# Chapter 2
# Platelet Rich Fibrin "PRF" and Regenerative Medicine: 'The Low-Speed Concept'

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## 2.1 Introduction

The multidisciplinary field of tissue engineering has tackled a wide variety of medical challenges over the years with the aim to predictably repair, regenerate or restore damaged and diseased tissues [1–4]. Defects frequently encountered are commonly produced by a variety of underlying conditions caused by congenital abnormalities, injury, disease and/or the effects of aging [1–4]. Many strategies have since been adapted to regenerate these tissues. One of (if not the) key component during the regenerative phases during wound healing is the absolute necessary for ingrowth of a vascular blood source capable of supporting and contributing to cellular function and the future development and maintenance of nutrients across this newly created blood supply [5]. Although normal biomaterial and tissue engineered scaffolds are typically avascular by nature, over 15 years ago a series of proposed motifs introduced blood concentrates as a regenerative modality in order to improve the vascular network to obtain successfully regenerated soft or hard tissues where lack of a blood supply was often at the forefront of the defect [5].

Wound healing is a complex biological process that includes the active participation of numerous cell types, a matrix consisting of extracellular matrix as well as soluble factors capable of facilitating regeneration. By nature, these are normal healing events that take place in response to normal tissue injury involving a cascade

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of complex, orderly and elaborate events [6]. Numerous studies have already demonstrated that the delivery of multiple growth factors in a well-controlled manner can enhance bone formation [7–9]. Generally, the events related to wound-healing are divided into four overlapping phases including hemostasis, inflammation, proliferation and remodeling [7–9]. One of the key players during these phases have been platelets, cells that have been shown to be important regulators of hemostasis through vascular and fibrin clot formation [6]. Ongoing studies over the past decades have revealed platelets are the responsible cell-type for the activation and release of important biomolecules including platelet-specific proteins and growth factors including platelet-derived growth factor (PDGF), coagulation factors, adhesion molecules, cytokines/chemokines and angiogenic factors capable of stimulating the proliferation and activation of cells involved in wound healing including fibroblasts, neutrophils, macrophages and mesenchymal stem cells (MSCs) [10]. For these reasons, it was proposed in the 1990s that platelet concentrates could be utilized and centrifuged to reach supra-physiological doses to achieve wound healing and tissue regeneration by facilitating angiogenesis. While numerous studies have previously demonstrated that the delivery of multiple growth factors can enhance new tissue formation, it has since been shown that more importantly blood vessel formation is tightly coupled with tissue regeneration, and that the ideal scenario for tissue regrowth is to deliver a multitude of growth factors designed to induce angiogenesis and tissue regeneration simultaneously in order to produce a vascularized remodelled/regenerated tissue fully vascularized and able to sustain itself long-term. Leading to the science behind platelet concentrates, a group of research begun to investigate platelet concentrates for tissue wound healing and regeneration in medicine beginning in the 1990s.

### *2.1.1 Brief History of Platelet Concentrates*

Although recently the use of platelet concentrates have gained tremendous momentum as a regenerative autologous source of growth factors utilized in various field of medicine (especially due to the more recent development of platelet rich fibrin (PRF)), it is important to note that their utilization spans over two decades in surgery [11]. It was originally proposed that leading to their preparation, a belief that concentrated platelets derived from autologous sources could be collected in plasma solutions later to be utilized in surgical sites could potentially release supra-physiological doses of growth factors capable of promoting local healing [12, 13]. Further work in the 1990s led to the popular working name ‘platelet rich plasma’ (PRP) which was introduced in the 1990s in dental medicine [14–16]. Since the goal of PRP was to collect the largest and highest quantities of growth factors from platelets, PRP was fabricated with a protocol lasting over 30 min of centrifugation cycles and requiring the use of anticoagulants to prevent clotting. The final composition of PRP contains over 95% platelets, known cells responsible for the active

secretion of growth factors involved in initiating wound healing of various cell types including osteoblasts, epithelial cells and connective tissue cells [14, 17].

Following a few years of use with PRP, several limitations were observed. Since the technique and the preparation required the additional use of bovine thrombin or $CaCl_2$ in addition to coagulation factors, it was found that these drastically reduced the healing process during the regenerative phase. Furthermore, the entire protocol was technique sensitive with several separation phases lasting sometimes upwards of 1 h making it inefficient for everyday medical purposes. Since PRP is liquid in nature, it was originally required as an agent to be combined with various other biomaterials, most notably bone grafting materials. Interestingly, very recent data from our laboratories has shown that growth factor release with PRP is released very early in the delivery phase whereas a preference would be to deliver growth factors over an extended period of time during the entire regenerative phase as opposed to a quick short burst [18–20]. All these limitations have led to the emergence of a second generation of platelet concentrates which takes advantage of the fact that without anti-coagulants, a fibrin matrix that incorporates the full set of growth factors trapped within its matrix and slowly released over time could be achieved [21]. Furthermore, PRF (which was later renamed leukocyte PRF or L-PRF) contains white blood cells, which have been shown to be key contributors to wound healing later described in this chapter.

### *2.1.2 From PRP to PRF*

Due to the reported limitations of PRP mainly derived from anti-coagulant incorporation, further research led by Dr. Joseph Choukroun in the early 2000s was focused at developing a second-generation platelet concentrate without utilizing anti-coagulation factors [22]. As such, a platelet concentrate lacking coagulation factors could be harvested from the upper layer of centrifugation tubes following single centrifugation cycles of 12 min at 2700 rpm (750 g). This formulation was termed platelet rich fibrin (PRF) owing to the fact it contained a fibrin matrix following centrifugation [23–26]. PRF (leukocyte-PRF or L-PRF) additionally contains white blood cells (WBCs) within the fibrin matrix; necessary cells involved in the wound healing process by improving defense immunity and secreting a large quantity of growth factors (Fig. 2.1) [27–32]. It's interesting to note that since WBCs are a combination of neutrophils and macrophages, they are always one of the first cell-types found in wounded infection sites as well as the first cell types in contact with biomaterials and thus play a major role in phagocytizing debris, microbes and necrotic tissue as well as directing the future regeneration of these tissues through release of cytokines and growth factors. As depicted in Fig. 2.2, macrophages areone of the three key cells found in PRF derived from the myeloid lineage (WBCs) and secrete a wide range of growth factors including transforming growth factor beta (TGF-beta), PDGF and vascular endothelial growth factor (VEGF) (Fig. 2.1). These cells, in combination with neutrophils and platelets, are

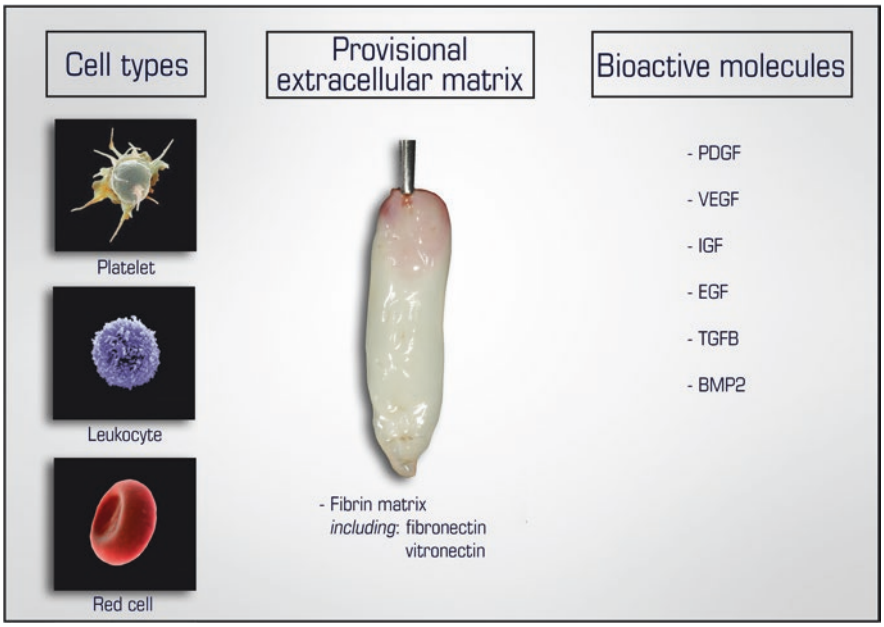


**Fig. 2.1** Natural components of PRF include (1) cell types (platelets, leukocytes and red blood cells), (2) a provisional extracellular matrix three-dimensional scaffold fabricated from autologous fibrin (including fibronectin and vitronectin) as well as (3) a wide array of over 100 bioactive molecules including most notably PDGF, VEGF, IGF, EGF, TGF-beta and BMP2 (reprinted with permission from Miron et al. 2016)

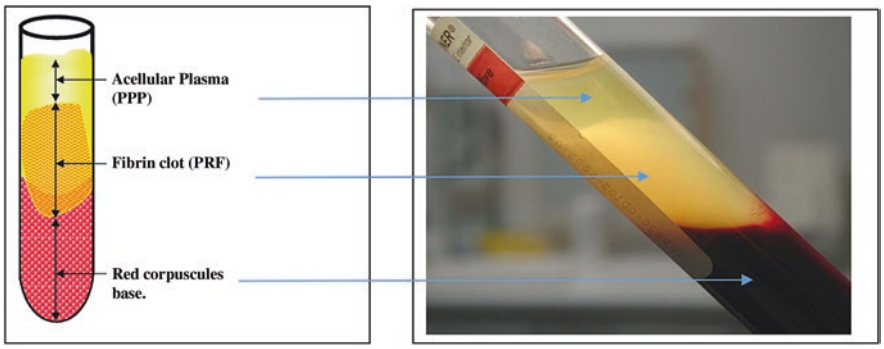


**Fig. 2.2** Fibrin clot in the tube after centrifugation

the main players in tissue wound healing and together (as opposed to solely with platelets in PRP) are able to further enhance new blood vessel formation (angiogenesis) which subsequently leads to new bone and tissue formation [23–26, 29]. To date, numerous studies have investigated the regenerative potential of PRF in various medical situations. With respect to tissue engineering, it has long been

proposed that in order to maximize the regenerative potential of various bioactive scaffolds, three components are essential to improve tissue repair including (1) a three-dimensional matrix capable of supporting tissue ingrowth, (2) locally harvested cells capable of influencing tissue growth and (3) bioactive growth factors capable of enhancing cell recruitment and differentiation within the biomaterial surface. With respect to PRF, all three of these properties are met whereby (1) fibrin serves as the scaffold surface material, (2) cells including leukocytes, macrophages, neutrophils and platelets attract and recruit future regenerative cells to the defect sites and (3) fibrin serves as a reservoir of growth factors that may be released over time from 10 to 14 days. Below we summarize these three components in sections and explain the rational of each.

1. Major Cell Types in PRF

A. Platelets

Platelets are one of the cornerstone cells found in PRF and the cells that were first collected in previous versions of platelet concentrates including PRP. Interestingly, in PRF, platelets are theoretically trapped massively within the fibrin network and their three-dimensional mesh allowing their slow and gradual release and associated growth factors over time [20]. Recent research has shown that blood alone is enough to drastically improve wound angiogenesis and tissue regeneration [33].

Platelets are constantly being formed in the bone marrow from megakaryocytes. They are discoidal and anuclear structures by nature and their lifespan is typically in the range of 8–10 days. Their cytoplasm contains many granules whose contents are secreted at the time of activation. Alpha-granules contain many proteins, both platelet specific (such as b-thromboglobulin) and non-platelet specific (fibronectin, thrombospondin, fibrinogen, and other factors of coagulation, growth promoters, fibrinolysis inhibitors, immunoglobulins, etc.) that have been shown to possess many functions during wound healing [34, 35]. Moreover, the platelet membrane is a phospholipid double layer into which receptors for many molecules are inserted (collagen, thrombin, etc.) and act to improve wound healing. Activation is fundamental to initiate and support haemostasis because of aggregation on the injured site and interactions with various coagulation mechanisms [34, 35].

B. Leukocytes

Leukocytes are the other major cell type found in PRF playing a prominent role in wound healing. Interestingly, the major difference between PRF (which has since been renamed L-PRF specifically due to its high leukocyte content) apart from the fact anti-coagulants are not utilized in PRF, is the fact that both PRP and PRGF (first generation platelet concentrates) either do not or contain very low numbers of leukocytes. The literature dealing with platelet concentrates often ignores the impact of leucocytes on tissue wound healing. Several studies have already pointed out the key role of leucocytes, both for their anti-infectious action and immune regulation [36–38]. Apart from their anti-infectious effect, leucocytes produce large amounts

of VEGF and PDGF amongst other growth factors. Additional VEGF, which stems from leucocytes, might be crucially important for the promotion of angiogenesis. The amount of white cells in PRF has been determined at around 50% and newer formulations of PRF have further shown ways to collect a higher number of leukocytes.

Interestingly, studies from basic sciences have revealed the potent and large impact of leukocytes on tissue regeneration [30, 32]. They additionally release growth factors and play a large role in immune defense, but also serve as key regulators controlling the ability for biomaterials to adapt to new environments. For instance, studies conducted following extraction of third molars has shown that a tenfold decrease in third molar osteomyelitis infections was detected simply by placing PRF scaffolds into extraction sockets [39]. Furthermore, in a separate study, patients receiving PRF report having less pain and requiring less analgesics when compared to control, most notably due to the defense of these immune cells preventing infection, promoting wound closure and naturally reducing swelling and pain felt by these patients [40].

Recent research has further shown that macrophages (derived from the white blood cell lineage with leukocytes) are the necessary driving force for new bone formation [41–45]. It has been shown that in certain in vitro culture conditions with osteoblasts, removal of macrophages led to a 23-fold decrease in osteoblast mineralization, drastically and convincingly demonstrating the pronounced impact of macrophages and WBCs in bone biology [43]. Furthermore, it has been shown that monocytes and macrophages are one of, if not the most important cell type during biomaterial integration into host tissues [46]. Therefore, the influence of leukocytes derived from PRF matrixes should not be under-estimated as numerous basic and animal studies have recently pointed to their vast importance in wound healing and tissue regeneration and long-term integration.

## 2. Platelet Rich Fibrin–PRF: A Natural Fibrin Matrix and Its Biological Properties

While PRF was first developed in France by Choukroun et al. in 2001 [22]. The lack of an anticoagulant made it so that the fibrin clot begins to form during the centrifugation process and when centrifugation tubes are removed, a fibrin clot can be observed as depicted in Fig. 2.2. Naturally this technology requires a centrifuge and a collection system present within the office since anti-coagulants are not utilized, clotting forms rapidly. Therefore, centrifugation must take place within seconds after blood harvesting. The original PRF protocol was first established with a very simple protocol: A blood sample is taken without anticoagulant in 10-mL tubes which is immediately centrifuged at 750 g for 12 min. The absence of anticoagulant implies the activation in a few minutes of most platelets of the blood sample in contact with the tube walls and the release of the coagulation cascades. Fibrinogen is initially concentrated in the upper layer of the tube, before the circulating thrombin transforms it into fibrin. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom of the tube and the acellular plasma at the top (PPP) (Fig. 2.2).

As previously mentioned, the success of this technique entirely depends on the speed of blood collection and its subsequent transfer to the centrifuge. Indeed, without anticoagulants, the blood samples start to coagulate almost immediately upon contact with the tube glass, and it takes a minimum of a few minutes of centrifugation to concentrate fibrinogen in the middle and upper part of the tube. Quick handling is the only way to obtain a clinically usable PRF matrix. If the duration required to collect blood and launch centrifugation is overly long, failure will occur. By driving out the fluids trapped in the fibrin matrix, practitioners will obtain very resistant autologous fibrin membranes.

## 2.2 What Is Fibrin?

Fibrin is the activated form of a plasmatic molecule called fibrinogen. This soluble fibrillary molecule is massively present both in plasma and in the platelet alpha-granules and plays a determining role in platelet aggregation during haemostasis. It is transformed into what resembles a biological glue capable of consolidating the initial platelet cluster, thus constituting a protective wall during coagulation. In fact, fibrinogen is the final substrate of all coagulation reactions. Being a soluble protein, fibrinogen is transformed into an insoluble fibrin by thrombin while the polymerized fibrin gel constitutes the first healing matrix of the injured site [47]. Studies from basic science have also pointed to the fact that fibrin alone (fabricated from various sources) is able to act as a provisional matrix allowing cell invasion and tissue regeneration [48–50].

3. Cytokines

Cytokines and growth factors have been observed released in high number from platelet alpha granules after clotting. They are active through specific cell receptors and play a predominant role in wound healing. One interesting finding that was recently discovered later described in this chapter is the effect of centrifugation times and speeds on growth factor release from PRF clots, most likely as a result in a higher number of leukocytes and more loosely dense PRF clot allowing better growth factor release from the PRF matrix over time. Below we describe the most commonly reported growth factors found in PRF.

– **TGFb-1:** Transforming growth factor b (TGFb) is a vast superfamily of more than 30 members known as fibrosis agents [51, 52]. The reference molecule from the TGFb superfamily is TGFb-1. In vitro research has demonstrated its effects are extremely variable according to the amount applied, the matrix environment and cell type in which applied. For example, it has been shown that it could stimulate the proliferation of osteoblasts just as easily as it could cause their inhibition [53]. Although its effects in terms of proliferation are highly variable, for the great majority of cell types, it constitutes the most powerful fibrosis agent among all cytokines and the growth factor commonly released from autogenous

bone during tissue repair and remodeling [52]. In other words, it induces a massive synthesis of matrix molecules such as collagen1 and fibronectin, whether by osteoblasts or fibroblasts. Thus, although its regulation mechanisms are particularly complex, TGFb-1 can be considered as an inflammation regulator through its capacity to induce fibrous cicatrisation.

- **PDGF:** PDGFs (platelet-derived growth factors) are essential regulators for the migration, proliferation, and survival of mesenchymal cell lineages. According to the distribution of their specific receptors, they are able to induce stimulation in these cells. This position of regulation node plays a fundamental role during the embryonic development and all tissue remodelling mechanisms. For this reason, PDGFs play a critical role in the mechanisms of physiologic healing and have been commercially available in a recombinant source (rhPDGF-BB) and FDA approved for the regeneration of various defects in medicine and dentistry. Interestingly, PDGF is naturally produced and accumulated in high quantities in PRF clots and are considered one of the important released molecules over time from PRF.
- **VEGF:** Vascular endothelial growth factor was previously isolated as the most potent growth factor leading to angiogenesis of tissues [54]. It has potent effects on tissue remodelling and the incorporation of VEGF alone into various bone biomaterials have demonstrated increases in new bone formation, thereby pointing to the fast and potent effects of VEGF [54].
- **The IGF axis**: Insulin-like growth factors (IGFs) I and II are positive regulators of proliferation and differentiation for most cell types, which act as cell-protective agents [55]. Although these cytokines are cell proliferative mediators, they also constitute the major axis of programmed cell death (apoptosis) regulation, by inducing survival signals protecting cells from many apoptotic stimuli. Moreover, even though IGFs are released during platelet degranulation, they are initially massively present in blood circulation [55].

***The combination of these three properties including (1) host cells, (2) a three-dimensional fibrin matrix and (3) cytokine and growth factor release from PRF membranes acts to synergistically lead to a fast and potent increase in tissue regeneration.***

## 2.3 Introducing the Low-Speed Concept

It is now more known that the most important factor for stimulation is not the amount of growth factors released but the maintenance of a low and constant gradient of growth factor delivery to the milieu. As the use of PRF has seen a continuous and study increase in regenerative medicine, there was great interest to determine if the clinical situations could be improved by optimizing centrifugation protocols to alter the PRF matrix. This hypothesis was derived from the fact that cells within the original PRF matrix were surprisingly found gathered at the bottom of the PRF

matrix [56]. Therefore it was found that centrifugation speeds (which naturally push cells towards the bottom of centrifugation tubes whereas the PRF is collected from the top one third) would benefit from slower speeds (g-force) to prevent from driving the cells downwards. This hypothesis was confirmed by a classical study by Ghanaati and co-workers whom showed that by decreasing centrifugation speeds from 2700 rpm (750 g) to 1300 rpm (200 g), a more optimal formulation of PRF could be created with a higher number of leukocytes more evenly distributed throughout the PRF matrix [56]. This new formulation of PRF was given the working name Advanced-PRF or A-PRF and is deemed natural evolution from over 13 years of research from the original L-PRF. It is now recognized that evidently the leukocytes were being pushed out of the fibrin clots unnecessarily down to the bottom of centrifugation tubes. More recently, it has further been shown in a recent study published in the Journal of Periodontology (August 2016) that both centrifugation speed and time could be reduced to further enhance growth factor release and cell performance from A-PRF.

One of the primary proposed reasons for a slower release of growth factors over time is the ability for the fibrin matrix to hold proteins within its fibrin network as well contain cells capable of further releasing growth factors into their surrounding micro-environment [57–61]. Therefore, if centrifugation protocols are optimized to contain more cells (most notably leukocytes), then evidently they will subsequently release more growth factors over a 10 day period as well as contribute to tissue defence, and biomaterial integration all factors necessary to further enhance tissue regeneration.

Another interesting observation has been that since centrifugation speeds have been drastically decreased from the first version of L-PRF, it was observed also that a liquid version of PRF could be obtained with even lower centrifugation speeds. This new formulation was given the working name 'Injectable-PRF' or I-PRF due to its hypothesized ability to be injected into defects or be combined with other biomaterials such as bone grafts or barrier membranes (in a similar fashion to PRP however without use of anti-coagulants) further improving tissue regeneration. While ongoing research is continuously underway, this new formulation of I-PRF has been shown to contain an increase in leukocytes and mesenchymal progenitor cells have also been detected utilizing lower centrifugation speeds which have been decreased from 2700 to 700 RPM (750 g to 60 g) for only 3 min. Below we summarize the effects of these two new formulations of 'smart' blood concentrates on leukcocyte number and VEGF growth factor quantity (Fig. 2.3).

1. Advanced Platelet Rich Fibrin: A-PRF

Considerable evidence has been accumulating demonstrating the pronounced and marked impact of white cells on vascularization and bone formation [36]. Furthermore, granulocytes have been shown to play an additional role on vascularization and improve the function of monocytes whom have been described by Soltan et al. to be the so-called "super cells for bone regeneration" [62]. Both cells are found in higher concentrations in A-PRF. Our understanding of the role of g-force on the loss of white cells during the spin cycle guided for new protocols to reduce

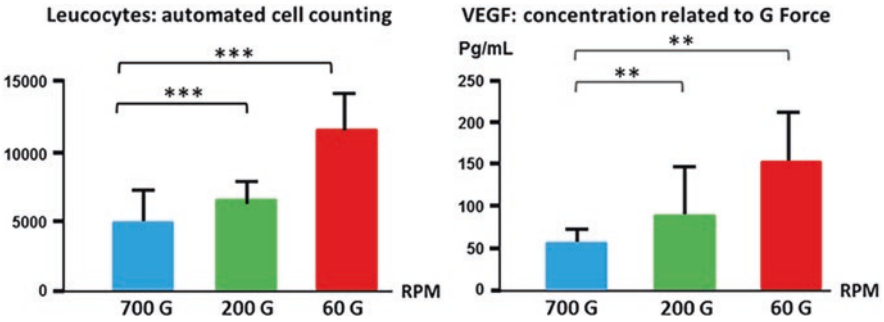


**Fig. 2.3** Higher number of leucocytes and VEGF found in PRF centrifuged at lower g-forces. Figures adapted from (**Choukroun J.** et al. *Injectable Platelet Rich Fibrin: A smart blood concentrate achieved by the low speed concept.* **J.Cell Communication Signaling** *in revision*)

the rpm to maintain a higher amount of white cells in the fibrin matrix. Furthermore, the introduction of a special glass tube that induced a more rapid clotting allowed a marked reduction in centrifugation time from 12 to 14 min down to 8 min, further reducing the lost number of leukocytes from high centrifugations speeds and times. This new fibrin clot is richer in white blood cells (Fig. 2.3), with a fibrin matrix that is less dense allowing the invasion and penetration of incoming cells to repopulate the matrix in an ongoing more rapid process [56]. The newer formulation of PRF (A-PRF+) has been shown to increase growth factor release of TGF-beta1, PDGF-AA, PDGF-AB, PDGF-BB, VEGF, IGF and EGF (Fig. 2.4). Furthermore, it has subsequently been shown that gingival fibroblasts in contact with A-PRF produce higher collagen levels and a significantly higher cell migration towards A-PRF+ was observed when compared to either PRP or L-PRF (Fig. 2.4).

2. Injectable Platelet Rich Fibrin: i-PRF

With the same concept of non-additive platelet derivatives, i-PRF was developed to fulfil the goal of acting as a regenerative agent that could be delivered in liquid formulation by drawing blood rapidly in a specific centrifugation tube at a very low speed of 700 rpm (60 g) for an even shorter centrifugation time (3 min). Here the objective was to centrifuge without anti-coagulants nor additives, yet maintain the ability to separate two layers as depicted in Fig. 2.5. This new formulation can be utilized for a variety of procedures including mixing with bone grafts to form a stable fibrin bone graft for improved handling after a short period of time (1–2 min) which improves graft stability (as can be envisioned during sinus lifting procedures with bone grafting materials to improve graft stability by avoiding the migration of granules into the maxillary cavity). Subsequently, I-PRF alone can be used for a variety of procedures when utilized alone including knee injections for the management of osteoarthritis, temporo-mandibular joint disorders as well as various procedures in facial aesthetics to improve collagen synthesis naturally. The principle for I-PRF remains the same—it contains a larger proportion of leukocytes and blood plasma proteins due to the ‘low-speed concept’; known inducers of vascularization and thus speed the rate at which wound healing can take place.

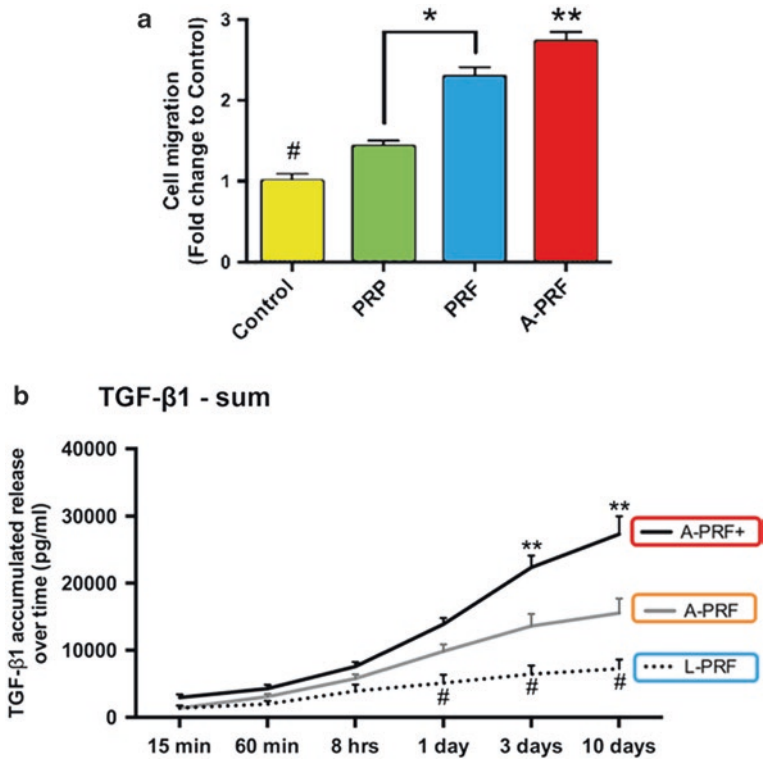


**Fig. 2.4** Higher number of gingival fibroblast cell migration from A-PRF when compared to PRF and PRP as well as higher growth factor released from the slow speed concept. (**a**) The cell migration assay shows a higher gingival fibroblasts migration from A-PRF when compared to PRF and PRP, (**b**) it is observable a higher growth factor (TGF-beta1) release when the slow speed concept is performed. Adapted with permission from Kobayashi et al. [2016]: *Optimized Platelet Rich Fibrin with the Low Speed Concept: Growth Factor Release, Biocompatibility and Cellular Response*. Accepted for publication in **Journal of Periodontology** (not yet online)

## 2.4 Clinical Use of PRF and Indications

The clinical uses of PRF have exploded across many fields of medicine and dentistry over the past 15 years since its original development. Most notably, PRF has had a major impact in soft tissue regeneration as well as various indications in dentistry where PRF can be utilized as a fast and easy procedure to aid in the regeneration of various common bone and soft tissue defects often encountered in daily clinical practice.

Our group recently performed two extensive systematic review articles to elucidate the effects of PRF on (1) soft tissue wound healing and (2) its use in dentistry.

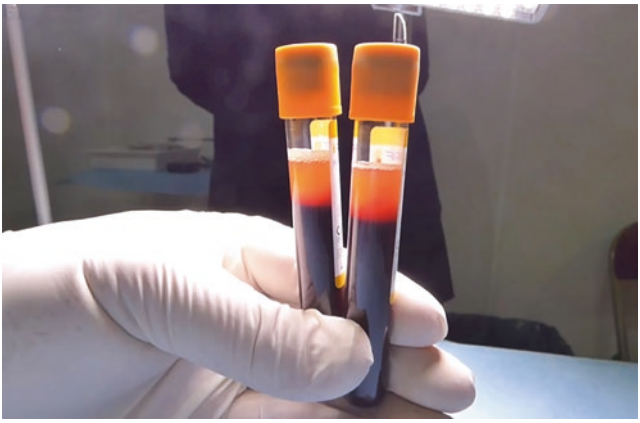

**Fig. 2.5** The newer formulation of I-PRF is a liquid formulation of PRF found in the top 1 mL layer of centrifugation tubes following a 700 rpm spin for 3 min. This liquid can be collected in a syringe and re-injected into defect sites or mixed with biomaterials to improve their bioactive properties

In total 164 articles were screened for soft tissue wound healing and publications were divided into (1) in vitro, (2) in vivo and (3) clinical studies. In summary, it was found that 86% of all included articles found a significant increase in tissue wound healing and regeneration when PRF was used when compared to their respective controls. Most notably however, the use of PRF has remarkably now been utilized in over 20 different clinical procedures in medicine and dentistry; 7 of which coming from the oral and maxillofacial region. In the dental field, the most commonly utilized use of PRF is for the treatment of extraction sockets [39, 63–65], gingival recessions [66–68] and palatal wound closure [69–71] with PRF being additionally utilized for the repair of potentially malignant lesions [72], regeneration of periodontal defects [73], hyperplastic gingival tissues [74] and in conjunction with periodontally accelerated osteogenic orthodontics [75]. In general medicine, the use of PRF has been successfully utilized for hard-to-heal leg ulcers including diabetic foot ulcers, venous leg ulcers and chronic leg ulcers [76–80]. Furthermore, PRF has been utilized for the management of hand ulcers [81], facial soft tissue defects [82], laparoscopic cholecystectomy [83], in plastic surgery for the treatment of deep nasolabial folds, volume-depleted midface regions, facial defects, superficial rhytids and acne scars [84], induction of dermal collagenesis [85], vaginal prolapse repair [86], urethracutaneous fistula repair [87, 88], during lipostructure surgical procedures [89], chronic rotator cuff tears [90] and acute traumatic ear drum perforations [91]. Thus, there is evidently growing use of PRF for the treatment of various medical procedures due to its ability to (1) speed revascularization of defect tissues and (2) to serve as a three-dimensional fibrin matrix capable of further enhancing wound healing.

Furthermore, a second systematic review focused only on the regenerative potential of PRF in dentistry found that of roughly 200 articles that were investigated

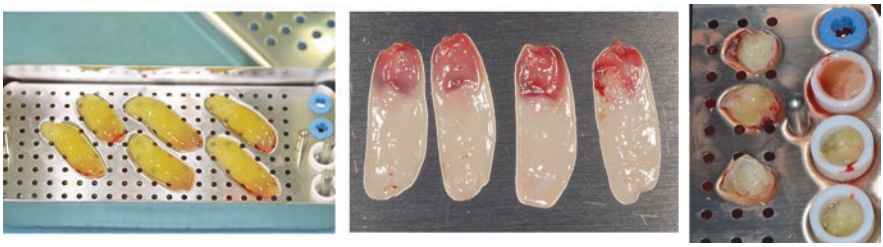

**Fig. 2.6** PRF clots formed to either make membranes or PRF plugs

(only clinical studies), the most commonly utilized uses of PRF were shown to be for (1) guided bone regeneration procedures and extraction socket healing, (2) sinus lift procedures, (3) for the treatment of gingival recessions and (4) for intrabony and furcation defect regeneration. Of all the known clinical applications of PRF, it is known that PRF accelerates tissue cicatrisation due to enhanced neovascularization and ability to defend against an infectious environment found in the oral cavity.

When it comes to soft tissue management and maturation utilizing PRF, three key elements have been encountered. PRF is able to simultaneously support the development of angiogenesis, immunity and epithelial coverage. Fibrin has been shown to act as the natural scaffold guiding angiogenesis which consists of the formation of new blood vessels inside the wound. Thus, the requirement of an extracellular matrix scaffold that allows the migration, division, and phenotypic change of endothelial cells has been clearly demonstrated leading to faster angiogenesis. Furthermore, the angiogenic property of PRF may further be partially explained by the high number of trapped cytokines found within the fibrin mesh. Here a variety of cytokines and ECM proteins have been found within PRF providing structural and functional support for the cells and tissues involved in the regeneration process consisting of several molecules including collagen, proteoglycans, heparin sulfate, chondroitin sulfate, hyaluronic acid, elastin, fibronectin, and laminin. A few plasma-derived proteins such as fibrin, thrombospondin, and fibronectin have also been reported as provisional ECM.

Regarding the clinic use of PRF in daily dental practice, PRF scaffolds may be utilized as both a tissue matrix/scaffold (provisional ECM) with the ability to simultaneously release growth factors over a 10 day period. The clots are prepared in a PRF metallic box which allows the slight compression of their clots into membranes or plugs to be later utilized as depicted in Fig. 2.6.

1. Socket Preservation

The most often utilized application for PRF in dental practice has been in the management of extraction sockets [39, 64, 92, 93]. After extraction, the socket may be filled with PRF plugs as depicted in Fig. 2.7 by utilizing the philosophy “as much as you can” into the extraction socket. Since PRF is a natural matrix including various wound healing cell-types, it provides the ability to increase and speed tissue regeneration. This technique furthermore does not necessitate the use of having to

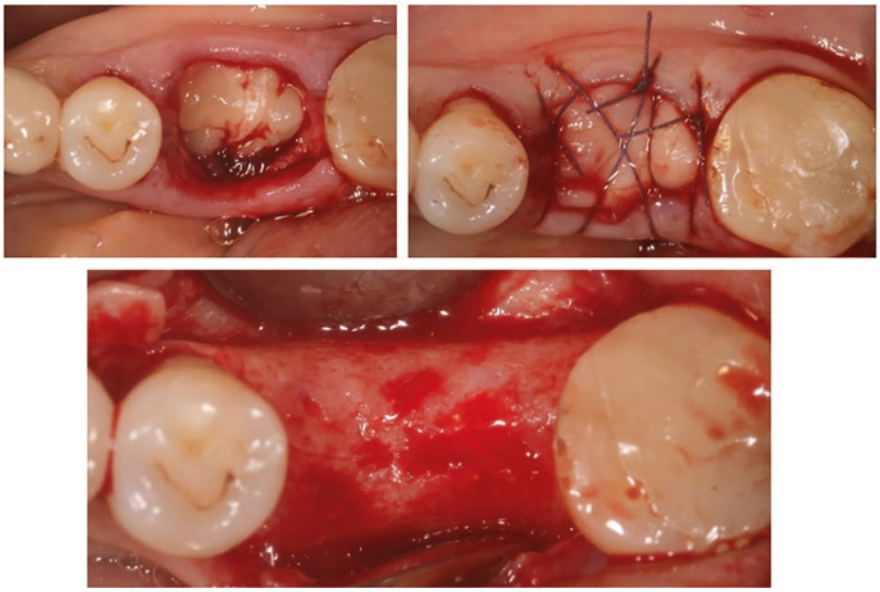

**Fig. 2.7** PRF plugs that have been utilized to fill an extraction socket followed by appropriate suture for PRF stability. After a 3 month healing period, new bone formation taking place prior to implant bed preparation

use another barrier membrane or other biomaterials to cover the flap as the PRF scaffolds may be left exposed. Sutures are simply used for stabilization purposed of the PRF matrix within the socket. Primary closure is not necessary as the material in the socket is fully natural. Over time, the fibrin matrix is transformed into new tissue: bone in the socket and soft tissue at the surface. The healing of the site is completed after 3 months. Further advantages of using PRF for socket preservation is the fact that reports have shown that PRF reduces osteomyelitits infections in third molar extraction sites approximately tenfold and decreases the amount of pain and analgesics taken as reported by patients [39, 64, 92, 93].

2. Sinus Lift

The principle for the use of PRF for sinus lifting is quite the same as for socket preservation, it acts as a provisional matrix of ECM proteins which provide quick vascularization due to its simultaneous incorporation of autologous growth factors. Here, PRF can be utilized alone or mixed with a bone grafting material. In such combination cases, PRF membranes may be cut into small fragments with scissors and mixed with a bone grafting material. However, as in the sockets, PRF is often utilized alone and many reports now point to the fact that PRF can act as a sole grafting material when utilized (1) during sinus lifting procedures with simultaneous implant placement and (2) preferably in narrow sinus [94–96]. Furthermore, PRF may be utilized for the repair of Shneiderian membranes, or to close the maxillary window during lateral sinus lifting procedures (Fig. 2.8).

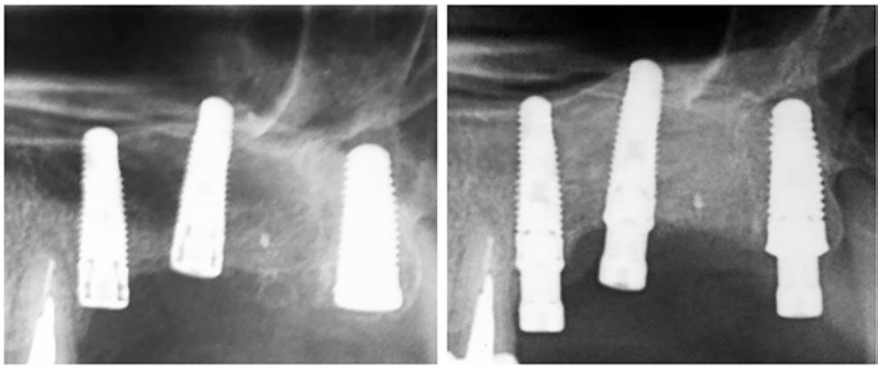

**Fig. 2.8** Implant placement into the sinus in combination with PRF. Notice the new bone formation taking place around the apical portion of implants after a 6 month healing period

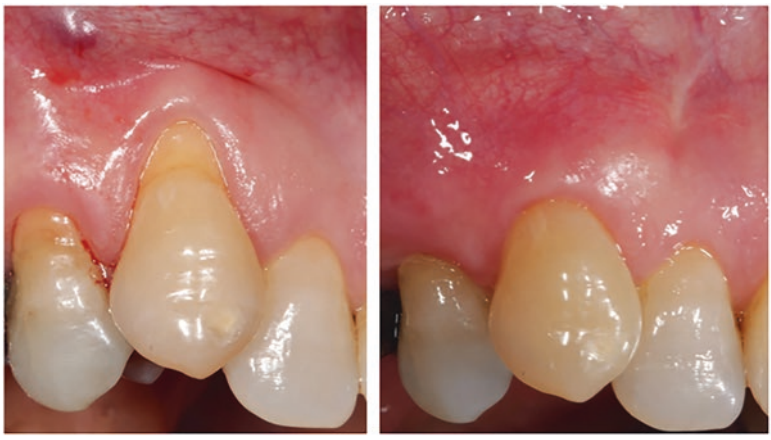

**Fig. 2.9** Gingival recession of upper canine treated with PRF alone. Notice the excellent wound healing properties of PRF following a 6 month healing period with revascularization of the underlying soft tissues

3. Soft Tissue Management: Gingival Recession Regeneration

The treatment of gingival recessions with PRF has also been a highly utilized regenerative procedure used by many periodontists. Over ten clinical studies have now shown that in Miller Class I and II defects, PRF can be utilized as a sole grafting material often carrying the ability to replace connective tissue grafts harvested from the palatal sites [67, 97–108]. Therefore, PRF may be used an alternative graft material for treating multiple adjacent recessions of the gingiva without a requirement of a second surgical site thereby reducing patient morbidity. In such procedures, it has commonly been reported that although PRF has the ability to significantly improve root coverage to similar levels as CTG, one remaining limitation is it does not necessarily improve the thickness of keratinized tissue. Therefore, in clinical situations

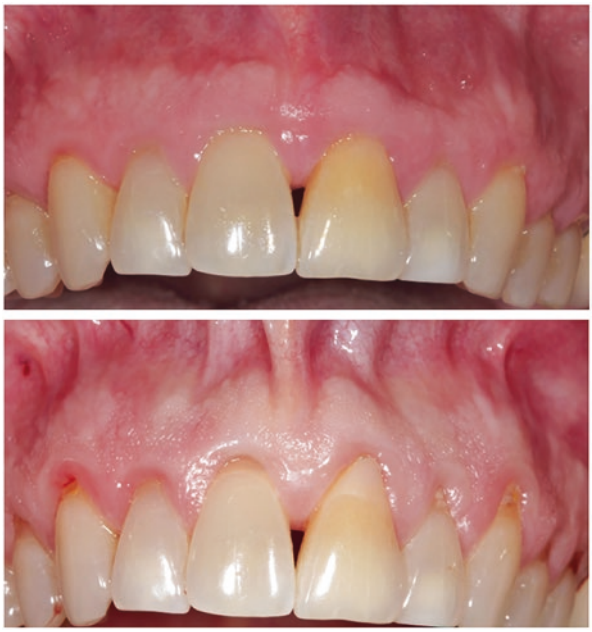

**Fig. 2.10** Multiple gingival recession of upper 8 maxillary teeth treated with PRF alone. Notice the excellent root coverage of all teeth treated with PRF following a 6 month healing period with great keratinized tissue

where keratinized tissue is lacking, PRF may then be combined with a CTG in order to improve tissue thickness while simultaneously improving tissue revascularization and regeneration (Figs. 2.9 and 2.10).

4. Intrabony Defect Regeneration with PRF

Another area of research receiving much attention in recent years has been regarding the use of platelet concentrates for periodontal regeneration of intrabony and furcation [59, 60, 73, 109–116]. As such, PRF alone or combined with bone grafts has also been utilized in a number of clinical studies showing improved results when compared to controls alone. Recent evidence suggests that PRF alone can be utilized for intrabony defect as successfully as various leading bone grafting materials including demineralized freeze-dried bone allografts (DFDBA) [117]. Furthermore, PRF has been shown in three studies to significantly improves the regeneration of Class II furcation defects [118–120].

## 2.5 Conclusion

The use of PRF in regenerative medicine has now seen a huge increase in its use across many fields of medicine due to its ease of use and low-associated costs while providing a completely autologous source of growth factor delivery. Furthermore, recent advancements in our understanding of the regenerative potential of PRF has

further allowed modifications to the centrifugation speeds and times (A-PRF) to further enhance its regenerative potential and bring to clinical practice a liquid formulation that is injectable during use (I-PRF).

After more than 15 years of research and more than 450 publications available in Medline, there continues to be growing evidence and support for its use. Future strategies are continuously being developed to further improve the clinical outcomes following regenerative procedures utilizing platelet concentrates.

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