Knee Surg Sports Traumatol Arthrosc (2008) 16:360–369
DOI 10.1007/s00167-007-0471-0

KNEE

The extracellular remodeling of free-soft-tissue autografts and allografts for reconstruction of the anterior cruciate ligament: a comparison study in a sheep model

M. Dustmann · T. Schmidt · I. Gangey · F. N. Unterhauser · A. Weiler · S. U. Scheffler

Received: 3 September 2007 / Accepted: 27 November 2007 / Published online: 9 January 2008

Abstract Our study was aimed to advance the currently limited knowledge about differences in the biological remodeling of free soft-tissue tendon allografts and autografts for ACL reconstruction. Allogenic and autologous ACL reconstructions were performed in a sheep model using the flexor digitalis superficialis tendon. After 6, 12 and 52 weeks the animals were sacrificed. We analyzed the collagen crimp formation and its relationship to expression of contractile myofibroblasts in both graft types. Additionally, structural properties and ap-laxity were compared during biomechanical testing. At 6 weeks only descriptive differences were found between autografts and allografts with a more organized crimp pattern and myofibroblast distribution in autografts. Significant differences in myofibroblast density and crimp formation were found after 12 weeks. At these early stages, the progress of remodeling in autografts was more advanced toward the central areas than in allografts. At 1 year, grafts in both study groups returned to an ACL-similar structure. Structural properties and ap-laxity did not vary significantly between auto- and allografts at early healing stages. However, at 52 weeks, failure loads, stiffness and ap-drawer test showed superior values for autograft ACL reconstruction. Extracellular remodeling of allografts develops slower than in autografts. Therefore, rehabilitation procedures will have to be adapted according to graft and patient selection. Postoperative treatment regimens from autograft primary ACL reconstruction should not be directly transferred to allograft ACL reconstructions.

Keywords Anterior cruciate ligament · Reconstruction · Remodeling · Autograft · Allograft

M. Dustmann · T. Schmidt · I. Gangey · F. N. Unterhauser · A. Weiler · S. U. Scheffler (✉)
Center for Musculoskeletal Surgery, Sports Traumatology and Arthroscopy Service, Charité, Virchow Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany
e-mail: sven.scheffler@charite.de

Introduction

Currently, the reconstruction of the anterior cruciate ligament (ACL) is the most common reconstructive procedure in orthopaedic practice [42]. The autologous patella tendon is still considered the graft of choice in many practices due to its osseous fixation methods. However, hamstring tendons and especially the semitendinosus tendon have gained increasing popularity in recent years as a graft alternative due to the reduced harvest morbidity [6, 36] and significantly improved fixation techniques.

Regardless of the graft type, autograft harvest is associated with varying degree of morbidity [44], which negatively impacts postoperative rehabilitation or may even limit full recovery after ACL reconstruction. Therefore, avoiding such morbidity by using allograft tissue might positively affect post operative rehabilitation. Further, some authors reported that improved cosmesis, reduced surgery and rehabilitation time, which are suggested to result into cost reduction, have been associated with allograft use for ACL reconstruction [4]. Due to these advantages, the demand for allografts has significantly increased in recent years, especially in the Anglo-American world [39]. However, there is an ongoing discussion about possible differences in the healing behavior between allograft and autograft ACL reconstructions, and whether these differences have clinical relevance, e.g. the post operative rehabilitation or time to return to full weight bearing.

The existing literature on comparison of auto- and allografts has shown contradictive results [17, 19, 32, 35]. There is only a very limited number of clinical studies that prospectively compared the outcome of autologous with allogenic ACL graft reconstruction [2, 28, 30]. The majority of studies examined only the clinical outcomes of allograft reconstructions with no control group available [15, 26, 33, 41]. It is difficult to directly compare these studies since they used different methods to evaluate the outcomes (choice of grafts, patient selection, primary vs. revision surgery, non-aggressive vs. aggressive rehabilitation). It also must be considered whether graft sterilization was conducted, since current sterilization techniques have been shown to negatively affect the biological healing and mechanical properties of the graft [5, 7, 17]. Non-sterilized allografts are currently considered to be the graft of choice due to the lack of inherent disadvantages of current sterilization techniques [29].

It is also important to note that basic science as well as clinical studies have exclusively focused on the patella tendon graft [1, 10, 17, 18, 25]. Furthermore, our literature search could not find any comparison studies of bone block free soft tissue grafts in primary ACL reconstruction. Also, little information is available on the time dependent changes of graft remodeling of allografts in comparison to those of the autologous tissues.

Recently, myofibroblasts have been identified as an important player for the reorganization of extracellular matrix during early tendon remodeling [23, 43]. However, until today, myofibroblast expression in allograft ACL reconstruction has not been examined. Possible differences in the restoration of the extracellular graft matrix between allo- and auto-grafts could yield direct consequences for how rehabilitation should be carried out following allograft ACL reconstruction.

The aim of this study was to analyze the extra cellular remodeling of autologous and allogenic non-sterilized bone block free soft tissue grafts in a sheep model and their biomechanical properties during the course of healing. We hypothesized that the remodeling of allogenic ACL grafts would show a time delay compared to that of the autologous ACL reconstruction.

Material and methods

In this study, 54 mature female merino-mix sheep underwent ACL reconstruction. The sheep were randomly divided into two groups: half of the animals underwent autologous ACL reconstruction and the other half received allogenic non-sterilized fresh frozen free soft tissue grafts (tendon of m. digitalis superfecialis; Fig. 1). Allogenic flexor tendons were harvested one week earlier from different sheep not involved in this study. Allografts were fresh frozen at −80°C and thawed at room temperature directly prior to the surgery.

The contralateral intact ACL of the 52 week specimens and native flexor tendons grafts of identical breed of sheep taken from a different study were used as controls.

All animal procedures were conducted according to the guidelines of the National Institute of Health for the use of laboratory animals. All animals were checked for bony maturity and normal health status by a veterinarian doctor.

Prior to surgery, anesthesia was induced with intravenous application of 20 mg/kg thiopental-sodium and, following intubations, maintained with isoflurane and nitrous oxide throughout the surgical procedure. Each animal received 0.5 mg fentanyl for intra-operative analgesia and 2.2 g amoxicillin clavulanate for perioperative antimicrobial prophylaxis. Each left hind limb was shaved and prepared in a standardized sterile fashion.

Surgical technique

The tendon of the superficial flexor digital muscle was harvested from each left hind limb as a free soft-tissue graft, providing a 6–8 cm long and 7 mm wide transplant. Each graft end was augmented with two No.2 Ethibond Excel polyester sutures (Ethicon, Inc.) in a whipstitch technique.

Following graft harvest, an open ACL reconstruction was performed on each left hind limb (Fig. 1). The joint was opened by a medial arthrotomy. Following transsection of the medial patellofemoral ligament (and reattachment during closure of the knee joint) and a small incision of the M. vastus lateralis, the patella was lateralized and the knee joint flexed. For better visualization a small part of the Hoffa fat pad was excised. The ACL was removed and its insertion site was identified and carefully debrided. In deep flexion, a guide pin was introduced into the femoral footprint of the ACL and overdrilled at a length of 20 mm to the diameter of the prepared graft. The graft was pulled into the tunnel and fixed at the femoral cortex with a fixation button (Flipptack®, Fa. Storz GmbH). The tibial tunnel was also prepared in an inside-out technique, where a guide-pin was placed at the center of the tibial footprint of the ACL and then overdrilled to the graft diameter. The graft was pulled into the tibial tunnel and a bone bridge was created approximately 1 cm distally from the tibial tunnel exit (Fig. 1).

The knee joint was then manually moved through ten cycles of maximum flexion and extension and final graft fixation was achieved by multiple knots onto the bone bridge at 30° of flexion under maximum manual tension. After relocation of the patella and re-fixation of the medial

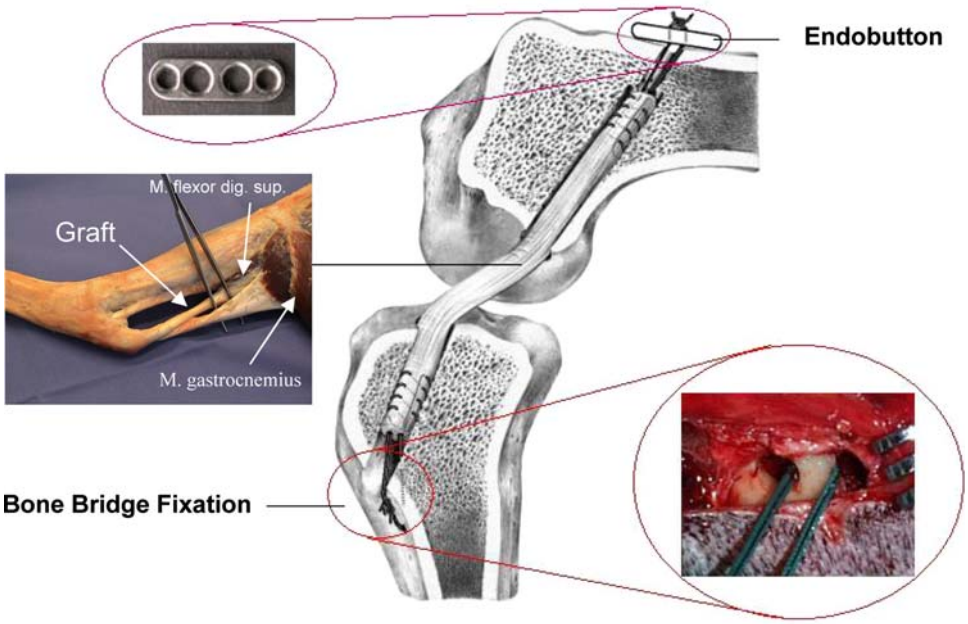

Fig. 1 ACL reconstruction technique with femoral cortical suture-button and tibial suture-bone bridge fixation of a free soft-tissue tendon graft (tendon of the flexor digital superficial muscle)

patellofemoral ligament, the joint capsule and the respective soft-tissue layers and skin were closed. Several sterile bandages were used for wound protection.

Animals were euthanized at 6, 12 and 52 weeks post operatively. Each group consisted of nine animals, in which seven underwent biomechanical testing and histological analysis and the remaining two animals were only assessed histologically.

First, the ACL reconstructed knee joint underwent biomechanical testing. Following failure testing, tissue from undamaged, intra-articular graft regions away from the rupture site, were harvested and longitudinal sections were embedded in paraffin. Intact contralateral knee joints and time-zero reconstructions were used as controls.

Immuno-histochemistry

To our knowledge, this is the first time that the autografts and allografts are compared by immuno-histochemical assessment of myofibroblast expression. These cells contain an isoform of actin, α-smooth-muscle Aktin (ASMA), which can be stained by a monoclonal antibody. Longitudinal sections embedded in paraffin were exclusively used for this analysis. All serial sections were mounted on slides and then deparaffinized, hydrated and pretreated with 0.1% protease (protease type XIV; Bacterial, Sigma-Aldrich, Steinheim, Germany). Immuno-histochemical staining of myofibroblasts was performed using a technique previously described in detail by Unterhauser et al. [38].

To avoid non-specific binding of the antibody, sections were briefly blocked with 10% normal horse serum (Vector Laboratories, Burlingame, CA, USA). Mouse anti-human ASMA monoclonal antibody (cat. no. M0851, Dako, Glostrup, Denmark) was applied, followed by tissues incubation with biotinylated horse anti-mouse IgG secondary antibody (Vector Laboratories). Slides were then incubated with an avidin-biotin complex (ABC kit; Vector Laboratories) linked with alkaline phosphatase as a reporter enzyme. Staining was visualized using neufuchsin as a chromogen. Finally, the sections were briefly counterstained with methylene green, dehydrated, and mounted in a xylolsoluble mount (Vitro-Clud, R. Langenbrinck, Emmendingen, Germany). Samples were incubated with TBS buffer on each slide to monitor for non-specific staining. Myofibroblasts were morphologically differentiated from pericytes that are typically found in vascular structures. These cells vary by their cell shape, the aforementioned proximity to vessels, and show a different distribution between matrix fibers.

Quantification of myofibroblast density was conducted on longitudinal sections using a digital video analysis system (KS 400 3.0, Carl Zeiss AG).

Polarization microscopy

In this study, polarization microscopy was used for assessment of collagen tertiary structure, e.g. its alignment and orientation. Collagen consists of long parallel running string molecules, which appear anisotropic in a polarization microscope. Collagen bundles usually display a wave-like structure, where wave frequency is defined as the collagen crimp.

Collagen crimp frequency (per mm) was calculated with customized software by using a standardized calibrated scale in ten regions of interest in longitudinal graft sections that were also used for myofibroblast quantification.

Biomechanical testing

Two testing conditions were applied during biomechanical analysis. First, anterior–posterior (ap) laxity was measured with all soft-tissue structures left intact. This was followed by a load-to-failure test of the femur-ACL graft-tibia complex. Prior to mechanical testing, all muscle was dissected from the femoral and tibial bones, which were potted in polymethyl-methacrylate and placed in aluminum clamps on the mechanical testing machine (Modell 1455, Fa. Zwick GmbH).

Anterior–posterior laxity was measured at 60° of flexion. After application of a 5 N preload, ten ap drawer cycles between +50 N to −50 N were performed at a cross-head speed of 2.5 mm/s. Final data analysis was drawn from the tenth cycle.

Prior to load-to-failure testing, graft cross-sectional area was calculated with a micrometer according to the technique by Ellis [9]. The knee was again mounted onto the material testing machine at 30° of flexion with the graft's longitudinal axis being aligned in the direction of loading (Fig. 2). After a preload of 5 N, failure testing was performed at a cross-head speed of 2.5 mm/sec. Failure load and stiffness (calculated between 30 and 90% of failure load) were recorded and analyzed with in-house software.

Statistics

The analysis of data for normal distribution using Kolmogorov-Smirnov test showed that the data in our study were not normally distributed. Therefore, a non-parametric test, the Mann-Whitney-U-rank sum-test was applied for statistical comparison of the data in the respective study groups. Level of significance was set at $P \leq 0.05$.

Results

One animal was lost directly following surgery. Autopsy by an in-house veterinarian doctor could not reveal cause of death. Two animals had to be excluded from the study because of one knee infection and one chronic effusion that prevented restoration of normal gait until three month after surgery. One animal suddenly died at 6 months post-surgery. Autopsy showed a Salmonella infection with massive liver infiltration. All animals were replaced by an appropriate number of sheep to maintain equal group sizes.

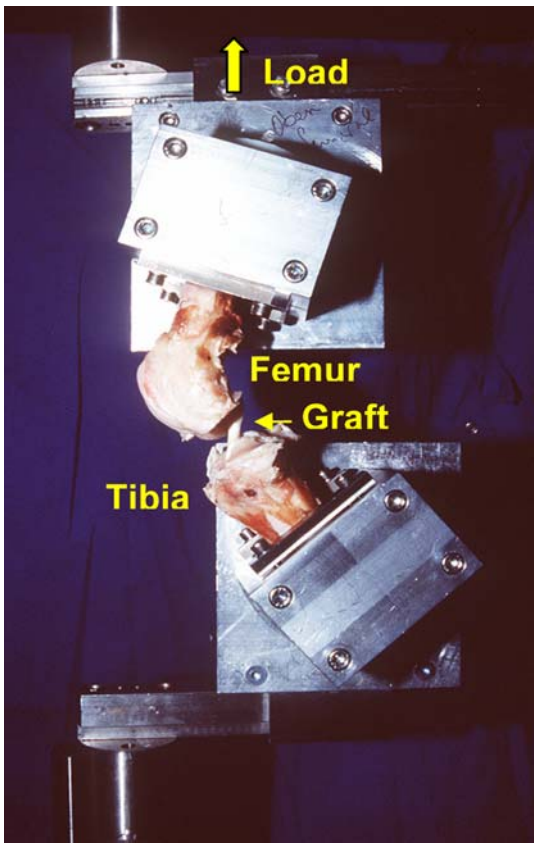

Fig. 2 Experimental setup for biomechanical analysis of structural properties from load-to-failure testing. The graft is aligned parallel to the load application direction

Myofibroblasts expression

At 6 weeks, we did not find significant differences in myofibroblast density between auto- and allograft ACL reconstructions, even though increased numbers were found in the autograft group (Figs. 3, 4). However, at 12 weeks myofibroblast density continued to increase with now significantly higher values in the autograft group ($P < 0.05$; Fig. 3). At 52 weeks, absolute values for myofibroblast density decreased and statistical differences between both groups disappeared.

Allografts showed a continuous increase in myofibroblast density during the postoperative course of 52 weeks, while autografts showed a peak at 12 weeks with a subsequent decrease at 52 weeks. During early healing cellular distribution was very irregular. The descriptive analysis at 6 weeks showed an influx of cells from proximal and distal as well as from the synovial sheets of the graft. Overall cellular distribution was more irregular in the allografts compared to the autograft reconstructions at 6 and 12 weeks of healing. As the time went on, cellular distribution became more regular along all graft regions. Also, cell shape changed from spindle-like cells as they are

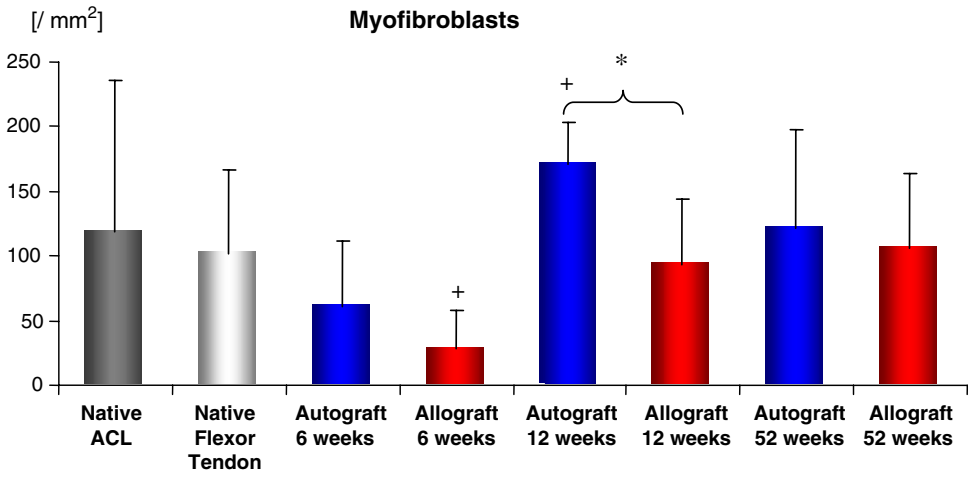

Fig. 3 Myofibroblast density was significantly different between study groups at 12 weeks only. In allografts a continuous increase in cell density was seen during healing time, while a decrease was found in autografts from 12 to 52 weeks postoperatively. (mean ± SD, "+" significantly different from native flexor tendon grafts, $P \leq 0.05$)

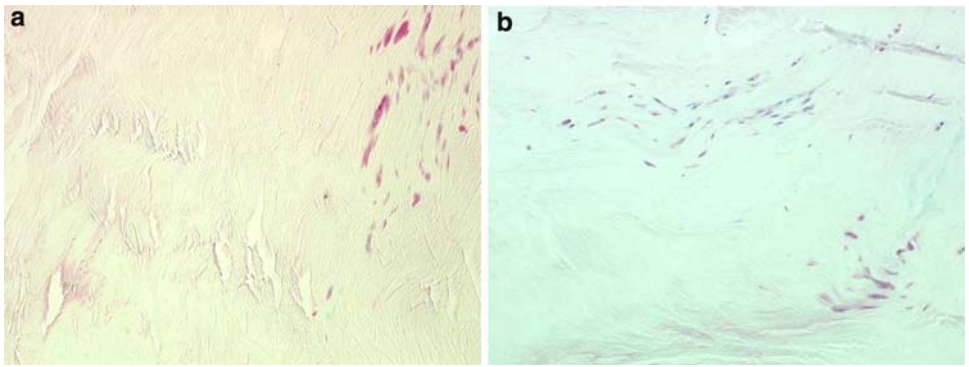

Fig. 4 Myofibroblast expression in 6 week specimens. Autograft (**a**), allograft (**b**): myofibroblasts migration from the periphery towards the acellular center of the graft (ASMA-stain, ×20)

predominantly found in native flexor tendon grafts to ovoid cell morphology, typically found in the native anterior cruciate ligament [8].

Polarization microscopy

No significant differences in crimp frequency were found between the study groups during early healing at 6 weeks. There was a significantly larger crimp length in the autograft group at 12 weeks. At one year postoperatively, differences in crimp length disappeared, showing similar values in the allografts and autografts (Fig. 7).

Time-dependent changes were consistent in the autograft group with a continuous decrease in crimp length until 52 weeks, while there was a slight, but not significant increase in allograft crimp length from 12 to 52 weeks of healing. Both study groups showed a significantly larger crimp length compared to the intact ACL at 6 and 12 weeks, with no differences at 52 weeks of healing.

Descriptive analysis of the extracellular matrix revealed a very non-homogeneous structure throughout all graft regions at 6 weeks in both groups (Fig. 5). At 12 weeks, crimp alignment became more homogeneous at the periphery and is slowly reorganized towards the center of the graft (Fig. 6). It is not until 52 weeks that a distinct organization in longitudinal septums could be seen in the graft tissues. However, since crimp length continued to vary substantially even after one year in the respective graft tissues, a full restoration of the organization of the extracellular matrix as seen in the native ACL was not achieved (Fig. 7).

Biomechanical analysis

No significant differences were seen in ap laxity between the study groups at 6 weeks of healing (allografts 5.7 ± 1.6 mm, autografts 5.6 ± 2.1 mm). Failure testing also showed no significant differences in the structural properties at this time point (Fig. 8a, b). All grafts in both groups failed by tunnel pullout.

Similar observations were made at 12 weeks (Fig. 8a, b). Six out of seven allografts and five out of seven autografts failed by intraligamentous graft rupture and the remaining one allograft and two autografts by tunnel pullout.

However, at 52 weeks, autograft reconstructions showed a significantly smaller ap-laxity (allografts 5.2 ± 1.3 mm, autografts 3.9 ± 1.1 mm), significantly higher failure loads and stiffness (Fig. 8). All grafts in either group failed by intraligamentous rupture.

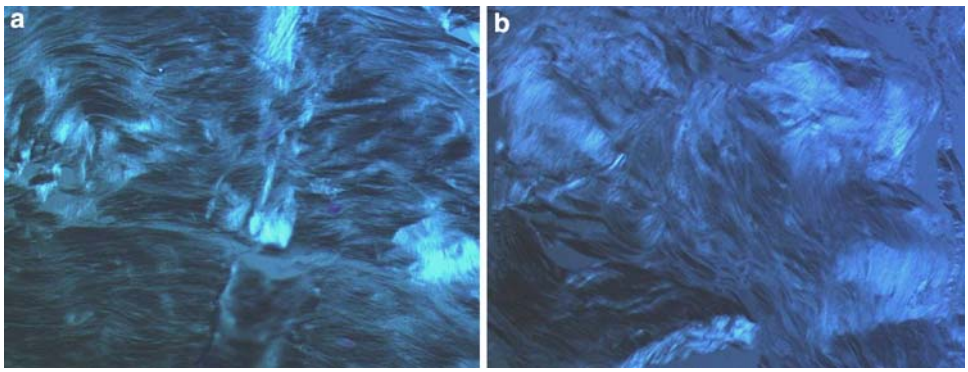

Fig. 5 Collagen-crimp at 6 weeks, autografts (**a**), allografts (**b**): the regular organization of the extracellular matrix (ECM) is completely lost. Allografts showed a more non-homogenous ECM than the allografts (×20)

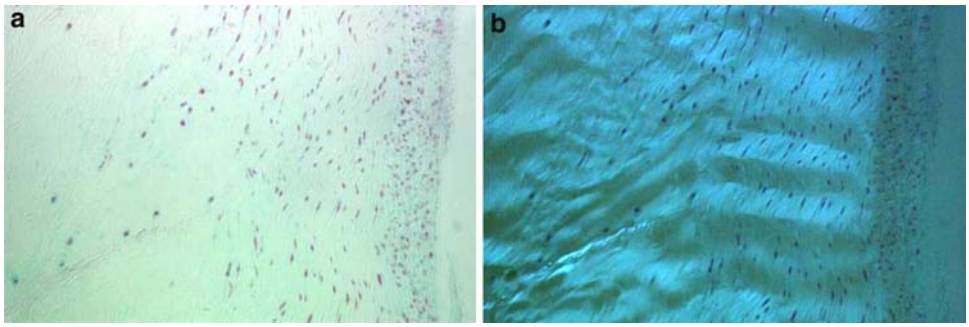

Fig. 6 Autografts after 12 weeks; (**a**) conventional microscopy picture of ASMA-stain, (**b**) identical region under polarized light. Cells align along the collagen matrix, infiltrating from the synovial membrane into the graft tissue. It shows a relationship between the myofibroblasts with crimp formation. Large acellular regions can be found next to hypercellular areas (×10)

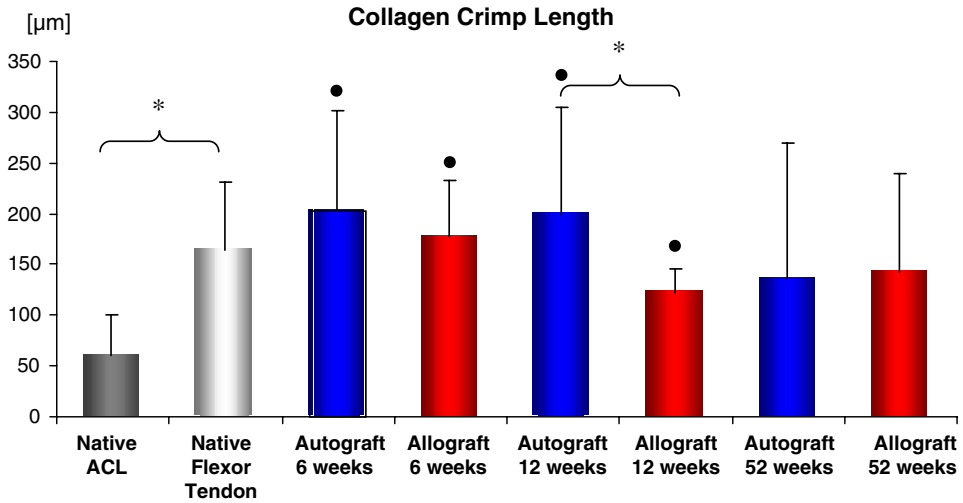

Fig. 7 Crimp frequency showed significant differences between auto- and allografts at 12 weeks only. At 52 weeks no differences were found of the respective study groups compared to the intact ACL. (mean ± SD, "*filled circle*" significantly different from the native ACL, $P \leq 0.05$)

In the autograft group we observed a significantly higher failure load ($P < 0.05$) and increased stiffness at 12 weeks compared to 6 weeks of healing, while there were no significant differences at these time points in the allograft group. Even after 52 weeks, only a slight increase in the allograft structural properties could be observed. In contrast, we found a significant improvement of the structural properties at the same time point in the autograft group (Fig. 8).

Neither ap-laxity (1.84 ± 0.53), nor structural properties of the intact ACL (failure load 1670.5 ± 375.6 N, stiffness 173.0 ± 19.6 N/mm) were fully restored in either group at 6, 12 or 52 weeks. Autografts retained a failure load of 38% and stiffness of 67% of the intact ACL compared to 18 and 38% respectively, at 1 year postoperatively (Fig. 8).

Discussion

Even though allografts continue to see an increasing demand and use in primary and revision ACL reconstruction, concerns have been raised whether the biological

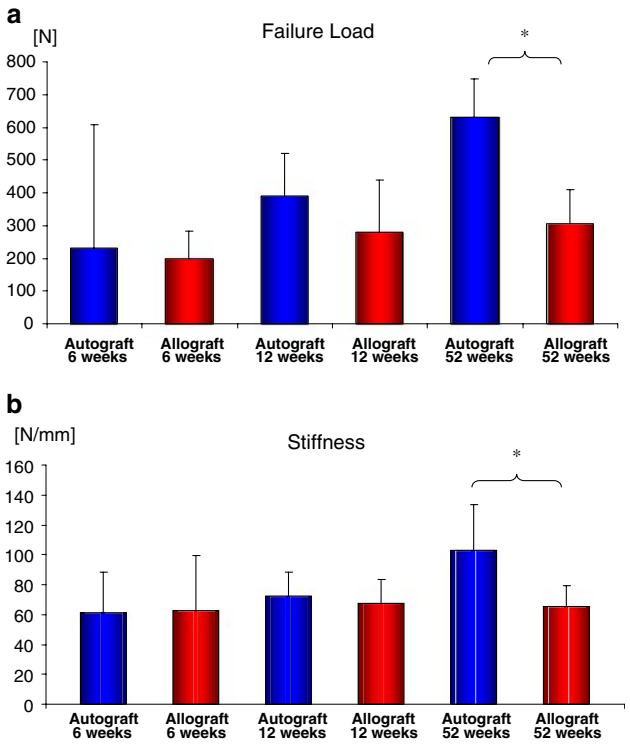

Fig. 8 Autograft structural properties increase more significantly than in allograft ACL reconstructions during the course of 52 weeks of healing. At 52 weeks significant differences can be found between auto- and allografts. (mean ± SD, $P \leq 0.05$)

behavior of allografts during the healing period will match that of the autografts.

Current literature shows inconsistent findings. Even though there is an increasing trend to use free tendon grafts, such as tibialis anterior or hamstring tendons, rather than bone- tendon-bone grafts, almost all authors exclusively analyzed the patellar tendon-bone graft [3, 10, 11, 13, 17–20, 25, 31]. There are no available data in the current literature that analyzed the healing behavior of free soft-tissue tendon allografts and there is a lack of in-vivo animal studies that have a sufficient number of specimens to allow for statistical comparison of biological properties during graft healing.

Hence, it was our aim to conduct a statistically sound study that compares the healing phases between bone-block free soft-tissue autografts and fresh-frozen non-sterilized allografts in a sheep model of ACL reconstruction.

To our knowledge, it is also the first time that an immuno-histochemical analysis of myofibroblast expression was conducted in allografts. These cells have been identified in the intact and ruptured human ACL and were postulated to be at least partially responsible for the crimping of the collagenous fibers [23]. Weiler et al. identified myofibroblasts in the healing ACL graft in sheep [43] and showed that these cells significantly affected the remodeling behavior of their grafts by exerting a contractile force onto the extracellular matrix [12, 21, 22]. Therefore, these cells can be considered as a possible indicator for the ligamentization process.

In the present study, overall remodeling activity of the autografts differed from allografts. While no substantial differences could yet be found quantitatively at 6 weeks, the most pronounced variations were found in histological analysis descriptively and quantitatively at 12 weeks among both graft types. There was a significantly higher expression of myofibroblasts and a faster restoration and improved organization of collagen crimp in the autograft reconstructions. Contrary to previous reports for autografts [43], we did not find an association between increased myofibroblasts density and tissue contraction, but observed a significantly longer collagen crimp in the autografts at 12 weeks. This might be explained by the intense remodeling activity of this early healing phase with the loss of extracellular matrix composition and alignment. It might not be until subsequent time points, when the ligamentization process with the restructuring of extracellular and cellular components progresses that a direct correlation of collagen crimp and myofibroblasts density could be seen.

The varying changes of myofibroblast expression and crimp pattern among both graft types did not affect structural properties of these grafts until one year postoperatively. Only at this time point significant higher failure loads and stiffness values as well as decreased ap-laxity were seen in the autografts. On the other hand, the substantial differences in histological appearance present in the early healing phase diminished.

Even though we did not see full restoration of the graft morphology to the level of the intact native ACL, the typical process of ligamentization with adaptation towards an ACL-like structure was detected. Statistically, no significant differences could be found for collagen crimp in either group compared to the native ACL at 52 weeks. Still, no conclusions can be drawn from this study, whether graft maturation progresses beyond 52 weeks of healing and if allografts will become indistinguishable from autografts at later time points.

It is interesting to note that findings reported in the existing literature disagree with some of our results. For example, other authors did not find slower time-dependent changes in remodeling of ACL allografts [25, 31]. Shino et al. even indicated a possible superiority of allografts due to the lack of harvest site morbidity seen with autograft use [34]. In a dog model, the same authors did not find any significant disadvantages in allografts as a replacement for the ACL [32]. Possible explanations for these discrepancies may lie in the differences in the experimental methodology. Shino et al. used BPTB allografts and analyzed them

at 3, 6, 15, 30 and 52 weeks. Autograft controls only existed for the 30 week group with no controls examined at early time points, when differences were detected in our study. Therefore, no conclusions can be drawn from this study concerning possible differences in time-dependent changes of auto- and allografts. Also, only descriptive histological and no quantitative analyses were performed, from which Shino concluded complete maturity of allografts at 52 weeks.

The majority of in-vivo animal studies confirmed earlier biological healing for autograft tissue. Jackson et al. found more substantial differences and increased inflammatory response with allograft ACL reconstruction compared to the intact ACL than with autografts at 6 weeks in a goat model [17]. Adequate statistical comparisons, however, were limited due to the low number of specimens (two per group) for histological and electron-microscopic analyses of their BPTB grafts. They found histological maturity of the allografts at 6 months of healing, while, in our study, allografts were only partially mature at 52 weeks. Biomechanically, the authors also observed that varying remodeling dynamic at early healing was associated with significantly reduced mechanical properties of the allografts at late time points of 6 months. This agrees with our findings at 52 weeks. This phenomenon might be explained by the fact that in our and Jackson's studies animals were full weight bearing during the early post-operative period (from the second postoperative weeks on), which coincides with the most vulnerable healing phase in allografts, and eventually results in impaired mechanical strength at later time points. These findings suggest that auto- and allograft ACL reconstructions should undergo different rehabilitation protocols customized to their healing phases to ensure long-term stability. This would be especially important to take into consideration since there is an increasing trend to use allografts in primary ACL reconstruction, where aggressive rehabilitation protocols are used and the lack of harvest site morbidity would even allow for shorter recovery time to return to full function compared to that in autografts.

Several clinical studies that compared autograft and allograft ACL reconstructions found increased failure rates [37], a higher incidence of pivoting [27, 30], and an increased anterior knee laxity when allografts were used [28, 40]. All authors used bone-block allografts, either BPTB or Achilles tendon.

A large clinical study of 268 patients by Gorschewsky et al. [10] found significantly higher re-rupture rates and increased anterior and rotational laxity in patients who received allograft ACL. Substantial harvest site morbidity was observed in the autograft group. In addition to the clinical examinations, 4 autografts and 14 allografts were available for histological analyses when respective patients underwent revision surgery after 5–20 months. Allografts showed reduced incorporation and less maturity of intra-articular graft portions. It is important to note that Gorschewsky et al. used allografts that underwent low dose gamma irradiation for sterilization prior to ACL reconstruction, which might have played a contributing role in their findings.

Yet, many published studies did not find any differences in the clinical outcome between autograft and allograft ACL reconstructions [2, 16, 19, 26]. However, these studies either compared revision ACL surgeries [27] or opted for non-aggressive rehabilitation protocols [11, 16, 26]. There is only one recently published study that prospectively examined primary auto- and allograft ACL reconstruction with aggressive rehabilitation and did not find significant differences in long-term stability and clinical outcome [2].

It is important to mention the limitations inherently present in our study as in any other study that uses an animal model. Even though the sheep has been shown to be an acceptable large animal model for analysis of ACL reconstruction, thanks to its anatomical and functional similarities with humans, results cannot be fully transferred to the human patient [14, 24]. Particularly, metabolic processes vary substantially between animals and humans, as it has been shown with faster wound healing in sheep. This might also affect the time-dependent changes of graft remodeling. Also, a standardized post operative rehabilitation with control for time of return to full weight bearing and restoration of motion, proprioception and function cannot be carried out in an animal model as it is usually done with human patients. Therefore, co-founding variables exist that might have had an impact on the differences between auto- and allografts in our study. Future studies will have to be designed to address and control for these factors, so that adaptations of postoperative rehabilitation might compensate for the delayed remodeling, found in allografts.

Conclusion

In this study, free soft-tissue allografts showed delayed remodeling of their extra-cellular matrix compared to autografts in ACL reconstruction. At 1 year postoperatively, autograft extra cellular morphology approached the intact ACL, while allografts displayed a lesser degree of graft maturity. The main differences between both graft types were observed during early healing, which resulted in significantly reduced mechanical properties at 1 year, when early weight bearing was tolerated.

Our results might have important clinical implications. It could be assumed that due to the delayed and prolonged

remodeling activity of allografts in ACL reconstruction early weight bearing might compromise long-term mechanical stability. The rehabilitation protocols for the patients who receive an allograft ACL should not be directly transferred from autograft reconstructions, but rather be specifically tailored to the graft type, healing time and the patient activity level.

References

1. Arnoczky SP, Warren RF, Ashlock MA (1986) Replacement of the anterior cruciate ligament using a patellar tendon allograft. An experimental study. J Bone Joint Surg Am 68(3):376–385
2. Bach BR Jr, Aadalen KJ, Dennis MG, Carreira DS, Bojchuk J, Hayden JK, Bush-Joseph CA (2005) Primary anterior cruciate ligament reconstruction using fresh-frozen, nonirradiated patellar tendon allograft: minimum 2-year follow-up. Am J Sports Med 33(2):284–292
3. Chang SK, Egami DK, Shaieb MD, Kan DM, Richardson AB (2003) Anterior cruciate ligament reconstruction: allograft versus autograft. Arthroscopy 19(5):453–462
4. Cole DW, Ginn TA, Chen GJ, Smith BP, Curl WW, Martin DF, Poehling GG (2005) Cost comparison of anterior cruciate ligament reconstruction: autograft versus allograft. Arthroscopy 21(7):786–790
5. Conway B, Tomford W, Mankin HJ, Hirsch MS, Schooley RT (1991) Radiosensitivity of HIV-1-potential application to sterilization of bone allografts. Aids 5(5):608–609
6. Corry IS, Webb JM, Clingeleffer AJ, Pinczewski LA (1999) Arthroscopic reconstruction of the anterior cruciate ligament. A comparison of patellar tendon autograft and four-strand hamstring tendon autograft. Am J Sports Med 27(4):444–454
7. Curran AR, Adams DJ, Gill JL, Steiner ME, Scheller AD (2004) The biomechanical effects of low-dose irradiation on bone-patellar tendon-bone allografts. Am J Sports Med 32(5):1131–1135
8. Duthon VB, Barea C, Abrassart S, Fasel JH, Fritschy D, Menetrey J (2006) Anatomy of the anterior cruciate ligament. Knee Surg Sports Traumatol Arthrosc 14(3):204–213
9. Ellis D (1968) Cross-sectional area measurements for tendon specimens: a comparison of several methods. J Biomech 2:175–186
10. Gorschewsky O, Browa A, Vogel U, Stauffer E (2002) Klinisch-histologischer Vergleich des allogenen und autologen Patellarsehnendrittels (bone-tendon-bone) zur Rekonstruktion des vorderen Kreuzbands. Unfallchirurg 105:703–714
11. Harner CD, Olson E, Irrgang JJ, Silverstein S, Fu FH, Silbey M (1996) Allograft versus autograft anterior cruciate ligament reconstruction: 3- to 5-year outcome. Clin Orthop 324:134–144
12. Harris AK, Stopak D, Wild P (1981) Fibroblast traction as a mechanism for collagen morphogenesis. Nature 290(5803):249–251
13. Harris NL, Indelicato PA, Bloomberg MS, Meister K, Wheeler DL (2002) Radiographic and histologic analysis of the tibial tunnel after allograft anterior cruciate ligament reconstruction in goats. Am J Sports Med 30(3):368–373
14. Hunt P, Scheffler SU, Unterhauser FN, Weiler A (2005) A model of soft-tissue graft anterior cruciate ligament reconstruction in sheep. Arch Orthop Trauma Surg 125(4):238–248
15. Indelicato PA, Linton RC, Huegel M (1992) The results of fresh-frozen patellar tendon allografts for chronic anterior cruciate ligament deficiency of the knee. Am J Sports Med 20(2):118–121
16. Indelli PF, Dillingham MF, Fanton GS, Schurman DJ (2004) Anterior cruciate ligament reconstruction using cryopreserved allografts. Clin Orthop Relat Res 420:268–275
17. Jackson DW, Grood ES, Goldstein JD, Rosen MA, Kurzweil PR, Cummings JF, Simon TM (1993) A comparison of patellar tendon autograft and allograft used for anterior cruciate ligament reconstruction in the goat model. Am J Sports Med 21(2):176–185
18. Kirkpatrick JS, Seaber AV, Glisson RR, Bassett FH 3rd (1996) Cryopreserved anterior cruciate ligament allografts in a canine model. J South Orthop Assoc 5(1):20–29
19. Kustos T, Balint L, Than P, Bardos T (2004) Comparative study of autograft or allograft in primary anterior cruciate ligament reconstruction. Int Orthop 28(5):290–293
20. Malinin TI, Levitt RL, Bashore C, Temple HT, Mnaymneh W (2002) A study of retrieved allografts used to replace anterior cruciate ligaments. Arthroscopy 18(2):163–170
21. Masur SK, Dewal HS, Dinh TT, Erenburg I, Petridou S (1996) Myofibroblasts differentiate from fibroblasts when plated at low density. Proc Natl Acad Sci USA 93:4219–4223
22. Mochitate K, Pawelek P, Grinnel F (1991) Stress relaxation of contracted coallagen gels: disruption of actin filament bundles, release of cell surface fibronectin, and down-regulation of DNA and protein synthesis. Exp Cell Res 193:198–207
23. Murray MM, Spector M (1999) Fibroblast distribution in the anteromedial bundle of the human anterior cruciate ligament: the presence of alpha-smooth muscle actin- positive cells. J Orthop Res 17(1):18–27
24. Murray MM, Weiler A, Spindler KP (2004) Interspecies variation in the fibroblast distribution of the anterior cruciate ligament. Am J Sports Med 32(6):1484–1491
25. Nikolaou PK, Seaber AV, Glisson RR, Ribbeck BM, Bassett FH 3rd (1986) Anterior cruciate ligament allograft transplantation. Long-term function, histology, revascularization, and operative technique. Am J Sports Med 14(5):348–360
26. Noyes FR, Barber-Westin SD (1996) Reconstruction of the anterior cruciate ligament with human allograft. Comparison of early and later results. J Bone Joint Surg Am 78(4):524–37
27. Peterson RK, Shelton WR, Bomboy AL (2001) Allograft versus autograft patellar tendon anterior cruciate ligament reconstruction: A 5-year follow-up. Arthroscopy 17(1):9–13
28. Poehling GG, Curl WW, Lee CA, Ginn TA, Rushing JT, Naughton MJ, Holden MB, Martin DF, Smith BP (2005) Analysis of outcomes of anterior cruciate ligament repair with 5-year follow-up: allograft versus autograft. Arthroscopy 21(7):774–785
29. Prodromos C, Joyce B, Shi K (2007) A meta-analysis of stability of autografts compared to allografts after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 15(7):851–856
30. Shelton WR, Papendick L, Dukes AD (1997) Autograft versus allograft anterior cruciate ligament reconstruction. Arthroscopy 13(4):446–449
31. Shino K, Horibe S (1991) Experimental ligament reconstruction by allogeneic tendon graft in a canine model. Acta Orthop Belg 57 Suppl 2(6):44–53
32. Shino K, Kawasaki T, Hirose H, Gotoh I, Inoue M, Ono K (1984) Replacement of the anterior cruciate ligament by an allogeneic tendon graft. An experimental study in the dog. J Bone Joint Surg Br 66(5):672–681
33. Shino K, Inoue M, Horibe S, Nagano J, Ono K (1988) Maturation of allograft tendons transplanted into the knee. An arthroscopic and histological study. J Bone Joint Surg Br 70(4):556–560
34. Shino K, Nakata K, Horibe S, Inoue M, Nakagawa S (1993) Quantitative evaluation after arthroscopic anterior cruciate ligament reconstruction. Allograft versus autograft. Am J Sports Med 21(4):609–616

35. Siebold R, Buelow J-U, Boes L, Ellerman A (2002) Allogene Transplantate zur Rekonstruktion des vorderen Kreuzbandes bei Primär—und Revisionseingriffen. Zentralbl Chir 127:850–854
36. Simonian PT, Harrison SD, Cooley VJ, Escabedo EM, Deneka DA, Larson RV (1997) Assessment of morbidity of semitendinosus and gracilis tendon harvest for ACL reconstruction. Am J Knee Surg 10(2):54–59
37. Stringham DR, Pelmas CJ, Burks RT, Newman AP, Marcus RL (1996) Comparison of anterior cruciate ligament reconstructions using patellar tendon autograft or allograft. Arthroscopy 12(4):414–421
38. Unterhauser FN, Bosch U, Zeichen J, Weiler A (2004) Alpha-smooth muscle actin containing contractile fibroblastic cells in human knee arthrofibrosis tissue. Winner of the AGA-DonJoy Award 2003. Arch Orthop Trauma Surg 124(9):585–591
39. Vangsness Jr C (2004) Overview of allograft soft tissue processing, in Bulletin AAOS, pp 33–36
40. Victor J, Bellemans J, Witvrouw E, Govaers K, Fabry G (1997) Graft selection in anterior cruciate ligament reconstruction–prospective analysis of patellar tendon autografts compared with allografts. Int Orthop 21(2):93–97
41. Vorlat P, Verdonk R, Arnauw G (1999) Long-term results of tendon allografts for anterior cruciate ligament replacement in revision surgery and in cases of combined complex injuries. Knee Surg Sports Traumatol Arthrosc 7(5):318–322
42. Weiler A, Scheffler S, Hoher J (2002) Transplant selection for primary replacement of the anterior cruciate ligament. Orthopade 31(8):731–740
43. Weiler A, Unterhauser FN, Bail H-J, Hüning M, Haas NP (2002) alpha-Smooth muscle actin is expressed by fibroblastic cells of the ovine anterior cruciate ligament and its free tendon graft during remodeling. J Orthop Res 20(2):310–317
44. Yasuda K, Tsujino J, Ohkoshi Y, Tanabe Y, Kaneda K (1995) Graft site morbidity with autogenous semitendinosus and gracilis tendons. Am J Sports Med 23(6):706–714