The Future: Optimizing the Healing Environment in Anterior Cruciate Ligament Reconstruction

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Abstract: The therapeutic use of autologous platelet-rich plasma constitutes a breakthrough in the stimulation and acceleration of soft-tissue healing and bone regeneration. Platelet-rich technologies seek to facilitate anterior cruciate ligament replacement by mimicking the native tissue and improving the adequacy of tissue function with appropriate cues, ultimately leading to better patient care. There are different technical protocols for preparing platelet-rich plasma, and the resultant products are typically heterogeneous; moreover, protocols for administrating the products in patients vary extensively. Poor standardization in the field makes full evaluation of different plasma products and establishing standards for the most beneficial applications of this technology difficult. This article presents the current data on the use of platelet-rich plasma in the reconstruction of the anterior cruciate ligament. Although the findings are not conclusive, the use of autologous platelet-rich plasma is shown to be safe, reproducible, and effective in mimicking the natural processes of soft tissue and bone healing. Platelet-rich technologies offer new opportunities for research and the application of anterior cruciate ligament tissue engineering.

Key Words: Knee, arthroscopy, platelet-rich therapies, healing, ACL reconstruction, tendon graft

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A torn anterior cruciate ligament (ACL) is a common injury, and is more highly typical among the active young population. It requires surgical intervention to stabilize the knee and prevent cartilage and meniscal injuries, which lead to degenerative joint disease.[1] 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 biologic challenges. It requires the application of mechanical knowledge as well as an understanding of how cells maintain 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 platelet-rich (PR) technologies seeks to facilitate ACL healing by mimicking the native tissue and improving the adequacy of tissue function with the appropriate cues, ultimately leading to better patient care.

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The therapeutic administration of PR plasma (PRP) extends to the treatment of multiple musculoskeletal disorders and to the regeneration and healing of a wide range of tissues in several medical and scientific fields.[2] In 2003, Sánchez et al[3] were the first to report the use of PRP for orthopedic-related problems. Furthermore, they conceived the "in situ" preparation of fully integrated bioactive grafts for ACL reconstruction using the now-traditional paradigm of tissue engineering.[4] Since then, PRP and methods for its application (ie, PR therapies) have been further developed, creating new opportunities for tissue repair in sports medicine[5,6] and even opening the door to novel therapies for the treatment of knee joint pathologies.[7] PRP is an autologous product prepared from the patient's own blood, hence it is safe and biocompatible[7,8]; in addition, clearance for marketing (510 k) is achievable without the need for randomized clinical trials and thus the product can be introduced rapidly into the clinical setting. This technology may offer real and clear possibilities of therapeutic application in some of the most important orthopedic problems. In short, the development of this technology could be of outstanding relevance both from social and medical points of view.

The future of ACL reconstruction is based on the fundamental understanding of the relationships among cells, scaffolds, and signaling factors, as this knowledge provides the basis for the development of biologic substitutes to restore, maintain, or improve function in ACL reconstruction.[9] PR therapies contribute to healing by serving as a source of growth factors and cytokines. On the basis of the potential of PR therapies in soft tissue and bone regeneration, we discuss the role of these therapies in enhancing tendon graft ligamentization and the regeneration of bone-bone or tendon-bone interfaces. As one example of an effort to reduce the morbidity associated with autografts, it is possible to introduce PRP to the donor region where the plasma can regenerate tissue.

PR TECHNOLOGIES FOR DELIVERING GROWTH FACTORS

Platelets are known for their role in hemostasis, where they help to prevent blood loss at sites of vascular injury. They also have a role in tissue repair and vascular remodeling as well as having key roles in the inflammatory and immune responses.[10] Blood platelets are produced in large numbers from megakaryocytes in the bone marrow. The normal platelet concentration is 150,000/μl to 350,000/μl. Anucleate platelets circulate for 7 to 10 days and mediate primary hemostasis. The platelets contain α-granules, and upon activation, they secrete multiple proteins such as platelet-derived growth factor (PDGF), transforming

growth factor (TGF-β1), platelet factor 4, vascular endothelial growth factor (VEGF), endostatins, angiopoietins, and thrombospondin-1.[11] PRP releases both angiogenic factors, including VEGF, which has been implicated in the promotion of angiogenesis, and antiangiogenic factors, such as platelet factor 4 and endostatins. The ratio of these factors under local conditions might determine the efficacy of the treatment.[12] Growth factor modulation occurs after the growth factor first binds to a specific cell-surface receptor. This binding triggers the process of signal transduction, during which the occupied receptor initiates a chain of intracellular signaling that results in protein synthesis that then influences tissue healing.

It is well known that tissue healing and remodeling is a complex cascade of biologic events controlled by a large list of cytokines and growth factors that influence various processes such as cell growth, angiogenesis, inflammation, and other mechanisms governing tissue healing and regeneration. Such complexity calls for refined restorative approaches that involve the delivery of multimolecular effective therapies tailored to the exact biologic state of the healing tissues. Autologous PR preparations are already being used as biologic systems for growth factor delivery and tissue engineering.[13] This concept involves enriching the injured site with the patient's own cellular signals, which have the ability to activate local cells as well as to attract precursor cells from the bloodstream, which then take part in the formation of new tissue.

The method of producing PRP determines its composition and concentration in terms of platelets, leukocytes, and erythrocytes in a given plasma volume; this make-up determines the molecules present in a given preparation, the molecular balance, and the concentrations of the respective molecules. There are 2 methods to produce PRP: the single spinning and double spinning procedures. When using the single spinning method, the platelet yield is 1 to 3 times baseline levels, whereas double spinning can yield levels 5 to 8 times baseline levels. Double spinning also concentrates leukocytes; hence, these products are more precisely called leukocyte/platelet concentrates.[14] Whether concentrated leukocytes have a positive or negative influence cannot be generalized for all tissues and clinical conditions, and this point remains a controversial issue that demands further investigation. A priori, we report several concerns regarding the presence of high levels of neutrophils containing MMP-8 (matrix metalloproteinase-8) and MMP-9; MMP-8 is also referred to as neutrophil collagenase and was once thought to be expressed exclusively in neutrophils and polymorphonuclear leukocytes.[15] The release of reactive oxygen species by neutrophils may also be relevant.[16]

The successful clinical application of PRP also depends on a clarification of the optimal readministration procedure, the volume of PRP used, and the frequency of the applications. For example, one possible use is for conditioning tendon grafts before implantation by injecting the activated liquid, which results in local fibrin matrix development and in proteins being readily extruded from the matrix in situ (Fig. 1). Alternatively, the fibrin scaffold can be formed ex vivo, and then implanted into the chosen site before retraction. In addition to providing initial cues for local cell activation and the homing of precursor cells to the injury site for differentiation, platelets and fibrin are also potent adhesive substrates for cells. We used fibrin scaffolds to enhance cicatrization of soft tissues at the donor site.

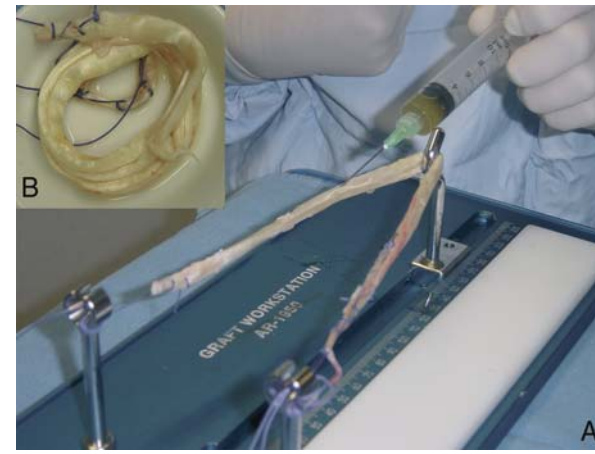

FIGURE 1. Treatment of the hamstring before implantation: (A) liquid-activated preparation rich in growth factor (PRGF) is injected within tendon fascicles; (B) the graft is soaked in PRGF before implantation.

THE ROLE OF PR THERAPIES IN ACL RECONSTRUCTION

Cell cultures[17,18] and animal research,[19–22] in addition to human clinical studies, drive the main hypotheses for the application of PR technology 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 PR therapies will help in donor site healing.

Healing of Bone-bone and Bone-tendon Interfaces

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 the bone plug-patella tendon-bone (BPTB) grafts than with the hamstring.[23] 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 accelerate bone fusion. Previous animal research and clinical experience showing enhanced bone regeneration in the maxillofacial and dentistry fields support this notion.[24,25] In orthopedics, Sánchez et al[26] evaluated the clinical safety and efficacy of using a particular PRP named PRGF (preparation rich in growth factors) for the treatment of 16 nonhypertrophic nonunions. The mean time from prior surgical treatment was 21 months (9 to 46 mo), and the mean time from surgery and/or PRGF application to union was 4.9 months (2 to 8 mo), with no associated complications. Although the application of PR therapies to enhance BPTB healing is promising, careful evaluation is required to show the true value of PRP application in these circumstances.

Hamstring fixation is considered problematic because it relies on slow tendon-bone incorporation within the tunnels. In normal anatomy, the ACL attaches to the surface of the bone, which contains both uncalcified and calcified fibrocartilage, via direct insertion. However, there are no sites in humans where a tendon goes into a bone

tunnel, and therefore, there is no native situation analogous to a tunnel-hamstring graft. Previous approaches aiming to promote bone ingrowth onto a tendon graft placed in a bone tunnel are reviewed elsewhere.[27] Most authors have investigated the influence of synthetic growth factors in the optimization and enhancement of graft-bone interface following common osteoinductive strategies. Rodeo et al[28] used a collagen sponge carrying rhBMP-2 in the graft-tunnel in an animal model, whereas Martinek et al[29] used gene therapy techniques. Chen and Anderson[30] attempted more extensive bone formation using periostium wrapped around the tendon graft in the bone tunnel.

At present, there is clinical evidence supporting the beneficial effect of PRP in bone and tendon healing separately, but not in tendon-bone healing, although it should be apparent that autologous growth factors released from PR scaffolds might influence tendon-bone healing at many levels. At a biologic level, healing seems to begin with proliferation of fibrovascular tissue and progress through the maturation of this matrix in the interface between tendon and bone. Typically, PRP application seeks to enhance the initial formation of highly vascular and cellular fibrous tissue that makes up the tendon-bone interface. Ideally, this tissue would mature to a more organized one with continuity of collagen fibers aligned along the direction of the pull of the muscle tendon unit between the tunnel and the graft. Previous research has demonstrated the anabolic effect of PR therapies on tendon cells by stimulating the synthesis of Collagen I, Collagen III and cartilage oligomerix matrix protein, which is believed to have an organizational role in the formation of collagenous matrices.[31,32] Therefore, the delivery of growth factors should promote collagen hypertrophy and growth factor expression from both the graft and host bone, which may ultimately determine the success of graft integration. Despite the significance of this hypothesis, clinical studies are lacking, as is clear histologic evidence showing Sharpey's fiber development that anchors the tendon to the bone.

In a preliminary study, Sánchez et al[4] described a procedure for treating the bone tunnels and conditioning the graft before implantation with PRGF; they compared a group of 50 patients treated with surgery and PRGF with another group of 50 patients who underwent surgery alone. The 2 groups were matched for age and graft type. The authors reported better integration of PRGF-treated grafts within the tunnels, as assessed by x-rays, and a larger number of completely stable knees in the PRGF group. Other authors[33,34] have explored the influence of autologous bone plugs, either alone or combined with PR therapies, on the promotion of femoral bone-tendon healing. They reported that bone plugs, but not PR therapies, significantly prevented femoral tunnel widening.

We use autologous bone blocks to enhance graft fixation within the tibial tunnel because the tibia is more mechanically demanding[35] than the femur. Our strategy focused on creating biphasic scaffolds made of 2 bone plugs with different sections (11 mm distal and 9 to 10 mm proximal) enrobed in fibrin, as shown in Figure 2. Typically, when making the tibial tunnel we perform a sequential trephine drilling with escalating diameter sections; initially we start distally with a trephine drill 11 mm wide and 20 mm long and then we change to 9 to 10 mm wide proximally. In doing so, the graft first penetrates a 9 to 10 mm wide tunnel and then expands to 11 mm. Here, the hypothesis states that mechanical strain causes internal deformations within the fibrin matrix affecting the migration and growth of cells that repopulate the fibrin and transform the temporary fibrin into collagen fibers (Fig. 3). We fix the hamstring at the level of the femoral tunnel with transcondylar screws and, once the graft is placed, activated liquid PRGF is injected into both tunnels to promote intraosseous healing. Figure 4 illustrates intraosseous bone healing 4 months after hamstring implantation was performed using PRGF-assisted protocols.

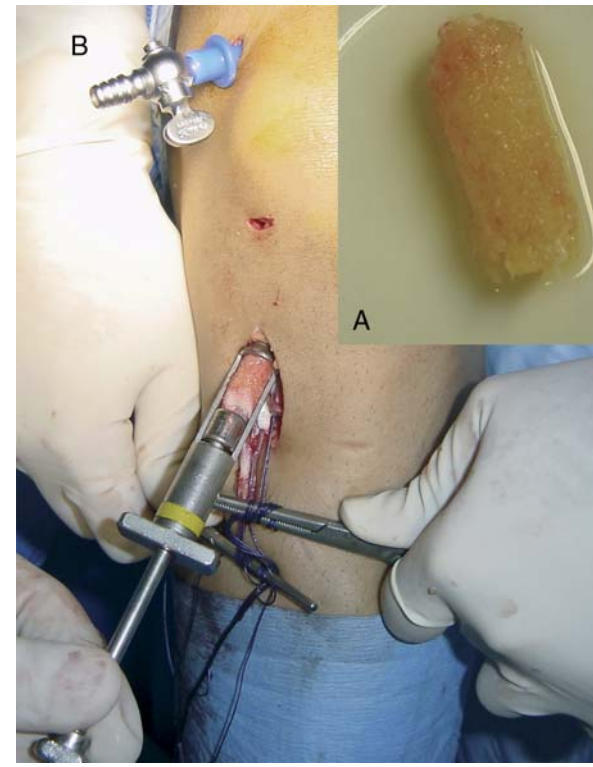

FIGURE 2. A, Bone plugs harvested from the tibia are soaked in preparation rich in growth factor before implantation; (B) insertion of bone plugs within the tibia tunnel.

Ligamentization and Growth Factors

Appropriate function of the ACL graft, essential for normal knee biomechanical functioning, entails successful intra-articular graft ligamentization. Recent research into the remodeling of tissues points to several growth factors that could improve ACL remodeling. Several researchers have focused on examining the effects of the local administration of single growth factors, such as PDGF-BB,[36] TGF-β1,[37,38] or VEGF[39]; theoretically, all of these factors may stimulate ligamentization. Nevertheless, limiting factors in these studies are related to the requirements for multiple signals to drive the remodeling process to completion, as well as the complex nature of the interdependent factors at play during the period of ligamentization.

One exciting option to enhance ligamentization is to transfer multiple cytokines and growth factors (including PDGF, TGF-β1, and VEGF, among others) to the graft simultaneously, 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,[40] but also to attract cells, such as endothelial or stem cells, from

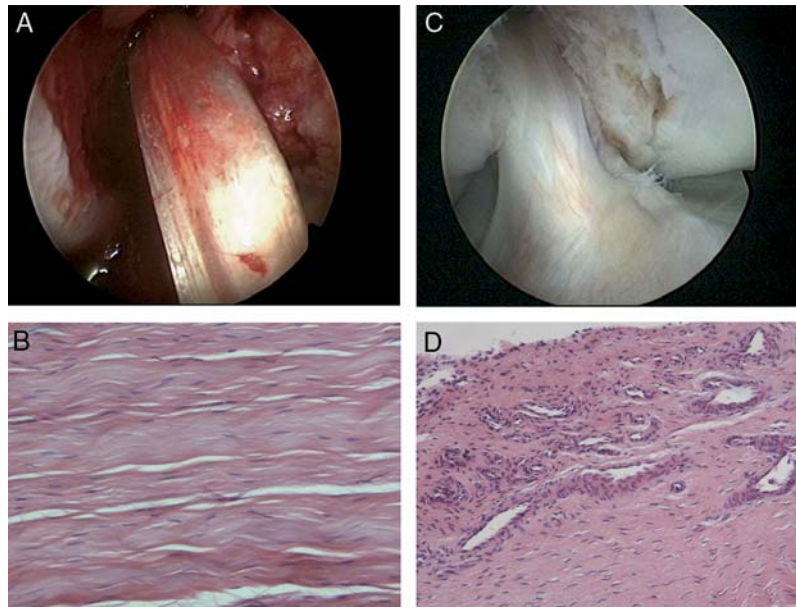

FIGURE 3. Gross morphology (A) and histology (B) of the hamstring tendon at the time of surgery; arthroscopic (C) and microscopic (D) appearance of the PRGF-treated graft 12 months after implantation. PRGF indicates preparation rich in growth factors.

adjacent niches (such as the synovium and/or the intrapatellar pad) to graft structures using the synovial fluid for passage.[41] 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 PRGF-treated and PRGF-untreated grafts during the remodeling period (6-24 mo). Gross morphology was evaluated on second-look arthroscopy focusing on graft thickness, apparent tension, and synovium coverage. The overall arthroscopic evaluation evidenced a higher percentage rated as excellent in the PRGF group (57% vs. 33%). No grafts were scored as poor in the PRGF group, but 20% of the controls showed poor morphology. At the same time, PRGF treatment influenced the histologic characteristics of the tendon graft, resulting in tissue that was more mature than in the controls. Histology displayed newly formed connective tissue enveloping the graft in 77.3% of PRGF-treated grafts and in 40% of controls.[42] On the basis of histologic findings, we suggest that the remodeling of PRGF-treated grafts involves the formation of synovial-like tissue enveloping the graft, eventually integrated in the remodeled tendon graft and conferring a similar appearance to the ACL (Fig. 2). Radice[43] and Orrego et al[33] independently confirmed the acceleration of the maturation of grafts treated with PRP, using a rating magnetic resonance imaging score to evaluate graft homogeneity. In these studies, the PRP product and the procedure for its application vary from the Sánchez approach. Radice[42] and Orrego et al[33] used a compressed gelatin sponge soaked with leukocyte and PR concentrate (GPS system by Biomet Biologic, Warsaw), which is then sutured to the intra-articular part of the graft.

Donor Site Repair

Autografts are associated with donor site morbidity in up to 40% of all patients.[44] In light of the high donor site morbidity, allografts have come into increasing use. A

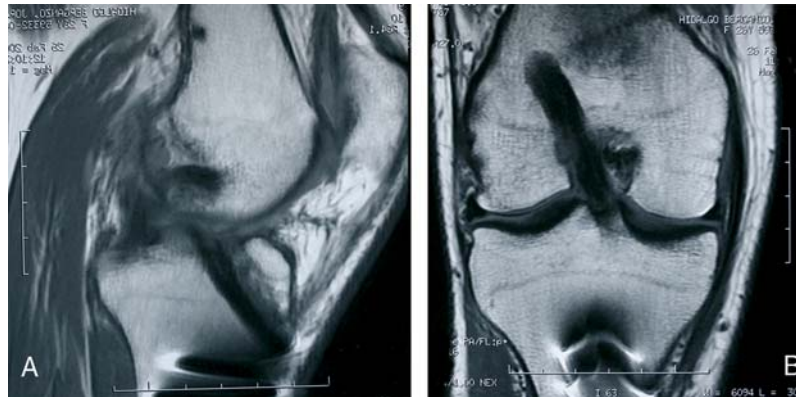

FIGURE 4. Magnetic resonance imaging aspect of the tibial (A) and femoral (B) tunnels 4 months after anterior cruciate ligament surgical reconstruction.

recent meta-analysis comparing allograft and autograft stability showed that allograft ACL reconstruction produced significantly lower levels of stability than that with autografts.[45] If it is possible to reduce donor site morbidity, autografts rather than allografts may offer greater advantages. Studies have confirmed that local application of PR preparations is especially important in conditions where healing is compromised due to tissue discontinuity. In fact, the dynamic and efficient process of wound healing involves a complex series of events including hemostasis, inflammation, granulation tissue formation, epithelialization, neovascularization, collagen synthesis, and wound contraction. Blood platelets have a major role in the initiation of wound healing. They adhere, aggregate, and release numerous growth factors, adhesive molecules, and lipids that regulate the migration, proliferation, and function of fibroblasts and endothelial cells. Preclinical and clinical studies with PRGF have shown that it can accelerate tissue repair in soft tissues via mechanisms involving the further synthesis of signaling proteins that participate in cell mitosis and angiogenesis.[46,47] Another important consideration is that in addition to stimulating the growth of new tissue, PRP application decreased pain and inflammation.[48–50]

CONCLUSIONS

The delivery of endogenous signaling molecules during ACL surgery may result in significant changes in the biologic function of the local cells. However, the production of tissue that is biologically, chemically, and mechanically normal will require a combination of strategies. Thus, combining the use of PR products with appropriate mechanical loading methods might yield both better tissue organization in the short term and enhanced mechanical properties in the long term, both of which are of paramount importance in young active patients. PR preparations are one of many in the novel biologic armamentarium of therapies as they promote the healing of tissue with a range of endogenous cell signaling factors. They provide a potential advantage for better and more predictable ligamentization and enhanced tendon-bone healing, suggesting an exciting role for PR therapies in ACL surgery. However, poor standardization in the field has hampered the evaluations of different plasma products and the establishment of standard beneficial application of this technology.

REFERENCES

1. Stergiou N, Ristanis S, Moraiti C, et al. Tibial rotation in anterior cruciate ligament (ACL)-deficient and ACL-reconstructed knees: a theoretical proposition for the development of osteoarthritis. *Sports Med*. 2007;37:601–613.
2. 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;28:4551–4560.
3. 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;35:1648–1652.
4. Sánchez M, Azofra J, Aizpurua B, et al. Use of autologous plasma rich in growth factors in arthroscopic surgery. *Cuadernos de Artroscopia*. 2003;10:12–19.
5. Creaney L, Hamilton B. Growth factor delivery methods in the management of sports injuries: the state of the play. *Br J Sports Med*. 2008;42:314–320.
6. Sánchez M, Anitua E, Orive G, et al. Platelet-rich therapies in the treatment of orthopedic sports injuries. *Sports Med*. 2009;39:1–10.
7. Sánchez M, Anitua E, Azofra J, et al. Intra-articular injection of an autologous preparations rich in growth factors for the treatment of knee OA: a retrospective cohort study. *Clin Exp Rheumatol*. 2008;26:910–913.
8. Everts PA, Devilee RJ, Brown Mahoney C, et al. Exogenous application of platelet-leukocyte gel during open subacromial decompression contributes to improved patient outcome. A prospective randomized double-blind study. *Eur Surg Res*. 2008;40:203–210.
9. Hutmacher DW, Cool S. Concepts of scaffold-based tissue engineering—the rationale to use solid free-form fabrication techniques. *J Cell Mol Med*. 2007;11:654–669.
10. Nurden AT, Nurden P, Sánchez M, et al. Platelets and wound healing. *Front Biosci*. 2008;13:3532–3548.
11. 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.
12. Anitua E, Sánchez M, Zalduendo MM, et al. Fibroblastic response to treatment with different preparations rich in growth factors. *Cell Proliferation*. 2009;42:162–170.
13. Anitua E, Sánchez M, Orive G, et al. Delivering growth factors for thearapeutics. *Trends Pharmacol Sci*. 2008;29:37–41.
14. Everts PA, Brown Mahoney C, Hoffmann JJ, et al. Platelet-rich plasma preparation using three devices: implications for platelet activation and platelet growth factor release. *Growth Factors*. 2006;24:165–171.
15. Bramono DS, Richmond JC, Weitzel PP, et al. Matrix metalloproteinases and their clinical applications in orthopaedics. *Clin Orthop Relat Res*. 2004;428:272–285.
16. Scott A, Khan KM, Roberts CR, et al. What do we mean by the term "inflammation"? A contemporary basic science update for sports medicine. *Br J Sports Med*. 2004;38:372–380.
17. 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;23:281–286.
18. Anitua E, Sanchez M, Nurden AT, et al. Reciprocal actions of platelet-secreted TGF-β1 on the production of VEGF and HGF by human tendon cells. *Plast Reconstr Surg*. 2007;119:950–959.
19. 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;77:285–293.
20. 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;24:820–830.
21. Murray MM, Spindler KP, Ballard P, et al. Enhanced histologic repair in a central wound in the anterior cruciate ligament with a collagen-platelet-rich plasma scaffold. *J Orthop Res*. 2007;25:1007–1017.
22. Fleming BC, Spindler KP, Palmer MP, et al. Collagen-platelet composites improve the biomechanical properties of healing anterior cruciate ligament grafts in a porcine model. *Am J Sports Med*. 2009;37:1554–1563.
23. Scheffler SU, Unterhauser FN, Weiler A. Graft remodeling and ligamentization after cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthoscop*. 2008;16:834–842.
24. Marx RE, Carlson ER, Eichstaedt RM, et al. Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998;85:638–646.
25. Anitua E. Plasma rich in growth factors: preliminary results of the use in the preparation of future sites for implants. *Int J Maxillofac*. 1999;14:529–535.
26. Sanchez M, Anitua E, Cugat R, et al. Nonunions treated with autologous preparations rich in growth factors. *J Orthop Trauma*. 2009;23:52–59.
27. Steiner ME, Murray MM, Rodeo SA. Strategies to improve anterior cruciate ligament healing and graft placement. *Am J Sports Med*. 2008;36:176–189.
28. Rodeo SA, Suzuki K, Deng XH, et al. Use of recombinant bone morphogenetic protein-2 to enhance tendon healing in a bone tunnel. *Am J Sports Med*. 1999;27:476–488.

29. Martinek V, Latterman C, Usas A et al. Enhancement of tendon-bone integration of anterior cruaciate ligament grafts with bone morphogenetic protein-2 gene transfer: a histological and biomechanical study. *J Bone Joint Surg Am*. 2002;84: 1123–1131.
30. Chen VW, Anderson K. Reconstruction of the anterior and posterior cruciate ligaments after recurrent anterior knee dislocation. *J Knee Surg*. 2003;16:179–181.
31. Schnabel LV, Mohammed HO, Miller BJ, et al. Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendon. *J Orthop Res*. 2007;25:230–240.
32. De Moss M, van der Windt A, Jahr H, et al. Can platelet-rich plasma enhance tendon repair? A cell culture study. *Am J Sports Med*. 2008;36:1171–1178.
33. Orrego M, Larrain C, Rosales J et al. Effects of platelet concentrate and a bone plug on the healing of hamstring tendons in a bone tunnel. *Arthroscopy*. 2008;24:1373–1380.
34. Silva A, Sampaio R. Anatomic ACL reconstruction: does the platelet-rich plasma accelerate tendon healing? *Knee Surg Sports Traumatol Arthrosc*. 2009;17:676–682.
35. Brand J Jr, Weiler A, Caborn DNM, et al. Graft fixation in cruciate ligament reconstruction. Current concepts. *Am J Sports Med*. 2000;28:761–774.
36. Weiler A, Förster C, Hunt P, et al. The influence of locally applied platelet-derived growth factor-BB on free tendon graft remodeling after anterior cruciate ligament reconstruction. *Am J Sports Med*. 2004;32:881–891.
37. Murray MM, Bennett R, Zhang X, et al. Cell outgrowth from the human ACL in vitro: regional variation and response to TGF-beta1. *J Orthop Res*. 2002;20:875–880.
38. Yamazaki S, Yasuda K, Tomita F, et al. The effect of transforming growth factor-beta1 on intraosseous healing of flexor tendon autograft replacement of anterior cruciate ligament in dogs. *Arthroscopy*. 2005;21:1034–1041.
39. Yoshikawa T, Tohyama H, Enomoto H, et al. Expression of vascular endothelial growth factor and angiogenesis in patellar tendon grafts in the early phase after anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthroscop*. 2006; 14:804–810.
40. Rughetti A, Giusti I, D'Ascenzo D, et al. Platelet gel release supernatant modulates the angiogenic capability of human endothelial cells. *Blood Transfus*. 2008;6:12–17.
41. Morito T, Muneta T, Hara K, et al. Synovial fluid-derived mesenchymal stem cells increase after intra-articular ligament injury in humans. *Rheumatology*. 2008;47:1137–1143.
42. Sánchez M, Anitua E, Azofra J, et al. Ligmentization of tendon grafts treated with an endogeneous preparation rich in growth factors gross morphology and history. *Arthroscopy*. 2009. In press.
43. Radice F. Uso de concentrado autólogo rico en factores de crecimiento en la reconstrucción del LCA. *Revista Argentina De Artroscopia*. 2008;15:31–40.
44. Kartus J, Movin T, Karlsson J. Donor-site morbidity and anterior knee problems after anterior cruciate ligament reconstruction using autografts. *Arthroscopy*. 2001;17: 971–980.
45. Prodromos C, Joyce B, Shi K. A meta-analysis of stability of autografts compared to allografts after anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthrosc*. 2007;15:851–856.
46. Sánchez M, Anitua E, Cole A, et al. Management of post-surgical Achilles tendon complications with a Preparation Rich in Growth Factors: a study of two-cases. *Injury Extra*. 2009; 40:11–15.
47. Anitua E, Aguirre JJ, Algorta J, et al. Effectiveness of autologous preparation rich in growth factors for the treatment of chronic cutaneous ulcers. *Biomed Mater Res B Appl Biomater*. 2008;84:415–421.
48. Zavadil DP, Satterlee CC, Costigan JM, et al. Autologous platelet gel and platelet-poor plasma reduce pain with total shoulder arthroplasty. *J Extra Corp Technol*. 2007:39:177–182.
49. Driver VR, Hanft J, Fylling CP, et al. A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. *Ostomy/Wound Manag*. 2006;52:68–87.
50. Randelli PS, Arrigoni P, Cabitza P, et al. Autologous platelet rich plasma for arthroscopic rotator cuff repair: a pilot study. *Disabil Rehabil*. 2008;30:1584–1589.

