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7 Therapeutic applications of the preparation rich in growth factors (PRGF)

Eduardo Anitua[1], Mikel Sánchez[2], Gorka Orive[1] and Isabel Andia[1]
[1]Biotechnology Institute I MAS D, c/ San Antonio 15, 01005 Vitoria, Spain
[2]Unidad de Cirugía Artroscópica "Mikel Sánchez", c/ La Esperanza
01005 Vitoria, Spain

Abstract

The technology of preparation rich in growth factors (PRGF) constitutes a relatively new biotechnology for the stimulation and acceleration of tissue healing and bone regeneration. Recent advances in the field have enabled the therapeutic application of this technology in surgery and in the treatment of a wide range of medical disorders including dentistry, oral implantology, orthopedics, ulcer treatment and tissue engineering among others. This book chapter aims to describe the important progress and the most significant therapeutic applications that have been accomplished in the field.

Correspondence/Reprint request: Dr. Eduardo Anitua, Biotechnology Institute I MAS D, c/ San Antonio 15, 01005 Vitoria, Spain. E-mail: eduardoanitua@eduardoanitua.com

Introduction

The period 2000-2010 has been named "the bone and joint decade" in an effort to bring the problems related with these disorders to the attention of scientists and to promote advancement in these fields [1]. The increasing incidence of bone, cartilage, tendon and ligament injuries is stimulating the development of less invasive procedures and accelerated treatments that in general reduce morbidity while enhance functional recovery. Many of these emerging technologies utilize or release growth factors and bioactive proteins at localized orthopaedic sites with the aim of triggering healing and regenerative processes.

The potential role of growth factors comes from the established knowledge that tissue repair process is a complex cascade of biological events controlled by a large list of cytokines, proteins and growth factors which provide signals at local injury sites, regulating the mechanisms and pathways that govern tissue wound healing and regeneration [2]. Therefore, the ability to release growth factors in a spatiotemporal manner mimicking the needs of the injured tissue has become a challenge in the scientific and medical field. The last few years have seen one important first, that is, the development of platelet rich-therapies [3,4]. The emergence and application of these platelet enriched preparations have revolutionized the field of regenerative medicine in part due to the repair capacities of the growth factors and proteins secreted by the platelets [5]. The easy preparation protocols, the biosafety and versatility of the platelet-based preparations and their reduced costs have also stimulated the research and interest by the scientific community.

This chapter will provide an overview of the potential therapeutic use of platelets and platelet rich technologies for the release of growth factors in the treatment of a wide range of diseases. For the purposes of this chapter, the role of platelets as growth factors reservoir units will be discussed. A detailed description of the potential therapeutic value of platelet rich technologies and especially of preparation rich in growth factors (PRGF) is followed by a section on applications of PRGF and platelet rich technology in numerous medical fields.

Growth factors as therapeutic agents

It is generally accepted that growth factors have an essential role in the healing process and in tissue formation. The cellular and molecular events resulting after a traumatic injury are mostly shared by the different tissues of the body. These events are controlled by a wide variety of different cytokines and growth factors acting locally as regulators of the most basic cell functions via endocrine, paracrine, autocrine and intracrine mechanisms. The growth factors influence many of the processes common to both tissue repair and

disease, including angiogenesis, chemotaxis, proliferation and the controlled synthesis and degradation of extracellular matrix proteins by binding to the extracellular domain of a target growth factor receptor that in turn activates the intracellular signal-transduction pathways [6].

In the last few decades, the biological roles of the different growth factors on these stages of tissue repair have been progressively elucidated at the cellular level (Figure 1).

Although the role of all the growth factors involved in tissue regeneration is only partially elucidated, the potential benefits of many of them have been demonstrated. For example, transforming growth factor-β (TGF-β) is a pleiotropic molecule with myriad of effects on nearly every cell type and tissue [7], platelet derived growth factor (PDGF) is a powerful mitogen for connective tissue cells [8], insulin-like growth factor (IGF-I) might promote the late-stage differentiation and activity of osteoblasts, and vascular endothelial growth factor (VEGF) induces endothelial cell proliferation and migration, thus initiating the angiogenic response [9].

Some of the molecules have been more intensively investigated and have recently accessed to the clinics. For example, two BMP-containing products

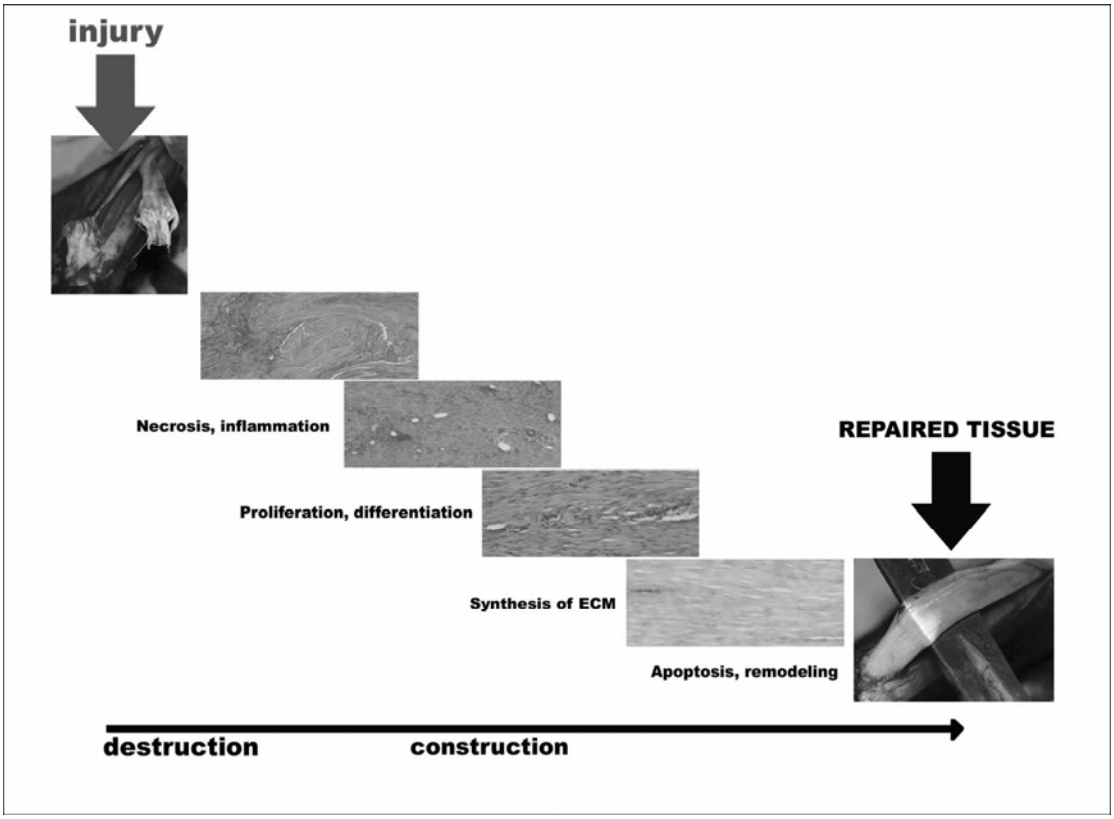

Figure 1. Biological roles of the growth factors in the different phases of the tissue repair process.

incorporated in a collagen sponge have been approved by several federal agencies for the treatment of long bone fractures. BMP-2 appear to be effective in the treatment of fresh fractures [10] and interbody spinal fusion whilst recombinant human BMP-7 (also known as Osteogenic protein-1) has shown efficacy in the treatment of non-unions [11]. In the field of chronic ulcer treatment, the recombinant human platelet derived growth factor BB (rh-PDGF-BB) was approved by the Food and Drug Administration to heal foot ulcers in diabetics and it has been commercially available since 1998 [12]. Another interesting approach under study includes the use of neurothrophic factors for the treatment of central nervous system degenerative disorders and blindness-threatening ocular diseases.

Despite the enormous efforts, the field has not lived up to expectations expressed a few years ago. It is difficult to determine why only few growth factors have been approved and commercialized for therapy in humans. One critical point is that a pool of growth factors, cytokines and proteins are likely to be required according to the complex intricacy of the healing and tissue repairing processes. Considering one specific growth factor as a magic bullet might only conduce to impaired tissue regeneration. Furthermore, regulating the kinetic release of all these multiple growth factors aiming to mimic as much as possible the natural injured tissue requirements during the different regeneration phases is of paramount importance. The reduced half-lives and local biodistribution of the growth factors may require in some therapeutic conditions their association or incorporation into biomaterials or drug delivery systems in order to better control their formulation and pharmacokinetics. Last but not least, the excessive cost of the synthetic growth factors and their immunogenic concerns are also major hurdles in this field.

These limitations have stimulated the research and development of novel formulations designed to release a pool of growth factors with biological roles in tissue regeneration. Using platelets as growth factor releasing tools emerges as a novel biological and biocompatible therapeutic approach.

Platelets as growth factor reservoirs

Platelets are known for their outstanding role in haemostasis as they prevent blood loss at sites of vascular injury. But what has gained the interest of the scientific and medical community is their capacity to store and release, upon activation, multiple growth factors and cytokines that promote wound healing and tissue regeneration. These growth factors are stored in different populations of granules, but most of them are secreted from alpha granules [13].

Alpha granules contain adhesive proteins such as fibronectin, vitronectin, fibrinogen and thrombospondin-1. A large list of mitogenic agents essential for wound repair and tissue regeneration is also stored in these granules. Examples

of mitogenic agents include PDGF, TGF-β, basic fibroblast growth factor (bFGF), platelet derived epidermal growth factor (PDEGF) and IGF-I among others. Additionally, they also contain a mixture of pro- and anti-angiogenic molecules that may promote or inhibit the neo-vascularization process.

Assuming the potential of platelets as biological systems for growth factor delivery, much effort has been devoted to properly formulate these cells into "therapeutic preparations" that could be clinically tested and used in numerous medical disorders. To address these issues, it is initially necessary to concentrate the platelets in the formulation in order to increase the growth factor content and improve the therapeutic effects. However, this may be carefully done as lower platelet concentrations can lead to suboptimal effects whereas higher concentrations might have an inhibitory effect [14]. Another critical issue is the activation process of the platelets within the formulation. This may have important implications in the subsequent therapeutic effects. This therapeutic strategy based on platelets should fulfill the same quality and biosafety standards that any other medicine. Additionally, a high versatility that may allow its application in different situations, reduced costs and high biocompatibility are also major requirements for this novel biological technology.

Platelet rich technologies: PRGF

In the past two decades, an increased understanding of the physiological roles of platelets in wound healing and after tissue injury has led to the idea of using platelets as therapeutic tools. Indeed, after fibrin glue was introduced in the early 1990s as a biomaterial with haemostatic and adhesive properties, an autologous modification of the fibrin to include platelets was reported [15]. This new preparation, known as platelet-rich plasma, consisted of a limited volume of plasma enriched in platelets which after activation released a pool of growth factors and proteins.

More recently, a novel preparation has emerged with the aim of addressing some of the limitations of conventional platelet rich plasmas. This new platelet based product is known as preparation rich in growth factors (PRGF) and it enables upon activation the formation of a three-dimensional and biocompatible fibrin scaffold and the local delivery of a myriad of growth factors and proteins that contribute to the accelerated postoperative wound healing and tissue repair [13]. One initial advantage of this type of preparation is that it is easily and rapidly obtained from patient's blood, and theoretically since the donor and receptor should be the same, the immunological concerns are circumvented. Additionally, the release of the biologically active agents via platelet de-granulation is induced by the addition of a standardized dose of calcium chloride, which permits a more sustained release of growth factors than thrombin [16]. Leukocyte content has been eliminated from PRGF with

the aim of avoiding the pro-inflammatory effects of the proteases and acid hydrolases contained in white blood cells [17]. This may be especially interesting in the blade sharp lesions made during the surgical approach to the target pathological tissues which are essentially aseptic. In this context, the metalloproteases secreted by leukocytes would provoke negative destroying effects.

As it has been recently demonstrated, the platelet-growth factor ratio and the subsequent therapeutic effects may also be manipulated pharmacologically. For example, Wallace et al. showed that orally administered platelet rich preparations accelerated gastric healing ulcer through presentation of VEGF [18]. The same authors suggested that platelet content of pro- versus anti-angiogenic factors might be regulated with several drugs including ticlopidine, NSAIDs or thrombopoietin which could alter the relative content of some growth factors versus others. The latter may have important clinical considerations as shifts in the serum levels of pro- versus anti-angiogenic factors can influence the healing of colon and stomach ulcers and have been suggested to be important in the repair of joint injuries in arthritis [19].

The angiogenic potential of PRGF is another advantage from a therapeutic point of view and neo-vascularization is a key process in tissue regeneration. Recently, we demonstrated the angiogenic potential of PRGF after the infiltration of the biological preparation in Achilles tendon in sheep. Results showed that in tendons injected with PRGF, there was an increase in cellularity and a change in cell morphology comparing with saline injections and untreated control tendons [20]. When PRGF was injected, tendon cells with an ovoid shape appeared aligned along the collagen fibers, showing organization along lines of tension. The latter contrasted with tendons injected with saline where disorganized and disordered cells accumulated in defined limited areas (Figure 2). Additionally, in tendons treated with PRGF, endotenons were more prominent through an accumulation of round cellular elements.

One potential field of interest for the combination of PRGF and biomaterials is bone grafting technology. The effectiveness of bone grafting can be enhanced by creating custom-made biomaterials that will meet specific structural and biological tissue requirements in different anatomical locations. In this context a wide array of composite biomaterials can be created by mixing PRGF with either artificial or natural biomaterials. For example, in oral implantology, dentists find difficult and challenging the manipulation and application of some bone augmentation materials and even autologous bone. By combining selected biomaterials with scaffold-like PRGF, it is possible to improve the handling and adaptation of the matrix to the injured tissue because the fibrin acts a biologic glue to hold together the matrix particles. This may have implications for some specific dental surgeries like sinus floor elevation [21-23].

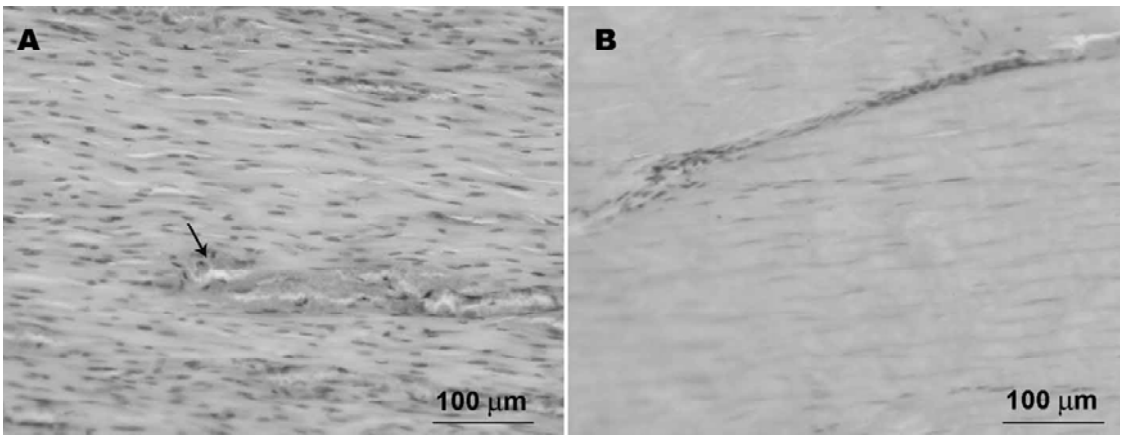

Figure 2. Angiogenic effects of the PRGF. a) histological sections of the Achilles tendons treated with PRGF and b) untreated tendon. Note the presence of blood vessel in the PRGF treated tendon (black arrows).

The mixture, encapsulation or immobilization of PRGF within biomaterials may also be a valid tool to alter the pharmacokinetics of the release proteins and growth factors. It has been reported that activation of platelets with bovine thrombin provokes an initial burst effect in which more than 95% of the stored growth factors are secreted within the first hour [24]. By using an acidic gelatin with an isoelectric point of 5.0, the growth factors released by the platelet rich product after its activation are immobilized and retained in the hydrogel through physicochemical interactions. The latter substantially alters growth factor kinetic profile as release will depend on hydrogel degradation [25]. Similar approaches have been described using collagen and calcium sulphate as biomaterials.

Therapeutic applications of PRGF technology

The technology of platelet rich preparation has been applied in different scientific and therapeutic fields including the treatment of chronic ulcers, treatment of musculoskeletal conditions and bone and soft tissue regeneration in dentistry and oral implantology among others.

Treatment of chronic ulcers

Historically, the first clinical application of platelet derived preparations was conducted in chronic leg ulcers where wounds were filled with collagen embedded in platelet secreted proteins [26,27]. This initial product, known as PDWHF (platelet-derived wound healing factors) stimulated the formation of the vascularised connective tissue found in healing wounds. Thereafter various other types of platelets products have been assayed in several pilot studies, case series and clinical trials [28-30].

The way growth factors are released to the injured tissue is a matter of debate which may conditioned the therapeutic potential of the formulation. For example, Stacey and colleagues reported that topical application of platelet lysates on the ulcers did not influence the healing [31]. The application of a fibrin scaffold like-PRGF, in which rapid clearance of growth factors and proteins is prevented, might shed light on this controversy. Recently, we successfully evaluated such a protocol consisting on coagulating the plasma in vivo within the bed ulcer and covering afterwards the whole area with a fibrin membrane prepared ex vivo (Figure 3). In a randomized open-label controlled pilot trial the effectiveness of this protocol in the treatment of chronic vascular ulcers was analyzed and compared with the standard therapy [32]. Results showed that at 8 weeks, the mean percentage of surface healed in the PRGF group was 73±22% whereas it was 21±34% in the control group ($P < 0.05$).

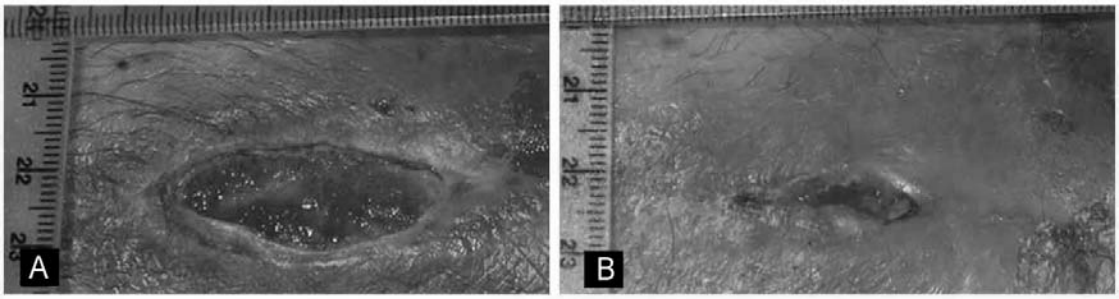

Figure 3. Evolution of a typical skin ulcer treated with PRGF: a) debrided ulcer before treatment, and b) eight weeks post-treatment.

Treatment of orthopaedic lesions

Soft tissue disorders including tendon, ligament and joint capsular injuries represent 45% of all the musculoskeletal injuries reported each year in the USA. Studies from primary care show that 16% of the general population suffers with shoulder pain [33] whereas elbow tendinopathy affects 1-2% of the population. The importance of this problem is substantial because the field of sports medicine influences million of people from athletes to people who participate in recreational sport or simply people who use exercise to stay healthy and active. Additionally, tendon injuries account for 30-50% of all injuries related to sports [34,35], often requiring surgical treatment.

The use of platelet rich preparations in this context might be focused on restoring the normal tissue composition while avoiding further degeneration. For example, application of platelet rich plasma and fibrin sealant peri-operatively in arthroplasty reduces blood transfusion requirements and the length of hospital stay, decreases the incidence of blood leakage and arthrofibrosis whereas improves the range of motion [36]. In another approach,

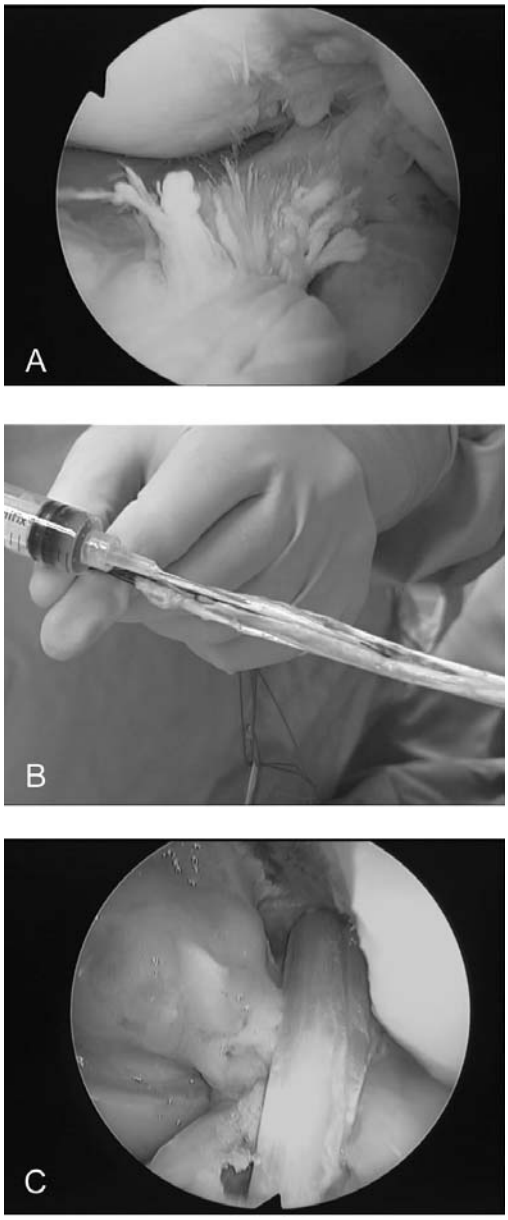

Figure 4. Use of PRGF during ligament reconstruction in the knee. a) image showing the rupture ends of the anterior cruciate ligament; b) PRGF is injected within a substitute graft tendon to enhance biological incorporation of the graft; c)PRGF has been introduced into the tibial and femoral tunnels during tendon graft fixing aiming to accelerate the subsequent healing of the tunnel.

we observed exciting results after intraarticular administration of PRGF in the arthroscopic treatment of an avulsion of articular cartilage [37]. A similar strategy has been applied by others, demonstrating that the treatment of full-thickness cartilage defects with PRGF enhances mechanical properties in a rabbit model [38].

The potential therapeutic value of PRGF has been also studied for the treatment of tendon injuries. Initially, we decided to evaluate the effects of the pool of growth factors released from PRGF on tendon cells. Results showed that human tendon cells increased their proliferation rate and were stimulated to release VEGF and HGF. The former will promote angiogenesis which is directly related with tendon healing capability while the latter is a potent anti-fibrotic agent that could reduce the scar formation around tendon tissues. Others have reported that injections of platelet rich plasma one week postoperatively increase tendon regenerate strength [39]. The clinical translation of this approach was assayed in 6 athletes. PRGF was injected among the tendon fibers after the tendon was sutured. After closing the paratenon and before closing the overlying skin, the affected area was covered with the fibrin scaffold. Results showed that those receiving the PRGF-therapy experienced a significant acceleration in functional recovery comparing with a matched group that followed conventional surgery [40].

Finally, much effort has been paid to the development of novel medical tools for repairing anterior cruciate ligament (ACL) injuries. ACL reconstructive surgery typically involves using a substitute graft and securing the respective ends of the graft to the walls of the tibial and femoral tunnels. In the last few years, several groups have attempted to fabricate tissue engineered ligaments using natural biomaterials and a wide-range of nanometer-sized artificial scaffolds [41-43]. Platelet rich preparation may bridge the gap between inactive scaffolds and cell biology adding to the scaffold structure the biologic stimulation necessary to get transformed into a functional remodeling tissue. This novel approach to create fully integrated bioactive grafts was proposed by our group assuming that released growth factors will provide the necessary biological cues for cell migration, proliferation, angiogenesis and remodelling (Figure 4) [44].

Bone regeneration

More than 6 million bone fractures are reported annually in the USA, from which 5-10% have impaired healing causing pain and disability. To improve results, scientists are making great efforts both to create bone substitutes and to develop ways of improving bone healing. The use of platelet rich preparations may help to fulfil some of these requirements, particularly as an aid to bone regeneration. In fact, in vitro studies have clearly demonstrated that platelet derived growth factors stimulate the proliferation of both human trabecular bone cells [45] and human osteoblast-like cells [46].

In one of the initial clinical studies involving PRGF in the field of dentistry, 20 patients who underwent tooth extraction because of periodontal disease or vertical fractures, were treated with and without PRGF. Results showed that in most of the patients receiving PRGF, bone regeneration was

extensive and the bone tissue was compact with well-organized trabeculae whereas in the control group connective tissue and little mature bone were found [4]. Another interesting potential of PRGF lies in its combination with dental implants to facilitate the anchorage and osseointegration of dental prostheses. Following a simple approach, titanium dental implants can be humidified with PRGF, creating a nano-membrane on the implant surface which increases the extent and quality of bone regeneration around the implants [22].

Another therapeutic approach involves the combination of platelet rich products with different bone matrices to improve the handling and adaptation of the matrix to the injured tissue. This is in part because these biological products may act as a biologic glue to hold together the matrix particles. Apart from facilitating the handling and manipulation, the combination of both materials may have synergistic effects in bone regeneration. As it was demonstrated in a striking fashion, the addition of platelet rich plasma to a natural deproteinized bovine bone induced a significant histomorphometric improvement at 1, 2 and 4 months [47]. Similarly, the combination of β-tricalcium phosphate with platelet rich plasma resulted in a more intense bone regeneration in the early healing phase [48].

Developing tissue engineered bone is another interesting approach for bone regeneration. This may be feasible after combining mesemchymal stem cells (MSCs) and scaffold-like platelet rich preparations. In fact, isolated cells, growth factors and biocompatible supporting scaffolds have generally been considered essential prerequisites to tissue engineering approaches. In the last few years, several attempts have been reported especially for bone regeneration, but also for cartilage and periodontal tissue engineering. For example, the potential bone regeneration capacity of a MSCs and platelet rich product mixture (MSC/PRP) was analysed and compared with other approaches including a natural deproteinized bovine bone, autologous bone and the platelet rich product alone [49]. Results show that MSC/PRP combination provided greater bone maturation and early stage bone regeneration from the viewpoint of histology and mechanical properties compared with the rest of the treatments. This mixed preparation has been also successfully applied for bone regeneration in several patients [50,51] The same group recently reported a similar strategy for periodontal tissue regeneration in one clinical case report, showing that this tissue engineering approach successfully reduced proving depth, improved attachment level and promoted bone defect filling in infrabony lesions [52].

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