



Potential of endogenous regenerative technology for *in situ* regenerative medicine

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ABSTRACT

Endogenous regenerative technology (Endoret) involves the use of patient's own biologically active proteins, growth factors and biomaterial scaffolds for therapeutic purposes. This technology provides a new approach for the stimulation and acceleration of tissue healing and bone regeneration. The versatility and biocompatibility of using patient-derived fibrin scaffold as an autologous, biocompatible and biodegradable drug delivery system open the door to a personalized medicine that is currently being used in numerous medical and scientific fields including dentistry, oral implantology, orthopaedics, ulcer treatment, sports medicine and tissue engineering among others. This review discusses the state of the art and new directions in the use of endogenous technology in the repair and regeneration of injured tissues by means of a controlled and local protein and growth factor delivery. The next generations of engineering strategies together with some of the most interesting therapeutic applications are discussed together with the future challenges in the field.

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1. Introduction

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The tissue repair process is a complex cascade of biological events controlled by numerous cytokines and growth factors that provides local signals at sites of injury. Just after a tissue injury occurs, a large number of intercellular and intracellular pathways are activated and

coordinated with the aim of restoring tissue integrity and homeostasis [1]. Cellular and humoral components, the inflammatory pathways and the blood coagulation cascade are activated in addition. A wide range of cells from the injured tissue and adjacent locations undergo marked changes in gene expression and phenotype, leading to cell proliferation, differentiation and migration [2,3]. The development of a new microvasculature and microcirculation is also critical for the homeostasis and correct tissue repair and regeneration. In fact, the absence of a suitable vasculature transporting oxygen, nutrients, soluble growth factors and biologically active proteins and numerous cell types to the injured tissue would imply the degeneration of the latter [1,4].

One pivotal discovery that has fuel the research in regenerative medicine and tissue engineering has been the role that cytokine and growth factors play in the process of tissue repair [5,6]. These molecules provide signals at local injury sites, regulating the mechanisms and pathways that govern wound healing and tissue regeneration [7]. Determining the roles that growth factors play in tissue repair and regeneration is as important as designing, developing and applying suitable formulations that release them with a spatiotemporal control. This is especially important as providing the injured tissue a milieu of biological signals may be desirable for the functional and accelerated repair of the tissue [8–11].

This knowledge has stimulated the rapid progress of tissue engineering. This field aims at repairing and restoring damaged tissue function employing three fundamental “tools”, namely cells, scaffolds and growth factors. [12,13]. The success of this approach relies on the delicate and dynamic interplay among these three components [14,15]. It has been reported that future generation of scaffolds will have to provide adequate mechanical and structural support, be biocompatible and bio-resorbable at a controllable degradation and resorption rate as well as actively guide and control cell attachment, migration, proliferation and differentiation. This may be achieved if the functions of scaffold are extended to supply biological signals able to guide and direct cell function through a combination of cue exposition and cytokine and growth factor controlled delivery [16].

In this review, we summarize the most interesting delivery technologies designed to permit the local and controlled spatiotemporal release of growth factors for tissue repair and regeneration. Especially, the progress that has been accomplished in the field of endogenous regenerative medicine will be described in detail. The use of patient's own growth factors and scaffolds for regenerative medicine will be highlighted, as some of the most interesting therapeutic applications of this technology including the treatment of chronic ulcers, bone and soft tissue regeneration in oral and maxillofacial surgery and oral implantology, the treatment of musculoskeletal injuries and disorders, and the development of tissue-engineered approaches among others.

2. Progress in the field of growth factor delivery

Although the role of all the growth factors involved in tissue regeneration is only partially elucidated, the biological effects on many of them have been understood during the last decade. This has opened the door to the potential evaluation of many different cytokines and growth factors as therapeutics in a wide range of medical disorders and for the repair or regeneration of many tissues [17–21]. Such a strategy comes from the determination that all the phases of tissue repair process are mediated and controlled by a pool of biologically active growth factors 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.

However, the current limited success of the current approaches using growth factors as therapeutics indicates that substantial challenges need to be addressed. 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 [22]. 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 pharmacokinetics [23,24]. Last but not least, the excessive cost of the synthetic growth factors and their immunogenic concerns are also major hurdles in this field.

Several new biomaterials and biomedical technologies are being explored with the aim of providing a control over growth factor release kinetics. One important challenge in the field has been to produce three-dimensional matrices and rendering them deliverable locally through minimally invasive techniques [25,26]. Some of these approaches are based on the combination of the growth factors and autologous, natural or synthetic biomaterials. The combination of polymers and growth factors can provide a controlled release into the local microenvironment to yield desirable concentrations over a period ranging from days to months. The new generation of biomaterials and technologies promises to allow greater control over cell fate and ultimately tissue structure and function. Some examples of polymers used for bioactive factor release include synthetic materials such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and their copolymers (PLGA) [27] and nitrocinnamate-derived polyethylene glycol (PEG-NC) hydrogel systems [28], natural polymers such as alginate [29–31] or gelatin [32] and autologous materials such as fibrin [33].

3. Endogenous regenerative technology (Endoret)

The field of medicine is advancing rapidly towards the development of personalized treatments and less invasive procedures that in general reduce morbidity while enhancing and accelerating functional recovery. These simple and cost efficient procedures may have a potential impact in reducing the economic costs for standard medical treatments. The use of endogenous growth factors and proteins for repair and regenerative purposes has opened a new way of understanding the regenerative medicine [22,34].

In the last few years, the concept of endogenous regenerative medicine is gaining the attention of scientists. The former involves using plasma and platelet-derived cytokines and biologically active factors as well as the naturally formed fibrin scaffold from patient's fluids. This technology is an evolution of the pioneering studies reported by Anitua [35], in which a 100% autologous preparation rich in biological mediators was reported for the first time in the literature as a new therapeutic tool to promote bone and soft tissue regeneration.

One pivotal aspect for the development of this technology has been the deep platelet characterization that has been realized in order to determine the most important growth factors and proteins contained within these cells but also the protocols that facilitate the manipulation and concentration of the cells safely [36,37].

3.1. The role of platelets: reservoirs of growth factors

Platelets prevent blood loss at sites of vascular injury by adhering, aggregating and creating a procoagulant surface that stimulates thrombin generation and fibrin formation. Recently, it has been reported that they play additional roles including promotion of tissue repair and regeneration, vascular remodelling and mediators in the inflammatory and immune responses [36,38,39]. Platelets release a pool of biologically active proteins and other substances that enable them to influence a range of processes promoting the recruitment, growth and

morphogenesis of cells. Such substances are either released or presented on the activated platelet surface or attached to the fibrin scaffold that is formed once the coagulation cascade is activated. As a consequence, the pool of growth factors and cytokines creates chemotactic gradients facilitating cell recruitment and constitute a storage pool that can be secondarily released by matrix metalloproteases (MMPs) active in the matrix. Most of the growth factors content is stored in the alpha-granules of the platelets. Upon activation of the platelets with calcium, proteins will be secreted by exocytosis by the formation of secretory vesicles that fuse with the plasma membrane allowing the liberation of their contents to the milieu [40]. Some of the most relevant proteins stored in platelet's alpha-granules are summarized in Table 1. Newly synthesized metabolites are released by diffusion across the membrane, while the activated platelet provides a catalytic surface for thrombin generation as well as releasing procoagulant microparticles by a process that has been related to apoptosis [41].

The large list of biological mediators stored in platelets includes for example the adhesive proteins fibrinogen, fibronectin, vitronectin and thrombospondin-1 (TSP-1). A subpopulation of these adhesive proteins becomes attached to platelet receptors during secretion. Fibrinogen may act as a mitogen, being first shown to potentiate the effect of interleukin-3 (IL-3) on human haematopoietic progenitor cells [42]. It also participates in wound repair and it promotes the mitogenic activity of PDGF [43]. Fibronectin mediates adhesive interactions and plays a central role in osteoblast survival, proliferation, differentiation, and matrix mineralization, as well as in bone formation [44–46].

Another protein from platelets able to form a complex with plasminogen and anchor it to collagen is osteonectin, a protein also secreted by osteoblasts [47]. Osteocalcin (also called bone Gla protein) is a low molecular weight vitamin K-dependent protein abundantly found in bone matrix [48,49]. Although previous studies report that osteocalcin was exclusively synthesized by osteoblasts and odontoblasts, other investigations showed that osteocalcin mRNA is not restricted to cells of mineralized tissues, but is also found in megakaryocytes and peripheral blood platelets [50]. It has been proposed that osteocalcin may act either as a cytokine or as a chemoattractant for osteoblasts, osteoclasts, and blood monocytes [51,52].

Among the stored growth factors essential for wound repair are platelet-derived growth factor (PDGF) with the -AB and -C isoforms predominating in platelets. In addition, transforming growth factor beta1 (TGFbeta1), vascular endothelial growth factor (VEGF, essentially VEGF-A), basic fibroblast growth factor (bFGF, also known as

FGF-2), hepatocyte growth factor (HGF), epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) are also present in platelets and their biological roles have been fully investigated [53–65].

PDGF is a powerful chemoattractant and stimulator of cell proliferation [66]. TGF-beta1 is a 2-chain polypeptide and is abundant in platelets as well as in bone. TGF-beta family members are important in wound repair and scarring. TGF-beta1 function is regulated by its activation from a secreted latent form; it may negatively influence angiogenesis although it promotes production of matrix proteins [67]. TGF-beta1 can promote connective tissue production by fibroblasts and stimulate tendon cells to produce VEGF [68] (25).

Last but not least, VEGF constitutes a family of proteins that act through a receptor of the kinase family expressed in endothelial cells to stimulate blood vessel formation [69]. VEGF exerts trophic effects on endothelial cells [70]. It can also be pro-inflammatory and stimulate the adhesion of leukocytes to endothelial cells.

3.2. Development of the technology for Endoret: from bench to bedside

One major challenge has been to transform the platelet potential into therapeutic formulations that can be handled, studied and administered easily by the scientists and clinicians. In particular, Plasma Rich in Growth Factor (PRGF) technology has enabled to transform the platelet and endogenous fibrin potential into almost 4 different formulations with therapeutic potential [10,22]. Therefore, PRGF technology has favoured the clinical translation of the endogenous regenerative technology from bench to bedside in a simple, easy and predictable way. These personalized formulations are obtained from each patient by simply controlling the elaboration protocol and coagulation degree of the samples (Fig. 1).

One initial formulation obtained from this technology involves the PRGF supernatant composed mainly of plasma and platelet growth factors and proteins and used as conventional eye-drop and cell culture media [71]. If the platelets are maintained in the formulation, a liquid PRGF is obtained. The latter has an initial liquid state but will progress into a 3-Dimensional (3D) fibrin scaffold rich in growth factors and proteins. This property is of major importance to facilitate the administration of the solutions and posterior conversion into a matrix-like structure. Some potential uses of this liquid solution include different modalities of surgeries, tissue infiltrations, bioactivation of dental implants by creating a biologically active nano-membrane on the titanium surfaces [72].

Table 1
Platelet α-granule protein and growth factor contents and their corresponding biological roles.

Classification	Protein	Biological effects
Adhesive proteins	VWF + pro-peptide, Fg, Fn, Vn, TSP-1, laminin-8 (alpha4- and alpha5-laminin subunits), SCUBE 1	Cell contact interactions, homeostasis and clotting, and extracellular matrix composition
Clotting factors and associated proteins	FactorV/Va, FactorXI-like protein, multimerin, protein S, high-molecular weight kininogen, antithrombin III, tissue factor pathway inhibitor (TFPI)1	Thrombin production and its regulation
Fibrinolytic factors and associated proteins	Plasminogen, PAI-1, u-PA, alpha2-antiplasmin, histidine-rich glycoprotein, TAFI, alpha2-macroglobulin	Plasmin production and vascular modelling
Proteases and anti-proteases	Tissue inhibitor of metalloproteinase 1–4 (TIMPs 1–4), metalloproteinase-1, -2, -4, -9, ADAMTS13, TACE, platelet inhibitor of FIX, protease nexin-2, C1 inhibitor, serpin proteinase inhibitor 8, alpha1-antitrypsin	Angiogenesis, vascular modelling, regulation of coagulation, and regulation of cellular behaviour
Growth factors	PDGF, TGF-beta1 and -beta2, EGF, IGF-1, VEGF (A and C), bFGF (FGF-2), HGF, BMP-2, -4, -6, CTGF	Chemotaxis, cell proliferation and differentiation, and angiogenesis
Chemokines, cytokines and others	RANTES, IL-8, MIP-1alpha, ENA-78, MCP-3, GRO-alpha, angiopoietin-1, IGF-BP3, IL-6sR, PF4, beta-TG, platelet basic protein, NAP-2, connective tissue-activating peptide III, HMG1B1, Fasl, LIGHT, TRAIL, SDF-1alpha, endostatins, osteonectin1, bone sialoprotein	Regulation of angiogenesis, vascular modelling, cellular interactions, and bone formation
Anti-microbial proteins	Thrombocidins	Bactericidal and fungicidal properties
Others	Chondroitin 4-sulfate, albumin, immunoglobulins, disabled-2, semaphorin 3A, PrPC	

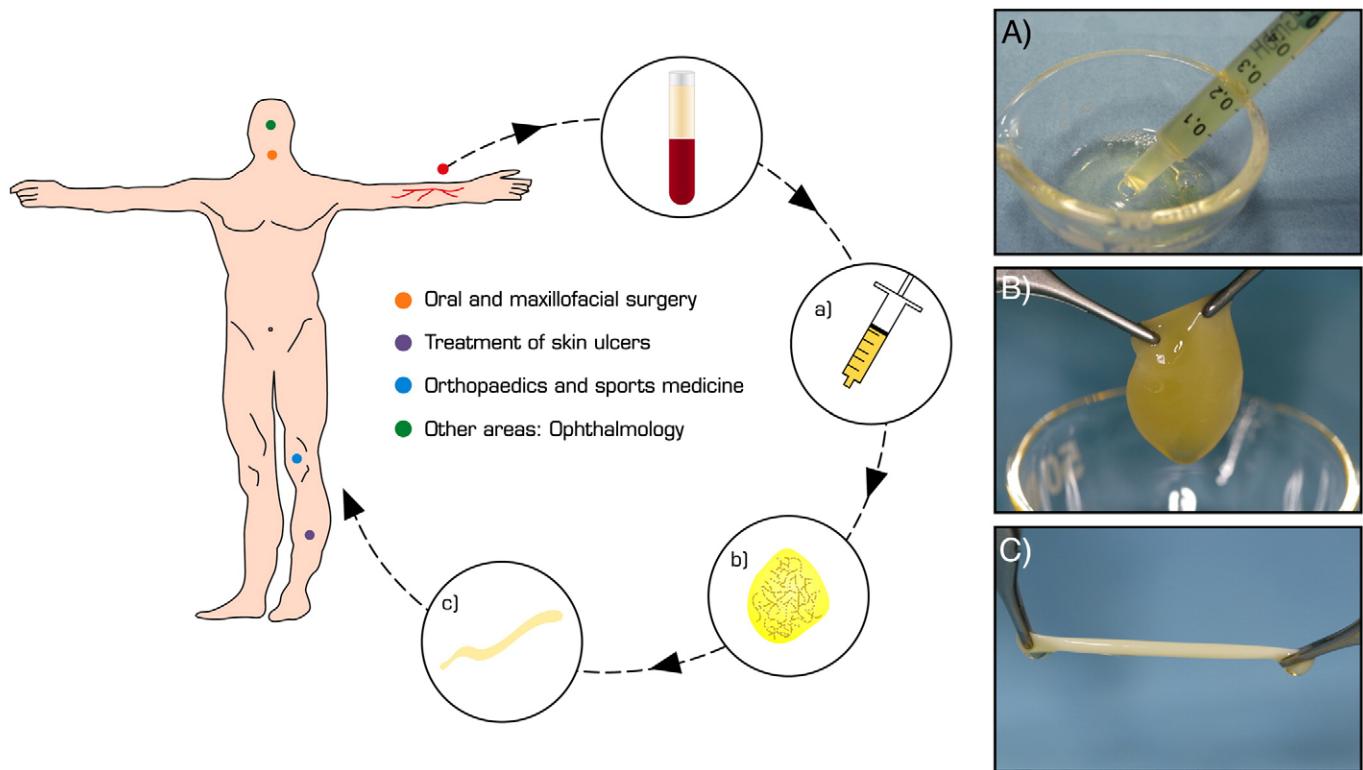


Fig. 1. The concept of endogenous regenerative technology consists on developing different therapeutic formulations obtained from the patient. Different formulations can be obtained by simply controlling the elaboration protocol and coagulation degree of the samples. These include A) the liquid plasma rich in growth factors (PRGF), B) the scaffold-like PRGF and C) the elastic and biocompatible fibrin. The versatility and biocompatibility of these endogenous formulations have opened the door to a personalized medicine that is currently being used in numerous medical and scientific fields including dentistry, oral implantology, orthopaedics, ulcer treatment, sports medicine and ophthalmology among others.

The scaffold-like structure is a three-dimensional structure composed of fibrillar and cellular components that can be easily prepared in the lab. Due to its nature, this scaffold can be used in ulcer treatment, regeneration of bone defects, and tissue engineering approaches among others. Finally, once the scaffold is retracted a biocompatible, elastic, dense and haemostatic fibrin material can be prepared. This natural biomaterial is biologically active as different platelet-derived growth factors and proteins are enclosed within the fibrin mesh providing an excellent tool to seal surgical defects while promoting the full epithelialization of soft tissues.

The versatility of these biological formulations can be widened when they combine with autologous tissues or biomaterials. For example, many biomaterials may offer increased stability and strength to the PRGF scaffold leading to an increased mechanical stability but at the same time the PRGF may facilitate the handling and manipulation of a large number of polymers. For example, in oral implantology, dentists find the manipulation and application of some bone augmentation materials and even autologous bone difficult and challenging. 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 as a biologic glue to hold together the matrix particles.

These endogenous formulations can also be combined with additional natural or synthetic biomaterials to provide a more tight control over growth factor pharmacokinetics and biodistribution. For instance, 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 [73]. Similar approaches have been described using collagen, calcium sulfate and alginate as biomaterials [74–76].

One major breakthrough of this technology is the possibility to obtain totally biocompatible, autologous and personalized formulations. This is in part only possible because calcium is used to activate the coagulation cascade and thus the release of growth factors and the formation of the 3D fibrin scaffold. Therefore, potentially toxic substances like bovine thrombin are avoided. In addition, calcium provides a reduced burst effect in comparison with thrombin, facilitating a more progressive growth factor release [77]. Interestingly, the dose of platelets in these endogenous formulations has been increased only 2-fold compared with platelet concentration in whole blood. This slight increase has been related with optimal biological benefit [78]. Leukocytes are not included in the different plasma formulations. It has been reported that the pro-inflammatory agents such as metalloproteases and the acid hydrolases contained in white blood cells may provoke negative tissue destroying effects [79].

3.3. Creating endogenous tissue-engineered approaches

Isolated cells, growth factors and biocompatible supporting scaffolds have generally been considered essential prerequisites to tissue engineering approaches. The endogenous PRGF formulations provide the basis of a 3D fibrin structure which maintains the regenerative space and serves as matrix for progenitor cells. This may facilitate its combination with somatic cells or mesenchymal or stromal stem cells for tissue engineering purposes. Ideally, if a tissue engineering approach that restores a bone defect is pretended, several key elements need to be addressed [80]: i) seed cells with a high osteogenic potential, ii) growth factors and iii) a three-dimensional scaffold which gives the construct sufficient mechanical properties for loading and facilitates vascularization. For the ideal construct, the seed cells should be autologous and easy to obtain with minimal donor site morbidity [81,82]; the osteogenic growth factor should be

easily produced with very low cost and poor immunogenicity; and the scaffold should be biodegradable and derived from homologous materials.

In the past few years, several research groups have explored the feasibility of endogenous fibrin matrices as tissue engineering scaffolds. For example, Ito and co-workers studied the potential of combining mesenchymal stem cells (MSCs) and a plasma-derived formulation compared with autogenous bone, a bone graft substitute and plasma-derived formulation alone to increase the rate of bone formation in mandible defects of dogs. The mixture of MSCs and a plasma-derived formulation provided greater bone maturation and early-stage bone regeneration, as shown by both histological examination and the testing of mechanical properties compared with the rest of the treatments [83]. Another group reported that this combination of endogenous growth factors, scaffolds and stem cells induced well-formed mature bone and neovascularization compared with a control group. Furthermore, their data demonstrated that fibrin scaffold enabled MSC proliferation without deforming cell structure, and that it was an excellent vehicle to hold and deliver cells to correct or reconstruct bone defects in a clinical setting [84]. In fact, this tissue engineering approach has successfully been applied for alveolar bone augmentation, with the simultaneous placement of implants in three human patients [85].

Recently, another interesting approach of tissue engineering using patient's own cells, growth factors and scaffold has been reported. This approach involves human adipose-derived stromal cells as seed cells, which can be easily obtained with large quantities and least donor site morbidity, facilitating its autologous application and the pool of growth factors and fibrin scaffold obtained from patient's plasma [86]. Results revealed that this tissue-engineered approach

formed bone structure in heterotopic site of nude mice. Furthermore, in normal healing wounds, adipose stem cells appear to enhance the healing process only when provided in a fibrin gel vehicle containing a number of complementary platelet-derived wound-healing trophic factors [87].

4. Therapeutic applications of endogenous regenerative technology

The ability to prepare an autologous source of growth factors and biocompatible fibrin scaffolds has stimulated the research and use of this technology in many different medical fields including dentistry, oral implantology, orthopaedics, ulcer treatment and eye disorders among others (Fig. 2). The next sections review some of the most interesting results of applying endogenous regenerative technology.

4.1. Oral and maxillofacial surgery and oral implantology

4.1.1. Bone regeneration: *in vitro* and *in vivo* results

Some of the initial evidences of the biological potential of the platelet-derived growth factors were observed in primary cultures of human osteoblasts and periodontally related cells [88,89]. The proliferation induced was significantly higher when compared with the effects exerted by the growth factors alone and even with a combination of them. Others have also observed that growth factors derived from platelets can stimulate the proliferation of different cells including human trabecular bone cells, human osteoblast-like cells, human stromal stem cells and human mesenchymal stem cells [90–94]. Recently, we evaluated the effects of PRGF supernatants for the expansion of human tenocytes [71,95]. Results showed that PRGF contains a pool of growth factors that significantly stimulated the

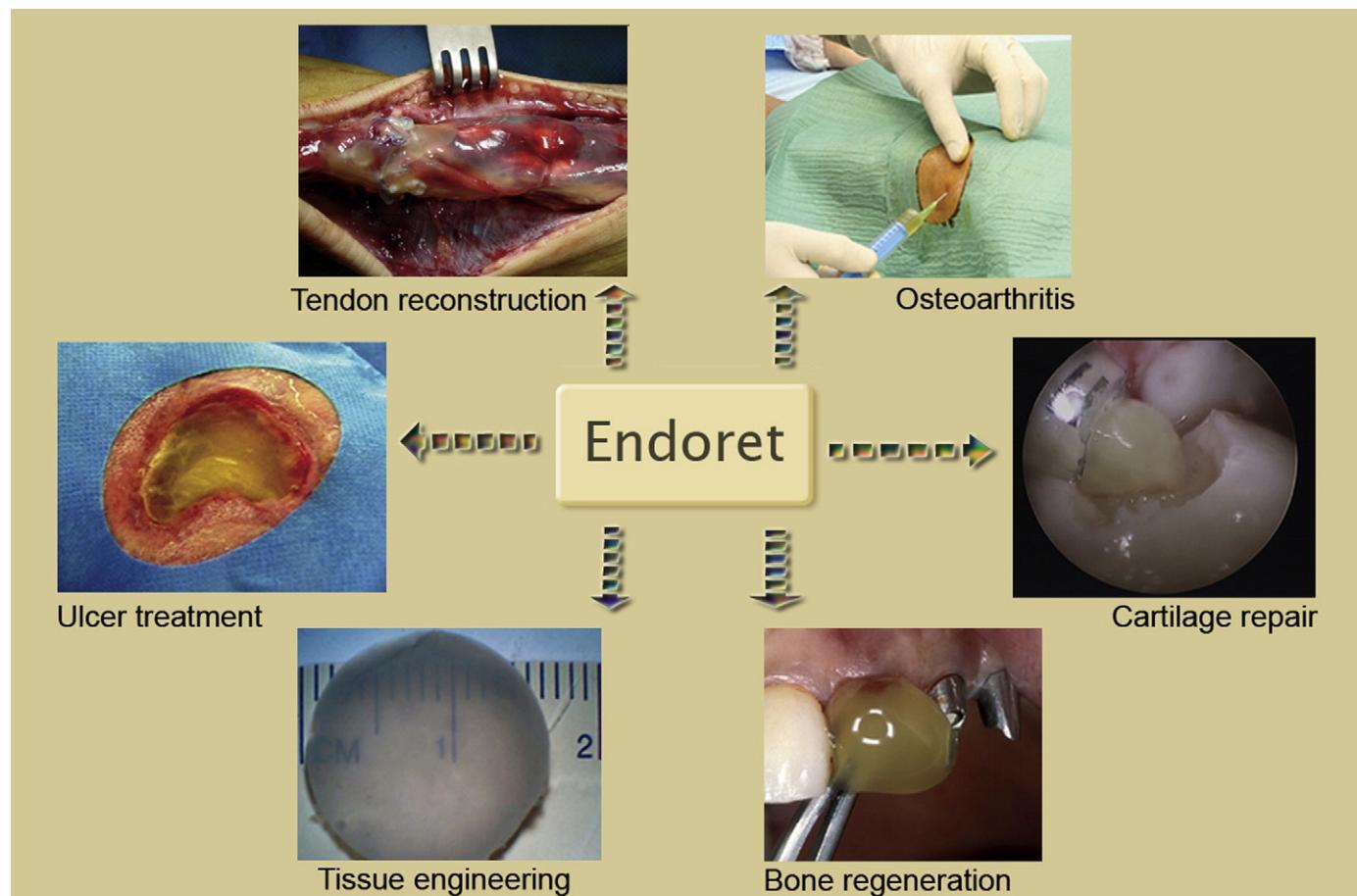


Fig. 2. Some examples of the therapeutic potential of endogenous regenerative technology.

proliferation of tenocytes and induced the paracrine secretion of both VEGF and HGF. The latter is particularly important as these agents play an active role in angiogenesis and as an anti-fibrotic molecule respectively. Another important consideration of this treatment is that cells return to their normal fate of proliferation once the platelet-derived growth factors are withdrawn, which is an obligatory biosafety requirement if the cells are to be transplanted into humans. Furthermore, potent angiogenic and mitogenic effects were observed *in vivo* [95].

Recent evidences support the use of endogenous liquid and scaffold-like formulation for bone to accelerate and promote bone regeneration and faster osseointegration of dental implants. In a set of experiments, artificial defects made in goat's tibiae were carefully filled with scaffold-like PRGF or blood (used as control). The histological analysis of the biopsies at 8 weeks revealed mature bone trabeculae when the endogenous fibrin scaffold rich in growth factors was used to fill the artificial defects and connective tissue with incipient signs of bone formation in the control group [96]. In a second study the potential of the endogenous liquid formulation rich in biological mediators to promote dental implant osseointegration was analyzed. Twenty six dental implants (13 humidified with the liquid biological solution and 13 without the formulation) were placed in the tibiae of goats. Histological and histomorphometrical results demonstrated that bioactivation of dental implants, that is, humidification of implant surface with the endogenous formulation increased the percentage of bone-implant contact in 84.7% [96]. Moreover, the whole surface of the implants treated with the biological solution was covered by newly formed bone whereas only the upper half was surrounded in control implants (Fig. 3). This liquid formulation is a biological tool to humidify dental implants before their insertion as the bioactivatable surface accelerates dental implant osseointegration. These effects can be explained in part by the polarity of the titanium surface and the negatively charged proteins present in the liquid PRGF such as vitronectin and fibronectin. The liquid PRGF may be adsorbed on the implant's surface, providing specific sites for cell adhesion. Fibronectin, is a well known adhesive protein which will enhance the formation of focal adhesions by osteoblasts [98,99].

4.1.2. Bone regeneration: clinical experience

The pioneering report that translated the potential of endogenous regenerative technology into the clinics was reported in 1999. That initial study involved 20 patients who underwent tooth extraction because of periodontal disease or vertical fractures [35]. The use of these 100% autologous formulations improved soft tissue epithelialization and bone regeneration was extensive. The bone tissue of patients receiving PRGF was compact with well-organized trabeculae whereas in the control group only connective tissue and little mature bone were found. The additional experimental evidences suggested potential clinical applications for both the 3D fibrin scaffold and the endogenous liquid for stimulating bone regeneration and bone apposition over implant surfaces. This may result interesting in the treatment of post-extraction defects in dentistry, especially when a complete regeneration of the alveolar bone and surrounding soft tissues is totally necessary to ensure the future success of the implant. In fact, in a recent study in fourteen patients who underwent tooth extraction in defects of different size and anatomical position, the potential role of the endogenous formulation to promote bone and adjacent soft tissue regeneration was evaluated [97]. Fresh extraction sockets from 7 patients were filled with scaffold-like PRGF and sealed with autologous fibrin and compared with control sockets from a matched group of patients in which the endogenous formulations were not used. Bone densitometry was measured in Hounsfields (HU). Results showed that bone density in the inner of the implant bed periphery was significantly higher in PRGF-treated subjects comparing with the control group (638.3 ± 70.8 HU versus 333.3 ± 160.2 HU; $P < 0.05$). Furthermore, filling the defects with the endogenous scaffold

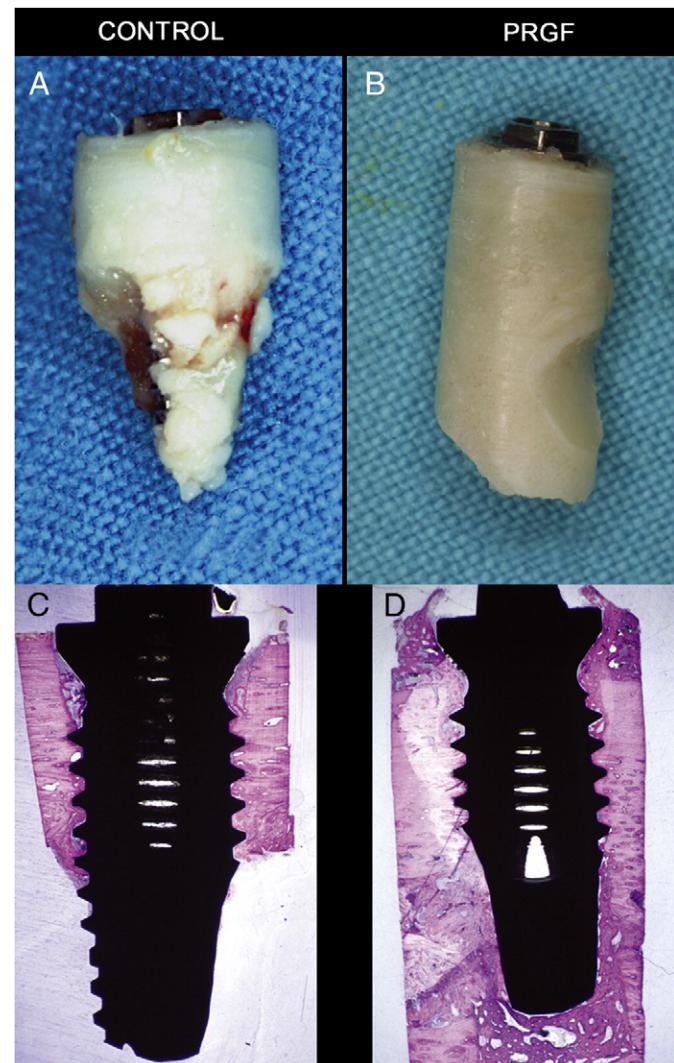


Fig. 3. Evaluation of the bioactivated dental implant surface with liquid PRGF. Representative macroscopic view eight weeks post-insertion of A) a dental implant without the biological solution and B) an implant coated with liquid PRGF. Photographs of the histological sections seen by light microscopy at eight weeks, C) the control implant had many threads exposed whereas D) the regenerated bone covered nearly all the threads in the PRGF-treated implant [96].

increased bone densitometry in more than 180% (565 ± 119.8 HU versus 201.2 ± 141.3 HU; $P < 0.05$).

Currently, the different endogenous formulations are being applied in surgical procedures for bone augmentation in dentistry such as split and crest expansion [100–102]. Another approach in which this technology can be applied with excellent results is the augmentation of the maxillary sinus floor. In general, dental implant insertion in the posterior region of the maxilla is a challenging procedure. Progressive resorption of both horizontal and vertical bone increases the cavity while reducing the thickness of the maxillary sinus floor [103,104]. The absence of upper molars may even increase bone resorption, leading to sinus pneumatization due to the increased osteoclast activity in Schneiderian membrane. These limitations may hamper implant installation and negatively affect successful osseointegration and stability of dental implants. In fact, several studies have reported that a higher rate of implant failures is observed in the upper jaw than other oral regions [105–107]. The most frequently applied procedure to re-establish an adequate bone volume and ridge height in the posterior maxilla is the augmentation of maxillary sinus floor. The latter involves the modification of the sinus cavity with the aim of generating enough bone volume inside a space previously being a

portion of the sinus cavity. In this approach the benefits derived from the use of the endogenous regenerative technology are many [108]. For example, the sinus lateral approach involves the separation of a sinus bone window which will be placed in its original anatomic position in a posterior phase of the surgery. Placing this bone window in the pool of biologically active growth factors may maintain the viability and functionality of the bone tissue. In the same approach, the autologous and biocompatible fibrin scaffold may be used as autologous sealant biomaterial in the case of Schneiderian membrane perforation. Furthermore, this scaffold can be easily combined with any bone augmentation biomaterial to create the final graft. This mixture facilitates the manipulation and administration of the graft, increasing the biosafety of the approach [108].

To properly evaluate the role of the Endoret in sinus floor augmentation, we carried out another case-series study in which the potential effect of the endogenous formulations was analyzed in 5 consecutive patients in which bilateral sinus lift augmentation was carried out. In fact, five patients received bilateral sinus floor augmentation, placing Endoret in one side and using the other as control [109]. Results showed that Endoret facilitated the surgical approach of sinus floor elevation. The control area was more inflamed than the area treated with the endogenous formulations. Patients referred also to an increased sensation of pain in the control area. The areas treated with the pool of autologous growth factors had more new vital bone than controls. In one of the patients, image processing revealed 21.4% new vital bone in the Endoret-treated area versus 8.4% in the control area whereas in another patient, 28.4% new vital bone was quantified in the Endoret-treated area compared with the 8.2% of the control side. The immunohistochemical analysis of the biopsies revealed that the number of blood vessels per mm² of connective tissue was 116 vessels in the sample treated with autologous growth factors and fibrin scaffold versus 7 in the control biopsy [109] (Fig. 4).

4.1.3. Improving implant stability and predictability

Another area of particular interest for the dentists is the concept of bioactivating dental implants with the liquid formulation to provide faster osseointegration and implant stability. Recent studies showed that after evaluating more than 5700 implants in 1060 patients, the survival of the biologically activated implants was superior to 99.2% [110]. In another intriguing 5-year retrospective study, more than 530 short implants humidified with the endogenous liquid solution were evaluated leading to a final survival rate of 99.2% [111].

The capacity of this growth factor rich solution to increase implant osseointegration is of special interest in one of the emerging protocols in dentistry, that is, the immediately loading of implants. This approach can be defined as a situation where the superstructure is attached to the implants no later than 72 h after surgery and the occlusion with the teeth of the opposite jaw [112,113]. Some potential advantages of immediately loading include the reduction of the number of surgical procedures and the total rehabilitation time, the absence of a temporary prosthesis between surgery and prosthetic rehabilitation and increased patient satisfaction [114–116]. Using an endogenous regenerative approach the survival of more than 1130 immediately loaded bioactivated implants was 99.3% [117].

4.2. Treatment of skin lesions: ulcers

More than 20.8 million persons in the US have diabetes mellitus; 2002 data estimates from the Centers for Disease Control and Prevention indicate that 82,000 lower-limb amputations were performed in persons with diabetes [118]. Characteristic pathological changes attributed to autonomic and sensory neuropathy, often combined with vascular disease, lead to a high-risk situation for the person with diabetes [119,120]. Persons who have had such pathology and experience trauma or infection are at high risk for developing ulceration of the foot or ankle. One study documented the causal

pathway of an amputation and found that in 81% of cases faulty wound healing contributed to amputation [121]. Healthcare practitioners should utilize wound treatments that can reduce the rate of faulty wound healing; thus, preventing amputations.

According to the American Diabetes Association, more than 60% of nontraumatic lower-limb amputations occur in people with diabetes; the rate of amputation for people with diabetes is 10 times higher than for people without diabetes. Nonhealing diabetic foot ulcers and the resulting potential amputations present significant costs to the healthcare system and reduce patient quality of life.

The goal of diabetic foot ulcer treatment is to obtain wound closure as expeditiously as possible. Accepted therapeutic objectives and standards of care for diabetic foot ulcers include wound debridement, pressure relief in the wound area, appropriate wound management (e.g., moist wound healing), infection management, ischemia management, medical management of comorbidities, and surgical management as needed. Platelet and plasma-derived proteins and growth factors have been used to treat wounds since 1985. In vivo prospective controlled studies as well as retrospective and cost effectiveness studies documenting the effect of this therapy have been published [122–129].

In 2001, an intriguing retrospective study analyzing the treatment results of 26,599 patients with diabetic neuropathic foot ulcers who had been treated with an autologous platelet releasate. The results suggest that platelet releasate provided with standardized care was more effective than standard care alone [130].

In a recent prospective, randomized, controlled, blinded, and multicenter clinical study the safety and efficacy of a platelet-derived formulation was evaluated for the treatment of nonhealing diabetic foot ulcers. 129 patients were recruited and randomized into two groups: the treatment group with the biological formulation or the control (saline gel). Results showed that 81.3% of the ulcers treated with the platelet-derived formulation and 42.1% of the control wounds healed [131]. Although authors from this study did not report side effects, the bovine thrombin used in this trial as platelet activator is considered a safety concern and it is almost prohibited in many countries worldwide.

To address this issue, we reported a new procedure to apply a 100% autologous formulation rich in growth factors. This protocol consisted on coagulating the endogenous formulation rich in platelets *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 [132]. Results showed that at 8 weeks, the mean percentage of surface healed in the group treated with the biological formulation was 73% whereas it was 21% in the control group.

4.3. Orthopaedics and sports medicine

The biosafety, versatility and biocompatibility of the Endoret have fueled the interest of sports physicians and orthopaedic surgeons. The following examples represent some of the most interesting current approaches in the treatment of acute and chronic sports injuries.

4.3.1. Tendon injuries

Injuries to tendons are becoming a widely distributed clinical concern. Studies from primary care show that 16% of the general population suffers with shoulder pain [133] whereas elbow tendinopathy affects 1–2% of the population. Assuming this, developing novel therapeutic tools to enhance and accelerate reconstruction and repair of musculoskeletal tissues is challenging. It has been reported that some growth factors can stimulate tendon repair after exogenous local application [134]. Recently, we reported that the pool of growth factors released from the endogenous 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

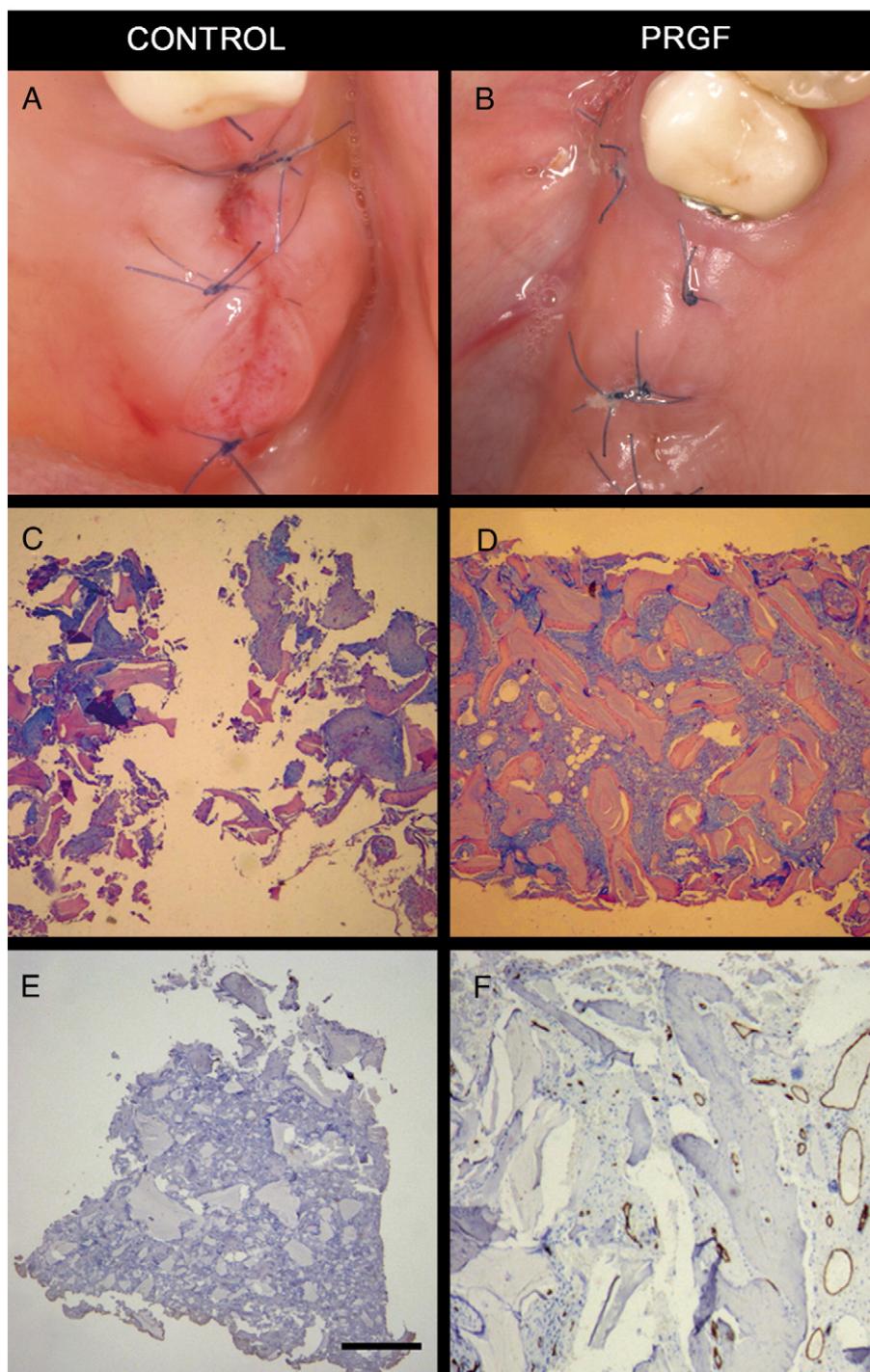


Fig. 4. Evaluation of the bone regenerative potential of PRGF technology. Bilateral sinus floor augmentation testing PRGF on one side and using the other as control. PRGF reduces the inflammatory level of the treated side. Soft tissue is more inflamed in the control area (A) than in the PRGF-treated area (B). Histological biopsies of each sinus obtained 5 months post-surgery. Note that the PRGF-treated biopsy (D) has more new bone and are more compact than the control (C). Biopsies are prepared using haematoxylin–eosin and Alcian blue staining. Scale bar: 500 µm. C,D) Immunohistochemical examination of vascular presence: E) control biopsy and F) sinus treated with PRGF [109].

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 [95]. Others have reported that injections of platelet rich plasma one week postoperatively increase tendon regenerate strength [135].

One typical problem in orthopaedic sports medicine is tendinopathy. It is considered as a syndrome characterized by tendon pain, localized tenderness and swelling that impair performance. Patellar tendinopathies are often associated with jumping sports such as basketball, volleyball and high jump [136] while tennis players and

golfers are more prone to medial and lateral epicondylitis [137]. The rationale of using Endoret in this physiopathological context may be related with the potential of autologous growth factors on restoring the normal tissue composition. Using ultrasound-guided injection of the platelet-derived solutions may offer an alternative treatment over palliative or operative treatments [138,139].

4.3.2. Joint injuries

It has been reported that 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 [140–142]. 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. Our group reported for the first time the use of a bioactive graft composed of the endogenous growth factors and fibrin scaffold. The latter will act as slow protein delivery system placed in the injured tissue. The released growth factors will provide the necessary biological cues for cell migration, proliferation, angiogenesis and remodelling [143] (Fig. 5). The autologous technology is also being 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 patient's own proteins intraarticularly in the arthroscopic treatment of an avulsion of articular cartilage in the knee was pioneered by our research group [144].

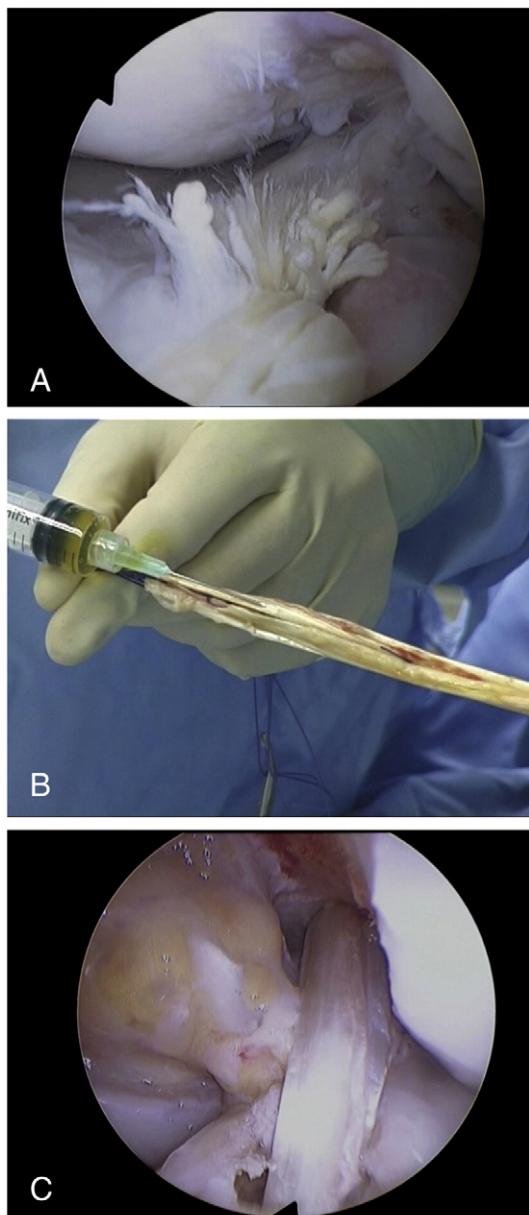


Fig. 5. Use of PRGF during ligament reconstruction in the knee. A) Image showing the rupture ends of the anterior cruciate ligament; B) PRGF is injected within a substitute graft tendon to enhance biological incorporation of the graft; C) PRGF has been introduced into the tibial and femoral tunnels during tendon graft fixing aiming to accelerate the subsequent healing of the tunnel.

Damage to the knee in an early stage of life can lead to osteoarthritis in a later stage, which is called post-traumatic or secondary osteoarthritis. It has been reported that >80% of American football players with a previous knee injury had evidence of osteoarthritis 10–30 years after retiring from competition [145]. Premature osteoarthritis is also a serious concern in the growing community of 'baby boomers' and recreational athletes who are too young for knee replacements. The preliminary clinical results showed that intra-articular injection of the autologous growth factors may be a new therapeutic option for osteoarthritis treatment in selected patients [146,147].

4.3.3. Muscle injuries

Treatment of muscle injuries usually implies physiotherapy, which uses physical modalities such as ice, electrotherapy, massage, mobilization, manipulation and exercise to optimize the healing process. The use of patient's own proteins is an alternative to conventional approaches, as the pool of biologically active mediators may accelerate muscle healing, reducing patient's injury time. In this approach the early blood clot is substituted with the platelet and fibrin scaffold, which maintains the regenerative space and provides supraphysiological concentrations of healing factors. In one study, after ultrasound-guided injections of PRGF in 22 muscle injuries of 20 high-level professional athletes, full recovery of functional capabilities was restored in as early as half of the expected recovery time [148].

4.4. Use of Endoret in other medical areas

The correct formulation of the endogenous growth factors enables the development of autologous eye-drops that can be used in many eye disorders. For example, in the treatment of patients suffering from dry eye symptoms, the use of autologous liquid solution rich in growth factors resulted to be very effective, improving both patient symptoms and major clinical signs [149]. In fact, symptoms improved significantly in 89% of the 18 patients and clear improvement on lachrymal meniscus and conjunctival hyperaemia were also observed. In addition, it has been reported that the autologous eye-drop promotes healing of dormant corneal ulcers even in eyes threatened by corneal perforation [150].

Another interesting approach of this technology is the use of platelet-derived growth factor for nerve regeneration purposes. Promising results were recently reported in peripheral nerve regeneration in a rat model [151]. In addition, its potential use as an adjunct to the staged mucosal advancement flap in the treatment of peri-anal fistulae has been evaluated. Ten patients with fistula tracts transversing from the middle third or upper part of the anal sphincter were treated for at least three months with non-cutting setons prior to definitive closure by the platelet-derived proteins [152]. Results showed that using endogenous proteins and fibrin scaffolds as an adjunct to staged mucosal advancement flap for the treatment of peri-anal cryptoglandular fistulae is a promising treatment modality and seems to establish a high healing rate. Last but not least, the use of this technology in burn wounds is being explored [153].

5. Concluding remarks

The use of endogenous proteins and biomaterials for therapeutics is revolutionizing many areas of medicine and surgery. The capacity to apply different autologous formulations with biological effects into the injured tissue represents a major advance in the concept of personalized medicine. The above examples represent some of the most interesting current approaches but authors believe this technology may see new exciting developments in the next few decades. To succeed, our group and others are actively working on characterizing the platelet secretome, improving the way to administer the pool of growth factors and designing novel interactions with biomaterials than might increase the versatility of the technology.

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