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Leading Opinion

The potential impact of the preparation rich in growth factors (PRGF) in different medical fields ☆

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Abstract

Platelet-rich preparations constitute a relatively new biotechnology for the stimulation and acceleration of tissue healing and bone regeneration. The versatility and biocompatibility of this approach has stimulated its therapeutic use in numerous medical and scientific fields including dentistry, oral implantology, orthopaedics, ulcer treatment, tissue engineering among others. Here we discuss the important progress that has been accomplished in the field of platelet-rich preparations in the last few years. Some of the most interesting therapeutic applications of this technology are discussed as are some of the limitations, future challenges and directions in the field.

Keywords: Growth factors; Platelets; Regenerative medicine; Tissue repair; Drug delivery; Biomaterials

1. Introduction

During the last decades, the scientific developments from cellular and molecular biology and the progressive understanding of wound healing and tissue regeneration processes have stimulated the research and development of novel multidisciplinary fields like regenerative medicine and tissue engineering. The increasing accumulation of knowledge is providing the fuel for clinical research and translational medicine. The idea of "biological solutions to biological and medical problems" is emerging as a new paradigm in medicine leading to the development of novel and more optimized biological preparations that might open new avenues in surgery and in the treatment of a wide range of diseases [1]. Such developments are coupled closely with advances in biomaterials which are leading to a variety of approaches that are widely used in numerous medical applications [2,3].

Additionally, the field of medicine is advancing rapidly towards the development of less invasive procedures and accelerated treatments that in general reduce morbidity while enhance functional recovery. These simple and cost-efficient procedures may have a potential impact in reducing the economic costs for standard medical treatments. The last few years have seen one important first, that is, the development of platelet-rich therapies [4,5]. 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 [6]. 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.

The present paper briefly reviews the important progress that has been accomplished in the field of platelet-rich preparations in the last few years. The main components of these preparations, the most exciting therapeutic applications and the existing current challenges will be discussed in detailed. Elucidating the molecular complexity of these products, determining the essential growth factors that

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determine the fate of a specific tissue, the criteria to establish the dosing, improving the pharmacokinetics and biodistribution of the released growth factors and developing tailored products for each pathological situation are but a few examples. More detailed and in-depth knowledge will provide novel opportunities for surgery and for accelerating and optimizing the quality of the repair tissues.

2. Role of growth factors in tissue repairing mechanisms

The mechanisms and pathways that govern tissue wound healing and tissue regeneration have been studied in great detail. The cellular and molecular events resulting after a traumatic injury are mostly shared by the different tissues of the body and include early and late inflammation phases, proliferation and migration of cells, angiogenesis, granulation tissue formation and finally matrix formation and remodelling [7,8]. Interestingly, this cascade of events is initiated immediately after injury by the secretion to the local environment of a pool of growth factors, cytokines and proteins from the serum and degranulating platelets. The initial formation of a blood clot characterized by cross-linked fibrin and by a variety of proteins such as vitronectin, fibronectin and thrombospondin prevents further bleeding, provides a barrier against invading pathogens and serves as a matrix for invading cells. In addition, this initial clot acts as a reservoir of growth factors required during the later stages of the healing process [9].

It is assumed that all the phases of tissue repair process are mediated and controlled by a wide range of growth factors and cytokines that modulate cell function through direct physical interactions with extracellular domain of transmembrane receptors. The latter transduce secondary signals, thereby controlling diverse aspects of subcellular biology. 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, platelet-derived growth factor (PDGF) is a powerful mitogen for connective tissue cells [10], transforming growth factor-β (TGF-β) stimulates osteoprogenitor cells to proliferate but also blocks in later stages cell differentiation and mineralization [11], 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 [12].

As a result of these and other discoveries, many growth factors and cytokines are being assayed as therapeutic molecules for the repair or regeneration of a wide range of tissues [13,14]. Nonetheless, according to the limited success of the current efforts, substantial challenges remain. 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.

3. Platelet-rich preparations

Platelets constitute a potential source of multiple autologous growth factors and proteins involved in tissue regeneration. However, although the potential effect of extracts from blood platelets in promoting cell growth was demonstrated 25 years ago [15], the choice of platelets as growth factor reservoir and thus the development of platelet-rich products was somehow fortuitous as the initial interest of platelets was related with the preparation of autologous fibrin glue with haemostatic and adhesive properties [16]. The initial rationale of platelet-rich products was to replace the blood clot with a preparation enriched in platelets which could, once activated, secrete a large pool of proteins and factors including PDGF, TGF-β, VEGF, IGF-I, hepatocyte growth factor (HGF), angiopoietins, platelet factor-4 (PF-4) and thrombospondin among others to the local milieu, driving the tissue regeneration mechanism. By getting rid of erythrocytes and leukocytes, the preparation would take full advantage of the concentrated platelets and the stored growth factors, enabling an accelerated wound healing and tissue regeneration [17]. Interestingly, activation of these preparations exerted via platelet degranulation and growth factor activation are not necessarily dependent events. For example, TGF-β1 is secreted mainly on its latent form and subsequently activated by a furin-like enzyme which is also released from platelets, being thereby platelet degranulation and growth factor activation independent events [18].

One potential advantage of platelet-rich preparations is that they are easily obtained from patient's blood after a simple centrifugation process. By regulating the processing technique, centrifugation parameters and activation protocol, it is possible to control the dose of growth factors and proteins that are released on activation [19]. However, the lack of a suitable standardisation and definition has provoked the appearance of many different platelet-rich products with controversial therapeutic effects. To succeed, it is necessary to optimize and standardize the product, to characterize the main components playing a key role in tissue regeneration and finally formulate a tailored preparation for each specific physiopathological situation.

In the quest for a better platelet-rich product, we developed the preparation rich in growth factors (PRGF) which is characterized by a more sustained release of growth factors as calcium chloride instead of thrombin is used [20], a moderated platelet concentration which has been related with optimal biological benefit [21] and the formation of a three-dimensional fibrin scaffold which maintains the regenerative space and serves as matrix for progenitor cells. 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 [22]. 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.

But what makes PRGF technology different from other platelet-enriched products is its versatility. In fact, four different formulations with therapeutic potential are obtained from the same patient's blood depending on the coagulation and activation degree of the samples (Fig. 1). The formulations include the PRGF supernatant used as conventional eye-drop and cell culture media [23], the liquid PRGF used in surgery and to bio-activate dental implant surfaces by creating a biologically active nano-membrane on the titanium surfaces [24], the scaffold-like PRGF composed of fibrillar and cellular components [25], used in ulcer treatment and tissue engineering approaches and the elastic, dense and haemostatic fibrin which is an excellent tool to seal the post-extraction sockets and to promote the full epithelialization of soft tissues. The notion that these preparations have only cell mitogenic effects is rather simplistic. In fact, the interest on this type of products is increasing as novel potential effects are being discovered. For example, recent reports suggest that PRGF exerts potent angiogenic effects (Fig. 2) [26] and also antibacterial effects against *Staphylococcus aureus* and *Escherichia coli* [27]. Platelet-rich products act also as anti-inflammatory agents by blocking monocyte chemotactic protein-1 (MCP-1) released from the monocytes and lipoxin A4 generation [28]. In addition, setononin, a neurotransmitter and hormone present in platelets, has been reported to directly mediate liver regeneration [29].

4. Combining platelet-rich preparations and biomaterials

The emergence of novel biomaterials is having an enormous impact on medicine. The combination of platelet-rich preparations and biomaterials is opening the door to novel therapeutic alternatives and improving pre-existing ones, increasing the versatility of this type of products (Fig. 3). Additionally, biomaterials may also improve the kinetic properties of the autologous platelet-rich preparations enabling a more slowly release of the stored growth factors.

One potential field of interest is the incorporation of platelet-rich products in bone grafting technology. This development is extremely relevant in different surgical

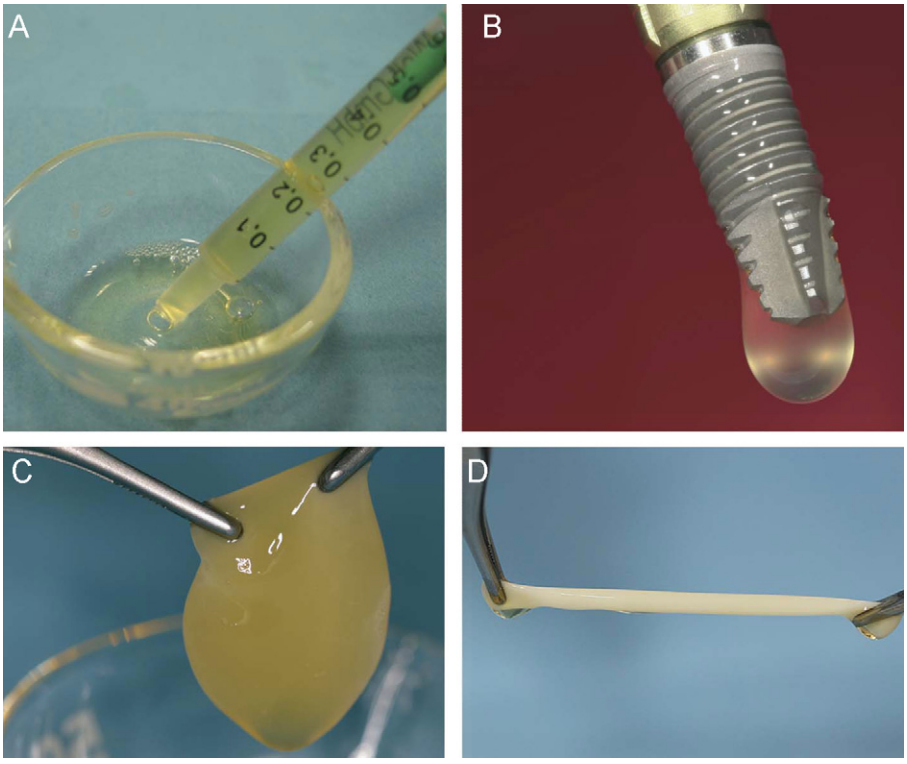

Fig. 1. Versatility of the PRGF technology: (A) the PRGF supernatent; (B) the liquid PRGF used to bio-activate dental implant surfaces; (C) the scaffold-like PRGF composed of fibrillar and cellular components and (D) the elastic, dense and haemostatic fibrin.

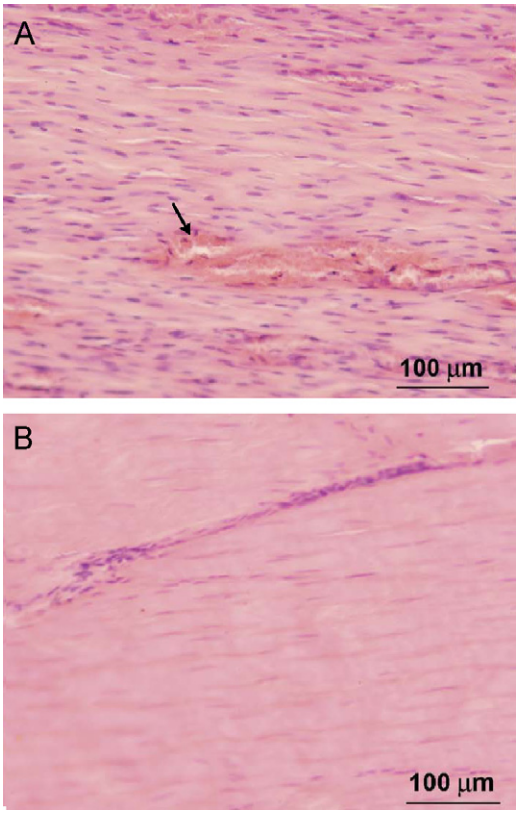

Fig. 2. Angiogenic effects of the PRGF: (A) histological sections of the Achilles tendons treated with PRGF and (B) untreated tendon. Note the presence of blood vessel in the PRGF treated tendon (black arrows) (adapted from Anitua et al. [26], Fig. 6A and D).

fields including orthopaedic and maxillofacial surgery and oral implantology. Actually, massive bone allografts are usually employed in reconstructive surgery to replace missing bone parts such as critical size defects. 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 (Fig. 3). For example, in oral implantology, dentists find difficult and challenging the manipulation and application of some bone augmentation materials such as Bio-Oss® 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 fibrin acts as a biologic glue to hold together the matrix particles. This may have implications for some specific dental surgeries like sinus floor elevation [30].

In the treatment of chronic ulcers, the cavity can be completely filled with activated PRGF allowing the fibrin matrix to develop within the ulcer bed. The effectiveness of the treatment is also determined by the selection of the most suitable dressing. In fact by further exploring PRGF interactions with hydrocolloid or alginate-based dressings, the therapeutic potential of the former may be enhanced, achieving the most effective treatment for each ulcer type.

Another interesting approach is to incorporate, mix or even encapsulate the PRGF on a biomaterial with the aim of altering 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 [31]. By using an acidic gelatin with an isoelectic 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 [32]. Similar approaches have been described using collagen and calcium sulphate as biomaterials [33,34].

Of particular note is the combination of mesemchymal stem cells (MSCs) and scaffold-like platelet-rich preparation for tissue engineering purposes. 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 analyzed and compared with other approaches including the natural deproteinized bovine bone known as Bio-Oss®, autologous bone and the platelet-rich product alone [35]. 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 [36,37]. 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 [38].

5. Therapeutic applications in different medical fields

Preparations enriched in platelets have been studied and applied with different scientific and therapeutic purposes including the treatment of chronic ulcers, bone and soft tissue regeneration in dentistry and oral implantology, treatment of musculoskeletal conditions, surgery and cell culture among others.

5.1. 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

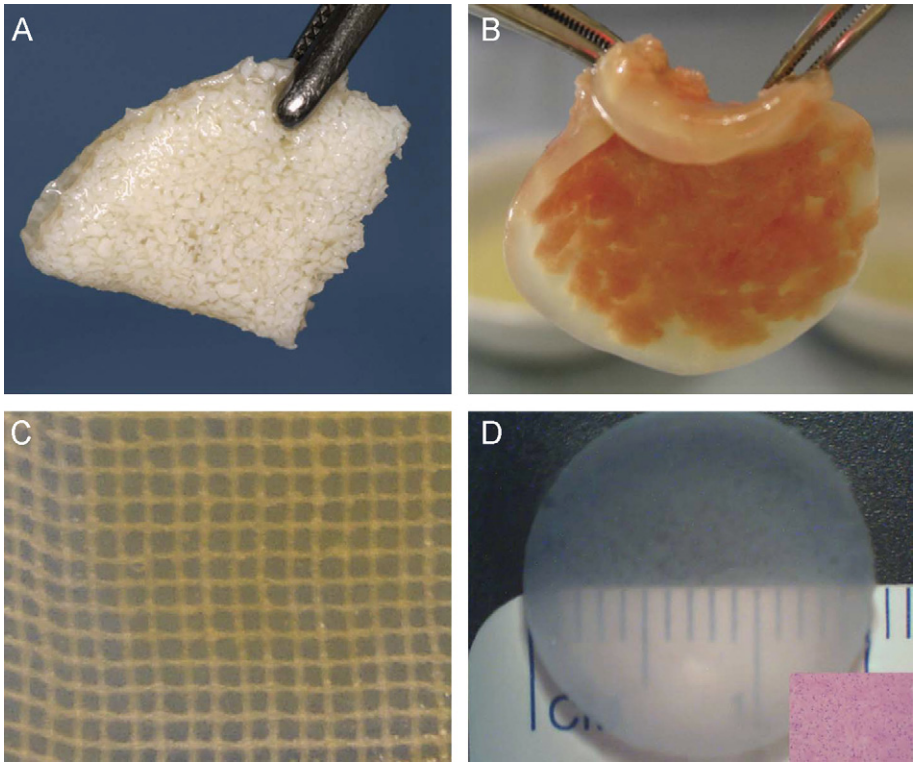

Fig. 3. Combining PRGF and biomaterials: (A) hydroxiapatite aggregated within the scaffold-like PRGF; (B) bone allograft particles embedded in the scaffold-like PRGF; (C) sterile gauze used as scaffold to hold the clotted PRGF and (D) mesemchymal stem cells cultured within a scaffold-like PRGF for tissue engineering purposes.

platelet-secreted proteins [39,40]. This initial product, known as platelet-derived wound healing factors (PDWHF) 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 [41–43].

One major cause for misleading and controversial results in the field might be related to the delivery mode of growth factors and proteins to the injured tissue. For example, Stacey and colleagues reported that topical application of platelet lysates on the ulcers did not influence the healing [44]. 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. 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 [45]. Results showed that at 8 weeks, the mean percentage of surface healed in the PRGF group was $73 \pm 22\%$ whereas it was $21 \pm 34\%$ in the control group ($P < 0.05$). Alternatively in shallow ulcers where topical application of scaffold like PRGF is unpractical, the use of sterile gauzes as scaffold to hold the clotted plasma may be an alternative.

5.2. Bone and soft tissue regeneration

Oral-bone loss caused mainly by periodontal disease is a major public health concern. Approximately 80% of the Americans suffer from some status of periodontal disease which may provoke loss of cementum and supporting bone tissue. Additionally, over 6 million bone fractures are reported annually only in the USA.

Researchers have become excited by the potential therapeutic effects of platelet-rich preparations in accelerating the regeneration of bone and soft tissues (Fig. 4). In a recent study, the addition of platelet-rich preparation to Bio-Oss® significantly increased bone density in non-critical size defects in rabbits [46]. Using a similar approach, Suba and colleagues demonstrated that the addition of platelet-rich plasma to β-tricalcium phosphate significantly increased the bone area percentage 6 weeks after grafting compared with the biomaterial alone (45.9% versus 30.8%). These differences were still significant 12 weeks after grafting (52.5% versus 49.4%) but not after 24 weeks, reinforcing the idea that the main function of platelet-rich products is to accelerate bone regeneration [47]. Interestingly, Kitoh et al. [48] observed that transplantation of bone marrow cells and platelet-rich plasma shortened the treatment period and reduced associated complications by accelerating new bone formation in distraction osteogenesis. Reducing the time needed for optimal bone and soft tissue regeneration will have major

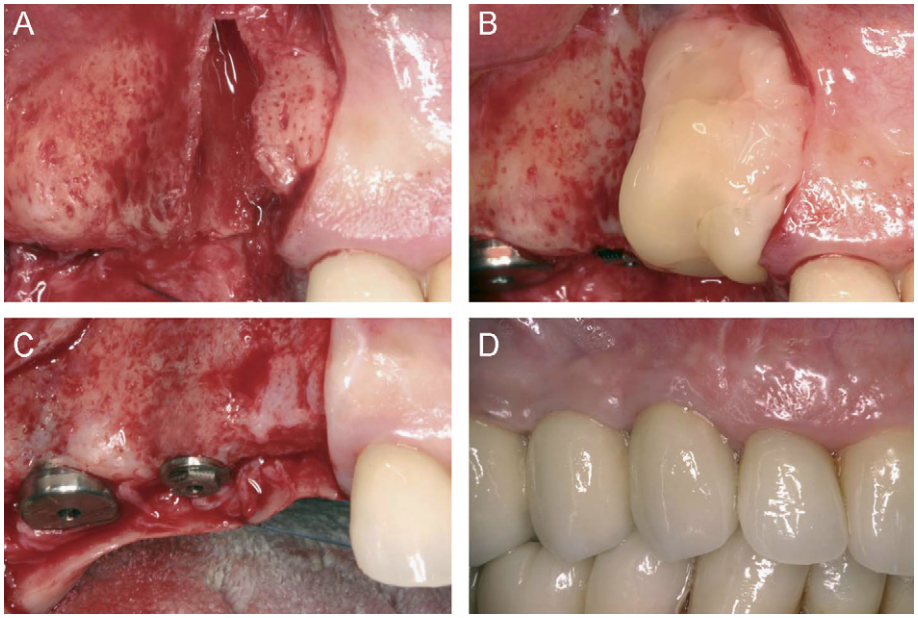

Fig. 4. Vertical fracture of a canine treated with scaffold-like PRGF: (A) image showing the vertical fracture of the canine; (B) the scaffold-like PRGF filling the defect; (C) bone regeneration of the site 12 weeks post-treatment; (D) final restoration.

impact on reducing treatment time and costs, accelerating patient recovery time-period, and improving patient's life quality.

However, despite these encouraging results, much work lies ahead to determine why platelet-rich products are extremely effective in some situations and ineffective in others. The use of standardized preparations, the critical size of the bone defects treated and the exact time when the potential effects are evaluated may be of paramount importance to address this issue.

Another interesting focus of interest lies on the combination of PRGF with dental implants with the aim of facilitating the bone–implant contact of the latter. The development of novel surfaces with increased osseointegration potential is garnering increased attention in the field of oral implantology. The humidification of titanium rough implant surfaces with activated liquid PRGF enables the formation of a biological nano-membrane (bioactivation of dental implants) composed of a fibrin scaffold containing fibronectin, osteonectin and vitronectin which provide specific sites for cell adhesion. Moreover, the release of the large list of growth factors from the activated platelets embedded within the fibrin scaffold will also promote accelerated bone apposition on the implant surface. Recently, we have observed that humidification of dental implants with liquid PRGF enhances the percentage of bone–implant contact in compared with implants without the biological preparation [24]. In addition, explantation of the implants revealed that the whole surface of the PRGF-treated implants was covered by newly formed bone whereas only the upper half was surrounded in control implants.

5.3. Treatment of orthopaedic lesions

Injuries to tendons and ligaments are becoming a widely distributed clinical concern. Studies from primary care show that 16% of the general population suffers with shoulder pain [49] whereas elbow tendinopathy affects 1–2% of the population. Additionally, rates of healing failure of the anterior cruciate ligament (ACL), even with surgical repair, range from 40% to 100%, resulting in medical costs of $1 billion only in the USA. Articular cartilage injuries often occur in conjunction with ACL injuries with symptoms that often cause disability by limiting employment, sports participation and activities of daily living. Assuming this, developing novel therapeutic tools to enhance and accelerate reconstruction and repair of musculoskeletal tissues is challenging.

The use of platelet-rich preparations in this context might be focused on restoring the normal tissue composition while avoiding further degeneration. Recently, we observed that the pool of growth factors released from PRGF increased the proliferation of human tendon cells significantly and stimulated them to produce factors such as 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 [24,26]. Others have reported that injections of platelet-rich plasma 1 week post-operatively increase tendon regenerate strength [50].

The clinical translation of this approach has been demonstrated in striking fashion by our group. As shown in Fig. 5, the activated PRGF is injected among the tendon

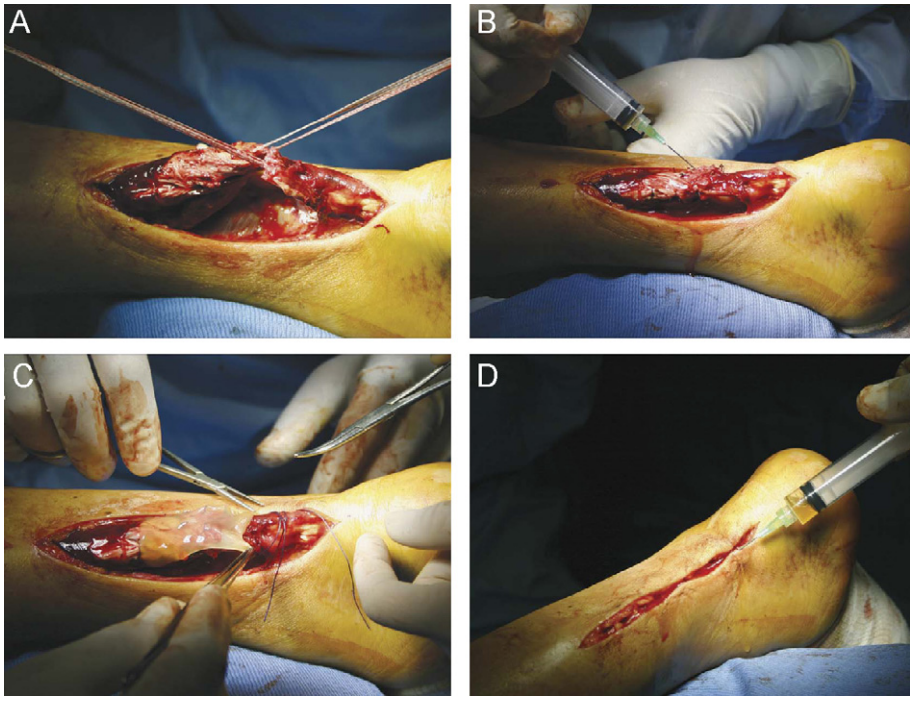

Fig. 5. Surgical procedure for repairing Achilles tendon tears: (A) image showing ruptured ends of the Achilles tendon being approximated and sutured; (B) injection of activated liquid PRGF within the fascicles; (C) affected area being covered with the scaffold-like PRGF prior to closure of the overlying skin and (D) subcutaneous infiltration of activated liquid PRGF before suturing. Adapted from Sánchez et al. [52], Fig. 1.

fibres after the tendon was sutured. After closing the paratenon and before closing the overlying skin, the affected area was covered with the fibrin scaffold. Using this surgical technique in six athletes, we observed a significant acceleration in functional recovery comparing with a matched group that followed conventional surgery [51]. Alternatively, the local delivery of this biological preparation by intratendinous injection under ultrasound guidance has also been proposed and successfully applied in the treatment of elbow tendinosis [52].

Increasing attention has been paid to the development of novel medical tools for repairing ACL injuries. 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 [53–55]. 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 remodelling tissue (Fig. 6). 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 [56]. Aiming to achieve successful fixation of the graft and prompt functional efficacy PRGF is also applied within both femoral and tibial bone tunnels created by the surgeons to secure the ends of the graft.

The treatment of articular injuries affecting avascular connective tissues such as chondral lacerations and meniscus tears is also challenging. The idea of using PRGF intraarticularly in the arthroscopic treatment of an avulsion of articular cartilage in the knee was pioneered by our research group [57]. More recently, the capacity of platelet-rich products to react as a cocktail of molecular cues inducing proliferation and differentiation has been successfully demonstrated [58]. Furthermore, the treatment of full-thickness cartilage defects with PRGF showed enhanced mechanical properties in a rabbit model [59].

Another focus of interest lies on the use of platelet-rich preparations in clinical therapies and surgical interventions. For example, current techniques for perforating calcified subchondral bone leads to poor functional fibrocartilage tissue which in general progresses to osteoarthritis. 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 [60]. Interestingly, the potential of PRGF to enhance the limited capacity of cartilage to repair itself has stimulated the hypothesis of treating degenerative joint conditions with autologous growth factors. In our modest opinion, the idea of improving osteoarthritis joint conditions with PRGF might constitute one of the greatest challenges ever investigated in this field.

5.4. Other therapeutic approaches

The potential therapeutic value and versatility of platelet-rich products has stimulated the research and

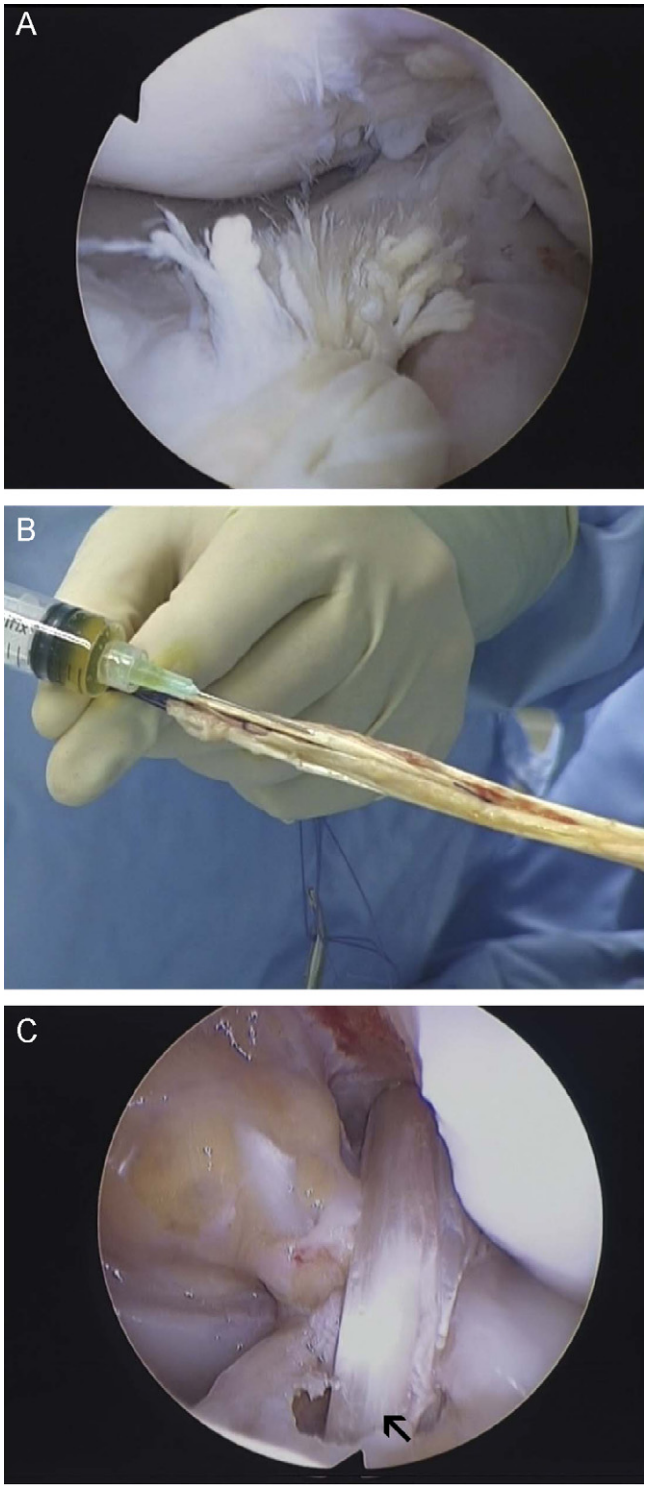

Fig. 6. Surgical procedure for ligament reconstruction: (A) image showing the rupture ends of the anterior cruciate ligament; (B) growth factors are transferred to the substitute tendon graft by injecting "ex-vivo" the activated liquid PRGF and (C) image showing the newly reconstructed ligament (black arrow).

application of this autologous preparation in other medical fields. For example, in the treatment of patients suffering from dry eye symptoms, the use of autologous platelet-rich plasma resulted to be very effective, improving both patient symptoms and major clinical signs [61]. In fact, symptoms improved significantly in 89% of the 18 patients and clear improvement on lachrymal meniscus and conjunctival hyperaemia were also observed. The same group has also reported that platelet-rich plasma promotes healing of dormant corneal ulcers even in eyes threatened by corneal perforation [62].

Other interesting recent approaches using this biotechnology include the successful application of platelet-rich plasma in peripheral nerve regeneration in a rat model [63] and the use of the pool of growth factors released by the platelets as a powerful substitute of foetal calf serum (FCS) in the culture of a wide range of cells, including fibroblasts and osteoblasts [64], mesenchymal stem cells [65], stromal cells [66].

6. Concluding remarks

Platelet-rich preparations represent a new biotechnology for the stimulation and acceleration of soft tissue healing and bone regeneration. The above examples represent some of the most interesting current approaches but authors believe this technology may see new exciting development in the next few decades. To succeed, some of the current challenges need to be addressed including the complete characterization of the platelet released factors and proteins, determining the intra-individual biological reasons that make some platelet-rich products more effective than others and designing novel interactions with biomaterials than might increase the versatility of the technology. These efforts point to a future where tailored platelet-rich products will be used for each specific medical purpose.

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