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Prospective Clinical Feasibility Study of a Poly-L-Lactic Acid Scaffold in Primary Anterior Cruciate Ligament Reconstruction with Three-Year Follow Up

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Abstract

Background: This study assessed the safety and feasibility of the L-C Ligament, a bioresorbable scaffold, for ACL reconstruction. This acellular scaffold is constructed of poly (Llactic) acid (PLLA) microfilaments that are three-dimensionally braided together to direct ligament regeneration.

Purpose: To evaluate patient outcomes up to 3-years follow-up following ACL reconstruction with the PLLA scaffold.

Methods: Fifteen patients with ruptures of the ACL (<18 weeks after injury) were enrolled in this prospective, first-in-human study. The PLLA scaffold was used to anatomically reconstruct the ACL using outside-in technique with titanium interference screws. The primary endpoint of the study was the incidence of graft ruptures. Secondary endpoints included device-related adverse event rates, subjective patient-reported outcomes (2000

IKDC scale, KOOS pain, Tegner, and Lysholm scores), clinical function (Lachman, KT-1000, pivot shift, anterior drawer, and single leg hop test), and imaging analyses (MRI and CT). In the case of graft rupture, biopsies were taken from the intra-articular region and processed for histological and molecular weight analysis.

Results: This firstinhuman study of primary ACL reconstruction, the PLLA scaffold demonstrated an improvement in patient-reported and clinical function scores at 12 months (IKDC: 90.1 ± 13.5; KOOS: 94.4 ± 10.7) over baseline (IKDC: 60.2 ± 12.7; KOOS: 75.6 ± 15.4). IKDC Physical Knee exam also showed the patients to have normal or nearly normal (Grade A or B) function at 12 months, with >80% of patients having normal laxity according to KT-1000 measurements. No infections, synovitis, or allergic reactions were reported for the duration of the study. However, review of MRI at 6 and 12

Months showed a hyper-intense signal indicative of chronic inflammation and in turn incomplete ligament maturation. After returning to activity and sport, five patients experienced graft ruptures between 12 and 36 months. Histological analysis of graft biopsies obtained during revision surgery revealed connective tissue ingrowth into the scaffold, presence of new vasculature, and a peripheral synovial layer. A chronic inflammatory response was observed adjacent to remnant polymer, with molecular weight analysis showed 87% loss at 35 months. The 10 remaining patients continue to report normal ACL function.

Conclusion: The first-in-man study of a PLLA scaffold for primary ACL reconstruction demonstrated the feasibility of a tissue-engineering approach; however, tissue remodeling was incomplete in this 15-patient cohort over 36 months. Five patients experienced ruptures between 12 and 36 months, suggesting insufficient load-bearing capacity of the new ligament tissue in the presence of a resorbing scaffold. Further innovation is required to optimize scaffold properties to achieve long-term clinical efficacy with a bioresorbable implant for ACL reconstruction.

INTRODUCTION

Anterior cruciate ligament (ACL) rupture is one of the most serious sports-related injuries, with over 200,000 ACL reconstructions performed each year in the US [1-8]. The ideal graft selection for ACL reconstruction continues to be vigorously debated. While the gold standard treatment option is auto graft, co-morbidities associated with the procedure include donor-site morbidity, pain, and permanent loss of function [9,10]. Allograft, the only alternative in the United States, is inconsistent in quality and prone to higher rates of rupture: auto graft 3.5% vs. Allograft: 8.9% at 2-year follow-up [11] and 8.3% vs. 26.5%[6] at 10-years follow-up. Moreover, a multi-center study demonstrated that less than 50% of patients return to pre-injury activity levels 2-years after ACL reconstruction with both treatment options [12].

To address the inconsistencies and shortcomings of tissue grafts, permanent prosthetic ligaments were developed in the

1970s and 80s, including the Kennedy Ligament Augmentation Device, Gore-Tex, Dacron, and Leeds-Keio implants. Although early clinical results demonstrated an improvement in knee stability, long-term data showed high complication and rupture rates [13-17]. Polymer wear particles within the joint space caused inflammatory synovitis. For implants designed to act as a scaffold (Gore-Tex, Leeds-Keio, Dacron) stress-shielding inhibited remodeling of new tissue into a functional ligament, contributing to the eventual failure of the implant [13,15-17]. Consequently, the use of permanent synthetic ligaments for ACL reconstruction in the US was abandoned in the 1990s, and there remains a clinical demand remains for an effective 'off-the-shelf' graft.

Tissue engineering approaches have the potential to develop an ideal solution for ACL reconstruction by creating bioresorbable scaffolds to mechanically stabilize the joint and facilitate the regeneration of new ligament tissue. The LC Ligament is a bioresorbable, poly(L-lactic) acid (PLLA) scaffold comprising a three-dimensional braid of highly aligned polymer microfilaments that mimic the multi-scale and hierarchical structure of the native ligament [18]. Two dense end regions facilitate mechanical fixation in the bone tunnels, while the central scaffold region is an open fibrous network designed to direct endogenous cell infiltration and matrix synthesis within the scaffold. In vitro degradation studies predicted gradual strength loss over a period of nearly 3 years, as the polymer resorbed, with the intent of providing long-term mechanical support during tissue regeneration (Figure 1). Preclinical work in an ovine model [19] showed a fully encapsulated scaffold, with new tissue infiltration contributing to an increase in graft strength between 6 and 12 months. New tissue surrounded PLLA fibers, extending through the center of the scaffold, with regions of cell and matrix alignment appearing similar to native ligament tissue.

Based on these findings, a first-inhuman study of 15 patients was initiated to evaluate the safety and feasibility of using the L-C Ligament for ACL reconstruction. The primary endpoint of the study was the incidence of graft ruptures at one year follow-up. Secondary endpoints included device related adverse event rates, subjective patient-reported outcomes (2000 IKDC scale, KOOS pain, Tegner, and Lysholm scores), clinical function (Lachman, KT-1000, pivot shift, anterior drawer, and single leg hop test), and imaging analyses (MRI and CT). This study reports on patient follow-up out to 3 years after ACL reconstruction.

MATERIAL AND METHODS

Competent authority and ethics committee approvals were granted in the Netherlands prior to initiating the study. The European clinical trial was registered on clinicaltrials.gov per the identifier NCT01634711. Written informed consent was obtained from all patients prior to participation in the trial.

Study Design

This study was designed as a prospective and consecutive clinical evaluation of the L-C Ligament in primary ACL reconstruction, and represents the first-in-human use of the device. Between June 2013 and April 2014, 15 patients were enrolled across two investigational sites in the Netherlands; Isala Klinieken, Zwolle, Netherlands (K.v.E.) and Martini Ziekenhuis, Groningen, Netherlands (R.W.B.). Study follow-up visits were scheduled post-operatively between 1 to 8 weeks, and at 3, 6, 12, 18, and 24 months. At 36 months, patients could elect for a telephone follow-up visit or undergo an office visit.

Inclusion and Exclusion Criteria

Patients between 18 and 45 years old with ruptures of the ACL, and could be scheduled for ACL reconstruction within 18 weeks (127 days) of injury, were eligible for the study. If a concomitant medial collateral ligament injury was present, the patient was eligible if the injury was ≤ Grade 2. Patients also had to have a baseline IKDC Subjective Evaluation score of ≤ 70, agree to not participate in sports for a minimum of 9 months post-procedure, and be willing to comply with post-operative rehabilitation and follow-up visits. Patients were excluded if: they had a prior ACL reconstruction or other surgical procedure on the affected knee, had previous or current ACL injury in the contralateral leg, required multi-ligament reconstruction, had prior fractures of the affected leg, malalignment with Varus thrust, signs of moderate to severe degenerative joint disease, presence of cartilage defects that required surgical intervention other than micro fracture, complete or partial posterior cruciate ligament tear, concomitant injuries to the knee or lower extremities requiring treatment

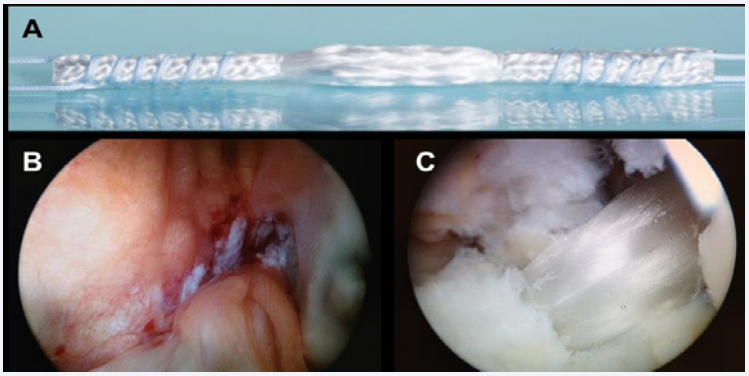

Figure 1 Images of the L-C Ligament (PLLA scaffold) prior to and during ACL reconstruction. (A) The scaffold contains three distinct regions, a porous central scaffold region for ligament regeneration and two dense end regions for robust interference screw fixation in the bone tunnels (shown here whip-stitched). Arthroscopic images taken (B) pre-operatively showing a torn ACL and (C) at the time of scaffold implantation. *Post-Operative Rehabilitation.*

not allowable under the inclusion criteria, medical condition that would interfere with study participation, active or latent infection in or about the affected knee, a confirmed connective tissue disorder, or a neuromuscular disorder that could engender unacceptable risk of knee instability, prosthesis fixation failure, or complications in postoperative care. Additionally, patients were excluded if they were a professional athlete engaged in active sport, were ≥ 6' 4" tall, were mentally compromised, pregnant, obese with a BMI ≥ 35, known allergy to PLLA, or any severe pain, swelling, or redness within 24 hours prior to surgery.

LC Ligament: PLLA Scaffold

The L-C Ligament was manufactured by Soft Tissue Regeneration, Inc. (New Haven, CT), and all biocompatibility and sterility testing was completed and verified prior to use in the study. The scaffold was composed of three-dimensionally braided PLLA microfilaments and measured approximately 12 cm in length and 10 mm in width (Figure 1A). A preclinical study in an ovine model demonstrated the regenerative capacity of the scaffold, with new tissue contributing to increased graft strength from 6 to 12 months, and sheep having intact and functional ACL 4 years beyond polymer degradation [19].

Surgical Technique

The L-C Ligament was implanted using an accepted surgical technique for a hamstring graft in an anatomic orientation, with outside-in interference metal screw fixation on the tibia and femur (Figure 1B-C). The remnant ACL stumps were minimally debrided, while allowing sufficient visualization for placement of the bone tunnels. Tibial and femoral bone tunnels (8mm in diameter) were independently drilled through the center of the tibial and femoral stumps, with the location as close to anatomical as possible. A rasp or shaver was used to chamfer the entrances of both tunnels to minimize potential impingement or abrasive damage to the device. To facilitate passing and tensioning of the L-C Ligament, both ends of the scaffold were whip-stitched with non-restorable #5 Ethibond suture using a tapered needle. The L-C Ligament was placed with equal lengths of the central region in the tibial and femoral tunnels (3 to 7 mm). Titanium interference screws (were used for fixation on the femur (8 mm x 25 mm, Smith & Nephew) and tibia (9 mm x 25 mm, Smith & Nephew). The final position of the LC Ligament within the femoral tunnel was with the tip of the device flush with or extending outside of the femoral cortex to ensure complete engagement with the interference screw. After placing the femoral screw outside-in over a guidewire, the device was manually tensioned at 10 to 20 degrees of knee flexion. The tibial screw was placed adjacent to the LC Ligament within the tibial tunnel, with the scaffold in the anatomic position. Remnant device outside the femoral or tibial cortex was trimmed to be flush with the surface of the bone and screw.

The patient was fitted with a hinged knee brace in the operating or recovery room, which was locked for 2 to 3 weeks until the patient could demonstrate the ability to straight leg raise. Physical therapy began the day after surgery with range of motion exercises focused on flexion and extension. When not exercising, patients were to keep the knee in extension; those with repaired meniscal tears were to keep the knee in extension for three weeks and limited to 90 degrees for 6 weeks. Closed chain rehabilitation started 14 days after surgery, after the patient had regained flexion and the pain had subsided, and continued for approximately 3 months. Subsequently, under the direction and supervision of a physical therapist, patients began open chain exercises. Running was not permitted for at least 3 months. Patients started isokinetic exercises after approximately 6 months. Non-professional or amateur athletes were not permitted to return to sport for at least 9 months and were required to have a score of ≥ 85% on the single leg hop test.

Outcome Measures

Adverse Events: Patients were continuously monitored for device-related and procedure-related adverse events for the duration of the study. If graft rupture was suspected, the length of time post-surgery and possible cause was reported. Study questionnaires and clinical function outcomes were documented, as described below.

Clinical Function: KT1000 and Anterior Drawer tests were performed to measure knee laxity. Pivot-Shift, Lachman, and Single-Leg Hop tests were performed as part of the IKDC Physical Evaluation. Clinical function assessments were performed pre-operatively, and at 6, 12, 18, and 24 months; examination was optional at 36 months.

Subjective Pain and Functional Assessment: The 2000 IKDC scoring system, Lysholm scale, Tegner activity scale, and KOO's pain scale were used to measure the symptoms, function, and/or sports activity level for each patient. Assessments were performed pre-and post-operatively, and at 3, 6, 12, 18, 24 and 36 months.

Imaging Analyses: X-Rays, MRI, and CT evaluations were performed and reviewed to assess: the position of the bone tunnels, tunnel-widening, development of OA, and indirect evidence of ligament tissue remodeling. X-Rays were obtained pre-operatively and during the procedure or prior to patient discharge. CT scans were obtained at the latter time point, and at the primary endpoint of 12 months. MRI was obtained pre-and post-operatively, and at 6, 12, 18, and 24 months; MRI was optional at 36 months. Note that at 12 months, six patient MRIs were omitted from analysis due to initial imaging artifacts caused by the titanium screws and one patient was omitted due to pregnancy. At 24 months, two of the 10 patients remaining in the study did not have MRI scans.

Histological & Molecular Weight Analysis: In the case of graft rupture, if patients elected to undergo revision surgery, the surgeon explanted or retrieved biopsies of the device, which was subsequently processed for histological analysis. Samples from the femoral side, midsubstance, or tibial side, were fixed, dehydrated, and embedded in paraffin blocks for sectioning. H&E staining was performed, and experienced pathologists assessed the cell and tissue response. Patients with graft ruptures did not complete any subsequent questionnaires or physical exams as part of the study. For molecular weight analysis, samples were dissolved in chloroform to perform size exclusion chromatography.

Statistical Analysis: Descriptive statistics including frequencies for categorical variables and means, medians,

standard deviations, and ranges for continuous variables were calculated. The study design was intended to evaluate the safety and feasibility of using the L-C Ligament and therefore not powered to perform statistical analysis.

RESULTS

Patient Demographics

Patient demographics are shown in (Table 1). A total of 15 patients (5 female, 10 male) were enrolled in this study, having a mean age of 27 years (range of 18 to 46 years). The mean pre-injury Tegner score was 8.1 (11 of 15 were Tegner 9) and the majority of patients (14 of 15) were playing sports at the time of injury. Eleven of 15 patients sustained a traumatic contact or non-contact ACL injury; the remaining four patients sustained a non-traumatic, sudden onset ACL injury. The time from injury to surgery averaged 88.2 days, and 7 of 15 patients underwent a concomitant partial meniscectomy during the ACL reconstruction procedure.

Outcome Measures

Adverse Events. A summary of adverse events is shown in Table 2. At 36 months follow-up, 13 adverse events specifically related to the device and/or procedure were reported. A total of five graft ruptures were reported; three occurred between 12 and 18 months (12.2, 13.6, and 16.1 months), one at 18.9 months, and one at 34.7 months. Four of the five patients elected for revision surgery. One partial graft rupture was reported at 31.7 months, as determined by MRI; the patient was provided with a CTI brace for sports and no additional treatment was undertaken. The non-serious adverse events included fracture of the femoral wall during the surgical procedure, effusion, a Cyclops lesion (confirmed by MRI), knee pain, and a meniscal tear.

Clinical Function: KT1000 testing at baseline, prior to surgery showed 53.8% of patients reported normal laxity (< 3 mm side-to-side difference) and 46.2% of patients reported unusually high or high laxity indicative of an ACL tear (3 to 5 mm, or > 5 mm side-to-side difference, respectively) (Table 3). At 6 months through 24 months follow-up, >80% of patients demonstrated normal examination with no laxity. IKDC Physical Evaluation Grades showed all patients had abnormal or severely abnormal knee function at baseline pre-op (Table 4), but regained normal or nearly normal knee function at 6 and 12 months post-ACL reconstruction. However, at 18 and 24 months, 25% (3 of 12) and 18.2% (2 of 11) of patients reported abnormal knee

Table 1: Demographic Data.

Demographic	N (or %)	Mean ± SD (or %)	Range
Age (years)	15	27 ± 7	18-46
Females (years)	5 (33.3%)	27 ± 7	19-39
Males (years)	10 (66.6%)	30 ± 9	18-46
BMI	15	22.9 ± 2.3	20.3-28.3
Tegner Pre-Injury	15	8.1 ± 1.7	3-9
Traumatic Injury	11 (73.3%)		
Time from Injury to Surgery (days)	15	88.2 ± 22.7	52-121
Concomitant Partial Meniscectomy	7 (46.7%)		

Table 2: Number of Adverse Events Related to Device and/or Procedure.

	0 – 30 days	31 days – 6 months	6 – 12 months	12 – 18 months	18 – 24 months	24 – 36 months
Bone Fracture	2	0	0	0	0	0
Cyclops Lesion	0	0	0	1	0	0
Effusion	0	1	0	0	0	0
Meniscal Tear	0	0	0	1	0	0
Knee Pain	0	1	0	0	1	0
Graft Rupture	0	0	0	3	1	1
Partial Graft Rupture	0	0	0	0	0	1

Table 3: KT1000- Part C (20lb): Anterior I-N.

	Baseline*	6 months	12 months	18 months	24 months
	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)
< 3 mm	7/13 (53.8%)	13/15 (86.7%)	12/14 (85.7%)	10/12 (83.3%)	8/10 (80%)
3 to 5 mm	5/13 (38.5%)	2/15 (13.3%)	2/14 (14.3%)	2/12 (16.7%)	2/10 (20%)
> 5 mm	1/13 (7.7%)	0/15 (0%)	0/14 (0%)	0/12 (0%)	0/10 (0%)

* Baseline KT1000 not conducted on two patients due to lack of equipment

function, respectively, indicating a slight decline in performance. The individual outcomes for the Lachman, Pivot Shift, and Single Leg Hop tests are summarized in Tables 1-3.

Subjective Pain and Functional Assessment: The results of patient-reported outcome measures are shown in (Table 5). IKDC scores showed that a majority of patients experienced a clinically meaningful improvement in function at 6, 12, 24 and 36 months according to Minimal Clinically Important Difference (MCID: ≥ 11.5 point improvement over baseline). IKDC and KOOS Pain scores also met the Patient Acceptable Symptom State scores (IKDC ≥ 80, PASS ≥ 90), indicating most patients were "feeling well" through 36 months follow-up (Table 4). Lysholm scores showed an improvement from "fair" knee function at baseline to "good" knee function, with the highest average score recorded at 12 months follow-up; however, a gradual decline in average score was observed, returning to "fair" knee function at 36 months follow-up. Tegner scores showed a marked increase in patient activity level from pre-injury baseline to 12 months after ACL reconstruction that remained stable through 36 months follow-up.

Table 4: Physical IKDC Evaluation Group Grades.

	Baseline	6 months	12 months*	18 months	24 months
	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)
Grade A – Normal	0/15 (0%)	7/15 (46.7%)	8/14 (57.1%)	3/12 (25%)	7/11 (63.6%)
Grade B – Nearly Normal	0/15 (0%)	8/15 (53.3%)	6/14 (42.9%)	3/12 (25%)	2/11 (18.2%)
Grade C – Abnormal	14/15 (93.3%)	0/15 (0%)	0/14 (0%)	3/12 (25%)	2/11 (18.2%)
Grade D – Severely Abnormal	1/15 (6.7%)	0/15 (0%)	0/14 (0%)	0/12 (0%)	0/11 (0%)

*One patient had a meniscus injury at 11 months and did not undergo a complete IKDC Exam;; only Lachman test was performed and given a Grade of B

Table 5: Patient-Reported Outcome Measures.

	Baseline	6 months	12 months	18 months	24 months	36 months
Questionnaire	mean ± SD (range)	mean ± SD (range)	mean ± SD (range)	mean ± SD (range)	mean ± SD (range)	mean ± SD (range)
N	15	15	15	12	11	9^
IKDC Score (0-100)	60.2 ± 12.7 (40.2-87.4)	82.8 ± 14.6 (51.7-98.9)	90.1 ± 13.5 (46-100)	83.8 ± 20.1 (36.8-100)	85.9 ± 16.5 (39.1-98.9)	81.8 ± 15.8 (48.3-96.6)
Lysholm	74.4 ± 13.2 (49-95)	91.7 ± 12.3 (52-100)	93.8 ± 8.6 (67-100)	89.4 ± 16.8 (47-100)	87.5 ± 16.1 (42-100)	82.7 ± 15.2 (49-95)
Tegner Post-Injury	2.7 ± 1.4 (0-5)	4.1 ± 1.0 (2-6)	7.3 ± 2 (3-10)	6.5 ± 2.3 (3-10)*	6.5 ± 2.5 (1-10)	6.4 ± 2.0 (3-9)
KOOS Pain	75.6 ± 15.4 (61.1-100)#	89.7 ± 15.1 (44.4-100)	94.4 ± 10.7 (58.3-100)	90.3 ± 17.7 (44.4-100)	93.2 ± 11.0 (61.1-100)	87.8 ± 17.2 (47.2-100)

*One patient's 18-month Tegner score of 3 was excluded due to pregnancy
only patients from one site had baseline scores available
^ One patient did not complete any questionnaires for the 3-year visit, but confirmed to be in good health

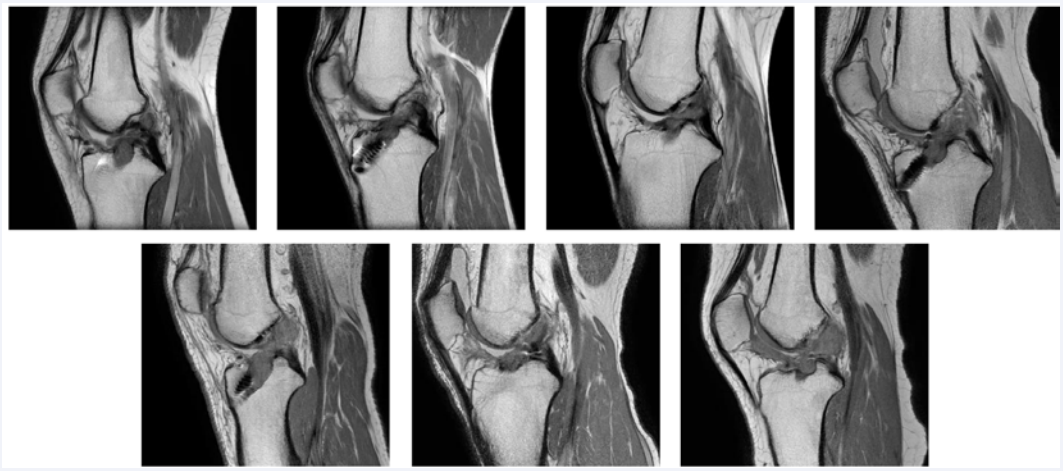

Figure 2 MRI taken at 12 months after ACL reconstruction. A hyper-intense signal was observed in the region of the graft, indicative of chronic inflammation and incomplete tissue maturation. A subset of patients also showed grafts with irregular contours and thinning (second image, upper row), as well as synovial effusion (lower row).

Imaging Analyses: X-Rays were normal and no signs of moderate or severe joint disease were found in any patients. CT scans showed no abnormalities with bone tunnel placement post-operatively, and at 12 months, average bone tunnel widening in the tibia and femur was 1.3 ± 0.9 mm and 1.3 ± 1.5 mm, respectively. Review of MRI at 6 and 12 months showed a hyper-intense signal indicative of chronic inflammation and incomplete ligament maturation (Figure 2). A subset of grafts also showed irregular contours and abnormal morphology with fibrous strands and thinning; two patients also showed extensive synovial effusion. For the 10 patients with intact grafts at 36 months, MRI taken at 18 and 24 months follow-up showed a thin ligament and persistent regional hyper-intensity for all patients remaining in the study without graft rupture (Figure 3).

Histology of Biopsies from Patients with Graft Ruptures: Graft biopsies were retrieved from the femoral side, midsubstance, and tibial side of the intra-articular region of the ruptured graft. An arthroscopic image of the graft at 12 months after ACL reconstruction showed soft tissue encapsulation of the scaffold with a continuous structure in place of the former ligament, having remnant PLLA fibers; however, the structure did not appear like mature ligament (Figure 2). Histological analysis (H&E) revealed a fully cellular zed scaffold, with connective tissue surrounding remnant PLLA fibers (Figure 4). Degradation of PLLA was measured as a decrease in molecular weight as a function of implantation time, reaching 86% molecular weight loss at 34.6 months (Figure 1). A peripheral synovial cell layer was observed across multiple patients, as well as the presence of new blood cells infiltrating the graft. In some regions, there was longitudinal alignment of collagen fibers; Immunohistochemistry performed for a subset of patient biopsies showed diffuse staining of collagen I and collagen III (Figure 3). A chronic inflammatory

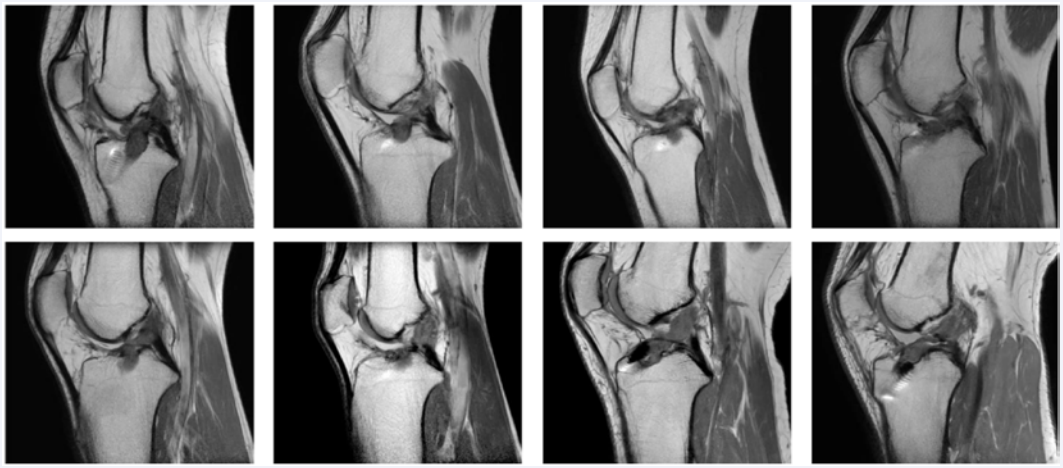

Figure 3 24 month MRI for patients having intact grafts at 36 months. MRI showed a thin ligament and persistent regional hyper-intensity; a small cyclops lesion was observed for one patient (top left image).

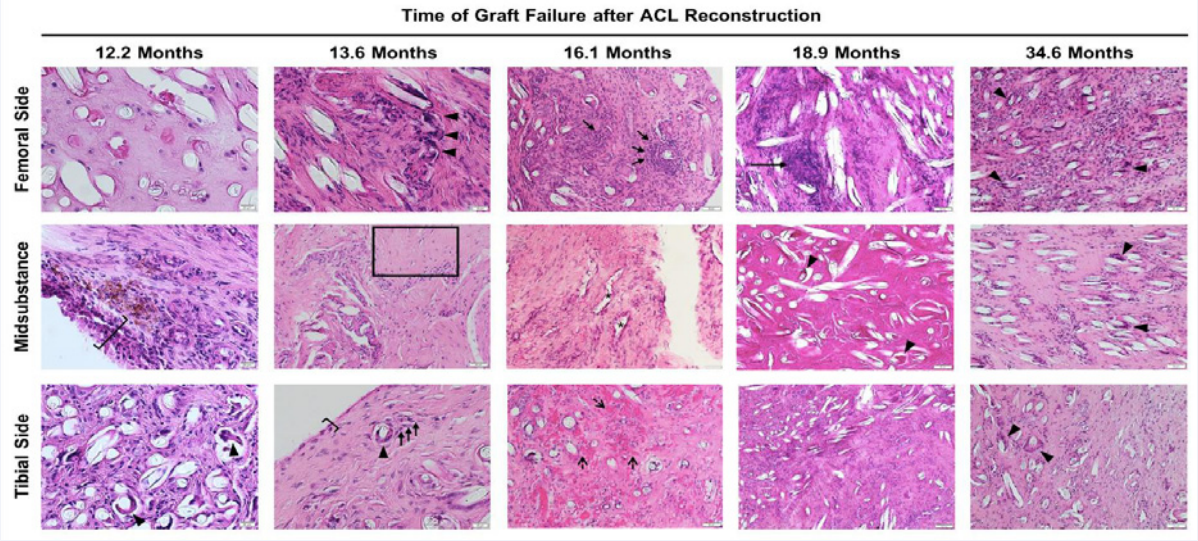

Figure 4 Histology of intra-articular graft biopsies. H&E staining revealed the PLLA scaffold fully infiltrated by cells and connective tissue. In some regions, collagen fiber alignment was observed ( ), as well as a peripheral synovial layer ([ ) and new vasculature (*). A chronic inflammatory response was seen in all biopsies, marked by the presence of foreign body cells ( ), macrophages and lymphocytes ( ), and eosinophils (-).

response was also observed, marked by foreign body giant cells, macrophages, lymphocytes, and eosinophils adjacent to PLLA fibers.

DISCUSSION

This firstin-human study demonstrated the safety of the PLLA scaffold for primary ACL reconstruction, with no infections, synovitis, or allergic reactions reported. In the near term of 12 months post-operatively, progressive improvement in knee function was observed, with all 15 patients having a functional ACL with normal activity. However, 5 of 15 patients (33%) experienced graft ruptures between 12 and 36 months; the remaining 10 patients continue to report normal ACL function and general satisfaction. Histological analysis of graft biopsies obtained at the time of revision surgery showed cells and connective tissue infiltrating the scaffold and surrounding PLLA fibers, but in the presence of a chronic inflammatory response to the remnant material. These results suggest that while the scaffold has the potential to regenerate new ligament tissue, the resulting neotissue did not possess sufficient strength during scaffold resorption to support the mechanical demands on the ACL, resulting in an increased risk of graft rupture.

Three factors that potentially contributed to the inconsistent clinical performance were identified: device stress shielding, slow resorption rate, and inflammatory response to PLLA. The absence of load-sharing results in stress-shielding of the nascent tissue by the scaffold, wherein the scaffold bears the majority of loads and thereby inhibits tissue maturation. While the time-zero strength of the scaffold matched values reported for hamstring and bone patellar-tendon-bone grafts (PLLA scaffold: 2291 ± 94 N, tissue grafts [20-24]:900 to 2300 N), the time-zero stiffness of the scaffold exceeded that of tissue grafts and likely stress-shielded the regenerating tissue (PLLA scaffold: 621 ± 80 N/mm, tissue grafts[20-24]: 130 to 360 N/mm). Accelerated in vitro degradation studies showed that scaffold strength gradually declines to approximately 50% of time-zero values after 18 weeks (equivalent to approximately 18 weeks under physiologic conditions); however, scaffold stiffness remains nearly constant during that same period (Figure 4). Histological analysis showed a disorganized extracellular matrix was observed throughout the length of the graft with few regions of collagen fiber alignment. Collagen type I and type III staining was also diffuse, and while unlike that of native ACL, is similar to patterns observed during development and after injury. In a young, healthy adult, collagen type I shows a distinct crimping and banding pattern in the ACL, while collagen type III is localized to the loose connective tissue that divides collagen fibrils [25]. In contrast, immature or healing tendons [26] and ligaments [27] show collagen type I and type III more equally distributed or expressed within the tissue. As the tissue remodels and matures, collagen type I eventually replaces collagen type III, with the latter acting as a template for collagen type I fibrillogenesis [26,28]. Correspondingly, MRI showed a gradual decrease of the signal intensity and swelling of the ACL graft during the first year, reflecting regular healing. However, in many patients, high signal intensity of the graft persisted past the 12 month time point. Although the histology and MRI showed evidence of a remodeling process, the overall immaturity of the tissue, combined with the weakening of polymer strength with increasing functional demand upon patient return to full-activity, likely led to graft overload and rupture.

Graft maturation not only depends on the mechanical properties of the scaffold, but also on the polymer resorption behavior and associated cell and tissue response. PLLA was chosen for scaffold construction due to its strength, long-term degradation profile [29, 30], biocompatibility [31-34], positive preclinical testing [19], and use in FDA-approved medical devices [35, 36]. In the form of fibers assembled into a 3D braid, the scaffold was designed to provide a durable, but temporary, framework where tissue could regenerate, remodel, and mature before total polymer resorption. However, the persistence of a foreign material can cause an adverse tissue and joint reaction, as previously described for synthetic and permanent ligament prosthetics of the past [13-17]. In this study, PLLA molecular weight decreased with increasing implantation time, showing 86% loss at 34.6 months, similar to predictions from in vitro degradation studies (Figure 1). Complete resorption of the polymer (i.e. mass loss) would not be expected for approximately 8 years (data not shown) and preclinical work showed remnant polymer in intra-articular histology at 4 years after ACL reconstruction [19]. These findings are consistent with the prolonged degradation of PLLA implants observed in pre-clinical models [29,37, 38] and clinical case studies [39]; however, it is important to note that PLLA degradation rates can vary widely based on polymer properties (e.g. molecular weight, crystallinity, polydispersity), implant form factor, and the local in vivo environment. While no synovitis was observed in any patients, histological analysis of ruptured grafts showed a chronic inflammatory response. Foreign body giant cells were observed adjacent to polymer fibers, along with macrophages and lymphocytes, which may have inhibited the remodeling process and maturation of the graft. Chronic inflammatory reactions have also been reported for other orthopedic PLLA implants, including spinal fusion cages [29], suture anchors [40], interference screws [38], and multilayer plates [37], persisting several years after implantation. These findings suggest that while PLLA is broadly considered biocompatible, in this application, its capacity to facilitate tissue regeneration may be limited.

While the objective of this study was the safety profile of the PLLA scaffold, the number of ruptures did not recommend further investigation to evaluate the efficacy of the device; however, 10 patients continue to have normal ACL function at 36 months. This outcome suggests there is the potential to regenerate functional ligament tissue using a tissue-engineering based approach. The 3D braided construction of the PLLA scaffold was designed to have continuous microfilaments from end-to-end, in order to allow infiltrating cells to traverse the entire length of the scaffold. Polymer microfilaments [41], as well as nanofibers [42-44], have been shown to facilitate cell elongation along the axis of the fiber and influence extracellular matrix synthesis and alignment. Additionally, a high surface area to volume ratio allows degradation byproducts to diffuse readily, limiting an acidic build-up or so-called ‘acid dumping’.

The ability of this scaffold to create a nascent ‘tissue bridge’ is supported by the histological analysis of retrieved biopsies, showing connective tissue ingrowth into the femoral side,

midsubstance, and tibial side regions of the intra-articular graft. However, further design improvements are required to achieve clinically acceptable outcomes comparable to auto grafts [45]. A scaffold having biomimetic mechanical properties (i.e. stiffness to tissue grafts) would improve load-sharing with nascent tissue as it grows into the device, and thereby better stimulate tissue remodeling into an organized and load-bearing structure. The use of a less inflammatory, bioresorbable polymer, along with a shorter degradation profile to facilitate early load transfer during rehabilitation, may also improve ligament regeneration and maturation. Future research should focus on tuning these scaffold features to optimize regeneration and better understand functional remodeling of the ACL.

This study has several limitations. A 15-patient cohort was chosen to evaluate the safety and feasibility of the L-C Ligament, and therefore was not powered to evaluate the efficacy of the device. The study was designed with the guidance of the FDA, with the goal to use this data to support a larger randomized clinical trial if patient outcomes were promising. In addition, due to the scope of this study, no control group was included to compare PLLA scaffold to either auto graft or allograft performance.

This first-inman study of the L-C Ligament, a PLLA scaffold for primary reconstruction of the ACL, demonstrated the feasibility of using an acellular, bioresorbable scaffold. However, tissue regeneration was insufficient in this 15-patient cohort, resulting in a clinically unacceptable rupture rate in this limited study. Five patients experienced ruptures between 12 and 36 months, suggesting insufficient load-bearing capacity of the new ligament tissue in the presence of a resorbing scaffold. These findings suggest that further innovation is required to optimize scaffold properties in order to achieve long-term efficacy with a bioresorbable implant for ACL reconstruction.

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