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REVIEW ARTICLE

Ten years of injectable platelet-rich fibrin

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Abstract

The use of platelet-rich fibrin (PRF) has seen widespread advantages over platelet-rich plasma (PRP) in many fields of medicine. However, until 2014, PRF remained clinically available only in its solid clotted form. Modifications to centrifugation protocols and tube technology have led to the development of a liquid injectable version of PRF (i-PRF). This narrative review takes a look back at the technological developments made throughout the past decade and further elaborates on their future clinical applications. Topics covered include improvements in isolation techniques and protocols, ways to further concentrate i-PRF, and the clinical impact and relevance of cooling i-PRF. Next, various uses of i-PRF are discussed, including its use in regenerative periodontology, implantology, endodontics, temporomandibular joint injections, and orthodontic tooth movement. Furthermore, various indications in medicine are also covered, including its use in sports injuries and osteoarthritis of various joints, treatment of diabetic ulcers/wound care, and facial esthetics and hair regrowth. Finally, future applications are discussed, mainly its use as a drug delivery vehicle for small biomolecules, such as growth factors, antibiotics, exosomes, and other medications that may benefit from the controlled and gradual release of biomolecules over time.

KEYWORDS
horizontal centrifugation, L-PRF, platelet-rich fibrin, tissue regeneration, wound healing

1 | INTRODUCTION

The use of platelet concentrates has gained tremendous popularity in the fields of regenerative medicine and dentistry owing to their ability to speed healing through improving neovascularization.[1] Platelet-rich plasma (PRP) is derived from peripheral blood following centrifugation with anticoagulants. Over the years, a second-generation platelet concentrate was developed with the main aim of anticoagulant removal. Termed platelet-rich fibrin (PRF), this second-generation platelet concentrate allows for faster and better

healing, with many studies demonstrating more optimized wound healing when compared to PRP.[1]

One of the advantages of PRP is that it is liquid in nature, allowing it to be easily combined with various bone biomaterials, most notably bone grafting materials, or utilized as an injectable regenerative agent for procedures such as those involving osteoarthritic joints. While many reports have demonstrated the benefit of using PRP for joint injections as well as when combined with bone grafts in dentistry, its full optimization cannot be achieved due to the use of anticoagulants. As reviewed in previous articles and a textbook,[1] clotting is an important step to healing, and the incorporation of anticoagulants during the PRP preparation process prohibits maximizing its regenerative potential.

Interestingly, as centrifugation speeds were reduced with the aim of optimizing PRF by better understanding the low-speed centrifugation concept (LSCC),[2] it was noted that a nonclotted liquid version of PRF could be obtained. This liquid PRF layer contained liquid fibrinogen and thrombin that was not yet converted to fibrin and was later given the working name injectable PRF for simplicity. If handled correctly, the clinician could rapidly harvest this liquid PRF layer and inject it into a defect area prior to clotting, which resulted in better wound healing owing to its ability to clot following injection and the slower and more gradual release of growth factors compared to PRP.[3]

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). This layer has since been utilized for injection into various joints/spaces similar to PRP but with the advantage of a longer growth factor release time. In addition, the concept of "sticky" bone was also developed. Importantly, a different type of tube (plastic) was needed to minimize clotting, as will be discussed later in this article.

Based on its beneficial use in clinical applications, an array of preclinical and clinical studies were carried out to further evaluate the regenerative potential of i-PRF when compared to PRP. In a publication titled "Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry?," our research group found that growth factor release from PRP was typically completed within the first hour, whereas injectable PRF had a much more gradual release of growth factors over time, similar to solid PRF (Figure 1).[4,5] In 2015, i-PRF was developed and initially studied using a very short and slow centrifugation protocol of 700 rpm (60 g) for 3–4 min using plastic PET tubes. Following this protocol, i-PRF remained liquid for approximately 15–20 min.[3] This new formulation was utilized for a variety of procedures, including mixing with bone grafts to form a stable fibrin graft with improved handling and graft stability.

A number of basic research studies have since demonstrated the regenerative potential of i-PRF compared to PRP.[3,6–10] While both formulations exhibited high biocompatibility with human gingival fibroblasts and significantly induced higher cell migration when compared to control tissue-cultured plastic in vitro,[3] i-PRF induced significantly greater cell migration, and mRNA levels of TGF-β and collagen 1 expression (Figure 2).[6–10]

2 | PITFALLS OF THE CURRENT METHODS UTILIZED TO QUANTIFY CELL TYPES IN PRF

One major pitfall in the quantification of PRP/PRF was addressed in 2019. As highlighted in our previous article titled "Optimization of Platelet Rich Fibrin," histological studies showed an uneven distribution of cell types in various fractions of PRF. Furthermore, many clinicians expressed confusion, mainly generated by commercial interests, regarding the importance of centrifugation protocols, RCF values versus rpm values, tube-rotor angulation and its effect on cell accumulation, rotor radius size, and tube composition in producing PRF.[1]

In 2019, our research team addressed these ongoing issues and proposed a novel method to quantify cell numbers and concentrations within PRF scaffolds following centrifugation by utilizing a sequential pipetting methodology (Figure S1: QR Code 1 SCAN ME).[11] In that study, 1 mL layers were sequentially pipetted from blood tubes, starting at the upper layer and moving toward the bottom of the tube until all 10 mL were harvested (Figure 3). Each of these 10 samples from each centrifugation tube was then sent for complete blood count (CBC) analysis to accurately and precisely quantify cell numbers within each 1 mL blood layer, which were then compared according to cell numbers and concentrations. This protocol allowed for a more accurate representation of the cell types in each layer following centrifugation.

3 | 2014–2018: OPTIMIZATION OF PROTOCOLS—A HISTORY LESSON OF i-PRF IMPROVEMENTS

Utilizing the abovementioned technique confirmed that the original i-PRF protocols developed in 2014 were unable to fully concentrate platelets in the upper plasma layer, with the majority of platelets and leukocytes remaining in the lower layers.[11] In summary, only a 2.07-fold increase in platelet concentrations and a 23% increase in leukocytes were observed utilizing the original protocol of 800 rpm for 3 min (Figure 4).[11] Thus, it was necessary to further optimize this protocol and enhance the tubes to maintain a liquid formulation of injectable PRF over time. Additionally, it was revealed that the use of horizontal centrifugation of PRF as opposed to standard fixed-angle devices led to a fourfold increase in cell concentration.[11]

4 | 2019–PRESENT: i-PRF VERSUS C-PRF—LESSONS LEARNED OVER THE YEARS

Following the systematic investigation of over 24 protocols utilizing horizontal centrifugation at various speeds and times,[12] our research

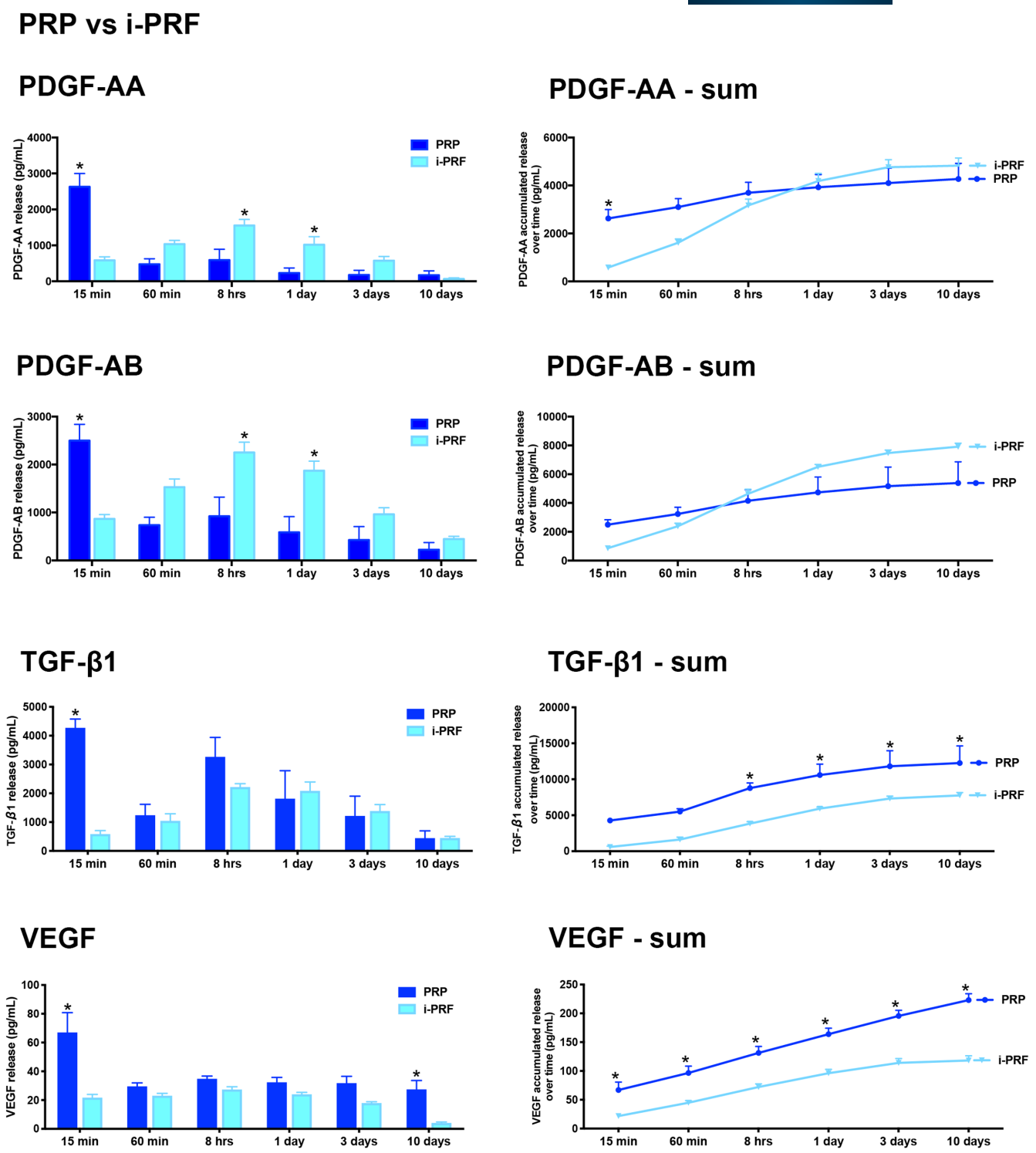

FIGURE 1 Growth factor release from i-PRF is compared with PRP at each time point for the growth factors PDGF-AA, -AB, TGF-B1, and VEGF over a 10-day period. Note the varying growth factor release from PRP and i-PRF. Some growth factors were more highly released from PRP, which posed many questions several years ago. Reprinted with permission from Miron et al.[3] i-PRF, injectable platelet-rich fibrin; PRP, platelet-rich plasma.

team found that it was much more beneficial to centrifuge samples at higher speeds and concentrate cells at the buffy coat layer as long as clotting would not occur. We therefore hypothesized that if we could specifically collect this 1 mL layer, we could create a much richer liquid PRF formulation in terms of cells and growth factors. In a study titled "A Novel Method for Harvesting Concentrated Platelet Rich Fibrin (C-PRF) with a 10-fold increase in Platelet and Leukocyte Yield,"[13] we addressed two specific questions: (1) In what total volume above the red blood corpuscle layer within the buffy coat were the majority of these cells located? (2) What final concentration

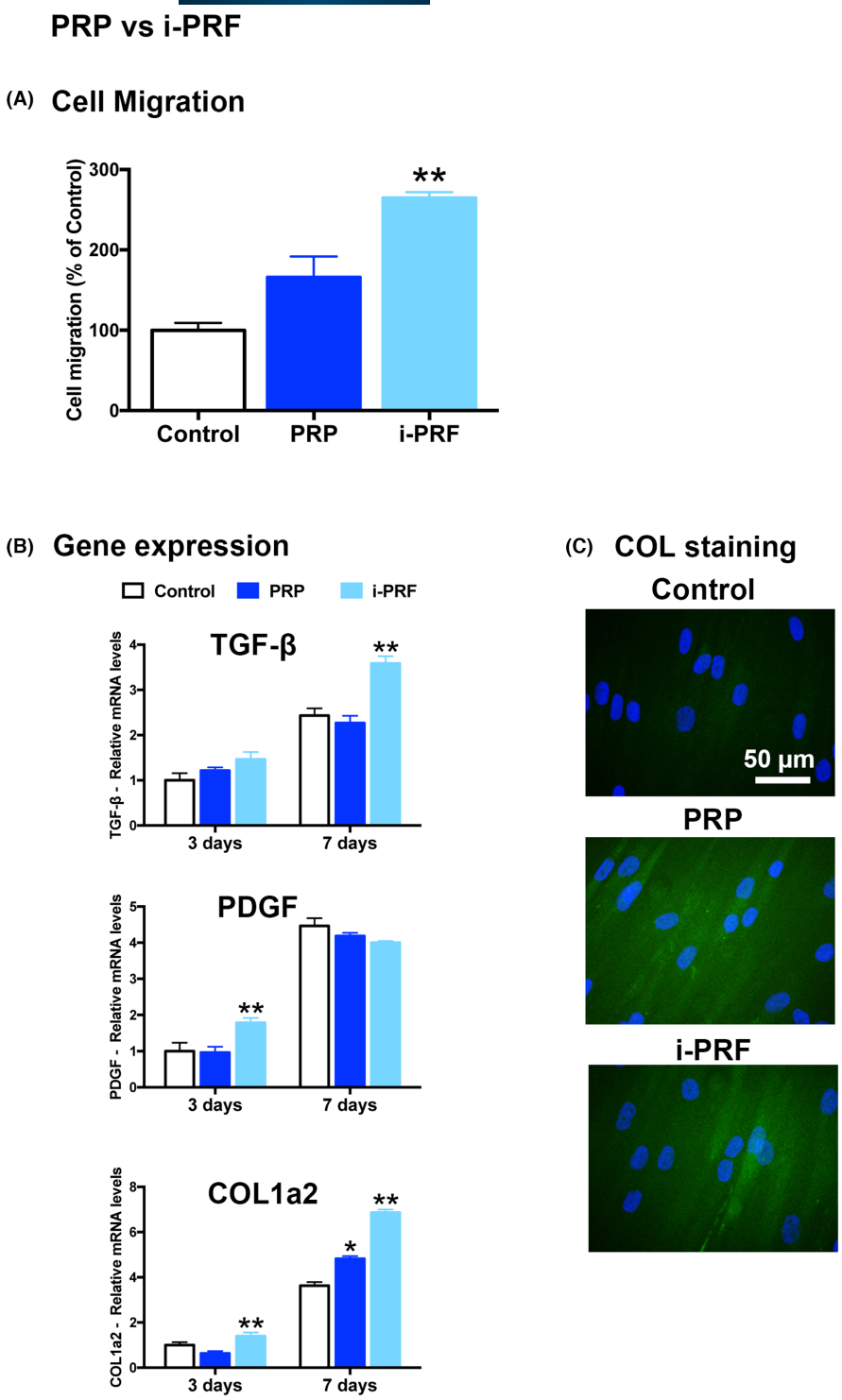

FIGURE 2 Human gingival fibroblast behavior when exposed to i-PRF versus PRP. (A) Cell migration, (B) gene expression, and (C) collagen synthesis. Reprinted with permission from Miron et al.[3] i-PRF, injectable platelet-rich fibrin; PRP, platelet-rich plasma.

could be obtained by collecting the cells found within this precise buffy coat region when compared to conventional i-PRF protocols?

Unlike in the previous study, however, we decided to quantify PRF using 100 µL sequential layers (i.e., 0.1 mL) to precisely investigate the location of cells (Figure 5). Since we had previously observed massive cell accumulation within the buffy layer in a 1 mL sample following the higher speed protocols, we aimed to precisely determine the volume in which these cells were located within this 1 mL layer directly above the red cell corpuscle layer. As such, we developed a novel methodological approach whereby 100 µL sequential layers were pipetted starting from ~1.2–1.5 mL above the buffy coat down to the red blood cell layer (Figure 5, depicted as +1 to +12 layers). Additionally, 3 layers were harvested within the red blood cell layer to determine the number of cells incorporated within this layer (Figure 5, depicted as layers −1 to −3). Each of these layers was sent for CBC analysis.

The second tube from each group was utilized to determine the final concentrations of the liquid version of the i-PRF yellow plasma layer. For L-PRF protocols, one tube was utilized to harvest 0.5 mL of concentrated PRF (defined as the 0.5 mL buffy coat layer directly above the red blood cell corpuscle layer). This concentrated buffy

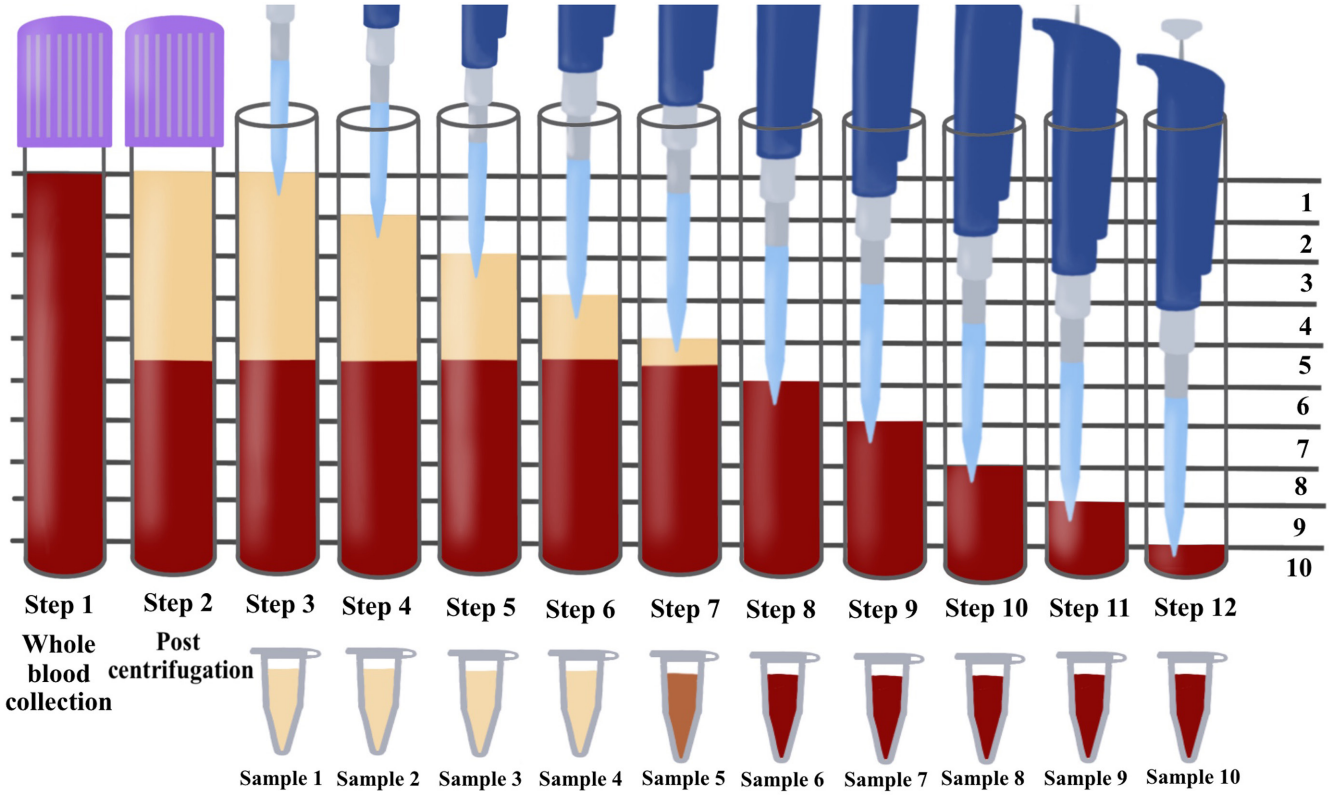

FIGURE 3 Illustration demonstrating the proposed novel method to quantify cell types following centrifugation of PRF. Currently, one of the limitations is that whole blood is compared to the total plasma concentration following centrifugation. However, this does not give a proper representation regarding the location of cells following centrifugation. By utilizing the proposed technique in this study by sequentially pipetting 1 mL of volume from the top layer downward, each of the 10 samples can be sent for CBC analysis to accurately determine the precise location of each cell type following centrifugation in various protocols. Note that one layer (in this case, layer 5) will contain some yellow plasma and red blood cells. This is the buffy coat, where a higher concentration of platelets is typically located. Reprinted with permission from Miron et al.[11] CBC, complete blood count; PRF, platelet-rich fibrin.

coat layer was termed concentrated PRF (C-PRF) (Figure 6). Similarly, 0.3 mL of C-PRF liquid was also harvested from this layer.[13]

In summary, we observed a 2.5-fold increase in platelets following the i-PRF protocol and only a slight increase in leukocytes. Figure 7 demonstrates that while the i-PRF protocol increases leukocyte numbers 1.23-fold, a marked and significant 4.62- and 7.34-fold increase was observed in the 0.5 mL and 0.3 mL C-PRF layers, respectively. In addition, while i-PRF protocols have typically been shown to increase platelet yields between 200 and 300%, the C-PRF protocols massively increased platelet yields by 1138% and 1687%, respectively (Figure 7). A similar trend was also observed for monocytes. Total values following averages are summarized in Table 1.

We also noted that some cells, especially leukocytes, were also found in an approximately 0.3 mL layer within the red buffy coat zone. Further research from other independent groups has also confirmed the advantages of collecting the red buffy coat zone when harvesting i-PRF.[14,15]

Thus, in summary, a new method to concentrate liquid PRF directly from the buffy coat layer using higher centrifugation speeds was proposed. Regarding the collection protocol, it is best to remove the upper platelet-poor plasma (PPP) layer first, followed by collection of the concentrated C-PRF layer (Figure 8). This protocol is currently used at 2000 RCF for 8 min utilizing hydrophobic blue plastic tubes (Figure S1: QR Code 2 SCAN ME).

5 | EFFECTS OF TUBE TECHNOLOGY AND COOLING ON THE CLOTTING ABILITY OF i-PRF

Given that hydrophilic tubes improve PRF clotting, it was hypothesized that if we could design a more hydrophobic tube, we could improve the length of time that PRF remained liquid. As such, our research group purposefully designed more hydrophobic tubes to avoid protein adsorption and platelet contact with the tube wall surfaces where clotting starts (Figure S1: QR Code 3 SCAN ME). The

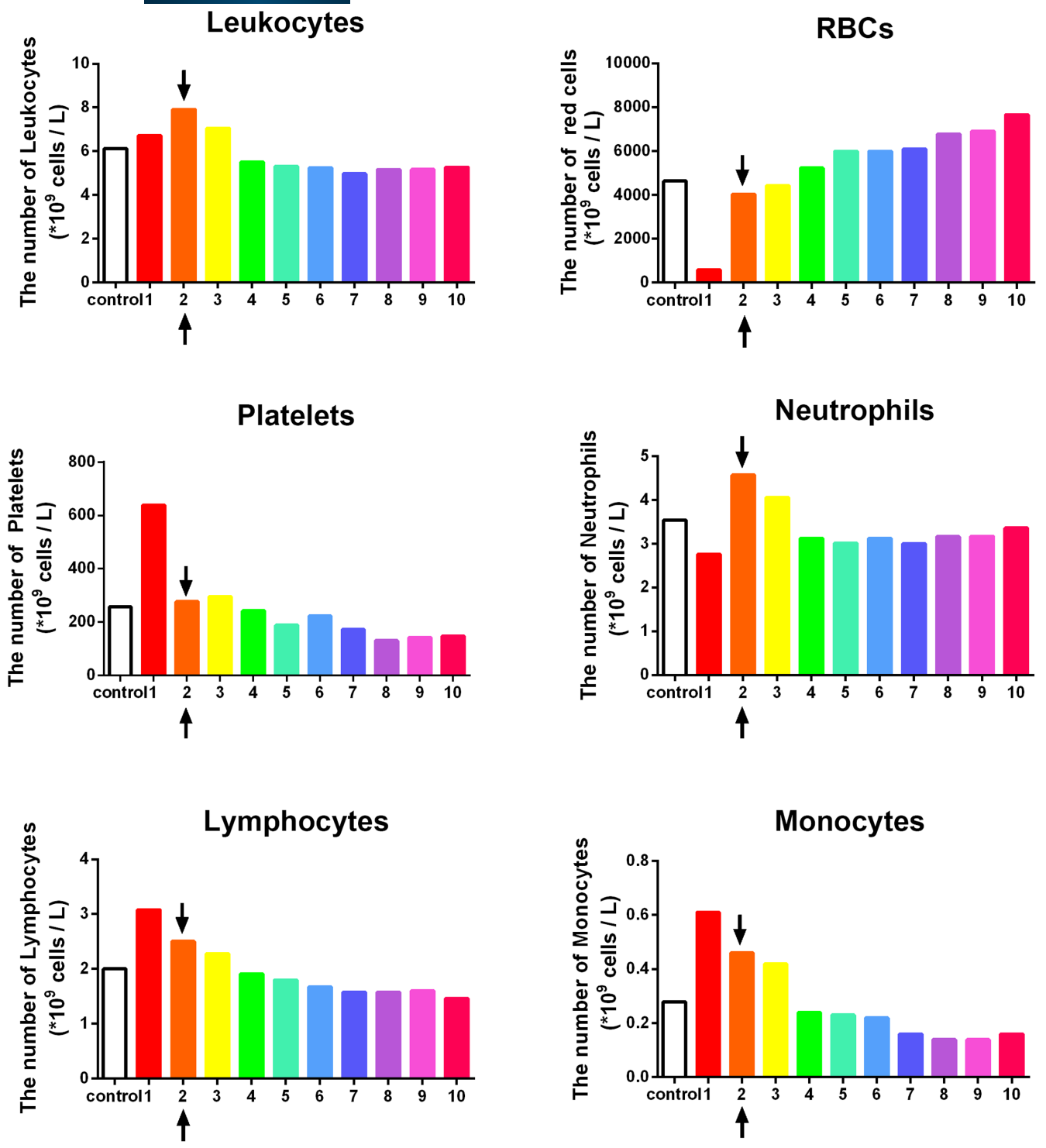

FIGURE 4 The concentration of cell types in each layer from 1 mL down to the 10th mL sample utilizing the liquid PRF process for PRF (i-PRF) protocol (800 rpm for 3 min; ~60 g). Note that very little change in platelet or leukocyte accumulation is observed when utilizing this centrifugation cycle. However, a slight increase in platelets and leukocytes was observed when compared to the control. Reprinted with permission from Miron et al.[11] i-PRF, injectable platelet-rich fibrin.

clinician could therefore maximize their working time with liquid i-PRF. In a study titled "Extending the working properties of liquid platelet-rich fibrin using chemically modified PET tubes and the Bio-Cool device,"[16] it was found that chemically modified PET tubes performed 37% better than control tubes and extended the working properties of liquid PRF by over 20 min. It is well understood that the conversion of liquid fibrinogen and thrombin to fibrin is an enzymatic process. Enzymes function well at body temperatures but lose their ability to function at colder temperatures. In 2019, it was hypothesized that by cooling plasma, the clinician could dramatically delay clotting and extend their working time using injectable PRF. By placing liquid PRF in a cooling device (Bio-Cool), it was observed

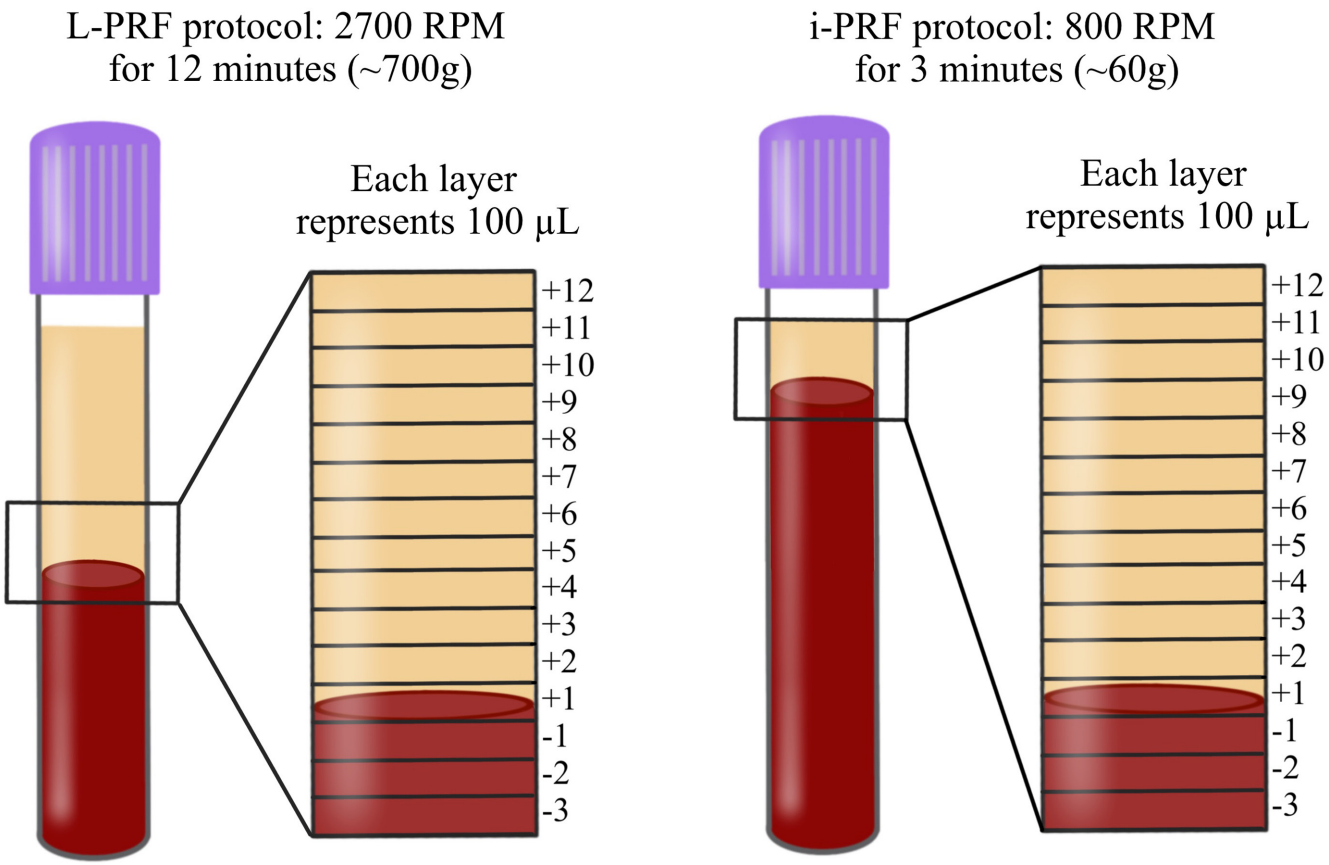

FIGURE 5 A second methodological illustration depicting the sequential harvesting technique. Briefly, because the majority of cells accumulate within 1 mL of the buffy coat, we sought to precisely investigate the total volume of liquid (mL) above the buffy coat cells. For the L-PRF protocols, 3.5 mL was removed, followed by sequential 100 µL layer pipetting and CBC analysis. Three layers in the red blood cell layer were also harvested. In comparison, all plasma layers of the i-PRF protocol were also harvested in 100 µL sequential layers. Three red blood cell layers (100 µL each) were also collected. Reprinted with permission from Miron et al.[13] CBC, complete blood count; i-PRF, injectable platelet-rich fibrin; L-PRF, liquid platelet-rich fibrin.

that liquid PRF would stay liquid for up to 4 h, marking a 270% improvement when compared to tubes at room temperature (Figure 9, Figure S1: QR Code 4 SCAN ME).[16] 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 in which to inject the final liquid PRF formulations. Clinicians may also benefit from placing PRF tubes in an incubator when faster clotting of PRF is preferred, such as for diabetic wound treatments or dentistry, as highlighted later in this article.

6 | BIOLOGICAL PROPERTIES OF i-PRF

Injectable PRF has regenerative properties similar to those of PRF, which have been extensively reviewed in many articles as well as a recent textbook.[1] In an extensive systematic review by Strauss and colleagues investigating the biological properties of PRF, 1746 studies were identified, of which 53 were included.[17] Since PRF is capable of improving angiogenesis in vivo, it was reported that PRF enhanced the proliferation, migration, adhesion, and osteogenic differentiation of a variety of cell types in addition to cell signaling activation. Furthermore, it was concluded that PRF reduced inflammation, suppressed osteoclastogenesis, and increased the expression of various growth factors in mesenchymal cells.[17]

Several interesting recent studies have further demonstrated that i-PRF also directs macrophage polarization from a proinflammatory M1 phenotype toward a pro-resolving M2 phenotype.[18] Since macrophages are extremely important cells during the healing process and can be involved in the secretion of either proinflammatory markers (M1) or pro-resolution markers (M2), their study is extremely relevant to PRF since they are present in high concentrations.[18]

In a study by Nasirzade et al., murine primary macrophages and a human macrophage cell line were exposed to saliva and lipopolysaccharides (LPSs) with and without PRF lysates.[18] The expression of the proinflammatory M1 marker genes interleukin-1beta (IL-1β) and interleukin-6 (IL-6) was significantly suppressed, and PRF-conditioned medium enhanced the expression of tissue resolution

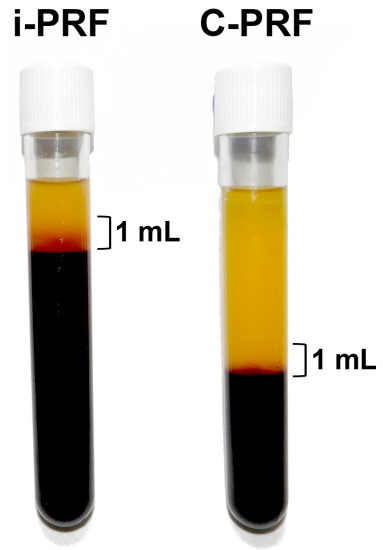

FIGURE 6 (A) visual representation of the layer separation following either i-PRF (300×*g* for 5 min) or C-PRF protocols (3000×*g* for 8 min). Harvested plasma was collected from the 1 mL layer above the RBC layer within the buffy coat region. Reprinted with permission from Fujioka-Kobayashi et al.[34] C-PRF, concentrated platelet-rich fibrin; i-PRF, injectable platelet-rich fibrin; RBC, red blood cell.

markers. PRF was concluded to have potent anti-inflammatory activity and to shift macrophage polarization from an M1 toward an M2 phenotype.[18] In a second study on this topic, i-PRF reduced the proinflammatory M1 phenotype of macrophages as well as the activated dendritic cells around muscle defects injected with bacterial suspensions.[19]

While the study of PRF on various cell types, such as immune cells, has only just begun, i-PRF has been shown to have effects on various immune cells and to act to reduce the proinflammatory response and further decrease the inflammatory process and response toward LPSs. Thus, this work may in part explain the observed clinical improvements in postoperative pain reported in the literature.[1]

7 | BIOLOGICAL ANTI-INFLAMMATORY AND ANTIBACTERIAL PROPERTIES OF PRF

Both the anti-inflammatory and antibacterial properties of PRF have been a topic of interest in recent years, owing to the clinical observation that PRF seems to reduce postoperative swelling and pain. In a study titled "Effects of liquid platelet-rich fibrin on the regenerative potential of hPDLCs cultured under inflammatory conditions,"[20] our group investigated the effects of PRF on human periodontal ligament cells (hPDLCs) under various inflammatory conditions. hPDLCs were investigated using a migration and proliferation assay. To investigate hPDLC differentiation, an alkaline phosphatase (ALP) assay and alizarin red staining were performed, and gene expression levels

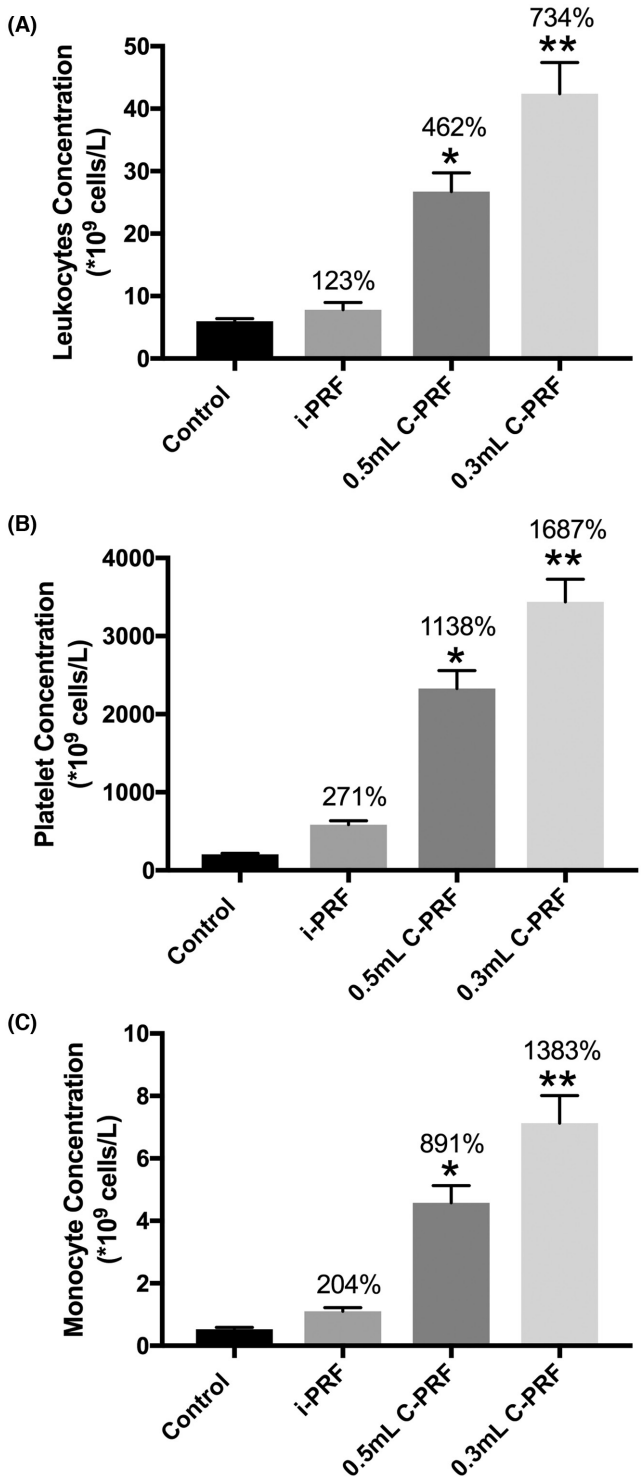

FIGURE 7 Concentration of (A) leukocytes, (B) platelets, and (C) monocytes following centrifugation using i-PRF protocols versus collecting 0.3–0.5 mL of C-PRF. Note that while i-PRF was typically responsible for a 1.2- to 2.5-fold increase in the various cell types following centrifugation, up to a 15-fold increase in platelet concentration could be achieved with C-PRF (* represents a significant difference when compared to i-PRF; ** represents significantly higher than all groups, $p < 0.05$). Reprinted with permission from Miron et al.[13] C-PRF, concentrated platelet-rich fibrin; i-PRF, injectable platelet-rich fibrin.

of *Runx2*, *Col1a1*, and *OCN* were determined. In addition, cells were cultured with LPSs to induce an inflammatory condition. The results of all assays were compared to those for PRP.[20]

Osteogenic differentiation demonstrated that liquid PRF induced significantly greater ALP activity and more mineralized nodules when compared to PRP and controls (Figure 10). According to the experimental timeline, cells were then pretreated with or without LPSs for 7 days to induce an inflammatory condition, and then liquid PRF was added to the culture medium for an additional incubation period of 7 days (Figure 10A). Immunofluorescence images demonstrated that LPSs induced more *p65* expression (a marker for inflammation) (Figure 10B), while the addition of liquid PRF decreased its expression level. Furthermore, other inflammation markers, including *IL-1β* and *TNF-α*, were also significantly downregulated, as confirmed by RT–PCR (Figure 10C). In a final experiment from the same study, i-PRF promoted the osteogenic differentiation of hPDLCs even when cultured in an inflammatory environment. In summary, these findings indicate that the anti-inflammatory effect and regenerative potential of liquid PRF can counterbalance the negative inflammatory effect induced by LPSs.[20] These findings are specific to the periodontal field since PRF has been shown to improve the regeneration of intrabony and furcation defects,[21] and this is hypothesized to be caused not only by an improvement in local growth factor concentrations but also by counterbalancing the inflammatory response induced by LPSs.

In a recent systematic review specific to injectable PRF, 18 in vitro studies, 5 animal studies, 6 case reports, and 31 clinical studies evaluated the effect of i-PRF on oral and maxillofacial soft and hard tissue regeneration.[22] The investigated studies verified the anti-inflammatory and antimicrobial efficacy and the positive effects of i-PRF application for wound, periodontal, bone, cartilage, and pulp regeneration (Figure 11),[22] which will be discussed later in this article.

8 | USE OF i-PRF FOR IMPROVED WOUND HEALING IN THE ORAL CAVITY

An in vitro study by Dohle and colleagues assessed the effect of i-PRF on angiogenesis and wound healing in vitro.[23] It was revealed that i-PRF increased factors associated with wound healing, including PDGF-BB, ICAM-1, and E-selectin, and upregulated the proangiogenic and growth factors VEGF, BMP2, and ALP.[23] In an animal study investigating submandibular salivary glands, Elsherbini et al. found that both i-PRF and melatonin increased regenerative capacity by significantly reducing caspase-3 and increasing vascular endothelial growth factors.[24] In another animal study conducted in 2020, Mu et al. found that the addition of i-PRF to a commonly utilized deproteinized bovine bone mineral (DBBM) improved growth factor release by nearly 2 weeks.[25]

Four clinical studies have investigated the use of i-PRF on wound healing in the oral cavity. In a clinical study by Kiziltoprak and colleagues, higher palatal wound epithelialization and lower bleeding and pain scores were found in the i-PRF group than in the control group.[26] Two further studies demonstrated that i-PRF injections significantly improved symptomatic oral lichen planus.[27,28] In a final clinical study, Negah and colleagues investigated i-PRF administration for the management of root resorption in 10 healthy patients.[29] It was reported that the clinical evaluation resulted in the resolution of signs and symptoms through a 12-month follow-up period in all cases. Moreover, the radiographic evaluation showed a marked decrease in the mean volume of internal inflammatory root resorption and periapical lesions.[29] Finally, an additional four studies found that i-PRF demonstrated antimicrobial efficacy against both periodontal and cariogenic pathogens.[30–33]

9 | USE OF i-PRF IN PERIODONTOLOGY

In total, five studies investigated the effects of i-PRF on periodontal regeneration. In 2017, Wang and colleagues compared the effects of i-PRF and PRP on human gingival fibroblasts cultured on titanium discs.[9] While both i-PRF and PRP showed excellent gingival fibroblast viability and biocompatibility, i-PRF showed significantly higher TGF-β, PDGF, fibronectin, and collagen type 1 levels than PRP.[9] A study by our group in 2020 further confirmed the regenerative potential of liquid C-PRF compared to i-PRF.[34] In that study, a significant increase in growth factor release was observed with the C-PRF obtained from the buffy coat layer following higher centrifugation protocols and significantly increased gingival fibroblast migration, proliferation, gene expression, and collagen I synthesis (Figure 12).[34] In another study published in 2020, Iozon and colleagues showed that i-PRF significantly increased gingival cell proliferation and osteogenic genes.[35] Last, Zheng et al.[20] and Thanasrisuebwong et al.[15] demonstrated that i-PRF improved human periodontal ligament cell proliferation, migration, biological differentiation, and mineralization when cultured with conditioned media including i-PRF. In the only animal study using i-PRF for periodontal regeneration, Aydinyurt and colleagues investigated the use of subgingival i-PRF injections for potential improvements in patients with periodontitis; however, no positive outcomes were observed.[36]

A number of clinical studies have investigated i-PRF for various clinical indications/applications. In a case study investigating the use of i-PRF with solid PRF and a bone graft, Lei et al. found that the combination approach improved alveolar bone gains and reduced pocket depths in a case of severe periodontal bone loss at a 15-month follow-up.[37] Six clinical studies have investigated the effect of i-PRF on periodontal parameters. In 2019, İzol and Üner investigated the effect of i-PRF on root coverage in free gingival graft surgery and found improved root coverage and increased new gingival tissue formation.[38] In 2020, Turer and colleagues used i-PRF in combination with connective tissue grafts for root coverage of gingival recessions.[39] It was found that the additional use of i-PRF resulted in a significant reduction in recession depth and an increase in keratinized tissue height compared

TABLE 1 Leukocyte, platelet, and monocyte concentrations in whole blood, i-PRF, 0.5 mL of C-PRF, and 0.3 mL of C-PRF (represented as $10^9 \times$ cells/L).

	Whole Blood	i-PRF	0.5 mL C-PRF	0.3 mL C-PRF	i-PRF% increase	0.5 mL C-PRF % increase	0.3 mL C-PRF% increase
Leukocytes	6.0	7.8	26.7	42.4	123.8	461.7	733.7
Platelets	206.3	586.0	2327.9	3437.6	270.6	1138.2	1687.3
Monocytes	0.5	1.1	4.6	7.1	203.8	891.5	1383.4

Note: The final three columns represent the percentage increase in leukocyte, platelet, and monocyte values when compared to baseline controls. While i-PRF protocols are able to achieve an approximately 1.2- to 2.7-fold increase in cell types, C-PRF protocols, especially 0.3 mL C-PRF, were able to massively concentrate leukocytes (>7-fold), platelets (>16-fold), and monocytes (>13-fold) when compared to controls. Reprinted with permission.[13]

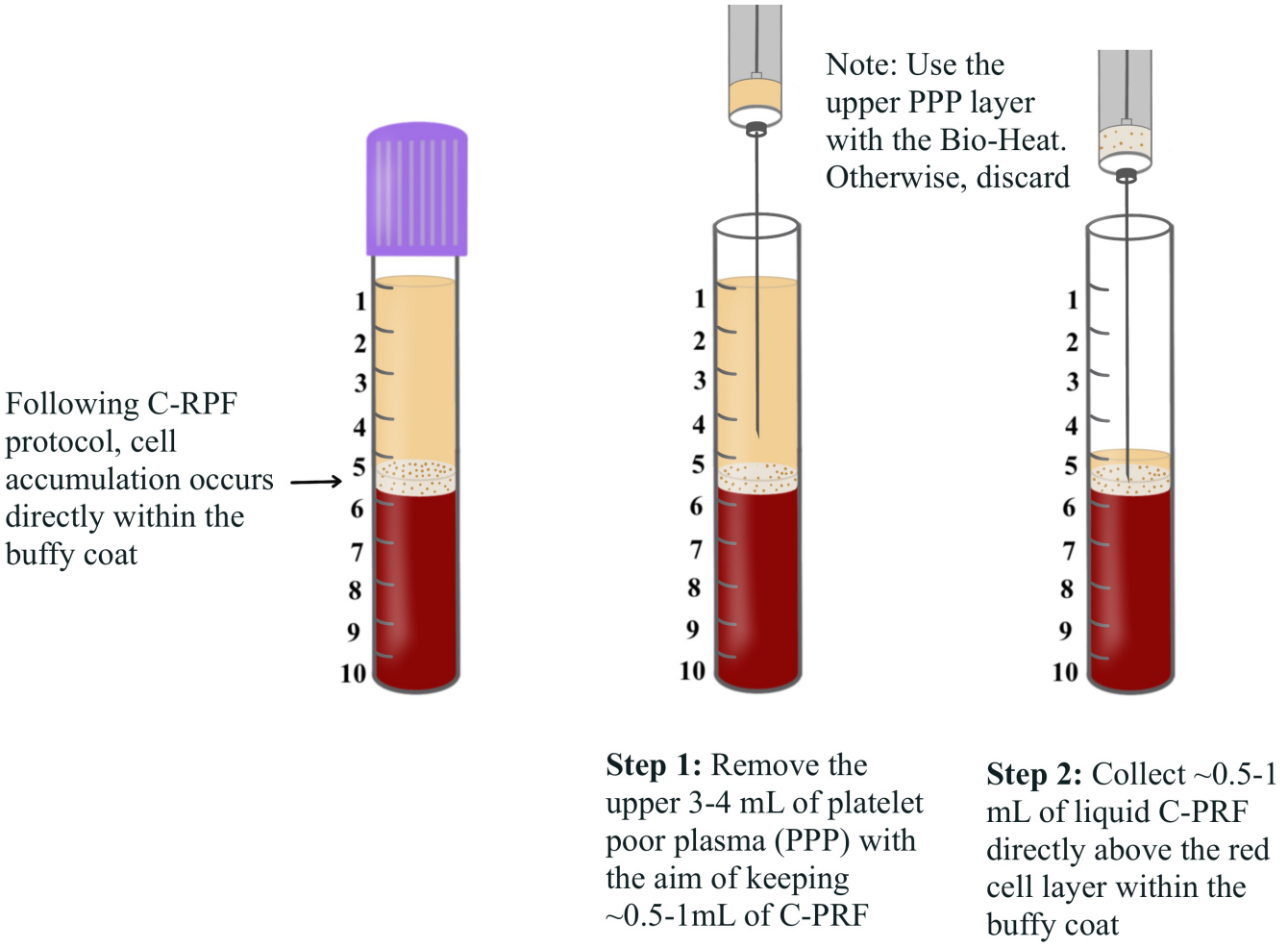

FIGURE 8 Methods to collect and concentrate C-PRF. Following centrifugation at higher speeds (2000 × ***g*** for 8 min), the majority of cells are located directly at the buffy coat layer. Instead of attempting to remove this layer using a long needle through the deep layers, it is highly advised to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

to CTG alone.[39] In another study, i-PRF was added to standard scaling and root planing for patients diagnosed with chronic periodontitis.[40] It was revealed that i-PRF improved the clinical attachment level, gingival margin levels, pocket depths, and bleeding on probing compared to SRP alone.[40] In a rather novel study, microneedling was performed to enhance the gingival thickness of the keratinized tissue width in thin periodontal phenotypes.[41] It was concluded that the combination of microneedling and i-PRF increased gingival thickness when compared to microneedling alone.[41] In a separate study, Kapa and colleagues investigated the use of i-PRF to create sticky bone in 16 patients with isolated Miller Class I and II recessions in the maxillary esthetic zone.[42] Clinical evaluation demonstrated that all treated cases achieved an increase in gingival thickness and keratinized tissue width, as

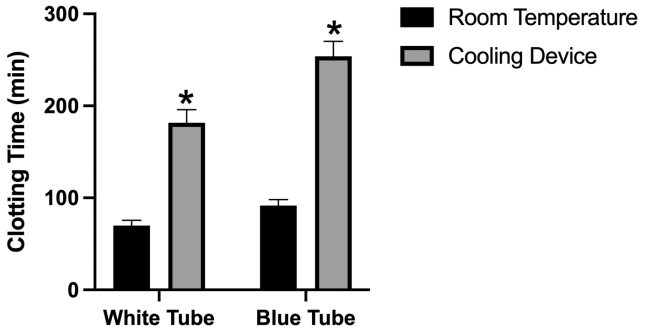

FIGURE 9 Bar graph representing the average clotting time of liquid platelet-rich fibrin in (1) white tubes at room temperature, (2) white tubes placed in the cooling device, (3) blue tubes at room temperature, and (4) blue tubes placed in the cooling device (*$p < 0.05$ indicates a significant difference between tubes placed at room temperature and the cooling device; $n = 30$). Reprinted with permission from Miron et al.[16]

well as a decrease in periodontal pockets and recessions.[42] Finally, i-PRF was used as an adjunct in subgingival irrigation of SRP in 15 patients with bilateral periodontal pockets (≥5 mm).[43] No differences were reported between the groups.[43] Naturally, much more data exist comparing standard solid PRF clots for various indications in regenerative dentistry when compared to i-PRF. Briefly, systematic reviews with meta-analysis have pointed to the fact that PRF seems to better improve soft tissue healing, including recession coverage and intrabony defect regeneration, when compared to bone augmentation, including alveolar ridge preservation and/or sinus grafting.[21,44–46]

10 | USE OF i-PRF fOR BONE REGENERATION

In total, 22 studies evaluated the use of i-PRF during bone regeneration, including eight in vitro studies,[10,23,47–52] three animal studies,[25,53,54] three case reports,[55–57] and nine clinical studies.[26,39,48,56–61]

In vitro studies have demonstrated that i-PRF exhibits better biological properties, including the ability of osteoblasts to proliferate, differentiate, and produce mineralized nodules on either tissue-culture plastic or titanium discs, than PRP, A-PRF, L-PRP, and freeze-dried homologous PRP.[10,23,49–51] Additionally, i-PRF has been added to bone grafting materials, and the characteristics of human osteoblasts have been significantly improved.[47,48] Our research team addressed the effects of adding i-PRF to sticky bone on the mechanical properties of the combination approach.[52] Solid PRF fragments + liquid PRF + a commonly utilized bovine bone graft had by far the fastest solidification period (over a 10-fold increase) as well as the most resistance to degradation (Figure S1: QR Code 5 SCAN ME). Scanning electron microscopy and tensile strength tests also revealed that the mechanical properties of this combination approach were significantly superior in strength when compared to bone grafts with PRF fragments or bone grafts with i-PRF alone and further induced the highest osteoblast migration and osteogenic differentiation, as confirmed by ALP, ARS, and real-time PCR (Figure 13).[52]

Three animal studies have evaluated the effects of i-PRF on bone formation. Two studies demonstrated that i-PRF was able to induce maxillary bone regeneration during sinus augmentation in a rabbit model.[25,53] More recently, Yuan et al. evaluated angiogenesis, osteogenesis, and bone mass using a bone graft with i-PRF in an extraction site model using male beagle dogs.[54] The combination of i-PRF with a biomaterial significantly enhanced angiogenesis and woven bone and reduced osteoclast activity in extraction sockets 2 weeks after the operation. Significant corticalization on the alveolar ridge crest was also reported at 8 weeks post-operation.[54]

To date, three case studies have evaluated i-PRF for bone regeneration.[55–57] In a case study by Chenchev et al.,[56] a combination of bone graft material with both solid PRF and i-PRF was used for ridge augmentation of the maxilla. New bone formation was apparent 4 months post-operatively with the ability to place a dental implant. In two other case reports, the effect of a combination of i-PRF and solid PRF with a bone graft appeared to improve bone augmentation either horizontally or vertically.[55,57]

Nine clinical studies[58–66] have investigated bone regeneration using i-PRF, mainly in case series. Positive outcomes have been reported investigating i-PRF in sinus grafting in combination with a collagen plug[60] and a deproteinized bovine bone graft,[59] horizontal ridge augmentation with a bone graft followed by implant placement,[58,65,66] in combination with an iliac bone graft on a patient with complete unilateral cleft alveolus[62] and alveolar clefts,[64] and for GBR with simultaneous implant placement using i-PRF and xenografts.[61] In one study by Isik and colleagues, an allograft combined with i-PRF was compared to autogenous block grafting with similar outcomes.[63] Thus, one of the major reported advantages of i-PRF in bone grafting is the improvement in graft material handling (Figure 13, Figure S1: QR Code 6 SCAN ME).

11 | CLINICAL USE OF i-PRF IN ENDODONTICS

Over the years, one of the desired goals in endodontics has been to discover novel means to regenerate the pulp and potentially utilize i-PRF as a drug delivery vehicle. In 2019, our team compared the regenerative capacity of i-PRF to that of PRP and found that i-PRF substantially increased the migration of dental pulp cells and significantly increased alkaline phosphatase activity and the expression

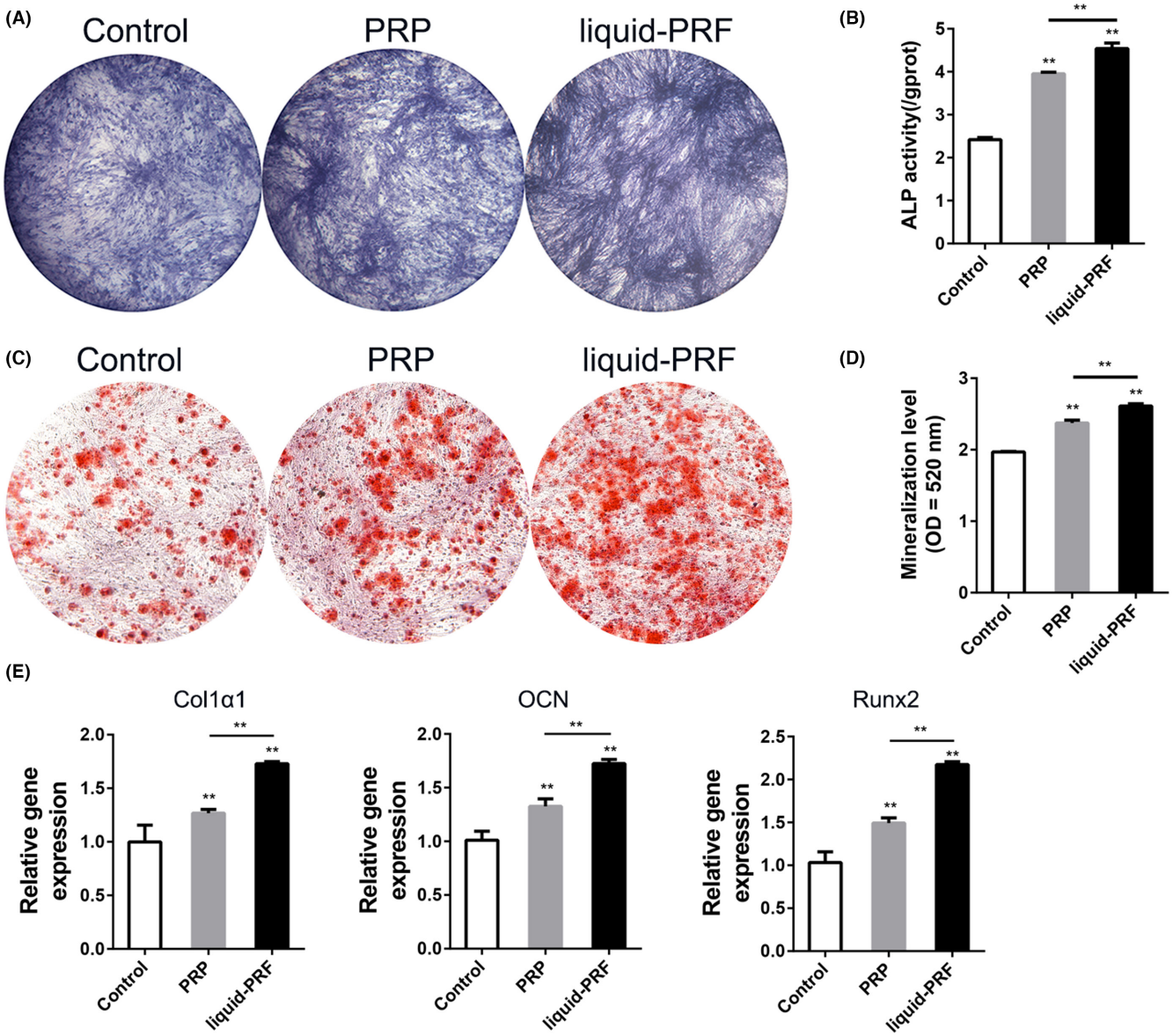

FIGURE 10 (A, B) Effects of PRP and liquid PRF on ALP activity were detected by (A) ALP staining and (B) ALP activity test. (C) Alizarin Red-S staining showed the mineralized nodules in each group after induction for 14 days. (D) Semiquantitative analysis of mineralization levels. (E) Relative gene expression levels of Col1a1, OCN, and Runx2 after treatment with PRP or liquid PRF for 14 days. Error bars correspond to the means ± SDs. Significant differences are indicated: $^{*}p < 0.05$; $^{**}p < 0.01$; ALP, alkaline phosphatase; hPDLCs, human periodontal ligament cells; ns, not statistically significant versus control group; PRF, liquid platelet-rich fibrin; PRP, liquid platelet-rich plasma.

of collagen type 1 and dentin matrix proteins.[7] Furthermore, Rafiee and colleagues evaluated the in vitro drug delivery profile of two prepared mixtures of triple antibiotics, metronidazole, ciprofloxacin, and minocycline, and observed sustained release for up to 14 days.[67] In a second study by the same group, the efficacy of i-PRF loaded with a triple antibiotic mixture was evaluated against *Enterococcus faecalis* and *Actinomyces naeslundii* biofilms in an infected root canal model. It was found that delivering a triple antibiotic mixture using i-PRF was most efficient at reducing bacterial loads when compared to other delivery methods.[31] Although these studies remain limited to in vitro testing, a number of clinicians have now utilized i-PRF for various applications in endodontic therapy, as highlighted in a recent textbook on the topic.[1] Other applications include direct pulp capping and the replantation of lost teeth (Figure S1: QR Code 7 SCAN ME and QR Code 8 SCAN ME).

12 | CLINICAL USE OF i-PRF FOR ORTHODONTIC TOOTH MOVEMENT

One recent trend has investigated the use of i-PRF for improvements in orthodontic tooth movement. As PRP/PRF in general has been

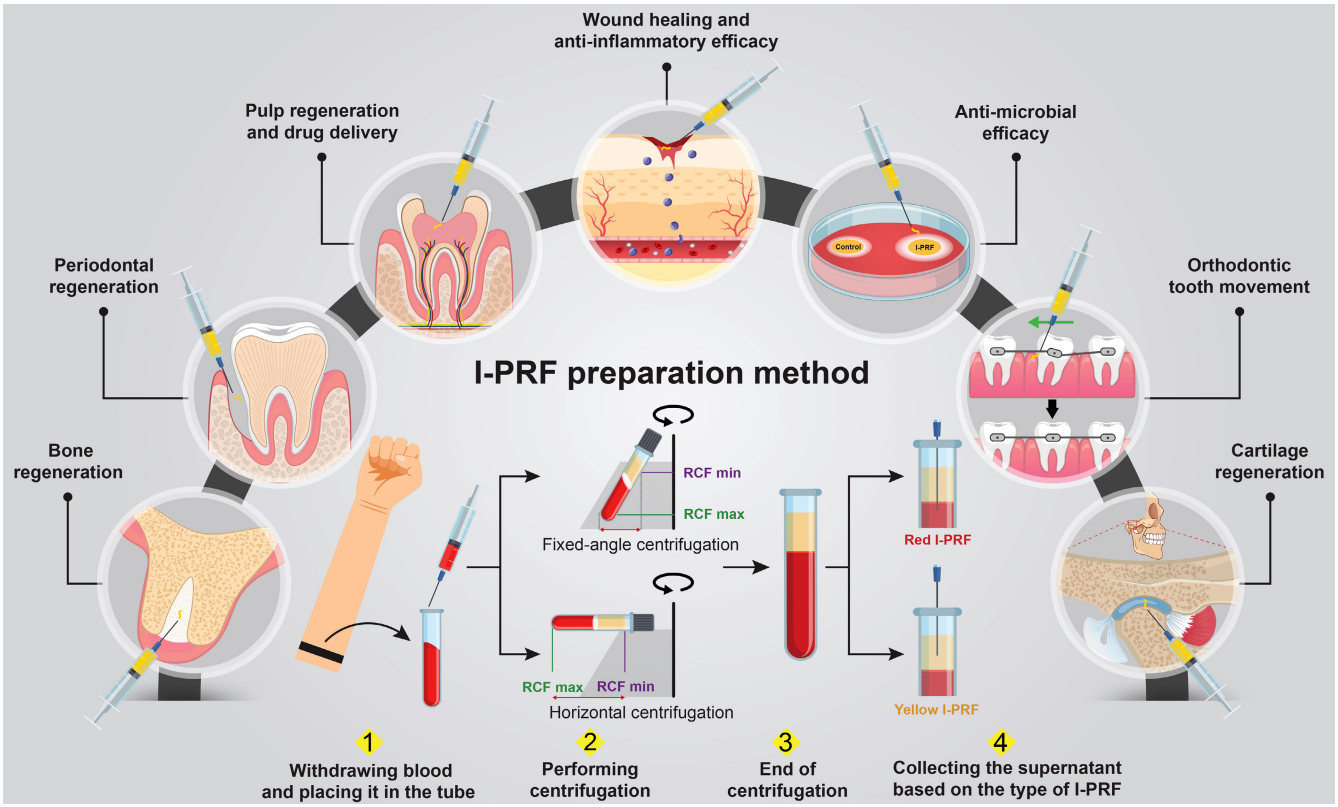

FIGURE 11 A schematic figure of the applications of injectable platelet-rich fibrin in soft and hard tissue regeneration in relation to oral and maxillofacial structures and its preparation method. Reprinted with permission from Farshidfar et al.[22]

shown to speed the metabolic activity of various cell types, it was hypothesized that tooth movement could potentially be achieved in a shortened period of time using i-PRF. To date, five clinical studies have investigated this concept.[68–72]

In summary, it was found that i-PRF could increase the retraction rate of maxillary incisors[68] and maxillary canines[70] and increase the levels of IL-1β, matrix metalloproteinase-8, and receptor activator of nuclear factor kappa-B ligand (RANKL) in the gingival crevicular fluid.[71] Other studies found no reported advantages using i-PRF, including a study by Karci and colleagues comparing piezocision with i-PRF injections during canine distalization,[69] and it did not improve the preservation of bone or root resorption in various orthodontic patients.[72]

13 | CLINICAL USE OF i-PRF for TMJ INJECTIONS

One area that has been highly researched is the use of i-PRF as an injection modality for the treatment of temporomandibular joint disorders. In 2018, a breakthrough study by Albilia and his colleagues found that patients suffering from TMJ dysfunction or pain could benefit from i-PRF injections.[73] In that study, patients were assessed after 8 weeks, 3 months, 6 months, and 12 months of follow-up, and the authors noticed a significant decline in pain scores due to possible remodeling of the damaged cartilage surfaces.[73] In 2020, Yuce and Komerik[74] compared intra-articular infiltrations with the commonly utilized hyaluronic acid (HA) to i-PRF. Pain values were significantly decreased in the i-PRF group compared to the HA group. Furthermore, the maximum mouth opening values in the i-PRF group were significantly greater than those in the HA group at 9- and 12-month intervals.[74] In 2021, the effectiveness of arthrocentesis in combination with or without i-PRF injection was investigated for internal derangement of the TMJ.[75] At the 3-month follow-up, substantial improvements in VAS and Helkimo clinical dysfunction scores were reported, along with improvements in the maximum incisal opening of the patients.[75] In another study, the effects of intra-articular i-PRF injections on the treatment of osteoarthritis of the TMJ were assessed.[76] In this study, it was revealed that while i-PRF did not alleviate TMJ pain after 6 months, repeated injections did positively impact maximal mouth opening. Ghoneim and colleagues also compared the effectiveness of arthrocentesis with/without i-PRF injections for the treatment of TMJ disc displacement and reduction.[77] A significant reduction in click sound and pain intensity and an increase in lateral movement and maximal mouth opening were reported in the i-PRF group.[77] Finally, the use of i-PRF was examined for the treatment of Wilkes stage III internal derangement, and arthrocentesis in combination with i-PRF injections was more effective than arthrocentesis alone or in combination with HA.[78]

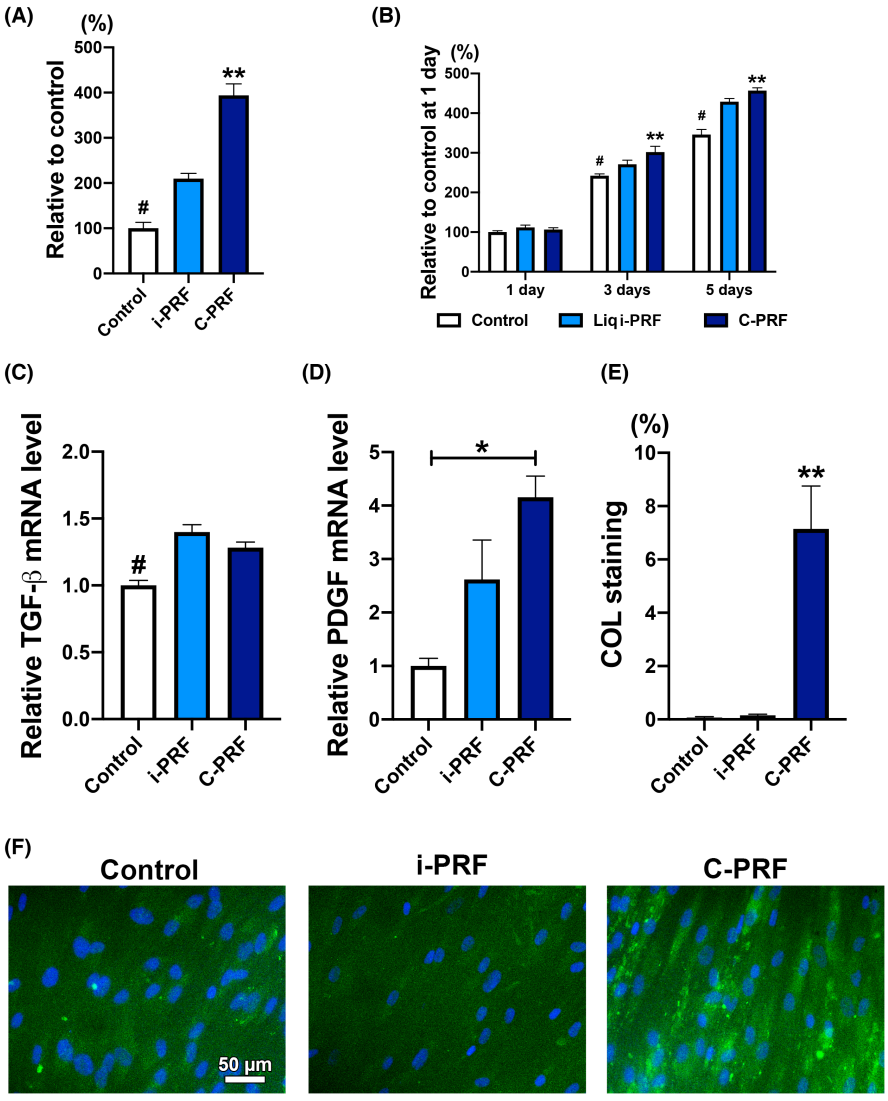

FIGURE 12 (A) Cell migration at 24 h and (B) cell proliferation at 1, 3, and 5 days in HGF-1 cells. (C, D) Real-time PCR analysis of the mRNA levels of (C) TGF-β and (D) PDGF in human gingival fibroblasts treated with i-PRF and C-PRF at 3 days. (E) Quantitative and (F) representative staining of collagen I at 14 days (data represent means ± SEs; * indicates significantly higher than the control group ($p < 0.05$), ** indicates significantly higher than all other groups ($p < 0.05$), and # indicates significantly lower than all groups ($p < 0.05$)). C-PRF, concentrated platelet-rich fibrin; i-PRF, injectable platelet-rich fibrin.

In summary, all studies observed an improvement with i-PRF use for the treatment of various TMJ disorders (Figure 14, Figure S1: QR Code 9 SCAN ME).

14 | MEDICAL APPLICATIONS

14.1 | Clinical use of i-PRF for cartilage regeneration and osteoarthritis

One heavily utilized use of PRP has been for cartilage degeneration and osteoarthritis of the knees.[79–83] In a preclinical study on the topic, our group evaluated the effect of i-PRF on cultivated chondrocytes and osteochondral regeneration in critical-sized osteochondral defects of the rabbit's knee in comparison to PRP.[6] Chondrocytes were first investigated for their ability to proliferate and differentiate in response to PRP and i-PRF. Thereafter, full-thickness, critical-sized osteochondral defects 5 mm in diameter and 5 mm in depth were created in the knee joint of 12 adult female New Zealand White rabbits. Defects were regenerated with either PRP or i-PRF and compared to the control group. Animals were sacrificed at 4 and 12 weeks post-operatively and evaluated histologically by macroscopic and microscopic examination for cartilage regeneration. i-PRF significantly promoted chondrocyte proliferation and mRNA levels of Sox9, collagen type II, and aggrecan when compared to PRP and the control group.[6] Histological analysis revealed that at 4 weeks, macroscopic ICRS scores from the i-PRF group were significantly enhanced when compared to the PRP and control groups. At 12 weeks post-surgery, the microscopic ICRS scores demonstrated that the i-PRF group experienced significantly improved cartilage regeneration when compared to the PRP group. In conclusion, the use of i-PRF significantly promoted chondrocyte activity and further improved cartilage regeneration compared to PRP. The histological results revealed early and better cartilage regeneration within 4 weeks post-operatively when i-PRF was utilized, and the results were maintained at 12 weeks.[6]

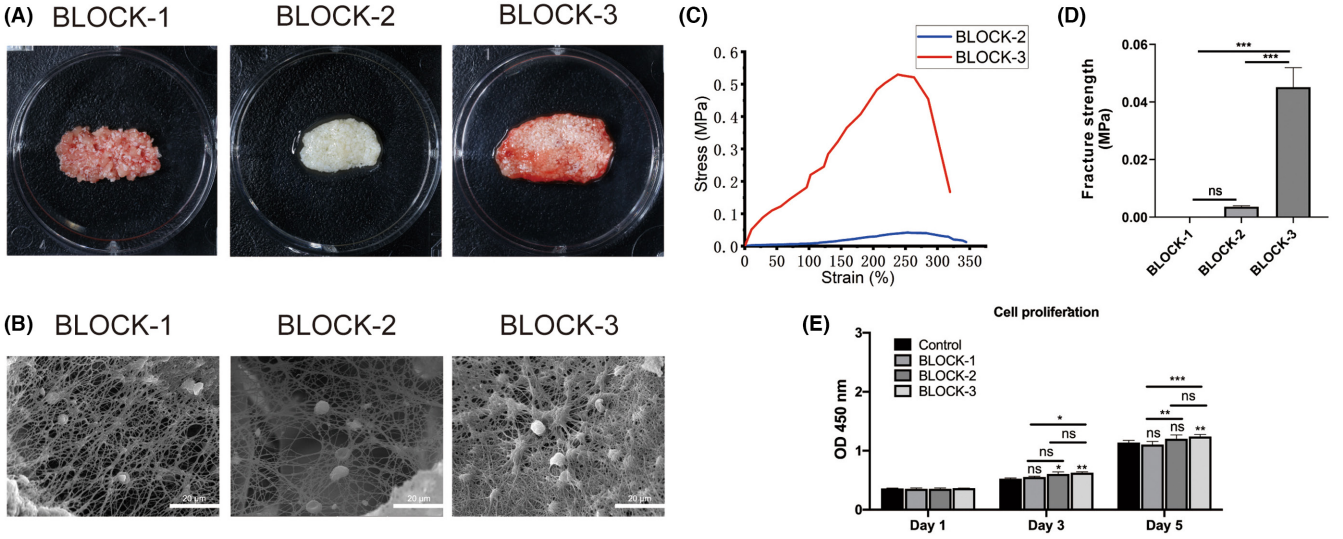

FIGURE 13 Characteristics of platelet-rich fibrin (PRF) blocks. (A–C) Photos of BLOCK-1, BLOCK-2, and BLOCK-3. (D–F) SEM images of BLOCK-1, BLOCK-2, and BLOCK-3. Scale bar = 20 μm. (G, H) The tensile resistance and fracture strength of BLOCK-1, BLOCK-2, and BLOCK-3 (samples were performed in triplicate). (I) Proliferation assay of osteoblasts at Days 1, 3, and 5 using control culture medium and conditioned culture medium from all three PRF blocks (* denotes $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$; samples were performed in triplicate with three independent experiments). Reprinted with permission from Feng et al.[52]

FIGURE 14 Use of liquid injectable platelet-rich fibrin for the treatment of various temporomandibular joint disorders. Reprinted with permission from Miron.[1]

Although clinical i-PRF studies are only commencing, a number of studies have successfully utilized solid PRF for cartilage repair. In 2019, Kemmochi et al. created a method to utilize PRF for meniscus tear repair.[84] In 2021, Screpis and colleagues utilized PRF to repair parameniscal cysts, describing their surgical technique.[85] In 2022, Narayanaswamy and Sha described a reproducible and simple way to harvest PRF and create and use a PRF clot, along with detailed instructions on how to integrate the clot with a meniscal repair arthroscopically.[86] Therefore, although studies remain in their infancy, various research groups have demonstrated promising results utilizing i-PRF for joint injections (Figure 15, Figure S1: QR Code 10 SCAN ME).

14.2 | Clinical use of i-PRF for wound care

Much like PRP, the additional use of growth factors concentrated in whole blood in wound care has been one avenue demonstrating promise for future research. In a study titled "Leucocyte- and platelet-rich fibrin (L-PRF) as a regenerative medicine strategy for the treatment of refractory leg ulcers: a prospective cohort study,"[87] Pinto and colleagues treated 44 consecutive patients with venous leg ulcers and found that topical applications of PRF on chronic ulcers recalcitrant to standard wound care promoted healing and wound closure in all patients following treatment.[87]

With the advancements made in liquid injectable PRF and a better understanding of protocols, the advantage of treating such defects using injectable liquid PRF is twofold. (1) The clinician can utilize liquid PRF prior to clot formation and create custom-shaped/sized membranes. Following the i-PRF protocol, the liquid PRF can be collected and redistributed into a custom-shaped tray (Figure 16A). After a typical 15-min waiting period, a clot is formed, and the custom shape can then be utilized (Figure 16B). This greatly facilitates its use when covering such wounds and better assures full wound coverage (Figure S1:

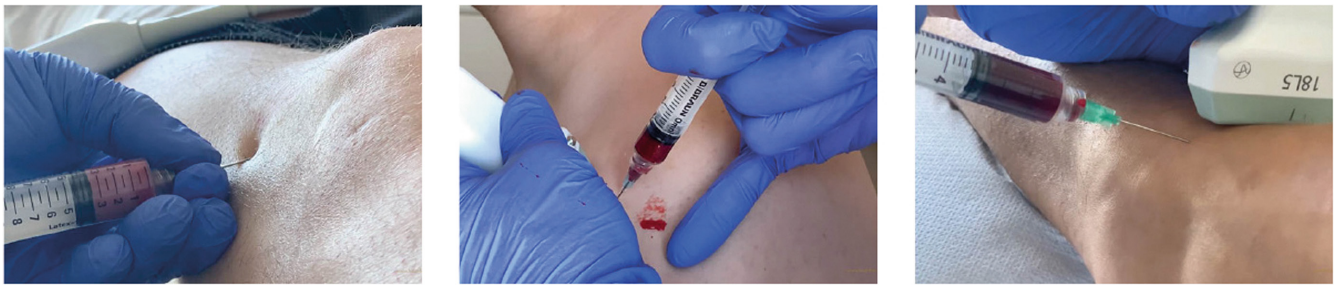

FIGURE 15 Use of liquid injectable platelet-rich fibrin for the treatment of various knee disorders, including osteoarthritis of the knee. Reprinted with permission from Miron.[1]

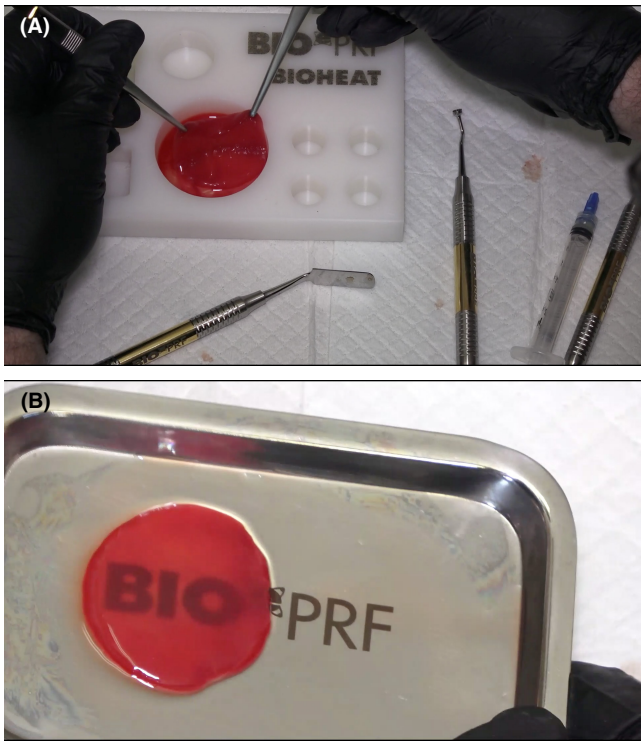

FIGURE 16 (A) Placement of liquid platelet-rich fibrin into a custom tray for 15 min until clotting. (B) Final Bio-Graft. Reprinted with permission from Miron2021.[1]

QR Code 11 SCAN ME). (2) A second advantage is that during the solidification process, additional small biomolecules (such as antibiotics) can be incorporated within the clot prior to clot formation and thereafter utilized clinically, as presented later in this article.

Thus, various ulcers, such as diabetic ulcers, are currently being treated routinely using Bio-Graft scaffolds created from i-PRF (Figure 17, Figure S1: QR Code 12 SCAN ME). Furthermore, large custom-shaped PRF membranes have also been utilized following cancer resection surgery (Figure S1: QR Code 13 SCAN ME). These findings highlight how the use of clotted PRF membranes of any size may be beneficial for various indications in medicine and dentistry.

15 | CLINICAL USE OF i-PRF IN FACIAL ESTHETICS AND HAIR REGROWTH

One rapidly growing field has been the use of PRF in facial esthetics. Given its ability to heal more rapidly and stimulate collagen production, a number of clinicians have begun to utilize the technology in the field of esthetic medicine, with an entire textbook dedicated to the topic.[88] In a preclinical study titled "Fluid platelet-rich fibrin stimulates greater dermal skin fibroblast cell migration, proliferation, and collagen synthesis when compared to platelet-rich plasma,"[8] our group demonstrated that although all platelet concentrates were nontoxic to cells, demonstrating high cell survival, skin fibroblasts migrated over 350% more in the i-PRF group when compared to the control and PRP groups (200% increase). i-PRF also significantly induced greater cell proliferation at 5 days. Although both PRP and fluid PRF induced significantly elevated cell mRNA levels of PDGF, it was observed that TGF-beta, collagen 1, and fibronectin mRNA levels were all significantly higher in the i-PRF group.[8]

In 2019, Karimi and Rockwell published an overview article on the topic, covering its immense potential in the field of cosmetic medicine.[89] Similarly, in 2021, Buzalaf and Levy published an overview article on using autologous platelet concentrates in facial esthetics.[90] It was reported that i-PRF was easier to obtain and seemed to induce greater collagen production than PRP.[90]

Regarding other uses of PRF in facial esthetics, several studies have noted that i-PRF is able to improve fat grafting and its stability.[91–93] *In a prospective single-center study, Hassan and colleagues found that i-PRF* significantly improved skin surface spots ($p = 0.01$) and pores ($p = 0.03$) at the 3-month follow-up. Other variables, such as skin texture, wrinkles, ultraviolet spots, and porphyrins, showed

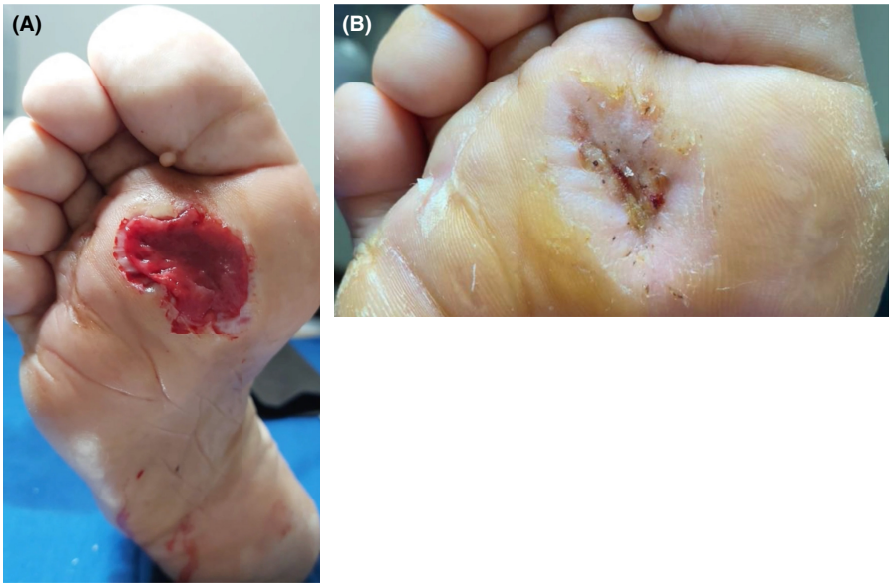

FIGURE 17 (A) Use and application of a Bio-Graft for the treatment of a hard-to-heal diabetic ulcer on the foot. (B) 3 weeks post-operatively. Case performed by Dr. Marco Castro-Pinto.

FIGURE 18 (A) Before and after a woman in her mid-40s received treatment for deep fine lines and wrinkles. (B) The patient was treated with some Bio-Filler injections (QR Code demonstrating a Bio-Filler injection into the tear trough area). Reprinted with permission from Miron.[1]

a numerical improvement.[94] The FACE-Q scales, which measure satisfaction with appearance, all showed a significant improvement from baseline, including satisfaction with skin ($p=0.002$), facial appearance ($p=0.025$), cheeks ($p=0.001$), lower face and jawline ($p=0.002$), and lips ($p=0.04$), with no major adverse effects.[94] In a recent study titled "Fluid platelet-rich fibrin (PRF) versus platelet-rich plasma (PRP) in the treatment of atrophic acne scars: A comparative study,"[95] acne scars were compared using a quartile grading scale and patient satisfaction reports. It was found that the therapeutic response was significantly higher in the PRF group when compared to PRP, either alone or combined with needling.[95] Thus, a number of studies have now reported the benefit of utilizing PRF in facial esthetics (Figure 18, Figure S1: QR Code 14 SCAN ME).

Much less research has been performed on hair regeneration using PRF. In 2021, a study by Lu et al. demonstrated that i-PRF facilitated hair follicle regeneration by promoting human dermal papilla cell proliferation, migration, and trichogenic inductivity.[96] A clinical study with only three patients reported the benefits of

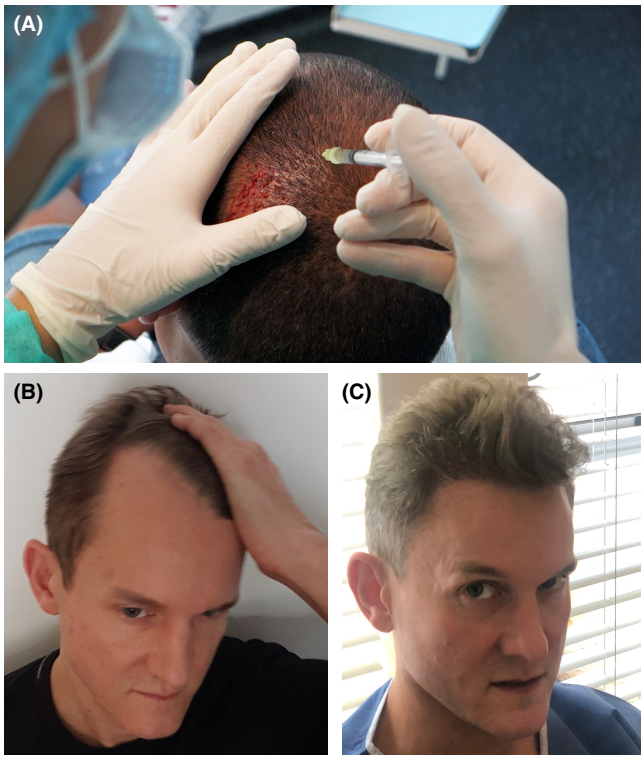

FIGURE 19 (A) Use of injectable platelet-rich fibrin (i-PRF) for hair regrowth and regeneration. (B, C) Before and after a man in his mid-20s received i-PRF injections and a hair transplant using liquid PRF. Reprinted with permission from Miron.[1]

i-PRF for the treatment of alopecia.[97] Notably, further improvements have also been reported when i-PRF is utilized to stimulate hair regeneration when compared to PRP (Figure 19, Figure S1: QR Code 15 SCAN ME).[88]

16 | USE OF i-PRF AS A DRUG DELIVERY VEHICLE

One main advantage of PRF over PRP has been its slow and gradual breakdown and release of growth factors over time. While PRP releases growth factors in a matter of minutes to hours once injected, PRF (owing to its clotted formulation) has a much longer and extended growth factor release over a typical 14-day period.[98] Therefore, many advantages exist in regard to drug delivery, as many growth factor carrier systems typically benefit from a longer and more gradual release over time.

In a study titled "Autologous liquid platelet rich fibrin: A novel drug delivery system," our research team envisioned the use of liquid injectable PRF as a drug delivery vehicle.[99] We therefore introduced the use of liquid PRF as an advanced local delivery system for small and large biomolecules. Both large (growth factors/cytokines and morphogenetic/angiogenic factors) and small (antibiotics, peptides, gene therapy, and anti-osteoporotic) molecules are considered potential target candidates for enhanced bone/cartilage tissue regeneration (Figure 20). Furthermore, liquid PRF was introduced as a potential carrier system for various cell types and nanosized particles that are capable of limiting/bypassing the immune system, such as in exosome therapy, minimizing potential foreign body reactions within host tissues following injection. A recent systematic review investigating PRF for the regeneration of intrabony defects found that the additional use of small biomolecules improved outcomes when compared to PRF alone.[21] Typically, in the field of regenerative periodontology, antibiotics, metformin, bisphosphonates, and statins have been investigated.[21] One can utilize the same concentration of antibiotics, such as minocycline, as that found in various commercial products (1 mg; Arrestin), yet instead of having various polymer-based biomaterials, one can utilize all-natural i-PRF as the delivery carrier system. Advantages further include the incorporation of leukocytes, cells that have antibacterial properties. Furthermore, an array of novel studies are ongoing in many fields of medicine combining i-PRF with exosome therapy. Two separate articles in this issue describe the full potential of exosomes in regenerative medicine and dentistry.

17 | FINAL THOUGHTS AND FUTURE TRENDS

This overview and narrative review provided many updates on the use of injectable PRF and its increasingly widespread use over the past 10 years. The following key messages should be derived from the article:

- The use of horizontal centrifugation of i-PRF has been shown to be significantly more capable of accumulating more cells and growth factors than conventional fixed-angle centrifugation.
- Liquid PRF derived from faster centrifugation protocols (C-PRF) greatly improves cell and growth factor concentrations when compared to the original protocols. Thus, a protocol of 2000 RCF for 8 min is recommended.
- Notably, following high-speed centrifugation, proteins such as fibrinogen are found in the uppermost layer. Therefore, ~0.5 CC from the uppermost layer should be collected, followed by ~0.5–1 CC from the buffy coat. Many cells are found in the red buffy coat zone.
- The use of more hydrophobic blue-top PRF tubes has been shown to significantly extend the working properties of liquid PRF. Since platelets adhere and begin the clotting cascade on the tube wall surfaces, the use of more water-repelling hydrophobic tubes has been shown to provide the clinician with more working time prior to clotting.
- Cooling i-PRF tubes within a Bio-Cool device has been shown to improve working time even more markedly, up to 4 h. Therefore, the combination of blue hydrophobic PET tubes kept within a cooling device is best able to maintain i-PRF in a liquid state.

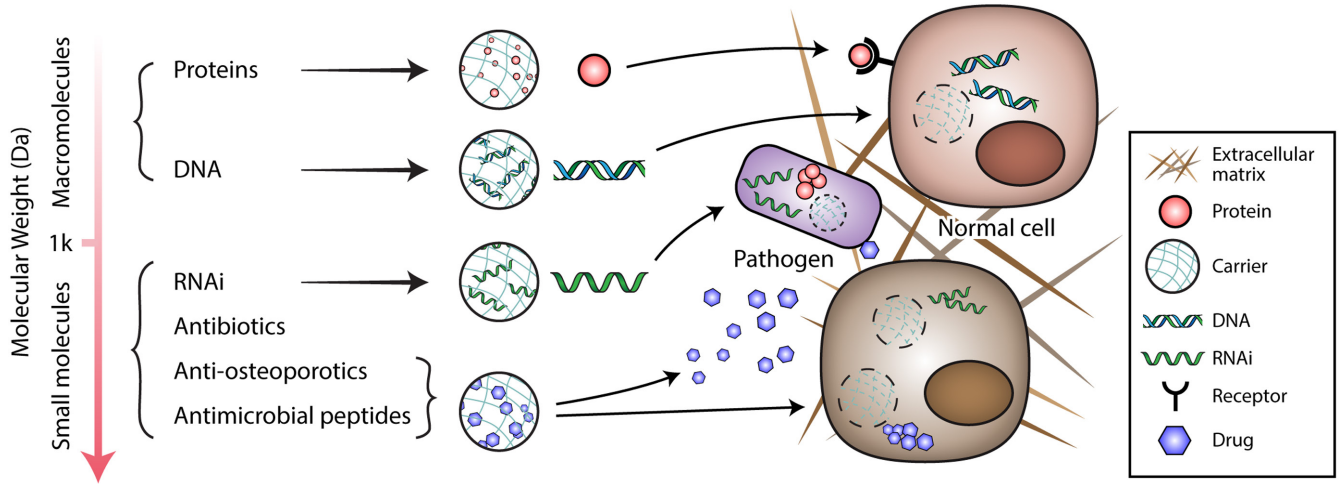

FIGURE 20 Classification and controlled release of biomolecules involved in therapeutic applications in CMF bone regeneration. Macromolecules, primarily proteins and DNA, can be loaded by carrier devices and released extracellularly or intracellularly to guide cell behaviors and regulate the bone healing process, whereas small molecules, herein defined as biomolecules with a molecular weight lower than 5 kDa, primarily target bone regeneration under compromised conditions, such as infection, skeletal malignancies, and metastases in CMF bone and osteoporosis (small molecule sizes). Figure modified from Ji et al.[100]

- A number of biological properties exist when utilizing i-PRF, including its regenerative properties, but it also exhibits benefits as an antibacterial and anti-inflammatory agent.
- Various uses of i-PRF have now been studied and reported in regenerative dentistry, including its use in periodontology, implantology, endodontics, temporomandibular joint injections, and orthodontic tooth movement.
- Additionally, various applications in medicine include its use in sports injuries and osteoarthritis of various joints, treatment of diabetic ulcers/wound care, facial esthetics, and hair regrowth.
- The ability to manipulate i-PRF prior to clotting allows the clinician to create custom-shaped clotted PRF membranes (Bio-Grafts) that may further be incorporated with various regenerative agents.
- Exciting new opportunities exist for utilizing i-PRF as a potent drug delivery vehicle for small biomolecules, such as additional growth factors, antibiotics, exosomes, and other medications that may benefit from controlled, gradual release over time.

One of the main goals of platelet concentrates is to deliver a concentrated source of growth factors derived from autologous whole blood. Over the past 10 years, many studies have supported the use of i-PRF in multiple fields of regenerative dentistry and medicine. Numerous studies now support i-PRF as a regenerative agent with the ability to promote cell migration, proliferation, differentiation, and collagen synthesis while also having the ability to reduce bacterial counts and lower inflammation. While substantial research is needed to validate its use in many fields of medicine and dentistry, the ability to delay clotting, create custom shapes and protocols, and utilize i-PRF as a scaffold for the delivery of small biomolecules opens many avenues for future research in the coming years.

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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