1

Platelet Rich Fibrin: A Second-Generation Platelet Concentrate

Joseph Choukroun and Richard J. Miron

Abstract

Almost two decades have passed since platelet rich fibrin (PRF) was first introduced. Initially, the primary objective was to develop a therapy where platelet concentrates could be introduced into wounds by effectively utilizing the body's natural healing capacity. This was achieved by collecting growth factors derived from blood in a natural way. Platelet rich plasma (PRP) and platelet rich growth factor (PRGF) had been commercialized, yet both contained secondary byproducts that were both unnatural and known inhibitors of wound healing. By removing these anti-coagulants and modifying centrifugation protocols, PRF was introduced some years later with the potential to markedly impact many fields of medicine including dentistry. Many aspects important for tissue regeneration have since been revealed including the important role of fibrin as well as the preferential release of growth factors over longer periods of time from PRF. Furthermore, by introducing a new set of cells into platelet concentrates (namely leukocytes), a marked impact on tissue regeneration and wound healing was observed. Over the past 5 years, further modifications to centrifugation speed and time have additionally improved PRF into a concept now known as the "low-speed centrifugation concept." Investigators began to modify surgical techniques to favorably treat patients with PRF with improved clinical outcomes. Together, many key opinion leaders from around the globe have been gathered to share their experiences and knowledge in many educational courses and seminars in what we now know as platelet rich fibrin. In this first chapter, we highlight the discovery of PRF and the studies leading to its first use in regenerative medicine. We focus specifically on its properties for wound healing and how its presented advantages over previous versions of platelet concentrates have favorably enhanced the regenerative potential of platelet concentrates in dentistry.

Highlights

- Introduction of Platelet Rich Fibrin
- Reasons for its invention two decades ago
- Its variations from the formally known platelet concentrate "platelet rich plasma" or "PRP"
- The first case treated with PRF
- Properties important for wound healing

1.1 Introduction

Wound healing is a complex biological process where many cellular events taking place simultaneously leading to the repair or regeneration of damaged tissues [1–4]. Many attempts have been made in the field of tissue regeneration with the aim of predictably repairing, regenerating, or restoring

Platelet Rich Fibrin in Regenerative Dentistry: Biological Background and Clinical Indications, First Edition.
Edited by Richard J. Miron and Joseph Choukroun.

damaged and diseased tissues [1–4]. These include strategies with foreign materials often derived from allografts, xenografts, or synthetically produced alloplasts to regenerate host tissues [1–4]. While many of these materials have shown promise in various aspects of regenerative medicine, it is important to note that all create a "foreign body reaction," whereby a foreign material is introduced into human host tissues.

Platelet concentrates collected from whole blood was first introduced over 20 years ago. The concept was developed with the aim of utilizing human blood proteins as a source of growth factors capable of supporting angiogenesis and tissue ingrowth based on the notion that blood supply is a prerequisite for tissue regeneration [5]. Four aspects of wound healing have since been described as key components for the successful regeneration of human tissues (Figure 1.1). These include 1) hemostasis, 2) inflammation, 3) proliferation, and 4) maturation. Each phase encompasses various cell types. One of the main disadvantages of currently utilized biomaterials in the field of tissue engineering is that the great majority are typically avascular by nature, and therefore do not provide the necessary vascular supply to fully obtain successful regeneration of either soft or hard tissues [5].

It must further be noted that in general, wound healing demands the complex interaction of various cell types with a three-dimensional extracellular matrix as well as soluble growth factors capable of facilitating regeneration [6]. Certainly, one area of research in dentistry that has gained tremendous momentum in recent years is that of recombinant growth factors where a number have been used to successfully regenerate either soft or hard tissues [7–9]. Table 1.1 provides a list of currently approved growth factors along with their individual roles in tissue regeneration and clinical indications supporting their use. Similarly, a number of barrier membranes with various functions and resorption properties have also been commonly utilized in regenerative dentistry formulated from either synthetic or animal-derived materials [10]. Lastly, many bone-grafting materials are brought to market every year, all characterized by their specific advantages and disadvantages during tissue regeneration. While each of the above-mentioned biomaterials have been shown to

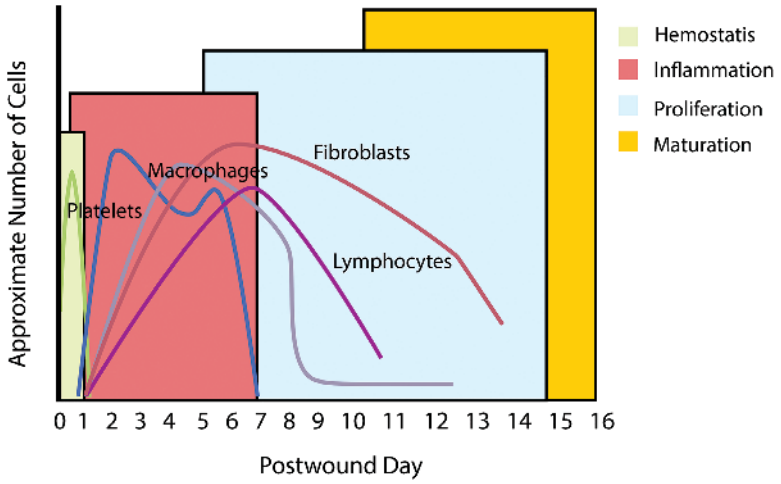

Figure 1.1 Four phases of wound healing including 1) hemostasis, 2) inflammation, 3) proliferation, and 4) maturation. Noteworthy are the overlaps between each of the phases and the population of cells found in each category. Whereas lymphocytes typically arise at 7 days, the ability for PRF to introduce a high number at day 0 acts to speed the regenerative phase during this process.

Table 1.1 List of growth factors used for the regeneration of periodontal intrabony defects with listed advantages and disadvantages.

Growth factor	Advantages	Disadvantages
Enamel Matrix Derivative	– Mimics the formation of root development – Amelogenin proteins improves PDL cell adhesion, proliferation and differentiation – Adsorbs to the root surface up to 4 weeks post-surgery – Histologically demonstrated as "true" periodontal regeneration with formation of Sharpey's fibers	– Gel formulation unable to prevent flap collapse – Adsorption to other materials uncertain
Platelet-Derived Growth Factor	– Growth factor with the strongest potential to recruit progenitor cells – Strong proliferative potential	– Necessitates a carrier system – No specific function in periodontal regeneration
Bone Morphogenetic Proteins	– Growth factor with the strongest potential to regenerate alveolar bone – Also some potential to recruit mesenchymal progenitor cells and induce cell proliferation	– Strong tendency to cause ankylosis – Lack of clinical trials demonstrating any use in periodontal regeneration
Platelet Rich Plasma and Fibrin	– Supernatural concentration of growth factors – Autologous source – Used for a variety of procedures and easily obtainable	– PRP contains anticoagulants – Typically requires the use of a bone grafting material to maintain volume
Growth and Differentiation Factor-5	– Recently demonstrated clinical safety and efficacy – Histologically shown to improve periodontal regeneration	– Less known about its mode of action – Need for more clinical trials demonstrating its validity

carry properties necessary for the repair and regeneration of various tissues found in the oral cavity, very few possess the potential to promote blood supply/angiogenesis directly to damaged tissues.

Wound healing has therefore previously been characterized as a four-stage process with overlapping phases [7–9]. What is noteworthy is the fact that platelets have been described as key components affecting the early phases of tissue regeneration important during hemostasis and fibrin clot formation [6]. Platelets have also been shown to secrete a number of important growth factors including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), coagulation factors, adhesion molecules, cytokines/chemokines, and a variety of other angiogenic factors capable of stimulating the proliferation and activation of cells involved in the wound healing process including fibroblasts, neutrophils, macrophages, and mesenchymal stem cells (MSCs) [11].

Interestingly, in the mid- to late 1990s, two separate strategies were adopted to regenerate human tissues based on these concepts. First, the main growth factor secreted from platelets (PDGF) was commercialized into a recombinant growth factor (rhPDGF-BB). This has since been FDA-approved for the regeneration of numerous tissues in the

human body including intrabony defects in the field of periodontology. A second strategy was proposed around the same time to collect supra-physiological doses of platelets by utilizing centrifugation. Since blood is naturally known to coagulate within minutes, the additional use of anti-coagulants was added to this process to maintain a liquid consistency of blood throughout this procedure. A positive correlation between platelet count and the regenerative phase was therefore observed for tissue wound healing. In fact, it has also been shown that the simple combination of bone grafting materials with blood alone is known to enhance angiogenesis and new bone formation of bone grafts when compared to implanted bone grafts alone that are not pre-coated [12]. Based on these findings, several research groups across many fields of medicine began in the 1990s to study the effects of various platelet concentrates for tissue wound healing by adapting various centrifugation techniques and protocols with the aim of improving tissue regeneration.

1.2 Brief history of platelet concentrates

It is interesting to point out that the use of platelet concentrates have dramatically increased in popularity over the past decade since the discovery of PRF. Despite this, it is important to understand that growth factors derived from blood had been used in medicine for over two decades [13]. These first attempts to use concentrated platelet growth factors was derived from the fact that supra-physiological doses could be obtained from platelets to promote wound healing during and following surgery [14,15]. These concepts were later established into what is now known as "platelet rich plasma" (PRP), which was later introduced in the 1990s in dentistry with leading clinician-scientists such as Whitman and Marx [16,17]. The main goal of PRP was to isolate the highest quantity of platelets and ultimately growth factors associated with their collection and re-use them during surgery. Typical protocols ranged in time from 30 minutes to more than 1 hour based on their respective collection methods. It has been well documented that their formulation contains over 95% platelets; cells having a direct effect on osteoblasts, connective tissue cells, periodontal ligament cells and epithelial cells [18,19].

Despite the growing success and use of PRP in the initial years following its launch, there were several reported limitations that prevented its full potential. The technique itself was lengthy and therefore required the additional use of anti-coagulant factors to prevent clotting using bovine thrombin or CaCl2, both known inhibitors of wound healing. These drawbacks in combination with the lengthy harvesting/centrifugation preparation times were then frequently being utilized in large maxillofacial surgeries, whereas the typical dental or medical practitioner was resistant to its use due to lengthy preparation times.

One of the other drawbacks of PRP was the fact that it was liquid by nature, and therefore required its combination with other biomaterials including bone grafts derived from human cadavers (allografts) or animal products (xenografts), thereby further combining its use with other "unnatural" products. Interestingly, very recent data from within our laboratories has pointed to the quick "burst" release of growth factors from PRP (Figure 1.2) [20]. It has since been suggested that a preferential release of growth factors may be obtained by a more slowly-releasing curve over time as opposed to a quick and short burst as found using PRP [20–22].

In summary, the combination of several of these limitations has forced others to investigate new modalities for successful regeneration. From this perspective, a second-generation platelet concentrate, without the use of anti-coagulants, was therefore developed with shorter preparation times termed platelet rich fibrin (PRF) [23]. During this harvesting procedure, many of the cells

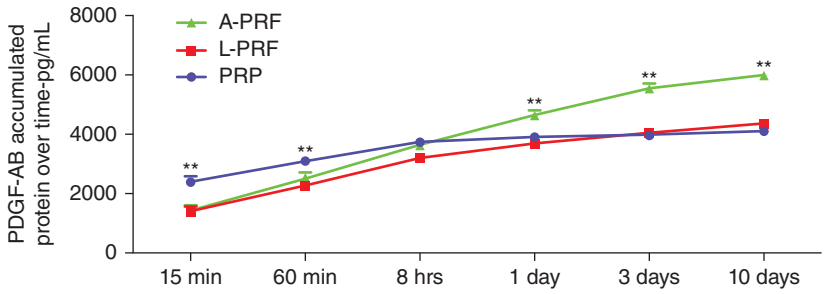

Figure 1.2 Growth factor release of PDGF-AB from A-PRF, L-PRF, and PRP. Notice the initial burst of growth factor increase from PRP; however, after a 10-day period, significantly higher growth factors are released from A-PRF. (** signifies p<0.01). Source: Kobayashi *et al.* 2016 [20]. Reproduced with permission of Springer.

(which now include additional leukocytes) were trapped within the fibrin matrix along with growth factors [24]. PRF (which was later renamed leukocyte PRF or L-PRF due to its additional leukocyte content) contains a variety of cells, which have individually been studied for their role in the regeneration process later described throughout this book.

1.3 The development of PRF from PRP

In the early 2000s, the focus of research in the Pain Clinic in Nice, France was to try and solve blood-flow–related issues to large ulcers often leaving patients with large chronic wounds that potentially resulted in amputation. At the time, certain research groups were suggesting that PRP, which was mainly utilized as a supra-physiological dose of blood-derived growth factors, could enhance wound healing. Despite this, a desire to develop a new platelet concentrate without the use of anti-coagulants (known inhibitors of wound healing) was a primary objective. With these concepts in mind, further research in the early 2000s was undertaken to develop what is now known as a second-generation platelet concentrate without utilizing anti-coagulation factors [23]. The protocol was developed using a simpler centrifugation protocol requiring only 1 cycle of 12 minutes at 2700 rpm (750 g). The original objective was to spin at high centrifugation speeds in order to phase separate the layers between the red corpuscle base and the overlaying clear liquid containing leukocytes and plasma. As no anti-coagulants were utilized, the resultant formulation came with a three-dimensional fibrin scaffold termed PRF [25–27]. PRF has now been highly researched with over 500 publications on its topic, many of which are discussed within this textbook.

Additional research from various groups around the world have since shown the marked impact of white blood cells found within the fibrin matrix and their involvement in the wound healing process. For these reasons, an improved defense to foreign pathogens has been observed when surgery is performed with PRF leading to the more favorable clinical results resulting in lower infection rates [28–33]. Additionally, macrophages and neutrophils contained within PRF are naturally one of the first cells found within infected wounds. For these reasons, the use of PRF during surgery increases their numbers at the initial stages of healing thereby playing a central role in the phagocytosis of debris, microbes and necrotic tissues, as well as directing the future regeneration of these tissues through release of cytokines and growth factors.

Three main components of PRF have been noted as being key components assisting in tissue regeneration. As illustrated in Figure 1.3, PRF not only contains host cells,

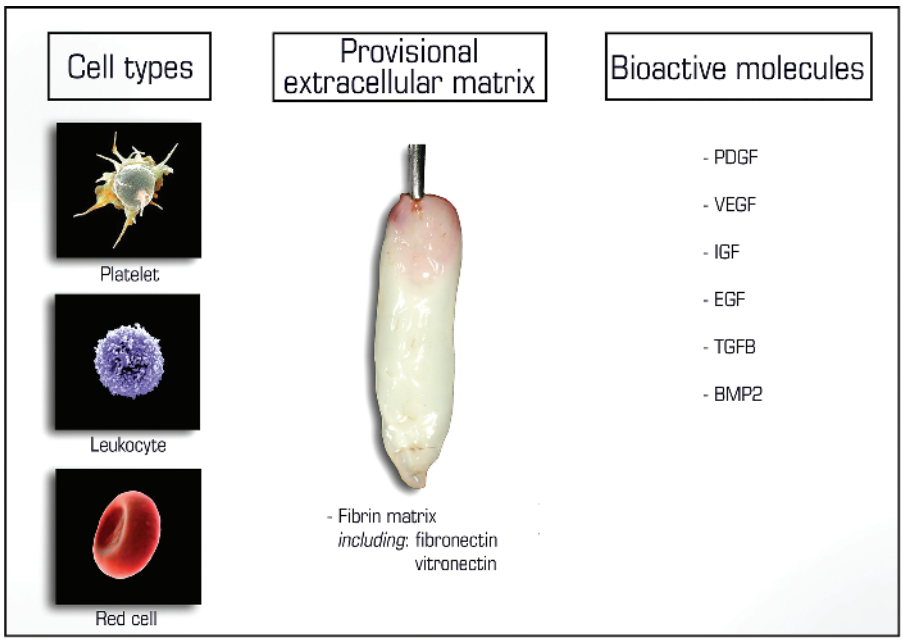

Figure 1.3 Three main components of PRF include 1) cell types (platelets, leukocytes, and red blood cells), 2) a provisional extracellular matrix three-dimensional scaffold fabricated from autologous fibrin (including fibronectin and vitronectin) and 3) a wide array of over 100 bioactive molecules including most notably PDGF, VEGF, IGF, EGF, TGF-beta, and BMP2. Source: Miron *et al.* 2016 [54]. Reproduced with permission of Elsevier.

but also contains a three-dimensional fibrin matrix containing various growth factors. These include transforming growth factor beta (TGF-beta), PDGF and VEGF, insulin growth factor (IGF), and epidermal growth factor (EGF). Recent research has more specifically shown how leukocytes (as opposed to platelets) are the main implicators in the tissue wound healing process capable of further enhancing new blood vessel formation (angiogenesis) and tissue formation [25–27, 30, 34].

It is also important to note that PRF has not solely been utilized in dentistry and much research has been dedicated to its use in various other fields of medicine. Recently, PRF has shown effectiveness for the clinical management of hard-to-heal leg ulcers including diabetic foot ulcers, venous leg ulcers, and chronic leg ulcers [35–39]. Furthermore, PRF has had positive outcomes for hand ulcers [40], facial soft tissue defects [41], laparoscopic cholecystectomy [42], deep nasolabial folds, volume-depleted midfacial regions, facial defects, superficial rhytids, and acne scars [43]. Its use has also been extended toward the induction of dermal collagenesis [44], vaginal prolapse repair [45], urethracutaneous fistula repair [46,47], lipostructure surgical procedures [48], chronic rotator cuff repair [49], and acute traumatic ear drum perforation healing [50]. It goes without further mention that by increasing blood flow to defect sites from various etiology, favorable wound healing and tissue regeneration may take place. We now know that PRF serves all three important criteria for tissue regeneration including 1) serving as a three-dimensional fibrin scaffold, 2) includes autologous cells such as leukocytes, macrophages, neutrophils, and platelets, and 3) serves as a reservoir of natural growth factors that may be released over a 10- to 14-day period. Research has now

demonstrated that each of these three individual components of tissue regeneration are important during wound healing with PRF.

1. **Major cell types in PRF**
   The aim of this introductory chapter is not to introduce the important cell-types found in PRF. This will be described later in Chapter 2. However, it is important to note that PRF contains a number of cells including platelets, leukocytes, macrophages, granulocytes, and neutrophils. Following the centrifugation cycle, the majority of these cells are trapped within the three-dimensional fibrin matrix. As stated previously, the addition of blood alone to bone biomaterials has been shown to drastically improve wound angiogenesis [12]. One of the main differences between PRF and previously utilized PRP is the incorporation of leukocytes in PRF. Several studies have shown their key importance during anti-infectious pathogen resistance as well as their implications in immune regulation [51–53]. Furthermore, they play a significant role during host tissue-to-biomaterial integration [31,33,54]. Due to the added benefits of leukocytes, it is not surprising to learn that extraction of third molars have specifically shown up to a 10-fold decrease in osteomyelitis infections as well as greater wound healing following simple placement of PRF into extraction sockets [55]. Therefore, the influence of autologous cells contained within PRF, most noteworthy leukocytes, should be considered a major advantage during regenerative therapy.
2. **A natural fibrin matrix and its biological properties**
   A second major difference between PRF and PRP as previously mentioned is the lack of anti-coagulants thus resulting in a fibrin matrix (Figure 1.4). Naturally without anti-coagulants blood will clot and for these reasons, centrifugation *must* take place immediately following blood collection. Initial protocols were established whereby 10-mL of blood was collected and centrifuged for 12 minutes at 2700 rpm (750g). In Chapter 3, the biological concept of utilizing lower centrifugation speeds and time will be discussed.

   Nevertheless, what was once thought to be simply a carrier for growth factors and cells, the fibrin matrix has since been shown to be a main feature of PRF. The PRF matrix acts as a key component of tissue wound healing as highlighted in more scientific detail in Chapter 2.
3. **Cytokines contained within PRF**
   The third primary advantage of PRF is the fact it contains natural growth factors found in blood. While their individual biological roles will be explained in the

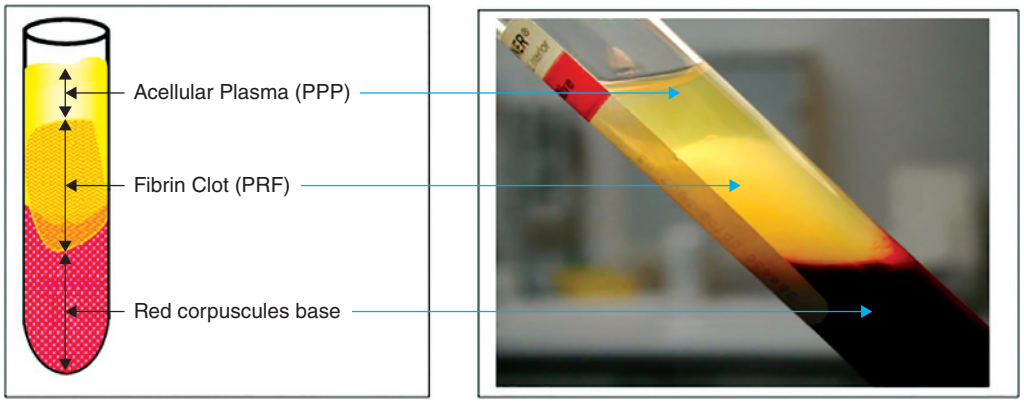

Figure 1.4 Platelet Rich Fibrin (PRF) clot formed in the upper third of glass tubes after centrifugation.

following chapter, PRF contains TGF-beta, a known agent responsible for the rapid proliferation of various cell types found in the oral cavity [56,57]. Its other major growth factor is PDGF, an essential regulator for the migration, proliferation, and survival of mesenchymal cells. A third important growth factor in PRF is VEGF responsible for angiogenesis and future blood flow to damaged tissues [58]. Other growth factors are epidermal growth factor and insulin-like growth factor, both regulators of the proliferation and differentiation of many cells types later described in Chapter 2.

The combination of 1) host cells, 2) a three-dimensional fibrin matrix and 3) growth factors contained within PRF act to synergistically enhance faster and more potent tissue wound healing and regeneration.

1.4 Effect of PRF on periosteum behavior

Following years of practice with the use of PRF, one biological property observed with almost every surgical technique has been its stimulation of the capacity of blood supply within the periosteum. From this point of view, direct contact of PRF with periosteum substantially improves the blood supply to the keratinized soft tissue favoring its thickness, as well as improves blood supply to the underlying bone tissues. This has been one of the key activities of PRF, whereby stimulation with growth factors over a long period of release.

1.5 The first case treated with PRF

The most appropriate way to conclude this first chapter is by introducing the concept of PRF utilized in regenerative medicine in the first years. Leg ulcers are a common reported problem in diabetic patients often resulting in amputation. In my pain clinic, a patient with obvious skin necrosis caused by Lyell syndrome with repeated failed antibiotic treatment was referred to me (Figure 1.5). From this perspective, patients were often directed to my pain clinic in Nice, France to receive treatment for pain. Over the years, science has shown that infection was often a secondary problem to poor blood supply. Therefore, to improve treatment outcomes, attempts were being made to see if PRF fibrin clots could be utilized to regenerate these defects (Figure 1.6). The idea was that by introducing supra-physiological doses of growth factors from blood, one could potentially re-introduce blood flow into these tissues. To our great interest, wounds that were initially covered with PRF and plastic "Saran" wrap began to heal in as early as 10 days, and infection had disappeared. By 30 days, great clinical improvements could be visualized and this was achieved utilizing PRF alone even in the absence of antibiotics (Figure 1.7). Similar clinical outcomes could also be observed following foot amputation where resulting wounds were extremely difficult to heal. The application of PRF alone could re-introduce blood flow into these defects, improving significantly tissue regeneration (Figures 1.8 and 1.9). Most interesting is at which point the body's natural ability proves to treat these defects in a physiological way with 100% naturally derived human blood.

Following these early treatments, it was obvious that the potential for PRF to be utilized across many fields of medicine was clear. The concept was later introduced to the dental field where a much larger number of regenerative procedures could be performed on a yearly basis. From there, expert clinicians have attempted to use PRF in various regenerative procedures in dentistry later discussed in this textbook and the field has been expanding ever since.

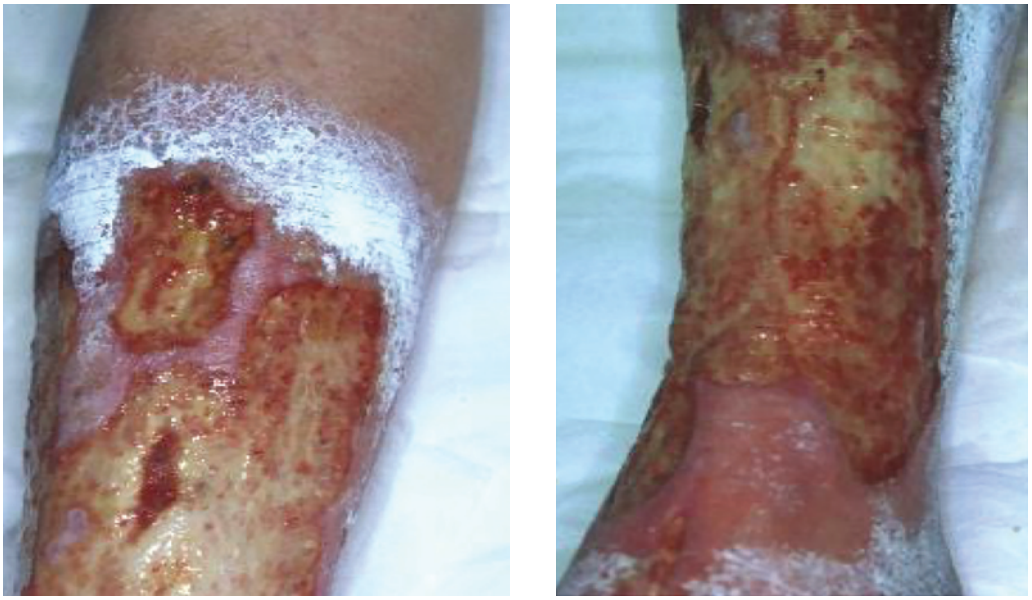

Figure 1.5 Patient presenting to the Pain Clinic in Nice, France with Lyell syndrome. Antibiotic therapy in such cases is not always effective (Case performed by Dr. Joseph Choukroun).

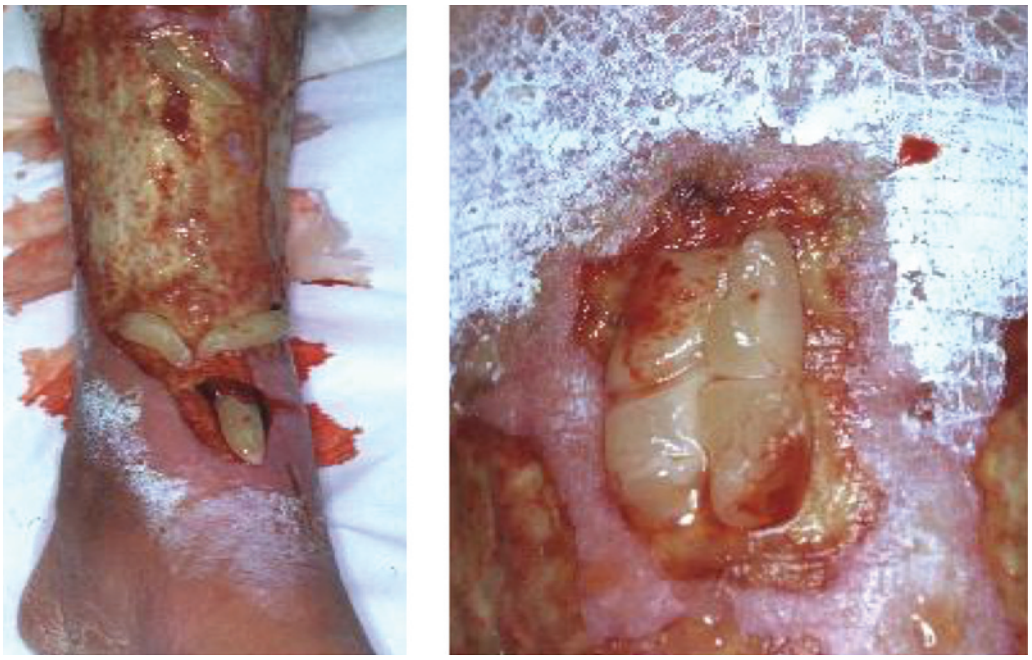

Figure 1.6 Patient from Figure 1.5 with Lyell syndrome treated with PRF. PRF membranes were placed on the defects, wrapped in a plastic wrap, and allowed to heal without use of antibiotic therapy (Case performed by Dr. Joseph Choukroun).

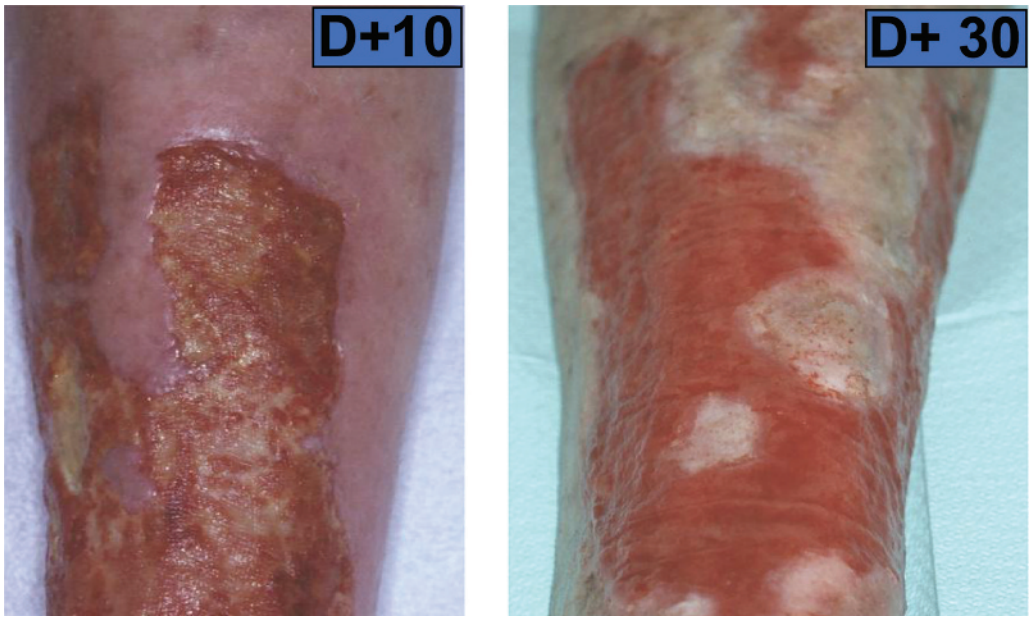

Figure 1.7 Patient from Figures 1.5 and 1.6 with Lyell syndrome treated with PRF. After 10 and 30 days of healing, notice the marked improvement in tissue revascularization and wound healing (Case performed by Dr. Joseph Choukroun).

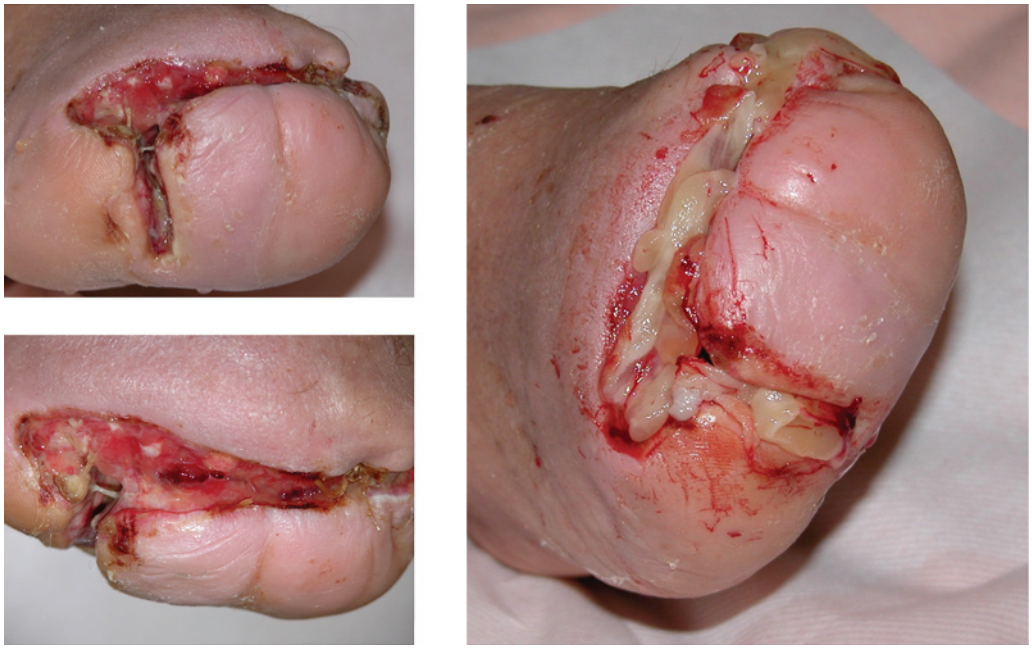

Figure 1.8 Diabetic foot amputation with infection after 15 days. Right photo demonstrates PRF clots that are applied to the wound (Case performed by Dr. Joseph Choukroun).

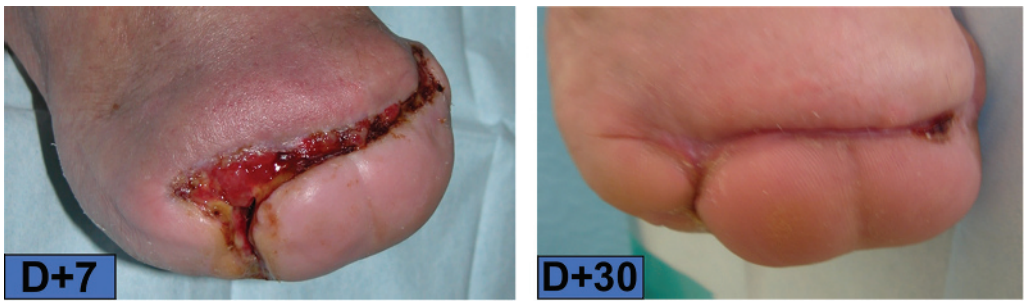

Figure 1.9 Diabetic foot amputation (patient from Figure 1.8) following 7 and 30 days of healing (Case performed by Dr. Joseph Choukroun).

1.6 Conclusion

The use of PRF has seen a large and steady increase in popularity since it was first introduced in medicine for the treatment of hard-to-heal leg ulcers and wounds. While described as a second-generation platelet concentrate, one of the main advantages of PRF is the fact that it is produces without use of anti-coagulants or other unnatural by-products that prevent the coagulation cascade and is therefore considered 100% autologous and natural. While PRF contains three important aspects for tissue wound healing, including 1) host cells, 2) a three-dimensional fibrin matrix, and 3) accumulation of growth factors, its synergistic effects has frequently been recognized in dentistry most notably for the healing of soft tissues. Future strategies to improve PRF formulations and techniques are continuously being investigated to further enhance the clinical outcomes following regenerative procedures utilizing this technology.

References

1 Coury AJ. Expediting the transition from replacement medicine to tissue engineering. Regenerative biomaterials. 2016;3(2):111–3.
2 Dai R, Wang Z, Samanipour R, Koo KI, Kim K. Adipose-Derived Stem Cells for Tissue Engineering and Regenerative Medicine Applications. Stem cells international. 2016;2016:6737345.
3 Rouwkema J, Khademhosseini A. Vascularization and Angiogenesis in Tissue Engineering: Beyond Creating Static Networks. Trends Biotechnol. 2016.
4 Zhu W, Ma X, Gou M, Mei D, Zhang K, Chen S. 3D printing of functional biomaterials for tissue engineering. Current opinion in biotechnology. 2016;40:103–12.
5 Upputuri PK, Sivasubramanian K, Mark CS, Pramanik M. Recent developments in vascular imaging techniques in tissue engineering and regenerative medicine. BioMed research international. 2015; 2015:783983.
6 Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010;89(3): 219–29.
7 Gosain A, DiPietro LA. Aging and wound healing. World journal of surgery. 2004;28(3):321–6.
8 Eming SA, Brachvogel B, Odorisio T, Koch M. Regulation of angiogenesis: wound healing as a model. Progress in histochemistry and cytochemistry. 2007;42(3):115–70.
9 Eming SA, Kaufmann J, Lohrer R, Krieg T. [Chronic wounds. Novel approaches in

research and therapy]. Der Hautarzt; Zeitschrift fur Dermatologie, Venerologie, und verwandte Gebiete. 2007;58(11): 939–44.

10 Zhang Y, Zhang X, Shi B, Miron R. Membranes for guided tissue and bone regeneration. Annals of Oral & Maxillofacial Surgery. 2013;1(1):10.

11 Nurden AT. Platelets, inflammation and tissue regeneration. Thrombosis and haemostasis. 2011;105 Suppl 1:S13–33.

12 Barbeck M, Najman S, Stojanovic S, Mitic Z, Zivkovic JM, Choukroun J, et al. Addition of blood to a phycogenic bone substitute leads to increased in vivo vascularization. Biomedical materials (Bristol, England). 2015;10(5):055007.

13 de Vries RA, de Bruin M, Marx JJ, Hart HC, Van de Wiel A. Viability of platelets collected by apheresis versus the platelet-rich plasma technique: a direct comparison. Transfusion science. 1993; 14(4):391–8.

14 Anfossi G, Trovati M, Mularoni E, Massucco P, Calcamuggi G, Emanuelli G. Influence of propranolol on platelet aggregation and thromboxane B2 production from platelet-rich plasma and whole blood. Prostaglandins, leukotrienes, and essential fatty acids. 1989;36(1):1–7.

15 Fijnheer R, Pietersz RN, de Korte D, Gouwerok CW, Dekker WJ, Reesink HW, et al. Platelet activation during preparation of platelet concentrates: a comparison of the platelet-rich plasma and the buffy coat methods. Transfusion. 1990;30(7):634–8.

16 Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. Journal of oral and maxillofacial surgery. 1997;55(11): 1294–9.

17 Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 1998;85(6): 638–46.

18 Jameson C. Autologous platelet concentrate for the production of platelet gel. Lab Med. 2007;38:39–42.

19 Marx RE. Platelet-rich plasma: evidence to support its use. Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons. 2004;62(4):489–96.

20 Kobayashi E, Fluckiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clinical oral investigations. 2016.

21 Lucarelli E, Beretta R, Dozza B, Tazzari PL, O'Connel SM, Ricci F, et al. A recently developed bifacial platelet-rich fibrin matrix. European cells & materials. 2010; 20:13–23.

22 Saluja H, Dehane V, Mahindra U. Platelet Rich fibrin: A second generation platelet concentrate and a new friend of oral and maxillofacial surgeons. Annals of maxillofacial surgery. 2011;1(1):53–7.

23 Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunité en paro-implantologie: le PRF. Implantodontie. 2001;42(55):e62.

24 Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. Journal of periodontology. 2010;81(4):546–55.

25 Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2006; 101(3):e56–60.

26 Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2006;101(3):e37–44.

27 Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2006;101(3):e45–50.

28 Martin P, Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. Trends in cell biology. 2005; 15(11):599–607.

29 Tsirogianni AK, Moutsopoulos NM, Moutsopoulos HM. Wound healing: immunological aspects. Injury. 2006;37 Suppl 1:S5–12.

30 Adamson R. Role of macrophages in normal wound healing: an overview. Journal of wound care. 2009;18(8):349–51.

31 Davis VL, Abukabda AB, Radio NM, Witt-Enderby PA, Clafshenkel WP, Cairone JV, et al. Platelet-rich preparations to improve healing. Part I: workable options for every size practice. The Journal of oral implantology. 2014;40(4):500–10.

32 Davis VL, Abukabda AB, Radio NM, Witt-Enderby PA, Clafshenkel WP, Cairone JV, et al. Platelet-rich preparations to improve healing. Part II: platelet activation and enrichment, leukocyte inclusion, and other selection criteria. The Journal of oral implantology. 2014;40(4):511–21.

33 Ghasemzadeh M, Hosseini E. Intravascular leukocyte migration through platelet thrombi: directing leukocytes to sites of vascular injury. Thrombosis and haemostasis. 2015;113(6):1224–35.

34 Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2006;101(3): e51–5.

35 Danielsen P, Jorgensen B, Karlsmark T, Jorgensen LN, Agren MS. Effect of topical autologous platelet-rich fibrin versus no intervention on epithelialization of donor sites and meshed split-thickness skin autografts: a randomized clinical trial. Plastic and reconstructive surgery. 2008;122(5):1431–40.

36 O'Connell SM, Impeduglia T, Hessler K, Wang XJ, Carroll RJ, Dardik H. Autologous platelet-rich fibrin matrix as cell therapy in the healing of chronic lower-extremity ulcers. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society. 2008;16(6):749–56.

37 Steenvoorde P, van Doorn LP, Naves C, Oskam J. Use of autologous platelet-rich fibrin on hard-to-heal wounds. Journal of wound care. 2008;17(2):60–3.

38 Jorgensen B, Karlsmark T, Vogensen H, Haase L, Lundquist R. A pilot study to evaluate the safety and clinical performance of Leucopatch, an autologous, additive-free, platelet-rich fibrin for the treatment of recalcitrant chronic wounds. The international journal of lower extremity wounds. 2011;10(4):218–23.

39 Londahl M, Tarnow L, Karlsmark T, Lundquist R, Nielsen AM, Michelsen M, et al. Use of an autologous leucocyte and platelet-rich fibrin patch on hard-to-heal DFUs: a pilot study. Journal of wound care. 2015;24(4):172–4, 6–8.

40 Chignon-Sicard B, Georgiou CA, Fontas E, David S, Dumas P, Ihrai T, et al. Efficacy of leukocyte- and platelet-rich fibrin in wound healing: a randomized controlled clinical trial. Plastic and reconstructive surgery. 2012;130(6):819e–29e.

41 Desai CB, Mahindra UR, Kini YK, Bakshi MK. Use of Platelet-Rich Fibrin over Skin Wounds: Modified Secondary Intention Healing. Journal of cutaneous and aesthetic surgery. 2013;6(1):35–7.

42 Danielsen PL, Agren MS, Jorgensen LN. Platelet-rich fibrin versus albumin in surgical wound repair: a randomized trial with paired design. Annals of surgery. 2010;251(5):825–31.

43 Sclafani AP. Safety, efficacy, and utility of platelet-rich fibrin matrix in facial plastic surgery. Archives of facial plastic surgery. 2011;13(4):247–51.

44 Sclafani AP, McCormick SA. Induction of dermal collagenesis, angiogenesis, and adipogenesis in human skin by injection of platelet-rich fibrin matrix. Archives of facial plastic surgery. 2012;14(2):132–6.

45 Gorlero F, Glorio M, Lorenzi P, Bruno-Franco M, Mazzei C. New approach in vaginal prolapse repair: mini-invasive surgery associated with application of platelet-rich fibrin. International urogynecology journal. 2012;23(6):715–22.

46 Soyer T, Cakmak M, Aslan MK, Senyucel MF, Kisa U. Use of autologous platelet rich fibrin in urethracutaneous fistula repair: preliminary report. International wound journal. 2013;10(3):345–7.

47 Guinot A, Arnaud A, Azzis O, Habonimana E, Jasienski S, Fremond B. Preliminary experience with the use of an autologous platelet-rich fibrin membrane for urethroplasty coverage in distal hypospadias surgery. Journal of pediatric urology. 2014;10(2):300–5.

48 Braccini F, Chignon-Sicard B, Volpei C, Choukroun J. Modern lipostructure: the use of platelet rich fibrin (PRF). Revue de laryngologie-otologie-rhinologie. 2013;134(4-5):231–5.

49 Zumstein MA, Rumian A, Lesbats V, Schaer M, Boileau P. Increased vascularization during early healing after biologic augmentation in repair of chronic rotator cuff tears using autologous leukocyte- and platelet-rich fibrin (L-PRF): a prospective randomized controlled pilot trial. Journal of shoulder and elbow surgery/American Shoulder and Elbow Surgeons [et al]. 2014;23(1):3–12.

50 Habesoglu M, Oysu C, Sahin S, Sahin-Yilmaz A, Korkmaz D, Tosun A, et al. Platelet-rich fibrin plays a role on healing of acute-traumatic ear drum perforation. The Journal of craniofacial surgery. 2014;25(6): 2056–8.

51 Kawazoe T, Kim HH. Tissue augmentation by white blood cell-containing platelet-rich plasma. Cell transplantation. 2012;21(2-3): 601–7.

52 Perut F, Filardo G, Mariani E, Cenacchi A, Pratelli L, Devescovi V, et al. Preparation method and growth factor content of platelet concentrate influence the osteogenic differentiation of bone marrow stromal cells. Cytotherapy. 2013;15(7): 830–9.

53 Pirraco RP, Reis RL, Marques AP. Effect of monocytes/macrophages on the early osteogenic differentiation of hBMSCs. Journal of tissue engineering and regenerative medicine. 2013;7(5):392–400.

54 Miron RJ, Bosshardt DD. OsteoMacs: Key players around bone biomaterials. Biomaterials. 2016;82:1–19.

55 Hoaglin DR, Lines GK. Prevention of localized osteitis in mandibular third-molar sites using platelet-rich fibrin. International journal of dentistry. 2013;2013:875380.

56 Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. The New England journal of medicine. 1994; 331(19):1286–92.

57 Bowen T, Jenkins RH, Fraser DJ. MicroRNAs, transforming growth factor beta-1, and tissue fibrosis. The Journal of pathology. 2013;229(2):274–85.

58 Shamloo A, Xu H, Heilshorn S. Mechanisms of vascular endothelial growth factor-induced pathfinding by endothelial sprouts in biomaterials. Tissue engineering Part A. 2012;18(3-4): 320–30.