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ORIGINAL ARTICLE

Extending the working properties of liquid platelet-rich fibrin using chemically modified PET tubes and the Bio-Cool device

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Abstract

Objectives Platelet-rich fibrin (PRF) has been utilized in regenerative medicine as a concentration of autologous platelets and growth factors that stimulates tissue regeneration. More recently, liquid-PRF (also called injectable-PRF; i-PRF) has been brought to market utilizing PET plastic tubes. Due to new advances made in tube technology, the first aim of the present study was to investigate the liquid consistency of liquid-PRF utilizing both standard and chemically modified PET plastic tubes. Furthermore, it is well known that the conversion of PRF into a fibrin matrix is derived from the temperature-controlled enzymatic process that converts liquid fibrinogen and thrombin to solid fibrin. This study also investigated for the first time the use of a cooling device (Bio-Cool) to extend the liquid working properties of liquid-PRF.

Materials and methods In total, 30 participants enrolled in this study. From each patient, four tubes of liquid-PRF were drawn, two standard white Vacuette tubes and two blue chemically modified hydrophobic tubes. Following centrifugation at 700 RCF-max for 8 min in a Bio-PRF horizontal centrifuge, one white and one blue tube were kept upright at room temperature, while the other white and blue tube were placed within the cooling device. Thereafter, the liquid-PRF layers were monitored over time until clotting occurred. Patient gender, age, and altitude above sea level (+ 5000 ft) were recorded and compared for clotting times.

Results The findings from the present study demonstrated that the chemically modified PET tubes performed 37% better than the control tubes (extended the working properties of liquid-PRF by over 20 min). Most surprisingly, tubes kept in the cooling device demonstrated an average of 90 min greater working time (270% improvement). While patients living at altitude did significantly improve the clotting ability of liquid-PRF, no differences were observed when comparing male vs female or younger vs older patients in liquid-PRF clotting times.

Conclusions Cooling of blood following centrifugation represented a 270% improvement in working properties of liquid-PRF. Optimization of liquid-PRF tubes utilizing chemically modified hydrophobic PET tubes also delayed the clotting process by 37%. Patient gender and age had little relevance on liquid-PRF.

Clinical relevance The present findings demonstrate for the first time that cooling of liquid-PRF is able to extend the working properties of liquid-PRF by over 90 min. Thus for clinicians performing longer clinical procedures, the cooling of blood may represent a viable strategy to improve the working time of liquid-PRF in clinical practice.

Keywords PRF · Bio-PRF · Cooling device · Wound healing · Platelet-rich fibrin

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Introduction

The use of platelet-rich fibrin (PRF) in regenerative medicine and dentistry has continued to see widespread use owing to its ability to rapidly stimulate angiogenesis leading to improvements in tissue regeneration [1]. Recently, various systematic reviews have demonstrated that PRF is able to promote intrabony defect regeneration [2], favors better soft tissue healing and clinical outcomes of recession coverage procedures [3], is able to limit dimensional

changes post-extraction [4], and is utilized for guided bone regeneration and sinus grafting procedures in implant dentistry [5]. While platelet-rich plasma (PRP) was initially developed in the late 1990s with widespread use in dentistry and medicine [6–10], drawbacks including its use of bovine thrombin and other limitations regarding its regenerative potential were also reported [6, 11, 12].

When anti-coagulants and clotting factors are removed from the centrifugation process, PRF (a second-generation platelet concentrate following the introduction of PRP) relies on the tube chemistry to either form a three-dimensional fibrin matrix or remain in a liquid state [13]. As a result, an array of clinical procedures have been developed by clinicians that either utilize the solid-PRF membrane form or liquid state depending on the clinical indication. In liquid form, PRF can be injected into joint spaces as well as mixed with various biomaterials such as bone grafts or collagen barrier membranes to improve their handling and effectiveness [13]. In solid state, PRF is utilized as a barrier to improve soft tissue healing [14] and also utilized in medicine for the treatment of hard-to-heal diabetic ulcers [15].

The liquid and solid properties of PRF depend on the properties and chemistry of the tubes that are used when drawing blood. For example, a solid clot will form if the tube is made of a material with hydrophilic properties such as glass, silica-coated plastic, or titanium [13, 16]. The opposite is true when the clinician favors the use of liquid-PRF whereby the tubes are made of a more hydrophobic material such as polyethylene terephthalate (PET) which delays adsorption of proteins to the tube wall surfaces where clotting begins. Simply put, the more hydrophobic the tube wall chemistry, the longer the liquid working time for clinicians to perform their clinical activities.

Furthermore, it is well known that the conversion of PRF into a fibrin matrix is derived from the temperature-controlled enzymatic process that converts liquid fibrinogen and thrombin to solid fibrin [13, 17, 18]. In 2019, the cooling device was introduced to delay clotting and extend the working properties of liquid-PRF by decreasing the enzymatic process and conversion of liquid fibrinogen and thrombin into a fibrin clot through a cooling process of blood. However to date, no data exists demonstrating its effectiveness or ability to prolong the working properties of liquid-PRF.

The aim of this study was therefore to study factors that affect blood coagulation in an attempt to extend the working properties of liquid-PRF. Several factors, including (1) tube chemistry, (2) temperature control, (3) age, (4) gender, and (5) altitude above sea level, were all studied to determine which factors most affect the clotting time and properties of liquid-PRF.

Materials and methods

Preparation of PRF

Blood samples were collected with the informed consent of 30 volunteer donors. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No ethical approval was required for this study because human samples were not identified, as previously described [19]. The donors were separated into 14 female patients and 16 male patients between the ages of 28 and 79. Half of the patients were composed of individuals residing at sea level in Sarasota Florida. The other half were collected at an altitude above 5000 ft in Longmont, Colorado. This was purposefully done to investigate the effects of altitude on clotting times. Patients with any obvious apparent blood disorder such as abnormal concentration of thrombin and factor XIII in plasma and patients with issues related to hyperglycemia and cigarette smoking were excluded from the study [20]. All patients were included if systemically healthy, non-smoking, and not taking any medications. The patients were then equally sub-sectioned into respective groups including (1) their age, (2) gender, and (3) altitude above sea level.

For each patient, four 10 ml tubes of whole blood were randomly collected from each individual patient, and centrifugation began thereafter. Two different types of PRF tubes were used to harvest blood without additives, a standard white PET Vacuette tube (Bio-PRF, Venice, Florida) along with a blue chemically modified hydrophobic PET tube (Bio-PRF). Following centrifugation at 700 RCF-max for 8 min in a Bio-PRF horizontal centrifuge, tubes were removed, one white tube and one blue tube were kept upright at room temperature, while the other white and blue tube were placed within a cooling device set at 8 °C (Bio-Cool, Bio-PRF). Thereafter, the liquid-PRF layers were monitored over time until clotting occurred. The final time point at which fibrinogen + thrombin converts into a solid PRF clot was then recorded. Thereafter, clotting times were compared between males and females, between age groups, and between high and low altitude.

Statistical analysis

All experiments were performed with each of the 30 patients**.** Means and standard errors were calculated, and data were analyzed for statistical significance using

one-way analysis and *t*-test for clotting times with GraphPad Prism 6.0 software (GraphPad Software, Inc., La Jolla, CA, USA; *p values < 0.05 were considered significant).

Results

Improvements in working properties of liquid-PRF using chemically modified PET tubes and the cooling device

First, clotting times were compared in 4 groups including 1) white tubes at room temperature, 2) blue tubes at room temperature, 3) white tubes placed in the cooling device, and 4) blue tubes placed in the cooling device. On average, the white tubes clotted in roughly 60 min, whereas the chemically modified blue tubes significantly delayed clotting to over 90 min (Fig. 1). More noteworthy, the cooling device was responsible for a roughly 2–threefold increase in clotting time, irrespective of tube type. It was therefore revealed that the cooling temperature more significantly impacted the working properties of liquid-PRF when compared to the tube type (Fig. 1).

Differences in clot formation of liquid-PRF from individuals living at low/high altitude

Thereafter, the data was sub-divided between individuals living at high (above 5000 ft) versus low (sea level) altitude. As demonstrated in Fig. 2A, each of the 4 tested groups demonstrated significantly longer clotting times at altitude when compared to sea level. The working properties of liquid-PRF were extended from an average of 49 min to an average of 86 min in the white tubes at room temperature group (average 37-min increase), whereas the blue tubes demonstrated an increase from 75 to 105 min (30 min). Altitude also increased the white tubes in the cooling device group from 145 to 209 min (65-min increase), whereas blue tubes placed in the cooling device increased clotting times from 184 to 307 min (123-min increase). Thus, altitude was able to significantly increase the working liquid properties of PRF by as much as 2 h (Fig. 2B).

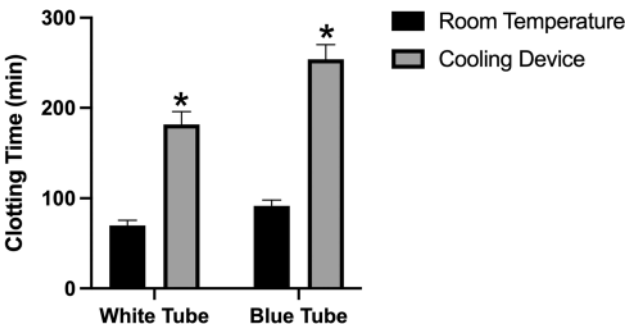

Fig. 1 Bar graph representing the average clotting time of liquid-PRF in (1) white tubes at room temperature, (2) white tubes placed in the cooling device, (3) blue tubes at room temperature, and (4) blue tubes placed in the cooling device. (*$p < 0.05$ indicates a significant difference between tubes placed at room temperature and the cooling device; $n = 30$)

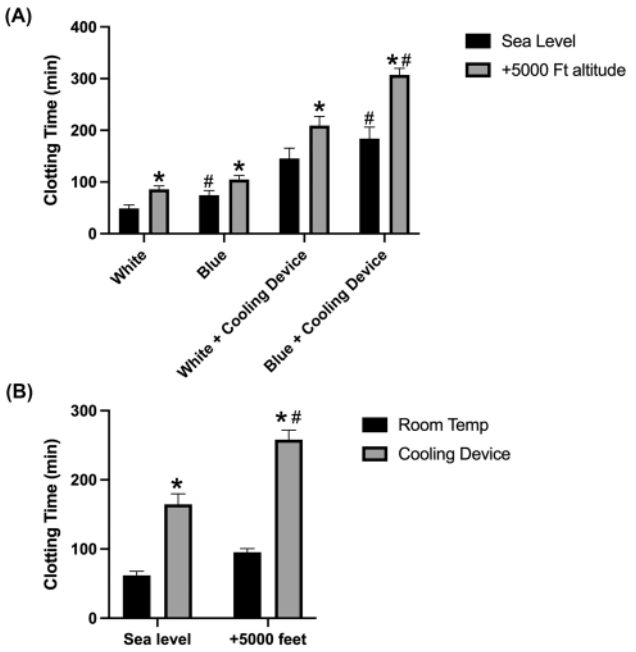

Fig. 2 Bar graph representing the average clotting time of liquid-PRF for individuals living at sea level and 5000-ft altitude. **A** In all treatment groups, altitude had a significant delay on clotting times. (*$p < 0.05$ indicates a significant difference between sea level and altitude; #$p < 0.05$ indicates a significant difference between white and blue tubes; $n = 30$). **B** Effect of the cooling device comparison at low and high altitude. (*$p < 0.05$ indicates a significant difference between tubes placed at room temperature and the cooling device; #$p < 0.05$ indicates a significant difference between sea level and altitude; $n = 30$)

Difference in clotting time between males/females and individuals younger vs older than 40

Next, the differences in clotting times between males and females as well as older vs younger individuals were compared. Figure 3A depicts the effects of gender on clotting time. No significant differences were observed between any of the tube types comparing males and females though a roughly 15–20% (non-significant) longer clotting time was observed in females. No differences were reported between younger and older individuals (Fig. 3B).

Percent increases investigating tube types, cooling device, age, gender, and altitude

In a final comparison, percent increases were reported to determine which factors affect clotting times most

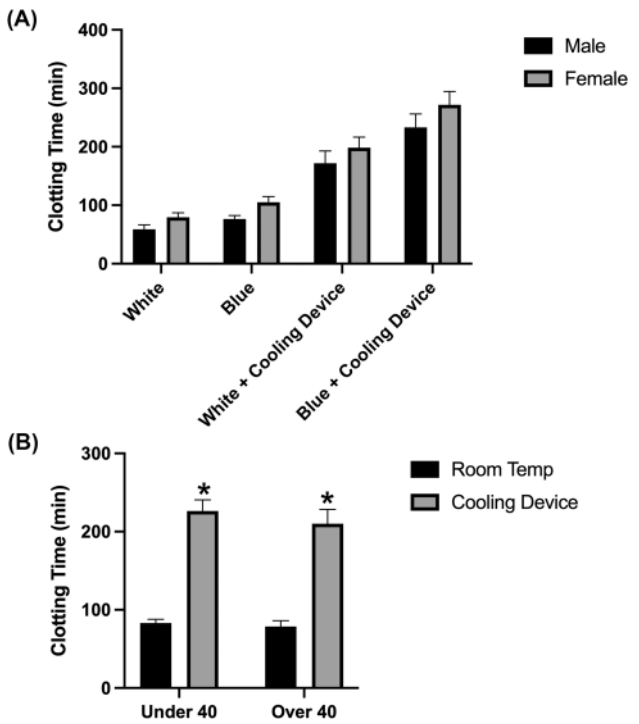

Fig. 3 Bar graph representing the average clotting time of liquid-PRF for gender (male vs female) and age (over/under 40). **A** No differences were reported in clotting times between males and females at all time points. **B** No differences were reported between patients older/younger than 40. (*$p < 0.05$ indicates a significant difference between tubes placed at room temperature and the cooling device; $n = 30$)

prominently. It was first observed that the chemically modified blue tubes increased clotting time by an average of 37% across all groups (Fig. 4A). Most prominently, the cooling device extended liquid-PRF properties by over 170% up to 270% initial baseline values (Fig. 4B). Altitude also had a significant effect on the clotting time of liquid-PRF increasing the liquid phase of PRF by 56% (Fig. 4C). Neither gender nor age had a significant impact on clotting times (Fig. 4D, E).

Discussion

This study was the first to investigate the impact of a novel chemically modified hydrophobic tube as well as the cooling device to delay the clotting times of liquid-PRF. Several other factors were also investigated in order to better understand the clinical variability that occurs when producing liquid-PRF.

Interestingly, it was found that the modification of the tube chemistry improved the liquid properties of PRF by over 20 min. Noteworthy as well, some patient samples are observed over a doubling in working time of liquid-PRF. This highlights the importance of better understanding and investigating the hydrophobic properties of PRF as to date; no single study has investigated this phenomenon. It has previously been reported that the adsorption of proteins to a biomaterial surface is the initiating event in the processes occurring when blood contacts a "foreign" surface leading inevitably to thrombus formation [21]. Over the years, accumulating knowledge of protein adsorption has demonstrated that blood proteins form layer compositions on the surfaces of biomaterials, leading to the initiation of coagulation, platelet adhesion, and activation [21]. Thus, the results from our study open an entire avenue of potential future research to better understand tube chemistry and its impact on the initiation/delay of fibrin clot formation from its precursors fibrinogen and thrombin. In our study, it was observed that the more hydrophobic plastic PET tubes repelled liquid from the sides of the tube walls, minimizing/delaying protein adsorption and ultimately the probability for a stable fibrin clot to form.

This study was also the first to demonstrate that remarkably, the most effective way to delay clotting was by use of the cooling device. In our study, the method of simply placing a liquid-PRF tube following centrifugation into the cooling device extended the working properties of liquid-PRF by over 90 min and represented a massive 270% improvement in liquid-PRF working time. On average and regardless of tube types utilized, PRF lasted over 3 h in its liquid state, long enough for practically any clinical application to take place without fear of clotting. Thus, it is well known that the conversion of fibrinogen and thrombin into a solid fibrin clot is largely dependent on enzymatic temperature control [22]. This is the first study to markedly demonstrate the significant clinical impact of the cooling device on the working properties of liquid-PRF.

We then studied the impact of patient variability on the properties of liquid-PRF. Previously, our research team investigated the variability in the macroscopic morphology/size of PRF membranes between patients using solid-PRF red tubes [23]. The rationale of this previous study was to determine the impact of patient age, gender, and time between blood draw and the start of centrifugation on the size outcomes of PRF membranes. It was revealed clearly that patients with higher percentages of red blood cells, such as males and younger individuals, had smaller PRF membranes owing to the greater difficulty in separating cells based on density using a centrifuge (since RBCs are higher in number, the blood is thicker, and thereby more gravitational force (g-force) is required to separate them). This was vastly different than their ability to induce clotting investigating the exact same patient populations.

In our study, we investigated 3 parameters that were hypothesized to potentially contribute to liquid-PRF formation: (1) patient gender, (2) patient age, and (3) patient

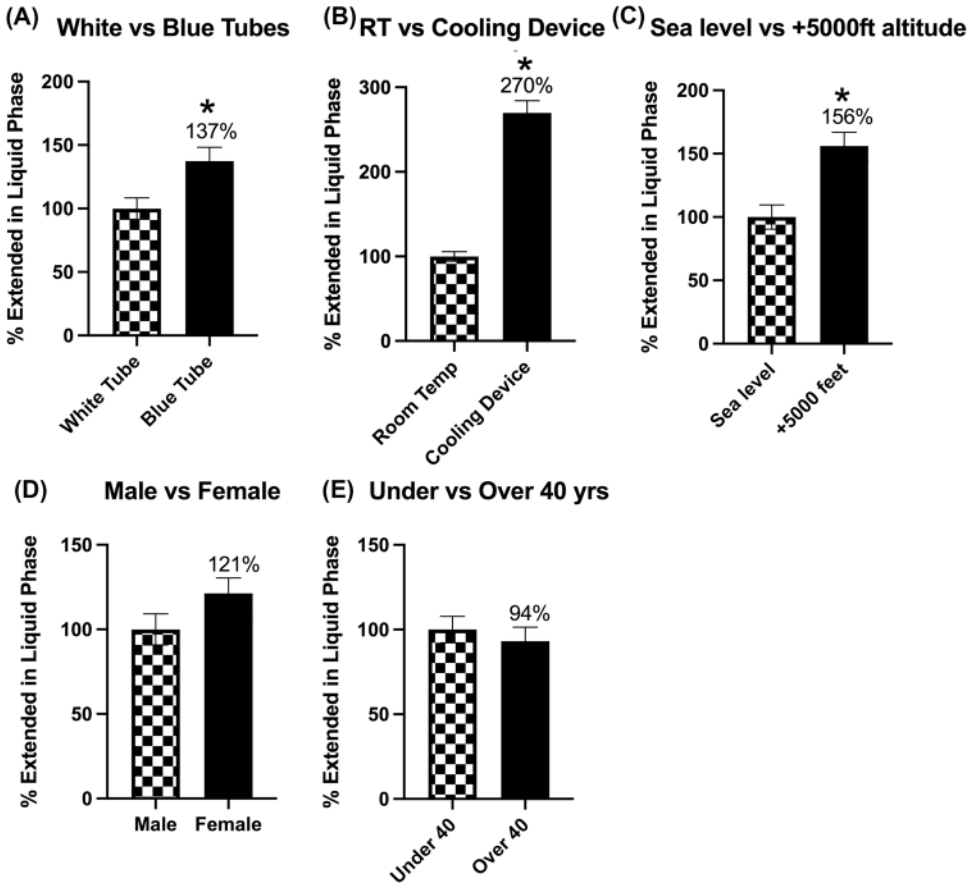

Fig. 4 Bar graph representing percent increases investigating **A** tube types, **B** the cooling device, **C** altitude, **D** patient gender, and **E** patient age. (*$p < 0.05$ indicates a significant difference between groups; $n = 30$)

altitude level above sea. Unlike in our previous study that groups demonstrated differences in PRF membrane size in both patient gender and age, the present study found no significant differences between either (Fig. 4). Thus, patient blood, whether female or male, old or young, tend to clot with equal efficiency. Nevertheless, one apparent difference was the effect on clot formation in patients living at altitude. Since half of the blood draws within this study were performed at altitude, it was noted that 56% improvement in the liquid phase of PRF was noted in patients living above 5000 ft at sea level. It remains unclear why such changes were observed, but noteworthy, a delay in clotting of PRF specifically was noted for individuals living full time at altitude.

Some of the limitations of this study are to better address the biological reasons for the observed changes which require future study. For instance, it is unknown what factors in individuals living at altitude may be affected based on the present findings. Furthermore, it remains relatively unknown to what effect temperature change does in fact reduce the proficiency of the enzymatic conversion of fibrinogen and thrombin to fibrin. These questions may further be evaluated in future basic research studies.

Furthermore, future research could also address other possibilities that may affect blood coagulation. For instance, it is known that smokers in general have higher blood viscosity which has been shown to lead to higher rates of ischemia and thrombosis [24]. It would be interesting to note if smokers have similar increases in clotting efficiency when liquid-PRF is harvested. Furthermore, it is well known and it has been hypothesized that certain vitamins/minerals and supplements delay clotting. It is suspected that such vitamins taken within 24 h of blood draw may also impact the clotting efficiency of liquid-PRF. Therefore, future research investigating additional parameters remains needed and of interest to better understand how to best optimize both solid-PRF and liquid-PRF in clinical practice.

Conclusion

In summary, the findings from the present study demonstrated that the chemically modified PET tubes performed 31% better than the control tubes at room temperature and 40% better when additionally the cooling device was utilized. In general however, the most significant improvement in delayed clotting efficiency was observed when either liquid-PRF tube was simply placed in the cooling device (270% increase in working time). While patients living at altitude did have a significant impact on delaying the clotting ability of liquid-PRF, both patient gender and patient age had

no impact on liquid-PRF clotting ability. Future research to better understand differences and variability in the patient population is further needed.

Declarations

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

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

Conflict of interest Richard J Miron holds intellectual property on the production of PRF. All other authors declare that they have no conflict of interest.

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