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REVIEW ARTICLE

Extended platelet-rich fibrin

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1 | INTRODUCTION

Platelet concentrates have been utilized in many fields of regenerative medicine owing to their ability to deliver supraphysiological concentrations of autologous platelets, leukocytes and growth factors.[1,2] While platelet-rich plasma (PRP) has been extensively employed as a first generation platelet concentrate, platelet rich-fibrin (PRF) has since been utilized with a better ability to release more growth factors over an extended period of time with superior clinical outcomes in various fields of medicine and dentistry.[3,4] PRF has proven to be an excellent biomaterial composed of autogenous cells and growth factors entrapped within a fibrin network that have been shown to breakdown more slowly over time when compared to traditional PRP.[4]

Nevertheless, one of the main reported drawbacks of PRF (and especially PRP) is its faster-than-ideal resorption rate characterized within a 2–3 week timeframe.[5] Because of its fast resorption rate, PRF membranes cannot be utilized as a barrier membrane similar to collagen owing to their inability to exclude soft tissues over an extended period of time. Interestingly, a number of recent studies have demonstrated that PRF could be significantly extended from 2 to 3 week resorption properties to greater than 4 months (extended-PRF; e-PRF) by heating a liquid platelet-poor plasma (PPP) layer (denaturing albumin) using Bio-Heat technology.[6]

The heated version of plasma, which has now been utilized in many areas of medicine and dentistry, has recently been the basis of intensive research endeavors across many regenerative labs owing to its extended working properties. Since one of the main limitations of PRP/PRF has historically been its short in vivo turnover rate, these extended PRF membranes can be used as substitutes for collagen membranes in guided bone regeneration (GBR) procedures requiring a typical "barrier" function that protects bone regeneration from faster growing soft tissues.[7]

Since PRF alone could not be utilized for such cases (fast resorption properties), an interesting attempt was made in 2015 where Kawase and his colleagues introduced a heat-compression technique with PRF membranes that aimed to heat PRF to make it last longer and extend its working properties.[7] It was observed that the heat-compression technique extended the degradation properties of PRF to greater than 3 weeks, significantly longer than the standard 2-week PRF membranes, which were completely resorbed.[7] This led to the hypothesis that heating platelet concentrates, specifically the platelet-poor plasma (PPP) layer rich in albumin, could favor a slower degradation rate when compared to traditional PRF.

To overcome the quick degradation properties of platelet concentrates and better maintain volume stability, the Alb-PRF protocol was developed. This protocol involves the use of the PPP layer (containing ~60% albumin) and heating it to 75°C for 10 min to allow for albumin denaturation as well as breaking of many weak linkages or bonds (e.g., hydrogen bonds) within its protein molecule. Following denaturation, the proteins are then restructured

TABLE 1 Investigated studies reporting on heated albumin gel fabricated under various protocols.

Authors (year)	Study type	Centrifugation parameters	Groups	Conclusions
Matthews-Brzozowska et al. (2017)[19]	Clinical study—case series	• Medifuge 200 centrifuge with activated plasma albumin gel (APAG system) • Protocols not disclosed	Case series consisting of CGF injections with APAG system	One of the first case series studies on the technology with authors giving an overview summary of the technology. It was concluded that "the physician performing the procedure can further adjust the density of the gel to the needs of the patient. Growth factors are released longer in a controlled manner, which results in stronger stimulation and regenerative effects."
Mourão et al. (2018)[10]	In vitro study	• Medifuge 200 centrifuge with activated plasma albumin gel (APAG system) • 75 C for 10 min	1. CGF + APAG	This preliminary study indicates that the protocol may provide autologous moldable and stable biomaterials for use as a soft tissue barrier, offering the basis for further research on its effectiveness for guided tissue regeneration.
Kargarpour et al. (2020)[14]	In vitro study	Swing-out rotor; Z 306 Hermle, Universal centrifuge, Wehingen, Germany 700*g* for 8 min 75°C for 10 min	1. Buffy Coat (BC) 2. Platelet poor plasma (PPP) 3. Alb-PRF 4. Buffy Coat	These results strengthen the evidence that not only the cell-rich C-PRF but also PPP comprise a TGF-β activity that is, however, heat sensitive. It thus becomes relevant to mix the heated PPP with the buffy coat C-PRF layer to regain TGF-β activity, as proposed during the preparation of Alb-PRF.
Kargarpour et al. (2020)[20]	In vitro study	Swing-out rotor; Z 306 Hermle, Universal centrifuge, Wehingen, Germany 400*g* for 12 min 75°C for 10 min	1. Buffy Coat (BC) 2. Platelet poor plasma (PPP) 3. Alb-gel 4. Buffy Coat	These findings suggest that PRF, PPP, and the buffy coat can neutralize hydrogen peroxide through the release of heat-sensitive catalase. This work supports the fact that non-heated platelet concentrates are able to reduce inflammation and decrease oxidative stress once implanted further supporting the combination of the heated albumin gel with PRF from the buffy coat.
Sun et al. (2020)[17]	Clinical case series of 24 patients	• Medifuge 200 centrifuge with activated plasma albumin gel (APAG system) • Protocols not disclosed	1. CGF alone group 2. plasma albumin gel (PAG) alone group 3. CGF + PAG	CGF combined with PAG can reduce the scar grading, anxiety of patients, and enhance patients' satisfaction and scar improvement in the treatment of patients with facial depressed scar. The combined CGF+ PAG injection, without significant adverse reactions, is better than single component injection and is worthy of clinical application.
Fujioka-Kobayashi et al. (2021)[5]	In vitro study	Bio-PRF centrifuge with Bio-Heat technology 700 RCF for 8 min 75°C for 10 min	1. Control tissue culture plastic 2. Alb-PRF	The present results indicate that Alb-PRF possesses regenerative properties induced by the slow and gradual release of growth factors found in liquid PRF via albumin gel degradation over a 10 day period. It further stimulates fibroblast collagen production and growth factor enhancement.
Gheno et al. (2021)[6]	In vivo study	Bio-PRF centrifuge with Bio-Heat technology 700 RCF for 8 min 75°C for 10 min	1. L-PRF 2. H-PRF 3. Alb-PRF	This study demonstrates a marked improvement in the membrane stability of Alb-PRF following ISO 10993-6/2016 when compared to standard PRF preparations. This indicates its future potential for use as a biological barrier membrane for GBR procedures with a long-lasting half-life, or as a biological filler material in aesthetic medicine applications.
Kargarpour et al. (2021)[16]	In vitro study	Swing-out rotor; Z 306 Hermle, Universal Centrifuge, Wehingen, Germany 2000*g* for 12 min 75°C for 10 min		These findings suggest that liquid PRF holds a potent in vitro heat-sensitive anti-inflammatory activity in macrophages that goes along with an inhibition of osteoclastogenesis.
Shirakata et al. (2021)[18]	In vivo study	Bio-PRF centrifuge with Bio-Heat technology 700 RCF for 8 min 75°C for 10 min	1. L-PRF 2. H-PRF 3. Alb-PRF	In the PRF-applied defects, new bone and new cementum formation occurred to varying degrees regardless of the protocols used to produce PRF. Particularly in the two-wall intrabony defects, new bone formation extended from the host bone toward the coronal region of the defects in the H-PRF applied sites compared with those in the OFD, F-PRF and Alb-PRF groups, and the H-PRF group showed the greatest amount of newly formed cementum.

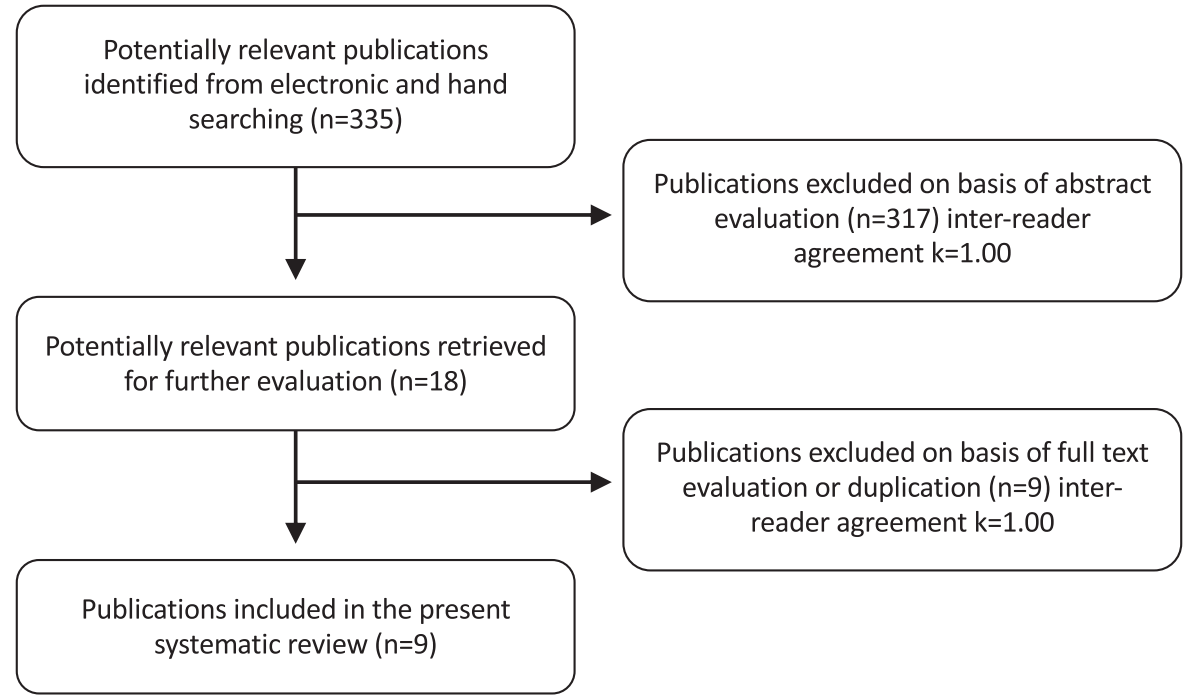

FIGURE 1 Flow chart of the screened relevant publications.

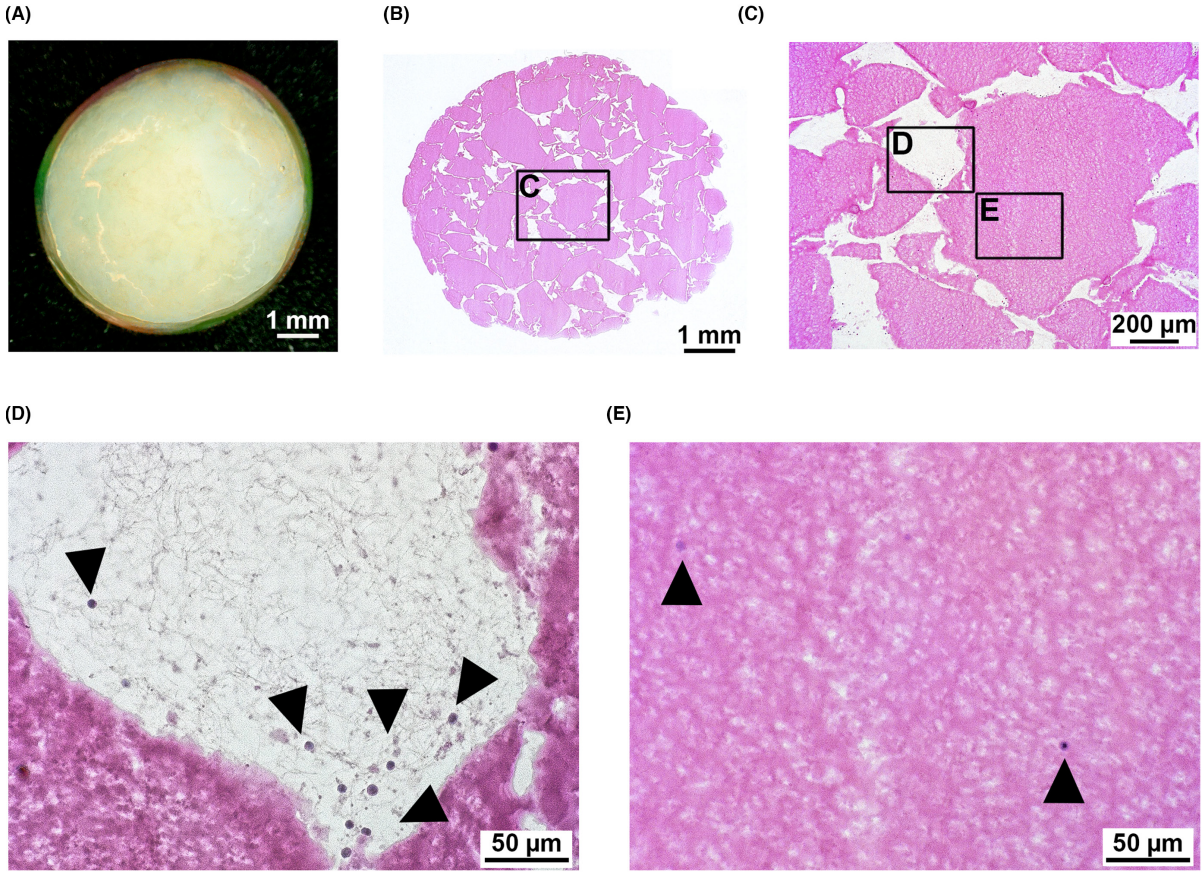

FIGURE 2 Microscopic observation of the e-PRF membrane. (A) A trimmed 8-mm e-PRF membrane sized with a biopsy punch. (B) H&E staining of the e-PRF section. (C) A high-magnification view of the image shown in (B). Two components, the eosin-stained filler particle-like structure and the matrix, were observed. (D) High-magnification view of the native liquid PRF portion shown in (C). Leukocytes were observed in the fibrin matrix. (E) High-magnification view of denatured liquid PPP (albumin gel) shown in (C). A dense fiber network was observed with few leukocytes. Reprinted with permission from Fujioka-Kobayashi et al.[5]

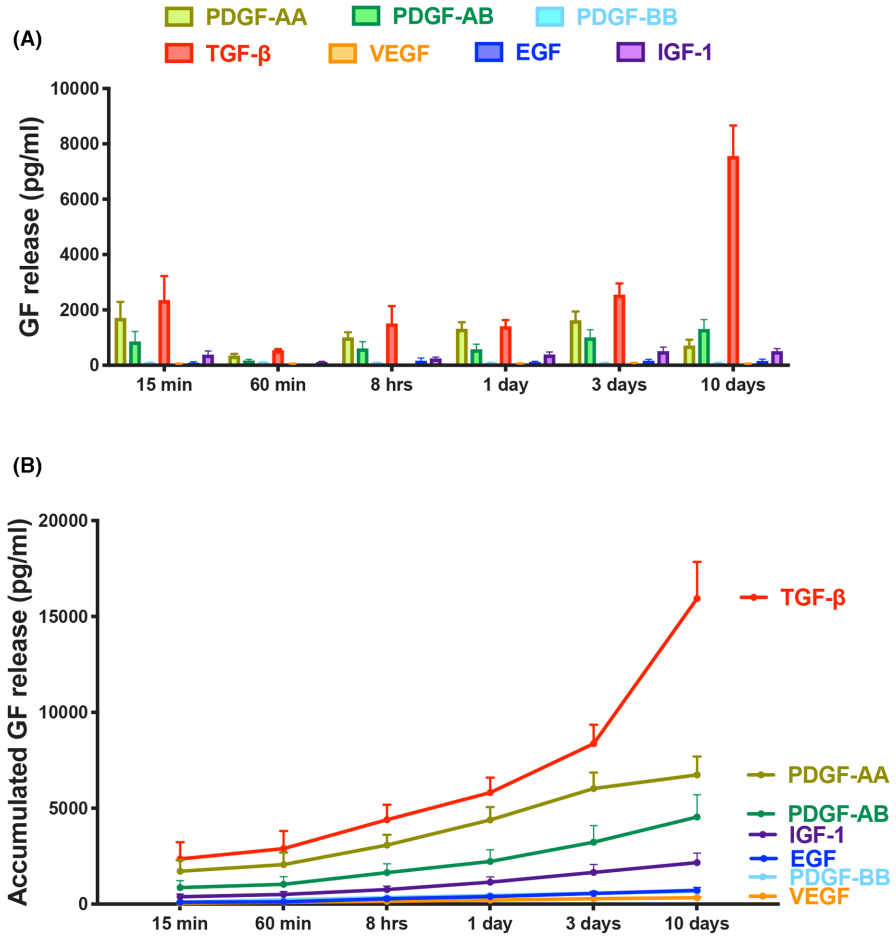

FIGURE 3 (A) ELISA protein quantification at each time point of PDGF-AA, PDGF-AB, PDGF-BB, TGF-β1, VEGF, EGF and IGF-1 over a 10-day period. (B) Total accumulated growth factor released over a 10-day period for PDGF-AA, PDGF-AB, PDGF-BB, TGF-β1, VEGF, EGF and IGF-1. Reprinted with permission from Fujioka-Kobayashi et al.[5]

in a more densely organized assembly that extends the resorption properties of PRF to up to 4–6 months (QR Code 1 SCAN ME).[8-11] However, the heating process also destroys cells/growth factors, and thereby, the platelet concentrates lose much of their regeneration potential. For these reasons and following heating, a concentrated PRF layer (C-PRF)[12,13] taken from the buffy coat (covered in the previous article titled "Ten Years of Injectable Platelet Rich Fibrin") is then mixed back into the heated PPP (albumin gel) once cooling has occurred (termed Alb-PRF or e-PRF for extended PRF).[5]

In view of previous research to date on this topic, the aim of this review article was to systematically review the literature and present the biological properties of this novel regenerative modality, including animal data demonstrating its superior resorption properties. Furthermore, the step-by-step processing of e-PRF as well as clinical studies to date on the topic are presented. Finally, the future field is discussed with potential future application and research endeavors of this novel technology as a replacement for collagen membranes in GTR/GBR procedures, closing lateral windows in sinus grafts and as a stable biological membrane during recession coverage procedures. Furthermore, Alb-PRF may also be injected as a regenerative biological filler lasting extended periods with advantages in joint injections, osteoarthritis and in the field of facial aesthetics.

2 | METHODS

2.1 | Development of a protocol

First, a systematic review of the topic was conducted. A protocol including all aspects of a systematic review methodology was developed prior to commencing the review. This included definition of the focused question; a defined search strategy; study inclusion criteria; determination of outcome measures; screening methods, data extraction and analysis; and data synthesis.

2.2 | Defining the focused question

The following focused question was defined: "What literature exists supporting the use of a heated albumin gel."

2.3 | Search strategy

Electronic and manual literature searches were conducted independently by two authors (RJM and CD) in several databases, including MEDLINE (OVID), EMBASE (OVID), Cochrane Central Register of Controlled Trials (Cochrane Library), Cochrane Oral Health Group Trials Register (Cochrane Library), Web of Science (Thomson Routers) and SciVerse (Elsevier). The electronic literature was searched for

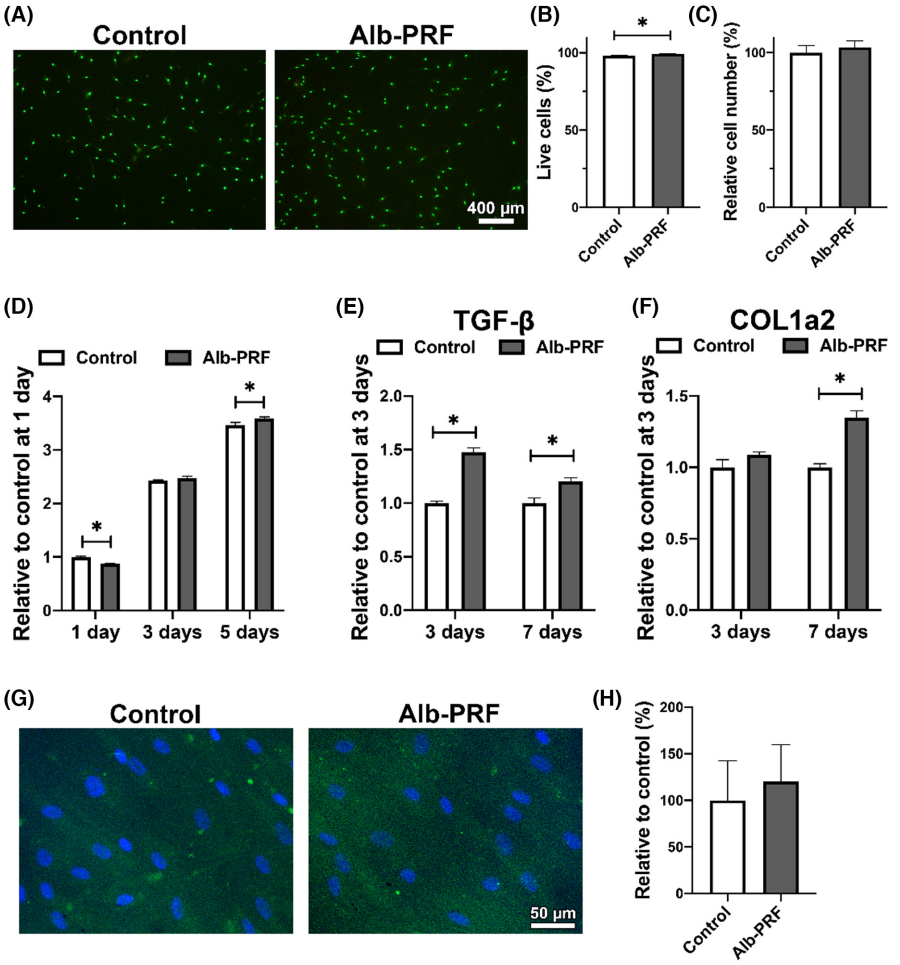

FIGURE 4 Cell behavior when stimulated with e-PRF. (A, B) Live/dead assay at 24 h of human gingival fibroblasts treated with e-PRF. (A) The merged fluorescent images of live/dead staining with viable cells appearing in green and dead cells in red. (B) Cell viability was quantified as the percentage of living cells. (C, D) Effects of e-PRF on human gingival fibroblast (C) cell migration at 24 h and (D) cell proliferation at 1, 3 and 5 days. (E, F) Real-time PCR of human gingival fibroblasts cultured with e-PRF at 3 and 7 days for mRNA levels of (E) TGF-β and (F) COL1a2. (G, H) Immunofluorescent collagen 1 (COL1) staining of human gingival fibroblasts treated with e-PRF at 14 days. (G) The merged fluorescent images of COL1 staining (green) with DAPI staining (blue). (H) Quantified values of COL staining in comparison with control samples. (* denotes a significant difference). Reprinted with permission from Fujioka-Kobayashi et al.[5]

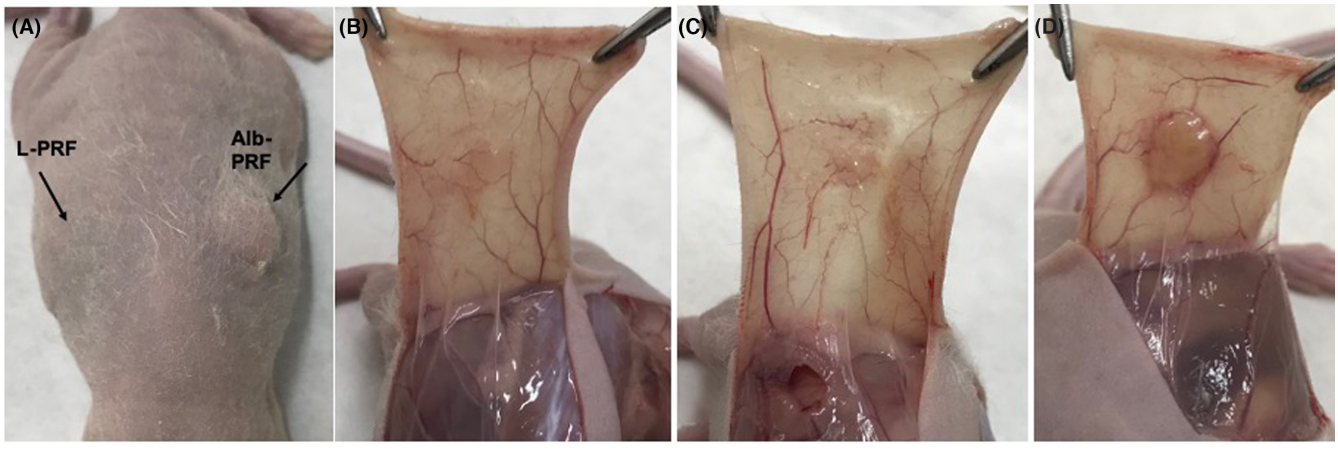

FIGURE 5 After 21 days of implantation, macroscopically, the volume on the animal's back was observed, referring to the e-PRF, which remained in place during all the experimental periods. (A) Animal's back with e-PRF volume (arrow); (B) L-PRF; (C) H-PRF; (D) e-PRF. Reprinted with permission from Gheno et al.[6]

articles published up to and including January 3rd, 2023: combinations of several search terms and search strategies were applied to identify appropriate studies. The following key words were searched individually: "Bio-Heat," "albumin gel," "albumin-PRF," "Alb-PRF," "Alb-CGF," "extended-PRF," "e-PRF," "Activated plasma albumin gel," and "APAG."

2.4 | Criteria for study selection and inclusion

The study selection considered only articles published in English describing in vitro, in vivo and human clinical studies evaluating the effect of an albumin heated gel pertinent to the field of regenerative medicine. All in vitro studies, in vivo data and human studies

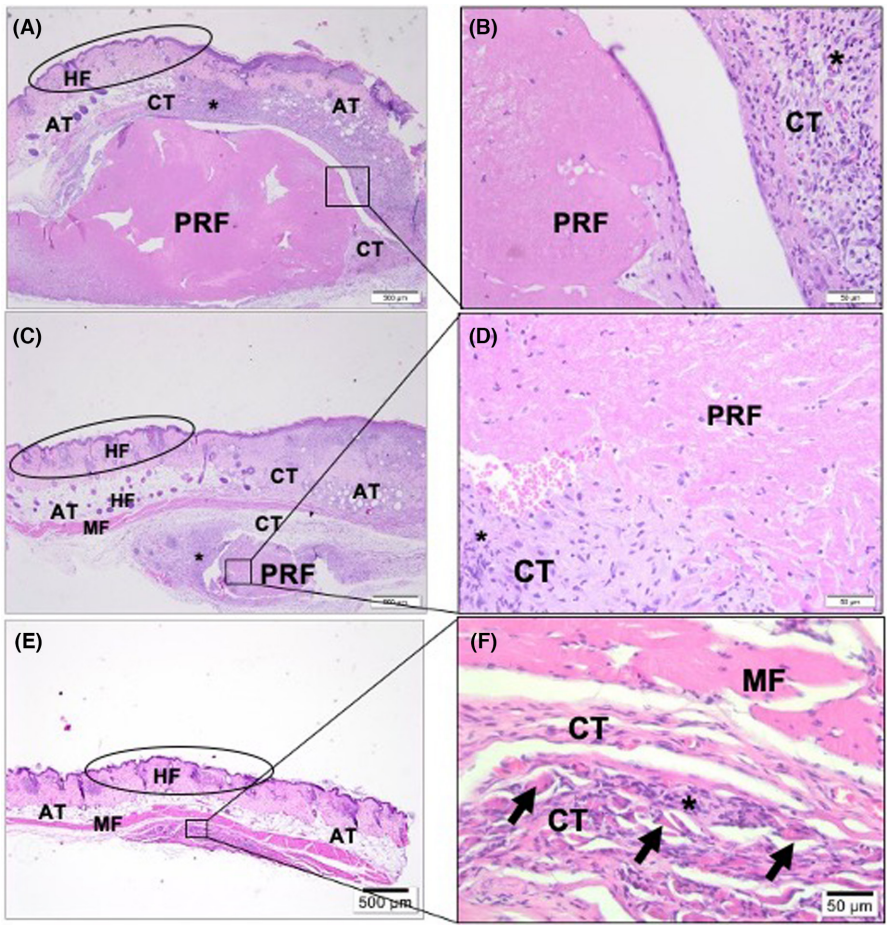

FIGURE 6 Photomicrographs of the L-PRF group. (A and B) circle: epidermis and papillary dermis with hair follicle (HF), recovering connective tissue (CT) with moderate and diffuse inflammatory cells (*) surrounding the PRF (PRF). Adipocyte tissue (AT) was noted above the epithelium. (C and D) circle: epidermis and papillary dermis with hair follicle (HF), recovering connective tissue (CT) with moderate and diffuse inflammatory cells (*) surrounding PRF (PRF). (E and F) circle: epidermis and papillary dermis with hair follicle (HF), recovering connective tissue (CT). Rare inflammatory cells among muscle fibers (mf) and adipocyte tissue (AT) were noted. PRF was not observed in this period. (A and B) 7 days; (C and D) 14 days; (E and F) 21 days. (A, C and E) 4× magnification, scale bar: 500 µm; (B, D and F) 40× magnification, scale bar: 50 µm. Stain: hematoxylin and eosin. Reprinted with permission from Gheno et al.[6]

reporting the effects of Alb-PRF were included. Human studies were not limited to randomized clinical trials.

2.5 | Outcome measure determination

The primary outcome of interest was the difference in effect when extending the biological or resorption properties of Alb-PRF. The outcome measures were separated into (1) in vitro studies, (2) animal studies and (3) clinical studies due to the heterogeneity of the studies. Since there was large variability in the outcomes measured by the various groups working across several fields of medicine, a meta-analysis was not considered. Outcomes are summarized in Table 1 for the various in vitro, in vivo and clinical studies according to the specific effect of Alb-PRF.

2.6 | Screening method

Titles and abstracts of the selected studies were independently screened by two reviewers (RJM and NEE) on January 3rd, 2023. The screening was based on the question "What literature exists supporting the use of a heated albumin gel for regenerative medical procedures." Full text articles were obtained if the response to the screening question was "yes" or "uncertain" in terms of support for such use. The level of agreement between reviewers was determined by kappa scores according to company software instructions (GraphPad Software, Inc., http://graphpad.com/quickcalcs/kappa1.cfm). Disagreement regarding inclusion was resolved by discussion between authors. For necessary missing data, the authors of the studies were contacted. Articles that did not address regenerative medicine were excluded.

2.7 | Data extraction and analysis

The following data were extracted: general characteristics (authors, year of publication); PRF centrifugation characteristics/protocols, albumin gel centrifugation characteristics/protocols, evaluation characteristics; methodological characteristics (study design, methodological quality); and conclusions. Because of the heterogeneity of the included studies (study design, in vitro versus animal versus clinical studies, investigated parameters, materials used, evaluation methods, outcome measures, observation periods), no mean differences could be calculated, and consequently, no quantitative data synthesis and meta-analysis could be performed. Instead, the data are reported in a systematic fashion characterizing all available literature to date with conclusions from each study.

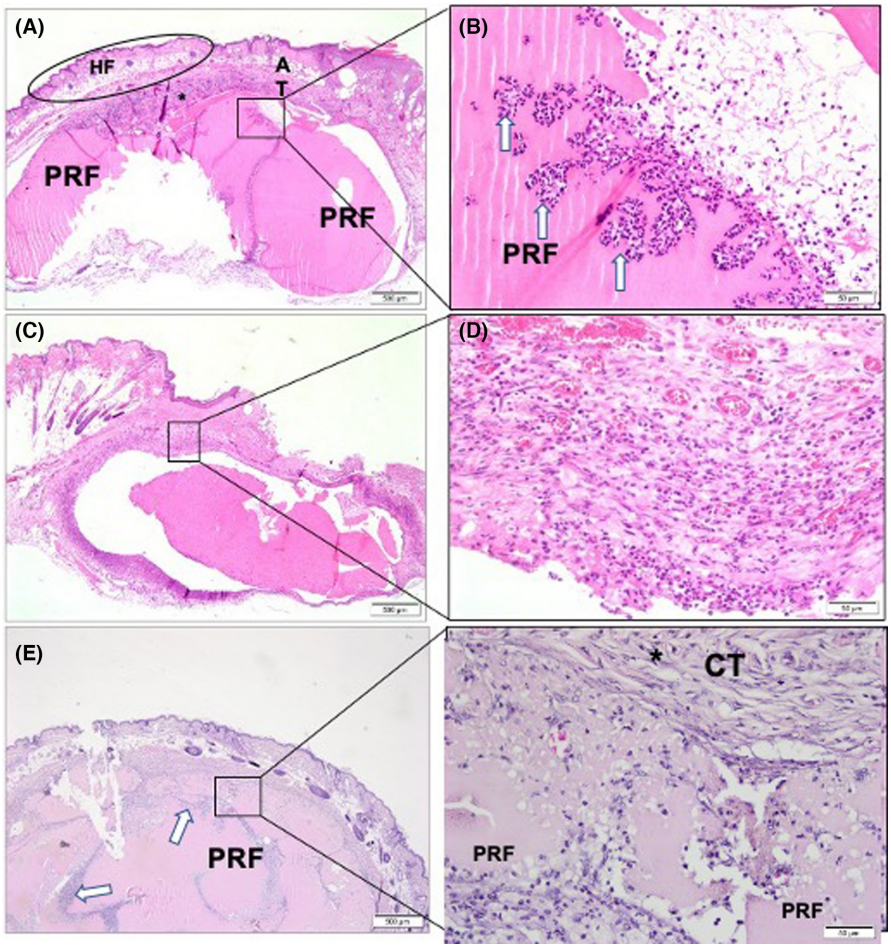

FIGURE 7 Photomicrographs of the e-PRF group. (A and B) circle: epidermis and papillary dermis with hair follicle (HF), recovering connective tissue (CT) with moderate and focal inflammatory cells (*) surrounding the PRF (PRF). Highlighting the presence of leukocyte groups inside the membrane (white arrow); (C and D) circle: epidermis and papillary dermis with hair follicle (HF), recovering connective tissue (CT) with moderate inflammatory cells (*) surrounding PRF (PRF); (E and F) circle: epidermis and papillary dermis with hair follicle (HF), recovering connective tissue (CT) with dispersed inflammatory cells (*) surrounding PRF (PRF); presence of leukocyte groups inside the membrane (white arrow) (A and B) 7 days; (C and D) 14 days; (E and F) 21 days. Stain: hematoxylin and eosin. Reprinted with permission from Gheno et al.[6]

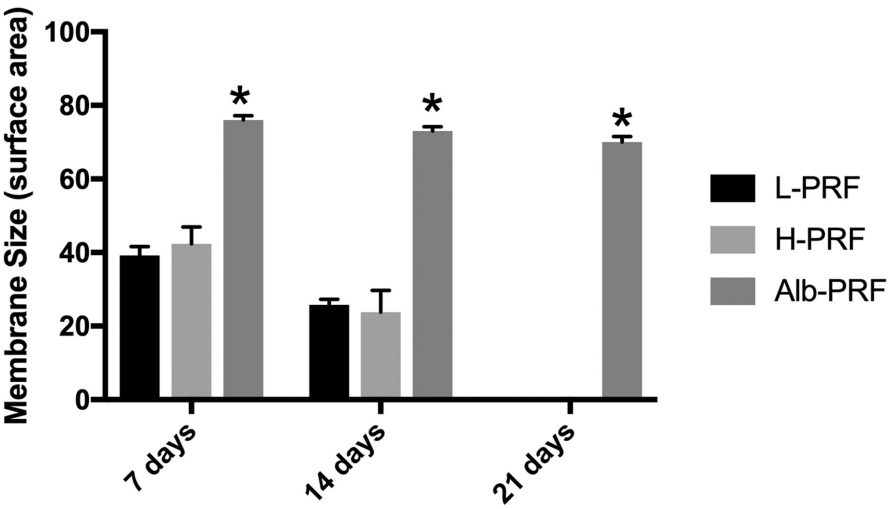

FIGURE 8 Quantification of the membrane surface area. Both the L-PRF and H-PRF groups exhibited complete resorption by Day 21. Alb-PRF demonstrated superior volume stability over time ($p < 0.05$; *significantly greater surface area when compared to all other groups). Reprinted with permission from Gheno et al.[6]

3 | RESULTS

3.1 | Systematic search

In total, 335 articles were initially screened (Figure 1; Table S1). Initial screens included 283 articles for the search term Bio-Heat,[14-16] 19 articles for albumin gel,[5,6,14,17] 6 articles for Alb-PRF,[5,6,14,15,16,18] 1 article for Alb-CGF,[10] 2 articles for extended PRF,[6] 2 articles for e-PRF,[5] 3 articles for activated plasma albumin gel,[10,19] and 19 articles for APAG.[10] Accordingly, references highlight articles selected after meeting the inclusion criteria from each search. Table 1 highlights the conclusions from each of the selected studies along with centrifugation parameters and heating times/temperatures. A total of 9 studies were included and further discussed below.

FIGURE 9 Image of the Bio-HEAT medical apparatus for the production of e-PRF. Syringes of various sizes may be loaded into the upper compartment, and thereafter, denaturing of albumin will occur at 75°C.

3.2 | In vitro biological characterization of a liquid platelet-rich fibrin mixture consisting of autologous albumin gel and liquid platelet-rich fibrin (Alb-PRF/e-PRF)

In a preclinical study investigating the biological characterization of liquid-PRF with heated albumin gel (Alb-PRF), Fujioka-Kobayashi et al.[5] investigated frozen sections of Alb-PRF at the microscopic level and demonstrated 2 structures, including denatured albumin gel particles and gelated liquid PRF (Figure 2A–C), with entrapment of leucocytes within the fibrin fibers (Figure 2D,E).

Growth factors released from Alb-PRF include PDGF, VEGS, TGF-β1, and EGF, and Alb-PRF released growth factors over a 10-day period (Figure 3).[5] Alb-PRF also demonstrated an ability to improve gingival fibroblast cell migration, proliferation and expression of collagen type 1 (Figure 4).[5] e-PRF induced a significant increase in cell numbers at Day 5 and improved the mRNA levels of TGF-β and COL1a2 at 7 days postseeding when compared to controls (Figure 4E).[5] Thus, it was concluded that e-PRF was highly biocompatible, had an extended ability to release growth factors over time and possessed the ability to improve gingival fibroblast cell behavior.[5]

Three further studies investigated the biological properties of Alb-PRF. Kargarpour et al.[14] found in an initial study that not only the cell-rich buffy coat PRF layer but also PPP comprise a TGF-β activity that was heat sensitive. It was therefore recommended and relevant to mix the heated PPP (albumin gel) back together with the concentrated buffy-coat PRF (C-PRF) layer to regain TGF-β activity, as proposed during the preparation of Alb-PRF. In a second study by the same group, it was also found that the same layers could neutralize hydrogen peroxide through the release of heat-sensitive catalase, thereby reducing inflammation and decreasing oxidative stress once implanted in vivo.[20] Last, the group found that the liquid-PRF component holds potent in vitro heat-sensitive anti-inflammatory activity in macrophages that coincides with an inhibition of osteoclastogenesis.[16]

3.3 | In vivo evaluation of the biocompatibility and biodegradation of Alb-PRF

A pioneering study, according to ISO 10993-6/2016, investigated for the first time the inflammatory reaction, biocompatibility and extended degradation properties of Alb-PRF when compared to standard leukocyte-PRF (L-PRF) and horizontal-PRF (H-PRF) at 7, 14 and 21 days in a nude mouse subcutaneous implantation model.[6]

After 21 days, it was observed macroscopically that the majority of samples implanted with standard PRF demonstrated significant or complete resorption, whereas the Alb-PRF group demonstrated only a slight change in volume dimension (Figure 5).[6] Of significance, Figure 5A demonstrates an animal that was subcutaneously implanted on either side of the animal midline with standard PRF and e-PRF. Complete resorption in the PRF group was observed, whereas a bolus remained in the opposite e-PRF group (Figure 5A). Figure 5B–D further demonstrates that the standard-PRF group demonstrated complete resorption at Day 21. The e-PRF group retained the presence of vascularization around the implanted biomaterial with volume stability over the entire study duration (Figure 5D).[6] Owing to the autologous nature of both of these implanted biomaterials, all demonstrated excellent immune cell reactions at all time points (Figures 6 and 7).[6]

Quantification of the membrane size surface area demonstrated that two standard PRF-type (L-PRF and H-PRF) groups lost approximately 50% volume when compared to the e-PRF group by 7 days (Figure 8).[6] Approximately 25% of the volume was lost by Day 14, and complete resorption was noted by Day 21. In contrast, from Day 7 to 21, the e-PRF group lost only 10% of its original volume, demonstrating superior volume stability over time (Figure 8).

3.4 | Clinical protocol to produce albumin gel plus platelet rich fibrin (e-PRF)

To extend the working properties of standard PRF, a specialized heating device (Bio-HEAT, Bio-PRF) is required (Figure 9). The e-PRF is produced by collecting peripheral blood using 9–10 mL

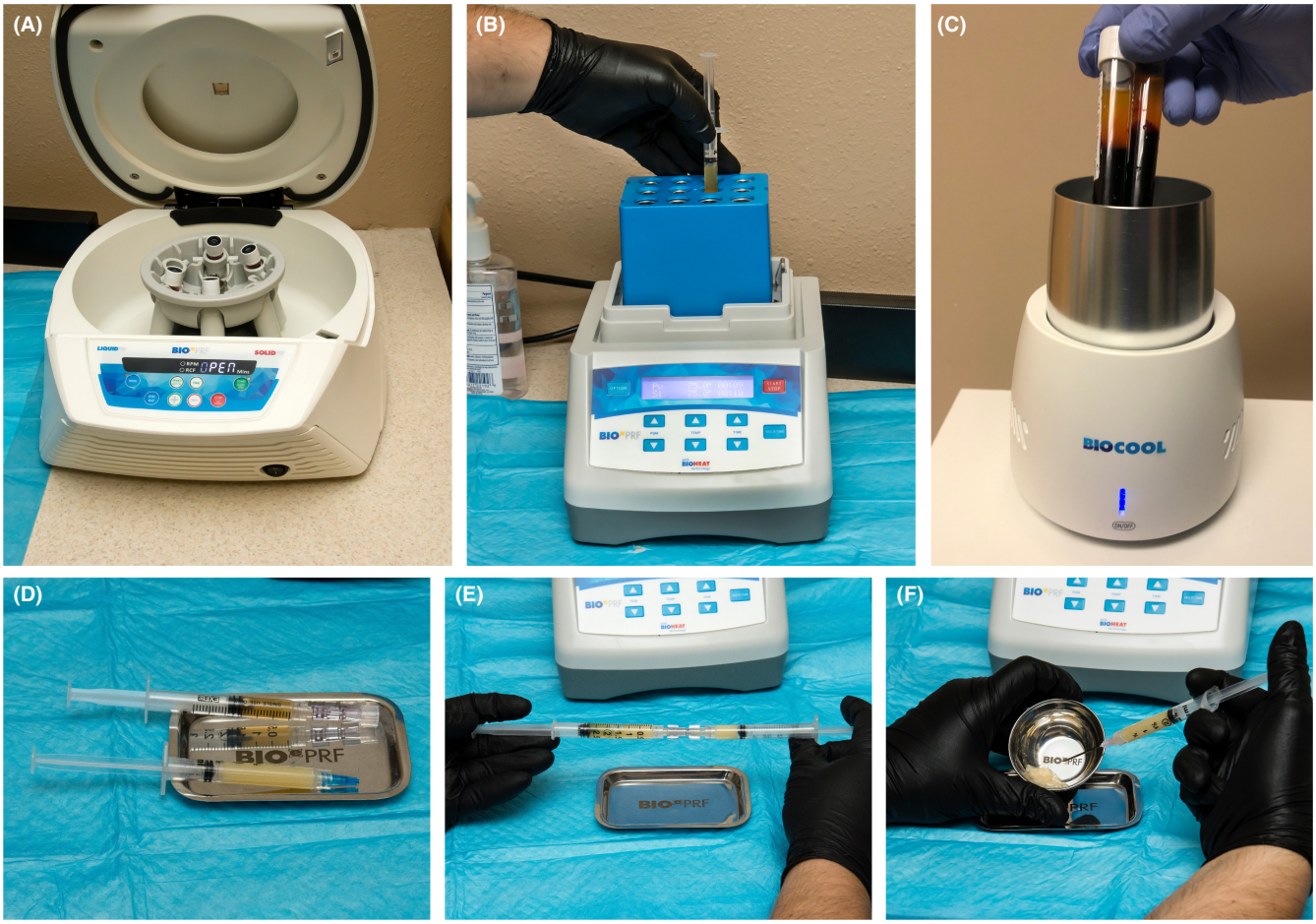

FIGURE 10 Step by step clinical demonstration for the production of e-PRF. (A) Venipuncture and blood collection followed by centrifugation. (B) Following centrifugation, the upper 2 cc of plasma is placed into the Bio-Heat medical device to heat the serum + platelet-poor plasma (PPP). Note that the machine must be preheated prior to use at 75°C for 10 min. (C) The remaining platelet-rich layer is kept in the Bio-Cool device to extend the clotting time. (D) Following 10 min of heating, clinical differences in color are observed between the liquid PRF (upper) and the albumin gel. (E) A luer-lock mixer device is attached to both the liquid PRF and albumin gel to mix the 2 components and create e-PRF. (F) e-PRF ready for use. Note the ability to inject it out of a syringe following adequate mixing. Reprinted from Davies and Miron.[28]

tubes without adding any additives, as overviewed in Figure 10. Following blood collection, the blood tubes were placed in a horizontal centrifuge (Bio-PRF) utilizing a 700–2000 *g* for the 8-min protocol (Figure 10A). It is important to best optimize PRF as presented in a recent article on the topic.[21] After processing, it is possible to observe separation of the blood layers into plasma and the remaining decanted red cells.

Two to four milliliters of the initial portion of plasma (platelet-poor plasma) is then collected with a syringe and placed into the Bio-Heat device (Figure 10B), while the other blood portions (buffy coat, liquid PRF, and red blood cells) are placed in the Bio-Cool device (Figure 10C). The syringes containing PPP are then inserted into a heating device (Bio-Heat) for human serum albumin denaturation plasma to produce the albumin gel (Figure 10B). After 10 min at an operating temperature of 75°C, the syringes are then removed and allowed to cool within the Bio-Cool device for 2 min. Liquid PRF from the buffy coat (C-PRF) is then collected.[12,13] Figure 10D demonstrates the noticeable color change between the albumin gel and standard liquid PRF. Thereafter, the albumin gel and the liquid-PRF are mixed together between syringes by passing back and forth (roughly 10×) using a female–female luer lock connector (Figure 10E). Thereafter, e-PRF can be utilized to inject autologous concentrations of growth factors, cells and heated albumin (Figure 10F). As an injectable filler, a 25G needle or 22G canula is recommended (Figure 11;

QR Code 2 SCAN ME). Alternatively, a similar process can be utilized to create a custom shape membrane with extended properties (e-PRF) lasting up to 4 months and utilized clinically as a substitute for

collagen membranes (QR Code 3 SCAN ME).

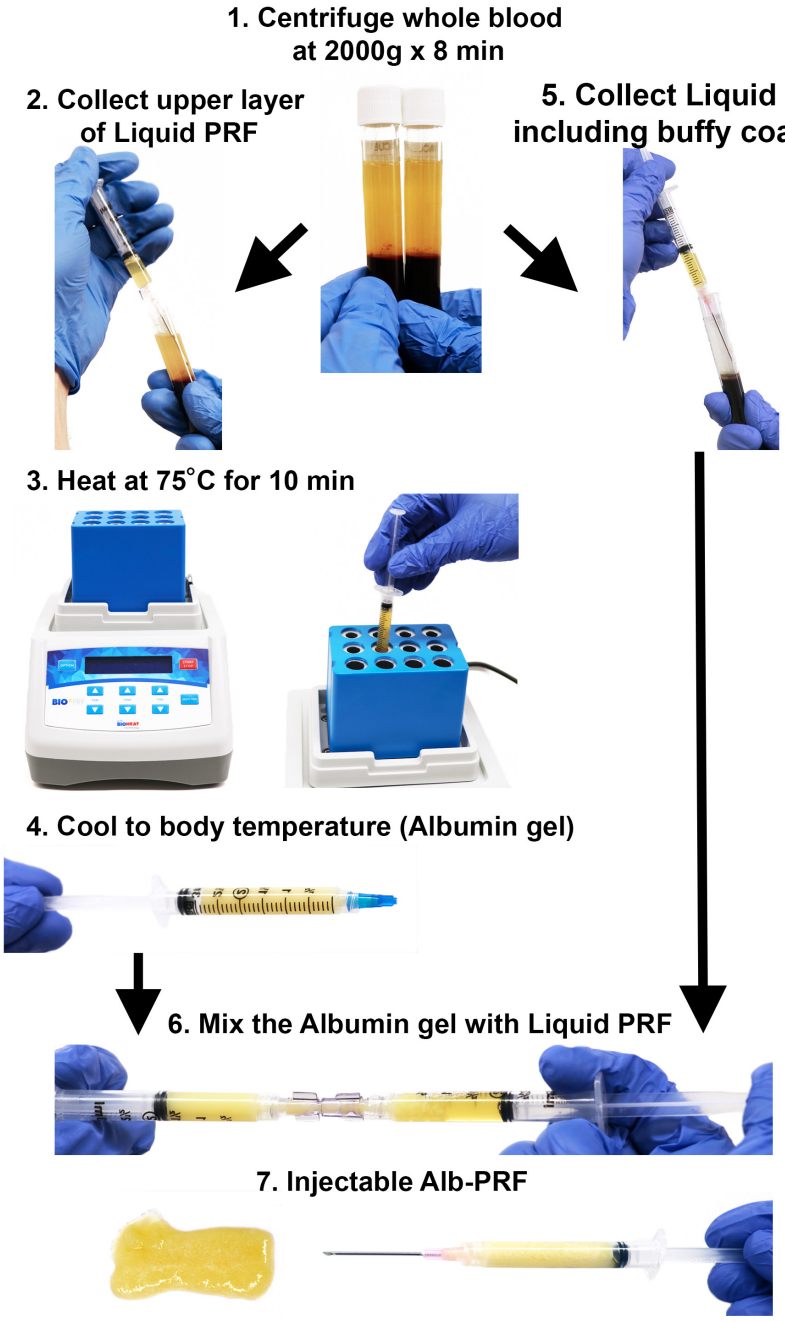

FIGURE 11 e-PRF preparation protocol. (1) Whole blood was centrifuged at 2000*g* for 8 min. The upper layer (yellow layer) shows the liquid plasma layer. (2) The uppermost layer of platelet-poor plasma (PPP) was collected in a syringe. (3) The collected PPP was heated in a heat block device at 75°C for 10 min and thereafter (4) cooled to room temperature for approximately 10 min. An injectable albumin gel was then prepared. (5) The liquid platelet-rich layer (liquid-PRF), including the buffy coat layer with accumulated platelets and leukocytes, was collected in a separate syringe. (6) The albumin gel and native liquid PRF were then thoroughly mixed by utilizing a female–female luer lock connector. (7) Injectable e-PRF in final ready form. Reprinted with permission from Fujioka-Kobayashi et al.[5]

3.5 | In vivo evaluation using Alb-PRF/e-PRF for periodontology and implant dentistry

One of the main areas where the e-PRF membranes were first clinically utilized and tested was as a substitute for collagen membranes (QR Code 1 SCAN ME). Therefore, several clinical case series were started whereby the e-PRF membranes were created and utilized for extraction site management to cover bone allografts following tooth removal similar to either a collagen membrane/plug or a PTFE membrane (Figure 12). This opportunity offers the clinician a 100% all-natural biomaterial with extended resorption properties that can also be left exposed in the oral cavity following placement. Notably, the e-PRF membranes may also be left exposed in the oral cavity, and their inclusion of supra-physiological concentrations of leukocytes allows for greater defense against incoming pathogens. QR Code 4 SCAN ME highlights a clinical example of an e-PRF membrane used, and Figure 12F highlights the much faster soft tissue closure ability of epithelial tissue over the e-PRF membrane when compared to alternative strategies.

Another example where e-PRF membranes have been utilized extensively is during lateral sinus lift procedures. Clinicians have been fabricating denser membranes from whole blood to either

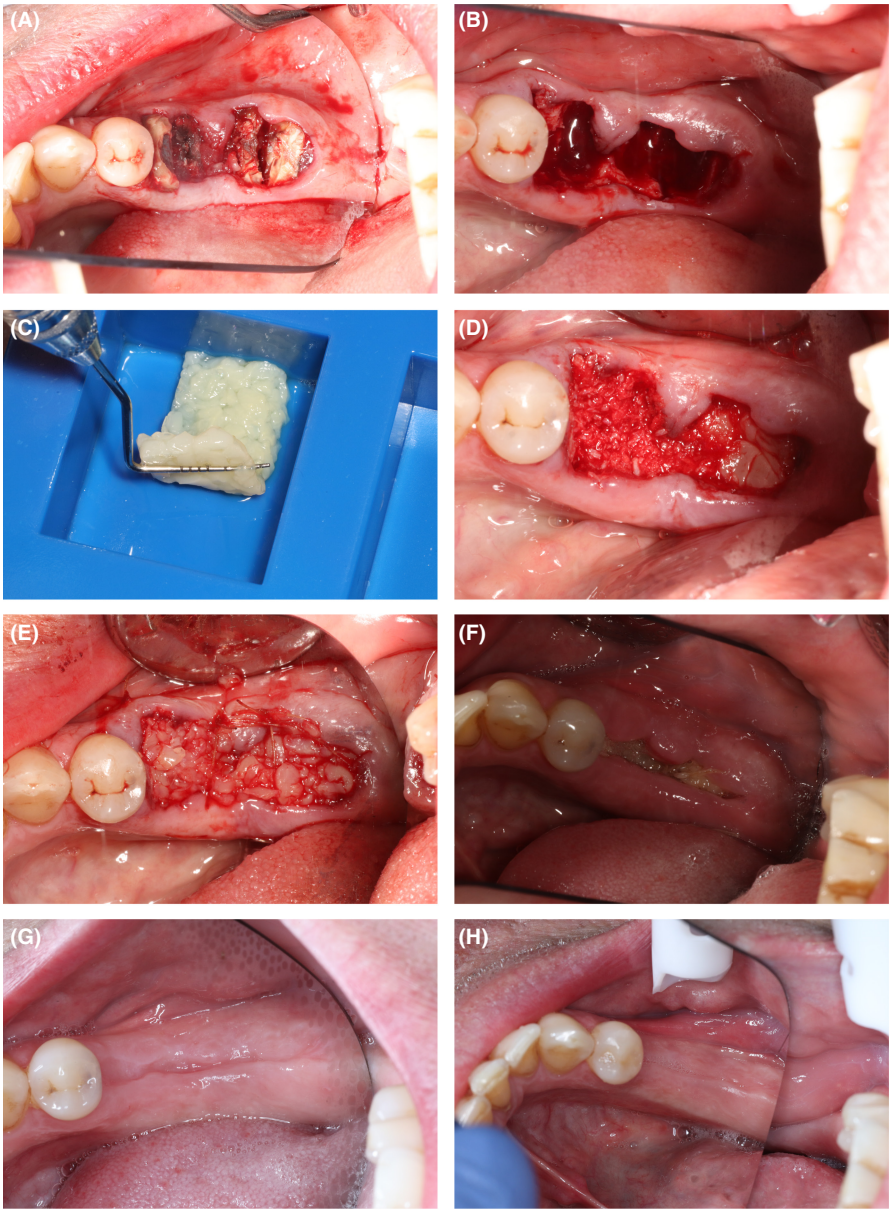

FIGURE 12 Clinical case using an e-PRF membrane as a substitute for a collagen barrier membrane following extraction site management. (A) Sectioning of teeth. (B) Following tooth extraction. (C) Allograft bone grafting with PRF fragments. (D) Fabrication of the e-PRF membrane following heat treatment at 75°C for 10 min in the Bio-Heat device. (E) e-PRF membrane in place with suturing (Video is found in QR Code 4 SCAN ME). (F) 1 week postoperatively. Note the excellent healing. (G) 8 weeks post-op. (H) 4 months post-op. Case performed by Dr Nathan Estrin.

repair small Schneiderian membrane perforations or to cover the lateral wall window (Figure 13, QR Code 5 SCAN ME). Finally, e-PRF membranes have been utilized extensively during GBR procedures (Figure 14, QR Code 6 SCAN ME). Here, such membranes may be much less foreign to soft tissues, with reports of improved biocompatibility. Figure 15 demonstrates a large GBR procedure where zygomatic implants were covered with e-PRF membranes. Finally, e-PRF membranes have also been utilized as a substitute for various collagen membranes during recession coverage procedures utilizing a vestibular tunneling approach (Figure 16, QR Code 7 SCAN ME).

3.6 | Clinical studies using e-PRF as an injectable Bio-Filler

To date, thousands of cases have utilized the technology of extending the working properties of platelet concentrates as a biological filler (Bio-Filler). One of the first approaches was to restore the facial

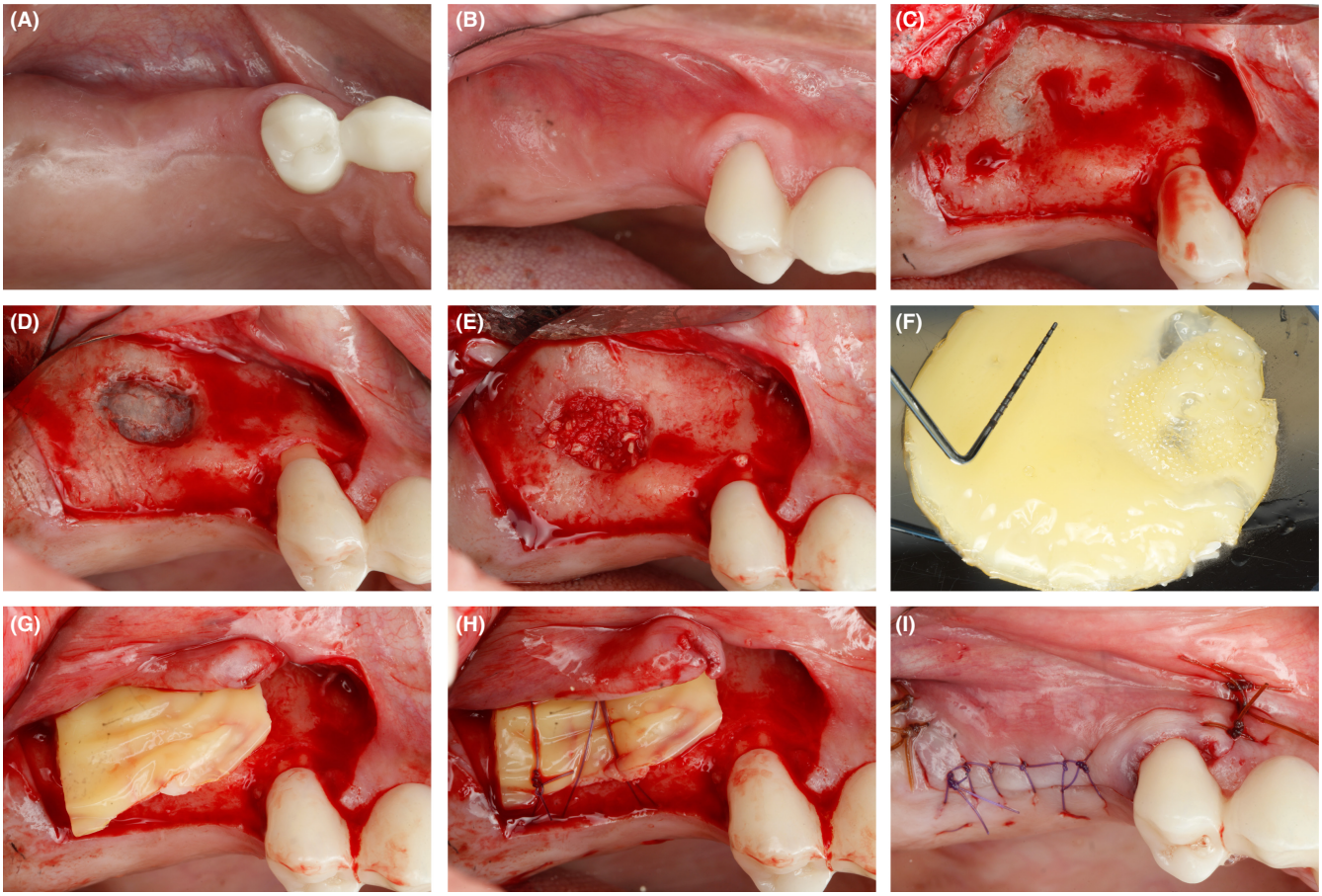

FIGURE 13 Use of the e-PRF membrane for lateral sinus grafting. (A, B) Preoperative images demonstrating deficient ridges requiring sinus grafting. (C) Full thickness flap elevation. Exposure of the lateral wall of the sinus. (D) Lateral sinus window preparation. (E) Placement of a particulate bone graft into the sinus cavity. An allograft and xenograft mixture was utilized. (F) Fabrication of an e-PRF membrane. (G) Placement of the e-PRF membrane over the lateral window. (H) Stabilization of the membrane using resorbable sutures. (I) Soft tissue closure. Case performed by Dr. Hussein Basma.

volume (Figures 17 and 18, QR Code 8 SCAN ME), and 2 pioneering studies investigated its use in various case series studies. In 2017, Matthews-Brzozowska et al.[19] highlighted the advantages of the first study on the topic by noting that the physician performing the procedure can adjust the density of the albumin gel to the needs of the patient. Growth factors were released longer in a controlled manner, which resulted in stronger stimulation and regenerative effects. Consequently, this made way to a variety of other protocols to utilize the technology but most importantly having the ability to adjust the gel via various heating methods as well as the ability to remix the liquid buffy coat layer of PRF into this albumin gel.[19]

Furthermore, Sun et al.[17] found that concentrated growth factor (CGF; another trademark that is equivalent to PRF) combined with albumin gel was better suited for reducing scars and enhancing patient satisfaction. The combined use of both modalities drastically improved either material used alone as the standard CGF and supplied the array of growth factors, whereas the albumin gel provided the long-lasting release and volume fill expected from a filler. Notably, the authors comment that this treatment was without significant adverse reactions, making it highly worthy of further clinical application.[17] Interestingly, Alb-PRF has been utilized for injections into deficient papilla with quite promising results, yielding up to 4 years of data (Figure 19, QR Code 9 SCAN ME). Last, Alb-PRF has also been utilized as a biological growth factor for joint and spinal injections such as diseased osteoarthritic joints and spinal injections (QR Code 10 SCAN ME).

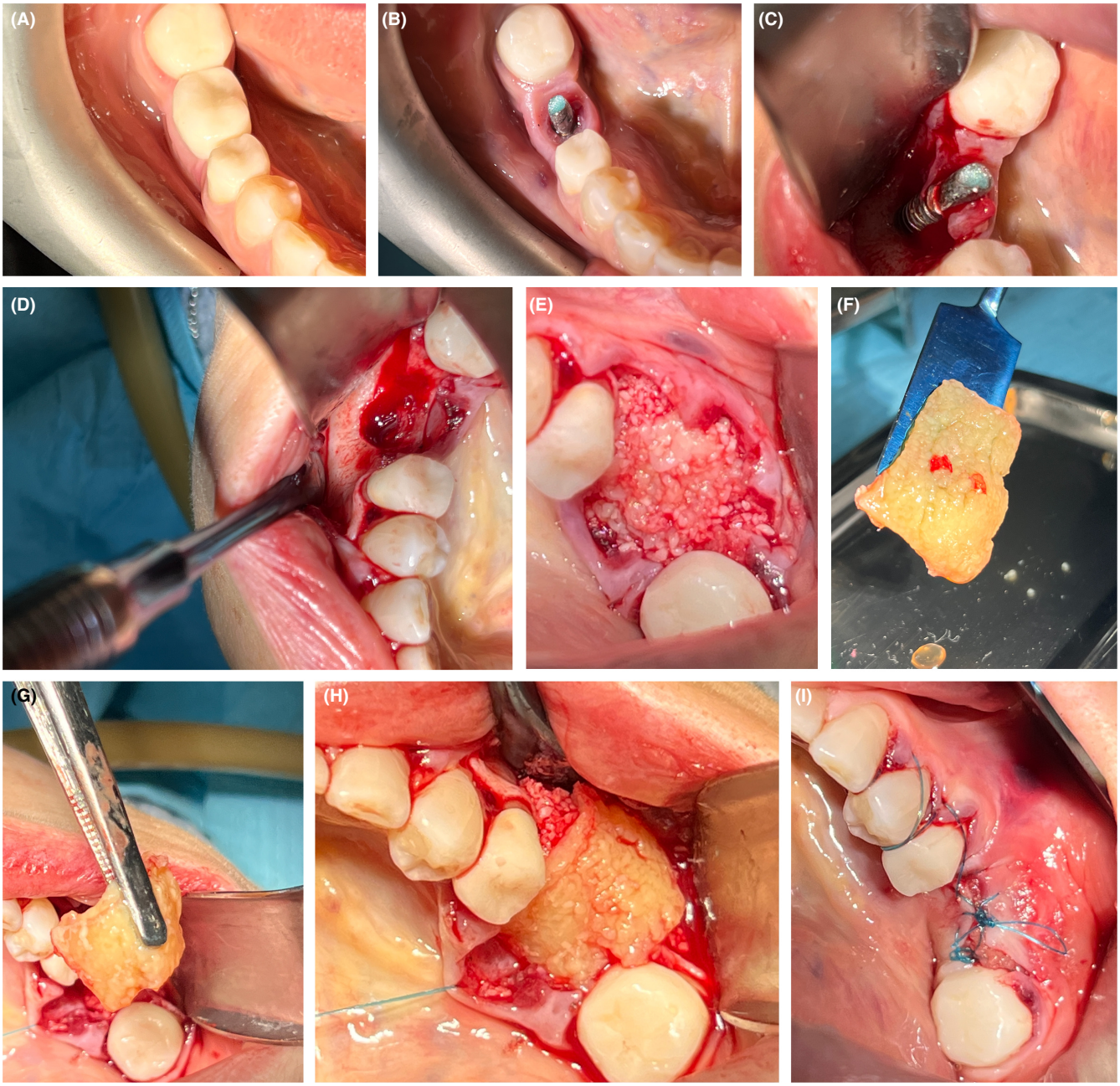

FIGURE 14 Use of the e-PRF membrane for GBR. (A, B) Lower first molar demonstrating deficiency of the buccal surface of the implant. (C) Flap elevation demonstrating complete loss of the buccal bone of this malpositioned implant. (D) Implant removal. (E) Grafting of the defect site with bone particles mixed with PRF fragments. (F) Fabrication of an e-PRF membrane. (G, H) Placement of the e-PRF membrane covering the grafted defect (Video is found in QR Code 6 SCAN ME). (I) Final soft tissue closure. Case performed by Dr. Alan Rene Espinoza.

4 | DISCUSSION

For over 3 decades, platelet concentrates have been utilized in regenerative medicine with the goal of concentrating cells and growth factors collected from peripheral blood. This easy-to-obtain technique allowed for a concentration of platelets, leukocytes and growth factors that favored the future revascularization of tissues, cell recruitment and cell proliferation, all of which promoted tissue regeneration.[2,22]

With the continual growth and discovery of new techniques and research endeavors, interest specifically in the field of platelet concentrates has certainly gravitated and received much attention in the

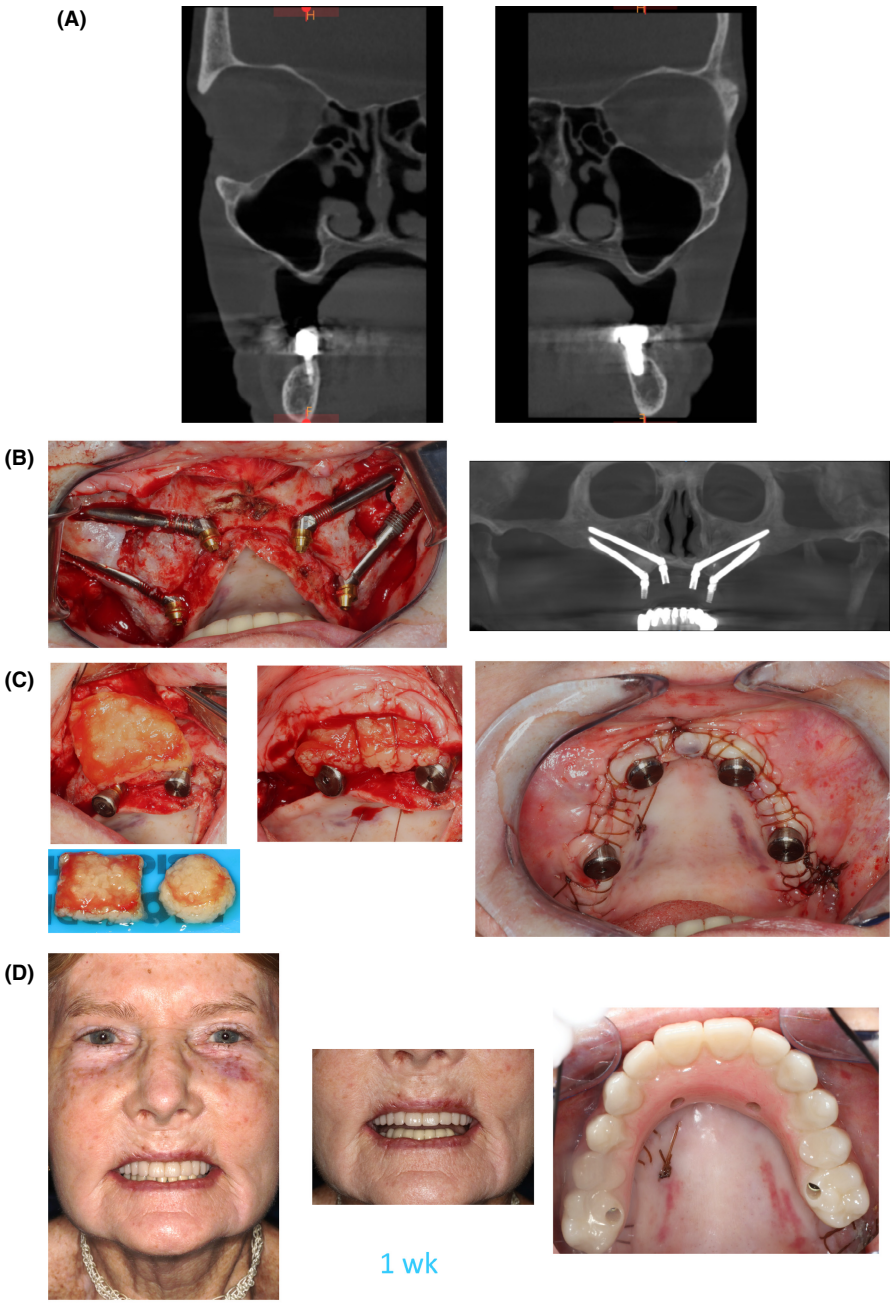

FIGURE 15 Use of e-PRF membranes in a full-arch zygomatic implant case. (A) CBCT images demonstrating fully pneumatized sinuses with minimal residual bone height (RBH). (B) Placement of four ZIs with significant exposure of the implants. (C) Fabrication of various e-PRF membranes to cover the implants. (D) One-week postoperative view demonstrating excellent healing and little postoperative inflammation. These cases are currently being followed 1 year postoperatively with excellent clinical success, without the need for a collagen barrier membrane. (Case performed by Dr Michael A. Pikos).

field of dentistry, orthopedics and aesthetic medicine.[23,24] For several years, however, all improvements and techniques related to PRP/PRF were focused on cell accumulation and growth factor release. PRF is still considered relatively "novel" despite being discovered 20 years ago. The main reason is that without the use of anticoagulants, a fibrin mesh could be obtained, thereby favoring a longer growth factor delivery vehicle, all via completely natural approaches. Furthermore, the ability of PRF to form a three-dimensional scaffold upon injection poses the additional advantage that it could be utilized as a growth factor delivery system.[25] While different protocols for autologous platelet concentrates have been proposed for various clinical applications, one of their main drawbacks has been short resorption properties typically limited to a 2-week timeframe.[26,27]

Recently, a novel technique was developed using fully natural blood, with the ability to extend the working properties of PRF upward of 4–6 months.[6] This product consists of denaturized serum albumin with an extended working time termed e-PRF commercially or Alb-PRF scientifically. The heating process denatures albumin, which allows for a modification in the secondary structure of the protein and transforms it into a tridimensional structure. During this heating process, new hydrogen and disulfide ligations are formed in the enzymes, which favors a larger tridimensional structure that improves the resorption properties of the albumin gel and drastically extends its stability over time. This in term creates a biomaterial derived from 100% whole blood, with extended resorption properties that last up

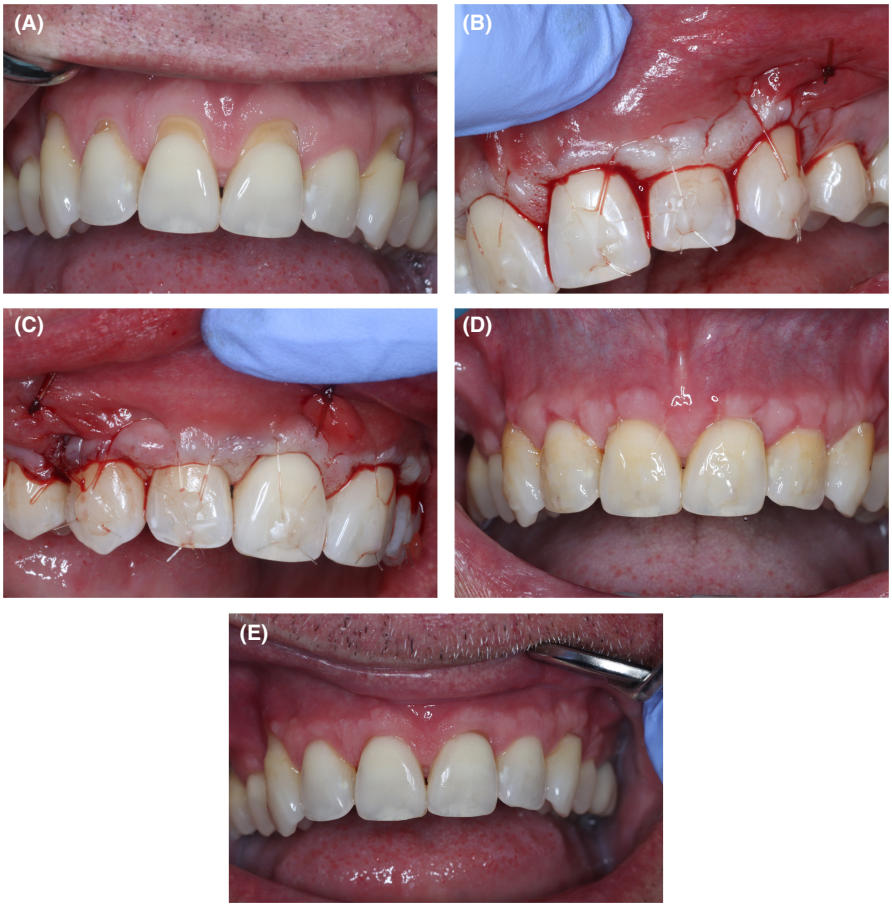

FIGURE 16 Use of e-PRF for the management of multiple gingival recessions. (A) Preoperative images demonstrating multiple anterior gingival recessions. (B, C) Postsurgical result following tunneling with e-PRF via a vestibular incision (Video is found in QR Code 7 SCAN ME). (D) 2 weeks post-op; note the excellent soft tissue healing. (E) 8 weeks post-op. Case performed by Dr Nathan Estrin.

FIGURE 17 (A) Mid-40-year-old female patient with pronounced Mariotte lines, deep nasolabial folds and an overall aged facial appearance. (B) Final outcome following 3 treatments with 100% natural approaches, including laser therapy (Smoothlase, Fotona), microneedling with PRF, and e-PRF injections. Case performed by Dr. Scott Delboccio. Reprinted with permission from Davies and Miron.[28]

to 6 months as opposed to 2 weeks. While albumin is the most abundant human plasma protein, responsible for more than 50% of the total protein present in the bloodstream, it is important to note that during denaturation, collected growth factors and cells also lose their activity and undergo apoptosis at high temperatures. Therefore, a novel protocol was developed following heating to reintroduce cells and growth factors back into the e-PRF (Figure 11).

5 | FUTURE RESEARCH ON e-PRF AND CONCLUSIONS

The findings from the current systematic review have shown that by heating the PPP layer from liquid-PRF, the biological resorption properties can be extended from a standard 2–3 week period toward 4–6 months. While this change in protocol adds approximately 10 min to standard PRF, it presents clear clinical benefits. It

FIGURE 18 A mid-40-year-old patient with volume loss under the eye in the trough area. The area was filled with e-PRF only. Images show the appearance (A) before and (B) after treatment. Case performed by Dr. Catherine Davies.

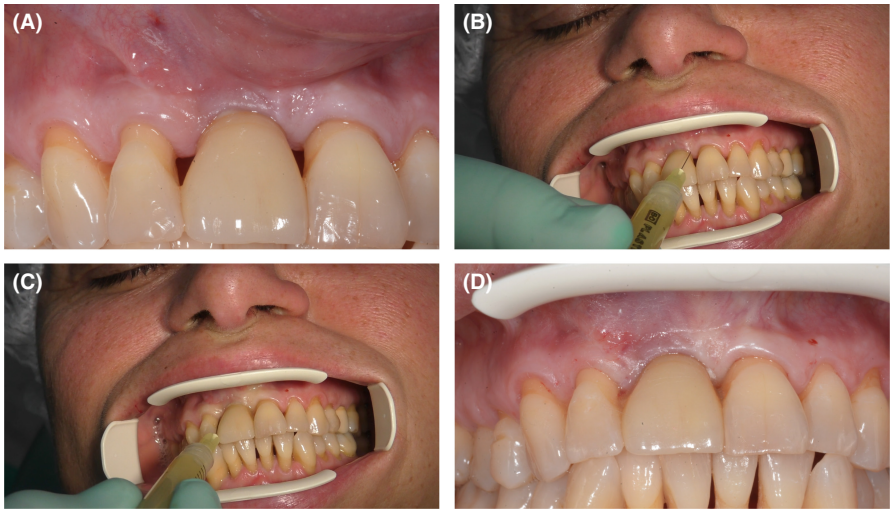

FIGURE 19 (A) Clinical photograph demonstrating maxillary anterior teeth with multiple black triangles. (B, C) Injection techniques of the Alb-PRF mixture into various papillas. (D) Final clinical photographs after injections

(Video found in QR Code 9 SCAN ME). Case performed by Dr Ezio Gheno.

therefore becomes possible to use e-PRF as a replacement option for standard collagen membranes used frequently in periodontology and implant dentistry or to utilize it as a true biological filler (i.e., Bio-Filler) derived from 100% autologous sources with drastically extended resorption properties.

One area of high interest is to further investigate and determine the degradation properties of e-PRF in various areas of the oral cavity, face and body. Preliminary data have suggested that e-PRF has different rates of degradation depending on the areas where it is implanted, and the degradation rate seems to be correlated with the vascularization potential within that area. For instance, it is known that the lips and surrounding area are fairly well vascularized. Preliminary data suggest that e-PRF tends to resorb more rapidly in high-vascularity tissues (such as lips) when compared to lower vascularity tissues (such as cheek bones, which form the trough area under the eyes) when injected as a facial filler.[28] Thus, future research is needed to specifically address the resorption properties of e-PRF in the various areas where it is commonly utilized in larger patient population sample sizes.

The present review article focuses on the current state of the art knowledge on e-PRF, including all the background studies from in vitro, in vivo and clinical research to date. e-PRF may be used as either a replacement for collagen membranes in dentistry or as an injectable platelet concentrate following mixing with female–female luer lock connectors, with resorption properties that extend as long as 4–6 months. e-PRF possesses excellent biocompatibility and has been shown to greatly enhance fibroblast cellular activity and collagen production via the release of blood-derived growth factors. While the available data thus far remains weak owing to the limited human studies thus far, it possesses great potential for further research in regenerative medicine. It therefore offers much future potential use as a biological and natural biomaterial in periodontology, implant dentistry, orthopedics, and aesthetic medicine. Future research is ongoing and focused on further extending the resorption properties of e-PRF with cross-linking agents as well as introducing small biomolecules into the mixture to further enhance tissue regeneration.

DATA AVAILABILITY STATEMENT

Data 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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