ELSEVIER

Operative Techniques in
Orthopaedics

Biological Approach to Anterior Cruciate Ligament Surgery

Mikel Sánchez, MD,* Juan Azofra, MD,* Nicolas Fiz, MD,* Beatriz Aizpurua, MD,* Jorge Guadilla, MD,* Eduardo Anitua, MD,† Isabel Andia, PhD,‡ and Nicola Maffulli, MD, PhD, FRCS(Orth)§

We present a novel technique for reconstruction of the anterior cruciate ligament with autologous ipsilateral hamstring tendons, assisted by platelet-rich plasma (PRP). PRP serves as a source of growth factors and cytokines to speed up the process of ligamentization and tendon-bone consolidation. Before implantation, the hamstring tendons are treated with liquid-activated PRP, so that a fibrin scaffold is formed within the tendons. The intratendinous location of the PRP scaffold was confirmed in Achilles tendons of sheep after injecting liquid-activated PRP stained with Indian blue ink. In the technique described, the tibial tunnel is produced using 2 trephines of different diameters, which allows to harvest 2 bone blocks with different diameters. The bone blocks are also treated with PRP before reimplantation. The tibial tunnel, which is wider distally, allows unconstrained positioning and drilling of the femoral socket within the isometric area. The graft is secured in the femur using transcondylar fixation. For tibial fixation, the PRP-treated bone blocks are introduced within the tunnel, firmly compacted, and left in the precise position that facilitated reconstruction of the tibial anatomy. Liquid-activated PRP is injected in both bone tunnels. To reduce morbidity associated with autografts, a platelet and fibrin scaffold is introduced in the donor region, and the preserved fascia of the pes anserinus is carefully sutured.

Oper Tech Orthop 22:64-70

KEYWORDS anterior cruciate ligament reconstruction, ligamentization, platelet-rich plasma, tibial fixation

In anterior cruciate ligament (ACL) lesions, reconstruction with autologous tendon grafts is the standard. However, restoring joint congruity, alignment, and stability does not prevent progressive changes producing degenerative joint disease and, eventually, symptomatic osteoarthritis.[1] Therefore, not only mechanical but also biological issues intrinsic to the peculiarity of the intra-articular microenvironment should be considered when reconstructing the torn ACL.

Successful ACL reconstruction relies on biomechanical and biological factors, including graft choice, correct position of the bone tunnels, and fixation of the graft, along with biological factors involved in graft maturation and graft-bone interface healing. The bone-patellar tendon-bone graft is widely used because graft stabilization is achieved more rapidly than with hamstring tendon autografts.[2] Hamstring fixation is considered problematic because it relies on relatively slow tendon-bone incorporation within the tunnels, and knee laxity may develop during the immediate postoperative period until biological fixation occurs. In addition, hamstring fixation is considered more challenging for the tibia than for the femur.

To overcome limitations of ACL surgery with hamstring, we describe a technique to produce the tibial tunnel with 2 sections that allow unconstrained femoral socket positioning through the transtibial approach, along with anatomical fix-

*Unidad de Cirugía Artroscópica UCA "Mikel Sánchez", Clínica USP-La Esperanza, Vitoria, Spain.
†Fundación Eduardo Anitua, Vitoria, Spain.
‡Instituto de Investigación BIOCRUCES, Osakidetza, Basque Health Service, Pza Cruces s/n, 48903 Barakaldo, Vizcaya, Spain.
§Centre for Sports and Exercise Medicine Mile End Hospital, Institute of Health Sciences Education, Barts and The London School of Medicine Dentistry, Queen Mary University of London, London, DG, England.
Competing interests: E.A. is the research director of Biotechnology Institute, a company that commercializes PRGF-Endoret a system for preparing PRP.
Address reprint requests to Isabel Andia, PhD, Research Department, Osakidetza, Basque Health Service, B° Arteaga 107, 48170 Zamudio, Spain. E-mail: iandia2010@hotmail.com

1048-6666/12/$-see front matter
doi:10.1053/j.oto.2011.09.006

ation of the graft. Biologically active substances may be applied to facilitate both early tendon-bone consolidation and intra-articular graft remodeling. Accordingly, we routinely use pure platelet-rich plasma (PRP) with a moderate concentration of platelets in ACL surgery as a source of growth factors (GFs) and cytokines,[3] a procedure that may improve results. This procedure can also be applied to ACL reconstruction with bone-patellar tendon-bone allografts or other autografts.

We describe an original procedure for ACL reconstruction with autologous ipsilateral hamstring tendons, using PRP and emphasizing the biological aspects of the reconstruction. We illustrate the use of a novel tibial tunnel guide for unconstrained drilling of the femoral socket using a transtibial approach. Biological intervention in ACL surgery involves treating the hamstring with PRP before implantation, and using PRP-treated bone blocks to achieve fixation of the tendon graft in the tibial tunnel and enhance tendon-bone healing. Finally, to reduce the morbidity associated with the donor site, PRP is introduced at the graft donor region.

Surgical Technique

PRP Preparation

For the preparation of PRP for ACL surgery, 65 mL of peripheral venous blood is withdrawn into 9-mL tubes containing 3.8% (wt/vol) sodium citrate before inducing anesthesia. Blood is centrifuged at 580*g* for 8 minutes at room temperature (PRGF-Endoret, Vitoria, Spain). The upper volume of plasma, which contains a similar platelet count to that of peripheral blood, is drawn off and deposited in a collection tube. After the addition of calcium chloride (10% wt/vol), the plasma is incubated at 37°C for 40-60 minutes in a glass dish, to allow for the formation of a biocompatible platelet and fibrin scaffold. The fibrin scaffold is applied at the musculotendinous junction after hamstring harvest.

The 2-mL plasma fraction, located just above the sedimented red blood cells, but not including the buffy coat, is collected in another tube. This plasma contains a moderate enrichment in platelets (2-3 fold the platelet count of peripheral blood) with scarce leukocytes.[4] To initiate clotting, calcium chloride is added to the liquid PRP aliquots just before administration. Six milliliters of PRP is injected within the tendon graft fascicles and 3 mL is injected into each bone tunnel after graft fixation, as explained later in the text. The remaining aliquots are applied at the portals during suturing.

Preparation of the Graft

Graft Harvest

The surgeon performs a 3-cm vertical incision, 2 cm medial to the anterior tibial tubercle and 5 cm below the joint line over the pes anserinus with the knee flexed to 70°. The surgeon identifies the tendons of the semitendinosus and gracilis under the fascia, splits the fascia in a slightly oblique direction, and strips the pes from the underlying tendons of the semitendinosus and gracilis, taking care not to interfere with the medial collateral ligament. After releasing the gracilis by blunt finger dissection, Kocher forceps are used to hold the tendon while a tape is looped around to allow the surgeon to grasp firmly and gain traction. The gracilis tendon is detached at the myotendinous junction with the tendon stripper while maintaining tension in a distal to proximal direction. The semitendinosus tendon is stripped in a similar manner after taking care to sever the fibrous bands connecting the tendon to the medial head of the gastrocnemius. The muscle attached to the proximal end of each tendon is removed with the edge of the scissors. Number 5 braided nonabsorbable sutures are then placed using Krackow stitches through the proximal end. The graft is released from the tibia, and a traction suture is passed over the pes.

Graft Conditioning

A 4-strand graft is sized to determine the diameter of the tunnels. Next, the graft is extended by tying the sutures over the posts on the graft master, as shown in Figure 1A. A total of 6 mL of liquid-activated PRP is injected along the graft (but not in the ends of the graft), parallel to the axis of the tendon. We punctured the tendon with the 22G needle mounted on a 10 mL syringe. In doing so, plasma is deposited throughout the length of the graft, following the direction of the collagen fibers. Once the tendon graft has been prepared in this way, we soak it in a bowl containing activated PRP (see Fig. 1B). After some time, the graft is covered with a gel-like envelope. To define the best working conditions for graft conditioning, we tested 3 syringe sizes, ie, 3 mL, 5 mL, and 10 mL. The goal in this particular circumstance was not to obtain injection accuracy but to spread the tendon graft with PRP. We selected 10-mL syringe size because, for identical force applied, the pressure was lower, the graft was not damaged, and PRP spread better. The location of injected PRP within a tendon graft, after injecting 5 mL of activated PRP stained with blue ink using a 10-mL syringe within the Achilles tendon of sheep, is shown in Figure 1C. After splitting the tendon longitudinally, we can observe clotted PRP surrounding tendon fibrils (Fig. 1D).

Tibial and Femoral Tunnel Placement

A guide designed by the main author (M.S.) in the late 1980s is used for tibial tunnel placement and drilling (Fig. 2A), although other commercially available guides can be used. The tibial tunnel should exit in the anteromedial aspect of the tibial footprint. The tip of the tibial drill guide is hooked at this point. Then, the drill guide sleeve is positioned above the pes anserinus and fixed at the point intersecting with the tibial footprint. When placed at this site, the length of the tibial tunnel will usually be 30-40 mm.

The tibial tunnel is drilled from outside-in with 11-mm-wide trephine, limiting the tunnel length to 20-25 mm. The first bone block is harvested with a bone plug extractor; the second bone block is harvested using a trephine 9-10 mm in diameter. The bone blocks are soaked in activated PRP until graft fixation (Fig. 2B).

The transtibial approach is used to place an anatomic femoral socket within the insertion footprint of the anteromedial femoral bundle. The anatomic point is placed on the lateral

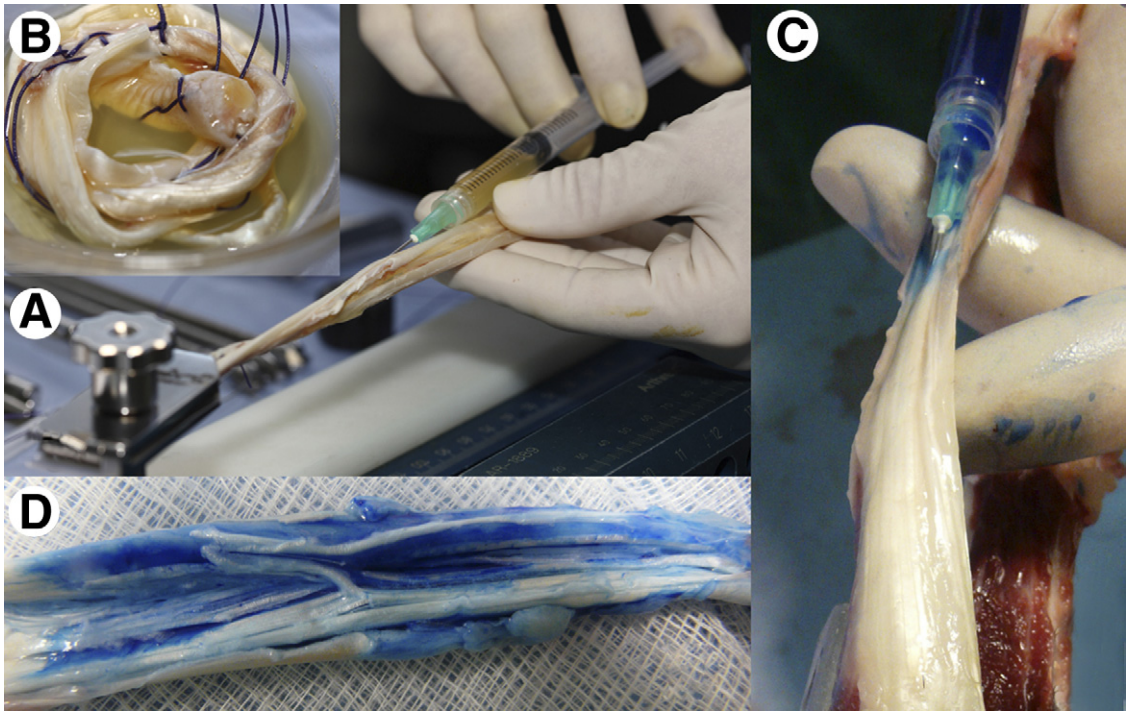

Figure 1 Hamstring conditioning before implantation. (A) The graft is pretensioned on the graft master, and 6 mL of liquid-activated PRP is injected parallel to the axis, producing several punctures. (B) After soaking the graft in liquid-activated PRP, it is covered with gelified PRP. (C) Using the same procedure, liquid-activated PRP stained with Indian blue ink is injected within the Achilles tendon of sheep post-mortem. (D) After opening the tendon longitudinally, the distribution of PRP within the tendon can be observed.

wall of the intercondylar notch at the 10-o'clock or 2-o'clock position in the right or left knee, respectively, with the knee flexed to approximately 90°-100°. The guide pin is hooked into the over-the-top position. The socket is then reamed to match the diameter of the tibial tunnel. The depth of the socket will be 30-35 mm. The shortcomings of the transtibial drilling method can be overcome because the 11-mm diameter of the distal part of the tibial tunnel allows the surgeon to reach the center of the femoral anteromedial insertion of the ACL without using the anteromedial portal.

Graft Fixation

The loop of the tendon graft is then inserted through the tibia and through the knee joint and suspended within the femoral tunnel using the transcondylar fixation system. After femoral fixation, the graft is tensioned manually. The position of the knee is not critical because tunnels are to be placed in the isometric area. The 4 free strands of the hamstring are fixed in the tibia with PRP-treated bone plugs within the tibial tunnels, and 2 spiked metal staples are placed in the anterior metaphysis of the tibia. The bone blocks are set up in the bone plug introducer (Fig. 2C), firmly impacted in the tunnel. Obviously, the distal bone block cannot pass the narrower proximal portion of the tunnel. Approximately 3 mL of liquid-activated PRP is injected within the femoral tunnel after graft fixation. Similarly, PRP is injected within the 4 free tendon ends fixed in the tibia.

Donor Site Treatment

Even if the hamstring donor site has relatively lower morbidity in comparison with the bone-patellar tendon-bone graft donor site, the hamstring area is treated with the platelets and fibrin scaffold prepared as described previously. The donor area is filled with fibrin scaffolds (Fig. 3A) and the fascia is closed carefully as shown in Figure 3B. An optimal environment for repair is achieved by keeping the fascia intact and taking advantage of the hemostatic and healing properties of the platelet and fibrin scaffold. Using this approach, we have observed less inflammation and the patients report little pain and discomfort.

Discussion

We have addressed 3 fundamental aspects of ACL surgery with hamstring, ie, graft ligamentization, anatomic tendon-bone fixation, and donor site treatment. The potential development of platelet-rich technologies,[5] along with biomechanical considerations, may help to enhance these processes. The various biological concepts are to deliver the appropriate cues close to target cells, to provide scaffold for cell adhesion, and also to promote cell migration.[6]

Graft Fixation

Graft fixation is the weakest link in ACL reconstruction, as knee laxity develops during the immediate postoperative pe-

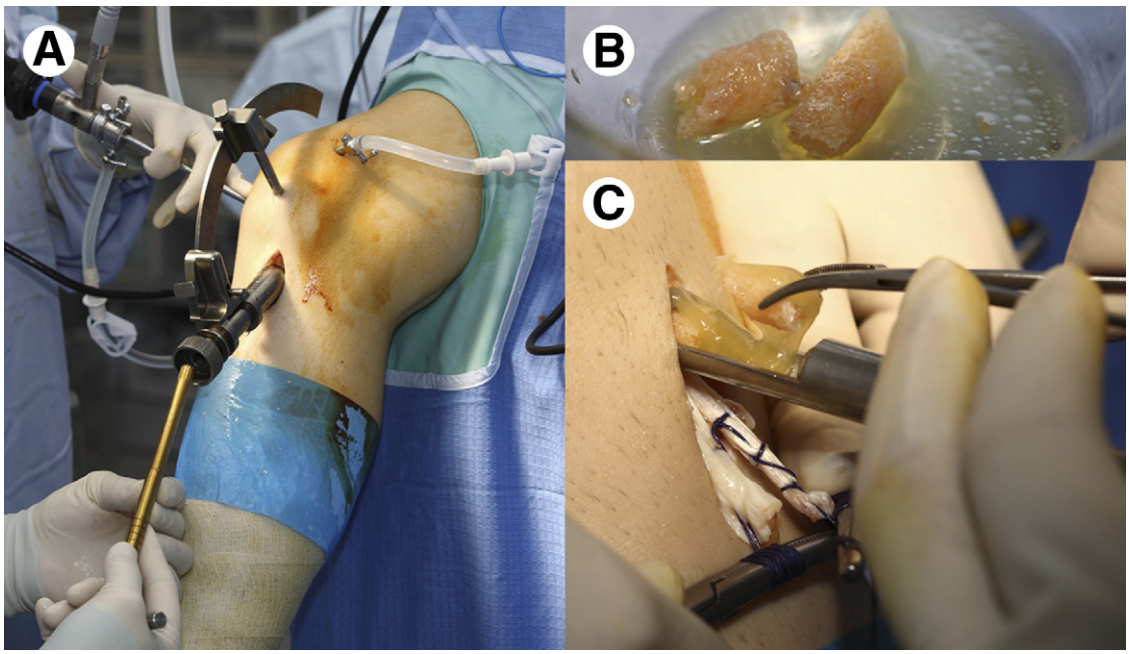

Figure 2 (A) Tibial tunnel production with a custom-made guide; the tibial tunnel is sequentially drilled from distal to proximal with escalating-diameter trephines. (B) Two bone plugs of different diameters have been harvested and are treated with PRP. (C) Insertion of the PRP-treated bone plug within the tibial tunnel.

riod until biologic fixation occurs within the bone tunnels. Classically, graft stabilization is achieved more rapidly with the bone-patella tendon-bone grafts than with the hamstring tendons. Hamstring fixation is considered more problematic because it relies on slow tendon-bone incorporation within the tunnels. To enhance hamstring fixation we have produced a tibial tunnel with 2 concentric diameters that facilitates transtibial drilling of the femoral socket and permits anatomic tibial graft fixation that allows undisturbed magnetic resonance imaging (MRI) evaluation and does not complicate second-look arthroscopy if required. This transtibial approach facilitates anatomic positioning in the femur. Moreover, we use autologous bone blocks soaked with PRP to enhance graft fixation within the tibial tunnel because the tibia is more mechanically demanding[7] than the femur. Certainly, the combined use of living cells (from bone and tendon), biologically active molecules (released from PRP), and structural scaffold (cancellous bone) to form a single construct is an application of the most common concepts underlying tissue engineering. Based on current knowledge in the field, the technique is expected to promote hamstring-bone fixation. However, there are no sites in humans where the tendon goes into a bone tunnel, and therefore, there is no native situation analogous to a tunnel-hamstring graft.

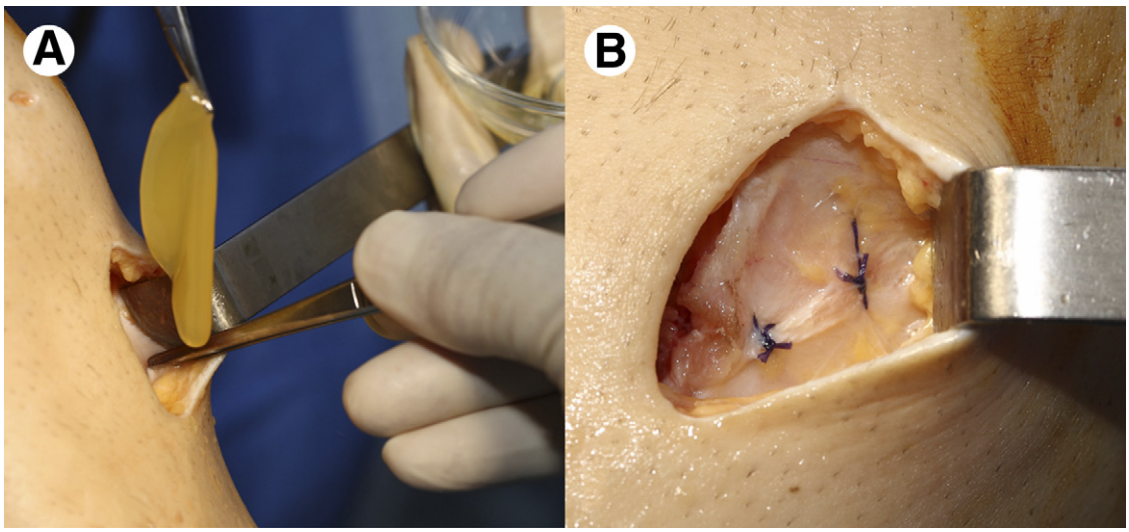

Figure 3 At the donor site, (A) PRP scaffolds are introduced within the donor area, and (B) the hamstring fascia is carefully sutured before closing the skin.

At present, there is clinical evidence supporting the beneficial effect of PRP in bone healing and tendon healing separately, but not in tendon-bone healing, although it should be apparent that autologous GFs released from PRP might influence tendon-bone healing at many levels. Healing appears to begin with proliferation of fibrovascular tissue and progress through maturation of this matrix 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. Previous research has demonstrated the anabolic effect of PRP therapies on tendon cells by stimulating the synthesis of collagen I, collagen III, and cartilage oligomeric matrix protein.[8,9] Therefore, the delivery of GFs should promote collagen hypertrophy and GF expression from both the graft and the host bone, which may ultimately determine the success of graft integration.

Despite the significance of this hypothesis, clinical evidences of graft-bone interface healing are lacking. As systematically reviewed recently,[10] 5 studies[11-15] have presented data on the effects of PRP on hamstring graft-bone interface healing. Most studies[11-14] used leukocyte- and platelet-rich plasma (L-PRP, 1-3 mL of leukocyte/platelet concentrate) clotted ex vivo; 3 of them did not evidence any significant effect on tendon-bone healing (Table 1), as assessed by MRI, but 1 study[14] reported enhanced vascularization in the PRP group. Another study did not show any beneficial effect of PRP in homologous patellar tendon-graft healing (bone-bone healing).[15] Overall, there is not enough evidence to form a clear conclusion, and apparently negative results could be attributed to optimization problems of both PRP formulation and the protocol for this application.

Ligamentization of Tendon Grafts

Appropriate function of the ACL graft, essential for normal knee function, entails successful intra-articular graft ligamentization. However the natural hamstring remodeling process is lengthy and takes more than 2 years, as shown recently.[21] One exciting option to accelerate and enhance ligamentization is to transfer multiple cytokines and GFs to the graft simultaneously by applying autologous PRP. Autografts could be loaded in situ with a balanced pool of signaling molecules. Therefore, we injected activated plasma within the fascicles and soaked the graft in PRP before implantation.[20] In doing so, the platelet-rich fibrin scaffold formed within the endotenons and also covered the entire surface of the graft. Recently, we investigated whether treating the graft with PRP as described previously produces faster ligamentization. The arthroscopic evaluation of the grafts and their histological examination at 6-24 months after ACL surgery gave us some insight into the biology and the remodeling of tendon grafts. The overall arthroscopic evaluation of PRP-treated grafts showed an excellent rating in 57% of the knees and a fair rating in 42.9%. Nontreated grafts showed an excellent rating in 33% of the knees, fair in 46.7%, and poor in 20%. In addition, PRP influenced the histology of the graft, resulting in tissue that was more mature than in the controls.[17] Five other studies[11,13,14,18,19] provided data on MRI assessment of maturation and confirmed the acceleration of the maturation of the grafts treated with PRP. These studies used different protocols, for example, they prepared a compressed gelatin sponge soaked with L-PRP, which is then sutured to the intra-articular part of the graft. This protocol is inspired from animal studies[22] that demonstrated a significant improvement in the graft structural properties and reduction on laxity, placing a collagen-platelet composite around the graft at the time of ACL reconstruction.

The underlying biological concept affirms that tissue healing and remodeling is a complex cascade of biological events controlled by many cytokines and GFs that influence various processes such as cell growth, angiogenesis, inflammation, and other mechanisms governing tissue healing and remodeling. Platelets embedded within the fibrin scaffold release cytokines and GFs, including platelet-derived growth factor, transforming growth factor beta 1, vascular endothelial growth factor, basic fibroblastic growth factor, hepatocyte growth factor, insulin-like growth factor 1, platelet factor 4(PF-4), and angiopoietin 1, among others.[23] However, so far no study has addressed which signals occupy key regulatory positions in the cascading sequence of events conducing to tendon-bone healing or graft ligamentization, an essential issue for PRP optimization. Nevertheless, a major debate in PRP therapies, intensified by commercial interests, is centered on the optimal level of platelets. Mastrangelo et al[24] compared PRP containing 5 times with PRP containing 3 times the baseline of platelets. This decrease did not alter the structural properties of the repaired ACL. Although the various PRPs differ somewhat in qualitative and quantitative composition, the differences between PRPs might be less important than the greater variability in patient response.

Donor Site Repair

Autografts are associated with donor site problems in up to 40% of patients.[25] In light of the high donor site morbidity, allografts are increasingly used. A recent meta-analysis comparing allograft and autograft showed that allograft ACL reconstruction produced significantly lower levels of stability than autografts.[26] In cases where it is possible to reduce donor site morbidity, autografts, rather than allografts, may offer greater advantages. Local application of PRP is especially important in conditions where healing is compromised because of tissue discontinuity. Recently, Cervellin et al[16] reported that L-PRP gel application at the donor site was able to significantly reduce knee pain in patients treated for ACL reconstruction with bone-patellar tendon-bone grafts in comparison with patients in whom PRP was not applied.

Platelet-rich technologies offer new opportunities for optimizing the healing environment in ACL reconstruction.[27] We have successfully used this procedure for more than 7 years; thus far, more than 600 patients have benefited from this form of ACL reconstruction, and we have never experienced any complication related to these procedures. Nevertheless, the development of novel autologous technologies for primary repair of the ACL remains a plausible goal. In fact, the usefulness of PRP augmentation to enhance primary re-

Table 1 Studies on PRP and ACL Reconstruction

Reference/Tendon Graft	Type of PRP/Activation	Design n Patients/Group	Outcome Measurements/ Time	Results	Level Evidence
Cervellin et al[16]/BPTB donor site	L-PRP (4-6×)/$CaCl_2$/thrombin clotted	RCT 20 patients/group	Donor site MRI, VISA/12 months	PRP: 70% bone gap filled and reduced pain control: 60% bone gap filled	I
Vogrin et al[14]/double looped hamstring	L-PRP (2-10×) $CaCl_2$/thrombin clotted	RCT Patient/group	MRI vascularization/4-6 weeks	Enhanced vascularization in the graft-bone interface	I
Sánchez et al[17]/hamstring	Pure-PRP (2-3×)/$CaCl_2$ activated	Case control 22 patients/PRP 15 patient/control	Gross morphology and ligamentization assessed by histology/6-24 months	PRP: improved macroscopic and microscopic graft ligamentization	III
Figueroa et al[13]/hamstring	L-PRP (2-10×)/$CaCl_2$/thrombin clotted	Case control 30 patient/PRP 20 patients/control	MRI/6 months	No difference in graft maturation and graft-bone interface healing	III
Radice et al[18]/BPTB or hamstring	L-PRP (4-6×)/$CaCl_2$/thrombin clotted	Case control 25 patients/group	MRI to assess graft homogeneity, VISA at 12 months	PRP required 48% of time to achieve same homogeneity than controls, PRP higher VISA	III
Silva et al[12]/double bundled hamstring	L-PRP (4-6×) thrombin clotted	Case control 10 patients/group, 4 groups	Graft integration assessed by MRI/3 months	No differences in graft-bone interface healing	III
Nin et al[15]/allograft BPTB	L-PRP (2×)/$CaCl_2$ activated	RCT 50 patients/group	IKDC, MRI, inflammatory variables	No differences in graft maturation, graft-bone interface healing, clinical, biomechanical variables	I
Orrego[11]/quadrupled hamstring	L-PRP (4-6×) thrombin clotted	RCT, 108 patients divided 4 groups: controls, PRP, bone plugs, and bone plugs + PRP)	MRI intra-articular maturation and graft-bone healing/3 and 6 months	No differences graft maturation, bone plugs prevented tunnel widening, PRP and bone plugs no differences in bone tunnel healing	II
Ventura et al[19]/quadrupled hamstring	L-PRP/thrombin clotted	RCT 10 patients/group	Tegner, KT-1000 CT/6 months	No differences Tegner, KT-1000 CT: significant difference graft homogeneity	II
Sánchez et al[20]/hamstring and BPTB	Pure PRP (2-3×) $CaCl_2$ activated	Case control 50 patients/group	Postoperative complications	PRP 6% vs controls 18%	III

PRP, platelet-rich plasma; L-PRP, leukocyte- and platelet-rich plasma; RCT, prospective randomized clinical trial; IKDC, International Knee Documentation Committee score; VISA, Victorian Institute of Sport Assessment Questionnaire; MRI, magnetic resonance imaging; BPTB, bone-patellar tendon-bone; CT, computerized tomography; KT, knee ligament arthrometer.

pair in animal models has been examined recently. Experimental findings indicated that either PRP or collagen scaffold were ineffective to supplement suture repair.[28,29] However, combining both PRP and collagen enhanced primary repair of the ACL in an in vivo porcine model.[22] Thus, primary suture repair in conjunction with collagen-platelet composites, performed after injury without delay, might be a novel treatment option for ACL injuries.[30]

Conclusions

There is tentative evidence that the use of PRP in ACL reconstruction is associated with enhanced ligamentization and donor site healing. However, heterogeneity in PRP formulations and variability of application protocols are found in most studies, leading to difficulty in interpreting the data. A better understanding of PRP biology will provide a framework for defining the optimal formulation in ACL applications. The present hurdles of PRP technologies must be overcome to generate protocols that may ultimately benefit ACL repair.

References

1. Oiestad BE, Holm I, Engebretsen L, et al: The association between radiographic knee osteoarthritis and knee symptoms, function and quality of life 10-15 years after anterior cruciate ligament reconstruction. Br J Sports Med 45:583-588, 2011
2. Scheffler SU, Unterhauser FN, Weiler A: Graft remodeling and ligamentization after cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthoscop 16:834-842, 2008
3. Anitua E, Sánchez M, Nurden AT, et al: New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol 24: 227-234, 2006
4. Sánchez M, Anitua E, Azofra J, et al: Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. Am J Sports Med 35:245-251, 2007
5. Sánchez M, Anitua E, Orive G, et al: Platelet-rich therapies in the treatment of orthopaedic sport injuries. Sports Med 39:345-354, 2009
6. Anitua E, Sánchez M, Orive G, et al: Delivering growth factors for therapeutics. Trends Pharmacol Sci 29:37-41, 2008
7. Brand J Jr, Weiler A, Caborn DN, et al: Graft fixation in cruciate ligament reconstruction. Am J Sports Med 28:761-774, 2000
8. Schnabel LV, Mohammed HO, Miller BJ, et al: Platelet rich plasma (PRP) enhances the anabolic gene expression patterns in flexor digitorum superficialis tendons. J Orthop Res 25:230-240, 2007
9. de Mos M, van der Windt AE, Jahr H, et al: Can platelet-rich plasma enhance tendon repair? A cell culture study. Am J Sports Med 36:1171-1178, 2008
10. Vavken P, Sadoghi P, Murray MM: The effect of platelet concentrates on graft maturation and graft-bone interface healing in anterior cruciate ligament reconstruction in human patients: A systematic review of controlled trials. Arthroscopy 27:1573-1583, 2011
11. 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 24:1373-1380, 2008
12. Silva A, Sampaio R: Anatomic ACL reconstruction: Does the platelet-rich plasma accelerate tendon healing? Knee Surg Sports Traumatol Arthrosc 17:676-682, 2009
13. Figueroa D, Melean P, Calvo R, et al: Magnetic resonance imaging evaluation of the integration and maturation of semitendinosus-gracilis graft in anterior cruciate ligament reconstruction using autologous platelet concentrate. Arthroscopy 26:1318-1325, 2010
14. Vogrin M, Rupreht M, Dinevski D, et al: Effects of a platelet gel on early graft revascularization after anterior cruciate ligament reconstruction: A prospective, randomized, double-blind, clinical trial. Eur Surg Res 45:77-85, 2010
15. Nin JR, Gasque GM, Azcárate AV, et al: Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? Arthroscopy 25: 1206-1213, 2009
16. Cervellin M, Girolamo L, Bait C, et al: Autologous platelet. Rich plasma gel to reduce donor-site morbidity after patellar tendon graft harvesting for anterior cruciate ligament reconstruction: A randomized, controlled clinical study. Knee Surg Sports Traumatol Artrosc 2011 Jun 16 (Epub ahead of print)
17. Sánchez M, Anitua E, Azofra J, et al: Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. Arthroscopy 26:470-480, 2010
18. Radice F, Yánez R, Gutiérrez V, et al: Comparison of magnetic resonance imaging findings in anterior cruciate ligament grafts with and without autologous platelet-derived growth factors. Arthroscopy 26: 50-57, 2010
19. Ventura A, Terzaghi C, Borgo E, et al: Use of growth factors in ACL surgery: Preliminary study. J Orthop Traumatol 6:76-79, 2005
20. Sánchez M, Azofra J, Aizpurúa B, et al: Use of autologous plasma rich in growth factors in arthroscopic surgery. Cuader Artrosc 10:12-19, 2003
21. Janssen RPA, van der Wijk J, Fiedler A, et al: Remodelling of human hamstring autografts after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 19:1299-1306, 2011
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 37:1554-1563, 2009
23. Nurden AT, Nurden P, Sanchez M, et al: Platelets and wound healing. Front Biosci 13:3532-3548, 2008
24. Mastrangelo AN, Vavken P, Fleming BC, et al: Reduced platelet concentration does not harm PRP effectiveness for ACL repair in a porcine in vivo model. J Orthop Res 29:1002-1007, 2011
25. Kartus J, Movin T, Karlsson J: Donor-site morbidity and anterior knee problems after anterior cruciate ligament reconstruction using autografts. Arthroscopy 17:971-980, 2001
26. 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 15:851-856, 2007
27. Sanchez M, Anitua E, Lopez-Vidriero E, et al: The future: Optimizing the healing environment in anterior cruciate ligament reconstruction. Sports Med Arthrosc 18:48-53, 2009
28. Murray MM, Palmer M, Abreu E, et al: Platelet-rich plasma alone is not sufficient to enhance suture repair of the ACL in skeletally immature animals: An in vivo study. J Orthop Res 27:639-645, 2009
29. Fleming BC, Magarian EM, Harrison SL, et al: Collagen scaffold supplementation does not improve the functional properties of the repaired anterior cruciate ligament. J Orthop Res 28:703-709, 2010
30. Magarian EM, Fleming BC, Harrison SL, et al: Delay of 2 or 6 weeks adversely affects the functional outcome of augmented primary repair of the porcine anterior cruciate ligament. Am J Sports Med 38:2528-2534, 2010