Ligamentization of Tendon Grafts Treated With an Endogenous Preparation Rich in Growth Factors: Gross Morphology and Histology

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Purpose: To investigate whether the application of a particular platelet-rich plasma preparation rich in growth factors (PRGF) during anterior cruciate ligament (ACL) surgery gives a potential advantage for better tendon graft ligamentization. **Methods:** This study included 37 volunteers who underwent either conventional (control group, n = 15) or PRGF-assisted (n = 22) ACL reconstruction with an autogenous hamstring and required second-look arthroscopy to remove hardware or loose bodies, treat meniscal tears or plica syndrome, or resect cyclops lesions at 6 to 24 months after ACL surgery. The gross morphologies of the grafts were evaluated on second-look arthroscopy by use of the full arthroscopic score (0 to 4 points) to evaluate graft thickness and apparent tension (0 to 2 points) plus synovial coverage (0 to 2 points). At the same time, biopsy specimens were harvested uniformly from the grafted tendons. In these specimens the histologic transformation of the tendon graft to ACL-like tissue was evaluated by use of the Ligament Tissue Maturity Index, and a score to assess the progression of new connective tissue enveloping the graft was created by use of 3 criteria previously used to characterize changes during ligament healing: cellularity, vascularity, and collagen properties. **Results:** The overall arthroscopic evaluation of PRGF-treated grafts showed an excellent rating in 57.1% of the knees (score of 4) and a fair rating in 42.9% (score of 2 or 3). In contrast, evaluation of untreated grafts showed an excellent rating in 33.3% of the knees, a fair rating in 46.7%, and a poor rating in 20% (score of 0 or 1). Overall, arthroscopic evaluations were not statistically different between PRGF and control groups ($P = .051$). PRGF treatment influenced the histologic characteristics of the tendon graft, resulting in tissue that was more mature than in controls ($P = .024$). Histologically evident newly formed connective tissue enveloping the graft was present in 77.3% of PRGF-treated grafts and 40% of controls. The appearance of the connective tissue envelope changed with increasing time from surgery. On the basis of the histologic findings, we suggest that the remodeling of PRGF-treated grafts involves the formation of synovial-like tissue enveloping the graft. This tissue is eventually integrated in the remodeled tendon graft, conferring a similar appearance to the normal ACL. **Conclusions:** The use of PRGF influenced the histologic characteristics of tendon grafts, resulting in more remodeling compared with untreated grafts. We have shown temporal histologic changes during the 6- to 24-month postoperative period of graft maturation, with newly formed connective tissue enveloping most grafts treated with PRGF. **Level of Evidence:** Level III, case-control study.

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Received March 13, 2009; accepted August 29, 2009.

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0749-8063/10/2604-9140$36.00/0

doi:10.1016/j.arthro.2009.08.019

At present, anterior cruciate ligament (ACL) reconstruction with autogenous grafts is both successful and predictable.[1] Nevertheless, a number of procedural factors, including graft options,[2] tunnel placement, tensioning, and fixation techniques,[3] are repeatedly being revised. Less studied are the biological aspects of the reconstruction, that is, intrasynovial remodeling and tendon-bone integration. Tendon graft remodeling, also termed ligamentization,[4] is of paramount importance to ACL repair because it improves, to some extent, the functionality and success of the ACL reconstruction. However, a major breakthrough in enhancing this process of ligamentization has not yet occurred.

Getting to the root of the process of enhancing the reconstruction requires identifying biological factors that may be determinants in ligamentization. A number of studies have focused on examining the effects of the local administration of single growth factors, such as platelet-derived growth factor BB,[5] transforming growth factor β1,[6,7] or vascular endothelial growth factor (VEGF),[8] all of which may stimulate ligamentization. The limiting factors in these prior studies are related to the requirements for multiple signals to drive the remodeling process to completion and the complex nature of the interdependent factors at play during the period of ligamentization and tendon-bone consolidation. One exciting option for enhancing ACL reconstruction is to transfer multiple cytokines and growth factors (including platelet-derived growth factor, transforming growth factor β1, and VEGF among others) simultaneously to the graft by applying an endogenous preparation rich in growth factors (PRGF).[9-11]

A few years ago, our group proposed the creation of "in situ" fully integrated bioactive grafts using the paradigm of tissue engineering.[12] Preclinical research and clinical activities show that the pool of growth factors released from PRGF significantly increases the proliferation of human tendon cells and stimulates these cells to produce factors such as VEGF and hepatocyte growth factor (HGF).[13,14] VEGF is expressed in the early phase after ACL reconstruction[8] and promotes angiogenesis, which is directly related to the tendon's remodeling capability. HGF activates both endothelial cell proliferation and migration and is a potent antifibrotic agent that could reduce scar formation around tendon tissues.[15] These results suggest that the increased availability of VEGF and HGF, which is induced by PRGF transfer to the graft, will promote the rapid formation of blood supply to the graft and enhance cell migration, thus contributing to rapid remodeling. Other authors have also suggested that locally injected platelet-rich plasma is an activator of circulation-derived stem cells.[16]

Overall, platelet-rich therapies seek to facilitate ACL replacements by mimicking the native tissue and improving the adequacy of tissue function by providing the appropriate cues. To meet this challenge, it is necessary to clarify the temporal sequence of biological mechanisms and identify the molecular enhancers and inhibitors involved herein. This goal involves tailoring plasma formulations to meet the needs of the healing graft, both in the intrasynovial portion and at the tendon-bone and tendon-tendon interfaces.

The purpose of this study was to determine whether the application of PRGF during ACL reconstruction with an autologous hamstring has any impact on the morphology and histology during remodeling over a period of 6 to 24 months. We hypothesized that PRGF may add a potential biological advantage to the autogenous graft to allow for better remodeling and more predictable ligamentization. To test this hypothesis, we compared the gross appearance and microscopic qualities of the PRGF-treated and untreated grafts during the remodeling period (6 to 24 months).

METHODS

Patients

Between January 2001 and July 2008, we performed 672 primary ACL reconstructions; 428 of these were performed with PRGF. We proposed PRGF treatment to all patients, except those aged under 16 years. Patients decided whether they were to receive PRGF treatment or not. The reasons for refusal were aversion to the idea of blood manipulation, economic factors, such as lack of reimbursement by insurance coverage, and declination of blood utilization for religious reasons.

During this period, 63 patient knees were available for second-look arthroscopy (36 PRGF-treated knees and 27 untreated knees). From this group of 63 patients, every patient who had an ACL reconstruction with autogenous hamstring and required second-look arthroscopy (n = 41) at 6 to 24 months after ACL reconstruction was asked to participate in the study. Thirty-seven consenting patients were included (PRGF group, n = 22; control group, n = 15). All knees were stable on examination and had no clinical symptoms of instability before biopsy. The study was approved by the institutional review

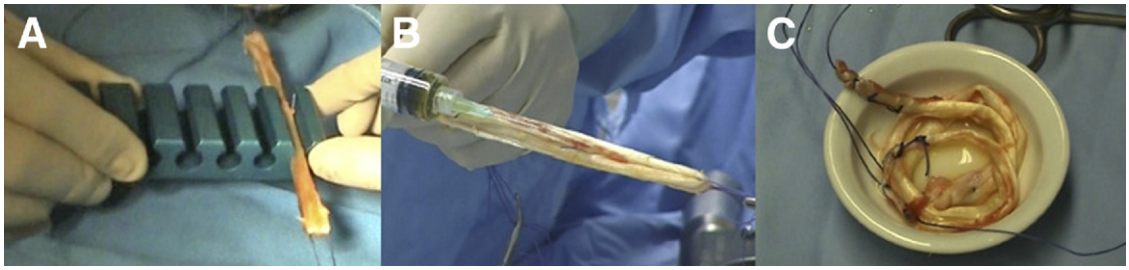

Figure 1. PRGF treatment of hamstring before implantation. (A) The tendons were stretched and sized (B) before injection of activated PRGF within the fascicles; (C) finally, they were soaked in PRGF, which resulted in fibrin covering the entire surface of the tendon.

board at the hospital, and informed consent was obtained from all patients.

PRGF Technology

The term "PRGF" identifies 100% of autologous products from the 1-step centrifugation process, in contrast to double-spin procedures, and sodium citrate and calcium chloride, but not exogenous thrombin, are used as an anticoagulant and an activator, respectively. Using a standardized dose of calcium chloride while avoiding the addition of exogenous thrombin grants control over the gel-liquid transformation and confers versatility to administration procedures. For the preparation of PRGF for ACL surgery, 65 mL of peripheral venous blood was withdrawn into 9-mL tubes containing 3.8% (wt/vol) sodium citrate. PRGF was prepared by centrifugation at 580*g* for 8 minutes at room temperature (BTI System II; BTI Biotechnology Institute, Vitoria, Spain). The upper volume of plasma, which contains a platelet count similar to that of peripheral blood, was drawn off and deposited in a collection tube. After the addition of calcium chloride (10% wt/vol), the plasma was incubated at 37°C for 40 to 60 minutes in a glass dish, allowing for the formation of a biocompatible fibrin with healing and hemostatic properties. The fibrin was applied at the musculotendinous junction after harvesting of the hamstring tendons.

The 2-mL plasma fraction, located just above the sedimented red blood cells but not including the buffy coat, was collected in another tube. This plasma contains a moderate enrichment in platelets (2- to 3-fold the platelet count of peripheral blood) with scarce leukocytes.[17,18] All of the procedures were performed in an A-class sterile hood.

To initiate clotting, calcium chloride was added to the liquid PRGF aliquots just before administration. Six milliliters was injected within the tendon graft fascicles as explained later, and the remaining aliquots were applied at the portals during suturing.

Treatment of Hamstring With PRGF Before Implantation

Hamstring tendons were harvested with an open-loop tendon stripper through a skin incision made 4 cm below the joint line. All muscle tissue was removed from the tendons, and the tendons were detached from the site of tibial insertion. The tendons were stretched and sized over a tube (Fig 1A), and then 6 mL of activated liquid PRGF was injected through a 22-gauge needle, with several punctures performed along the graft length (Fig 1B). In doing so, fibrin developed within the fascicles and worked as a safe and reproducible delivery system, which allowed for minimal dispersion of growth factors.[14] Afterward, the graft was soaked in PRGF until implantation; this resulted in PRGF-fibrin covering the entire surface of the hamstring tendons (Fig 1C).

Surgical Technique

ACL surgery was performed by the standard procedure. In brief, a drill guide was used for the correct placement of the femoral and tibial tunnels created by use of a 9- to 11-mm-wide trephine drill. Commonly, when we make the tibial tunnel, we perform sequential trephine drilling with escalating-diameter sections; at first, we started distally with a trephine drill that was 11 mm wide and 20 mm long, and then we changed to a drill 9 to 10 mm wide proximally. The graft was fitted into the femoral tunnel and held in place proximally, by transcondylar screw fixation. The graft was held under tension and inserted in the tibial tunnel; it was then fixed distally with the PRGF-treated bone plug and 2 metal staples. After graft fixation, activated liquid PRGF was applied to both

the femoral and tibial bone tunnels to enhance healing between the native bone and tendon.

Second-Look Arthroscopic Examination

A senior experienced surgeon performed all second-look arthroscopies through standard lateral parapatellar portals. The indications for second-look arthroscopy were meniscal tears in 5 patients; loose bodies, plica syndrome, hardware removal, or chondral defects in 12 patients; and symptomatic cyclops lesions in 20 patients. All arthroscopic procedures were recorded. The videos were critically reviewed to assess the presence or absence of synovial coverage over the graft, as well as to grade apparent tension and graft thickness. The details of the arthroscopic evaluation used in this study have been previously described.[19] The graft thickness and apparent tension of a reconstructed graft are evaluated as follows: a score of 2 indicates a sufficiently thick graft with no laceration or elongation, a score of 1 indicates a sufficiently thick graft with partial laceration or a relatively thin graft with no laceration or elongation, and a score of 0 indicates a graft with obvious elongation. Synovium coverage was graded as follows: completely covered (score 2), partially covered (score 1), or almost not covered (score 0). Thus a graft having a total score of 4 points was categorized as excellent, a graft having a total score of 2 or 3 points as fair, and a graft having a total score of 0 points or 1 point as poor. Two independent blinded observers evaluated the arthroscopic images of the transplanted tendons in consensus.

Histologic Examination

All of the biopsy specimens were harvested through the anteromedial portal by the senior surgeon using 3-mm basket forceps. The forceps was positioned directly under the femoral condyle; this positioning enabled the forceps to reach the same area of the graft at any time in all patients. The specimens (approximately 3 mm thick by 5 to 10 mm long) were fixed in formalin and embedded in paraffin blocks. Five-micrometer longitudinal sections were obtained with a microtome and stained with H&E and Alcian blue for visualization with light microscopy. For histologic evaluation, images were obtained at low- and high-power magnification with a Leica DFC 300 FX digital camera (Leica Microsystems, Wetzlar, Germany) coupled to a Leica DMLB light microscope.

In many specimens we found that the remodeled graft was enveloped by newly formed connective tissue. Both tissues became the subjects of our work. Because of their different appearances under light microscopy, we evaluated the 2 tissues separately by applying specific scores. The Ligament Tissue Maturity Index of Murray et al.[20] was adapted and used to evaluate the ligamentization of tendon grafts according to the following 3 criteria: (1) cellular aspects including cell density, nuclear shape, and orientation; (2) extracellular matrix characteristics, such as crimp and the presence of glycosaminoglycans; and (3) vascular features including blood vessel density and maturity (total score, 28 points). As a reference for these criteria, we used the hamstring tendons from 2 patients undergoing ACL surgery but not otherwise included in the study. When the Maturity Index was about 7 points, the specimen was judged to have substantial similarities to the ACL.

A maturity score to assess the newly formed enveloping connective tissue was created by use of the following 3 criteria, which were previously used by Murray et al.[20] to assess the maturity of a tissue: cell number and orientation, vessel abundance, and the looseness or tightness of the collagen of the extracellular matrix (total score, 16 points). By use of these criteria, in contrast to the Ligament Tissue Maturity Index,[20] higher scores depict immature connective tissue with a loosely woven matrix and a high degree of vascularization. Three independent observers unaware of the treatment and postsurgical timing examined all of the samples and scored the specimens on average.

Statistical Analyses

Data are expressed as mean ± SD. Differences in arthroscopic evaluations were tested by use of the Mann-Whitney U test. Pearson correlation analyses were used to examine relations between histologic scores and time of ligamentization. The Levene test was used to check the homogeneity of variances, and analysis of variance was then used to assess the effect of treatment on the histologic scores over time. Post hoc analyses were carried out by use of the Bonferroni test. Statistical differences between groups were considered significant at $P < .05$. Statistical analyses were performed by use of SPSS software, version 15.0 (SPSS, Chicago, IL).

RESULTS

Characteristics of Study Patients

The study was observational and performed in a private setting; we proposed the use of PRGF to all

patients, but ultimately, the patients decided whether PRGF was applied. Of 37 evaluated patients, 22 had been previously treated with PRGF during the arthroscopic ACL grafting. Baseline demographic characteristics were similar between the 2 groups; there were 26 men and 11 women with a mean age of 28 years, ranging from 18 to 48 years. The presence of a symptomatic cyclops lesion was the main reason for second-look arthroscopy; 7 control knees (46%) and 13 PRGF-treated knees (59%) sustained cyclops lesions. Cyclops lesions were present in 6% of the ACLs, with no differences between control knees and PRGF-treated knees. Three control knees (twenty percent) and two of the PRGF-treated knees (nine percent) presented because of meniscal tears. Four control patients and three PRGF-treated patients had loose bodies and/or chondral lesions, and removal of metal staples was necessary in three PRGF-treated patients. One patient in each group presented with plica syndrome.

Arthroscopic Evaluations

The mean period from ACL surgery to second-look arthroscopy and histologic analysis was 15 months (SD, 6), with a range of 6 to 25 months; this interval was similar in the PRGF group (14 ± 6 months) and control group (17 ± 5 months). Arthroscopic assessments are shown in Table 1. The overall evaluation of PRGF-treated grafts showed an excellent rating in 57.1% of knees and a fair rating in 42.9%. Untreated grafts showed an excellent rating in 33.3% of knees, fair in 46.7%, and poor in 20%. We could not ascertain a statistically significant difference between the 2 groups using the nonparametric Mann-Whitney $P = .051$.

Table 1. *Arthroscopic Evaluation of PRGF-Treated and Untreated Tendon Grafts*

Arthroscopic Evaluation	% of Grafts (n)	
	Control	PRGF
Synovium coverage		
Completely covered	33.3 (5)	61.9 (13)
Partially covered	46.7 (7)	38.1 (8)
Almost not covered	20.0 (3)	—
Thickness and apparent tension		
No elongation or laceration	40.0 (6)	71.4 (15)
Partial laceration of thick graft or no laceration of thin graft	53.3 (8)	28.6 (6)
Complete tear or obvious elongation	6.7 (1)	—
Overall evaluation		
Excellent	33.3 (5)	57.1 (12)
Fair	46.7 (7)	42.9 (9)
Poor	20.0 (3)	—

NOTE. We could not ascertain any significant difference between the 2 groups, Mann-Whitney $P = .051$.

Histology

Ligamentization of Tendon: PRGF treatment significantly influenced the histologic characteristics of the tendon grafts, resulting in more remodeling; the Maturity Index was 12.0 points (95% confidence interval [CI], 10.9 to 13.1) for PRGF-treated tendons versus 14 points (95% CI, 12.7 to 15.4) for control tendons ($P = .024$). Furthermore, the presence of symptomatic cyclops lesions was associated with the biological state of the graft, as evidenced by histology. Those patients sustaining symptomatic cyclops lesions had more immature grafts; tendon grafts in the knees with cyclops lesions scored 14.0 points (95% CI, 12.9 to 15.2), and those without cyclops lesions scored 11.9 points (95% CI, 10.7 to 13.2) ($P = .017$).

As shown in Fig 2, the morphology and microstructure of native tendons contrasted with those of tendons that had undergone ligamentization after 14 to 18 months. Of note, at the time of surgery, the uniform linear collagen orientation and the spindle-shaped morphology of the graft (Figs 2A and 2B) differed from the remodeled collagen and the ovoid cells present after ligamentization (Figs 2C-2F). More tenuous but discernible differences were observed between untreated tendons (Figs 2C and 2D) and PRGF-treated tendons (Figs 2E and 2F). Flat synovial cells were evident in most specimens (34 of 37) in both control grafts and PRGF-treated grafts.

New Connective Tissue: Interestingly, we observed newly formed connective tissue enveloping most grafts treated with PRGF (77%, n = 17). In contrast, this tissue was present in fewer than half of the untreated grafts (40%, n = 6). The quality of the connective tissue changed with increasing time from surgery; it was denser with more well-oriented cells after longer maturation periods. The temporal outcome of this tissue was more predictable in PRGF-treated grafts (n = 17), as suggested by the marked Pearson degree of correlation with time ($r = -0.5838$, $P = .0139$). No significant correlation was found in the control group ($r = 0.2213$, n = 6, $P = .6735$). The Pearson degree of correlation persisted but was less marked for analysis of the whole group (i.e., PRGF and controls) ($r = -0.413$, n = 22, $P = .050$).

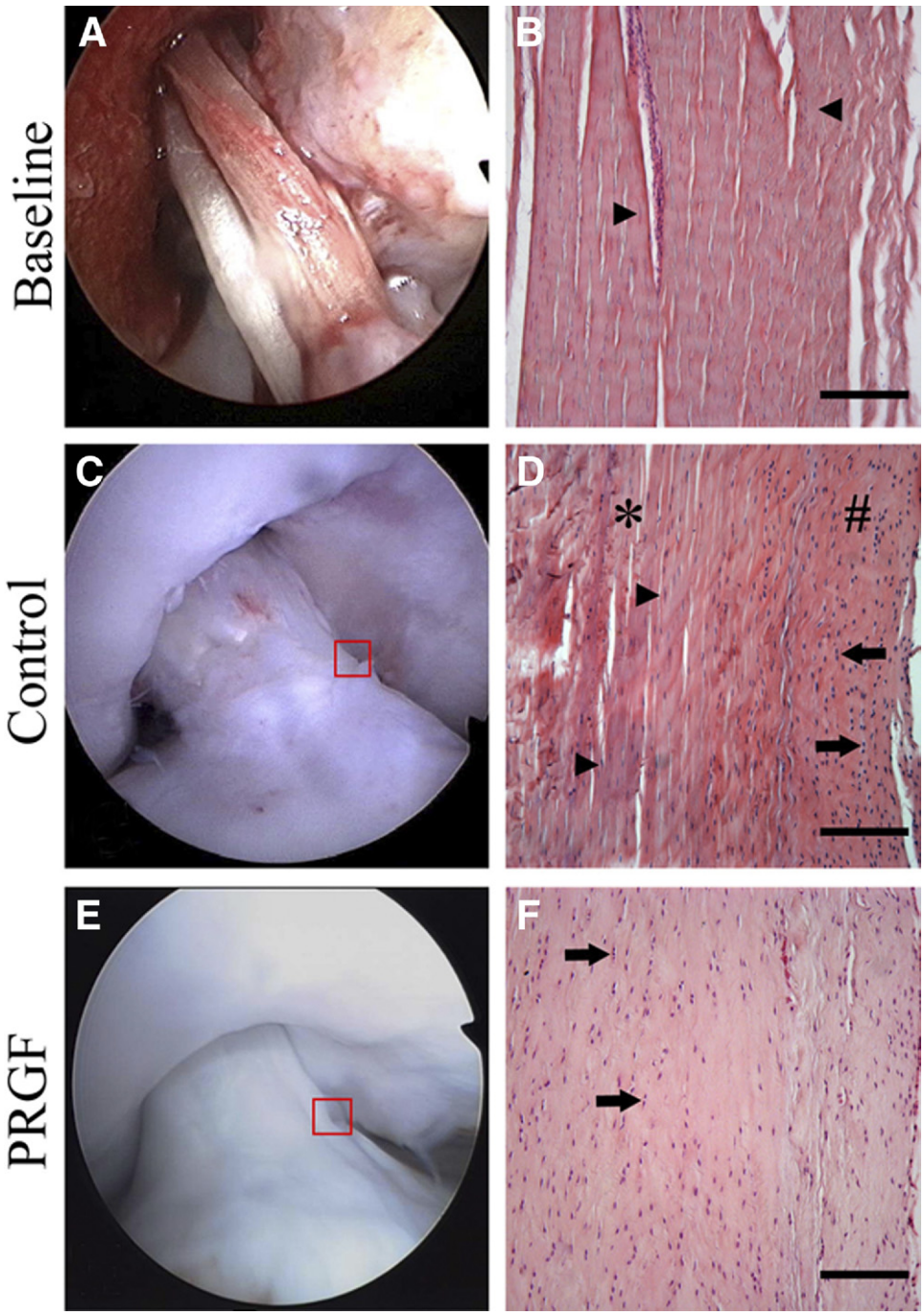

Figure 2. Gross morphology and histology of hamstring tendon grafts (A, B) at surgery and (C-F) after ligamentization. (B) At the time of surgery, the microstructure of the tendon graft shows a uniform aspect of strongly oriented collagenous matrix with interspersed spindle cells (arrowheads). The intrasynovial transformation (C, D) at 18 months (untreated graft) and (E, F) at 16 months (PRGF-treated graft) can be observed. The microstructure of the untreated graft is not uniform and shows 2 well-differentiated areas: the remodeling graft (asterisk) and the connective tissue (pound sign). These areas are identified by the cell shapes and the extent of matrix remodeling. The PRGF-treated graft is more uniformly remodeled and shows cells arranged in columns (arrows) similar to the native ACL. The red square in C and E shows the position of the sample harvesting. (Scale bars in B, D, and F, 200 μm.)

Proposition for Remodeling of PRGF-Treated Grafts

Although the process of remodeling is really a continuum, it can be divided into 3 stages for ease of description based on the temporal histologic changes found (Fig 3). During stage I, the period of 6 to 12 months after graft implantation, a new synovial-like enveloping tissue was found in the periphery of the graft; it had metabolically active cells (plump cells) lying over a loosely woven and highly vascularized matrix. At the same time, the tendon grafts seemed to be unchanged, revealing a tendinous structure with normal collagen alignment and crimp pattern; a concurrent high cell density was clearly visible within the original graft, suggesting remodeling activity (Figs 3A-3C). At this stage, newly formed enveloping tissue was evident in 9 of 11 PRGF-treated grafts (82%); in contrast, 2 of 5 control grafts (40%) were found to have this enveloping tissue.

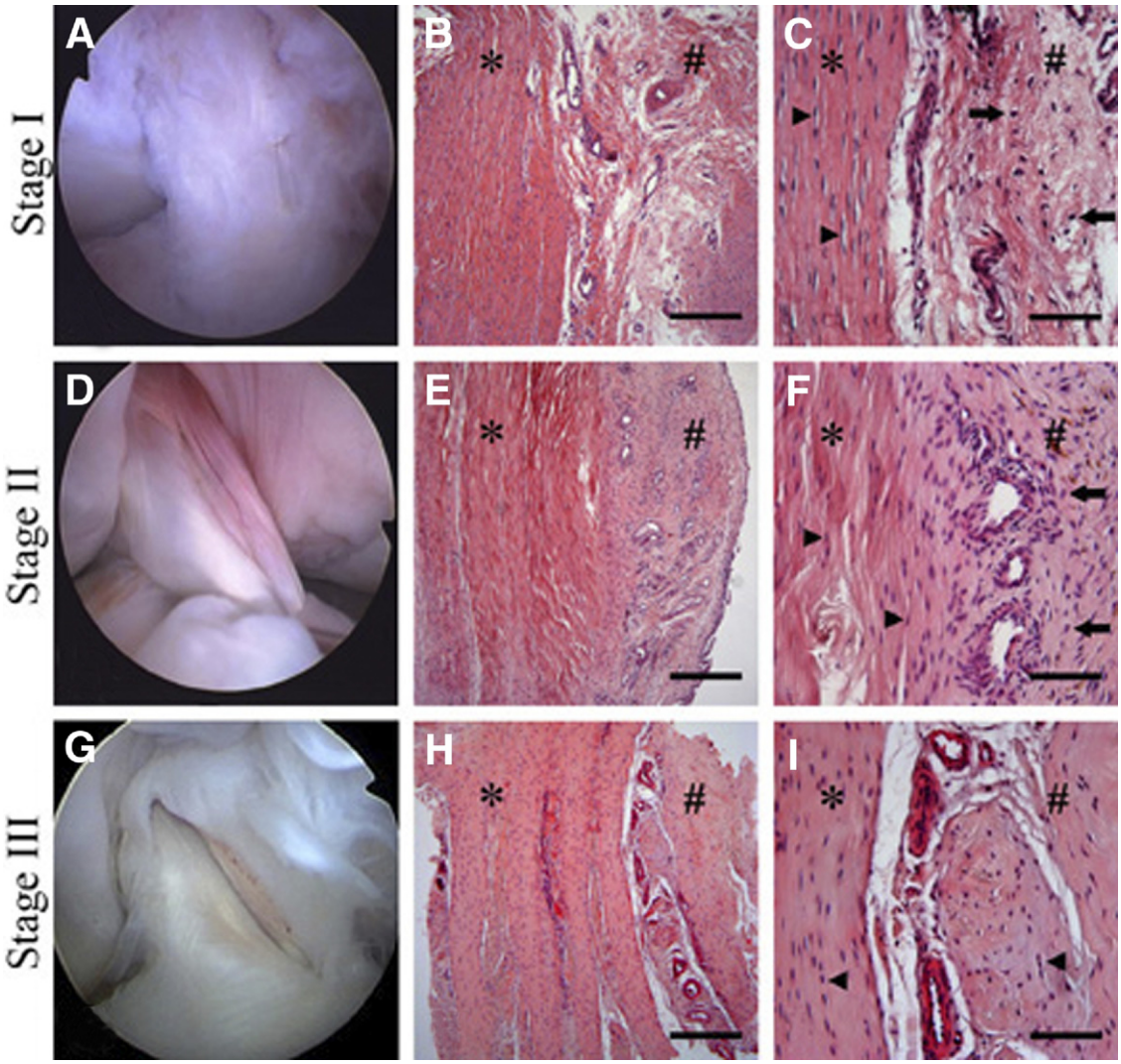

Figure 3. Arthroscopic appearance through anterolateral portal and underlying microstructure of representative hamstring at (A-C) stage I (7 months), (D-F) stage II (14 months), and (G-I) stage III (24 months) after implantation. During the earlier remodeling stages (I and II), longitudinal microphotographs showed 2 dissimilar tissues: the remodeling graft (asterisk) with spindle cells (arrowheads in C and F) and the newly formed covering tissue (pound sign) with active ovoid cells (arrows in C and F) and high vessel density. At stage II, the enveloping connective tissue had progressed to a denser, highly vascularized tissue with partially orientated cells (pound sign). In the third stage, the 2 tissues became hardly distinguishable with an appearance similar to the mature ligament, as shown by less active cells arranged in columns (arrowheads in I) and the presence of arterioles. (Scale bars in B, E, and H, 400 μm; scale bars in C, F, and I, 100 μm.)

During stage II, the period of 13 to 18 months after grafting, the newly formed enveloping tissue progressed to a denser tissue, displaying a thicker but still immature collagen matrix with partially orientated cells. Vascularity remained high within the enveloping tissue, and some vessels were evident within the original graft (Figs 3D-3F). At this stage, 5 of 6 specimens in the PRGF group and 1 of 4 control specimens were found to have the new connective tissue. During stage III, the period of 19 to 24 months after grafting, the enveloping tissue was present in 3 of 5 PRGF-treated grafts and in 3 of 6 control grafts. At this time, the connective tissue and the original graft could hardly be perceived as different; histologically, most specimens were characterized by well-oriented cells arranged in columns within the mature collagen matrix (Figs 3G-3I).

DISCUSSION

We investigated whether treating the graft with endogenous PRGF during ACL surgery yields a potential advantage for better and more predictable ligamentization. The arthroscopic evaluation of the grafts in parallel with the histologic examination at 6 to 24 months after ACL surgery gave us insight into the biology and the remodeling of ACL substitutes. The overall arthroscopic evaluation of the ACLs showed a higher percentage rated as excellent in the PRGF group than in the control group (57% *v* 33%). No grafts were scored as poor in the PRGF group, but 20% of the control grafts showed poor morphology. At the same time, PRGF treatment was associated with the histologic properties of the grafted tendon, and our results indicated further ligamentization of the PRGF-treated graft. Other authors have reported on the enhancing effect of platelet-rich plasma on the graft maturation process, as evaluated by magnetic resonance imaging signal intensity.[21]

The most common reason for second-look arthroscopy was the presence of symptomatic cyclops lesions; although etiologic factors for this phenomenon have not been identified thus far,[22] we found that the presence of cyclops lesions was associated with the biological state of the ACL substitute, in particular, more immature grafts. There was no association between PRGF treatment and the presence of cyclops lesions. Viability of cells from the native tendon has been a matter of debate in the literature.[23] The goal of administering PRGF injections within the fascicles of the autogenous tendon was to preserve the cell viability and encourage tendon cells to maintain the collagen structure, thus possibly helping to prevent the risk of graft elongation during knee rehabilitation.[24] Other attempts to optimize the structural properties of the graft and decrease knee laxity have been described by Fleming et al.,[25] who placed a collagen-platelet composite around the graft at the time of ACL reconstruction in a porcine model.

Previous basic research has indicated that autogenous tendon cells treated with PRGF have the potential for proliferation, collagen preservation, and remodeling.[13,14,18] Moreover, PRGF injections within the Achilles fascicles in a sheep model induced angiogenesis along with high cell density.[14] Assuming that these results are valid, PRGF injection within the tendon scaffold before implantation is appealing from a biological point of view because it enhances angiogenesis and the long-term survival of the graft. In support of these findings, throughout this study, we observed remodeling activity in well-preserved tissue without signs of necrosis. This lack of necrosis does not exclude the possibility that other specimens harvested from deeper areas of the grafted tendon might present with some other features over time.

PRGF also offers the possibility of delivering cues for cell migration and proliferation to the non-homogeneous cell population of the synovial capsule.[26,27] In keeping with this hypothesis, the microstructure of the tendon grafts changed over the studied time and was different in PRGF-treated and untreated grafts. In most PRGF-treated knees, the graft was enveloped by newly formed tissue that consisted of cells and vessels and appeared to be actively remodeled over time. The traditional paradigm for graft colonization assumes that cells migrate from the synovium and/or the infrapatellar pad to graft structures using the synovial fluid for passage.[28,29] Accordingly, recent work has shown that cells are present in great quantities (100-fold) in the synovial fluid of subjects with ligament injuries compared with normal subjects.[30] The present study may show that migratory cells first infiltrate the periphery and then gradually occupy the graft before revascularization and collagen reorganization occur. These observations suggest that the lack of sufficient covering tissue may be associated with less predictable and/or decreased ligamentization. In accordance with this hypothesis, previous research using intravital microscopy in animals indicated that only tendons implanted with preserved connective tissues showed high viability, whereas those that were completely stripped appeared to be subvital.[31] Classic descriptions of ligamentization in humans have not clearly

addressed this issue.[23,32] Some authors have referred to the formation of a synovial membrane during the ligamentization process,[33] but this term identifies the lining layer of flat synovial cells. In our study every specimen displayed this synovial rim of lining cells. However, most PRGF-treated grafts but few control grafts also showed a thick covering tissue beneath this cell layer. This connective tissue envelope had a synovial appearance in the early stage of ligamentization (6 to 12 months) and progressed through remodeling and maturation to an ACL-like structure. When it comes to describing the endpoints of the ligamentization, results from animal models have depicted different times and stages than human studies.[34] The structure of PRGF-treated grafts may resemble the structure of the normal ACL after 18 to 24 months (stage III), but whether these ACL substitutes entirely replicate the full mechanical properties of the intact ACL merits further investigation.

There is some confusion regarding the composition and terminology associated with platelet-rich plasmas, which impedes any distinction between the different systems and products obtained thereby.[35] The system of producing platelet-rich plasmas determines the composition and concentration in terms of platelets, leukocytes, and erythrocytes in a given plasma volume. Therefore relevant parameters vary between the various platelet-rich plasmas, which have different molecular compositions and dissimilar biological effects.[36] Double spinning, in contrast to single spinning, yields higher levels of platelets and leukocytes (5 to 8 times the baseline levels). PRGF is prepared by a single-spin method, is completely endogenous, and contains a moderate enrichment in platelets. Moreover, the leukocyte content is eliminated with the aim of avoiding the proinflammatory effects of the proteases and acid hydroxylases contained in neutrophils.[37] The release of biologically active agents by platelet degranulation is induced by the addition of a standardized dose of calcium chloride, to permit a more sustained release of growth factors than the addition of exogenous thrombin and to confer versatility to the administration procedures.

Clinical results are often controversial and may be attributed both to different products under the same name and to the procedures of re-administration to the patient. The demanding rationale for progress in the field involves describing the main characteristics of the product and observing the main features of the procedure of re-administration to the patient in each specific condition. Silva and Sampaio[38] have recently explored the influence of platelet-rich therapies in promoting the healing of femoral bone-tendon interfaces by magnetic resonance signal intensity assessments. In this case the lack of positive results may be attributed to several crucial factors concerning the reduced number of patients per group, the product itself, and/or the protocol of administration (i.e., platelet-rich plasma was not activated before application).

Our approach involves a different product and procedure of application compared with previous studies. The versatility of the PRGF technology is that it not only treats the graft before implantation but also occupies the graft donor site with the healing fibrin matrix. In addition, biological anchors were created by mixing PRGF with the autogenous bone plugs obtained during the procedure. Finally, at the end of surgery, we applied PRGF during suturing to help in healing the portals. We inserted the bone plugs in the tibial tunnel, whereas other authors fit compacted bone plugs in the femoral tunnel.[21] This insertion of bone plugs prevented tunnel widening. Curiously, the same authors could not find any further improvement after injecting 1 mL of platelet concentrate into the femoral tunnels between the strands of the graft.[21]

The delivery of endogenous signaling molecules during ACL surgery may result in significant changes in biological functions of the local cells. However, the production of tissue that is biologically, chemically, and mechanically normal requires a combination of strategies. Thus combining the use of platelet-rich products with appropriate mechanical loading regimens might yield both better tissue organization in the short term and enhanced mechanical properties, which are of paramount importance in young active patients.[39]

There are some limitations to this study. First, all of the patients required a second-look arthroscopy, which indicates a less-than-normal postoperative outcome. Because the reasons for second-look arthroscopy did not include graft tears or failure of reconstruction, we assumed that the causes for second-look arthroscopy did not interfere with ligamentization. Another limitation of this study is the small number of patients available over the broad range of time; a larger number of patients would have allowed for more reliable comparisons to be performed at each time point (6, 12, 18, and 24 months). The small number of patients also limited the statistical power, and we could not ascertain any statistical difference between treatment groups concerning morphology. Another limitation is that we could not randomize the patients to a particular treatment group, because the study was performed in a private setting and many patients specifically asked for PRGF treatment.

This study also had other weaknesses common to human studies of this nature. Only the periphery of the graft could be examined microscopically for ethical reasons, and thus the study did not shed any light on the biological differences occurring in the core of the graft. In addition, the study was limited to autologous hamstrings, which contain living cells, and the results cannot be extrapolated to homologous allografts or xenografts. These limitations should be considered when interpreting the results of this study.

CONCLUSIONS

The use of PRGF influenced the histologic characteristics of tendon grafts, resulting in more remodeling compared with untreated grafts. We have shown temporal histologic changes during the 6- to 24-month postoperative period of graft maturation, with newly formed connective tissue enveloping most grafts treated with PRGF.

Acknowledgment: The authors thank Miren Sánchez for her expert assistance in surgery and Jose J. Aguirre for his statistical advice.

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