Histological features of the ACL remnant in partial tears

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Abstract

Background: The aim of this study was to investigate the histological features of the remaining fibers bridging the femur and tibia in partial ACL tears.

Methods: Twenty-six ACL remnants were harvested from patients who had arthroscopic criteria concordant with a partial tear. Histological analysis includes cellularity, blood vessel density evaluation and characterization of the femoral bony insertion morphology. Immunohistochemical studies were carried out to determine cells positive for α-smooth actin and for mechanoreceptor detection.

Results: In these samples, a normal femoral insertion of the remnant was present in 22.7% of the cases. In 54% of the samples, substantial areas of hypercellularity were observed. Myofibroblasts were the predominant cell type and numerous cells positive for α-smooth actin were detected at immunostaining. Blood vessel density was increased in hypercellularity areas and in the synovial sheet. Free nerve endings and few Golgi or Ruffini corpuscles were detected in 41% of the specimens. The cellularity was correlated to the time between injury to surgery (p = 0.001).

Conclusion: Competent histological structures including a well-vascularized synovial sheet, numerous fibroblasts and myofibroblasts and mechanoreceptors were found in ACL remnants. These histological findings bring additional knowledge towards the preservation of the ACL remnant in partial tears when ACL reconstruction or augmentation is considered.

Clinical relevance: Descriptive laboratory study.

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1. Introduction

Over the past 15 years, knowledge of the anterior cruciate ligament (ACL) has evolved considerably. Identification of two bundles [1], a more precise description of the femoral and tibial insertion site areas [2,3] and a better understanding of the biomechanical anteroposterior and rotatory function of each bundle [4,5] have been established. This knowledge has led to improvements of techniques for ACL reconstruction with the emergence of double-bundle reconstruction and more recently selective bundle reconstruction in partial tears [4,6,7] or remnant preserving techniques [8,9]. Despite these evolving techniques, the significance of remnant preservation for selective reconstruction is an issue of current controversy [10]. A uniform definition of a partial ACL tear does not exist and its diagnosis remains clinically challenging [11,12]. Several potential advantages have been advocated for partial ACL reconstruction: vascular supply of the graft, faster remodeling [13] and increased proprioception [14] may come from the ligament remnant leading to enhanced healing. These potential advantages should be linked to the histological structure of the preserved tissue. Murray et al. [15] described four different histological phases after complete ACL rupture in humans but the histological analysis of partial ACL tears has not been performed yet, to our knowledge.
The purpose of this study was to describe the histological features of a continuous ACL remnant in a partial ACL tear. The hypothesis was that the partially torn ACL would demonstrate a transient healing response after the initial trauma and contain competent histological structures.

2. Materials and methods

A multicenter prospective study was performed. It received National Ethics Committee (no. 2011-A00109-25) and IRB approval. All patients provided informed written consent before surgery. All patients with clinically suspected partial ACL tear were pre-included.

Pre-inclusion criteria were as follows:

- Clinical examination with a firm end point at the Lachman test and/or a negative pivot shift test in patients having a history of trauma and symptoms of functional instability. This represented the indication of an ACL reconstruction in this series.
- MRI demonstrating high signal intensity in the ACL with some continuous fibers observable and/or a wavy course or a thinning aspect of the remaining fibers.

At surgery, if the tear was considered as macroscopically partial during arthroscopy (ACL fibers bridging the femur to the tibia in the posterolateral bundle insertion sites areas, Fig. 1), patients were included in the study. Patients with a complete ACL rupture or with AM bundle intact and PL bundle tear were excluded.

Among 301 cases, 26 patients were finally included. The remaining tissue of the ACL (considered as a partial ACL tear) was harvested in one piece as follows: a gouge was used first to carefully detach the remaining fibers from its femoral insertion with the bony attachment. The sample was detached from its tibial insertion with arthroscopic scissors and extracted through the anteromedial portal using arthroscopic forceps (although the objective was to analyze the femoral insertion and the mid-portion of the ACL remnant, the bony insertion of the tibial side was not harvested). It was marked with a thread at the femoral attachment in order to differentiate the lower from the upper side. It was fixed in 10% neutral buffered formalin and sent to the lab. After harvesting, a standard anatomic single-bundle ACL reconstruction was performed. The median age of the patients at the time of surgery was 28 years (range, 18 to 45 years). The median time between injury and surgery was 103 days (range, 7 to 473 days). At preoperative ligament testing, a firm endpoint was noted in all 26 patients with a mean side-to-side instrumented (KT-1000 and/or Rolimeter) anterior laxity of 5 mm (min 3, max 7). During arthroscopy, the macroscopic appearance of the ACL remnant appeared intact in 15 patients, and continuous but macroscopically altered (heterogeneity of the surface of the ligament remnant) in 11 patients.

2.1. Histological analysis

The harvested specimens were embedded in paraffin and sectioned longitudinally (along the longest axis of the sample) in 4-μm-thick sections. Sections were stained with hematoxylin, eosin and saffron for histological analysis by a pathologist (Figs. 2aa n db). Three sections were prepared for each sample (anterior, middle and posterior side of the sample). The same pathologist (C.B.) reviewed all the slides. Immunohistochemical studies were carried out and sections were deparaffinized, enzymatically pretreated and incubated with primary antibodies (Actin HHF 35, clone MU 090UC, Biogenex, dilution 1/50; PS100 clone Z0311, Dako, dilution 1/400) overnight at 4 °C (Benchmark XT, Ventana Medical Systems, Arizona, USA). Following this incubation, sections were incubated with Envision system for 30 min at room temperature. Staining each sample without adding anti-human primary antibody was performed as a negative control. Finally, samples were incubated with diaminobenzidine peroxidase substrate to give a brown reaction product. The sections were counterstained with hematoxylin and mounted with a plastic medium. The samples were then examined under a light microscope (Leica). The histological appearance of the ACL remnant was compared with normal femoral junction (Fig. 3) and the results were reported as grade 0 (no change), grade 1 (mild alteration), grade 2 (moderate alteration), grade 3 (severe alteration).

Fig. 1. Macroscopically torn AM bundle with intact PL bundle in a partial ACL tear.

Fig. 2. Resection of an entire remaining PL bundle including the femoral bone block. The femoral side (F) and tibial side (T) are identified before sagittal section (white dotted arrow) and paraffin embedding (Fig. 2a). Histological appearance of a 2.8-cm length ACL remnant (HES stain, original magnification ×1), with the femoral insertion (star); and an area of hypercellularity (arrow), (Fig. 2b).

Fig. 3. Normal femoral junction with four distinctive layers from the top to the bottom a part of bone (A), calcified fibrocartilage (B), uncalcified fibrocartilage (C) and ACL ligament (D) (HES stain, original magnification ×20).
brown stain, counterstained with hematoxylin and mounted with coverslips.

On each sample, the bone-to-ligament junction was morphologically analyzed based on HES staining with a classic four layers of transition: a part of bone, calcified fibrocartilage, uncalcified fibrocartilage and ACL bundle ligament (Fig. 3). They were classified as intact with normal four layers and torn if a lesion was found (detachment between layers).

Hypercellularity of the sample was considered as positive when hypercellularity areas covered more than 10% of the total sample area with a cell density superior to 700 cells per mm² (in a normal ACL, there is a mean of 500 cells per mm² [16] around the mid-portion). In these areas of hypercellularity, myoncartilaginous cell type with an ovoid or spheroid nuclear morphology (Fig. 4a) contrasting with low cellularity areas composed of a few fusiform fibroblasts embedded in an abundant extracellular matrix.

The number of squares covered by the cells was manually counted on each sample. They were classified based on HES staining with a classic four layers of transition: type I as spherical or ovoid Ruffini corpuscle, type II as the columnar concentric circular Pacini corpuscle, type III as spindle-shaped Golgi corpuscle and type IV as non-myelinated free nerve ending.

<table>
<thead>
<tr>
<th>No. of sample</th>
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<th>AP side to side laxity (mm)</th>
<th>Macroscopic appearance of the PL remnant</th>
<th>Cell density (mean per mm²)</th>
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2.2. Statistical analysis

All values are expressed as the mean ± standard deviation. Independent Student t-test was used to identify differences between the mean values of continuous variables of different groups. Bivariate correlation coefficients (Pearson r) were calculated to evaluate possible association between variables. A p value of 0.01 was taken as the level of significance.

3. Results

The mean length of the sample harvested was 2.52 cm (1.7–3.5 cm), depending essentially on the amount of bone present on the femoral side (0–1.1 cm). These measurements were done on histologic specimens.

The summary of the results is presented in Table 1. There was no correlation between the macroscopic appearance of the ACL remnant (torn or intact remnant defined under arthroscopy before harvesting) and the histologic findings ($r = 0.34$, $p > 0.4$).

Due to problems encountered during the arthroscopic sample harvesting, 4 samples were improper for femoral bundle insertion evaluation. In the 22 remaining samples, 5 cases (22.7%) presented a normal histological femoral insertion. Among these cases, 3 were macroscopically-arthroscopically intact and 2 altered.

In 14 cases (54%), there was a significant increase of cells and vessels with areas of hypercellularity covering 20 to 80% of the samples surface (hypercellularity group). The hypercellularity was mainly located in the femoral portion in 4 cases, and in the mid-portion in 10 cases. In these areas of hypercellularity, myofibroblasts were the predominant cell type with an ovoid or spheroid nuclear morphology (Fig. 4a) contrasting with low cellularity areas composed of a few fusiform fibroblasts embedded in an abundant extracellular matrix.
Nerve endings were present and underlined by PS100 immunostaining (Fig. 6). On HE slide. In the healing areas characterized by high cellular density, numerous free (hypercellularity group). Thickening of the walls, were observed when the time to surgery was less than 3 months. In the response group, the intimal hyperplasia, proliferation of the smooth muscle cells and thickening of the walls were observed when the time to surgery was less than 3 months (hypercellularity group).

For these 14 cases, the histological features were consistent with an active healing process in response to the initial injury. It may correspond to the phase C described by Murray et al. [15]. In the other 12 cases (46%), there were no significant cellular healing responses. These samples exhibited a low cellular density (<500/mm²) and fibroblasts with an elongated nuclear shape (low cellularity group).

The mean time between injury to surgery was 216 days (±128) in the low cellular response group and 86 days (±41) in the hypercellularity group (p < 0.01). The Pearson correlation coefficient and p value demonstrated a negative correlation between the injury to surgery time and the cellularity (r = −0.64, p = 0.0017). There were no correlations between the pre-operative laxity and the cellularity (r = −0.22, p = 0.3).

Continued inflammation with lymphocyte infiltration, hemosiderin deposit and dilatation of arterioles with intimal hyperplasia, proliferation of the smooth muscle cells and thickening of the walls, were observed when the time to surgery was less than 3 months (hypercellularity group).

Few corpuscles of Golgi or Ruffini were seen. Pacini corpuscles were never detected on HE slide. In the healing areas characterized by high cellular density, numerous free nerve endings were present and underlined by PS100 immunostaining (Fig. 6).

4. Discussion

The present study reports for the first time the histology of a continuous remnant after “partial” ACL tears. Among the samples, 54% presented an active healing process (phase B-C), whereas in 46% of the cases, no active healing response was observed. The mean time between injury and surgery was significantly different between these two groups. Moreover, the degree of cellularity inside the sample was statistically correlated to the delay between injury and surgery even for the samples with a normal femoral insertion. As the degree of cellularity is considered of importance in the healing process, a short delay between injury and surgery may be recommended. This finding was somewhat different from Young et al. [19]. They demonstrated a significant decrease in extracellular proteoglycan matrix and collagen levels of samples harvested in completely ruptured ACL when compared to intact ACL. Moreover, no correlation was found between time since rupture and proteoglycan or collagen distribution. It would be interesting to compare these findings: cell concentration and extracellular collagen level in normal ACL, partially torn ACL, ruptured ACL.

Murray et al. [15] described four phases of healing response of the retracted remnant after a complete rupture of the ACL. Two weeks after the rupture were characterized by an inflammatory phase. Week three to week eight corresponded to the epiligamentous regeneration phase. The proliferative phase occurred after eight weeks. The final phase was defined by the remodeling and maturation of the remnants with retraction of the ligament. The authors reported a cellular and blood vessel density increasing until the twentieth week after the injury and a decrease over time. In the present study, most of the samples were harvested before 20 weeks, corresponding to the proliferative phase described by Murray et al. [15].

Preservation of the ACL remnant is proposed to have several advantages. It may contribute to anterior knee stability [20] and improves biomechanical strength in the immediate post-operative period of the ACL reconstruction [21–23]. For Mifune et al. [24], an increase in cellularity and angiogenesis was observed in the augmented grafts compared with the conventionally reconstructed grafts in a rat model. Also, biomechanical testing showed that failure to load was significantly higher in the augmentation group compared with the conventional reconstruction group. Histological examination of human ACL remnant demonstrates an intrinsic potential for healing, with an intact vascular support provided by the synovial layer and an ability for fibroblasts to synthesize collagen [26].

Murray and Spector [17] demonstrated that cells in the human ACL retain their ability to migrate into an adjacent scaffold in vitro, four weeks after a complete rupture. The presence of proprioceptive neuroreceptors in the ACL remnant and their possible reinnervation of ACL grafts have been published by many authors [22,27,28].

Collagen matrix (Fig. 4b). Immunohistochemical analysis revealed α-smooth muscle actin-containing fibroblasts, and numerous blood vessels in areas of hypercellularity in all specimens. Blood vessel density was also increased in the sub-synovial tissue (Fig. 5).

Area of hypercellularity with numerous myofibroblasts and ovoid nuclear (arrows) (Fig. 4a). Area of low cellularity composed of fusiform fibroblast (star) embedded in abundant collagen matrix (Fig. 4b) (HES stain, original magnification ×40).

Immunohistochemical analysis revealed α-smooth muscle actin in sub-synovial tissue with high vessel density with brown stain (arrow). On surface, synovial cell layer is present (star) (antibody actin, original magnification ×20).

Immunohistochemical analysis with PS100 where brown designates a positive stain. In this area of hypercellularity, some free nerve endings (arrows) and ovoid Ruffini corpuscles (stars) are present (antibody PS100, original magnification ×40).
Mechanoreceptors in the ACL are proposed to play a role in preserving and restoring joint stability. Therefore, it may be expected that the more the tibial remnant is kept intact, the better the preservation of proprioceptive function [14,29]. ACL remnants may accelerate the cellular proliferation and revascularization of the grafted tendon [30]. In a recent canine model study, Matsumoto et al. [31] elucidated that transplantation of ACL-ruptured tissue, which was sutured to the tibial side of the graft, contributed to early tendon-bone healing of ACL reconstruction. For the authors, ACL ruptured tissue has a therapeutic potential in promoting an appropriate environment for tendon-to-bone healing in bone tunnels of ACL reconstruction.

The clinical outcome of studies investigating ACL augmentation in patients with preserved remnants are encouraging. Nevertheless, there are few studies comparing this technique to a standard ACL reconstruction [7,26,32–35].

There are some limitations of this study; in particular, the small number of samples. Both the ruptured “AM” fibers at their femoral insertion and the “intact PL bundle” fibers were harvested in one piece. Under histological examination, we were not able to distinguish between these two tissues. The ACL is a continuum of inter-connected fibers, and thus the apparent “partial” injury involving only the AM bundle is an oversimplification. There is likely injury throughout the substance of the ligament, with a continuum (remnant) from more severe injury to the AM portion and less to the PL portion. No control group can be added to the study design (resection of an untorn ACL in young patients is not ethically acceptable).

5. Conclusion

Competent histological structures including a well-vascularized synovial sheet, numerous fibroblasts and myofibroblasts and mechanoreceptors were found in ACL remnants. These histological findings bring additional knowledge towards the preservation of the ACL remnant in partial tears when ACL reconstruction or augmentation is considered.

Conflict of interest

None for all authors.

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