Platelet Rich Plasma (PRP) Biotechnology: Concepts and Therapeutic Applications in Orthopedics and Sports Medicine

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1. Introduction

Regenerative medicine is the augmentation or substitution of diseased or injured cells or tissues by one of two means: (1) an improvement in the ability of endogenous cells to reform damaged tissue or (2) the use of exogenous cells or tissues to replace damaged cells or tissues. Advances in regenerative medicine essentially depend on improving our understanding of cell biology and molecular signaling. Cell signaling is complex and incompletely understood due to the multiple interactions and cross-talk among system components. The human body has some 100 trillion cells, which in the healthy state coordinate their actions through an exchange of chemical signals to maintain body homeostasis. Every cell phenotype secretes signaling proteins that influence their own behavior (autocrine) or the behavior of other neighboring cells (paracrine) through interactions with specific transmembrane receptors located in the cellular membrane. Currently, a great deal of research is directed towards improving our understanding of intercellular communication and the intracellular transduction of these signals; in the field of regenerative medicine, this knowledge will help to disentangle the mysteries of tissue repair and to achieve proper tissue repair and regeneration. Moreover, to reach this goal we must integrate all the information and understanding derived from basic research into novel therapies that yield quicker and more efficient tissue regeneration.

Within the last decade, the development of platelet-rich plasma (PRP) technology has emerged. The impact of the discoveries regarding the potential of PRP healing has fueled the optimism about autologous regenerative medicine. Indeed, the emergence and application of PRP technology, i.e., autologous molecular pool, has revolutionized the field of regenerative medicine in part due to the repair capacities of growth factors (GFs) and cytokines secreted by platelets. The easy preparation protocols, biosafety and versatility of PRP preparations have stimulated translational research and interest by both the scientific and medical communities. PRP therapies represent a major breakthrough in the treatment of many medical conditions and are currently one of the hottest topics in regenerative medicine because of their important implications for our future health. Discovery and contributions in the field have not only improved the clinical treatment of many patients

with different clinical conditions but, from a multimolecular perspective, have opened the field of PRP science to cellular and molecular exploration of healing mechanisms. This technology provides the opportunity of moving molecular knowledge off the shelves and into practice, making it relevant in a clinical context, and achieves a true marriage between what we have learned through research and clinical applications.

This chapter will provide an overview of the potential therapeutic use of platelets and plasma for the release of signaling proteins in regenerative medicine. For the purposes of this chapter, the basic principles of healing and the role of platelets as molecular reservoirs will be discussed. A detailed description of the potential technological relevance of PRP biotechnology is followed by a section on applications of PRP therapies in numerous clinical conditions and medical fields with a special emphasis on orthopedics and sports medicine. There is no question that the key to both future advances in PRP science and its application in the treatment of disease and trauma lies in a better understanding of repair processes.

2. A picture of healing mechanisms

The most effective way to improve tissue repair is to understand normal healing mechanisms after a perturbation due to disease, which then becomes the basis for improving patient care and health. Healing mechanisms are, to a great extent, shared by the different tissues of the body and can be depicted by overlapping and successive phases characterized by a preponderance of cell signaling from various systems. The spatially and temporally dynamic nature of healing mechanisms presents a challenge to the identification of critical mechanisms. Firstly, hemostasis is accomplished through a network of processes that include the platelet system and the coagulation cascade; such processes arrest bleeding and set in motion the inflammatory response.

2.1 Early inflammatory response

Inflammation and blood coagulation are intimately linked. Acute inflammation, the complex systemic early defense system, is the first reaction of the innate immune system (platelet, leukocytes and macrophages) to injury. Direct exposure of cells to physical, mechanical or chemical trauma has immunological consequences relative to the degree of injury, i.e., the apoptotic or necrotic condition of resident fibroblasts. Accordingly, local regulatory mechanisms adjust the magnitude of the response so that inflammatory processes are localized to areas of damage, and the amount and duration of immune cell infiltration are adequate to phagocyte apoptotic/necrotic cells. In addition, endothelial cells, which are actively involved in healing, limit clot formation to the sites of injury. Activated platelets and leukocytes within this clot then release growth factors and numerous cytokines, establishing the onset of inflammation.

Eventually, spatially and temporally changing patterns of various leukocyte subsets transmigrate across the endothelium. Circulating neutrophils are rapidly captured by selectins that are presented by endothelial cells; they then invade the wounded tissue in response to chemical signals. The lifespan of neutrophils in the injured tissue is about two days, during which they perceive signals from the environment and respond by secreting cytokines (Borregaard et al., 2007). Furthermore, neutrophils release stored substances carried in different granule subsets, including reactive oxygen species, cationic peptides or

proteases. The key role of neutrophils is to clear the early rush of contaminating bacteria; in a sterile wound, such as surgical incisions that are experimentally induced, neutrophil absence does not perturb the healing process.

Monocyte recruitment and infiltration at the injury site happens days later and is highly regulated by adhesion molecules expressed by endothelial cells and by chemokines and other substances released by platelets, neutrophils (Soehnlein et al., 2009) and apoptotic/necrotic cells (Nathan, 2006). Commanded by signals present in the environment, monocytes turn into macrophages (the dedicated phagocytes) and induce major changes in gene expression and cell function. Indeed, the severity of tissue injury may determine the different states of macrophage activation. "Innate" activation occurs through lipolysaccharide or interferon-γ (IFN-γ) and is associated with a pro-inflammatory state [the production of interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Alternatively, "classical" activation occurs through IL-4/IL-23 and is associated with the synthesis of healing factors including transforming growth factors (TGF-β and TGF–α), basic fibroblastic growth factor (bFGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) (Krysko et al., 2006).

Recent research suggests that these features of inflammation may determine the difference between efficient repair and the failure to repair. For example, in animal experiments, neutropenia accelerated the closure of incision wounds (Dovi et al., 2003) but did not affect the healing of surgically repaired tendons. Macrophage depletion impaired skin wounding by reducing collagen deposition and angiogenesis and also impaired the response to wounding in diabetic mice. Other studies suggest that targeting macrophage activation may provide a new therapeutic approach to protect tissues from ischemia and promote repair. Notwithstanding, macrophage depletion significantly improved the morphology and biomechanical properties of the tendon-bone interface after experimental anterior cruciate ligament (ACL) surgery. Thus, there are large gaps in the understanding of how neutrophils and macrophages influence repair. The difficulty in understanding the inflammatory response stems, in part, from biological redundancy: one molecule may have several functional roles, and different molecules may perform overlapping functions.

2.2 Trophic phase

New tissue formation occurs 2-10 days after injury and is characterized by cellular proliferation and the migration of different cell types. New blood vessels are formed by a process known as angiogenesis, and later, the sprouts of capillaries along with fibroblasts and macrophages replace the fibrin matrix with granulation tissue that forms the new substrate for cell migration

2.2.1 Cell proliferation and migration

The proliferative phase begins with the formation of a fibrin, fibronectin glycosiaminoglycan, and hyaluronic acid matrix that is initially populated with macrophages and platelets. The various cytokines secreted by these cells enhance cell migration into the site using the fibrin and fibronectin matrix as a scaffold. Progenitors of differentiated cell types, such as bone, cartilage, muscle, nerve sheath and connective tissue cells, are thought to contribute to a collection of proliferating progenitor cells. Alternatively, progenitor stem-cell-like for tissue

niches migrate, divide and differentiate into tissue fibroblasts. Fibroblasts move through the extracellular matrix by binding fibronectin, vitronectin and fibrin via their arginine-glycine-aspartic acid amino acid sequence recognized by their integrin receptors. The fibroblasts proliferate in response to GFs and cytokines and become the predominant cell type by the third to fifth day following injury. Fibroblasts also secrete extracellular zinc dependent endopeptidases called metalloproteinases (MMPs), which facilitate their movement through the matrix and help with the removal of damaged matrix components. Once the fibroblasts have entered the wound, they produce collagen, proteoglycans and other components. Fibroblast activities are predominantly regulated by GFs such as PDGF and TGF-β. PDGF secreted by platelets and macrophages stimulates fibroblast proliferation, chemotaxis and collagenase expression. TGF-β has pleiotropic actions that are context-dependent.

2.2.2 Angiogenesis

Angiogenesis occurs with the formation of new capillary networks through endothelial cell migration and division. Endothelial cells are activated to initiate angiogenesis such that new blood vessels are initiated to promote blood flow to support the high metabolic activity in the newly deposited tissue. Angiogenesis is regulated by a combination of local stimulatory factors such as VEGF and anti-angiogenic factors such as angiostatin, endostatin, and thrombospondin. Local factors that stimulate angiogenesis include low oxygen tension, low pH and high lactate levels. Soluble mediators such as bFGF, HGF, TGF- β and VEGF also stimulate endothelial cells to produce vessels. Tissue oxygen levels directly regulate angiogenesis through hypoxia inducible factor (HIF), which binds oxygen. When there is a decrease in oxygen levels surrounding capillary endothelial cells, HIF-1 levels increase and stimulate VEGF transcription to promote angiogenesis. Animal studies have shed some light on the natural pattern of GF expression during this stage. For example, signaling of VEGF-A via the endothelial receptors VEGFR1 and VEGFR2 is present at the healing site early after tissue injury. Other growth factors including TGF-β, PDGF-BB, and angiopoietin-1, which are important for vessel stabilization, are expressed later at the healing site.

The new vasculature allows the delivery of nutrients and the removal of by-products. As noted above, granulation tissue consists of a dense network of blood vessels and capillaries, elevated cellular density of fibroblasts and macrophages and randomly organized collagen fibers. The metabolic rate of this tissue is high and reflects the activity required for cell migration, division and protein synthesis, which emphasizes the importance of adequate nutrition and oxygen to properly heal the wound. Granulation tissue is particularly abundant and accompanies the process of wound healing by secondary intention.

2.2.3 Synthesis of the extracellular matrix

The high concentration of growth factors and cytokines initially secreted by platelets and leukocytes and later amplified by macrophages induces a rapid increase in specific cell populations, including migrating fibroblasts and resident cells. The number of stromal cells increases in parallel with angiogenesis, which is readily evident in the hypoxic environment produced by the injury. So, the production of extracellular matrix molecules grows in proportion with increasing cell number.

Growth factors, including TGF-β1, PDGF, BDNF, bFGF and type-I insulin-like growth factor (IGF-I), function at various stages during the healing process and produce different

outcomes depending on the conditions. For instance, PDGF, a chemotactic and mitotic factor for fibroblasts, also induces the synthesis of collagen type I. TGF-β1, which peaks early in injuries, is essential for the recruitment and maintenance of progenitor cells during neo-tissue formation, and its function might be necessary during healing. Additionally, the interactions of TGF-β1 with other TGF-β isoforms, namely TGF-β2 and TGF-β3, mediate which type of collagen is synthesized in the healing tissue. IGF-I anabolic and anti-apoptotic activities are regulated by IGF-I binding proteins (BP), IGFBP-2, IGFBP-3, and IGFBP-4, which are also present in the early healing response. In both humans and animals, the expression of IGF-I and TGF-β1 preceded the stimulation of collagen synthesis, a relevant issue in tissue healing. The bioactivity of these growth factors is regulated not only at the receptor level but through activation of TGF-β complex and IGF-binding proteins.

2.3 Tissue remodeling and scarring

Finally, the tissue enters into the last phase of healing, a long remodeling phase in which granulation tissue matures into a scar. Collagen accumulation reaches a maximum at 2-3 weeks after injury, and the transition to remodeling begins. There is a balance between synthesis, deposition and degradation during this phase. Small capillaries aggregate into larger blood vessels, and there is an overall decrease in the water content of the wound. Similarly, cell density and the overall metabolic activity of the wound decrease. The most dramatic change occurs in the overall type, amount and organization of the collagen fibers, resulting in an increased tensile strength of the tissue. Initially, there is increased deposition of collagen type III, also referred to as reticular collagen, that is gradually replaced by collagen type I. Collagen fibers are cross-linked by the enzyme lysyl oxidase, which is secreted by fibroblasts in the extracellular matrix. The normal adult 4:1 ratio of type I to type III collagen is restored during remodeling. Equilibrium is established as new collagen is formed and collagen type III is degraded. The MMPs, collagenases, gelatinases and stromelysins, control the degradation of extracellular matrix components to facilitate cell migration into the wound, angiogenesis and overall tissue remodeling.

In each of the described healing phases, the specific signaling activity is silenced or counterbalanced by other endogenous signals that serve to limit the duration and to promote progression to a new stage. During all these stages, local and migratory cells synthesize different patterns of GFs and cytokines in an attempt to cope with the temporal demands of the healing tissue. Consequently, therapeutic approaches to manipulate healing may need to integrate multiple cell types and large signaling networks that are necessary for the dynamic communication between cells. The need to target various signaling pathways simultaneously demands the administration of a balanced combination of mediators instead of administering a purified isolated protein, which could not cope with the multiple requirements of the injured tissue. Therefore, the ability to release signaling molecules in a spatiotemporal manner that mimics the needs of the injured tissue has become a challenge in the scientific and medical fields.

2.4 Pathologic tissue healing

There are many categories of impediments for wound healing. These include local or systemic impediments. The former include tissue viability, seroma and/or hematoma,

infection, insufficient blood supply and/or mechanical factors. For example, adequate blood supply must exist to provide nourishment and oxygenation to healing tissues. A lack of blood supply may lead to tissue ischemia and an increased risk of infection. Tissues do not heal if there are more than 10^5 bacteria per gram of tissue. Hence, necrotic tissue must be debrided to avoid the risk of infection and because it interferes with normal healing.

Mostly clinical differences between chronic and acute healing tissues are thought to be explained in part by alterations in the local biochemical environment. The observation that increased amounts of TGF-β1 were present in hypertrophic scars led to clinical efforts to block scar formation through administration of antibodies against TGF-β1 and other pro-inflammatory mediators. Recent evidence also suggests that changes in the molecular environment of the cells in the wound may change the fate of healing tissues or organs. It is becoming increasingly apparent that growth factors and cytokines play a variety of key roles during normal tissue repair, and many have also been shown to act therapeutically in situations where normal healing is impaired. Although tremendous strides have been made in delineating the myriad of factors involved in normal and pathological healing, it has become clear that single-agent therapies, such as administration of growth factors, have only a moderate impact on tissue repair in the clinical setting, probably due to the redundancy and plasticity of the components of tissue repair or their rapid degradation at the injured site.

In this context, the emergence of PRP biotechnology as a way to harness tissue regeneration for medical needs has fueled the optimism surrounding cell-signaling based regenerative medicine. A deeper understanding will accelerate the development of PRP therapies.

3. Platelets: Molecular contribution to healing

The ultimate solution to tissue healing is likely to be the administration of multimolecular preparations with the ability to elaborate the full complexity of biological signaling, including all the environmental cues that are needed to regulate the biological mechanisms described above. Platelets are a natural source of growth factors and cytokines involved in tissue healing. Until now, it has not been possible to provide a pool of molecular signals and the temporary cell scaffold necessary to initiate healing in the same therapeutic agent. Substantial progress in the understanding of platelet biology has revealed much about the complexity of PRP therapies. Additional insights come from combining the information from the plasma proteome.

3.1 Platelet biology

Our understanding of fundamental aspects of platelet biology and function has been enriched in the last decades. Platelets are discoid cellular elements that are heterogeneous in size and have the smallest density of all blood cells, at 2 μm in diameter (a leukocyte is about 20 μm in diameter). They are anucleate and originate in the bone marrow as bulges along the length of pseudopodial extensions of megakaryocytes. The so termed proplatelets are then fragmented into individual platelets and released into the blood stream where they travel for about 7-10 days before removal from circulation after senescence and are replaced with younger platelets possessing greater functional capabilities. Platelets are replete with secretory granules, which are critical to platelet function. Among the three types of granules,

dense granules, alpha-granules and lysosomes, the alpha-granule is the most abundant. There are approximately 50-80 alpha-granules per platelet, although they are heterogeneous with regard to cargo (Villeneuve et al., 2009). For example, anti-angiogenic proteins are packaged in different alpha-granules subpopulations than pro-angiogenic proteins. Moreover, there is some evidence that secretion of pro- versus anti-angiogenic stores may be agonist-specific (Italiano et al., 2008). The total protein content of platelets includes not only soluble proteins that are released into the extracellular space but also membrane bound proteins that become expressed on the platelet surface. Many of the proteins found in α-granules are also present in plasma. In a recent survey of the platelet membrane proteome, 629 membrane proteins were detected (Maynard et al., 2007). Overall proteomic studies suggest that more than 1048 soluble proteins are present in the supernatant of platelets.

3.2 Platelet function

Not long ago, platelets were merely considered to function as haemostatic agents. However, as researchers broadened their understanding of platelets, many more facets were identified. Around 1980, platelets were recognized for their healing function. More than a decade later, the involvement of platelets in angiogenesis was discovered. Subsequently, Folkman showed that angiogenesis regulating proteins were selectively pumped into the budding pro-platelets from the mother megakaryocyte and that PF-4 is captured by platelets in tumor-bearing animals. Further developments using PRPs as a therapeutic biotechnology in the past few years have allowed the direct observation of platelet secretomes, not within, but outside the blood stream that interact with various injured tissues and organs.

In the physiological process of wound healing, platelets embedded within blood clots serve as a primary source of biologically active factors. Therefore, the PRP concept is straightforward. As platelets are a major source of healing factors within blood clots, the idea that concentrating platelets at the injured site could accelerate and optimize healing mechanisms opens the door for the development of PRP therapies. For example, typically after muscle strains or contusion, the hematoma that originates as a consequence of vessel disruption contains about 94% red blood cells, a small amount of platelets (4%) and less than 1% leukocytes. The rational for the use of PRPs involves replacing the blood clot with adhesive PRP, thus minimizing the presence of red blood cells (about 95% in volume) while increasing platelet concentration at the injury site. In doing so, we would achieve a supra-physiological concentration of platelet and plasma proteins that accelerates the repair process by direct or indirect mechanisms, i.e., by attracting immune cells via chemotaxis or enhancing further synthesis of healing proteins by local cells. Moreover, the ability to release these signaling factors in a spatiotemporal manner using the fibrin scaffold perfectly meets the needs of the injured tissue over time.

However, the present knowledge of both PRP therapies and healing mechanisms needs to be better explored to translate such knowledge into improved biological therapies.

4. Platelet-rich plasma biotechnology: New tools for tissue repair

4.1 The history: A three decades perspective

From a historical point of view, the first blood bank PRP preparations began during the 1960s and become routine preparations through the 1970s. In the 1980s, the advent of regenerative medicine aiming to rapidly translate the science into patient care using the patient's own

resources opened the door to the use of platelets as vehicles for the delivery of a balanced pool of healing factors. At that time, platelets were found to release wound healing substances that initiated the repair of injured tissues and vessels in cutaneous ulcers (Margolis et al., 2001). Later in the 1990s, platelets were introduced into maxillofacial surgery as autologous modifications of potent adhesives known as fibrin glues. The use of platelets was particularly fortuitous given that the main initial interest was to take advantage of the adhesive and haemostatic properties of the homologous fibrin during bone surgery. A realization of the clinical potential of PRP-therapies followed the positive clinical observations, such as enhanced bone formation and anti-inflammatory functions, during oral and maxillofacial applications (Whitman et al., 1997; Marx et al., 1998; Anitua E, 1999). At the beginning of the millennium, PRP was used for the first time to treat knee injuries in arthroscopic surgery (Sánchez et al., 2003 a and b), and later it was extended to the treatment of tendons (Sánchez et al., 2007), muscles injuries (Sánchez et al., 2005), osteoarthritic knees (Sánchez et al., 2008) and hips (Sánchez et al., 2011) and for use in chondropathies (Kon et al., 2010). Below we show the temporal sequence of the development of PRP therapies.
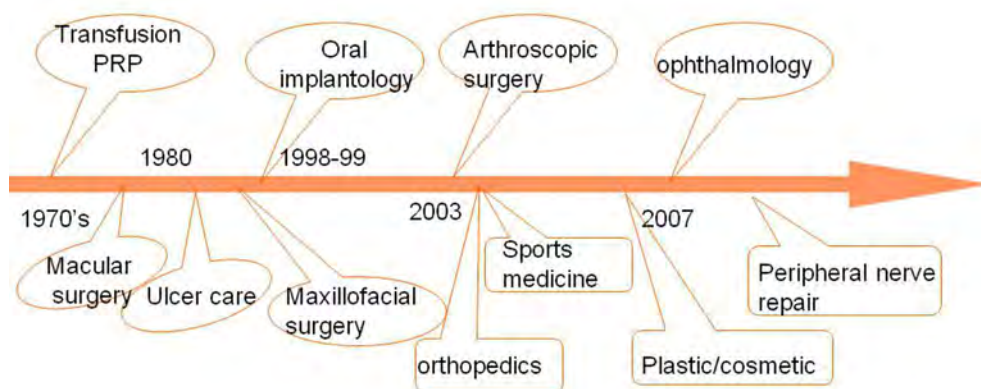

Fig. 1. Temporal sequence of the development of PRP technologies

4.2 Terminology

Long before any therapeutic application was imagined, the term PRP, which described plasma with a platelet count above the peripheral blood, was coined by hematologists. In 2007, the novel connotation of PRP was introduced to the Medical Subject Heading database (MeSH): PRP refers to a product consisting of PLATELETS concentrated in a limited volume of PLASMA used in various surgical tissue regeneration procedures where the GROWTH FACTORS in the platelets enhance wound healing and regeneration. At present (2011), the field is growing more complex, and the primacy of growth factors is now shared by new classes of platelet released biomolecules, which are also critical in healing.

4.3 PRP preparation

Peripheral blood is the supply source for the preparation of PRPs; the mean number of circulating platelets is 200,000 plt/µl. For PRP preparation, peripheral blood is drawn from the

patient under sterile conditions, with or without anticoagulants, and the plasma is prepared by centrifugation or filtration. The volume can be adapted to the clinical needs, ranging from 10 to 100 mL. Essentially, the methods of producing PRPs determine the composition and concentration of leukocytes, erythrocytes and platelets in a given plasma volume. There are three methods: 1) the double spinning method using automated machines and commercial kits, 2) the single spinning method using conventional laboratory centrifuges followed by manual PRP separation, and 3) selective blood filtration using commercially available technology. Single spinning yields a 1-3 fold change in platelet concentration over baseline levels, and double spinning yields a 4-8 fold change in platelet concentration over baseline levels. Double spinning also concentrates leukocytes. Accordingly, platelet concentrates have been categorized as pure platelet-rich plasma (P-PRP), in which leukocytes are purposely eliminated from the PRP, and leukocyte and platelet-rich plasma (L-PRP), which contains a high concentration of leukocytes (Dohan et al., 2009).
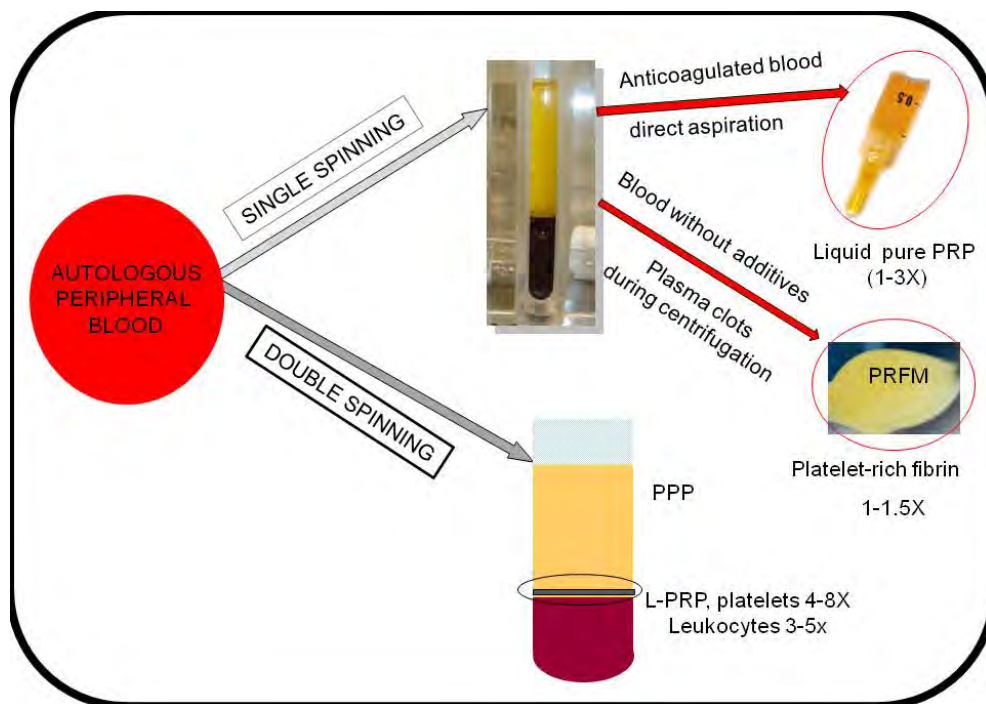

Fig. 2. Methods of producing PRP determine the composition and concentration of leukocytes, and platelets in a given plasma volume

At present, there is much debate surrounding four central questions of clinicians: (1) is the number of platelets important, (2) is the presence of leukocytes important, (3) when should PRP be activated, and (4) how should PRP be activated. The clinical variability observed throughout the studies points out that some techniques might not produce a sufficient number of functional platelets to produce the expected outcome. Similarly, there is no

consistency in the methods of application of this therapy, the timing of treatment, the number of injections per series or the volume of injections. This has precluded the establishment of the standards necessary to integrate the extensive relevant literature in basic and clinical science. For example, double spinning techniques yield a PRP concentrate with a volume of about 10% of the volume of blood withdrawn (i.e., 20 mL of whole blood would result in 2 mL of PRP). In contrast, 40-50% of the blood volume is obtained after single spinning. Also, each method leads to a different product with differing biological properties and potential uses. Currently, it is unclear whether these differences have any clinical relevance. Some authors have suggested that PRP preparations containing only moderately elevated platelet concentrations induce optimal biological benefit, whereas lower platelet concentrations produce suboptimal effects and higher concentrations produce inhibitory effects. According to others, the 'therapeutic dose' of PRP is at least 4-6 times higher than the normal platelet count. To add to the discussion, the actual growth factor content does not correlate with the platelet count in whole blood or in PRP when leukocytes are present in the preparation, and there is no evidence that gender or age affects platelet count or growth factor concentrations. However, age may influence the number of receptors on local cells interacting with the plasma signals.

4.4 PRP activation and fibrin delivery

Because these procedures are considered to be an autograft by the regulatory authorities of most countries, the plasma should be prepared and immediately used at the point of care, and the plasma should not be stored. Prior to application, platelets can be slowly activated by setting in motion the coagulation cascade with the addition of calcium chloride, a necessary cofactor for prothrombin conversion to thrombin. Alternatively, coagulation and platelets can be instantly activated by adding a standard solution of bovine or human thrombin with 10% calcium chloride to the PRP. After plasma activation, the fibrin scaffold can be formed *in vivo* or *ex vivo*: the latter is suitable for implantation in surgery or in ulcer care and provides a gradual release of growth factors in the area where it has been applied. Depending upon the activation mechanism, induced by $CaCl_2$, collagen or thrombin can achieve a sudden burst of GFs or a gradual release. Indeed, a central question in biology and cell signaling is how extracellular factors elicit a complex set of signaling events to achieve specific cellular functions.

Figure below shows fibrin which is a natural biopolymer involved in the coagulation cascade formed upon fibrinogen cleavage by thrombin. It acts as a reservoir for growth factors, cells and enzymes during wound healing and provides a scaffold for the synthesis of the extracellular matrix. Fibrin scaffolds provide nature's cues for tissue regeneration. Fibrin is a key scaffold material for the delivery of biomolecules, and it mimics natural processes and provides adequate exposure time to maximize biological interactions.

The kinetics of signaling may be influenced not only by distinct cell surface receptors but also by the method that their cognate ligands are secreted or delivered. A receptor may be acutely activated by an immediate increase in ligand concentration, a process mimicked in most pharmacological studies. In many cellular processes in vivo, however, cells encounter a gradual increase in the concentration of extracellular factors, i.e., constitutively secreted factors need to accumulate over time to reach a threshold set by the affinity of the receptor.
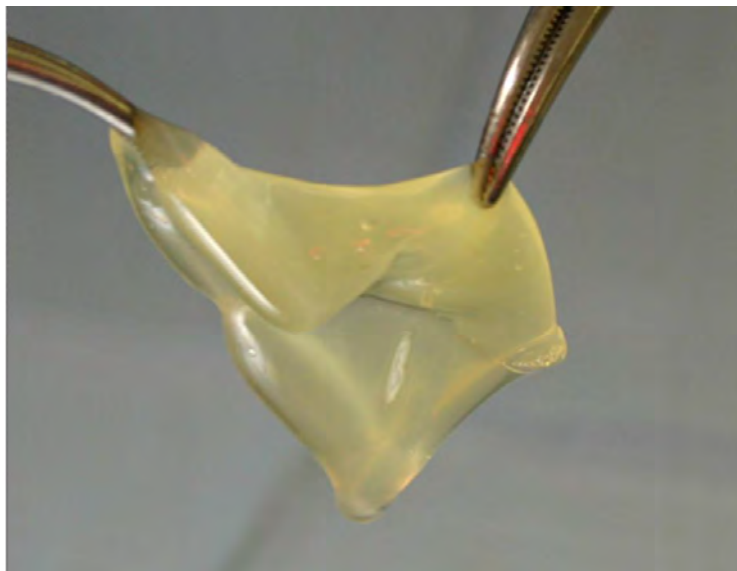
Fig. 3. Fibrin is a temporary scaffold for cell adhesion at the injured site and also functions as a vehicle for the delivery of growth factors and cytokines

Questions about safety still linger regarding the routine use of PRP. Any concerns regarding the transmission of diseases such as HIV, hepatitis, or Creutzfeldt-Jakob disease, or of the development of immunogenic reactions, a concern with the use of allografts or xenografts, are by definition not applicable due to the autologous nature of PRP. However, some systems use purified bovine thrombin to activate the platelets. This may produce coagulopathies, and most commercial systems now use recombinant human thrombin.

Some authors have raised the issue of genetic instability and have hypothesized that the use of PRP may lead to the development of neoplasms. Growth factors act on receptors located on the cell membranes rather than on the cell nucleus and activate normal gene expression via intracellular signaling proteins, which promote normal, not abnormal, gene expression. Growth factors are not directly mutagenic, and their activities in normal wound healing are highly regulated by various feedback control mechanisms. Furthermore, up to now, no systemic effect on circulating growth factors has been shown after PRP application.

Some antimicrobial activity of PRP (platelet-leukocyte gel) against *Staphylococcus aureus* has been shown in vitro and in vivo, although it is not comparable to systemic antibiotic treatment.

4.5 Technological relevance of PRP biotechnology

The medical industry is benefiting from a robust demand for technologically advanced products that accommodate the increasingly active baby boomer (people born between the 1940s and 1960s) life style as well as the sedentary lifestyle that accompanies the escalating levels of obesity. Expansion is rapidly occurring in the bone growth factor and protein segments, termed orthobiologics. In fact, PRP technologies are now very

important in at least two main market segments: (1) bone repair, which includes the use of regular PRP or PRP composites made of PRP mixed with structural biomaterials or bone grafts and (2) soft tissue repair, which includes the development of techniques for applying PRPs and the surgical tools needed to correctly apply the different physical-chemical configurations of the biomaterial. An estimated 30% of new products under development are "combo products" that involve medical devices embedded with pharmaceutical or biologics components. Ambulatory PRP treatments benefit from point-of-care ultrasonography; ultrasound guidance improves success in PRP per-cutaneous and intra-articular procedures.

Recently, the medical industry has realized the potential of autologous products. Thus, although not fully developed yet, autologous technologies are readily available, and the present leading firms that control the orthopedic industry and market, Zimmer Holdings, Stryker, Biomet, Arthrex, DePuy, Smith & Nephew and Synthes, have introduced PRP devices. In the last few years, several semi-automatic machines have been developed for the centrifugal separation of PRP for therapeutic use. The process of PRP preparation is relatively straight forward and can be performed in the clinic or in the operating room. In most cases, it can be completed within minutes. The cost to both medical practitioners and patients varies widely depending on the method used to produce the PRP.

5. Therapeutic applications

The versatility and biocompatibility of PRP biotechnology has stimulated its therapeutic use in many different fields (see Figure below), including orthopedics, sports medicine, ophthalmology, dentistry, and cosmetic, plastic and maxillofacial surgery. Here we present some of the most interesting therapeutic applications with a special emphasis on musculoskeletal applications.
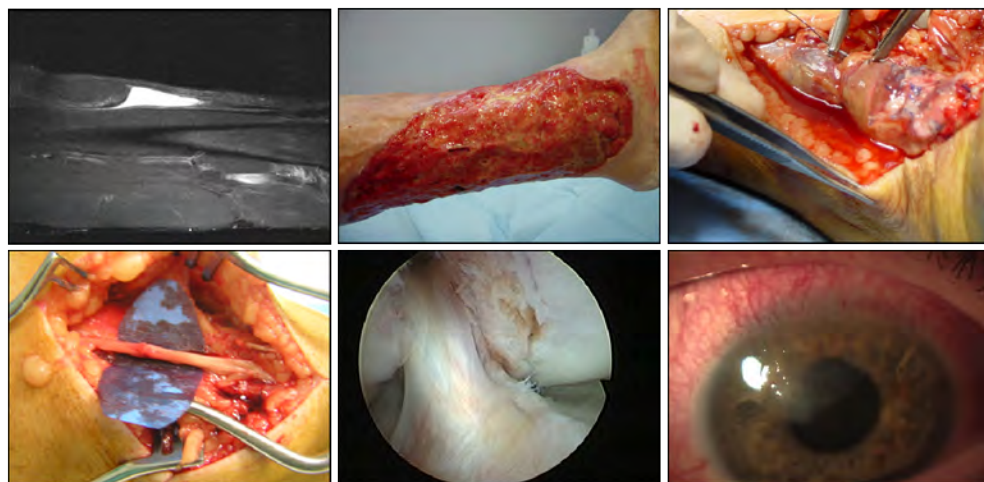

Fig. 4. Application of PRP therapies in the different medical fields: management of muscle injuries in sports medicine, open orthopedic surgery, arthroscopic surgery, ulcer care, peripheral nerve repair or the treatment of corneal ulcers in ophthalmology

Demand for musculoskeletal care

Musculoskeletal disorders, which affect millions of people worldwide, can lead to chronic pain and physical disability. According to leading pain experts, more people around the world experience musculoskeletal pain than any other type of pain (Global year against musculoskeletal pain, Oct 2009-Oct 2010, www.iasp-pain.org). In the United States alone, 2.5 million orthopedic reconstructions, including bone, cartilage, ligament and tendon reconstructions, are performed annually. With an aging population and a prolonged life expectancy, an increase in the number of patients suffering from musculoskeletal disorders such as osteoporosis and arthritis are expected in the future. The former is currently suffered by 10 million Americans over the age of 50, and the latter is a chronic musculoskeletal disease that affects 1 in 3 adult Americans. In addition, every year sporting activities result in a variety of injuries to cartilage, ligaments and especially muscles. Taken together, these musculoskeletal diseases increase patient morbidity and disability and the social and economical consequences are staggering. As a result, the period of 2000-2010 has been named "the bone and joint decade" in an effort to increase the attention of scientists regarding the problems related to these disorders and to promote advancement in these fields [Anitua et al., 2006].

5.1 Orthopedic applications

5.1.1 Bone repair

More than 6 million bone fractures are reported annually in the USA, of which 5-10% have impaired healing that causes pain and disability. To improve patient-care results, scientists are making great efforts to create bone substitutes and to develop ways of improving bone healing. The use of platelet rich preparations may help to fulfill 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 human trabecular bone cells and the differentiation of human osteoblast-like cells. Studies have confirmed that the local application of PRPs is especially important in pathological conditions in which bone healing is weakened due to an inadequate blood supply, such as that observed in atrophic nonunion fractures. Both percutaneous injection and surgical augmentation with freshly prepared PRP have been shown to normalize fracture callus (Sánchez et al., 2008). The hundreds of soluble proteins released from both plasma and platelets include VEGF-A, PDGF, FGF, EGF, HGF, and IGF. These angiogenic activators collectively promote vessel wall permeability and promote the growth and proliferation of endothelial cells (Nurden et al., 2008).These findings are consistent with those seen in diabetic patients with a Charcot foot who showed improved healing and fewer complications after ankle fusion treated with fresh PRP. In contrast, previously frozen and thawed PRP supplementation in long bone nonunions treated with external fixation failed to provide clinical usefulness. In orthopedic trauma to date, there are not enough clinical studies to make definite conclusions. However, in some clinical conditions, the development of newly grown bone may be a realistic target if PRP is applied with cells or scaffolds. In fact, 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 PRP with either artificial or natural biomaterials. Moreover, the use of PRP

improves the handling, adhesion and adaptation of the composite graft. 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 on bone regeneration. For example, when patients with solitary bone cysts were treated with allogenic grafts and PRP, the cysts were filled with newly formed bone after 12 months (Pedzizs et al., 2010). In a randomized control trial among people undergoing a medial, opening-wedge osteotomy of the proximal tibia, the use of an allograft with PRP showed better radiographic osseointegration at all stages of follow-up (Dallari D et al., 2007)

Developing engineered tissue is another interesting approach for bone regeneration. This may be feasible after combining mesenchymal stem cells (MSCs) and scaffold-like platelet rich plasma 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 an MSC and platelet rich plasma mixture (MSC/PRP) was analyzed and compared with other approaches, including a natural deproteinized bovine bone, an autologous bone and the platelet rich product alone. Compared with the other treatments, the results of histology and mechanical properties showed that the MSC/PRP combination provided greater bone maturation and early stage bone regeneration. This mixed preparation has also been successfully used for bone regeneration in several patients. Encouraging results were observed in clinical studies exclusively concerning children. For instance, in the distraction of long bones, Kitoh et al. (2007) reported less complications in children treated with PRP plus MSCs than in children that did not receive PRP and MSC augmentation. The same authors reported an enhanced healing index in a controlled series of children with achondroplasia or hypochondroplasia undergoing limb-lengthening procedures. Even so, achieving control of bone healing is difficult, and the challenges associated with PRP therapies are enormous, extending beyond the present knowledge.

5.1.2 Joint repair

Arthroscopy is a minimally invasive procedure that allows doctors to treat joint injuries and disease through small incisions in the skin. The concept of not having to perform extensive soft tissue dissection is appealing because the recovery is quicker and less painful than open techniques. The use of PRP in arthroscopic surgery was initially introduced by Sanchez et al. (2003) in the treatment of a cartilage avulsion in the knee of a young athlete and in the reconstruction of the anterior cruciate ligament, as explained below (Sánchez et al., 2003). In recent work, Guadilla et al. (2011) showed how the arthroscopic management of the femoral head may be enhanced by the application of PRP in several ways. First, by grafting the necrotic area with trabecular bone mixed with PRP to induce angiogenesis and to enhance cell survival and function. Second, platelet-rich plasma can be applied within the intra-articular space to improve the conditions of synovial cells, chondrocytes, and subchondral osteoblasts [Andia et al, 2011].

Other authors have shown that the perioperative application of platelet rich plasma and fibrin sealant in arthroplasties reduces blood transfusion requirements, the length of the

hospital stay, and the incidence of blood leakage and arthrofibrosis while it improves the range of motion. Another step forward would be to explore the analgesic and anti-inflammatory effects of PRP. Additional potential benefits, including blood loss, shorter hospital stay, and faster recovery time, should also be investigated.

PRP and cartilage engineering ex-vivo: Evidence of the effects of PRP on cellular proliferation and differentiation comes mainly from studies of tissue engineering. For example, chondral lesions represent a clinical challenge due to the limited capacity of chondrocytes to proliferate in vivo. Thus, autologous cells can be harvested from a small tissue biopsy and sufficiently expanded ex-vivo for re-implantation. When articular chondrocytes are the cellular source, PRP improves ex-vivo proliferation but also causes de-differentiation. Importantly, PRP-expanded cells retain their capacity to re-differentiate and synthesize cartilage-specific proteins when transferred to a 3D environment.

The cultivation of stem cells is another alternative that is under clinical investigation for the treatment of osteoarthritis; given their capacity to differentiate into chondrocytes and secrete a wide array of biologically active factors that support cell proliferation and tissue formation. The sources of these stem cells include the bone marrow and the synovial fluid. In addition, the Hoffa fat pad contains stem cells with chondrogenic potential. Stem cells derived from the meniscus, synovium, Hoffa fat, synovial fat and ACL share similar gene expression profiles. Culturing these cells under hypoxic conditions has been shown to enhance their differentiation into cartilage-like tissue.

To avoid contact of the cells with bovine products and to implement GMP-compatible protocols, PRP releasates or lysates provide a feasible alternative to fetal calf or bovine serum in the expansion of these cells for cartilage engineering purposes. The addition of PRP (compared to fetal calf serum) improves cellular expansion and imparts a differentiation capacity towards the osteogenic, chondrogenic and adipogenic lineage. In addition, PRPs can be used as carriers for chondrocyte delivery during re-implantation.

Osteoarthritis

The dramatic increase in the incidence and prevalence of joint pathology over the past two decades has focused attention on therapeutic interventions that can reverse or ameliorate progressive joint damage and pathology. Degenerative osteoarthritis (OA) is the most common form of arthritis and affects nearly 27 million adults in the US (Lawrence et al., 2008). Despite the vast amount of molecular knowledge accrued during the last few years, a major breakthrough in OA therapy has not emerged. A large part of the problem is that researchers do not know enough about the biology of OA to identify the right targets. The disease is the result of a long chain of events, but some of the links in that chain are still a mystery; nobody is certain which link to cut in order to stop disease progression. Limiting factors in the current efforts are to some extent attributed to a poor understanding of the molecular basis of the disease progression and the lack of dynamic biomarkers that reflect specific biological or pathological processes. Hence, with the exception of surgery, all approaches are merely palliative. The conservative management of OA and chondropathies with PRP biotechnologies is becoming increasingly popular, but clinical evidence is preliminary and modest and is limited mostly to observational case studies that have used patient-reported outcomes as end points. Our preliminary clinical results in a retrospective cohort study of knee OA showed that intra-articular injection of PRP decreased pain and

enhanced function compared to hyaluronic acid (HA) injections (Sánchez et al., 2008). In a case series study that involved 115 young patients with low degrees of articular degeneration in the knee, Kon et al. (2010) reported reduced pain and improved function that was maintained at 12 months but not 24 months after treatment (Filardo et al., 2011). Sampson et al. (2010), also in a small case series (n=13), reported significant pain and symptom relief but did not find any significant change in the daily activities or quality of life of the patients treated. PRP injections for hip OA produced clinically significant reductions in pain and function, although this was only seen in 40% of the patients studied (Sánchez et al., 2011). When discussing PRP therapies, differences between the preparations and the re-administration procedures used should be acknowledged. Although pure PRP and leukocyte PRP formulations are not comparable in terms of leukocyte content, platelet count or plasma volume, the resulting improvements in pain and function were not exclusive to any one formulation. The pursuit to identify a unifying therapy for OA would be enhanced by refining the end points in future clinical studies.

5.2 Sport medicine

Sports related soft tissue injuries cause athletes to lose a significant amount of time from their sport and represent a significant burden to society in terms of health care resources, personal disability and activity restriction. In 2002, an estimated 15.8 billion dollars in total health care expenditures was used for the medical management of these injuries (Yu WW 2005). Soft tissue disorders, including muscle, tendon, ligament and joint capsular injuries, represent more than 50% of all the musculoskeletal injuries reported each year in the USA. Primary care studies have shown that 16% of the general population suffers from shoulder pain, whereas elbow tendinopathy affects 1-2% of the population. The importance of this problem is substantial because the field of sports medicine influences millions of people from athletes to those who participate in recreational sports or simply exercise to stay healthy and active.

5.2.1 Muscle injuries

Muscle injuries resulting from extrinsic or intrinsic mechanisms are extremely common in sports, accounting for about 35-45% of all injuries. Contact sports and sports that require the generation of large eccentric forces present the highest risk. The vulnerability of soccer players to strains and contusions is a substantial problem for professional players and their clubs; such injuries involve significant time lost from training and competition. Due to the increasing demands of the competitive soccer season, muscle treatments able to accelerate the recovery time without adversely affecting the recurrence rate (i.e., those that can minimize the scarring response) are of paramount importance [Andia et al, 2011].

At present, no drugs have been developed that hasten the restoration of muscle function after injury. Therefore, in the absence of any available evidence-based treatments, injection therapies may be an important option to help professional athletes. At the 2nd World Congress of Regenerative Medicine, Sanchez (2005) reported for the first time the application of leukocyte-free PRP to 21 muscle injuries of different severities and different anatomical locations. Small tears progressed well with a single application, whereas more severe tears required 2-3 ultrasound-guided injections. The injected volume depended on tear severity. These athletes, who played in first division teams of the Spanish Soccer

League, resumed normal training activities in half the time needed by matched historical controls. Using the same leukocyte-free PRP preparation, Wee (2009) reported good outcomes (1 week to return to pre-injury activities) after three weekly US-guided injections to treat an adductor longus strain in a professional bodybuilder.

Another autologous blood derived biotechnology is named ACS (Autologous Conditioned Serum). This technology consists of an autologous liquid serum conditioned by the incubation of whole blood with glass beads. It contains signaling proteins that include interleukin-1b (IL-1b), tumor necrosis factor-alpha (TNF-a), IL-7, FGF-2, interleukin 1 receptor antagonist (IL-1Ra), HGF, platelet derived growth factor (PDGF-AB), transforming growth factor (TGF-β1) and IGF-1. Wright-Carpenter (2004) assessed the effects of ACS injections in a non-blinded, non-randomized case control study. The experimental group was treated with ACS, and the control group, which was analyzed retrospectively, included patients who had received Traumeel®/Actovegin®. Traumeel is a homeopathic formulation that contains both botanical and mineral ingredients in homeopathic concentrations. It is purported to suppress the release of inflammatory mediators and to stimulate the release of anti-inflammatory cytokines. Actovegin is a deproteinized calve blood hemodialysate that consists of a physiological mix of amino acids. The RICE principle was employed for initial care in both groups. The primary measured outcome was the time needed to resume full sporting activities. The experimental group returned to competition after 16.6 days, whereas the control group took 22.3 days. In addition, MRI scans taken at 16 days in both groups confirmed that regression of the edema/bleeding was faster in the ACS group. Both treatments were safe.

5.2.2 Tendon pathology

Chronic pain in tendons is very common and studies show that overuse, underloading and overloading, all contribute to tendon injuries and pain. More than 30-50% of the injuries among professional and recreational athletes are overuse tendon injuries resulting in the onset of pain and discomfort. Data collected from sedentary people showed that tendinosis is not necessarily a consequence of overuse. Nevertheless, the odds of having tendinopathy among elite endurance athletes are one in two (Kujala et al., 2005). Thus, the development of innovative strategies to treat tendon injuries is an essential task, but it requires a more thorough understanding of the underlying cellular and molecular mechanisms. The use of platelet rich preparations in this context may be focused on restoring the normal tissue composition while avoiding further degeneration. When we evaluated the effects of the pool of growth factors released from PRP on tendon cells, the results showed that human tendon cells increased their proliferation rate and were stimulated to release VEGF and HGF. The former promotes angiogenesis, which is directly related with tendon healing capability; the latter is a potent antifibrotic agent that can reduce scar formation around tendon tissues. Other studies have reported that injections of platelet rich plasma one week postoperatively increased tendon regenerate strength. The clinical translation of this approach was assayed in a pioneer study involving professional and recreational athletes. PRP was injected into 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. The results showed that those receiving the PRP-therapy experienced a significant acceleration in functional recovery compared with a matched group that underwent conventional surgery. Moreover, the effects induced by PRP therapies had long-term consequences such as

decreased cross-sectional area of the Achilles tendon after 18 months [Sánchez et al., 2007]. The feasibility and biosafety of PRP therapies made their application possible not only in surgeries but in the conservative management of tendon problems.

Currently, conservative management with PRP injections and its research attention are increasing [Andia et al, 2011]. Recently, three studies on PRP injection, of which two were on patients with chronic patellar tendinopathy [62,65] receiving three injections of leukocyte-platelet concentrate (double centrifugation), were reported. Significant improvements in the Tegner scores were described in one of the two studies. In addition, improvement in pain and function was reported after a single PRP injection in patients with epycondylitis [Mishra et al., 2006]. More recently, two double-blind, randomized clinical trials were performed on patients with lateral epycondylitis [Peerbooms et al., 2010] and chronic Achilles tendinopathy [De Vos et al., 2010], respectively. In both studies, the experimental treatment consisted of a single injection of an identical buffered PRP. The clinical results were significant for patients with lateral epycondylitis, for which PRP reduced pain and improved function. In contrast, in patients with Achilles tendinopathy, PRP injection did not reduce pain or improve activity [De Vos et al., 2010]. It seems improbable that a single injection could stop or reverse an ongoing degenerative process. Instead repeated injections appear to be more efficient in degenerative pathologies. No complications were reported after PRP treatments.

5.2.3 ACL reconstruction

Finally, a great deal of effort has been paid to the development of novel medical tools for the repair of injured anterior cruciate ligaments (ACL). The ACL is one of the four major ligaments connecting the bones of the human knee. A torn ACL is a common injury and is typical among the active younger population. The injury requires surgical intervention to stabilize the knee and to prevent cartilage and meniscal injuries, which lead to degenerative joint disease. ACL reconstruction, namely ACL tissue engineering, involves the manipulation of cells and tissues to replace the injured ligament; this process is a complex undertaking and involves many mechanical and biological challenges. It requires both the application of mechanical knowledge and an understanding of how cells are maintained and grow into functioning tissues to replace defective or injured ligaments. At present, the most common options in ACL replacement are allografts or autografts. A novel approach using PRP technologies seeks to facilitate ACL healing by mimicking the native tissue and improving tissue function with the appropriate cues (see Figure below), ultimately leading to better patient care.

Cell cultures and animal research, in addition to human clinical studies, drive the main hypotheses for the application of PRP biotechnology in ACL reconstruction. These applications involve first promoting bone-bone and bone-tendon healing, and second, influencing the pattern of change within the autograft body (ligamentization). Finally, the application of PRP-therapies will help in donor site healing. Graft fixation is the weakest link in ACL reconstruction because knee laxity develops during the immediate postoperative period until biologic fixation occurs within the bone tunnel. Classically, graft stabilization is achieved more rapidly with a bone plug-patella tendon-bone (BPTB) graft than with the hamstring. The BPTB graft becomes anchored to the bone wall via appositional bone formation, and in these circumstances, the use of PRP may aid in the formation of the callus and may accelerate bone fusion (Sánchez et al., 2010). In a

preliminary study, Sánchez et al. (2003) described a procedure for treating bone tunnels and for conditioning the graft prior to implantation with PRP. They compared a group of 50 patients treated with surgery and pure PRP with another group of 50 patients who underwent surgery alone. The two groups were matched for age and graft type. The authors reported better integration of PRP-treated grafts within the tunnels, as assessed by X-rays, and a larger number of completely stable knees in the PRP group. Other authors have explored the influence of autologous bone plugs, either alone or combined with PRP therapies, on the promotion of femoral bone-tendon healing. They reported that bone plugs, but not PRP-therapies, significantly prevented femoral tunnel widening.
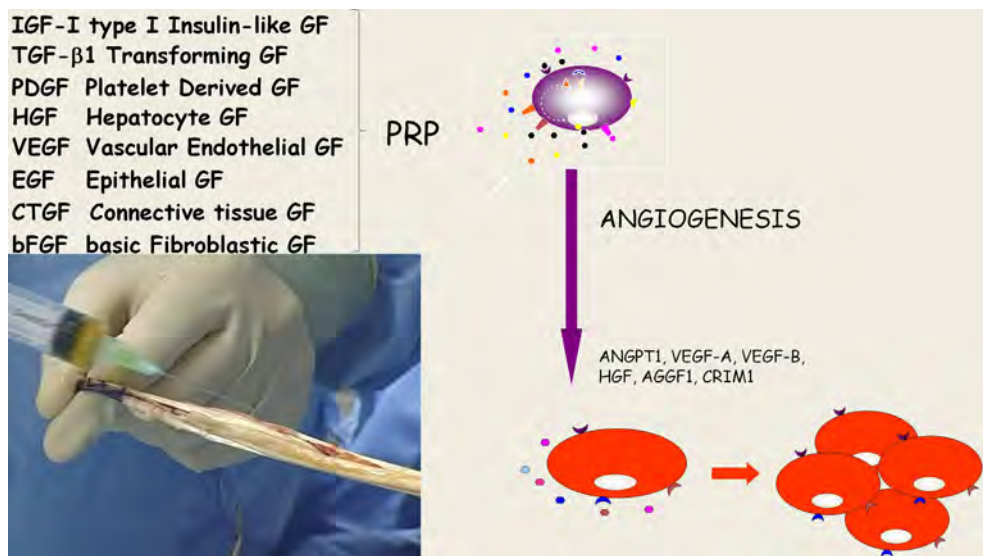

Fig. 5. Transfer of autologous GFs and cytokines to the tendon graft, applying the principles of tissue engineering and using PRP biotechnology to estimulate biological mechanisms such as angiogenesis.

The appropriate function of ACL grafts, essential for normal knee biomechanical functioning, entails a successful intra-articular graft ligamentization. One exciting option to enhance ligamentization is to simultaneously transfer multiple cytokines and growth factors (including PDGF, TGF-β1 and VEGF, among others) to the graft by applying an endogenous PRP. Autografts could be loaded in situ with a balanced pool of signaling molecules. These molecules would have the potential to not only activate the graft tenocytes but also to attract cells, such as endothelial or stem cells, from adjacent niches (such as the synovium and/or the intrapatellar pad) to the graft structures using the synovial fluid for passage. The corroboration and clinical translation of this notion may be enhanced healing and intrasynovial adaptation of the tendon graft to the synovial milieu. Recently, we have compared the gross appearance and microscopic qualities of the PRP-treated and untreated grafts during the remodeling period (6-24 months). Gross morphology was evaluated using second-look arthroscopy focusing on graft thickness, apparent tension and synovium coverage. The overall arthroscopic evaluation provided evidence that a higher percentage of

the grafts rated as excellent in the PRP group (57% versus 33%). No grafts were scored as poor in the PRP group, but 20% of the controls showed poor morphology. At the same time, PRP treatment influenced the histological characteristics of the tendon graft, which resulted in tissue that was more mature than in the controls. Histology displayed newly formed connective tissue enveloping the graft in 77.3% of the PRP-treated grafts and in 40% of the controls (Sanchez et al., 2010). Other authors have used a compressed gelatin sponge soaked with leukocyte and platelet-rich concentrate (GPS system by Biomet Biologic, Warsaw, USA) sutured to the intra-articular part of the graft, which confirmed the acceleration of the maturation of the grafts treated with PRP as assessed by magnetic resonance imaging.

5.3 Cutaneous ulcers

Clinical differences between acute and chronic wounds are in part explained by alterations in the local biochemical environment. For example, acute wounds are associated with a greater mitogenic activity than chronic wounds.
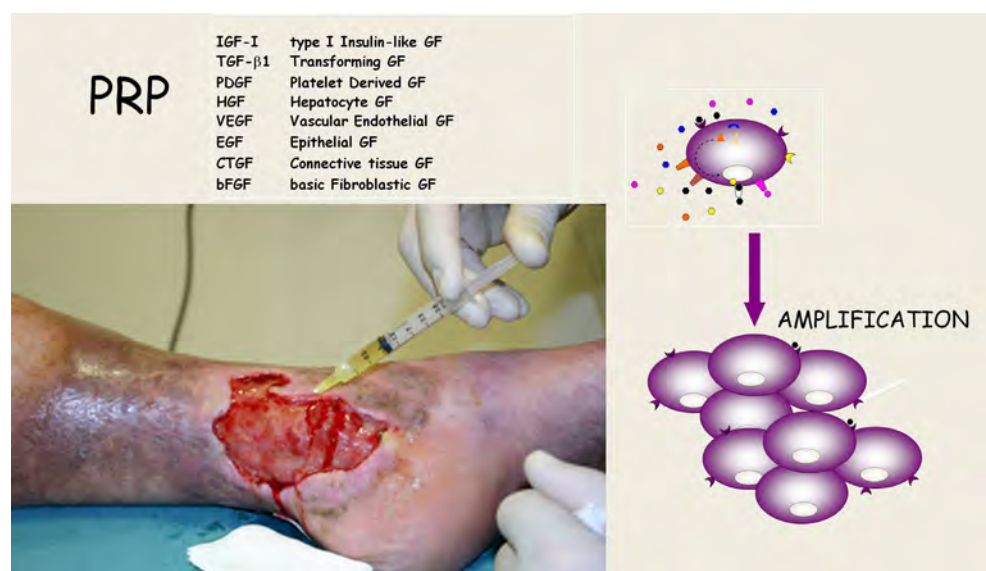

Fig. 6. Chronic ulcers are treated with several applications of PRP in order to enhance cell proliferation, and the formation of granulation tissue

Chronic wounds are associated with a higher level of pro-inflammatory cytokines than acute wounds. As chronic wounds begin to heal, they progress to a less inflammatory state. Elevated protease activities in chronic wounds may directly contribute to poor healing by degrading the proteins necessary for normal wound healing. Chronic wounds can be defined as those failing to proceed through an orderly and timely process to produce anatomic and functional integrity. Practically, a chronic wound is one that has failed to heal within 3 months. The cellular, biochemical and molecular events that characterize chronic wounds have been well defined, including a prolonged inflammatory phase, cellular senescence, deficiency of growth factors and/or their receptors, deficient fibrin production and

high levels of proteases. In normally healing wounds, acute inflammation with neutrophil infiltration brings neutrophil-derived matrix protease enzymes that debride the wound and pave the way for new tissue deposition and remodeling. In chronic wounds, the orderliness of the healing process is disrupted by some underlying abnormality that prolongs the inflammatory phase and produces a cascade of tissue responses that perpetuates the non-healing state. Repeated trauma, foreign bodies, pressure necrosis, infection, ischemia and tissue hypoxia also amplify the chronic inflammatory state, which is characterized by excess neutrophils, macrophages and lymphocytes. Fragments of dead tissue, bacterial products and foreign bodies are powerful chemoattractants that sustain a continuous influx of inflammatory cells, which in turn produce a variety of growth factors, cytokines, and matrix-degrading enzymes. Among the most potent of these enzymes are elastase and MMPs, which are present in large quantities in chronic wounds. Given the low levels of TIMPs, the MMP/TIMP balance is distorted; thus, the excess of proteolytic enzymes shifts the balance towards ECM destruction and the degradation of signaling proteins. Therefore, any effective intervention must include a strategy for disrupting this cycle and setting the wound on a permanent path towards healing. Historically, the first clinical application of platelet derived preparations was conducted in chronic leg ulcers in which wounds were filled with collagen embedded in platelet secreted proteins. This initial product, known as PDWHF (platelet-derived wound healing factors) stimulated the formation of the vascularized connective tissue found in healing wounds. Thereafter, various other types of platelet products have been assayed in several pilot studies, case series and clinical trials.

Growth factors are crucial for timely wound healing; inadequate levels of growth factors may be an important factor contributing to the chronicity of the wound, which may be degraded in excess by cellular or bacterial proteases. Initially, Margolis et al. (2001) showed that platelet releasates were more effective than standard therapy. Subsequently, PRP formulations were refined and primarily applied as fibrin membranes for the treatment of non-healing ulcers. More recently, the use of PRP in the management of chronic diabetic foot ulcers has been successful (Setta HS 2011). Moreover, PRP provides advantages in skin grafting for recalcitrant ulcers (Chem Tim et al., 2010). Allogenic platelet preparations have been used recently to treat recalcitrant ulcers in very elderly hypomobile patients for whom autologous blood processing may be difficult (Greppi et al., 2011). Finally, the use of PRP gel resulted in an improved quality of life and a lower cost of care over a 5-year period than other treatment modalities for patients with non-healing diabetic foot ulcers. Although actual treatment outcomes may differ from those modeled, PRP gel represents a potentially attractive treatment alternative for insurers and health care providers to address the cost burden and health effects of non-healing diabetic foot ulcers (Dougherty EJ 2008)

5.4 Other therapeutic approaches

The potential therapeutic value and versatility of platelet rich products has stimulated research in additional medical fields. PRP biotechnology holds promise as a healing preparation in surgical procedures and in the treatment of many different diseases. The use of PRPs for cell delivery and tissue engineering permit insights into the development of novel therapies. For example, autologous fat grafting, also known as fat transfer or fat injection, has long been a staple of cosmetic and reconstructive surgery. Fat grafts have proven very effective in the reconstruction of soft tissue defects, particularly for facial plastic and reconstructive

procedures. However, there has always been one significant disadvantage associated with autologous fat grafting: the unpredictable and often inconsistent graft survival rate. Promising new evidence has shown that PRP can enhance the fat graft survival rate. Moreover, nasolabial folds, superficial rhytids and acne scars have been successfully treated with injections of autologous PR fibrin matrices (Sclafani AP 2010). Additionally, PRP can be associated with novel dermatologic procedures as an aid in healing. For example, PRP is an effective method for enhancing wound healing and reducing transient adverse effects after fractional carbon dioxide laser resurfacing (Na et al., 2011).

Another remarkable application of PRP is in ophthalmology. Several successful examples include the use of PRP releasates as eye drops for the treatment of a broad spectrum of corneal persistent epithelial defects (Lopez-plandolit et al., 2010). Furthermore, the use of autologous platelet rich plasma was shown to be very effective in the treatment of patients suffering from dry eye symptoms; it improved both patient symptoms and major clinical signs [Alio et al., 2007]. Platelet rich plasma also promotes healing of dormant corneal ulcers even in eyes that are threatened by corneal perforation, and it is a reliable and effective therapeutic tool for the enhancement of epithelial wound healing on the ocular surface.
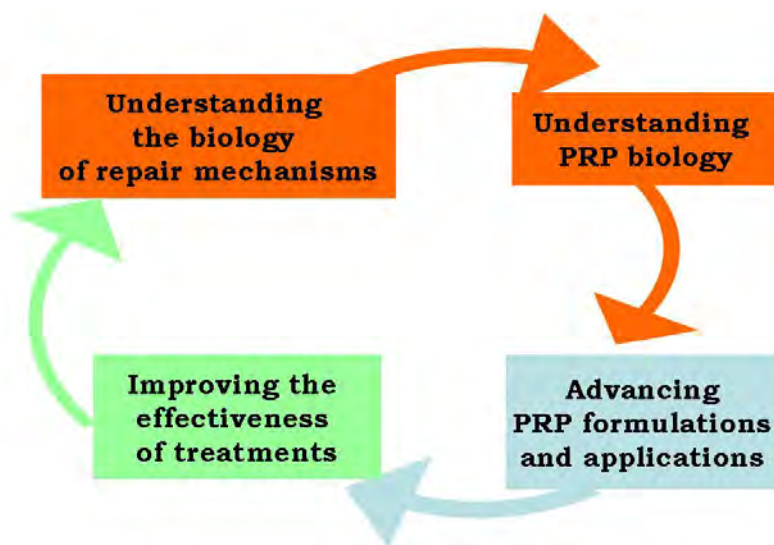

Fig. 7. The four domains of PRP science. Improved understanding of the biology of PRPs and repair mechanisms have emerged as a potential way of improving PRP formulations and applications. The identification of critical molecules that interact with healing will be critical in developing new approaches to treatments.

Other interesting recent approaches using PRP biotechnology include the successful application of platelet rich plasma in peripheral nerve regeneration (Sariguney et al., 2008) and the use of PRP biotechnology to treat damaged myocardial tissue. Utilizing a murine myocardial permanent ligation and ischemia/reperfusion model, a proprietary PRP formulation demonstrated a positive effect in left ventricular cardiac function. The use of PRP for skin rejuvenation is another application of PRP biotechnology.

6. Conclusion

Realistically, a substantial amount of research is needed to bring PRP technologies to the bedside, as clinical and laboratory findings that indicate its potential benefits must be followed by comprehensive clinical studies to demonstrate efficacy. Demonstrating effectiveness in different pathologies will be critical for the widespread adoption of PRP technology, including re-imbursement. Below is a schematic representation that illustrates the four domains of PRP science.

Because of the safety of these products, basic science, clinical discovery and patient-oriented research should be interdependent rather than successive steps. The substantial challenges of incorporating such research into clinical care must be pursued if the potential of PRPs is to be realized. Although PRP therapies have many compositions and procedures for application, they all try to maximize the cell signals that may enhance tissue healing. Our increased understanding of the healing mechanisms that result in tissue repair is paving the way towards the optimization of healing therapies

7. Acknowledgements

8. References

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