Preliminary Development of a Collagen–PLA Composite for ACL Reconstruction

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ABSTRACT: Our laboratory is developing resorbable composite implants for reconstruction of the anterior cruciate ligament (ACL) of the knee. Composites were fabricated by embedding parallel collagen fibers within a poly(lactic acid) (PLA) or collagen matrix. The mechanical properties, resorption rates, and subcutaneous tissue reactions were determined for both types of composites. The tensile strength and modulus of collagen–PLA composites were twice that of collagen–collagen composites. Subcutaneous fibrous tissue ingrowth was improved and implant resorption was slightly delayed in the collagen–PLA composites. ACL reconstruction surgeries were performed in rabbits using collagen–PLA composite implants. After 4 weeks, neoligament tissue was observed in seven of eight implants; however, four neoligaments had ruptured either in the midsubstance ($n = 2$) or at the bone tunnel interface ($n = 2$). These results and our previous work suggest that resorbable polymeric composite scaffolds are potentially useful for ACL reconstruction if the implants can be protected from excessive mechanical loading during formation of host neoligament tissue. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **63:** 1423–1428, 1997

Key words: anterior cruciate ligament; collagen fibers; poly(lactic acid); tissue engineering; biomaterial; composite; scaffolds

INTRODUCTION

Injury to the anterior cruciate ligament (ACL) of the knee can result in disability and progressive degeneration of other structures in the joint. A ruptured ACL must be surgically reconstructed to restore normal joint function, as primary repair has a high failure rate. Although ACL anatomy, structure, biomechanics, and healing have been extensively studied, there is still no biological graft or permanent prosthesis ideally suited for ACL reconstruction.[1,2] Our laboratory is developing resorbable biomaterials for use as scaffolds on which cells synthesize neoligament tissue to replace the ACL. The ideal scaffold would provide high strength initially, then gradually degrade, transferring mechanical loads to neoligament tissue (Fig. 1). This "tissue engineering" approach is potentially useful for regeneration of a variety of tissues and organs.[3]

Scaffolds for tissue engineering applications can be natural extracellular matrix-derived biopolymers (such as collagen), synthetic resorbable polymers (such as aliphatic polyesters), or hybrid combinations of natural and synthetic polymers. Composite collagenous scaffolds (collagen fibers in a collagen matrix) can induce neotendon formation in the Achilles tendon[4,5] and neoligament formation in the ACL of rabbits.[6] In our previous ACL reconstruction study,[6] about one-half of the collagen composites ruptured before adequate neoligament tissue was formed, in part due to sur-

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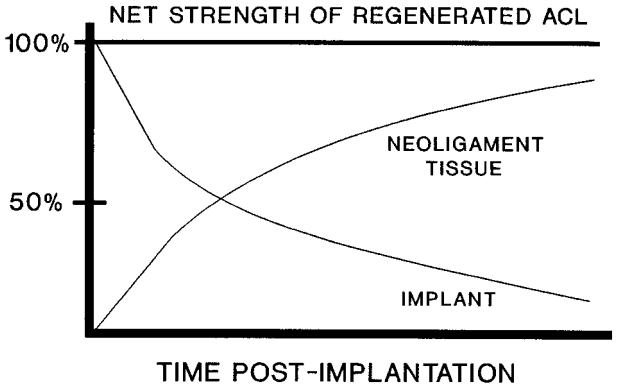

Figure 1 Ideal strength vs. time profile for a bone–ligament–bone complex surgically reconstructed using a resorbable "scaffold" implant. The gradual strength loss of the implant is offset by strength gain in the neoligament tissue. As a result, the net strength of the bone–ligament–bone complex remains high and relatively constant.

gical factors. Our goal was to improve the initial strength, strength retention, and rate of neoligament formation associated with resorbable composites for ACL reconstruction.

The strength and resorption rate of collagen fibers can be improved by varying their diameter[7] and by crosslinking.[8] Although extensively crosslinked collagen fibers are very strong, they may resorb too slowly, resulting in chronic inflammation after implantation.[5] Another way to improve the properties of these composites is to modify the *matrix* surrounding the collagen fibers. In this study, we fabricated collagen fiber-based composites using either collagen or a synthetic aliphatic polyester [poly(lactic acid)[9] (PLA)] as the matrix. We compared the initial mechanical properties and subcutaneous resorption rates for collagen–PLA and collagen–collagen composites. We also utilized a surgical model in rabbits to assess the feasibility of using collagen–PLA composites for ACL reconstruction.

MATERIALS AND METHODS

Fabrication of Composites

Collagen fibers (dry diameter 50–70 μm) were made by extrusion of an acidic 1% (w/v) type I bovine dermal collagen dispersion into fiber formation buffer (pH 7.5, 37°C) as previously described.[4–8] Fibers were rinsed in alcohol and distilled water and dried under tension overnight at room temperature. Fibers were crosslinked by dehydrothermal-cyanamide treatment[10] as previously described.[7] Fibers were heated to 110°C for 3 days under high vacuum (<0.1 micron Hg), then exposed to a saturated solution of cyanamide (a carbodiimide; Sigma, St. Louis, MO) for 24 h at room temperature.

Fiber bundles were prepared by aligning 200 or 500 collagen fibers in parallel. Composites were made by using either collagen or PLA as the matrix around the aligned collagen fibers. Composites contained approximately 50% fiber and 50% matrix (w/w). The collagen matrix was applied by dipping the collagen fibers within a 1% (w/v) acidic bovine dermal collagen dispersion and air-drying. PLA (Medisorb 100 L, DuPont, Wilmington, DE) was applied by dipping the collagen fibers in a 10% (w/v) solution of PLA in chloroform and drying under vacuum overnight at room temperature (Fig. 2). The PLA[11] was 100% poly-L-lactide (residual monomer content approximately 1%) with a weight-average molecular weight of 100+ M, an inherent viscosity of 0.9 dL/g, and a specific gravity of 1.55. The glass transition and melting temperatures were approximately 55–60°C and 170–175°C, respectively.

Mechanical Properties of Composites

Saline-soaked composites (n = 10) containing 500 collagen fibers with either the collagen or PLA matrix were tested in tension to failure on an Instron Model 4204 materials tester (Instron Corp., Canton, MA). The sample gauge length was 10 mm, and the elongation rate was 100 mm per min

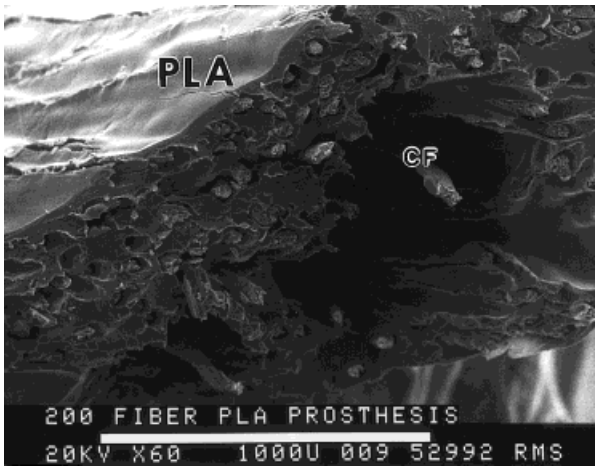

Figure 2 Scanning electron micrograph of the collagen–PLA composite. The collagen fibers (CF) were loosely surrounded by the PLA matrix (bar = 1,000 μm).

(1000% strain/min). Structural properties (peak load [N], deformation [mm], and stiffness [N/mm]) were obtained from the load-deformation curves. Material properties (ultimate tensile strength [MPa], strain [%], and modulus [MPa]) were determined by normalizing the structural properties by the dimensions of each sample. Cross-sectional areas were measured using Vernier calipers; two perpendicular measurements were made and an elliptical cross section was assumed.

Subcutaneous Implants

Composites for subcutaneous implantation contained 200 collagen fibers and either the collagen or PLA matrix. Samples of 1 cm length were implanted subcutaneously in anesthetized male New Zealand white rabbits. For all surgeries, NIH guidelines for the care and use of laboratory animals were observed.[12] Implants were retrieved at 2 and 4 weeks postimplantation ($n = 5$). The number of collagen fibers remaining intact was obtained by analyzing hematoxylin and eosin-stained cross sections of the implants under a Nikon light microscope as previously described.[13]

ACL Reconstruction Surgery

In a previous study, we reconstructed the ACL in rabbits using collagen fiber–collagen matrix implants.[6] In the present study, eight ACL reconstruction surgeries were performed using collagen fiber–PLA matrix composites in skeletally mature New Zealand white rabbits. Each implant contained 500 collagen fibers embedded in the PLA matrix, with PLA plugs on the ends. An 8 in. length of 4-0 Prolene suture (Ethicon, Somerville, NJ) with a straight needle on the end was attached to each PLA plug for surgical fixation of the composite. Prior to surgery, implants were sterilized in Exspor™ chemosterilant (Alcide Corp., Norwalk, CT) for 1 h and rinsed in sterile saline overnight. The animals were anesthetized and the hind limbs were shaved and prepared for sterile surgery.

All surgeries were performed by an orthopedic surgeon in our group (A. J. T.). The ACL was removed by sharp dissection at the tibial and femoral attachment sites. The fat pad was left intact. A 1.5 mm-diameter bone tunnel was created through the lateral femoral condyle and the tibia (exiting at the anatomic ACL attachment sites) using a minidriver drill. A sterile collagen–PLA composite (length 4 cm) was placed in the joint and through both bone tunnels at the anatomic attachment sites of the removed ACL. The ends of the composite were secured (using a 4-0 Prolene suture) to the periosteum of the femur and the tibia with the composite under tension [see Fig. 5(A)]. The patella was reduced, and the joint capsule and skin were closed with 4-0 Prolene suture using a running simple stitch. The limb was covered with gauze for several days to protect the incision sites.

Animals were returned to individual cages with unrestricted activity and given food and water *ad libitum*. Tetracycline was given orally for 2 weeks postimplantation to prevent infection. Animals were sacrificed at 4 weeks postimplantation by general anesthesia followed by intracardiac injection of pentobarbitol sodium (Webster, Sterling, MA). Neoligament tissue was evaluated by gross observation and by examination of paraffin-embedded, hematoxylin- and eosin-stained histological slides from neoligament midsubstance and surgical bone tunnel samples.

Statistical Analyses

Analysis of variance was performed using Statgraphics® software (Rockville, MD) to determine the effects of matrix composition (collagen vs. PLA) on the initial mechanical properties and subcutaneous resorption rates of the composites. Differences between individual groups were considered significant for $P < .05$.

RESULTS

Mechanical Properties of Composites

Compared to collagen–collagen composites (Fig. 3), collagen–PLA composites had significantly greater structural properties (breaking load 40 ± 5 Newtons; stiffness 14 ± 3 Newtons/mm), and material properties (ultimate tensile strength 13 ± 1 MPa; modulus 37 ± 9 MPa). The strain at failure was decreased for the collagen–PLA composites.

Subcutaneous Resorption and Tissue Reaction

The subcutaneous resorption rate of the implants was based on the number of collagen fibers remaining intact at 2 and 4 weeks postimplantation (Fig. 4). Approximately 75% of the implanted collagen fibers remained intact at 2 weeks; less than 50% of the implanted collagen fibers remained in-

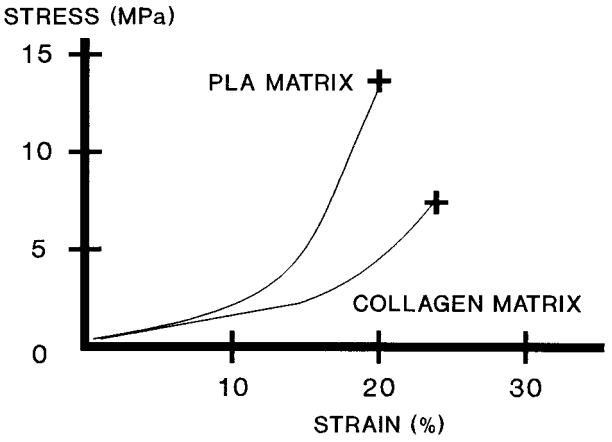

Figure 3 Material properties of resorbable composites, prior to implantation. The ultimate tensile strength and modulus of the collagen–PLA composites were significantly greater than for the collagen–collagen composites.

tact at 4 weeks. At both time periods, the collagen–PLA composites had more collagen fibers remaining intact; however, this difference was not statistically significant.

Histological analysis of retrieved subcutaneous implants revealed degrading composites surrounded by fibrous tissue and similar amounts of inflammatory cells. Collagen–collagen composites were well encapsulated and had limited fibrous tissue ingrowth. Collagen–PLA composites appeared to have more fibrous tissue ingrowth reaching the center of the implants. Polarized light microscopy revealed that the PLA network was largely intact at 4 weeks postimplantation.

ACL Reconstruction Surgery

In one of the eight operated knees, neoligament tissue failed to form in response to the collagen–PLA composite implant. In seven of the eight operated knees, neoligament formation was induced by implantation of the collagen–PLA composite (Fig. 5). The neoligament tissue appeared glistening and white, similar to normal ligament tissue [Fig. 5(B)]. Three of the seven neoligaments were completely intact between the tibia and the femur when the joint was opened at 4 weeks postimplantation. Two neoligaments had broken in their midsubstance; two neoligaments had broken at their interface with the surgical bone tunnel.

Histological sections from the neoligament midsubstance showed that the implants were largely degraded and replaced by host neoligament tissue and inflammatory cells. Nearly all of the collagen fibers were degraded by 4 weeks postimplantation, while the PLA matrix remained partially intact [Fig. 5(C)]. Within the surgical bone tunnels, new bone and soft tissue were found around and within the composite implants [Fig. 5(D)].

DISCUSSION

Both natural[4–6] and synthetic[14,15] resorbable polymers have been used as scaffolds for tendon or ligament reconstruction. Natural, extracellular matrix-derived polymers such as collagen have advantageous biological properties that synthetic polymers lack. On the other hand, synthetic polymers are cost-effective, exhibit less batch-to-batch variability, and have physicochemical properties which are readily modified to suit specific applications. It is likely that *hybrid* biomaterials combining natural and synthetic polymers will be needed to satisfy the rigorous physical and biological requirements for a resorbable ligament reconstruction device. To begin testing this hypothesis, we developed hybrid composites consisting of natural fibers (collagen) embedded within in a synthetic matrix (PLA).

The ultimate tensile strength of the collagen–PLA composites (13 MPa) was about one-third of the strength of the ACL (38 MPa).[16] Collagen–PLA composites had significantly greater strength and modulus than those of the collagen–collagen composites. This may be due to the greater strength and modulus of the PLA matrix

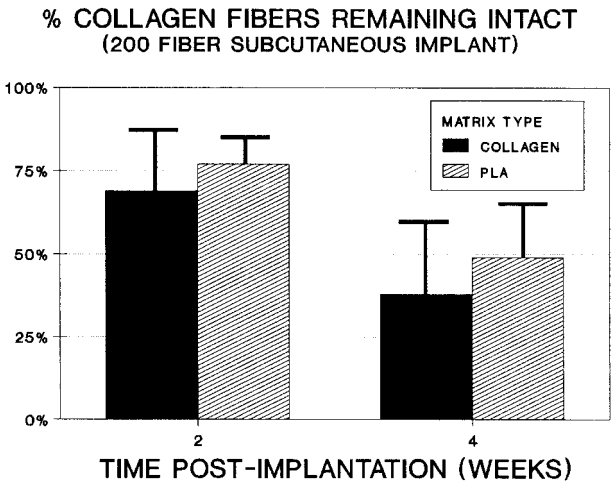

Figure 4 Collagen fiber resorption rate following subcutaneous implantation in rabbits. The slight delay in fiber resorption due to application of the PLA matrix was not statistically significant.

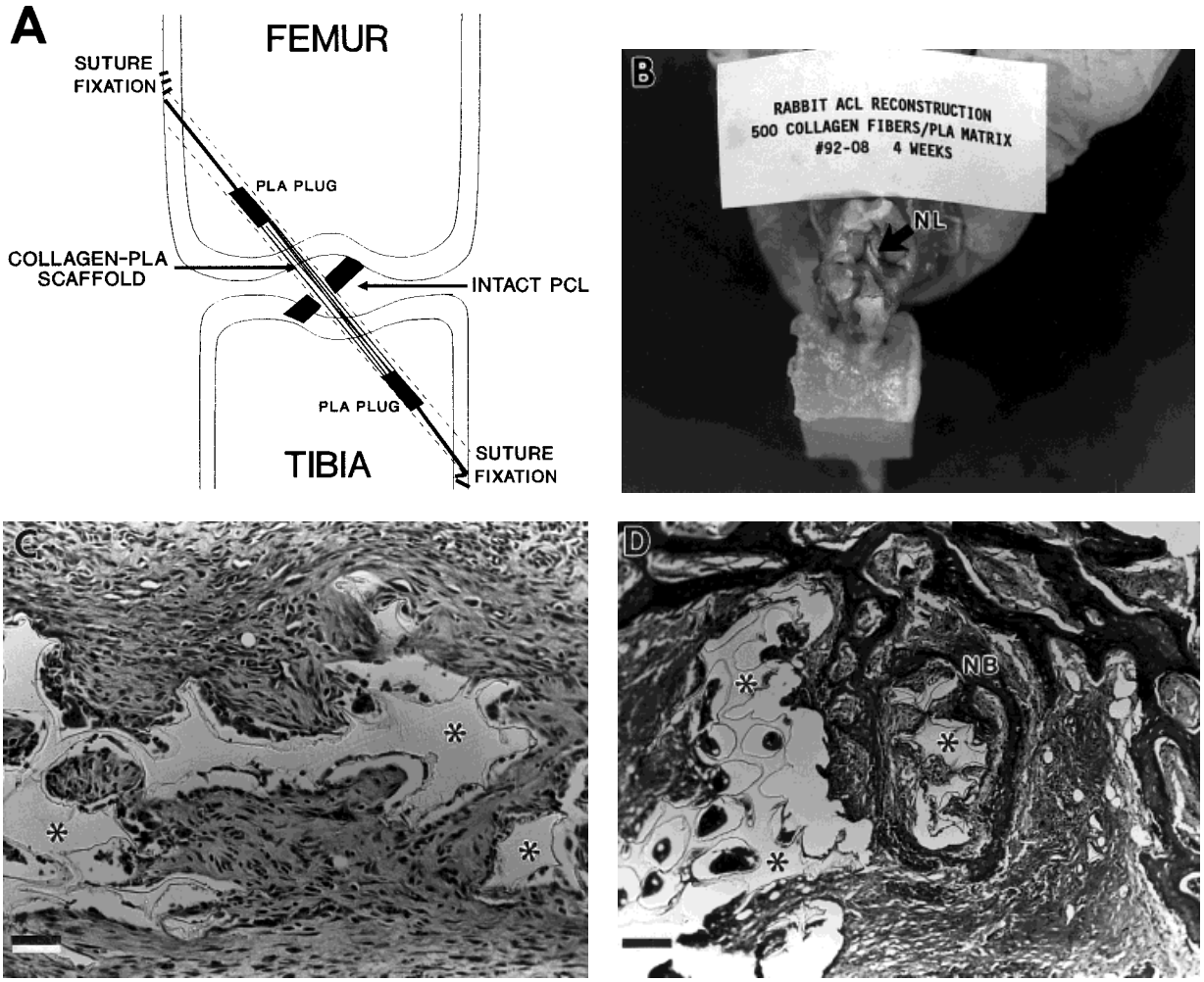

Figure 5 ACL reconstruction surgery in rabbits: (A) the collagen–PLA composite was placed through femoral and tibial bone tunnels at the anatomic attachment sites of the removed ACL; (B) example of intact neoligament tissue (NL; arrow) connecting the tibia to the femur at the anatomic attachment sites of the surgically removed ACL; (C) neoligament tissue was composed of host fibrous and inflammatory tissue infiltrating the degrading composite scaffold (* = PLA; bar = 25 μm); (D) in the bone tunnel, new bone (NB) and osteoid tissue infiltrated the degrading composite scaffold (* = PLA; bar = 50 μm).

(57 and 2100 MPa, respectively)[11] compared to the uncrosslinked collagen matrix (5 and 10 MPa, respectively).[7] It is also possible that the PLA matrix limits wetting and swelling of the collagen fibers. We previously found that dry collagen fibers have about three to five times the strength of wet collagen fibers, depending on the fiber diameter and crosslinking method.[7]

Histological evaluation of subcutaneous implants at 2 or 4 weeks indicated that more collagen fibers remained intact within the PLA matrix compared to the collagen matrix; however, this difference in fiber mass resorption was not statistically significant. We did not measure composite strength retention in this study. Since the PLA matrix was still largely intact after 4 weeks implantation, the strength retention profile may be improved for collagen–PLA composites compared to collagen–collagen composites. Studies are underway to determine whether PLA (or other synthetic matrix materials) improve the strength retention profile for collagen fiber-based implants *in vitro* and *in vivo*.

In our short-term ACL reconstruction study, the ligament was completely removed and replaced by a collagen–PLA composite which induced neoligament formation in seven of eight treated knees (after excision, the rabbit ACL is

incapable of spontaneous regeneration).[17] Only three of the seven neoligaments, however, were completely intact when the animals were sacrificed at 4 weeks postimplantation, consistent with our previous study.[6] Gross and histological observations of explants indicated that the mode of failure was most likely due to excessive loading of the joint or mechanical shearing where the implants emerged from the bone tunnels. Thus, it is likely that the performance of these composites can be enhanced by smoothing sharp bone tunnel edges and protecting the reconstructed knee from excessive loads. This can be accomplished by temporarily or partially immobilizing the treated knee and leaving the untreated leg to bear mechanical loads.

Results of this study and our previous work[6–8,13,18] suggest that ACL reconstruction using a resorbable device is feasible; however, the resorbable composites described here are not optimal for ACL reconstruction. For example, we no longer use dehydrothermal treatment to crosslink collagen fibers, since we showed that ultraviolet light crosslinked collagen fibers are equally strong[8] and much more stable in the presence of nonspecific proteolytic enzymes.[19] Furthermore, PLA is not the ideal polymer for this application for several reasons: collagen and PLA were difficult to combine, probably due to the relative hydrophobicity of PLA. Scanning electron microscopy results suggested poor bonding between collagen fibers and the PLA matrix. Other polymeric matrices may provide improved fiber–matrix interfacial bonding and higher composite strengths. Finally, recent reports suggest that in the long term PLA implants may cause resorption of bone.[20]

We are currently developing "second generation" hybrid devices combining ultraviolet light crosslinked collagen fibers[8,19] with other resorbable polymeric matrices. We also plan to combine synthetic resorbable fibers (providing high strength) with extracellular matrix-derived coatings (providing biocompatibility for cells)[18] to optimize both the bulk and surface properties of resorbable composite scaffolds for ACL reconstruction.

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