Platelet-rich plasma and its derivatives as promising bioactive materials for regenerative medicine: basic principles and concepts underlying recent advances

Article *in* Odontology · June 2015

DOI: 10.1007/s10266-015-0209-2

1 author:

Tomoyuki Kawase
Niigata University
99 PUBLICATIONS **1,919** CITATIONS

SEE PROFILE

Odontology (2015) 103:126–135
DOI 10.1007/s10266-015-0209-2

REVIEW ARTICLE

Platelet-rich plasma and its derivatives as promising bioactive materials for regenerative medicine: basic principles and concepts underlying recent advances

Tomoyuki Kawase[1,2]

Received: 23 January 2015 / Accepted: 16 May 2015 / Published online: 4 June 2015

Abstract Over the past decade, platelet-rich plasma (PRP), a platelet-concentrated plasma fraction, has been widely investigated and applied to regenerative medicine. The clinical utility of PRP is supported by evidence that PRP contains high concentrations of platelet-related growth factors and normal concentrations of plasma-derived fibrinogen, both of which contribute synergistically to the regenerative process. Additionally, its superior cost-efficacy versus conventional therapies is attractive to many clinicians. However, current disadvantages of PRP include a relatively complicated preparation procedure and variable operator-dependent efficacy. An additional disadvantage is the use of bovine thrombin, an animal-derived biological, as a coagulant. Many of these disadvantages are overcome by recent advances in preparation procedures and devices; for example, Joseph Choukroun simplified the platelet-rich fibrin preparation procedure and improved handling efficiency without the aid of animal-derived factors. With advancements in cell processing technology, there has been a general shift in cell therapy from autologous to allogeneic treatment; however, autologous PRP therapy will not easily be replaced by allogeneic treatment in the near future. Therefore, to provide more predictable regenerative therapy outcomes using autologous PRP, further investigations should address developing a standardized procedure for PRP preparation to augment its efficacy and potency, independent of donor variability. We would then propose that operators and clinicians prepare PRP according to the standardized protocol and to carefully evaluate the clinical scenario (i.e., recipient factors comprising skeletal defects) to determine which factor(s) should be added to PRP preparations. This careful approach will lead to improved clinical outcomes for patients.

Keywords Platelet-rich plasma · Platelet-rich fibrin · Quality control · Standardization · Regenerative medicine

Introduction

Platelet-rich plasma (PRP) is a platelet-concentrated plasma fraction. As demonstrated in previous studies [1–6], growth factors derived from platelets are present and concentrated in PRP preparations (Table 1). By contrast, growth factors derived from other cell lineages, such as colony-stimulating factors and hepatocyte-growth factors, as well as plasma-derived components such as albumin and fibrinogen, are not as concentrated. Recently, PRP has been increasingly used for the regeneration and reconstruction of skeletal and connective tissues in the periodontal and maxillofacial fields [7, 8]. In this review article, we present recent advances in the development and modification of platelet-derived biomaterials and discuss their future use by focusing on a standardized preparation of these platelet-derived biomaterials.

Historical background

Advances in platelet-derived biomaterials for regenerative medicine are illustrated in chronological order in Fig. 1. As a biomaterial, PRP was first applied as a "*glue*" in surgical

✉ Tomoyuki Kawase
kawase@dent.niigata-u.ac.jp

[1] Division of Oral Bioengineering, Institute of Medicine and Dentistry, Niigata University, Niigata 951-8514, Japan

[2] Advanced Research Center, The Nippon Dental University School of Life Dentistry at Niigata, Niigata 951-8580, Japan

Table 1 Major growth factors and cytokines contained in platelet-rich plasma

Category	Factor	Biological function
Growth factors	PDGF	Stimulate cellular growth, proliferation, healing, and cellular differentiation through regulation of a variety of cellular processes
	TGF-β	
	IGF-I	
	VEGF	
	EGF	
Angiogenic factors	VEGF	Exert a fundamental role in the process of blood vessel formation
Pro-inflammatory cytokines	IL-1	Promote systemic inflammation
	IL-6	
	TNF-α	
Other factors	Serotonin	Influence the biological aspects of wound healing
	Histamine	
	Dopamine	
	Calcium	
	Adenosine	

PDGF platelet-derived growth factor, *TGF-β* transforming growth factor-β, *IGF-I* insulin-like growth factor-I, *VEGF* vascular endothelial growth factor, *EGF* epithelial growth factor, *IL-1* interleukin-1, *IL-6* interleukin-6, *TNF-α* tumor necrosis factor-α

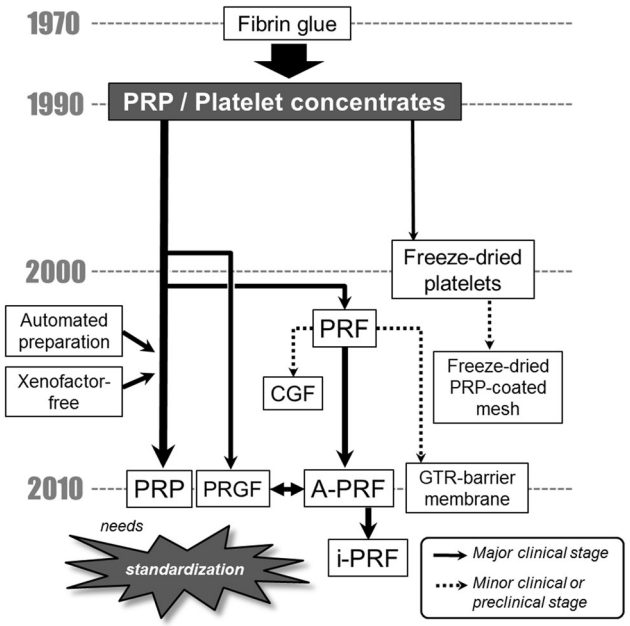

Fig. 1 A chronological table illustrating recent advances in the development of PRP and its derivatives. Note 1: The protocols for preparation of PRP and PRGF are modified by individual clinicians based on the scientific evidence available at the time. Therefore, protocols for preparation of PRP and PRGF should be standardized to allow for appropriate comparisons of clinical data between international laboratories/investigators. Note 2: PRGF is characterized by the elimination of leukocytes to suppress their pro-inflammatory effect, whereas leukocytes are enriched in A-PRF to exploit their antibacterial and osteoinductive actions. *PRP* platelet-rich plasma, *PRF* platelet-rich fibrin, *CGF* concentrated growth factors, *PRGF* plasma-rich in growth factors, *A-PRF* advanced-platelet-rich fibrin, *i-PRF* injectable-platelet-rich fibrin, *GTR* guided tissue regeneration

operations in the 1970s and is essentially identical to the present-day fibrin glue [9]. Currently, fibrin glue is generally prepared from platelet-poor plasma (PPP) [10]; several different protocols for this preparation now exist; therefore, the end products have been characterized and are distinct from one another. Based on results from earlier reports [9] and its application in surgical fields, fibrin glue is generally considered to have a positive effect on tissue repair and regeneration [11, 12].

From the earliest reports on the efficacy of fibrin glue, almost 30 years elapsed since PRP was identified as a promising reservoir of growth factors, and subsequently, began to be applied in regenerative medicine. Breakthrough studies in the late 1990s and early 2000s found that PRP facilitated skeletal regeneration [13–15]. Based on the theory that multiple growth factors involved in tissue regeneration are highly concentrated in PRP, Marx et al. tested the feasibility of using PRP in alveolar ridge augmentation and proposed PRP to be effective in the field of oral and maxillofacial surgery [16].

Due to the inherent liquid-form of PRP, preparations are converted to a gel-form prior to clinical use. This conversion was achieved by adding bovine thrombin to PRP preparations to minimize the rapid diffusion of growth factors at the site of application. This procedure continues to be a conventional method for clotting as reported in several review articles [17–19]. In 2006, Choukroun and co-workers developed a novel technique for the purpose of eliminating xenofactors [20]. Using this technique, liquid PRP clotting was achieved by stimulating only the endogenous coagulation pathway. As a result, this technique has simplified the PRP preparation

protocol. This so-called "*second generation of PRP*," designated as platelet-rich fibrin (PRF), has been increasingly used as a PRP substitute in regenerative medicine. Other favorable developments will be introduced in the "Innovation surrounding the clinical use of PRP" section.

Major factors in PRP

As described in the "Historical background" section, fibrinogen and fibrin are also components of PRP. Although most studies have focused primarily on growth factors, fibrinogen and its converted form, fibrin, also play key roles in tissue repair and regeneration [21, 22]. We confirmed these functions by in vitro cell culture experiments [23]. Fibrinogen and fibrin, in conjunction with growth factors, effectively support cell adhesion and proliferation by increasing collagen production.

The mechanism of action may be explained by the specific characteristics of fibrin. Fibrin functions as an "*adhesive*" scaffolding material for adherent cells to concentrate at the site of tissue regeneration in vivo or to increase the number of cells in in vitro studies [22, 23]. Additionally, fibrin functions as an "*adhesive*" carrier for growth factors to control their release and sustain their bioactivity for longer time periods [24, 25].

Importantly, the concentration ratio of fibrinogen to thrombin influences the mechanical and chemical properties of the PRP clot. As thrombin concentration increases, relatively thinner, more tightly packed fibrin strands are formed [25–27]. By contrast, low thrombin concentrations yield clots with thicker fibers, fewer branch points, and larger pores [25]. Fibrin fiber diameter affects the amount of surface area available for cell interactions during platelet activation as well as for cell adhesion in tissue regeneration [28]. Micro-pores composed of thin fibrin fibers that form within clots can function as scaffolds for cell proliferation, migration, and differentiation, as well as for delivery of growth factors. Furthermore, crosslinking between fibrin fibers mechanically stabilizes the architecture of fibrin networks and limits the fibrinolytic activity of plasmin [27]. Therefore, fibrin clots with structural crosslinking would be expected to have greater stability and clot duration against fibrinolysis. Overall, the quality and quantity of fibrin fibers, in addition to growth factors, affect the potency and efficacy of PRP both directly and indirectly in tissue regeneration and repair.

Clinical advantages of PRP

Although the effects of PRP on skeletal regeneration remain unclear and controversial, connective tissue wounds, can be successfully and predictably regenerated or repaired. From a clinical point of view, PRP has several remarkable advantages. The primary advantage of PRP is in its non-stimulative and bioactivity-supporting role. Compared to other synthetic biomaterials, PRP augments physiologic healing or regenerative processes without interfering with their homeostatic balance [9, 29]. For example, when applied to the regeneration or repair of periodontal tissue, PRP does not ankylose teeth, as has been observed in bone morphogenetic protein treatment [30, 31]. In addition, PRP rarely causes complications such as membrane exposure, an unwanted outcome that has been observed in cases using non-biodegradable (Gore-Tex®) or biodegradable barrier membranes [32, 33].

A second advantage of PRP is its outstanding cost to benefit ratio. While some investigators have recently suggested substituting recombinant growth factors for PRP, the cost for a PRP preparation is definitively less expensive than the combination of other biomaterials and recombinant growth factors. If a similar growth factor cocktail is prepared from individual recombinant growth factors, the cost would be at least 10 times higher than that of an equivalent PRP preparation. Recent advances in biotechnology and protein engineering have, to some extent, improved the quality of recombinant growth factors by overcoming barriers of bacterial expression systems such as the lack of post-translational modification mechanisms and limited capacity for disulfide bond formation [34]. However, this does not necessarily imply that all commercially available recombinant growth factors are as efficient or as potent as native growth factors. Therefore, recombinant growth factor cocktails are cost-prohibitive and are not currently ideal substitutes for PRP.

Third, PRP preparations for regeneration and repair procedures can be prepared on-site in a timely manner. PRP preparations do not require factory-like machines or expensive devices. In Japan, a new regenerative medicine law was established in 2014 and has since been enforced (November, 2014). Under this law, PRP preparations should be prepared at a Clean Bench or in a Safety Cabinet. The initial investment for producing PRP preparations, which includes centrifuges, would not exceed 700,000 yen (approximately US$8,400 in December, 2014) at the minimum. Therefore, in our opinion, it would be affordable for most private clinics to invest long term in basic facilities to produce PRP preparations.

A fourth advantage of using autologous PRP is the extremely low risk for infection. In allogeneic PRP, even after appropriate donor screening tests for pathogenic bacteria and viruses, the risk of transmission of unknown infections to recipients remains. In addition, the possibility of allogeneic PRP preparation-induced allergic and anaphylactic reactions cannot be ruled out, as can occur after platelet transfusions [35].

These major advantages support the clinical use of autologous PRP preparations in regeneration and repair processes.

Clinical disadvantages of PRP

The first disadvantage of PRP is the variable preparation quality of the technique-sensitive preparation method. The second disadvantage is the time-consuming nature of the PRP preparation method, which usually requires at least 30 min. These disadvantages may be stressful for some clinicians. The third disadvantage is the continued use of bovine thrombin for clotting the liquid preparation of PRP [17–19]. In this regard, concerns have been raised as to the transmission of unknown infections from animal-derived biologicals, such as bovine thrombin, to PRP recipients [36].

Nevertheless, in an effort to overcome the first two disadvantages (i.e., technique-sensitive quality and time/labor-intensive PRP preparations), automated preparation systems have been newly developed. Furthermore, some of the automated systems are capable of generating autologous thrombin for clotting PRP preparations, which addresses the third concern of animal-derived biologicals. Thus, at present, PRP can be prepared with safer and more user-friendly methods to be used by clinicians in a variety of fields. Subsequent challenges focus on expanding the clinical applications of PRP by highlighting particular characteristics of PRP. These issues will be discussed further in the following sections.

Innovation surrounding the clinical use of PRP

To overcome its disadvantages and to enhance the performance of PRP, several research groups and manufacturers have attempted to develop novel devices to modify PRP preparations.

Automated preparation

Many improvements and modifications have reduced the time-consuming process and frustration associated with on-site preparation of PRP. The development of various automated PRP preparation systems, regarded as a highly successful innovation, has been marketed by several manufacturers: Harvest, 2M Engineering, Thermogenesis, CaridianBCT, and Magellan. However, the purpose of this section is not to evaluate those products. Instead, we suggest that individual users carefully examine the performance and operability of different systems.

Other minor modifications have improved centrifugation and autologous thrombin preparation and have reinforced anti-bacterial action. In the immediate years following the use of PRP by Marx et al. [13, 16], many investigators have attempted to optimize the speed and efficiency at which platelets were collected using a double-centrifugation procedure. In a previous study [1], we reported that the optimal speed for centrifugation was 2400 rpm ($950g$) $\times$ 10 min and 3600 rpm ($2200g$) $\times$ 15 min for the first and second spin, respectively. Importantly, it should be noted that although collecting platelets is faster and more efficient, faster centrifugation speeds beyond an optimum level may over-activate platelets and result in the release and diffusion of growth factors into other fractions [37]. Optimization of these conditions has been considered in automated PRP preparation systems; when choosing manual preparation, individual clinical operators should determine optimal conditions based on the recovery of key growth factors.

To eliminate bovine thrombin from the clotting process, human thrombin, especially autologous thrombin, is a more optimal replacement. In Japan, human thrombin extracted from donor blood is marketed as a hemostatic agent and could be subject to "*off-label use*" for clotting PRP preparations, in a broad sense, under the Medical Practitioners Law or the Dental Practitioners Law. Yet, rather than an allogeneic source, autologous thrombin is highly recommended and preferable. Since Harvest and other manufacturers produce automated thrombin preparation kits along with the PRP preparation systems described above, clinical operators may easily prepare autologous thrombin using those systems. It is also possible to manually prepare autologous thrombin using a separate device. Alternatively, the supernatant of PRF preparations, in which thrombin activity is found, may be used as an autologous thrombin preparation.

Anti-microbial properties

PRP has often been demonstrated to suppress the growth of particular species of bacteria such as *Staphylococcus aureus* [38–40]. In addition, a recent study suggests that PRP may provide an early protection against bacterial contamination during surgical procedures [41]. However, it has also been reported that PRP promotes growth of *Pseudomonas* [39]. In a preliminary animal study [42], we confirmed that PRP has the potential to protect wounds from infection and simultaneously suppress severe inflammatory reactions. Yet, in a preliminary in vitro study using *Escherichia coli* (Kawase et al., unpublished observations), we also determined that the anti-bacterial action of freeze-dried PRP is very weak despite the presence of β-defensin-2, a cysteine-rich cationic low molecular weight anti-microbial peptide [43]. Burnouf et al. recently suggested that the PRP bactericidal or anti-microbial activity

against particular bacterial strains, such as *E. coli, Pseudomonas aeruginosa, Klebsiella pneumonia,* and *S. aureus*, is carried by plasma components rather than by platelets or white blood cells [44]. Therefore, we would caution that although the anti-bacterial action of PRP is favorable, it should not be overestimated or considered comparable in efficacy to antibiotic therapy.

Plasma rich in growth factors (PRGF)

PRGF was developed by Anitua et al. in 1999 [45, 46]. In our opinion, PRGF is considered a minor variation of PRP. However, according to Anitua and colleagues, PRGF can be distinguished from other PRP preparations by its purely autologous and biocompatible formulation obtained through a one-step centrifugation process using sodium citrate as the anticoagulant and calcium chloride as the platelet activator and as the coagulant [47]. Furthermore, PRGF has moderate platelet concentrations and does not contain white blood cells. Therefore, PRGF avoids the potential pro-inflammatory effects of proteases and acid hydrolases, both components of white blood cells [47]. The differences between PRP and PRGF fractions are illustrated in Fig. 2. The clinical significance of these observations has yet to be clearly demonstrated by independent investigators.

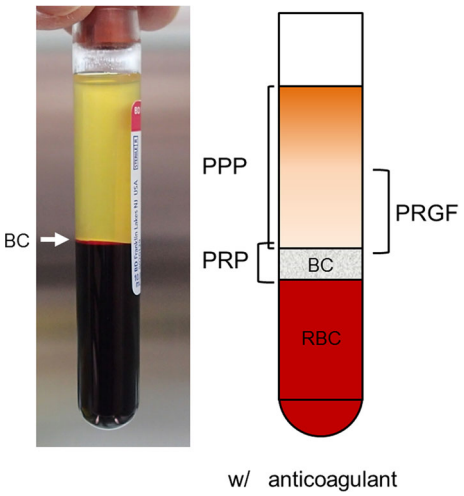

Fig. 2 Differences between platelet-rich plasma and plasma rich in growth factors. *BC* buffy coat, *RBC* red blood cell fraction

PRF

PRF is a fibrin meshwork that contains platelets, white blood cells, serum, and concentrated growth factors [20]. The macroscopic (A) and scanning electron micrograph (SEM) (B) composition of PRF are shown in Fig. 3. As described in preceding sections, the primary advantage of PRF is its simple preparation protocol that does not require anti-coagulants or coagulants, reduces the number of centrifugation steps, and eliminates the process of fractionation. Thus, this preparation protocol allows clinical operators to avoid more time-consuming and technique-sensitive fractionation processes and also reduces patient waiting time. For these reasons, PRF has been increasingly utilized around the world.

In the case of PRP, individual-based variation in effectiveness is explained by differences in the preparation procedure as well as by performance-based variability between individual operators. In contrast, the "*de facto standard*" procedure of PRF preparation minimizes the variability incurred by centrifuge speed and operator skill, and enables operators to provide PRF preparations of consistent quality to patients. Therefore, compared to PRP preparations, it is theoretically possible to compare the

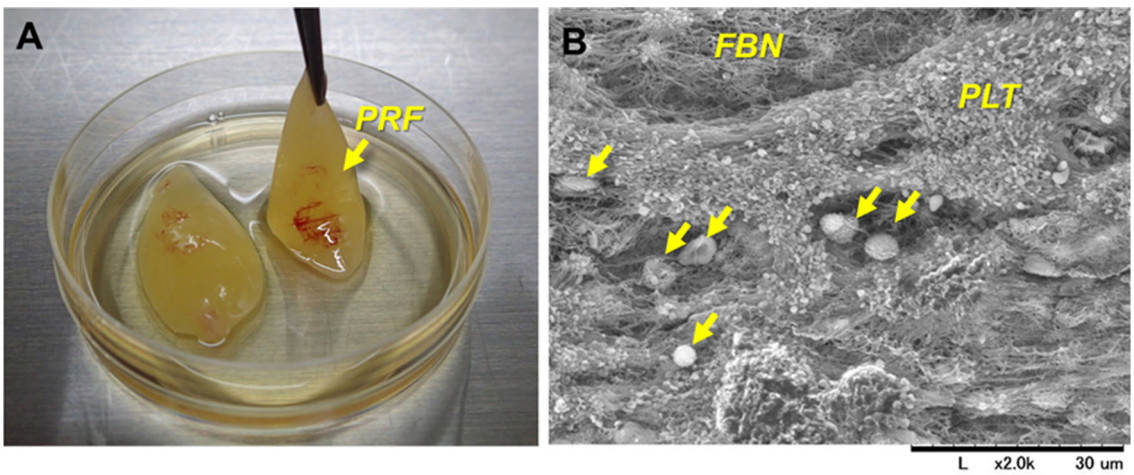

Fig. 3 Macroscopic (**a**) and SEM (**b**) observations of platelet-rich fibrin. *Arrows* represent leukocytes. *FBN* fibrin fibers, *PLT* platelets

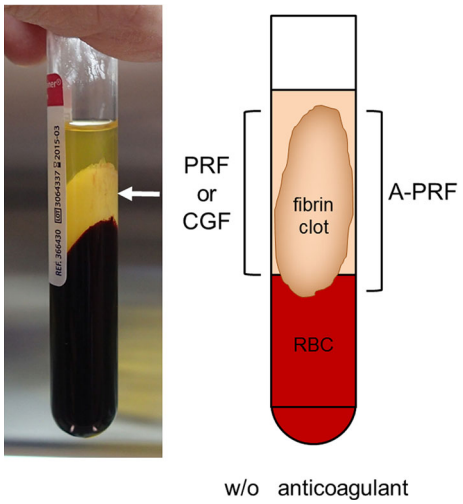

Fig. 4 Differences between platelet-rich fibrin/concentrated growth factors and advanced-platelet-rich fibrin

clinical effectiveness of PRF preparations of similar caliber that are obtained from different hospitals and clinics.

Choukroun's group and others have theorized and further modified PRF to produce A-PRF (leukocyte-enriched, advanced type), i-PRF (injectable type), and CGF (concentrated growth factors) [48–50]. Although the preparation of CGF adopts a seamless two-step centrifuge speed control, CGF is almost identical to PRF. The differences between PRF/CGF and A-PRF fractions are shown in Fig. 4. Both the A-PRF and i-PRF preparations are characterized by platelets, leukocytes, circulating stem cells, and endothelial cells concentrated in the fibrin clot [48]. By reducing the centrifuge speed, leukocyte infiltration into the red blood cell fraction is minimized. Furthermore, the red blood cell fraction adjacent to the fibrin clot is not completely eliminated. In fact, Choukroun considered these PRF modifications as "*blood concentrates*" and not "*platelet concentrates*" [48].

Particular leukocytes, such as monocytes, are generally believed to be involved in the anti-bacterial activity of PRP. In contrast to Anitua's suggestion that leukocytes should be eliminated from PRP [47], Choukroun notes the beneficial effects of monocytes on bone growth, vascularization, and the production of vascular endothelial growth factor (VEGF). Therefore, it is implied that these PRF modifications have enhanced bactericidal and skeletal regenerative properties due to a greater number of monocytes and endothelial cells. The clinical advantages of these PRF modifications have yet to be verified by independent investigators.

Freeze-dried PRP

In order to preserve and enhance the stability of PRP and its derivatives, freeze-drying technology has been explored in the literature. In 2001, Wolkers et al. first developed freeze-dried platelets for the treatment of bleeding problems [51]. By adding trehalose, platelets could be preserved for longer periods of time (presumably for months) without significant damage. The innovative prehydration process in freeze-drying procedures and the rehydration process for clinical application enabled the return of membrane and protein components to activity levels that are remarkably similar to those of fresh platelets [52]. These biomaterials are now utilized in battlefield clinics for the treatment of injured soldiers [53]. To build on this innovation, Pietramaggiori et al. demonstrated that carrier-free, freeze-dried human PRP facilitated wound healing without severe side effects in a chronic wound model in diabetic mice [54, 55].

To improve the handling efficiency of PRP preparations in regenerative therapy, we recently developed hybrid biomaterials by combining PRP and biodegradable polyglactin 910 mesh or collagen sponge [42, 56]. This hybrid material is expected to allow clinicians to avoid time-consuming and technique-sensitive procedures for on-site PRP preparation and to facilitate more rapid response to emergency use in various surgical fields, including periodontal and oral surgeries. This material can be cut to an appropriate size and adapted to the site of injury. In vitro and preclinical animal studies [42, 56] demonstrated that this hybrid material stimulates cell proliferation and facilitates wound healing even after a month-long preservation.

It is generally believed that PRP should be prepared and applied immediately after drawing blood from the patient. This is based on the principle that autologous PRP is safer in that it eliminates disease transmission and immunogenicity, which may be more likely to occur using non-autologous PRP. In addition to the well-known biological and medical advantages of immediate preparation and application, the economical advantage is that neither freezers nor other facilities are required for preservation. However, we emphasize that freeze-dried PRP preparations from a selected allogeneic source, under strictly performed quality control, can be a better alternative to freshly prepared autologous PRP. We expect that freeze-drying technology will further expand the clinical application of PRP in the future.

PRF barrier membrane

It has been a challenge to fabricate a barrier membrane using PRF. Guided tissue regeneration (GTR) therapy protects the space for periodontal tissue regeneration from epithelial down-growth using barrier membranes [57]. These membranes are classified by their biodegradability, and most are essentially bio-inert, except for collagen

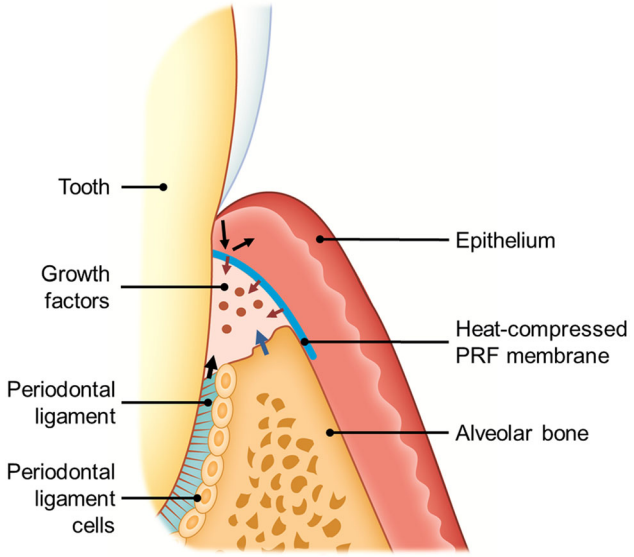

Fig. 5 Scheme of proposed functions of the heat-compressed platelet-rich fibrin membrane in guided tissue regeneration therapy

membranes [58, 59]. However, if a barrier membrane were bioactive and facilitated wound healing, it would be a convenient replacement for conventional treatment that combines the use of growth factors, scaffolds, and spacers.

A promising candidate for use as a barrier membrane is a heat-compressed PRF membrane [60]. Compressed PRF membranes biodegrade at the site of implantation within approximately 10 days. However, GTR membranes are expected to function for at least 4–8 weeks or longer. To increase PRF membrane stability, we recently developed a heat-compression technique that is expected to reduce the surface area and increase cross-linkage between fibrin fibers within PRF preparations [60]. As expected, this heat-compressed material was more resistant to biodegradation. In addition, this material facilitated fibroblast and/or myofibroblast proliferation as well as collagen deposition. These results suggest that the heat-compressed PRF membrane functions not only as a barrier membrane but also as a carrier of growth factors to facilitate wound healing. When applied to GTR therapies, we propose that this material will facilitate skeletal regeneration as illustrated in Fig. 5.

Platelet lysate

Platelet lysate has recently been developed as a substitute for fetal bovine serum (FBS) in the expansion of stem cells [61–63]. Platelet lysate is prepared by freeze-thawing, centrifugation, and filtration of platelet concentrates. The resulting extracts contain high levels of the growth factors stored in the platelets. Compared to FBS, the advantage of platelet lysate is the effect of immature stem cell expansion without inducing maturation. Therefore, in a sense, platelet lysate does not directly contribute to regenerative therapy but rather directly supports cell processing prior to stem cell therapy.

Several manufacturers have developed and marketed platelet lysates for laboratory use only (not for clinical use). Currently, a kit for the preparation of platelet lysate is also commercially available. In a clinical setting, even if prepared from allogeneic sources, platelet lysate may be useful to minimize immunological rejection of allogeneic platelets and leukocytes. In addition, if fibrin clot formation is not favorable, platelet lysate may be used as an alternative means, in combination with appropriate carrier materials or polymer compounds such as polyethylene glycol (PEG) and gelatin hydrogel [64, 65], in regenerative therapies.

Recipient factors that influence clinical outcomes of platelet-rich plasma therapy

The most critical factor affecting the success of PRP and its derivatives in regenerative therapy is the quality of PRP preparations. As described in preceding sections, preparation quality can be improved with standardized preparation procedures and well-trained operators or automated preparation systems. Nonetheless, it is essentially impossible to significantly augment the efficacy and potency of individual PRP preparations.

In regard to recipient factors, the conditions of alveolar bone defects vary with individual patients. Specific factors found in alveolar bone defects are important for achieving successful therapy using PRP preparations. According to the gold triangle standards for tissue engineering [66], stem cells (or appropriate progenitor cells), scaffolds, and growth factors continuously and cooperatively function to build tissues and organs during in vitro cell processing. This principle can also be applied to tissue regeneration in vivo. For example, if the injury site does not contain sufficient numbers of vitalized mesenchymal stem cells (or osteoblastic progenitor cells), a PRP preparation may not effectively facilitate alveolar bone regeneration.

In addition to stem cells, scaffolds, and growth factors, we believe that "*vascularization*" should be included as the fourth factor in the gold standard for tissue regeneration in vivo (Fig. 6). For example, if neovascularization is not promoted at the injury site, stem cells will not be effective in skeletal regeneration in response to the scaffolds and growth factors provided by PRP. Therefore, we suggest that clinicians carefully evaluate whether individual clinical cases are suitable for applications of PRP by focusing on stem/progenitor cells and vascular endothelial cells,

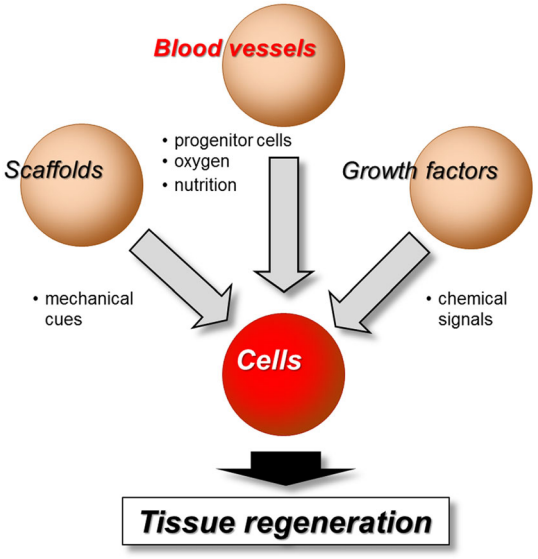

Fig. 6 Four major factors (cells, scaffolds, growth factors, blood vessels) involved in tissue regeneration

neither of which are significantly provided by PRP preparations.

Future perspective: autologous or allogeneic PRP preparations?

The optimization of individual- and sample-dependent efficacy and potency of PRP preparations remains to be addressed in future investigations. These issues are common for all biologicals. From a pharmaceutical perspective, perhaps a more effective approach would be to collect and pool highly bioactive PRP preparations obtained from selected donors. This approach is classified as an "*allogeneic cell therapy*." Using this approach, although appropriate quality control minimizes the risk of disease transmission and other adverse effects, a longer time period is required to ensure quality results from clinical trials and to obtain approval from the appropriate regulatory agencies. Perhaps, more importantly, these processes would certainly increase the cost of PRP products at the industrial production level. However, to reduce the total cost of cell processing, cell therapy is now shifting from autologous to allogeneic use. Therefore, clinicians may need to consider the use of allogeneic PRP products in future regenerative therapies.

Alternatively, it is theoretically possible to use PRP, prepared on-site from selected healthy donors, directly in non-donor patient recipients. Although recent advances in preservation technology have expanded the applicability of PRP, thus increasing the therapeutic uses of PRP [42, 51, 54, 56], to our knowledge, it is practically impossible to find suitable on-site donors and to ensure the safety of individual samples in a timely manner in a clinical setting. However, if on-site preparation and immediate use of allogeneic PRP is pursued, well-trained operators and clinicians should perform the preparation and quality control of PRP preparations according to guidelines or standardized protocols for both medical and economic reasons. Such protocols are not yet standardized internationally, but individual research groups have recently proposed various standardized protocols for the preparation of autologous PRP [6, 28, 37]. Additionally, the International Cellular Medical Society has released guidelines for the use of PRP [67]. This current trend implies that there is an increasing need for international standardized protocols for operators/clinicians and their societies. Therefore, these protocols and guidelines will hopefully be expanded to the preparation of allogeneic PRP and established in the near future.

Concluding remarks and our proposal

As described in the previous section, there are many future perspectives on the preparation and use of PRP. However, we believe autologous use will continue to be the conventional method in the near future, as autologous preparation remains the safest and most economical choice for both patients and clinicians. In order to minimize individual-based variations in efficacy and potency, it is necessary to have standardized protocols to produce the most clinically efficacious preparations. In some cases, it may be necessary to eliminate inappropriate blood samples (i.e., patient donors). Furthermore, to optimize clinical results, we propose the rigorous determination of any insufficient recipient factors at the site of regeneration/repair (i.e., donors in autologous therapy) and the delivery of these insufficient factors (e.g., calcium phosphate bone substitutes or stem/progenitor cells), together with platelet-derived biomaterials, to these sites. The careful preparation and quality control of PRP, in combination with the rigorous evaluation of recipient implantation sites, will lead to improved clinical outcomes for patients.

Acknowledgments The author expresses special thanks to Prof. Kazuhiro Okuda (Department of Periodontology, Institute of Medicine and Dentistry, Niigata University), Prof. Takaaki Tanaka (Department of Materials Science and Technology, Niigata University), and Prof. Larry F. Wolff (Department of Periodontology, University of Minnesota School of Dentistry) for their helpful comments on this article. This project was financially supported by a Grant-in-Aid for scientific research from JSPS KAKENHI Grant Numbers 24390443 and 24390465.

Conflict of interest The author declares no competing financial interests.

References

1. Okuda K, Kawase T, Momose M, et al. Platelet-rich plasma contains high levels of platelet-derived growth factor and transforming growth factor-beta and modulates the proliferation of periodontally related cells in vitro. J Periodontol. 2003;74:849–57.
2. Frechette JP, Martineau I, Gagnon G. Platelet-rich plasmas: growth factor content and roles in wound healing. J Dent Res. 2005;84:434–9.
3. Weibrich G, Kleis WK, Hafner G. Growth factor levels in the platelet-rich plasma produced by 2 different methods: curasan-type PRP kit versus PCCS PRP system. Int J Oral Maxillofac Implants. 2002;17:184–90.
4. El-Sharkawy H, Kantarci A, Deady J, et al. Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. J Periodontol. 2007;78:661–9.
5. Zhang N, Wu YP, Qian SJ, et al. Research progress in the mechanism of effect of PRP in bone deficiency healing. Sci World J. 2013;2013:134582.
6. Amable P, Carias RB, Teixeira MV, et al. Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. Stem Cell Res Ther. 2013;4:67.
7. Alsousou J, Ali A, Willett K, Harrison P. The role of platelet-rich plasma in tissue regeneration. Platelets. 2013;24:173–82.
8. Redler LH, Thompson SA, Hsu SH, Ahmad CS, Levine WN. Platelet-rich plasma therapy: a systematic literature review and evidence for clinical use. Physician Sports Med. 2011;39:42–51.
9. Naik B, Karunakar P, Jayadev M, Marshal VR. Role of platelet rich fibrin in wound healing: a critical review. J Conserv Dent. 2013;16:284–93.
10. Burnouf T, Goubran HA, Chen TM, et al. Blood-derived biomaterials and platelet growth factors in regenerative medicine. Blood Rev. 2013;27:77–89.
11. Stuart JD, Morgan RF, Kenney JG. Single-donor fibrin glue for hand burns. Ann Plast Surg. 1990;24:524–7.
12. Currie LJ, Sharpe JR, Martin R. The use of fibrin glue in skin grafts and tissue-engineered skin replacements: a review. Plast Reconstr Surg. 2001;108:1713–26.
13. Marx RE, Carlson ER, Eichstaedt RM, et al. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology. 1998;85:638–46.
14. Kassolis JD, Rosen PS, Reynolds MA. Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: case series. J Periodontol. 2000;71:1654–61.
15. Robiony M, Polini F, Costa F, Politi M. Osteogenesis distraction and platelet-rich plasma for bone restoration of the severely atrophic mandible: preliminary results. J Oral Maxillofac Surg. 2002;60:630–5.
16. Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg. 2004;62:489–96.
17. Alsousou J, Thompson M, Hulley P, Noble A, Willett K. The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery: a review of the literature. J Bone Joint Surg Br. 2009;91:987–96.
18. Davis VL, Abukabda AB, Radio NM, et al. Platelet-rich preparations to improve healing. Part II: platelet activation and enrichment, leukocyte inclusion, and other selection criteria. J Oral Implantol. 2014;40:511–21.
19. Lee KS, Wilson JJ, Rabago DP, et al. Musculoskeletal applications of platelet-rich plasma: fad or future? Am J Roentgenol. 2011;196:628–36.
20. 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 Endodontology. 2006;101:e37–44.
21. Clark RA. Fibrin and wound healing. Ann N Y Acad Sci. 2001;936:355–67.
22. Xie X, Zhang C, Tuan RS. Biology of platelet-rich plasma and its clinical application in cartilage repair. Arthritis Res Ther. 2014;16:204.
23. Kawase T, Okuda K, Wolff LF, Yoshie H. Platelet-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro. J Periodontol. 2003;74:858–64.
24. Kobayashi M, Kawase T, Horimizu M, et al. A proposed protocol for the standardized preparation of PRF membranes for clinical use. Biologicals. 2012;40:323–9.
25. Janmey PA, Winer JP, Weisel JW. Fibrin gels and their clinical and bioengineering applications. J R Soc Interface. 2009;6:1–10.
26. Ryan EA, Mockros LF, Weisel JW, Lorand L. Structural origins of fibrin clot rheology. Biophys J. 1999;77:2813–26.
27. Wolberg AS. Thrombin generation and fibrin clot structure. Blood Rev. 2007;21:131–42.
28. Perez AG, Rodrigues AA, Luzo AC, et al. Fibrin network architectures in pure platelet-rich plasma as characterized by fiber radius and correlated with clotting time. J Mater Sci Mater Med. 2014;25:1967–77.
29. Hamid MS, Yusof A, Mohamed Ali AR. Platelet-rich plasma (PRP) for acute muscle injury: a systematic review. PLoS One. 2014;9:e90538.
30. Wikesjo UM, Sorensen RG, Kinoshita A, Jian Li X, Wozney JM. Periodontal repair in dogs: effect of recombinant human bone morphogenetic protein-12 (rhBMP-12) on regeneration of alveolar bone and periodontal attachment. J Clin Periodontol. 2004;31:662–70.
31. Selvig KA, Sorensen RG, Wozney JM, Wikesjo UM. Bone repair following recombinant human bone morphogenetic protein-2 stimulated periodontal regeneration. J Periodontol. 2002;73:1020–9.
32. Murphy KG. Postoperative healing complications associated with Gore-Tex periodontal material. Part I. Incidence and characterization. Int J Periodontics Restor Dent. 1995;15:363–75.
33. Hitti RA, Kerns DG. Guided bone regeneration in the oral cavity: a review. Open Pathol J. 2011;5:33–45.
34. Kamionka M. Engineering of therapeutic proteins production in *Escherichia coli*. Curr Pharm Biotechnol. 2011;12:268–74.
35. Kiefel V. Reactions induced by platelet transfusions. Transfus Med Hemother. 2008;35:354–8.
36. Webb CA. Platelet-rich plasma update: clinical use in musculoskeletal care. http://www.rheumatologynetwork.com/articles/platelet-rich-plasma-update-clinical-use-musculoskeletal-care 2012. Accessed 23 Feb 2015.
37. Marques LF, Stessuk T, Camargo IC, et al. Platelet-rich plasma (PRP): methodological aspects and clinical applications. Platelets. 2015;26:101–13.
38. Intravia J, Allen DA, Durant TJ, et al. In vitro evaluation of the anti-bacterial effect of two preparations of platelet rich plasma compared with cefazolin and whole blood. Muscles, Ligaments Tendons J. 2014;4:79–84.
39. Sutter WW. PRP: indications for intra-articular use. https://www.acvs.org/files/proceedings/2011/data/papers/041.pdf. Accessed date 12 Dec 2014.
40. Wu X, Ren J, Yuan Y, et al. Antimicrobial properties of single-donor-derived, platelet-leukocyte fibrin for fistula occlusion: an in vitro study. Platelets. 2013;24:632–6.
41. Mariani E, Filardo G, Canella V, et al. Platelet-rich plasma affects bacterial growth in vitro. Cytotherapy. 2014;16:1294–304.

42. Nakajima Y, Kawase T, Kobayashi M, et al. Bioactivity of freeze-dried platelet-rich plasma in an adsorbed form on a biodegradable polymer material. Platelets. 2012;23:594–603.
43. Tohidnezhad M, Varoga D, Wruck CJ, et al. Platelets display potent antimicrobial activity and release human beta-defensin 2. Platelets. 2012;23:217–23.
44. Burnouf T, Chou ML, Wu YW, Su CY, Lee LW. Antimicrobial activity of platelet (PLT)-poor plasma, PLT-rich plasma, PLT gel, and solvent/detergent-treated PLT lysate biomaterials against wound bacteria. Transfusion. 2013;53:138–46.
45. 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;14:529–35.
46. Anitua E. The use of plasma-rich growth factors (PRGF) in oral surgery. Pract Proced Aesthet Dent. 2001;13:487–93.
47. Anitua E, Sanchez M, Orive G. The importance of understanding what is platelet-rich growth factor (PRGF) and what is not. J Shoulder Elb Surg. 2011;20:e23–4 **e4**.
48. Choukroun J. Advanced PRF &i-PRF: platelet concentrates or blood concentrates? J Periodont Med Clin Pract. 2014;1:3.
49. Ghanaati S, Booms P, Orlowska A, et al. Advanced platelet-rich fibrin (A-PRF)—a new concept for cell-based tissue engineering by means of inflammatory cells. J Oral Implantol. 2014;40(6):679.
50. Rodella LF, Favero G, Boninsegna R, et al. Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. Microsc Res Tech. 2011;74:772–7.
51. Wolkers WF, Walker NJ, Tablin F, Crowe JH. Human platelets loaded with trehalose survive freeze-drying. Cryobiology. 2001;42:79–87.
52. Crowe JH, Tablin F, Wolkers WF, et al. Stabilization of membranes in human platelets freeze-dried with trehalose. Chem Phys Lipids. 2003;122:41–52.
53. Brumfiel G. Cell biology: just add water. Nature. 2004;428:14–5.
54. Pietramaggiori G, Kaipainen A, Czeczuga JM, Wagner CT, Orgill DP. Freeze-dried platelet-rich plasma shows beneficial healing properties in chronic wounds. Wound Repair Regen. 2006;14:573–80.
55. Sum R, Hager S, Pietramaggiori G, et al. Wound-healing properties of trehalose-stabilized freeze-dried outdated platelets. Transfusion. 2007;47:672–9.
56. Horimizu M, Kawase T, Nakajima Y, et al. An improved freeze-dried PRP-coated biodegradable material suitable for connective tissue regenerative therapy. Cryobiology. 2013;66:223–32.
57. Wolff LF. Guided tissue regeneration in periodontal therapy. Northwest Dent. 2000;79(23–8):40.
58. Bottino MC, Thomas V, Schmidt G, et al. Recent advances in the development of GTR/GBR membranes for periodontal regeneration–a materials perspective. Dent Mater. 2012;28:703–21.
59. Stoecklin-Wasmer C, Rutjes AW, da Costa BR, et al. Absorbable collagen membranes for periodontal regeneration: a systematic review. J Dent Res. 2013;92:773–81.
60. Kawase T, Kamiya M, Kobayashi M, et al. The heat-compression technique for the conversion of platelet-rich fibrin preparation to a barrier membrane with a reduced rate of biodegradation. J Biomed Mater Res B Appl Biomater. 2015;103:825–31.
61. Bieback K. Platelet lysate as replacement for fetal bovine serum in mesenchymal stromal cell cultures. Transfus Med Hemother. 2013;40:326–35.
62. Shih DT, Burnouf T. Preparation, quality criteria, and properties of human blood platelet lysate supplements for ex vivo stem cell expansion. New Biotechnol. 2015;32:199–211.
63. Rubio-Azpeitia E, Andia I. Partnership between platelet-rich plasma and mesenchymal stem cells: in vitro experience. Muscles Ligaments Tendons J. 2014;4:52–62.
64. Robinson SN, Talmadge JE. Sustained release of growth factors. In Vivo (Athens, Greece). 2002;16:535–40.
65. Yamamoto M, Ikada Y, Tabata Y. Controlled release of growth factors based on biodegradation of gelatin hydrogel. J Biomater Sci Polym Ed. 2001;12:77–88.
66. Kawase T, Okuda K, Nagata M, Yoshie H. The cell-multilayered periosteal sheet—a promising osteogenic and osteoinductive grafting material. New Trends Tissue Eng Regen Med. Prof. Hideharu Hibi (Ed.). 2014:19–35.
67. Harmon K, Hanson R, Bowen J, et al. Guidelines for the use of platelet rich plasma. http://www.cellmedicinesociety.org/attachments/206_ICMS%20-%20Guidelines%20for%20the%20use%20of%20Platelet%20Rich%20Plasma%20-%20Draft.pdf. Accessed date 12 Dec 2014.