Vol. 00 No. 00 ■■ 2021

Autologous platelet concentrates in oral surgery: protocols, properties, and clinical applications

Laura O'Sullivan, BDS, MFDS RCSEd, PDTLHE,[a] and
Rícheal Ní Ríordáin, MBBS, BDS, MA (Higher Ed), PhD, MFD, FFD, FDS(OM)[b,c]

Autologous platelet concentrates (APCs) are a relatively new phenomenon, with initial reports of their regenerative potential published as recently as 1998. Despite their relative infancy, a huge body of evidence exists in support of their capacity to promote osseous and soft tissue regeneration through the physiologic processes of platelet activation and subsequent growth factor release. APCs have transformed many areas of healthcare and are now considered an essential component of the surgical milieu. In this narrative review, we explore the evolution of autologous platelet therapies with a particular emphasis on their contemporary applications in oral surgery, which rather fittingly was the first specialty to report the regenerative potential of APCs. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;000:1−9)

The last 20 years have seen huge advances in the development and understanding of the role of autologous platelet concentrates (APCs) in many areas of healthcare. Such is the predictability of their regenerative properties that APCs are now widely considered a fundamental treatment modality in oral and maxillofacial surgery, orthopedics, sports medicine, and ophthalmology. Their popularity is enhanced by their wide availability, ease of use, and cost-effectiveness. Despite a plethora of published literature on the topic, efforts to date to develop a simplified classification of autologous platelet products have been unsuccessful, instead generating a minefield of complex nomenclatures that are challenging to understand.

With clinical interest in APCs continually growing, procurement of production systems has become increasingly commercially driven, and there remains little guidance for clinicians in deciding which system is best suited to their individual needs. In recognition of this, we present a comprehensive review of the relevant literature in a succinct format for the interested clinician. This review provides a summary of the evolution of autologous platelet therapies through the years, describing their physiologic properties, preparation protocols, and clinical applications in an oral surgery context.

[a]Specialty trainee, Oral Surgery, Cork University Dental School and Hospital, University College Cork, Cork, Ireland.
[b]Consultant/Senior Lecturer, Oral Medicine, Cork University Dental School and Hospital, University College Cork, Cork, Ireland.
[c]Honorary Associate Professor, Oral Medicine, University College London, London Eastman Dental Institute, London, United Kingdom.
Received for publication Mar 14, 2021; returned for revision May 19, 2021; accepted for publication May 27, 2021.

2212-4403/$-see front matter
https://doi.org/10.1016/j.oooo.2021.05.013

BACKGROUND

The use of autologous blood products in surgery was first reported in 1954 by Kingsley, who used "platelet-rich human plasma" for its hemostatic and adhesive properties.[1,2] In 1970, Matras[3] introduced the concept of fibrin glue by demonstrating enhanced healing of skin wounds in a rat model. She went on to describe the hemostatic and tissue-healing properties of fibrin glue when used in oral and maxillofacial surgery, and later reported its applications in microvascular and microneural surgery. In 1994, Tayapongsak et al.[4] reported a 97% success rate in overcoming separation of fragments of particulate cancellous bone and marrow grafts by using autologous fibrin adhesive as a surgical adjunct during mandibular reconstruction surgery.

One of the many disadvantages of the fibrin glue system is the potential for blood-borne virus transmission due to the use of donor cryoprecipitate, with at least one case of human immunodeficiency virus transmission reported in the literature.[5,6] Fibrinogen can alternatively be sourced from autologous plasma, but this requires donation of blood by patients up to 3 weeks before surgery. This concentrated fibrinogen is cleaved by exogenous bovine thrombin in the presence of calcium to form fibrin in the final coagulation cascade (Figure 1). The fibrin strands are then cross-linked to form a stable fibrin clot in the presence of factor XIII.

In an effort to address the shortcomings of fibrin glue, Whitman et al.[5] later described platelet gel as a more favorable autologous alternative. Its advantages

Statement of Clinical Relevance

Autologous platelet concentrates (APCs) have been lauded by some as revolutionary in the field of regenerative medicine, and knowledge of the properties and applications of APCs should therefore be considered "essential" knowledge for the modern-day clinician.

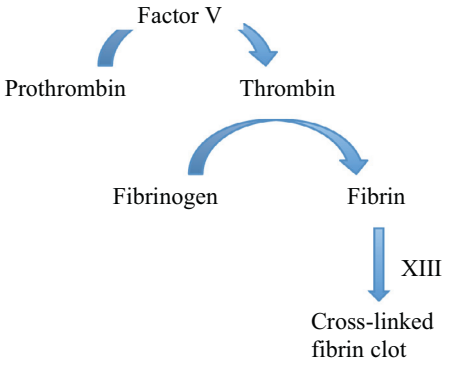

Fig. 1. Final common pathway of the coagulation cascade.

included a high concentration of platelets, native concentration of fibrinogen, and collection of 1 unit of whole blood (450 mL) immediately preoperatively, negating the need for advance predonation of blood. They documented a 2-stage centrifugation process, which allows selective siphoning of erythrocytes and platelet-poor plasma, leaving behind platelet-rich plasma. A disadvantage of this technique is the need for exogenous bovine thrombin to activate the platelet gel mixture, as is the case with fibrin glue preparation.

This initial inception of autologous blood products into the surgical armamentarium sparked much further research into the selective sequestration of platelets from autologous blood samples and the possibility that these resulting platelet concentrates might confer a physiologic advantage in the healing of hard and soft tissue defects. Thus was born the era of autologous platelet-rich therapies.

PLATELETS

Platelets are small anucleate cell fragments derived from megakaryocytes[7] with a lifespan of 9 to 10 days.[8] They are the smallest of the blood cells, with an average diameter of 2 to 5 μm, thickness of 0.5 μm, and numbering 150-400 $\times$ 10^9 in the average individual.[9] The role of platelets in hemostasis and thrombosis was recognized as far back as 1882 by Bizzozero, who described the adherence of platelets to sites of blood vessel injury and formation of platelet aggregates to begin the repair process.[10]

The internal structure of platelets has been studied at length with the aid of electron microscopy, cell fractionation, and platelet release studies.[7] Platelets contain many of the cytoplasmic organelles common to most eukaryotic cells (Figure 2), but in contrast, these organelles are primarily secretory in function, releasing their contents readily in response to signals such as collagen, thrombin, and thromboxane A2. In the 1960s, two additional platelet-specific secretory organelles were identified as being central to the processes of hemostasis, thrombosis, inflammation, angiogenesis, host defence,

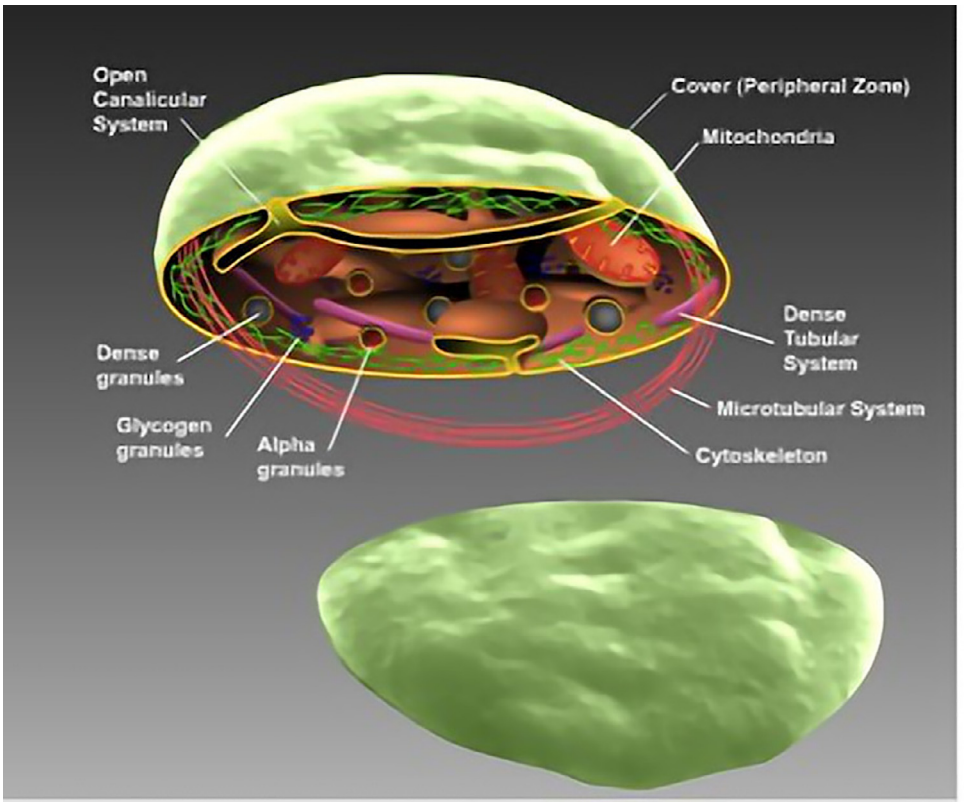

Fig. 2. Internal platelet structure (http://www.platelet-research.org/).

and mitogenesis: α-granules and dense (δ) granules. α-Granules are the most abundant of all platelet organelles, with the average platelet boasting between 50 and 80. They play a fundamental role in protein synthesis, storage, and release; these proteins (growth factors, membrane proteins, chemokines, adhesion proteins, immune mediators) are either suspended within the α-granule or bound to its membrane. Dense granules are less populous, with an average of 3-8 per human platelet. They are the primary source of adenosine diphosphate (ADP) during hemostasis, one of the main drivers of platelet aggregation and activation.[9]

Platelet activation

Platelet activation is a typical physiologic response to contact between platelet surface receptors and exposed collagen in injured tissue such as a blood vessel. The process stimulates migration of α- and δ-granules to release their contents either by fusion with the platelet membrane or via the open canalicular system. The flood of local coagulation factors, particularly factor V, promotes the production of thrombin (Figure 1). A concomitant increase in intracellular Ca^{2+} concentration promotes further granule secretion. This complex interplay of platelet activation, fibrinogen release, thrombin production, and growth factor secretion culminates in the formation of a stable fibrin clot. Over 300 different proteins have been identified during α-granule secretion.[9] At the same time, dense granules release a host of proteins, each with important effects: ADP enhances platelet−platelet aggregation, serotonin enhances vascular tone, and Ca^{2+} ions promote thrombin formation and further granule secretion.

Growth factors are fundamental to the successes of regenerative medicine, being hailed as a "biological solution to biological and medical problems."[11] A summary of the fundamental growth factors released during platelet activation is presented in Table I.

Table I. Summary of growth factors released during platelet activation and their physiological properties

Growth factor	*Function*
PDGF	• Initiates connective tissue healing • Mitogenesis of connective tissue cells • Macrophage activation
TGF-β	• Chemotaxis and mitogenesis of osteoprogenitor cells • Stimulates collagen deposition by osteoblasts • Inhibits osteoclast formation and bone deposition
VEGF	• Promotes angiogenesis through endothelial cell proliferation and migration
EGF	• Promotes chemotaxis and angiogenesis of endothelial cells • Promotes mesenchymal cell mitosis
IGF	• Regulates late-stage differentiation and activity of osteoblasts • Regulates apoptosis
HGF	• Potent inducer of angiogenesis • Antifibrotic properties
FGF	• Promotes endothelial cell chemotaxis and mitogenesis

EGF, epidermal growth factor; *FGF*, fibroblast growth factor; *HGF*, hepatocyte growth factor; *IGF*, insulin-like growth factor; *PDGF*, platelet-derived growth factor; *TGF*-β, transforming growth factor β; *VEGF*, vascular endothelial growth factor.

AUTOLOGOUS PLATELET CONCENTRATES TABLE II

Platelet-rich plasma

Following the earlier work of Whitman et al.[5] into the standardization of autologous platelet gel preparation, Marx et al.[12] took things a step in further in 1998 by describing their protocol for platelet-rich plasma (PRP) preparation in patients undergoing mandibular reconstruction under general anesthesia:

1. Centrifugation at 5600 rpm generating 3 distinct layers: 180 mL of erythrocytes, 70 mL of PRP with leukocytes ("buffy coat"), and 200 mL of platelet-poor plasma (PPP)
2. Removal of PPP
3. Slower centrifugation cycle of PRP at 2400 rpm to refine separation from erythrocytes
4. Return of PPP and erythrocytes to the patient via a central venous catheter or peripheral venous access

Marx et al.[12] reported accelerated and enhanced bone deposition in their cohort of 88 patients who underwent reconstruction of mandibular defects with adjunctive use of PRP at graft sites.

PRP deserves recognition for paving the way for further advances in autologous platelet therapies and for standardizing the preparation protocol in line with that previously described by Whitman et al.[5] The inconvenience of patients having to attend several days or weeks before surgery to donate a blood sample was overcome by obtaining blood samples immediately preoperatively. Both platelet gel and PRP permit a "command gelification" by the manual addition of a calcium-based activator to the platelet concentrate. This allows surgeons to apply PRP/platelet gel as required to the surgical site at the desired time.

Although the above advances cannot be disputed, there were certain disadvantages to the original PRP technique that limited its accessibility for widespread use: the need for a complex cell separator system such as the Electro Medics 500 (Medtronic)[12] often

Table II. Summary of properties of main autologous platelet concentrates

Properties	*PRP*	*PRGF*	*L-PRF*
Category	First generation	First generation	Second generation
Volume of blood	450 mL	36 mL	40 mL
Centrifuge system	Electro Medics 500 (Medtronic)	System V centrifuge (Endoret)	Process tabletop PC-02 centrifuge (Nice, France)
Centrifugation speed	5600 rpm 2400 rpm	580g	2700 rpm
Time	20-30 min	8 min	12 min
Volume of platelet-rich concentrate	70 mL	8 mL	PRF "clot"
Leukocytes	Yes	No	Yes

Protocols represent those originally reported in the literature and have likely been updated.
L-PRF, leukocyte and platelet-rich fibrin; *PRGF*, plasma rich in growth factors; *PRP*, platelet-rich plasma.

requiring help from a hematologist, the large quantity of blood (400-450 mL) required to produce approximately 70 mL of PRP, additional material costs (catheters, central venous lines), 2 centrifugation cycles making the process time-consuming, and the need for bovine exogenous thrombin to activate the mixture.

Plasma rich in growth factors

Completing the complement of first-generation platelet concentrates is a preparation known as plasma rich in growth factors (PRGF), which is produced commercially by Biotechnology Institute (BTI) under the trademark Endoret® and differs from other APCs by selectively excluding leukocytes from the product during preparation. The commercial Endoret® system is suitable for use in a primary care setting and consists of a system V centrifuge, PLASMATERM H, work rack, activation containers, and digital timer unit, all of which are reusable (Figure 3). Single-use KMU15 kits containing the necessary materials for venipuncture and fractionation are available to purchase. A 4-stage preparation protocol is described for Endoret®:

1. Blood collection: Four 9-mL tubes of venous blood are collected from the patient. Blood tubes are available with and without sodium citrate anticoagulant.
2. Centrifugation: Blood is spun at 580*g* for 8 minutes. This slow speed results in fractionation of the various blood cell constituents mainly by their specific gravities,[13] the order of layers or "fractions" from the bottom of the tube to the top being the red cell fraction, white cell fraction, and platelet fraction, respectively. This single-step centrifugation process results in better-defined fractions with less crossover, optimizing the separation of the leukocyte-rich "buffy coat" (distinct from the buffy coat described in PRP) from the platelet-rich "fraction 2" component immediately above. This near-total elimination of leukocytes from the product is the hallmark of Endoret®.
3. Fractionation: Tubes are transferred to the work rack, and all caps are removed. Markings are placed with an ink pen as shown in Figure 4 to demarcate the hematocrit, buffy coat (leukocytes), fraction 2 (platelet-rich component), and fraction 1 (plasma-poor component). Fractionation is carried out using a plasma transfer device to collect all fraction 1 volumes from each tube. This is repeated for the fraction 2 component in each tube. Fractionation should be carried out immediately after centrifugation to prevent delay-related overlap of the various fractions. The protocol produces a consistent 2-mL volume of platelet-rich fraction 2 immediately above the buffy coat following centrifugation[14,15] (Figure 4).
4. Activation: This step is only necessary when tubes containing anticoagulant have been used and involves the addition of 10% calcium chloride activator to fraction 1 and fraction 2. The amount of activator is dependent on the volume of plasma in each fraction, with 1 unit of activator added per 0.5 mL of plasma; assuming a total volume of 8 mL of fraction 2 is collected during preparation, then 16 units of calcium chloride are added to the tube to activate it. Each fraction is then transferred to a separate "activation dish" and heated in the PLASMATERM H device at body temperature for 10 to 15 minutes until they assume a jelly-like consistency.

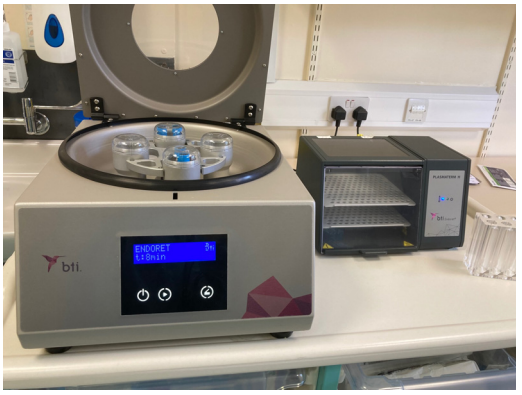

Fig. 3. Endoret® centrifuge loaded with blood bottles before spinning (photograph taken by Laura O'Sullivan October 30, 2020).

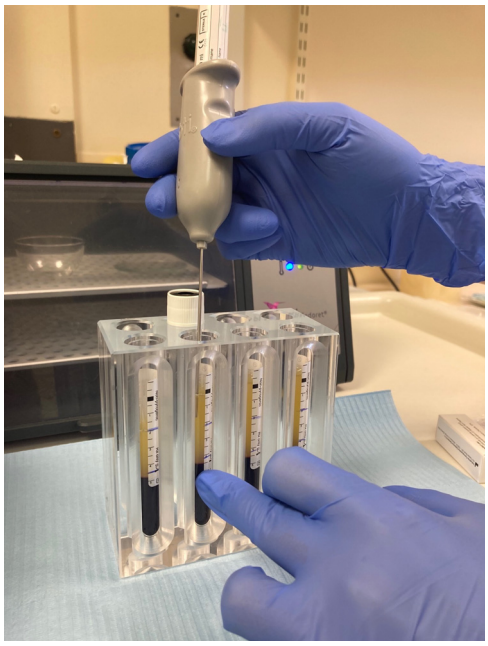

Fig. 4. Fractionation using plasma transfer device (PTD) (photograph taken by Laura O'Sullivan October 30, 2020).

The fraction 2 "clot" is rich in platelets, whereas the fraction 1 "membrane" is a condensed fibrin structure with far less cellular entrapment. In their study conducted to investigate the effects of anticoagulant and antiplatelet drugs on the preparation of PRGF, Anitua et al.[14] found that for patients taking warfarin, the time to clot formation was significantly longer than for non-warfarinized patients.

Leukocyte and platelet-rich fibrin

This second-generation platelet concentrate was developed in an effort to simplify the preparation process of APCs, negating the need for manual fractionation and activation. Its first clinical application was in the management of a patient with persistent Lyell syndrome (toxic epidermal necrolysis) affecting the lower leg. Marked improvement in healing was observed 30 days after initial leukocyte and platelet-rich fibrin (L-PRF) treatment.[16]

Choukroun's single-step preparation protocol involves obtaining a venous blood sample in 9- or 10-mL tubes with no anticoagulant. These samples are centrifuged immediately for 12 minutes at 750*g* using a Process tabletop PC-02 centrifuge (Nice, France) or a similar setup. The absence of anticoagulant means platelet activation and fibrin polymerization commence almost immediately.[17] Centrifugation produces a 3-layered suspension: erythrocytes at the base of the tube, acellular plasma at the top, and a dense fibrin clot suspended in the middle. The clot is removed with tweezers and compressed with gauze or similar material for ease of application to the surgical site. The success of this protocol relies on quick handling of the blood samples; any delay between venipuncture and centrifugation will result in failure of the technique.[17]

PROPERTIES OF APCS

Leukocyte exclusion during APC preparation remains a contentious issue, with no agreed consensus on the subject. One argument in favor of exclusion is the production of a more homogeneous and reproducible platelet product.[18] One in vitro study investigating the activity of human fibroblasts and osteoblasts treated with PRP and PRGF, under both normal and inflammatory conditions, showed consistently elevated release of proinflammatory cytokines such as interleukin (IL)-6, IL-8, tumor necrosis factor-α, and IL-1β in the PRP-treated cell groups. Although cytokines have a role to play in the inflammatory process and in fighting infection, excessive production can be destructive to surrounding tissues.[19] Concerns have also been raised about the potential for extracellular matrix destruction by neutrophils due to the release of matrix-degrading enzymes as well as reactive oxygen species that destroy healthy and injured tissues.[18]

Nishiyama et al.[13] investigated the composition of PRGF fraction 2 versus PRP by collecting venous blood from 7 healthy volunteers. Using an automated hematology analyzer, they were able to show near total elimination of erythrocytes and leukocytes from PRGF preparations, whereas the leukocyte count was increased 5.5-fold in the case of PRP. Platelets were concentrated by a factor of 2.84 in PRGF and 8.79 in PRP, whereas actual numbers of platelets per preparation were slightly higher in the former.

The regenerative properties of APCs, via growth factor release, are effected through migration and proliferation of native osteoblasts and fibroblasts, which are concentrated at the site of APC application through the formation of a biological 3-dimensional fibrin scaffold during platelet activation, which localizes these cellular components and growth factors at the site. Optimization of cellular proliferation has been the focus of much research, with one in vitro study demonstrating optimum proliferation of fibroblasts and osteoblasts at a platelet concentration 2.5 times that seen in whole blood. Concentrations of platelets above this level showed a negative effect on cellular proliferation and impairment of osteoblast function.[20] Nishiyama et al.'s[13] findings confirm that platelet concentrations in PRGF are in the optimum range for maximum regenerative potential.

The potential antibacterial effects of PRGF against methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus* and methicillin-sensitive and methicillin-resistant strains of *Staphylococcus*

epidermidis have been studied in vitro.[21] Although wound infections are typically polymicrobial in nature, staphylococci are believed to be major players in the etiology of delayed healing and infection in both acute and chronic wounds.[22] Using blood samples from 5 healthy volunteers, Anitua et al.[21] demonstrated a strong bacteriostatic effect of PRGF preparations during the first 4 hours after incubation, with staphylococcal populations tending to recover once again after 8 hours. Similar results have been reported for in vitro studies of PRP, which showed antibacterial activity against *S. aureus* and *Escherichia coli*.[23] Although the antibacterial effect of PRGF has yet to be fully evaluated in vivo, it is reasonable to extrapolate the role that PRGF preparations might play in prophylaxis against surgical site infection.

FORMULATIONS

The versatility of APCs stems from the many permutations of preparations that can be produced from a single blood sample. Using PRGF as an example, the 4 different formulations are as follows:

1. Liquid PRGF: nonactivated PRP

Indications

- Facial rejuvenation/wrinkle reduction (mesotherapy)
- Implant surface coating to promote "bioactivation" and enhance osseointegration
- Combination with autogenous or exogenous bone graft material to improve handling

2. Supernatant: the "leftover" fluid after PRGF activation

Indications

- Mouth ulcers
- Eye drops

3. Fraction 2 clot: the platelet-rich scaffold (PRP/PRGF)

Indications

- Extraction socket healing
- Treatment of medication-related osteonecrosis of the jaw (MRONJ)

4. Fraction 1 membrane: PPP

- Dental implant membrane
- Sinus lift surgery membrane

APPLICATIONS IN DIFFERENT MEDICAL FIELDS

Vascular surgery

Successful treatment of refractory leg ulcers with APCs has been widely reported in the literature. In one small-scale study, superior healing of leg ulcers treated with PRGF fraction 2 clots was reported compared to debridement with 0.9% normal saline alone. Mean healed skin surface area, the primary outcome measure, at 8 weeks was 72.94% (standard deviation [SD], 22.25) in the PRGF group (n = 5) and 21.48% (SD, 33.56) in the control group (n = 4).[24]

Similarly, a prospective cohort study of 44 patients undergoing refractory leg ulcer debridement as described above, with placement of L-PRF clots over the entire surface area, showed excellent success rates in achieving total wound closure of small ulcers (≤10cm diameter) at 8 weeks and significant wound reduction in larger ulcers (≥10cm) at up to 15 weeks.[25]

Sports medicine and orthopedic surgery

APCs have clinically proven benefits in functional recovery following tendon and ligament repairs.[11] In one cohort study of patients undergoing surgical Achilles tendon repair with and without autologous PRGF treatment before wound closure, the authors were able to demonstrate significant differences in the time to training activity resumption and the rate at which range of motion was recovered, favoring PRGF-treated cases.[26] Improvements in overall tendon strength and regeneration have also been demonstrated by injection of PRP at surgical sites 1 week postoperatively in similar cases.[27] Ultrasound-guided intratendinous injection of PRGF has been applied successfully in the management of elbow tendinosis.[28] PRGF has also been used in patellar tendon repair surgery and has been pioneered by the BTI research group for the arthroscopic management of articular cartilage avulsion injuries in knee joints.[29]

Ophthalmology

Alio et al.[30] investigated the role of PRP in the management of dry eye symptoms in a case series of 386 patients, a condition estimated to affect 4% to 30% of the population. Treatment consisted of autologous PRP eye drops applied at a dose of 1 drop 6 times per day for 6 weeks, with 87.5% of patients reporting improvement in their symptoms.

APPLICATIONS IN ORAL SURGERY

Extraction socket healing

A Cochrane review published in 2020 looked for the first time at adjunctive use of APCs in mandibular third

molar sockets, citing reduced incidence of dry socket 7 days postoperatively at APC-treated sites. No difference in facial swelling or postoperative pain experience were detected between groups. Authors of the review included 4 studies in their analysis and graded the available evidence as being at high or unclear risk of bias.[31] Other systematic reviews in the literature appear to support improvement in clinical outcomes such as trismus, soft tissue healing, and facial swelling at APC-treated extraction sites.[32-34] Conclusions appear to differ with regard to postoperative pain and inflammation. A further systematic review looking specifically at the effects of PRGF in postextraction sockets presented a qualitative analysis of 8 studies, reporting reduced postoperative pain and incidence of postoperative complications at PRGF-treated sites.[35]

Dental implantology

More rapid and enhanced bone healing is observed with use of PRGF at implant osteotomy sites, with the technique also producing superior soft tissue healing.[36] Recent advances have also led to clinicians combining PRGF with exogenous bone-grafting material such as Bio-Oss® (Geistlich Pharma) to improve the handling and adaptation at donor sites.[18] One of the major challenges in dental implantology is the atrophic maxilla, and a retrospective case series published by Anitua et al. suggested a promising role of PRGF in the augmentation of vertical bone height at this site.[37] Their cohort of 26 patients underwent transcrestal sinus elevation and PRGF plug insertion at the base of each osteotomy site before placement of a total of 41 implants. They observed a sustained increase in vertical bone height 3 years postoperatively.[37]

MRONJ

MRONJ is a relatively new phenomenon, having first been described by Marx in 2003,[38] and is a condition that is unique to patients with a history of bisphosphonate or antiresorptive/antiangiogenic therapy. A discussion of the pathophysiology of MRONJ is beyond the scope of this review. Currently, no universally agreed protocol exists for the prevention and therapeutic management of MRONJ in at-risk patients. Scoletta et al.[39] reported favorable results for the prevention of MRONJ in patients receiving intravenous bisphosphonates, with prophylactic placement of PRGF in extraction sockets and avoiding mucoperiosteal flaps where possible. In a retrospective case series of 32 patients undergoing treatment with intravenous bisphosphonates, Mozzati et al.[40] reported total mucosal coverage with absence of exposed bone in all cases of surgical debridement and placement of PRGF, with a minimum follow-up of 48 months. A case series of 12 sites of MRONJ and osteoradionecrosis investigated the effect of piezoelectric agitation of necrotic sites before L-PRF placement with unconvincing results (67% sites demonstrating complete mucosal healing at 12 months), possibly influenced by the small sample size.[41]

Other

APCs have also demonstrated success in the management of recurrent aphthous stomatitis and refractory oral lichen planus, in the promotion of apexification (root end closure) in immature teeth requiring endodontic treatment, and in the management of gingival recession defects, leading to restoration of keratinized mucosa across exposed root surfaces.[42]

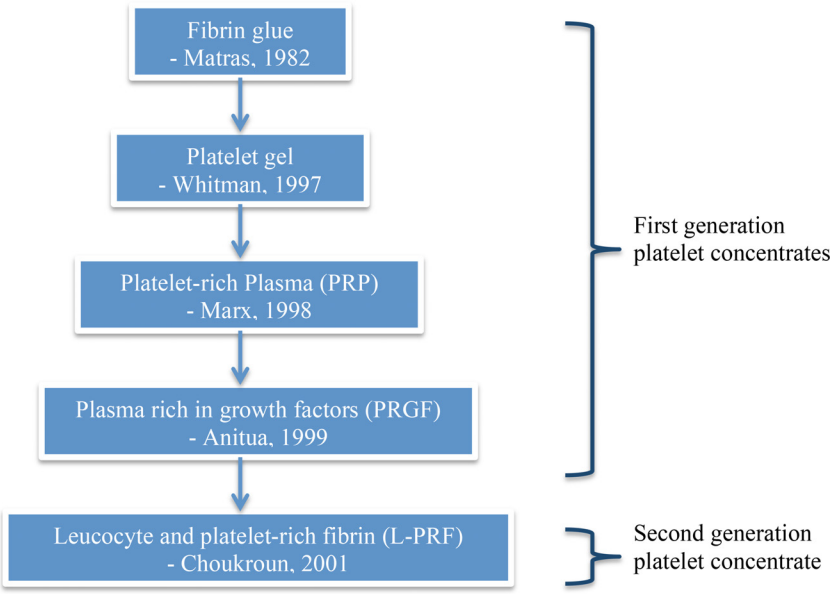

Fig. 5. Flowchart presentation of timeline of autologous platelet concentrate evolution.

CONCLUSIONS

Previous efforts to categorize APCs according to leukocyte content and fibrin matrix structure have yet to garner universal acclaim.[43] The terminologies arising from this proposed classification (P-PRP, L-PRP, L-PRF, L-PRP) have been superseded by yet more subcategories, including iPRF[44] (injectable PRF), aPRF[45] (advanced PRF), and cPRP[17] (concentrated PRP). These subcategories represent the range of formulations produced by altering centrifugation protocols, with up to 24 protocols described in the literature for PRF alone.[46]

The superfluous terminologies have been described as "disingenuous" by some experts who claim "PRP is PRP, whatever way you look at it."[47] There is concern in the scientific community that underlying commercial interests may lead to a cycle of reinventing the wheel. Ultimately, all APCs achieve the same end goal of regeneration, with relatively minor differences in preparation protocols and composition. Although original protocols have been discussed here as far as possible for their historical interest (Figure 5), we emphasize that companies continue to update and refine their protocols in line with latest advances and technology.

This narrative review has demonstrated the significant contribution of APCs to the field of regenerative medicine in recent years. It is clear that APCs will continue to play an increasingly pivotal role in improving patient outcomes following surgical intervention. Further research exploring clinical and patient-centered outcomes in this patient cohort would be most welcome.

REFERENCES

1. Kingsley CS. Blood coagulation; evidence of an antagonist to factor VI in platelet-rich human plasma. *Nature*. 1954;173:723-724.
2. Mozzati M, Martinasso G, Pol R, et al. The impact of plasma rich in growth factors on clinical and biological factors involved in healing processes after third molar extraction. *J Biomed Mater Res A*. 2010;95:741-746.
3. Matras H. The use of fibrin sealant in oral and maxillofacial surgery. *J Oral Maxillofac Surg*. 1982;40:617-622.
4. Tayapongsak P, O'Brien DA, Monteiro CB, Arceo-Diaz LY. Autologous fibrin adhesive in mandibular reconstruction with particulate cancellous bone and marrow. *J Oral Maxillofac Surg*. 1994;52:161-166.
5. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *J Oral Maxillofac Surg*. 1997;55:1294-1299.
6. Wilson SM, Pell P, Donegan EA. HIV-1 transmission following the use of cryoprecipitated fibrinogen as gel/adhesive. *Transfusion*. 1991;31:51S.
7. Yadav S, Storrie B. The cellular basis of platelet secretion: emerging structure/function relationships. *Platelets*. 2017;28:108-118.
8. Ogundipe OK, Ugboko VI, Owotade FJ. Can autologous platelet-rich plasma gel enhance healing after surgical extraction of mandibular third molars? *J Oral Maxillofac Surg*. 2011;69:2305-2310.
9. Gremmel T, Frelinger AL 3rd, Michelson AD. Platelet physiology. *Semin Thromb Hemost*. 2016;42:191-204.
10. de Gaetano G, Cerletti C. Platelet adhesion and aggregation and fibrin formation in flowing blood: a historical contribution by Giulio Bizzozero. *Platelets*. 2002;13:85-89.
11. Anitua E, Sánchez M, Orive G, Andía I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. *Biomaterials*. 2007;28:4551-4560.
12. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998;85:638-646.
13. Nishiyama K, Okudera T, Watanabe T, et al. Basic characteristics of plasma rich in growth factors (PRGF): blood cell components and biological effects. *Clin Exp Dent Res*. 2016;2:96-103.
14. Anitua E, Troya M, Zalduendo M, Orive G. Effects of antiaggregant, anti-inflammatory and anti-coagulant drug consumption on the preparation and therapeutic potential of plasma rich in growth factors (PRGF). *Growth Factors*. 2015;33:57-64.
15. Anitua E, Murias-Freijo A, Alkhraisat MH, Orive G. Clinical, radiographical, and histological outcomes of plasma rich in growth factors in extraction socket: a randomized controlled clinical trial. *Clin Oral Investig*. 2015;19:589-600.
16. Miron RJ, Choukroun J, eds. *Platelet Rich Fibrin in Regenerative Dentistry: Biological Background and Clinical Indications*, Hoboken, NJ: Wiley-Blackwell; 2017.
17. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101:e37-e44.
18. Anitua E, Sánchez M, Nurden AT, Nurden P, Orive G, Andía I. New insights into and novel applications for platelet-rich fibrin therapies. *Trends Biotechnol*. 2006;24:227-234.
19. Anitua E, Zalduendo M, Troya M, Padilla S, Orive G. Leukocyte inclusion within a platelet rich plasma-derived fibrin scaffold stimulates a more pro-inflammatory environment and alters fibrin properties. *PLoS One*. 2015;10:e0121713.
20. Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. *Clin Oral Implants Res*. 2006;17:212-219.
21. Anitua E, Alonso R, Girbau C, Aguirre JJ, Muruzabal F, Orive G. Antibacterial effect of plasma rich in growth factors (PRGF®-Endoret®) against *Staphylococcus aureus* and *Staphylococcus epidermidis* strains. *Clin Exp Dermatol*. 2012;37:652-657.
22. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev*. 2001;14:244-269.
23. Bielecki TM, Gazdzik TS, Arendt J, Szczepanski T, Król W, Wielkoszynski T. Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances: an in vitro study. *J Bone Joint Surg Br*. 2007;89:417-420.
24. Anitua E, Aguirre JJ, Algorta J, et al. Effectiveness of autologous preparation rich in growth factors for the treatment of chronic cutaneous ulcers. *J Biomed Mater Res B*. 2007;84:415-421.
25. Pinto NR, Ubilla M, Zamora Y, Del Rio V, Dohan Ehrenfest DM, Quirynen M. Leucocyte- and platelet-rich fibrin (L-PRF) as a regenerative medicine strategy for the treatment of refractory leg ulcers: a prospective cohort study. *Platelets*. 2018;29:468-475.
26. Sánchez M, Anitua E, Azofra J, Andía I, Padilla S, Mujika I. Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. *Am J Sports Med*. 2007;35:245-251.
27. Virchenko O, Aspenberg P. How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay

between early regeneration and mechanical stimulation. *Acta Orthop*. 2006;77:806-812.
28. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *Am J Sports Med*. 2006;34:1774-1778.
29. Sánchez M, Anitua E, Orive G, Mujika I, Andia I. Platelet-rich therapies in the treatment of orthopaedic sport injuries. *Sports Med*. 2009;39:345-354.
30. Alio JL, Rodriguez AE, Ferreira-Oliveira R, Wróbel-Dudzińska D, Abdelghany AA. Treatment of dry eye disease with autologous platelet-rich plasma: a prospective, interventional, non-randomized study. *Ophthalmol Ther*. 2017;6:285-293.
31. Bailey E, Kashbour W, Shah N, Worthington HV, Renton TF, Coulthard P. Surgical techniques for the removal of mandibular wisdom teeth. *Cochrane Database Syst Rev*. 2020;7:Cd004345.
32. Del Fabbro M, Bortolin M, Taschieri S. Is autologous platelet concentrate beneficial for post-extraction socket healing? A systematic review. *Int J Oral Maxillofac Surg*. 2011;40:891-900.
33. Moraschini V, Barboza ES. Effect of autologous platelet concentrates for alveolar socket preservation: a systematic review. *Int J Oral Maxillofac Surg*. 2015;44:632-641.
34. Del Fabbro M, Bucchi C, Lolato A, Corbella S, Testori T, Taschieri S. Healing of postextraction sockets preserved with autologous platelet concentrates: a systematic review and meta-analysis. *J Oral Maxillofac Surg*. 2017;75:1601-1615.
35. Del Fabbro M, Panda S, Taschieri S. Adjunctive use of plasma rich in growth factors for improving alveolar socket healing: a systematic review. *J Evid Based Dent Pract*. 2019;19:166-176.
36. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofac Implants*. 1999;14:529-535.
37. Anitua E, Flores J, Alkhraisat MH. Transcrestal sinus lift using platelet concentrates in association to short implant placement: a retrospective study of augmented bone height remodeling. *Clin Implant Dent Relat Res*. 2016;18:993-1002.
38. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. *J Oral Maxillofac Surg*. 2003;61:1115-1117.
39. Scoletta M, Arata V, Arduino PG, et al. Tooth extractions in intravenous bisphosphonate-treated patients: a refined protocol. *J Oral Maxillofac Surg*. 2013;71:994-999.
40. Mozzati M, Gallesio G, Arata V, Pol R, Scoletta M. Platelet-rich therapies in the treatment of intravenous bisphosphonate-related osteonecrosis of the jaw: a report of 32 cases. *Oral Oncol*. 2012;48:469-474.
41. Bilimoria R, Young H, Patel D, Kwok J. The role of piezoelectric surgery and platelet-rich fibrin in treatment of ORN and MRONJ: a clinical case series. *Oral Surg*. 2018;11:136-143.
42. Glavina A, Vucicevic Boras V, Gabric D, Susic M, Granic M, Pelivan I. Plasma rich in growth factors in dentistry. *Australas Med J*. 2017;10:497-501.
43. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol*. 2009;27:158-167.
44. Miron RJ, Fujioka-Kobayashi M, Hernandez M, et al. Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry? *Clin Oral Investig*. 2017;21:2619-2627.
45. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. *Clin Oral Investig*. 2016;20:2353-2360.
46. Miron RJ, Chai J, Fujioka-Kobayashi M, Sculean A, Zhang Y. Evaluation of 24 protocols for the production of platelet-rich fibrin. *BMC Oral Health*. 2020;20:310.
47. PRP – putting the record straight. *Br Dent J*. 2018;225:1085. https://www.nature.com/articles/sj.bdj.2018.1141#citeas.

Reprint requests:

Laura O'Sullivan, BDS, MFDS, RCSEd, PDTLHE
Department of Oral Surgery
Cork University Dental School and Hospital
Wilton Cork
Republic of Ireland
T12 E8YV.
laura.osullivan@ucc.ie