entire PRP/PRF plasma layer, sequential pipetting of 1 mL samples was conducted following each centrifugation protocol (Figure 5). This allowed our research group to investigate 10 samples per 10 mL PRF tube and locate precisely where the various cell types were going following different centrifugation protocols. Unlike a fixed angle centrifugation system whereby the tubes are actually inserted at an angle of ~45°, horizontal centrifugation (often referred to as swing-out bucket centrifugation) actually allows tubes to swing out to 90° (horizontally) once they are in rotation. Amazingly, the original PRP systems developed by Harvest and Marx used this technology (and still do).

This represented a marked ability to greatly concentrate cells found within PRF, which were previously being accumulated primarily on the back distal surfaces of PRF tubes. The major disadvantage of fixed angle centrifugation is that during the spin cycles, cells are typically driven along the back wall of centrifugation tubes at high g-forces with relative difficulty separating properly according to their cell density (Figure 6). This also exposes cells to higher compressive forces against the back wall, and cells must then separate by traveling either up or down the inclined/angled centrifugation slope based on their respective cell density differences. Since red blood cells are larger and heavier than platelets and leukocytes, they travel downward, whereas lighter platelets travel toward the top of the tube where PRF is collected. This makes it relatively difficult for small cell types such as platelets and leukocytes to reach the upper layers since they get caught underneath RBCs, especially because RBCs outnumber WBCs in particular, typically by 1000 times in standard whole blood.

Additionally, a study titled "Histological comparison of Platelet rich fibrin clots prepared by fixed-angle versus horizontal centrifugation"[12] investigated both L-PRF prepared on a fixed angle centrifuge machine as well as H-PRF prepared using horizontal centrifugation. It was consistently observed that L-PRF clots demonstrated an uneven sloped separation between the upper plasma and the bottom red blood cell (RBC) layers according to the angle of the rotor. Interestingly, red dots were also observed, through SEM and histology, on the back distal walls of the tubes in the upper layers, consisting of aggregates of RBCs, leukocytes, and platelets. Clots produced on the horizontal centrifuge showed much more even cell layer distribution/separation along the tube surfaces.

Therefore, and in summary, the use of fixed angle centrifugation was not able to achieve optimal concentrations of platelets or leukocytes as a result of their fixed angle system design, and the easy switch toward producing PRF via horizontal centrifugation

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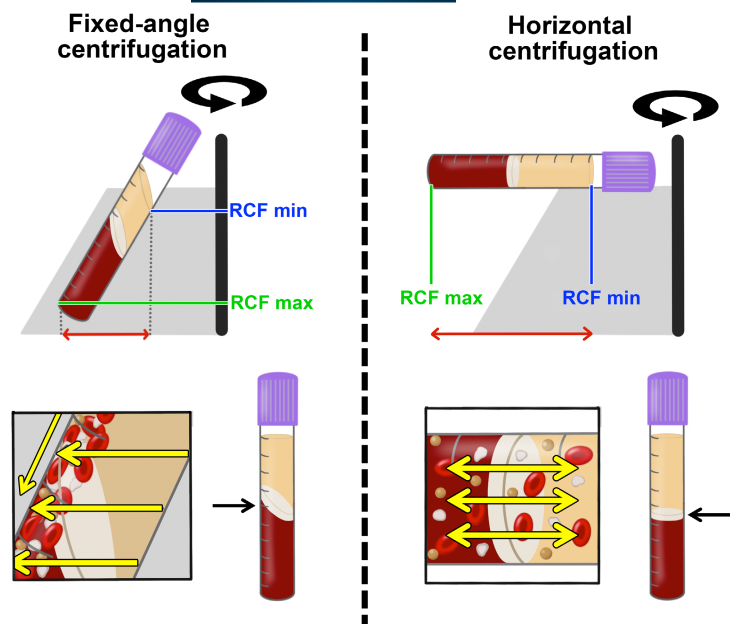

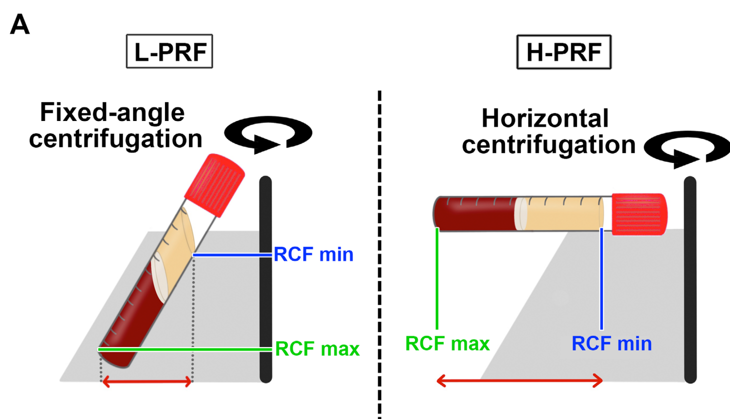

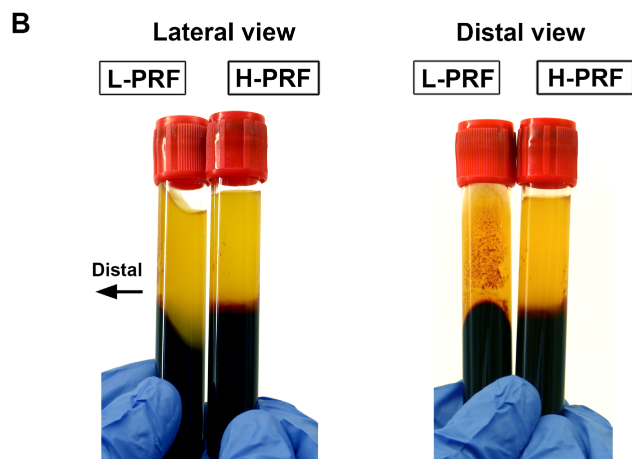

FIGURE 6 Illustrations comparing fixed angle and horizontal centrifuges. (A) With horizontal centrifugation, a greater separation of blood layers based on density is achieved owing to the greater difference in RCF-min and RCF-max. Following centrifugation on fixed angle centrifuges, blood layers do not separate evenly, and as a result, angled blood separation is observed. In contrast, horizontal centrifugation produces even separation. Owing to the large RCF values (~200–700 g), the cells are pushed toward the outside and downward. On a fixed-angle centrifuge, cells are pushed toward the back of centrifugation tubes and then downward/upward based on cell density. These g-forces produce additional shear stress on cells as they separate based on density along the back walls of centrifugation tubes. In contrast, horizontal centrifugation allows for the free movement of cells to separate into their appropriate layers based on density, allowing for better cell separation as well as less trauma/shear stress on cells. (B) Visual representation of layer separation following either L-PRF or H-PRF protocols. L-PRF clots are prepared with a sloped shape, and multiple red dots are often observed on the distal surface of PRF tubes, while H-PRF is prepared with a horizontal layer separation between the upper plasma and lower red corpuscle layer. Reprinted from Fujioka-Kobayashi et al.[39]

led to cell concentrations that were up to four times greater (QR

Code 5 SCAN ME). Table 1 highlights differences between the two systems.

7 | OPTIMIZATION OF PROTOCOLS USING HORIZONTAL CENTRIFUGATION—24 INVESTIGATED PROTOCOLS

Another main drawback of PRF therapy over the years has been the lack of optimization of protocols. As previously stated in a recent textbook on the topic, most studies to date have focused

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TABLE 1 Major differences between fixed angle and horizontal centrifugation.

	Fixed angle centrifugation	Horizontal centrifugation
Optimized use	FA centrifugation is more useful for pelleting of matter including cells to the bottom of tubes	Horizontal centrifugation most useful for separating cells/matter based on density
Separation of cells	Cells accumulate against the back walls of PRF tubes	Cells are evenly distributed throughout the upper layers
Cell concentration	Capable of concentrating platelets. Not effective at separating leukocytes	Up to 4 times more cells and growth factor concentration; especially of leukocytes
Cell damage	Owing to higher g-forces and fixed angles, cells are pushed toward the back walls and damaged. Clinicians can even visually see RBC accumulation along the back walls of tubes	Since cells are not driven toward the back walls, significantly lower chance of cell damage as a result of cells separating throughout the center of tubes
Protocols to create sticky bone	Most protocols combining liquid i-PRF and solid PRF requires the removal of liquid PRF tubes within 3–5 min and thereafter to re-spin the solid PRF tubes. Otherwise, a high chance of clotting may occur within the tubes	Both liquid and solid PRF tubes can be spun at the same time and same protocol. This is owing to the fact that cells are not driven toward the back walls of tubes that initiates clot formation
Protocols to create albumin gel	Not possible owing to the faster clotting times caused by fixed angle centrifugation	The protocol requires a horizontal centrifuge for optimization

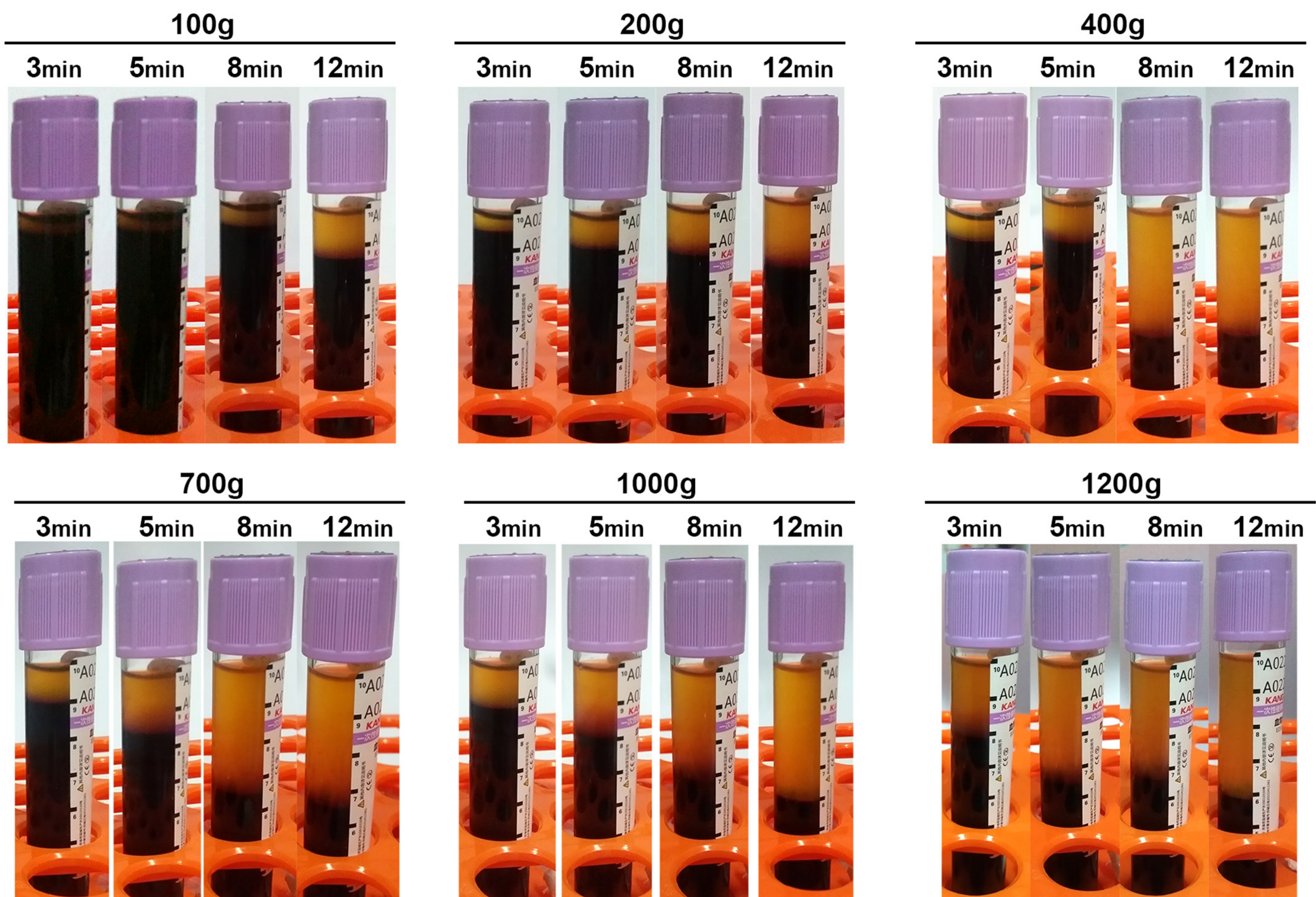

FIGURE 7 Clinical image demonstrating the plasma layer separation for the 24 protocols investigated in the study. Note that while some protocols reveal roughly identical plasma layer separation, the underlying cellular content in the various protocols may be drastically different. Reprinted with permission from Miron et al.[13]

on investigating two or three protocols given various research articles.[8]

The desire and emphasis of our research group around the year 2021 was to precisely optimize the production of PRF by systematically investigating 24 different protocols (Figure 7).[13] All protocols were compared utilizing our layer-by-layer method to quantify cells using the 1 mL sequential pipetting technique from the upper layer downward until all 10 mL were harvested. In total,

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960 complete blood counts (CBCs) were investigated. Both solid- and liquid-based PRF protocols were investigated following 24 protocols involving six relative centrifugal force (RCF) values (100, 200, 400, 700, 1000, and 1200 RCF) at 4 centrifugation times (3, 5, 8, and 12 min).[13]

Following these studies, it was observed that centrifugation at 700 RCF for 8 min was best able to evenly distribute cells in the upper plasma layer. This is the standard protocol used for solid PRF membranes/clots. Centrifugation at 200–300 RCF for 5 min represented a more optimized version of the standard injectable-PRF protocol developed in 2016–2017. It was further revealed that the most effective way to concentrate liquid PRF was by developing a high-speed protocol with centrifugation at 2000 RCF for 8 min. The PRF generated was termed concentrated PRF (C-PRF) (QR-Code 6,

).

8 | MAXIMIZATION OF PLATELETS AT THE BUFFY COAT USING C-PRF PROTOCOLS

Another misconception that exists is that lower centrifugation speeds are the most effective way to concentrate cell types, which is known as the low-speed centrifugation concept (LSCC). In a study published in 2020 titled "Improved growth factor delivery and cellular activity using concentrated platelet-rich fibrin (C-PRF) when compared to traditional injectable (i-PRF) protocols", it was demonstrated that much higher concentrations of cells could be obtained at much higher RCF values by sending cells specifically to the buffy coat region.[14] There was an approximately 10-fold increase in baseline concentrations of platelets and WBCs (when compared to 2-4X in i-PRF) found specifically in this 0.3–0.5-mL buffy coat layer directly above the RBC corpuscle layer.[14] The PRF obtained specifically from this harvesting technique was given the working name concentrated PRF (C-PRF). Figure 8 shows a clinical photograph of standard liquid PRF protocols versus those of C-PRF. It was shown in a subsequent study that C-PRF also exhibited higher growth factor release as well as superior cellular activity (QR Code 7

).[15]

In clinical practice, it is best to harvest C-PRF by first removing the upper 4–5 mL of the platelet-poor plasma layer and discarding it. Thereafter, the remaining 0.5–1 mL C-PRF layer can be taken much more easily. It is much more difficult to take this buffy coat zone with 5 mL over top of this layer as often, and it is harder to concentrate (Figure 9).

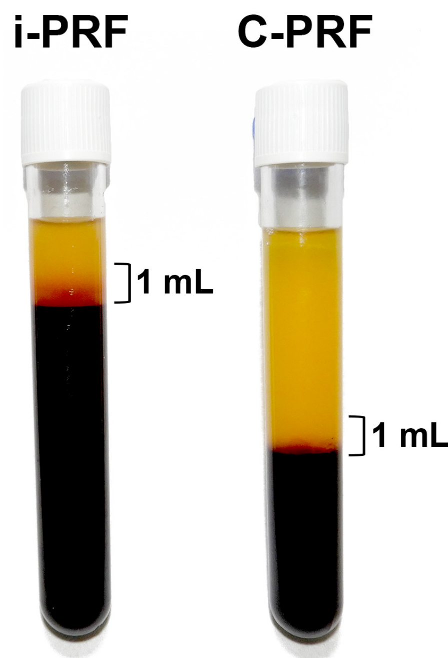

FIGURE 8 Visual representation of layer separation following either the i-PRF (300 × g for 5 min) or C-PRF protocol (3000 × g for 8 min). Plasma was collected from the buffy coat region within the 1-mL layer above the RBC layer.

9 | RED-TOP TUBES: CHEMICALLY MODIFIED TUBES WITHOUT THE ADDITION OF CHEMICAL ADDITIVES

Surprisingly, perhaps the biggest misconception regarding the production of PRF is mostly related to the optimization of PRF tubes. Many clinicians still assume that all red-top tubes are the same, and this fallacy may have the greatest effect on the final production of PRF when compared to all other factors. PRF tubes have a massive implication in the final outcomes of both solid PRF and liquid PRF. Simply, more hydrophilic tubes (red-top tubes, typically plain glass, or silica-coated plastic tubes) have been shown to be more effective for the production of solid PRF, whereas hydrophobic tubes (white- or blue-top tubes, typically PET plastic tubes) are utilized for the production of liquid PRF. The more hydrophilic the surface, the better-quality clot will occur (and the better the fibrin mesh), whereas the more hydrophobic, the more PRF will remain liquid in nature. This is very much in line with recent research that has shown that modified implant surfaces that are fabricated to be more hydrophilic (SLA-active, Nobel-active) have also been shown to improve osseointegration.[16–19] Since these surfaces are more hydrophilic, blood is more attracted to their surface, platelets degranulate, and the clotting cascade occurs more rapidly. This is the same phenomenon that occurs on the walls of PRF tubes.

Unfortunately, the majority of tubes utilized for the production of PRF are in fact not purposefully designed for PRF; they are generally laboratory test tubes utilized for lab testing that have since been brought to market and utilized for human clinical applications (many

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Note: Use the upper PPP layer with the Bio-Heat. Otherwise, discard

Following C-RPF protocol, cell accumulation occurs directly within the buffy coat

Step 1: Remove the upper 3-4 mL of platelet poor plasma (PPP) with the aim of keeping ~0.5-1mL of C-PRF

Step 2: Collect ~0.5-1 mL of liquid C-PRF directly above the red cell layer within the buffy coat

FIGURE 9 Method to collect and concentrate C-PRF. Following centrifugation at higher speeds (2000×g for 8min), the majority of cells are located directly at the buffy coat layer. Instead of attempting to remove this layer with a long needle into the deep layers, it is highly advised to first remove the upper 4mL layer of platelet-poor plasma (PPP), followed by collection of the concentrated platelet-rich (C-PRF) buffy coat layer.

without FDA/CE clearance). In a recent technical note on this topic, it was discovered that many PRF tubes are filled with chemical additives, such as silica/silicone, with unpredictable clinical outcomes. Figure 10 shows an experiment performed by a Japanese research group highlighting the use of A-PRF using silica-coated plastic tubes.[20] The remnants observed following the experiment are shown candidly in Figure 10, and unless otherwise corrected, would end up in patients. This section of the paper could very well be expanded into a separate full article,[21] but we will simply showcase recent modifications to tubes

(QR Code 8 SCAN ME).

Under natural wound healing conditions, once an injury occurs, the blood begins to condense, and endogenous and exogenous coagulation pathways are activated to repair damaged blood vessels.[22–24] During PRF clot formation in tubes, the main pathway to activate blood is via endogenous coagulation pathways associated with "surface contact" once blood interacts with the wall of the centrifuge tube.[25] The endogenous coagulation pathway relies on different enzyme groups that result in the activation of Factor XII to FXIIa,[26] leading to the formation of a solidified clot entrapped with a higher concentration of immune cells and growth factors.[24,27–30] Thus, platelets have great implications for the formation of blood clots (or PRF clots) through adhesion to various modalities (damaged endothelium, tube walls, aggregating with other platelets) and providing coagulation reaction steps that are all triggered by platelet adherence and activation.[22,31,32] Therefore, if the strategy to develop platelet concentrate therapy is to increase the regenerative potential of platelets in blood, it is clear that a higher number of activated platelets results in larger PRF clots and better biological properties.[22] Therefore, proper design of PRF tubes will certainly pave the way to more optimized PRF tubes and, more importantly, removing the chemical additives found in many PRF tubes today designed to help with clotting.

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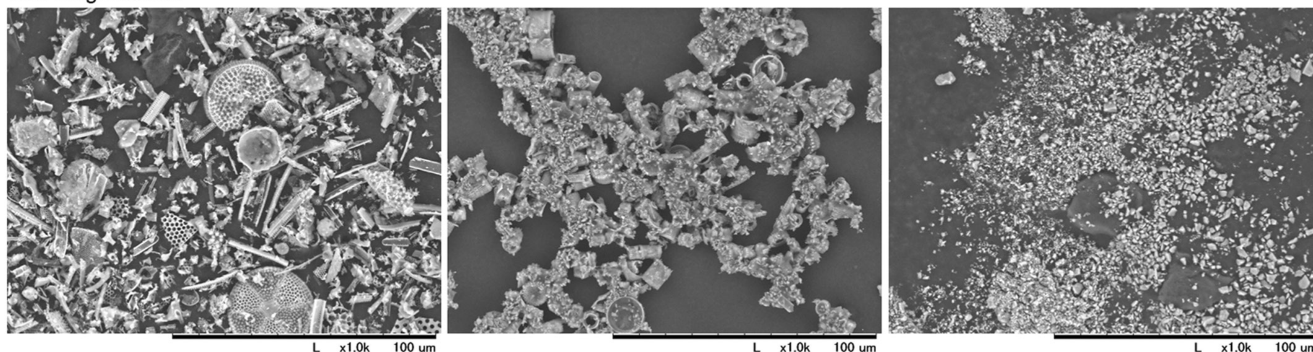

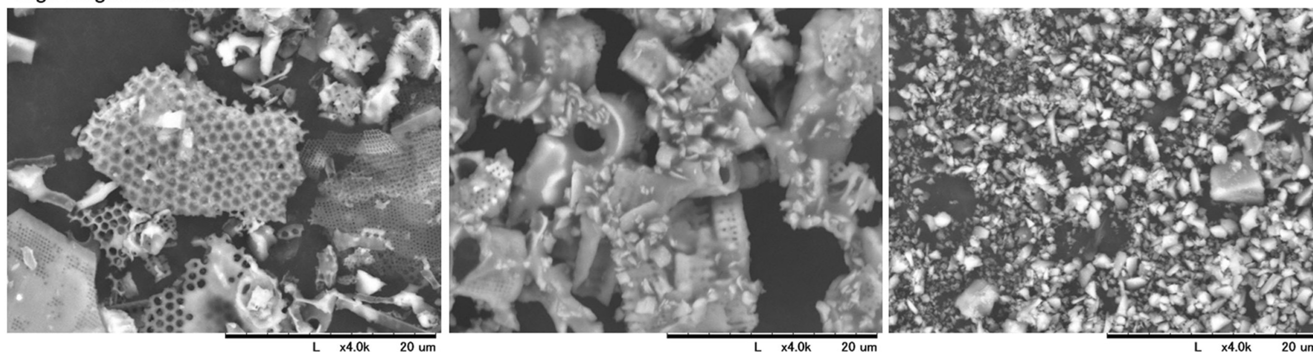

FIGURE 10 In this experiment, PRF clots were produced in three different commercially available tubes containing silica. Following centrifugation, clots were removed, the PRF clots were enzymatically digested, and "leftover" silica particles were visually assessed by scanning electron microscopy (SEM). SEM observations of silica microparticles contained in (A) neotubes, (B) Vacuette tubes and (C) Venoject II tubes at low (top) and high (bottom) magnification. Note the high incorporation of silica microparticles detached from PRF tube walls into PRF clots. Reprinted with permission from Tsujino et al.[20]

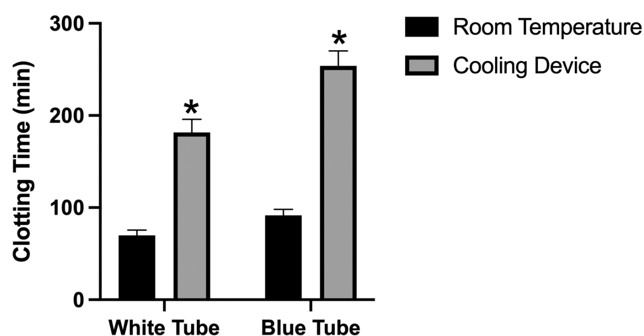

FIGURE 11 Bar graph representing the average clotting time of liquid PRF in (1) white tubes at room temperature, (2) white tubes placed in the cooling device, (3) blue tubes at room temperature, and (4) blue tubes placed in the cooling device ($^*p<0.05$ indicates a significant difference between tubes placed at room temperature and the cooling device; $n=30$). Reprinted with permission by Miron et al.[33]

10 | BLUE-TOP TUBES: EXTENDING LIQUID PRF WITH CHEMICALLY MODIFIED PET TUBES

Following the understanding that hydrophilic tubes improve PRF clotting, research also began by our group investigating hydrophobic tubes designed to avoid contact with tube walls to purposefully delay clotting. In a study titled "Extending the working properties of liquid platelet-rich fibrin using chemically modified PET tubes and the Bio-Cool device",[33] it was found that the chemically modified PET tubes performed 37% better than the control tubes and extended the working properties of liquid PRF by over 20 min (Figure 11). Most surprisingly, tubes kept in the cooling device demonstrated an average of 90 min greater working time (270% improvement).[33]

11 | HEATING AND COOLING PLATELET-RICH FIBRIN: EFFECTS ON CLOTTING TIME

Around the year 2019, devices were also beginning to be created to improve/delay clotting based on temperature. Since a fibrin clot is made from its precursors fibrinogen and thrombin, it was found that like most enzymes, they do not function quite as effectively at lower temperatures. Therefore, by placing liquid PRF tubes in a cooling device (a Bio-Cool), it was observed that liquid PRF would stay liquid for up to 4 h, marking a 270% improvement when compared to standard

tubes at room temperature (Figure 11; QR Code 9).[33]

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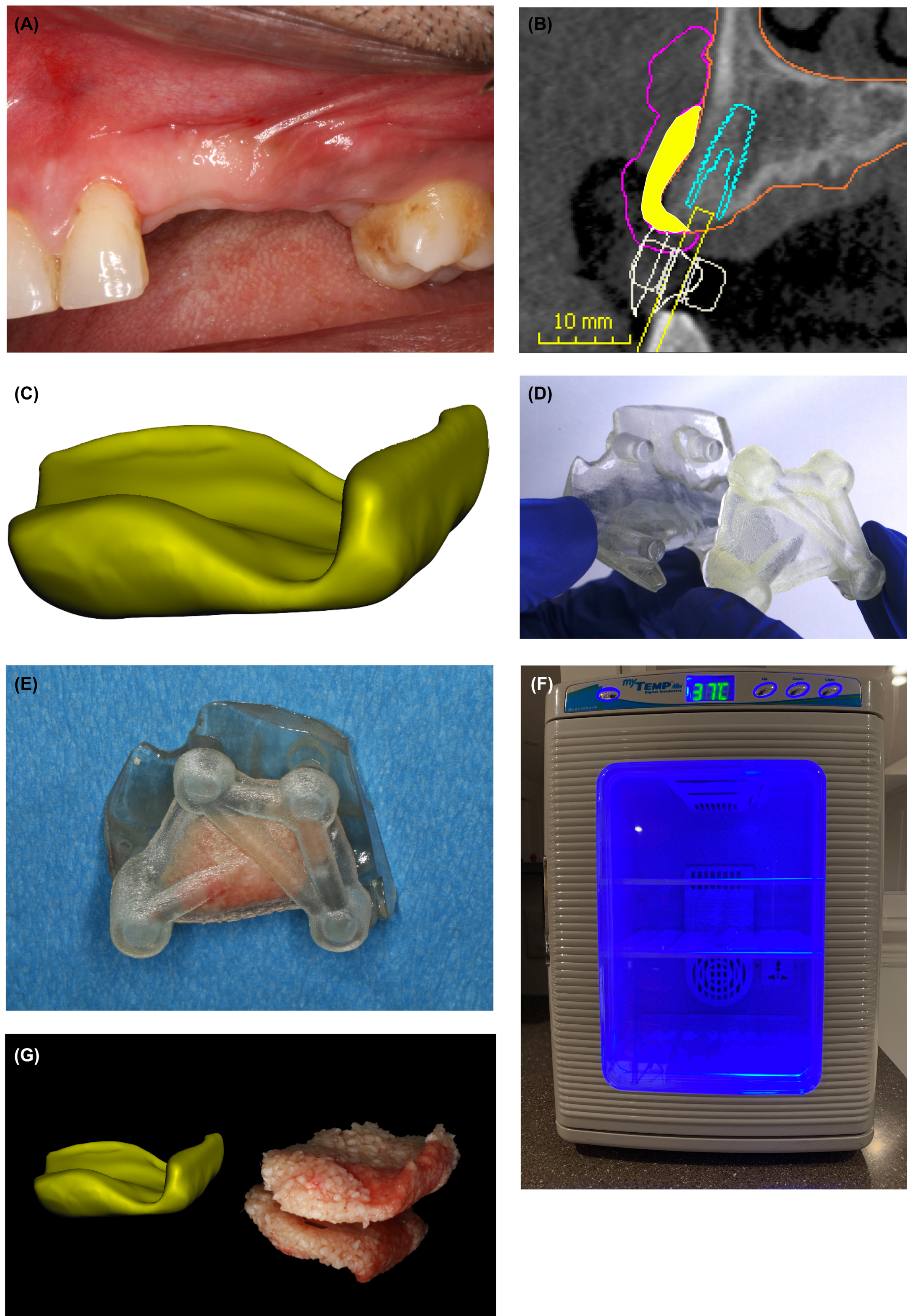

FIGURE 12 (A) Preoperative image of the edentulous ridge. (B) CBCT DICOM cross-sectional view showing the digital planning of the guides, molds, implant, and bone graft shown in yellow. (C) Custom 3D-rendered digital bone graft. (D) Bone mold orientation for seating is shown with the 3D printed models. (E) Sticky bone inserted into the bone mold. (F) Bone mold with incorporated sticky bone placed into an incubator at 37°C. (G) After 15 min, note the custom-made 3D bone graft. (H) Occlusal view of the custom bone graft in place as planned digitally. Case performed by Naheed Mohamed.

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Therefore, procedures performed not only in dentistry but also in other fields, such as orthopedic joint injections and facial esthetics, benefit tremendously from having a longer working period to inject the final liquid PRF formulations.

While cooling certainly delays clotting nearly 3-fold, it has also been observed that by simply heating PRF to a body temperature of 37°C in an incubator, PRF clots much more effectively and densely. Figure 12 demonstrates a customized 3D fabricated bone scaffold whereby bone allograft particles were premixed with liquid PRF and placed in an incubator to improve clotting and the clotting efficiency of PRF. Following a 10-min period, a custom-shaped 3D bone allograft block made from particles was utilized very conveniently owing to its custom shape and the density of the PRF "block" graft (Figure 12). Furthermore, by heating to temperatures above the denaturization of albumin, it is possible to extend the working properties of PRF from a 1–2 week resorption period to upward of 4 months (covered in the next article of this issue).

12 | FINAL THOUGHTS AND FUTURE TRENDS

This article highlights many advancements made in recent years but also depicts very simple ways to improve the production of PRF. The following key messages should be derived from the article:

- The report of the RPM value when producing PRF is entirely useless. Clinicians and scientists should focus on reporting RCF values at the max of the tube to better standardize PRF protocols.
- While the application of the low-speed centrifugation concept (LSCC) certainly leads to more evenly distributed cells across various PRF layers, horizontal centrifugation of PRF led to cell concentrations that were up to four times greater. Therefore, PRF should not be produced using fixed angle (45-degree-angled) devices.
- It was once thought that the most optimized method to collect PRF was via extremely low spin cycles of 3–5 min. This was mainly achieved owing to the shortcomings of horizontal centrifugation. In fact, faster speeds produced using horizontal centrifugation with a thin buffy coat zone extremely rich in cells (termed concentrated PRF or C-PRF) is the most optimized method to date to concentrate cells ~10×.
- The tubes used to produce PRF potentially have an even greater impact on the final size outcomes and regenerative ability of PRF when compared to the device/protocols utilized. Many of the tubes have been found to contain chemical additives that have since been shown to negatively impact cells found in the oral cavity, thereby decreasing the regenerative potential of PRF.
- The temperature at which PRF is kept post-spin cycle has the ability to either greatly increase clotting efficiency or delay clotting. Temperature modulation devices are relatively inexpensive pieces of equipment that can greatly impact clinical practice.

Various systematic reviews have now covered the ability for PRF to improve recession coverage, intrabony defect regeneration, alveolar ridge augmentation, and sinus graft.[34-37] In fact, an entire future periodontology 2000 issue will focus on the use of PRF in periodontology and implant dentistry.

Future trends are aimed at further extending the working properties of PRF using a higher heat-temperature protocol that can extend PRF lifetime in the body from 2 weeks to 4–6 months (QR Code

10

). This will be covered in the subsequent article. Furthermore, for additional improvements and uses of liquid PRF in various arenas of regenerative medicine, liquid PRF has more recently been utilized as a drug delivery system for various clinical applications. Therefore, advanced therapeutic compounds such as small biomolecules, antibiotics, and/or additional growth factors such as exosomes have been incorporated prior to clot formation and delivered to human tissues to further enhance tissue regeneration and attenuate post-inflammatory responses. These will be enthusiastically addressed in the two upcoming articles.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Yufeng Zhang https://orcid.org/0000-0001-8702-5291

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