Remodeling of ACL Allografts is Inhibited by Peracetic Acid Sterilization

Sven U. Scheffler MD, Johannes Gonnermann CMD,
Julia Kamp CMD, Dorothea Przybilla,
Axel Pruss MD

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Abstract Sterilization of allografts for anterior cruciate ligament (ACL) reconstruction has become an important prerequisite to prevent disease transmission. However, current sterilization techniques impair the biological or mechanical properties of such treated grafts. Peracetic acid (PAA) has been successfully used to sterilize bone allografts without these disadvantages and does not impair the mechanical properties of soft tissue grafts in vitro. We asked whether PAA sterilization would influence recellularization, restoration of crimp length and pattern, and revascularization of ACL grafts during early healing. We used an in vivo sheep model for open ACL reconstruction. We also correlated the histologic findings with the

restoration of anteroposterior stability and structural properties during load-to-failure testing. PAA slowed remodeling activity at 6 and 12 weeks compared to nonsterilized allografts and autografts. The mechanical properties of PAA grafts were also reduced compared to these control groups at both time points. We conclude PAA sterilization currently should not be used to sterilize soft tissue grafts typically used in ACL reconstruction.

Introduction

Allografts have recently gained popularity in orthopaedic sports medicine, especially in primary and revision reconstruction of the anterior cruciate ligament (ACL) [2]. Allografts eliminate harvest morbidity and reportedly allow faster rehabilitation, earlier return to full activity, and possible cost reduction [4, 10, 25]. Disadvantages of using allografts, however, include potential disease transmission and prolonged graft healing [7, 11, 22, 26, 31, 33]. Even though the risk of infection following allograft transplantation is very small, fatal incidences have been reported [31]. As a consequence, different sterilization techniques have been developed to eliminate infectious pathogens from the graft before transplantation. These include irradiation [32], chemical processing [15], and antibiotic soaks [29]. However, most current sterilization procedures have inherent disadvantages affecting biological properties and mechanical function of the graft. Gamma irradiation reduces the mechanical strength of ACL allografts when used at levels required to eliminate bacterial as well as viral pathogens [6, 18]. Antibiotic soaks reportedly provide only surface sterilization and chemical processing techniques, such as with ethylene oxide, have provoked synovial inflammation and have been abandoned [32].

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S. U. Scheffler (✉), J. Gonnermann, J. Kamp
Sports Medicine & Arthroscopy Service, Department for
Orthopaedic Surgery and Traumatology, Center for
Musculoskeletal Surgery, Charité University Medicine Berlin,
Charité Platz 1, 10117 Berlin, Germany
e-mail: sven.scheffler@charite.de

D. Przybilla
Institute for Laboratory Medicine and Pathobiochemistry,
Charité University Medicine Berlin, Berlin, Germany

A. Pruss
Tissue Bank Institute of Transfusion Medicine, Charité
University Medicine Berlin, Berlin, Germany

Peracetic acid (PAA) has been used since the early 1980s [28] mainly to sterilize bone allografts. Several preliminary in vitro studies suggest the feasibility of this technique with no adverse effects on the structural and mechanical properties as well as the possibility of full biological incorporation of such treated bone grafts [16, 17]. This technique has also been applied to soft tissue grafts. For example, full sterilization of Achilles tendon was achieved with 0.2% PAA solution [28]. Analyses of the mechanical function of bone-patellar tendon-bone grafts in vitro revealed no adverse effects of PAA sterilization compared to nonsterilized grafts [21]. However, cellularity and vascularity are substantially upregulated during the first 3 months of healing [23]. However, cellularity and vascularity are substantially upregulated during the first 3 months of healing in ACL autografts. The extent of remodeling activity is seen in reorganization and/or dissolution of the extracellular matrix [3, 14, 23], including the crimp length and crimp pattern. The remodeling behavior of PAA sterilized allografts in-vivo is unknown.

We first hypothesized there would be no time-dependent differences in cellularity, vascularity, and crimp length and pattern of PAA-sterilized ACL allografts compared to nonsterilized allografts or autografts during early healing in an animal model. We also hypothesized PAA-sterilized and nonsterilized ACL allografts would exhibit similar anteroposterior knee stability and structural properties.

Materials and Methods

We used 16 mature female merino-breed sheep (2–3 years old) to ascertain the effects of PAA sterilization at 6 ($n = 8$) and 12 weeks ($n = 8$) of healing. In our previous study [22], we used the identical ACL reconstruction model. We compared cellularity, vascularity, crimp length/pattern, and mechanical properties between eight nonsterilized fresh-frozen allografts, eight autografts, and eight intact ACLs at 6 and 12 weeks postoperatively. The data from the previous study were used as controls for the PAA-sterilized allografts investigated in this current study. The number of specimens used in this study was determined by performing a power analysis on preliminary data to estimate the required sample size to ensure 80% power ($\alpha = 0.05$; $\beta = 0.2$). All animal procedures were conducted according to the guidelines of the National Institute of Health (Germany) for the use of laboratory animals. All animals were checked for bony maturity by dental status and preoperative radiograph of the knee. Normal health status was confirmed by a veterinarian.

The first two animals received PAA-sterilized long flexor tendon grafts previously harvested from sheep not included in this study. In the other animals, the long flexor

Fig. 1 The superficial long flexor tendon was used as an allograft for ACL reconstruction.

tendon of the superficial flexor digital muscle was harvested as a free soft tissue graft from each left hind limb prior to ipsilateral ACL reconstruction (Fig. 1). It was placed in sterile bags and immediately transported on ice to the local tissue bank where PAA sterilization was conducted no later than 6 hours after harvest. Afterwards, grafts were stored for at least 6 days at -18°C . Sterilization was carried out under highest safety and sterility standards in “class A in B” laboratories as outlined in the European guideline for Good Manufacturing Practice of Human Tissue (Annex 1 ECGMP). All procedures occurred under aseptic conditions with constant laminar airflow. Grafts were consecutively rinsed under high pressure to completely remove blood remnants from the graft tissue using sterile water at 37°C for 30 minutes. Any remaining fat was removed by placing the tissue into a mixture of chloroform (extra pure, 99.4%) and methanol (for analysis, 99.8%) (v/v, 2/1) under constant agitation (laboratory shaker THYS 2, MLW, Leipzig, Germany) for 2 hours while the delipidating solution was changed every 30 minutes. Tissues were sonicated eight times with methanol in an ultrasonic bath (Sonorex RK 510 H, Bandelin Electronic, Berlin, Germany) for 15 minutes to completely remove any residual chloroform. Methanol was removed by flushing the tissues twice with sterile deionized water. The sterilization procedure was carried out under constant agitation (laboratory shaker THYS 2, MLW, Leipzig, Germany) under low pressure (200 mbar) at room temperature in a desiccator for 4 hours (Fig. 1). Lipid-free transplants were covered with PAA solution (v/v, 1/7.5). PAA solution consisted of 2% peracetic acid (Kesla-Chemie, Wolfen), 96% ethanol (Merck, Darmstadt) and aqua ad iniectabilia (Ampuwa; Fresenius, Bad Homburg, Germany) (ratio v/v/v 2/1/1), providing a final sterilization solution of 1% PAA. PAA was removed by washing the

grafts six times for 20 minutes with Soerensen buffer. This was followed by rinsing the grafts with aqua ad injectabilia twice. At the end of the procedure, the absence of peracetic acid was confirmed by a Reflectoquant[®] PAA test (Merck Eurolab GmbH, Darmstadt) with a sensitivity of 5 ppm. Grafts were dried in sterile air and stored in sterile bags at -18°C until time of transplantation. No antibiotic solution was added.

On the day of surgery, the PAA-sterilized graft was transported to the surgical animal facilities, thawed at room temperature, and then augmented with two #2 Ethibond Excel polyester sutures (Ethicon, Inc., Piscataway, NJ, USA) at each end. The graft length was between 60 and 70 mm.

An open ACL reconstruction was performed on each left hind limb as previously described (Fig. 2) [22, 34]. All surgery was performed by one individual (SS). The joint was opened by a medial arthrotomy, the ACL excised, and the knee was brought into deep flexion for placement of a guide pin in the femoral anatomical footprint of the ACL. The guide pin was overdrilled to a depth of 20 mm matching the diameter of the prepared graft. Graft fixation was achieved at the femoral cortex with a fixation button (Flipptack[®], Fa. Karl Storz GmbH, Tuttlingen, Germany). The tibial tunnel was placed in an identical fashion into the tibial footprint of the ACL and drilled through the tibial cortex. The graft was fixed by placing multiple knots of the augmented graft sutures onto a bone bridge that was created 1 cm distally to the exit of the tibial tunnel. Before final fixation, the knee was moved through several cycles of full flexion and extension to eliminate slack of the graft sutures.

All animals were immediately allowed full weight bearing with no limitation of range of motion. Anti-inflammatory drugs (Finadyne[®] [1%, Essex Pharma GmbH, Munich, Germany] 1 mg/kg s.c.) were given during the first 3 postoperative days. Wound status and gait pattern were recorded daily. The animals' gaits were subjectively

assessed by the same person and graded from 1 (no weight bearing) to 5 (full weight bearing with no signs of limping). All animals were released to an outside farm without any restriction of motion 2 weeks postoperatively.

The animals were sacrificed at 6 or 12 weeks and the left knee was removed, leaving the skin and all soft tissue structures intact. The knee was inspected intraarticularly for inflammation, effusion, the synovial coverage of the ACL graft, the status of the cartilage, and any degenerative changes.

To address our first hypothesis of whether biological remodeling was delayed in PAA grafts, we histologically examined cellularity, vascularity, and crimp length and pattern. Two researchers (JG, JK) performed all histological analyses independently, blinded to time of sacrifice and specimen numbers. Tissue samples were immediately fixed in 4% formalin and embedded in paraffin. Serial cuts 4- μm thick were prepared and mounted on slides with 3% silane (Sigma Chemical, St. Louis, MO, USA). A high-resolution microscope (Leica DMRB, Leica GmbH, Bensheim, Germany) linked to a digital image analysis system (KS 400 Imaging System, Release 3.0, Carl Zeiss Vision, Eching, Germany) was used for histological analysis. Hematoxylin and eosin and Masson-Goldner trichrome stains were used for assessment of cellularity, cell morphology, crimp length and pattern, and appearance of foreign-body, giant, and inflammatory cells in the PAA grafts. Cellularity (as mean cell density per mm^2) was quantified in 10 regions of interest (ROI) (0.06 mm^2 each) randomly chosen in each of two longitudinal sections per specimen in regular intervals along the complete section length.

During tendon remodeling dissolution and reorganization of collagen bundles occur, which can be quantified by the changes of their typical wave-like structure observed in soft tissue grafts. The wave-like structure of collagen bundles can be visualized due to its anisotropy with polarized light microscopy. Collagen crimp length was defined as the wave frequency (μm) of collagen bundles measured in the same ROIs (0.06 mm^2) using a calibrated scale with the digital image analysis system. Descriptive documentation of fiber alignment and orientation was also reported to provide further insights into the remodeling behavior.

One of us (JK) performed immunohistochemical analysis to quantify vascular density as previously described by Unterhauser et al. [30]. Two transverse sections of intact midsubstance graft tissue per specimen were used for quantitative analysis of vascularity. Transverse sections were subdivided into a subsynovial (Sub), an intermediate (Mid), and a central (Cnt) region. In each subregion, five representative regions of interest (0.06 mm^2) were identified and the number of vessel cross-sections was counted

Fig. 2 A schematic of open ACL reconstruction procedure is shown.

with a clear positive signal after immunostaining. The endothelial surface cells of blood vessels were immunostained with rabbit antihuman von Willebrand factor (DAKO, Glostrup, Denmark) in transverse sections. The tissue samples were hydrated and pretreated with 0.1% protease (type XIV, bacterial, Sigma Aldrich Chemie GmbH, Steinheim, Germany) for 10 minutes at 37°C. Ten-percent normal horse serum (Vector Laboratories Inc., Burlington, CA, USA) was used for 20 minutes to block nonspecific binding sites at room temperature. The antibody was diluted 1:200 and added to the tissue samples overnight in a humidity chamber at 4°C. The samples were then rinsed in tris-buffered saline and incubated with biotinylated horse antimouse immunoglobulin G secondary antibody (Vector Laboratories) for 30 minutes. This was followed by incubation with an avidin-biotin complex (ABC kit; Vector Laboratories) linked with alkaline phosphatase as a reporter enzyme. Staining was achieved with Neufuchsin as a chromogen. Tissue samples were counterstained with methylene-green for a few seconds, dehydrated, and mounted in a xylol-soluble mount (Vitro-Clud, R Langenbrinck, Emmendingen, Germany).

To test our second hypothesis and to evaluate the mechanical function of PAA grafts, two loading conditions were simulated on a material testing machine (model 1455, Zwick GmbH, Ulm, Germany): an anteroposterior drawer test and a load-to-failure test of the femur-ACL graft-tibia complex. AP drawer testing of the ACL reconstructed knee was performed (1) with all soft-tissue structures left intact, (2) with only the ACL allograft and PCL intact, and (3) as

an anterior drawer test with only the ACL allograft. All tests were performed at 60° of flexion with all motions restrained except the AP translation.

After preloading the knee with 5 N, an AP load of ± 50 N was applied perpendicular to the longitudinal axis of the tibia 10 times at a speed of 120 mm/min. AP laxity was recorded from the tenth cycle for each specimen (Table 1). The knees were then removed from the mechanical testing machine and the diameter of the ACL allografts were calculated according to a technique described by Ellis [8].

The joints were remounted at 30° of flexion and the longitudinal axis of the ACL allograft aligned parallel to the loading direction of the testing apparatus. After applying a preload of 5 N to the femur-ACL graft-tibia complex, a load-to-failure test was carried out at a speed of 120 mm/min (Table 2). The failure mode and a load-displacement curve were recorded and the structural properties, such as failure load and stiffness (in the linear region between 30% and 90% of the maximum load), as well as stress at failure as a mechanical property of the graft tissue were analyzed using in-house software.

A Shapiro-Wilk W test showed the experimental data were not distributed normally. Therefore, a nonparametric Mann-Whitney U test was used to compare cellularity, vascularity, and crimp length between PAA allografts and the previously published data for nonsterilized allografts, autografts, and the intact ACL. Anterior-posterior laxity and structural properties were also analyzed for differences among the aforementioned groups using the Mann-Whitney U test.

Table 1. Cellularity and crimp length at 6 and 12 weeks of healing

Variable		PAA allografts	ns-allografts [6, 21]	Autografts [6, 21]	Intact ACL [21]
Cells (/mm ²)	6 weeks	109 $\pm$ 111*	134 $\pm$ 72 [†] *	226 $\pm$ 61 [‡]	569 $\pm$ 92 [§]
	12 weeks	240 $\pm$ 238 [†]	656 $\pm$ 464	547 $\pm$ 264	—
Crimp length (μ m)	6 weeks	278 $\pm$ 123 [†]	177 $\pm$ 57 [‡]	203 $\pm$ 98 [‡]	60 $\pm$ 41
	12 weeks	309 $\pm$ 75 [†]	122 $\pm$ 24 [‡]	199 $\pm$ 106 [‡]	—

* Different from autografts ($p < 0.05$); [†]different from ns allografts ($p < 0.05$); [‡]different from the intact ACL ($p < 0.05$); (Stain, Masson Goldner's trichrome; original magnification $\times 200$); [§]different from ns-allografts and autografts ($p < 0.05$).

Table 2. Anteroposterior laxity following drawer testing at ± 50 N

Variable	PAA allografts		Fresh-frozen allografts [§]		Autografts [§]		Intact ACL [§]
Time of healing (wks)	6	12	6	12	6	12	—
Complete knee joint (mm)	5.1 $\pm$ 3.1	4.8 $\pm$ 1.5	5.7 $\pm$ 1.6	5.4 $\pm$ 1.2	6.0 $\pm$ 2.2	6.1 $\pm$ 0.6	2.7 $\pm$ 1.2 [‡]
ACL graft/PCL (mm)	9.2 $\pm$ 5.7	11.6 $\pm$ 5 [†]	7.4 $\pm$ 3.7	8.2 $\pm$ 2.6	8.7 $\pm$ 4.9	7.7 $\pm$ 1.4	3.9 $\pm$ 3.2 [‡]
ACL graft (mm)	6.6 $\pm$ 2.6 [†]	8.3 $\pm$ 4.7 [†]	4.1 $\pm$ 3.5	1.3 $\pm$ 0.3	1.4 $\pm$ 0.4	1.1 $\pm$ 0.2	1.6 $\pm$ 1.7 [‡]

* $p < 0.05$, significant larger than fresh-frozen allografts; [†] $p < 0.05$, significantly larger than autografts; [‡] $p < 0.05$, significantly smaller than all study groups; [§]All data presented for these groups have been previously published [21], using identical material and methods as for the PAA allografts.

Results

All animals in all experimental groups could fully weight bear and had normal gait pattern at 2 weeks postoperatively. At time of sacrifice, all animals showed free range of motion with no detectable effusion and no inflammatory reaction was present in either the PAA or control groups. We observed fibrotic ganglion cyst-like tissue at the tibial tunnel entrance in 10 of 16 PAA specimens, which was not observed in any of the control groups. These animals had no loss of motion compared to animals without such changes.

At 6 weeks postoperatively, cellularity of PAA allografts was similar to that in nonsterilized allografts ($p = 0.234$), but was lower than in the autograft group ($p = 0.01$) and the intact ACL ($p < 0.001$). PAA-treated allografts showed little influx of cells into the periphery, with vast areas of the grafts being acellular (Fig. 3). At 12 weeks, slightly increased ($p = 0.139$) cellularity was observed in the PAA group, which was lower than in nonsterilized allografts ($p = 0.04$), autografts ($p = 0.023$), and in the intact ACL ($p = 0.036$) (Table 1) (Fig. 3).

We observed lower vascularity of PAA allografts than in nonsterilized allografts (sub $p = 0.031$, mid $p = 0.008$, cnt $p = 0.01$), autografts (sub $p = 0.008$, med $p = 0.008$, cnt $p = 0.001$) and the intact ACL (Sub, $61.9 \pm 31.7/\text{mm}^2$; Mid, $53.6 \pm 23.4/\text{mm}^2$; Cnt, $27 \pm 13/\text{mm}^2$) (sub $p = 0.002$, mid $p = 0.001$, cnt $p = 0.001$) in all graft regions at 6 weeks (Fig. 4A). Sparse vascularity was confined to the periphery of the PAA-sterilized graft tissue. At 12 weeks, vascularity remained lower compared to nonsterilized allografts (mid $p = 0.008$, cnt $p = 0.024$) and autografts (sub $p = 0.000$, mid $p = 0.000$, cnt $p = 0.015$) in all graft regions with the exception of the subsynovial region in the PAA group that did not differ from nonsterilized allografts (Fig. 4B)

($p = 0.113$). We observed no differences between the PAA group and the intact ACLs Sub ($p = 0.470$) and Mid ($p = 0.210$) region, but lower values were seen in the Cnt ($p = 0.031$) region. Qualitatively, nonsterilized allo- and autografts showed overall hypervascularity compared to PAA allografts, which in turn looked similar to the intact ACL.

Crimp length of PAA allografts at 6 weeks was similar to that in nonsterilized allografts ($p = 0.093$) and autografts ($p = 0.074$). We observed longer PAA crimp length than in the intact ACL ($p = 0.001$) at this time point (Table 1) (Fig. 5). Qualitatively, both nonsterilized allo- and autografts had lost their typical crimp pattern in large areas of the graft tissue with reorganization of crimp pattern at the periphery and small areas of intact crimp pattern remaining in the graft center. PAA-sterilized grafts, in contrast, displayed only small areas of dissolving crimp, while in large parts of the grafts crimp pattern remained unchanged. At 12 weeks, crimp length was longer than in nonsterilized allografts ($p = 0.001$) and autografts ($p = 0.055$) as well as the intact ACL ($p = 0.001$) (Table 1, Fig. 5). Qualitatively, both nonsterilized allografts and autografts showed continuing high crimp turnover at 12 weeks, while crimp pattern of PAA grafts remained more homogeneous, although more irregular than at 6 weeks.

At 6 weeks, we observed similar anterior-posterior stability in PAA and nonsterilized allograft ACL reconstructions for each of the three testing conditions (all soft tissue structures intact, ACL graft/PCL intact, ACL graft only) (Table 2). When comparing PAA allografts with autograft ACL reconstructions, a larger ($p = 0.010$) anterior drawer was found for the PAA group with only the ACL graft left intact, while no differences were found for the other two test settings (Table 2) ($p > 0.05$). At

Fig. 3A–F Histologic samples demonstrate the reduced cellularity of (A) PAA grafts at 6 weeks and (B) 12 weeks, compared to (C) nonsterilized allografts at 6 weeks (stain, hematoxylin and eosin; original magnification $\times 100$) and (D) 12 weeks (stain, Masson Goldner's trichrome; original magnification $\times 200$) of healing, and (E) nonsterilized autografts at 6 weeks (stain, hematoxylin and eosin; original magnification $\times 100$) and (F) 12 weeks (stain, Masson Goldner's trichrome; original magnification $\times 200$) of healing.

Fig. 4A–B PAA grafts showed reduced revascularization compared to nonsterilized allo- and autografts in all regions of the grafts at (A) 6 weeks and (B) 12 weeks of healing.

12 weeks we observed a larger ($p = 0.003$) anterior drawer (only ACL graft intact) in the PAA-sterilized compared to the nonsterilized allograft reconstructions. In comparison to the autograft reconstructions, PAA grafts had a larger ($p = 0.023$) anterior-posterior translation with the ACL graft and PCL left intact and a larger ($p = 0.003$) anterior drawer with only the ACL graft left intact (Table 2).

Fig. 5A–F Photomicrographs of the crimp pattern are shown. The increased crimp length of PAA allografts and their lack of remodeling activity are displayed at (A) 6 weeks [$\times 100$] and (B) 12 weeks [$\times 50$] of healing. (C) In comparison, the nonsterilized allografts are shown in conventional photomicrographs under polarized light at 6 weeks [$\times 100$] and (D) 12 weeks [$\times 20$], and the (E) nonsterilized autografts are shown at 6 weeks [$\times 100$] and (F) 12 weeks [$\times 20$].

At 6 weeks, stiffness (ST) and failure loads (FL) of the PAA allografts were lower than in nonsterilized allografts (ST, $p = 0.147$; FL, $p = 0.529$) and autografts (ST, $p = 0.083$; FL, $p = 0.083$) (Table 3). Stress values of PAA allografts (Table 3) did not vary substantially from nonsterilized allografts ($p = 0.607$) and autografts ($p = 0.606$). One PAA-sterilized graft already failed partially during anterior drawer testing, therefore, no load-to-failure test was performed. Two grafts failed by intraligamentous rupture, while the remaining five failed by graft pullout from the tibial (two) and femoral (three) tunnels. In all of the pullout failures, grafts tore at the graft suture interface. All nonsterilized allo- and autografts failed by graft pullout with no specimens failing during anterior-posterior drawer testing. At 12 weeks stiffness ($p = 0.01$; $p = 0.003$), failure load ($p = 0.030$; $p = 0.030$) and stress ($p = 0.018$; $p = 0.030$) were lower for PAA grafts compared to nonsterilized allografts and autografts (Table 3). Four PAA grafts failed during anterior drawer testing and were not available for load-to-failure analysis. The remaining four PAA grafts failed by intraligamentous rupture, two in the midsubstance, and two close to its femoral insertion site. In the fresh-frozen allografts and autografts no failures prior to load-to-failure testing occurred. In six of seven allografts, failure mode was intraligamentous graft rupture and one tunnel pullout. In the autograft group five of seven failed by intraligamentous rupture and two by tunnel pullout.

Discussion

Allograft use in ACL surgery has substantially increased in recent years, not only in revision, but also in primary ACL reconstruction [29]. However, reports of disease

Table 3. Structural properties after load-to-failure testing

Variable	PAA allografts		Fresh-frozen Allografts [21]		Autografts [21]		Intact ACL [21]
Time (weeks)	6	12	6	12	6	12	—
Stiffness (N/mm)	34.1 ± 14*	43.1 ± 16.5*	62.5 ± 36.9	67.8 ± 15.6	61.2 ± 27.5	72.6 ± 15.9	173 ± 19.6‡
Failure load (N)	161.1 ± 77.3†	107.9 ± 40.8*	199.4 ± 129.7	280.5 ± 116.3	232.4 ± 82.5	391.5 ± 160.1	1670.5 ± 375.6‡
Stress (MPa)	9.8 ± 7.5	5.0 ± 1.5*	7.6 ± 5.9	11.45 ± 6.02	7.2 ± 3.5	10 ± 3.4	87.9 ± 26.0‡

* $p < 0.05$, significant lower than fresh-frozen allografts and autografts at respective time points; † $p < 0.05$, significantly lower than autografts;

‡ $p < 0.05$, significantly higher than all study groups.

transmission following ACL reconstruction with nonsterilized allografts, even though very limited in number, underline the importance of graft sterilization [31]. Furthermore, current sterilization techniques have been associated with certain disadvantages, such as interference with biological healing or reduction of mechanical properties of such treated grafts [5, 6, 13, 18, 19, 24, 27]. Peracetic acid has been successfully used to sterilized bone allografts [16, 17], and PAA sterilization does not alter the material, structural, and viscoelastic properties of human bone-patellar tendon-bone graft in vitro [21]. We therefore hypothesized PAA allografts would exhibit similar biological changes in terms of cellularity, vascularity, and crimp structure, and provide equivalent mechanical function compared to nonsterilized allografts and autologous ACL reconstructions during early healing.

It is important to mention the limitations of this study before considering use in humans. First, as in any animal study, it is almost impossible to fully control the postoperative weight bearing or apply specific rehabilitation protocols as it is the case in human patients, which could have affected our results. Second, we only evaluated early healing of the PAA-treated ACL grafts. Maybe a certain recovery of biological and mechanical function would have been possible, when longer healing times were considered. However, an increasing number of primary ACL reconstructions use allografts in combination with accelerated rehabilitation protocol, since this procedure avoids graft harvest morbidity. Therefore, the increased early weight bearing during the first 3 months of accelerated rehabilitation could be detrimental for the clinical outcome of patients with PAA-sterilized ACL allografts.

Based on our data the first hypothesis must be rejected: PAA sterilization delayed or even partial inhibited the biological remodeling of PAA grafts. This led to impaired functional knee stability and reduced structural properties of PAA grafts during subsequent healing up to 3 months. Our finding of inhibited graft remodeling due to PAA sterilization confirms findings of several other authors' [1, 12, 35] and our own report [22] of nonsterilized allo- and autograft ACL reconstructions in vivo. Both graft types undergo a maturation process, which is characterized by

overall hypocellularity and hypervascularity as early as 6 weeks postoperatively, and a significant recellularization and revascularization that occurs from the periphery towards the center of the grafts [22]. By 12 weeks, the cellularity of the intact ACL was restored in autografts [1, 12, 35] and allografts [1, 12, 35] and that hypervascularity remained. This graft maturation has been defined as the ligamentization process [1, 12, 35]. In contrast, PAA graft cellularity only slightly increased during early healing and did not recover to values of the intact ACL, while graft vascularity never exceeded the values for the intact ACL and substantially lagged behind nonsterilized ACL allo- and autografts [1, 12, 35]. The longer crimp of ACL auto- and allografts than in the intact ACL, appears characteristic for the time of simultaneous degradation and reorganization of the extracellular matrix [7]. Restoration of the intact ACL crimp length occurs with continuing graft maturity by 1 year postoperatively [7]. However, our quantitative analysis of crimp length of PAA grafts remains inconclusive. It is unclear whether the substantial crimp elongation of the PAA grafts was a result of inhibited healing or just a delay of remodeling, since no later time points were evaluated. Nonetheless, our qualitative analysis of the crimp pattern suggests the extent of crimp degradation and reorganization was substantially less in PAA grafts than what had been previously shown for nonsterilized allo- and autografts [7], providing further evidence of impaired remodeling activity.

Mechanical testing of PAA grafts also revealed a negative effect of PAA sterilization on mechanical function. Several authors have reported recovery of anterior knee laxity [22, 34] and structural properties [9, 20, 22, 34] from 6 to 12 weeks in various animal models for autologous and allogeneic ACL reconstruction, but anterior knee laxity in PAA reconstructed knees became even greater from 6 to 12 weeks, while structural properties deteriorated during this time period.

We are uncertain as to what caused the compromised remodeling activity in PAA-sterilized allografts. All grafts were extensively washed with water directly after PAA treatment for removal of the agent and were analyzed for any remains of peracetic acid at the end of the sterilization

procedure. No peracetic acid was noted in detectable amounts in any of the allografts before transplantation. PAA is a very unstable substance that dissolves continuously into acetic acid while releasing oxygen and heat. Therefore, it is unlikely PAA itself was causing the changes in biological remodeling activity at the respective time points. However, it is possible ultrastructural graft changes occurred after PAA sterilization and, consequently, impaired the healing process.

We suggest caution be exercised when considering using PAA-sterilized allografts for ACL reconstruction. Careful clinical followup examinations of patients who already have had ACL reconstruction with PAA-sterilized grafts is warranted to substantiate our findings. Only then final recommendations can be given, whether PAA sterilization is a safe and appropriate technique for soft tissue grafts in ACL reconstruction.

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