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Platelet-Rich Therapies in the Treatment of Orthopaedic Sport Injuries

Mikel Sánchez,[1] *Eduardo Anitua*,[2] *Gorka Orive*,[2] *Iñigo Mujika*[3] and *Isabel Andia*[2]

1 Unidad de Cirugía Artroscópica ''Mikel Sánchez'', La Esperanza, Vitoria, Spain
2 Biotechnology Institute IMASD, San Antonio, Vitoria, Spain
3 USP Araba Sport Clinic, Vitoria, Spain

Abstract

Biomedical sciences have made major advances in understanding how tissues repair, and the signalling mechanisms required to achieve this goal are progressively being dissected. Advances in the understanding of tissue repair mechanisms and the pivotal role of growth factors have stimulated the use of platelet-rich therapies by orthopaedic surgeons and sports physicians, mainly with the aim of stimulating and enhancing tissue healing. Autologous activated platelets retained in fibrin matrices are used as a source of molecular signals that control cell fate, including cell growth, cell differentiation and the synthesis of diverse functional proteins. Thus far, platelet-rich technologies have spawned additional ambitious endeavours, including surgical and non-surgical treatments in sports orthopaedics. Reconstruction of anterior cruciate ligament and tendon surgery and treatment of joint injuries, tendinopathy or muscle tears are but a few examples of the potential applications of this technology in the field of orthopaedic sports medicine. In the present article, some of the most important therapeutic applications using these approaches – especially preparation rich in growth factor (PRGF) technology – are presented, as are some of the limitations, anti-doping concerns and future challenges in the field. In view of a general state of confusion, the concept of platelet-rich plasma needs rigorous definition associated with well characterized products and re-administration procedures. There is evidence that reconstruction of anterior cruciate ligament and tendon surgery combined with PRGF enhances healing and functional recovery; clinical evidence is also appearing in the literature regarding treatment of tendinopathies and osteoarthritis. Currently, the challenge lies in conducting randomized, controlled clinical trials to determine the essential qualities of these technologies. If anti-doping agencies clarify their regulatory guidelines, robust studies in athletes are expected to emerge. Although much research work lies ahead, the current knowledge points to a future in which platelet-rich therapies will continue improving existing conventional approaches to treatment of sports injuries.

The field of sports medicine is currently expanding at a high pace, influencing millions of people from athletes to active individuals who participate in recreational sport or simply use exercise to stay healthy and active.[1,2] Physical activity and maintenance of good exercise habits are

promoted by current health systems in the industrialized countries because of the clear link between participating in sport and being fit and healthy. However, while the number of sports practitioners increases, the rate of sports injuries distressing the musculoskeletal tissues is growing and becoming a real problem.[3] For instance, Australia has recently reported 3.7 cases of medically treated sports injuries per 100 persons, with the costs increasing to $A1.5 billion (year of costing 2001) per year.[1]

The magnitude of the problem has stimulated significant research efforts focused on injury prevention, identifying ways to minimize risks and to promote protective behaviours.[4,5] Other endeavours target biological questions aiming to provide effective methods for helping the injured athlete/patient to recover within the shortest possible time frame. Those performing basic science believe that understanding the molecular basis of healing mechanisms is at the heart of the development of novel and rational approaches to treat injury. The progressive understanding of mechanisms required for successful tissue repair[6,7] has set the basis for the possibility of making injured tissues heal faster.[8] Among the emerging technologies for enhancing and accelerating tissue healing, a biocompatible and cost-effective approach, broadly referred to as platelet-rich therapy, involves the use of autologous activated platelets retained in fibrin matrices as a source of growth factors released from a three-dimensional scaffold.[9,10]

The present paper briefly addresses the main features of these autologous preparations, the most exciting therapeutic applications in sports medicine and the existing current challenges.

1. Tissue Repair and Growth Factors

A primary consideration for understanding a therapeutic treatment focused on accelerating healing is to learn from the physiological processes of wound healing and tissue repair. Tissue repair involves a number of complex cellular and molecular events participating through different coordinated phases that are to a great extent shared by the different tissues of the body. Subsequent to haemostasis, what primarily emerges is the notion that cells can initiate tissue repair by proliferating, a phase that involves either local cells or undifferentiated cells migrating into the injured area.[11] Concomitantly, physiological angiogenesis (provision of efficient concentration of nutrients and oxygen) takes place, which is critical to guarantee the survival of whole cell number. As proliferation and differentiation phases progress, the predominant cell in the wound site is responsible for producing the new extracellular matrix needed to restore the structure and the functionality of the injured tissue.[12] The challenge in orthopaedic sports medicine is to achieve tissue repair through a new well organized extracellular matrix, which ideally would reach the high mechanical performance and functional levels of the non-injured tissue.[13]

It is assumed that the above-mentioned stages of the tissue repair process are mediated and controlled by a wide range of growth factors and cytokines that modulate cell function through direct physical interactions with the extracellular domain of transmembrane receptors.[6] The latter transduce secondary signals, thereby controlling diverse aspects of subcellular biology. Although the roles of all the growth factors involved in tissue regeneration are only partially elucidated, the potential benefits of many of them have been demonstrated. For example, platelet-derived growth factor (PDGF) is a powerful mitogen for connective tissue cells,[14] transforming growth factor-β (TGFβ) is not only morphogenic but is also strongly implicated in collagen synthesis,[15] type I insulin-like growth factor (IGF-I) is critical for cell survival, growth and metabolism,[16] and the cooperative actions of vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) induce endothelial cell proliferation and migration, thus initiating the angiogenic response.[17]

Ultimately, therapeutic approaches to manipulate healing may need to integrate multiple cell types and large signalling networks necessary for the dynamic communication between cells.[18] The need to target various signalling pathways demands the administration of a balanced combination of mediators instead of administering a

purified isolated growth factor, which could not cope with the multiple requirements of the injured tissue. The delivery of autologous growth factors to the injured tissue may result in significant changes in biological function of the local cells. However, producing biologically, chemically and mechanically normal tissue will require a combination of strategies. So combining the use of platelet-rich products with appropriate mechanical loading regimens might yield better tissue organization in a shorter time and enhanced mechanical properties,[19] which are of paramount importance in sports medicine.

2. Biological Delivery of Growth Factors: Platelet-Rich Therapies

The advent of regenerative medicine, aiming to rapidly translate the science into patient care using patients' own resources, has opened the door to new approaches never imagined before. For example, the use of platelets as vehicles for the delivery of a balanced pool of healing factors has become a new therapeutic treatment since the late 1990s. At that time, platelet-rich plasmas (PRPs) were introduced as autologous modifications of potent adhesives known as fibrin glues.[20,21] The use of platelets as a source of growth factors was particularly fortuitous given that the main initial interest was to take advantage of the adhesive and haemostatic properties of fibrin. Realization of the clinical value of platelet-rich therapies arose from clinical observations such as enhanced bone formation and anti-inflammatory function after oral and maxillofacial applications.[22,23] These anti-inflammatory and antibacterial effects are attributed mainly to the presence of platelets in these preparations.[24,25] Platelets are produced in large numbers from megakaryocytes in the bone marrow. Anucleate platelets circulate for 7–10 days and mediate primary haemostasis. Upon coagulation, platelets secrete a pool of growth factors and other cytokines involved in healing.[26]

Since platelets are renowned as the major sources of healing factors within blood clots, the idea that concentrating them at the injured site could somewhat accelerate and optimize the healing mechanisms set the rationale for the development of PRP. Indeed, platelets carry α-granules and upon activation secrete multiple molecules such as PDGF, $TGF\beta_1$, platelet factor 4 (PF4), VEGF, endostatins, angiopoietins and thrombospondin-1.[26,27] In addition to providing initial cues for homing of precursor cells to the injury and differentiation, platelets and fibrin are also known to be potent adhesive substrates for cells. As reviewed elsewhere, the outcome of healing is influenced by fibrin structure (thickness of the fibres, number of branch points, the porosity and permeability of the clot) at the wound site.[28] The addition of a platelet-rich preparation at the injury site will accelerate the natural healing process and provide additional support for the binding of not only platelets but also, among others, endothelial cells, smooth muscle cells, fibroblasts, keratinocytes and incoming stem cells. Quite surprising is the recently recognized ability of platelets to reduce pain. The molecular basis of how platelets can influence pain is unknown; one possible explanation is protease-induced release of protease-activated receptor-4 peptides from platelets that have anti-nociceptive properties.[29] Not to be forgotten among the advantages in using platelet-rich therapies is the antibactericidal effects of the antibacterial and fungicidal proteins stored in platelets, which may help to prevent infection.[30,31]

Most commercial protocols for producing plasma are derived from the same general principle of blood spinning, and the products thereby obtained are denominated by a common terminology, i.e. PRP or platelet concentrates. However, the composition of these products differs widely, both qualitatively and quantitatively. PRP is an excessively general and vague concept, which demands more precise terms. Since a critical difference relies on accompaniment of concentrated leukocyte in the preparation, other authors[32] suggest the term 'platelet-leukocyte-rich plasma' (PLRP) for the non-activated product rich in platelets and leukocytes, and platelet-leukocyte gel (PLG) for the same product after its activation. Whether leukocytes have a positive or negative influence cannot be generalized for all tissues and clinical conditions, and remains a

controversial issue that demands further investigation. *A priori*, we report several concerns regarding the presence of neutrophils, which contain matrix metalloproteinase (MMP)-8 (referred to as neutrophil collagenase, once thought to be expressed exclusively in neutrophils and polymorphonuclear leukocytes) and also express MMP-9.[33] The release of reactive oxygen species by neutrophils may also be relevant. In fact, the paradigm suggests that neutrophils infiltrate injured tissue and in the process of assisting removal of disrupted tissue, exacerbate or increase the original damage.[34,35] In addition, there are *in vitro* data demonstrating that neutrophils can injure skeletal myotubes.[36] The available evidence, therefore, suggests that infiltrating neutrophils in injured skeletal muscle can act as a cytotoxic agent causing secondary destruction to muscle.[37]

Additionally, the characteristics of the product and the re-administration procedures to patients/athletes may also differ widely, raising controversial opinions regarding their therapeutic value.[38] In view of the general state of confusion, it is necessary to use a precisely defined terminology associated with well characterized products[39] while justifying the application procedures. The preparation rich in growth factor (PRGF) is an alternative technology developed by our group to formulate and use platelets as growth factor and protein reservoir units. The term 'PRGF' identifies 100% autologous and biocompatible products elaborated using a one-step centrifugation process, and sodium citrate and calcium chloride as anticoagulant and activator, respectively.[9] The latter is critical to achieve a sustained release of growth factors; moreover, using a standardized dose of calcium chloride, while avoiding the addition of exogenous thrombin, grants control over the liquid-gel (fibrin matrix) transformation and confers versatility on administration procedures.[40] For example, one possibility is to inject the activated liquid, a process that will result in local fibrin matrix development and proteins being readily extruded from the matrix *in situ*. Alternatively, the fibrin scaffold can be formed *ex vivo* and then implanted in the chosen site prior to retraction. Furthermore, if the fibrin matrix is incubated *ex vivo* for 40 minutes or so, the fibrin structure is modified in such a way that it retracts and turns into an elastic dense membrane. The latter can be used to enhance cicatrization of soft tissues.[41] As shown in figure 1, the preferred administration procedure depends on the specific management of each medical condition. Being aware of possible undesired effects provoked by proinflammatory proteases and acid hydrolases released from leukocytes, we exclude them from PRGF preparations. Neutrophils could be particularly detrimental for the injured tissue,[9] especially in muscle strains[36] or joint infiltrations, as discussed above.[33] Ultimately, the biosafety and versatility of this approach has inspired and stimulated its therapeutic use in a wide range of medical and scientific fields,[42,43] and to an outstanding degree in orthopaedics and sports medicine.

3. Platelet-Rich Therapies in Orthopaedic Sports Medicine

The emergence of PRPs as a cutting edge technology in the treatment of sports injuries appears intriguing. The easy preparation protocols and the biosafety and versatility of the platelet-based preparations and their reduced cost have stimulated 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.

3.1 Tendons

One interesting focus of research with platelet-rich therapies is tendon repair. Tendons consume comparatively low energy by themselves, resulting in a low metabolic rate that entails slow healing after injury. However, it has been shown that several growth factors can stimulate tendon repair after exogenous local application.[44] Considering that the pool of plasma and platelet-secreted factors may potentially benefit tendon repair, we have undertaken *in vitro* studies on tenocytes, the dominant cell type in tendons and also responsible for tendon physiological or

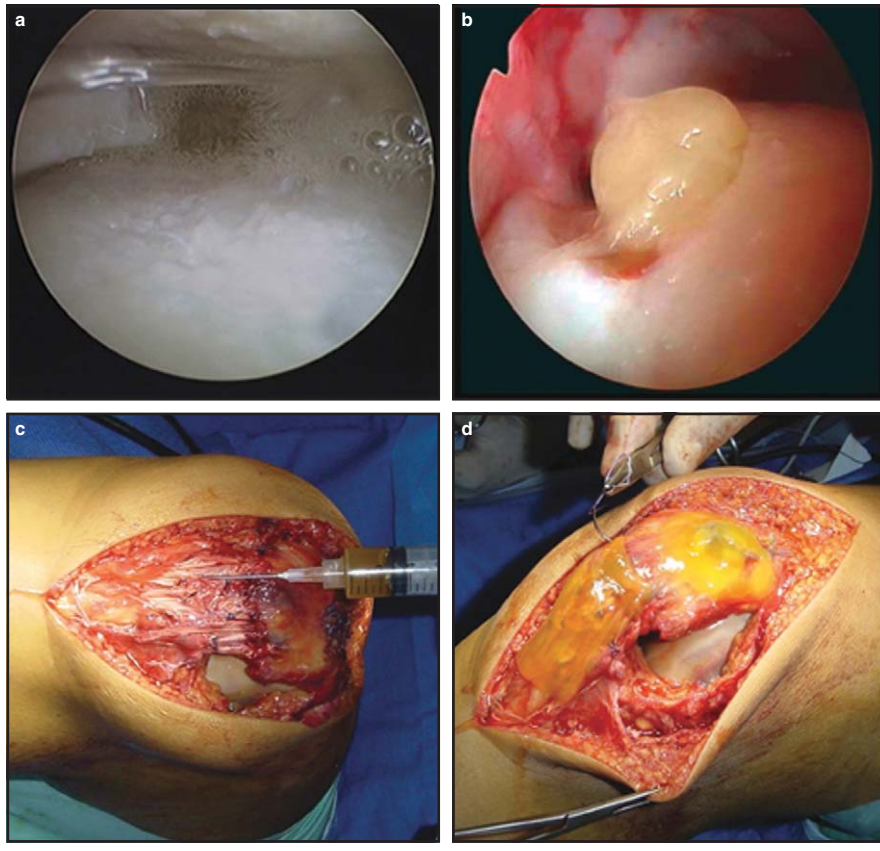

Fig. 1. Relevant procedures for growth factor and protein delivery in orthopaedic sports injuries: (**a**) the liquid-activated preparation rich in growth factor (PRGF) can be infiltrated within the capsular joint in patients with osteoarthritis; (**b**) PRGF fibrin scaffold is implanted in a cartilage defect by arthroscopy; (**c**) PRGF is injected in the injured tendon tissue to restore the biological environment; (**d**) the surgically treated patellar tendon is covered with dense fibrin membranes to enhance soft tissue healing.

pathological response to changes in the biological and mechanical environment. We have observed that the pool of growth factors released from an autologous PRGF increased the proliferation of human tendon cells significantly and stimulated them to produce growth factors such as VEGF and HGF.[45] The cooperative paracrine action of these growth factors will promote angiogenesis that is directly related to tendon healing capability and tendon graft integration. Additionally, since HGF is a potent antifibrotic agent, its secretion may help to reduce the scar formation around tendon tissues.[46] Further research in a sheep model showed that repetitive injection of PRGF within Achilles tendon fascicles triggered a healing response assessed by increased cell number and angiogenesis, and did not provoke fibrosis.[47,48] Other researchers have reported that injections of PRP 1 week postoperatively increased tendon regeneration and strength.[49] Recently, it has been observed that locally injected PRP is useful as an activator of circulation-derived cells for enhancement of the initial tendon healing process.[11]

This basic information gave us insight into how PRGF may benefit tendon healing when applied clinically. In operative treatment for serious structural damage such as partial or complete tendon tears, healing can be enhanced by PRGF application during the surgical procedure.

Using this ground-breaking surgical approach in athletes, we observed a significant acceleration in functional recovery after surgical repair of ruptured Achilles tendon compared with a matched group who had conventional surgery.[50] In addition, we found that the early stimulatory effect induced by PRGF can also have long-term consequences (after >18 months) such as decreased cross-sectional area in Achilles tendons treated with PRGF compared with conventional surgery.

Typically, tendon injuries are traumatic and acute but in many cases they become chronic. 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. It is often assumed that the latter is one of the most exasperating problems for patients and physicians in orthopaedic sport medicine. Being related to repetitive movements and overuse, the location of such injuries is sport specific. Patellar tendinopathies, for instance, are often associated with jumping sports such as basketball, volleyball and high jump,[51] while tennis players and golfers are more prone to medial and lateral epicondylitis.[52] The use of PRP in this context might be focused on restoring the normal tissue composition while avoiding further degeneration. In these conditions, ultrasound-guided PRP injection may offer an alternative treatment over palliative or operative treatments. Such treatment was evaluated in a cohort study by Mishra et al.,[53] reporting a reduction of pain in PRP treatment of chronic severe elbow tendinosis. Curiously, others have reported that autologous blood injection under ultrasound guidance appears to be an effective treatment of severe medial epicondylitis, commonly known as golfer's elbow, as shown by pain reduction and an index of elbow performance.[54]

3.2 Joint Injuries

People who participate in sports have an increased risk of joint damage. High levels of impact and torsional loading disrupt articulations, provoking a wide array of joint disorders. For instance, anterior cruciate ligament (ACL) injuries are usually traumatic and sports related,[55,56] with approximately 100 000 ACL reconstructions performed in the US each year[57] at a cost just under $US1 billion (year of costing 1998) per year.[58] Reconstruction is the surgical treatment of choice, as direct primary repair has been shown to result in persistent laxity and instability of the knee. In view of the clinical relevance of the problem, several groups have attempted to fabricate tissue-engineered ligaments using natural biomaterials and a wide range of nanometre-sized artificial scaffolds.[59] Deriving knowledge from preclinical research and clinical activities in a synergistic fashion showed the ways to assist ACL reconstructive surgery introducing PRGF technology. In this context, PRGF may bridge the gap between inactive scaffolds and cell biology, offering to the scaffold structure the biological stimulation necessary to become transformed into a functional remodelling tissue.[60] This novel approach to creating fully integrated bioactive grafts was proposed by our group 6 years ago,[61] using the traditional paradigm of *in vivo* tissue engineering in which platelet-rich fibrin was infiltrated to transfer growth factors to autologous or homologous grafts, therein providing biological cues for cell migration, proliferation, angiogenesis and remodelling. The increased bioavailability of angiogenic factors released from PRGF will promote a rapid blood supply to the graft, contributing to a rapid remodelling.[45] Aiming to achieve successful fixation of the graft and prompt functional efficacy, PRGF is also applied within both femoral and tibial bone tunnels created by the surgeons to secure the ends of the graft. Moreover, to provide early strength and optimal healing we used biological anchors created by mixing PRGF with the autologous bone plugs obtained during the procedure. Using this approach it is possible to enhance the long-term anchoring of the graft to the bone.

In another approach we observed exciting results after intra-articular administration of PRGF in the arthroscopic treatment of an avulsion of articular cartilage in the knee of a young soccer player.[62] Full-thickness cartilage defects treated with PRGF showed enhanced mechanical

properties in a rabbit model.[63] Other groups[64,65] have reported the ability of PRP to support chondrogenesis and the healing of meniscal defects by implanting PRP combined with cultured cells in animal models. These results are stimulating, since both the avascular cartilage and meniscus have limited or no chances for proper functional repair.

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. The latter is a common medical problem for athletes with a history of joint injury. 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.[66] Competitive soccer players present with an increased prevalence of osteoarthritis in the lower extremity joints compared with age-matched controls.[67] Premature osteoarthritis is also a serious concern in the growing community of 'baby boomers' and recreational athletes who are too young for knee replacements. Platelet-rich therapies may be promising in these populations. Basic research performed in our laboratory showed that intra-articular PRGF might be beneficial in restoring hyaluronic acid concentration within the joint and switching angiogenesis to a more balanced status, although it does not halt the effects of interleukin-1b on synovial cells.[68] Correspondingly, our preliminary clinical results showed that intra-articular injection of PRGF may be a new therapeutic option for osteoarthritis treatment in selected patients.[69,70] However, this outstanding development is still in its infancy and continued research is needed to ascertain the potential value and the underlying biological effects. Accordingly, we have launched a randomized, multicentre clinical trial to evaluate the therapeutic effect of PRGF infiltrations within the osteoarthritic knee.[71]

3.3 Muscle Tears

A muscle strain typically keeps athletes out of action for several weeks, and sports clubs employ fulltime medical staff in order to minimize this down-time. Conventionally, treatment comprises physiotherapy, which uses physical modalities such as ice, electrotherapy, massage, mobilization, manipulation and exercise to optimize the healing process. In these cases, platelet-rich therapies are applied as an alternative to conventional approaches, because of the promise of accelerating muscle healing and reducing a player's injury time. In this approach the early blood clot is substituted with platelet-rich fibrin, which maintains the regenerative space, provides supraphysiological concentrations of healing factors, and acts as the primer of the overtaking healing phases. In this context, special attention should be given to the composition of PRP products. As discussed in section 2, when preparing PRGF we avoid the presence of leukocytes, since neutrophils can exacerbate or increase the original muscle damage.[34,35] 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. Furthermore, fibrosis did not appear in any of the treated cases and no re-injuries occurred in any athlete after resuming their normal sports activities.[72]

4. Future Directions

Platelet-rich therapies represent a new biomedical technology for the stimulation and acceleration of healing and tissue regeneration. The above examples represent only some of the interesting current approaches, but the authors believe this technology may see new exciting developments in the next few decades.

One important consideration for future expansion of the field is to clarify any possible concern related to the biosafety of this approach, and especially any anti-doping concern. The recent World Anti-Doping Agency code, which prohibits all use of growth factor therapies in elite sport, has provoked distress among sports medicine practitioners. A detailed analysis of the prohibited S2 section reveals that only IGF-I may have a possible connection with the platelet-rich therapies described in the article.[73] However, there are at least two compelling reasons that eliminate

any anti-doping concern from the therapeutic use of PRGF. Firstly, the doses of IGF-I released from the PRGF are sub-therapeutic,[46] in terms of inducing systemic anabolic actions, by a factor of between 500 and 1000 (100 ng vs 160 μg). Secondly, the availability of most IGF-I is modulated by a binding protein[74] (IGFBP3) and only 1% of the total IGF-I released from the PRGF is unbound, and therefore biologically available and active, ready to bind to receptors IGF-IRs inducing biological outcomes. Additionally, the short half-life (10 minutes) of this unbound and active IGF-I makes an alteration in systemic levels unlikely. In any case, such a controversy may also affect other accepted therapeutic approaches, including the use of autologous tissue such as tendon grafts in the ACL reconstructions or bone grafts, as both applications imply the release of IGF-I.[75,76]

Therefore, the authors believe that it is necessary for regulatory and anti-doping agencies to refine and clarify their regulatory guidelines and prohibited lists in order to avoid confusion and to raise awareness of the current clinical utility of these types of products in general and especially of PRGF.

5. Conclusions

In summary, recent advances in the understanding of the pivotal role of growth factors in tissue repair mechanisms have opened new perspectives for the use of platelet-rich therapies to enhance tissue healing after orthopaedic sports injuries. PRPs applied at the injury site accelerate the physiological healing process, provide support for cellular binding, reduce pain and have anti-inflammatory and antibacterial effects. Proper characterization of available products and application procedures is needed before these therapies can be widely applied in the context of sports medicine. One of the most thoroughly analysed and researched of such products is PRGF, which has shown therapeutic value in tendon repair and treatment of joint injuries, and promising results are available in the treatment of osteoarthritis and muscle tears. Although much research work lies ahead, the current knowledge points to a future in which platelet-rich therapies will continue improving existing conventional approaches to patient care.

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Eduardo Anitua, Gorka Orive and Isabel Andia work in the Research Department of the Biotechnology Institute, a dental implant company that markets a system for preparing PRP for therapeutic use.

References

1. Cassel EP, Finch CF, Stathakis VZ. Epidemiology of medically treated sport and active recreation injuries in the Latrobe Valley, Victoria, Australia. Br J Sports Med 2003; 37: 405-9
2. Fanelli GC, Orcutt DR, Harris JD. The athletically active middle aged arthritic knee: introduction, demographics, sports participation epidemiology and types of injuries seen. Sports Med Arthroscop Rev 2003; 11 (2): 98-101
3. Timpka T, Ekstrand J, Svanström L. From sports injury prevention to safety promotion in sports. Sports Med 2006; 36 (9): 733-45
4. Fredericson M, Misra AK. Epidemiology and aetiology of marathon running injuries. Sports Med 2007; 37: 437-9
5. Goldber AS, Moroz L, Smith A, et al. Injury surveillance in young athletes. Sports Med 2007; 37: 265-78
6. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev 2003; 83: 835-70
7. Langer R, Tirell DA. Designing materials for biology and medicine. Nature 2004; 428: 487-92
8. Tabata Y. Tissue regeneration based on growth factor release. Tissue Eng 2003; 9: S5-15
9. Anitua E, Sanchez M, Nurden AT, et al. New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol 2006; 24: 227-34
10. Anitua E, Sánchez M, Orive G, et al. Therapeutic applications of the preparation rich in growth factors (PRGF). In: Jie Hu, editor. Recent advances in biomaterial research. Kerala: Transworld Research Network, 2008: 141-53
11. Kajikawa Y, Morihara T, Sakamoto H, et al. Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. J Cell Physiol 2008; 59: 1329-39
12. Hoffman A, Gross G. Tendon and ligament engineering in the adult organism: mesenchymal stem cells and gene-therapeutic approaches. Int Orthop 2007; 31: 791-7
13. Rehfeldt F, Engler AJ, Eckardt A, et al. Cell responses to the mechanochemical microenvironment: implications for regenerative medicine and drug delivery. Adv Drug Deliv Rev 2007; 59: 1329-39
14. Heldin CH, Westermark B. Mechanism of action and *in vivo* role of platelet derived growth factor. Physiol Rev 1999; 79 (4): 1283-316

15. Shi Y, Massague J. Mechanisms of TGF-beta signalling from cell membrane to the nucleus. Cell 2003; 113 (6): 685-700
16. Danielpour D, Song K. Cross-talk between IGF-I and TGF-beta signaling pathways. Cytokine Growth Factor Rev 2006; 17: 59-74
17. Gerritsen ME, Tomlinson JE, Zlot C, et al. Using gene expression profiling to identify the molecular basis of the synergistic actions of hepatocyte growth factor and vascular endothelial growth factor in human endothelial cells. Br J Pharmacol 2003; 140: 595-10
18. Gianchandani EP, Brautigan DL, Papin JA. Systems analyses characterize integrated functions of biochemical networks. Trends Biochem Sci 2006; 31: 284-91
19. Aspenberg P. Stimulation of tendon repair: mechanical loading, GDFs and platelets. A mini-review. Acta Orthop 2007; 31 (6): 783-9
20. Gibble J, Ness P. Fibrin glue: the perfect operative sealant? Transfusion 1990; 30: 741-7
21. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg 1997; 55 (11): 1294-99
22. Anitua E. Plasma rich in growth factors: preliminary results of the use in the preparation of future sites for implants. Int J Oral Maxillofac Implants 1999; 14: 529-35
23. Marx RE, Carlson ER, Eichstaedt RN, et al. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endodon 1998; 85: 638-46
24. El-Sharkawy H, Kantarci A, Deady J, et al. Platelet rich plasma: growth factors and pro- and anti-inflammatory properties. J Periodontol 2007; 78: 661-9
25. Bielecki TM, Gazdzik TS, Arendt J, et al. Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances. J Bone Joint Surg 2007; 89: 417-20
26. Anitua E, Andía I, Ardanza B, et al. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost 2004; 91: 4-15
27. Nurden AT, Nurden P, Sanchez M, et al. Platelet and wound healing. Front Biosci 2008; 13: 3532-48
28. Laurens N, Koolwijk P, De Maat MPM. Fibrin structure and wound healing. J Thromb Haemost 2006; 4: 932-9
29. Asfaha S, Cena N, Houl S, et al. Protease-activated receptor-4: a novel mechanism of inflammatory pain modulation. Br J Pharmacol 2006; 150: 176-85
30. Trowbridge CC, Stammers AH, Woods E, et al. Use of platelet gel and its effects on infection in cardiac surgery. J Extra Corpor Technol 2005; 37 (4): 381-6
31. Tang YQ, Yeaman MR, Selsted ME. Antimicrobial peptides from human platelets. Infect Immun 2002; 70: 6515-7
32. Everts AM, Zundert AV, Schönberger JPAM, et al. What do we use: platelet-rich plasma or platelet leukocyte gel? J Biomed Mat Res A 2008; 85: 1135-6
33. Bramono DS, Richmond JC, Weitzel PI, et al. Matrix metalloproteinases and their clinical applications in orthopaedics. Clin Orthop Relat Res 2004; 428: 272-85
34. Tidball JG. Inflammatory cell response to acute muscle injury. Med Sci Sports Exerc 1995; 27: 1022-32
35. Tidball JG. Inflammatory processes in muscle injury and repair. Am J Physiol Regul Integr Comp Physiol 2005; 288: R345-53
36. Pizza FX, McLoughlin TJ, McGregor SJ, et al. Neutrophils injured cultured skeletal myotubes. Am J Physiol Cell Physiol 2001; 281: C353-41
37. Schneider BP, Tiidus PM. Neutrophil infiltration in exercise-injured skeletal muscle. Sports Med 2007; 37 (10): 837-56
38. Veillet CJ, McKee MD. Growth factors-BMPs, DBMs, and buffy coat products: are there any proven differences amongst them? Injury Int J Care Injured 2007; 38 Suppl. 1: S38-48
39. Anitua E, Sánchez M, Orive G, et al. Shedding light in the controversial terminology for platelet rich products. J Biomed Mat Res A. In press
40. Anitua E, Sanchez M, Orive G, et al. Delivering growth factors for therapeutics. Trends Pharmacol Sci 2008; 29 (1): 37-41
41. Anitua E, Aguirre JJ, Algorta J, et al. Effectiveness of autologous preparation rich in growth factors for the treatment of chronic cutaneous ulcers. J Biomed Mater Res B: Appl Biomater 2008; 84B: 415-21
42. Anitua E, Sanchez M, Orive G, et al. The potential impact of the preparations rich in growth factors (PRGF) in different medical fields. Biomaterials 2007; 28: 4551-60
43. Creaney L, Hamilton B. Growth factor delivery methods in the management of sports injuries: the state of the play. Br J Sports Med 2008; 42: 314-20
44. Molloy T, Wang Y, Murrell GAC. The roles of growth factors in tendon and ligament healing. Sports Med 2003; 33: 381-94
45. Anitua E, Andia I, Sanchez M, et al. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. J Orthop Res 2005; 23: 281-6
46. Conway K, Price P, Harding KG, et al. The molecular and clinical impact of hepatocyte growth factor, its receptors, activators, and inhibitors in wound healing. Wound Rep Reg 2006; 14: 2-10
47. Anitua E, Sanchez M, Nurden AT, et al. Autologous fibrin matrices: a potential source of biological mediators that modulate tendon cell activities. J Biomed Mat Res A 2006; 77: 285-93
48. Anitua E, Sanchez M, Nurden AT, et al. Reciprocal actions of platelet-secreted TGF-beta1 on the production of VEGF and HGF by human tendon cells. Plast Reconstr Surg 2007; 119: 950-9
49. Virchenko O, Aspenberg P. How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay between early regeneration and mechanical stimulation. Acta Orthop 2006; 77 (5): 806-12
50. Sanchez M, Anitua E, Azofra J, et al. Comparison of surgically repaired Achilles tendon tears using PRGF. Am J Sports Med 2007; 35 (2): 245-51
51. Peers KH, Lysens RJ. Patellar tendinopathy in athletes. Sports Med 2005; 35 (1): 71-87
52. Field LD, Savoie FH. Common elbow injuries in sport. Sports Med 1998; 26 (3): 193-205
53. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med 2006; 34 (11): 1774-8

54. Suresh SPS, Ali KE, Jones H, et al. Medial epicondylitis: is ultrasound guided autologous blood injection an effective treatment? Br J Sports Med 2006; 40: 935-9
55. Griffin LY. Noncontact anterior cruciate ligament injuries: risk factors and prevention strategies. J Am Acad Orthop Surg 2008; 8: 141-50
56. Johnson DL, Urban Jr WP, et al. Articular cartilage changes seen with magnetic resonance imaging detected bone bruises associated with acute anterior cruciate ligament rupture. Am J Sports Med 1998; 26 (3): 409-14
57. Brown CH, Carson EW. Revision anterior cruciate ligament surgery. Clin Sports Med 1999; 18: 109-17
58. Pennisi E. Tending tender tendons. Science 2002; 295: 1011
59. Vunjak-Novakovic G, Altman G, Horan R, et al. Tissue engineering of ligaments. Ann Rev Biomed Eng 2004; 6: 131-56
60. Murray MM, Spindler KP, Devin C, et al. Use of a collagen-platelet rich plasma scaffold to stimulate healing of a central defect in the canine ACL. J Orthop Res 2006; 24 (4): 820-30
61. Sánchez M, Azofra J, Aizpurua B, et al. Use of autologous plasma rich in growth factors in arthroscopic surgery. Cuadernos Artroscop 2003; 10: 12-9
62. Sánchez M, Azofra J, Anitua E, et al. Plasma rich in growth factors to treat an articular cartilage avulsion: a case report. Med Sci Sports Exerc 2003; 35: 1648-52
63. Cugat R, Carrillo JM, Serra I, et al. Articular cartilage defects reconstruction by plasma rich in growth factors. In: Zanasi S, Brittberg M, Marcacci M, editors. Basic science, clinical repair and reconstruction of articular cartilage defects: current status and prospects. Bologna: Timeo Editore, 2006: 801-7
64. Akeda K, An HS, Okuma M, et al. Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis. Osteoarthritis Cartilage 2007; 14: 1272-80
65. Ishida K, Kuroda R, Miwa M, et al. The regenerative effects of platelet-rich plasma on meniscal cells *in vitro* and its *in vivo* application with biodegradable gelatin hydrogel. Tissue Eng 2007; 13 (5): 1103-12
66. Rall K, McElroy G, Keats TE. A study of the long-term effects of football injury in the knee. Mol Med 1984; 61: 435-38
67. Shephard GJ, Banks AJ, Ryan WG. Ex-professional association footballers have an increased prevalence of osteoarthritis of the hip compared with age-matched controls despite not having sustained notable hip injuries. Br J Sports Med 2003; 37: 80-1
68. Anitua E, Sanchez M, Nurden AT, et al. Platelet-released growth factors enhance the secretion of hyaluronic acid and induce hepatocyte growth factor production by synovial fibroblasts from arthritic patients. Rheumatology 2007; 46: 1769-72
69. Anitua E, Sanchez M, Azofra J, et al. Autologous preparation rich in growth factors for treating knee osteoarthritis: clinical-biological study [abstract]. World congress on osteoarthritis: osteoarthritis cartilage; 2007 Dec; Fort Lauderdale (FL), 15 Suppl. C: 6-9 C218
70. Sanchez M, Anitua E, Azofra J, et al. Clinical benefits of using an autologous preparation rich in growth factors to treat knee osteoarthritis [abstract]. World congress on osteoarthritis: osteoarthritis cartilage; 2007 Dec; Fort Lauderdale (FL), 15 Suppl. C: 6-9 C148
71. Sánchez M, Anitua E, Azofra J, et al. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study. Clin Exp Rheumatol 2008; 26: 910-3
72. Sanchez M, Anitua E, Andia I. Application of autologous growth factors on skeletal muscle healing [oral presentation]. 2nd International Conference on regenerative medicine 2005 [online]. Available from URL: http://regmed2005.abstract-management.De/overview/?ID = 314 [Accessed 2008 Jan 8]
73. World Anti-Doping Agency [online]. Available from URL: http://www.wada-ama.org [Accessed 2008 Mar 16]
74. Denley A, Cosgrove LJ, Booker GW, et al. Molecular interactions of the IGF system. Cytokine Growth Factor Rev 2005; 16: 421-39
75. Olesen JL, Heinemeier KM, Langberg H, et al. Expression, content and localization of insulin-like growth factor I in human Achilles tendon. Connect Tissue Rep 2006; 47: 200-6
76. Wildemann B, Kadow-Romacker A, Pruss A, et al. Quantification of growth factors in allogenic bone grafts extracted with three different methods. Cell Tissue Bank 2007; 8 (2): 104-14

Correspondence: Dr *Isabel Andia*, c/o Leonardo Da Vinci 14, 01510 Minano, Alava, Spain.
E-mail: Isabel.andia@bti-imasd.com