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Repair of experimental Achilles tenotomy with porcine renal capsule material in a rat model

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Abstract Porcine small intestinal submucosa (SIS) is a collagenous acellular matrix which has found substantial utility as a tissue growth scaffold. In the present study, the utility of porcine renal capsule matrix (RCM) was compared to SIS in a rat Achilles tenotomy repair model. Groups of rats underwent surgical tenotomy followed by either no repair, repair with a SIS graft, or repair with a RCM graft. The weight-bearing ability of the manipulated limb was evaluated for 10 days following surgery using a subjective scale. Tenotomy sites sampled 28 days after surgery were numerically graded for degree of histologic change. There were no statistically significant differences between groups with respect to return to weight-bearing ability ($p \geq 0.05$) or degree of histologic change ($p \geq 0.001$); however, a non-significant trend suggested that rats treated with SIS or RCM experienced a faster return to limb function than untreated rats, and RCM-treated rats had slightly higher scores for degree of histologic change, suggesting a more rapid repair of the tenotomy site than in SIS-treated or untreated rats. The harvested tenotomy sites in all treatment groups were characterized by marked fibroplasia and presence of macrophages. Remnants of SIS surrounded by macrophages and multi-nucleated giant cells were still present in some rats, however remnants of RCM were not observed, suggesting more rapid incorporation of RCM. The results show that RCM is equivalent to SIS as a material for repair of Achilles tendon injury and merits further study in other tendon injury models.

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Background

Achilles tendon rupture is a relatively common injury which results in a prolonged healing process and significant disability and lost time for productive activity. Though the injury associated with Achilles tendon rupture is sometimes a result of athletic activity, middle-aged individuals are particularly prone to the injury [1].

Clinical approaches to Achilles tendon rupture often involve surgical repair, though some investigators have argued that nonsurgical approaches to clinical management of Achilles tendon rupture result in outcomes equivalent to that seen with surgical intervention [2–5]. The standard surgical method involves suturing the remaining tendon stumps together followed by functional aftercare [2, 6]. However, in cases where insufficient tendon remains, surgical repair presents a significant challenge. In this regard, biomaterials for use as a tendon graft are of substantial value.

A variety of products have been examined for utility as tendon graft materials. For example, artificial polymers have been examined as possible materials to promote healing of tendon defects. Biodegradable poly(epsilon-caprolactone) films were shown to hasten recovery of function in rats subjected

to surgical Achilles tendon defect, though untreated rats with the same defect gained function which was not significantly different by 28 days following surgery [7]. Similarly, polyhydroxyethyl methacrylate (pHEMA) membranes promoted functional recovery in rabbits subjected to surgical flexor tendon defect, with comparable recovery between pHEMA-treated and untreated rabbits by 12 weeks post-surgery [8]. Polypropylene mesh allowed ingrowth of morphologically normal collagen bundles in a rabbit model of Achilles tendon rupture [9, 10]. Poly(epsilon-caprolactone) biodegradable film was used to fill the gap in a rat tenotomy model, with functional recovery of the operated limb within 28 days of injury and repair, however there was no histological evidence that the biomaterial enhanced tendon repair vis-à-vis a group which did not have the injured tendon repaired [7]. A copolymer of dimethyltrimethlene carbonate and trimethylene carbonate resorbed slowly, with fibers of the material still present at the repair site 26 weeks after injury and repair [11].

An optimal material for the repair of Achilles tendon rupture would be readily available, and allow fast, natural bridging of tendinous defects by host cells and with concurrent resorption of the material. In this regard, materials derived from biological sources have shown promise. Though gastrocnemius fascial flaps have been used successfully in reconstructive repair of injured Achilles tendons [12], this approach has been associated with postoperative complications, including re-rupture of the tendon, deep infection of the surgical wound site, delayed wound healing, and deep venous thrombosis [13]. Repair of Achilles tendon defects in dogs resulted in bridging of the defect with organized collagen-rich connective tissue and complete incorporation of the biomaterial within 8 weeks of implantation [14].

Biomaterials derived from animals have also been used for tendon repair grafts. Porcine small intestinal mucosa (SIS) used as an Achilles tendon graft repair material in dogs showed the remodeled SIS neotendon to be stonger than the musculotendinous origin and repair tissue of dogs not treated with a biomaterial by 12 weeks following surgery [14]. The SIS grafts were characterized by host cell infiltration and neovascularization. In contrast, SIS initially improved strength in a similar rabbit model, though by 16 weeks following surgery there was no difference in mechanical strength between SIS-repaired and non-repaired tendons [15].

In the study described here, we compared the use of a novel biomaterial, porcine renal capsule matrix (RCM) to SIS small intestinal submucosa (SIS) as a biological graft material for repair of Achilles tendon rupture. We have previously shown RCM to have comparable properties to SIS as a wound healing material [16]. The clinical progress and histological character of surgically created Achilles tendon rupture and repair with either RCM or SIS were compared as measures of the suitability of RCM as a tendon defect repair material.

Materials and methods

Animals

A total of 40 specific pathogen-free 250–300 g female Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) were used in this study. The animals were maintained on Purina Rodent Lab Chow (Purina, Inc., Richmond, IN) and tap water provided ad libitum. The studies described here were approved by the University of Notre Dame Institutional Animal Care and Use Committee and were conducted in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Test material

Renal capsule was dissected from mature pig kidneys immediately following slaughter. It was thoroughly rinsed under running tap water and disinfected using a dilute solution of peracetic acid in ethanol to remove potential contaminating bacteria and viruses [17]. Following disinfection, the RCM was rinsed in high purity water to remove the acid, lyophilized into a sheet form, and subsequently sterilized prior to implantation using ethylene oxide gas.

SIS was chosen for comparison to RCM since both are extracellular matrices derived from natural biologic sources. The SIS was prepared as previously described by harvesting porcine jejunum and placing 10- to 20-cm lengths into saline solution [18–20]. Following removal of all mesenteric tissues, the jejunal segment was everted and the tunica mucosa abraded using a longitudinal wiping motion with a scalpel handle and moistened gauze. The serosa and tunica muscularis were then gently removed using the same procedure. The remaining tissue was disinfected with peracetic acid, rinsed extensively in high purity water, and sterilized using ethylene oxide prior to implantation.

Experimental design

Rats were anesthetized with an intramuscular dose of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg). Using aseptic technique, each rat had a 5 mm section of the right Achilles tendon excised. Ten rats had the remaining tendon ends rejoined by a RCM graft sutured into place; ten rats had the tendon rejoined with a SIS graft; in ten rats no attempt was made to repair the ruptured tendon; and ten rats underwent no surgical manipulation.

Rats were observed daily following surgery and subjectively graded for ability to bear weight on the surgically-manipulated limb. A grading system was used in which 0 = non-weight bearing on the manipulated limb; 1 = mildly weight bearing; 2 = moderately weight bearing; and 3 = fully weight-bearing.

The rats were sacrificed by inhalation of carbon dioxide 28 days following surgery. Tissue was harvested from the surgical site, fixed overnight in 10% neutral buffered formalin and transferred to 70% ethanol. Excised tissue was embedded in paraffin and then sectioned at 3–4 μm and stained with hematoxylin and eosin. Tissues were evaluated descriptively and graded using a subjective histologic grading system as described below.

Histologic grading systems

A subjective grading system was used to score the degree of histologic change and integration of the remaining native Achilles tendon into regenerated tissue. This scale assigned points for: (1) the degree of attachment of the remaining native Achilles tendon into the regenerated tissue (0 = no attachment; 1 = 25% of the border attached; 2 = 50% of the border attached; 3 = 75% of the border attached; and 4 = 100% of the border attached); (2) the degree of fibroplasia (0 = no fibroplasia; 1 = minimal fibroplasia; 2 = moderate fibroplasia; and 3 = marked fibroplasia); and (3) presence of biomaterial (0 = biomaterial present with inflammatory cells; 1 = biomaterial present with minimal inflammatory response; and 2 = no biomaterial present). A maximum score of 9.0 represented an optimal regenerative response.

Statistical analysis

Data were analyzed by one-way analysis of variance, followed by the Multiple *t*-test for comparisons. Significance was set at $p \leq 0.05$. Data are presented as mean histologic and weight bearing scores (±SEM).

Results

Post-surgical weight-bearing ability

The ability of rats to bear weight on the surgically-manipulated limb is summarized in Table 1. All groups of rats had scores showing minimal to moderate weight-bearing ability beginning at the first day following surgery and progressing to full weight-bearing ability by 10 days after surgery. While the scores through day 6 are greater for RCM-treated animals compared to those treated with SIS or undergoing no repair of the ruptured tendon, the group differences were not statistically significant ($p \geq 0.05$) at any time point for surgically manipulated rats.

Degree of histologic change

The degree of histologic change observed on tissue sections stained with hematoxylin and eosin are summarized in Table 2. There were no significant differences between mean histologic scores of SIS-treated rats; RCM-treated rats; or rats which underwent surgery but without repair ($p \leq 0.001$); however, the mean scores of rats from these groups were all significantly lower ($p \leq 0.05$) than the mean histologic change score for rats which did not undergo surgical manipulation.

Tissue from RCM-treated rats was characterized by abundant fibroblastic tissue which blended into the remnant of the original tendon (Fig. 1). Occasional

Table 1 Weight bearing scores

Day after sugery	SIS	RCM	No repair
1	1.6 (0.84)	1.8 (0.92)	1.2 (0.79)
2	2.1 (0.74)	2.2 (0.79)	1.6 (0.52)
3	2.0 (0.67)	2.5 (0.53)	1.8 (0.42)
4	2.5 (0.53)	2.7 (0.48)	1.9 (0.57)
5	2.5 (0.53)	2.7 (0.48)	2.1 (0.57)
6	2.7 (0.48)	2.8 (0.42)	2.5 (0.53)
7	2.9 (0.32)	2.9 (0.42)	2.7 (0.38)
8	2.9 (0.16)	2.9 (0.10)	2.7 (0.38)
9	2.8 (0.12)	2.9 (0.10)	2.9 (0.16)
10	3.0 (0)	3.0 (0)	3.0 (0)

Rats were observed daily for the first 10 days following surgery and subjectively graded for ability to bear weight on the surgically-manipulated limb. A grading system was used in which 0 = non-weight bearing; 1 = mildly weight bearing; 2 = moderately weight bearing; and 3 = fully weight bearing. Mean scores are presented with standard deviations in parentheses. Scores for control rats not undergoing surgery were 3.0 at all time points. There were no significant differences between surgically manipulated groups at any time point ($p > 0.05$)

Table 2 Histologic scores of rat Achilles tendons

Group	Score
No surgery	9.0 (0)
SIS-repair	6.9 (1.4)
RCM-repair	7.2 (0.79)
Surgery, no repair	6.7 (0.95)

Tendons were harvested 28 days after surgery and graded for histologic character using the scale described in Table 1. Scores represent group means out of a total possible score of 9.0; standard deviations in parentheses. Mean scores between SIS-treated rats, RCM-treated rats, and surgery/no repair rats were not significantly different ($p \leq 0.001$); however scores of all three of these groups were significantly lower than rats which did not undergo surgery ($p \leq 0.05$)

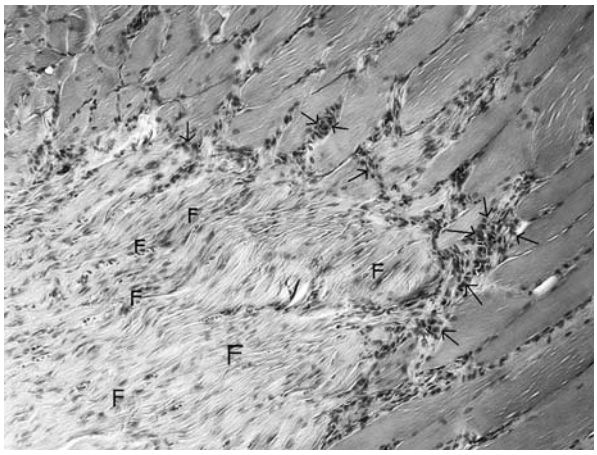

Fig. 1 Complete blending of regenerated tissue (*lower left quadrant*) into native Achilles tendon tissue in a rat which underwent repair of Achilles tendon defect with RCM. A moderate number of macrophages (*arrows*) are present at the interface of muscle and fibroplasia (F) in the repairing tissue. Stained with H & E, 100×

nests of macrophages and fibroblasts were present and presumably represented responses to remnants of RCM, though such remnants were not observed. Along the implant/tendon interface, marked mononuclear inflammation was present (Fig. 2). The histologic character of tissue from SIS-6treated rats was similar to that from RCM-treated rats, though fewer polymorphonuclear cells were present. In some samples, nests of macrophages and giant cells surrounded fragments of the SIS (Fig. 3) with occasional polymorphonuclear cells. In rats which underwent surgical tendon rupture without repair, few macrophages were present, though numerous fibroblasts were observed (Fig. 4). In contrast, rats which did not undergo experimental Achilles tenotomy did not show any inflammation or presence of fibroblasts at the tendon/muscle interface (Fig. 5).

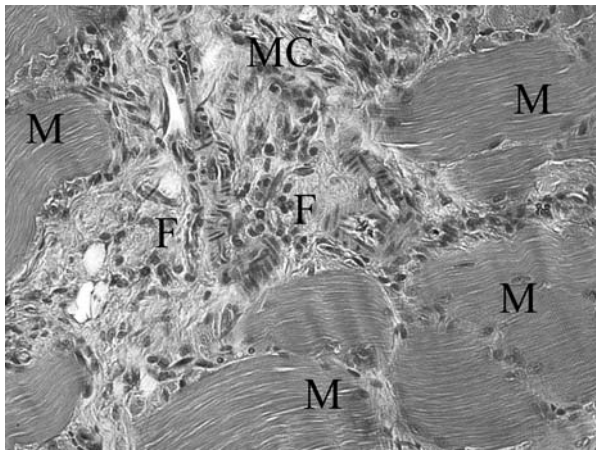

Fig. 2 Focus of mononuclear inflammation at interface of implant/tendon surface in a rat which underwent repair of Achilles tendon defect with RCM. Normal muscle (M) surrounds a focus of macrophages (MC) and fibroplasia (F). Stained with H & E, 200×

Discussion

In the present study we evaluated a novel biomaterial, RCM, for its utility as a tendon repair material in comparison to SIS. In the present study, RCM was found to invoke similar reparative properties to SIS. We found that repair of the tendon defect with either RCM or SIS resulted in quick return to function, with no significant difference between rats which underwent surgical repair and those in which the defect was not repaired. Further, both RCM- and SIS-repair were characterized by a strong fibroblastic response. In SIS-treated rats, some samples demonstrated nests of macrophages and giant cells surrounding remnants of

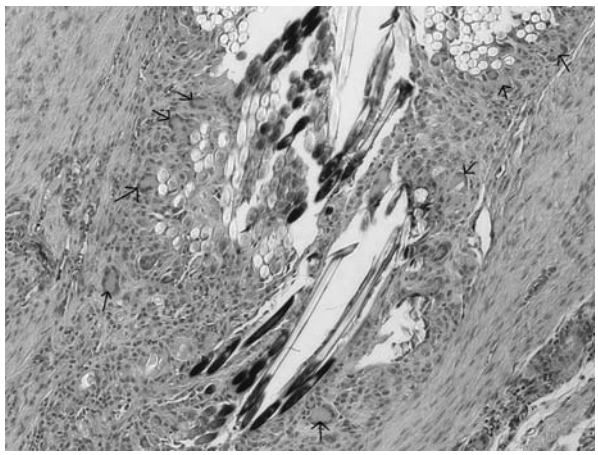

Fig. 3 Remnant of SIS biomaterial in a rat 28 days after undergoing repair of Achilles tendon defect. The remaining biomaterial is surrounded by macrophages with occasional giant cells (*arrows*). Stained with H & E, 100×

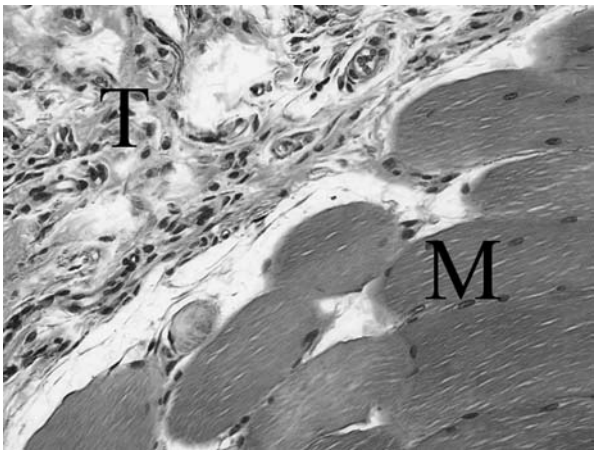

Fig. 4 Interface of repairing tendon (T) and muscle (M) from rat which underwent tenotomy without repair. The repairing tendon lacks a firm attachment to the muscle as found in rats which underwent repair with either RCM (Fig. 1) or SIS (Fig. 3). Stained with H&E, 200×

the biomaterial; in contrast, while several small nests of macrophages and fibroblasts were observed in RCM-treated rats, no remaining biomaterial was observed, suggesting that RCM may be incorporated more rapidly when used for repair of Achilles tendon transection. Though the degree of histological repair suggested a favorable trend for RCM, differences were not significant between any of the groups which underwent surgical tenotomy. While tendon strength measurements were not part of our experimental design, Badylak et al. [14] found that Achilles tendon defects repaired with SIS were stronger than those which were left unrepaired. Indeed, scar tissue formed during the process of injured tendons left to heal spontaneously is mechanically inferior to repaired tendon and more susceptible to further damage [21]. Further, total immobilization slowed healing of SIS-repaired segmental Achilles tenectomy with a resultant increase in mononuclear inflammation and deposition of extracellular matrix [22].

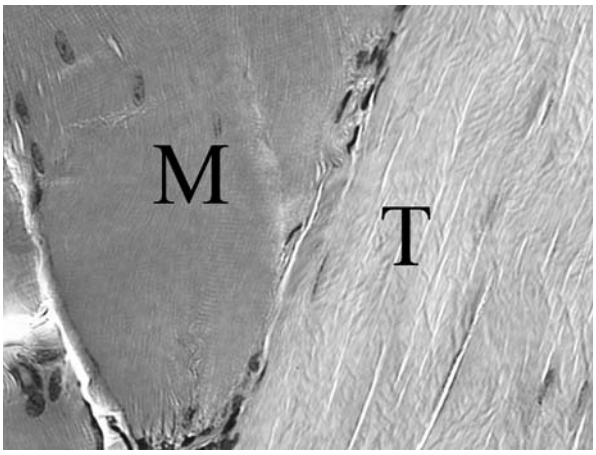

Fig. 5 Interface of tendon (T) and muscle (M) from rat which did not undergo experimental tenotomy. There is a lack of inflammation and fibroplasia. Note the close alignment of muscle with tendon. Stained with H & E, 200×

While the biochemical composition of RCM is not yet fully defined, SIS is characterized as a collagen-glycosaminoglycan (GAG) material [23] that has been shown to have intrinsic FGF-2 and TGF-β activities [24, 25]. Both FGF-2 and TGF-β are believed to play important roles in the healing of tendons and ligaments. Cell migration and proliferation was stimulated by FGF-2 in cultured rat patellar tendons [26], and *in vivo* studies have confirmed the in vitro findings in rat and dog models of tendon injury [27–29]. TGF-β has been shown to promote a number of processes associated with tendon healing, including cell migration [30], fibronectin binding interactions [31], regulation of proteinases [32], and stimulation of collagen production [33]. Further, members of the TGF-β superfamily increased the force at failure by 39% when administered to the transected Achilles tendons of rats [34, 35]. Heparin chains may directly stimulate angiogenesis or may act as part of a proteoglycan to stimulate the angiogenic effects of FGF-2 [36]. Dermatan sulfate, as a component of several different proteoglycans, interacts with TGF-β1 [37] and may help to control matrix formation and remodeling during the later phases of healing. In addition to regulating the function of TGF-β1, dermatan sulfate-containing proteoglycans regulate the structure of the extracellular matrix by controlling collagen fibril size, orientation, and deposition. Likely, it is the influence of FGF-2 and TGF-β which account, at least partially, for the repair response stimulated by SIS. It is reasonable that RCM may have FGF-2 and TGF-β activities similar to SIS which might, in turn, account for the similarity in the repair response following tenotomy. Further work will be needed to characterize the biochemical composition of RCM.

SIS has been shown to serve as a bioscaffold for tissue replacement in a variety of sites [14, 38–41], and RCM stimulated dermal wound repair in a manner similar to SIS in a rat model [16]. In the present study, RCM behaved similarly to SIS with respect to tendon healing. Neither biomaterial had significantly greater rates of return to weight-bearing function of the repaired limb, nor histological scores. However, the data suggest that RCM may be more readily incorporated in the rat Achilles tenotomy model, thus it seems reasonable that RCM may also hold promise for clinical application. For these reasons, we believe that

RCM represents a useful biomaterial for repair of Achilles tendon rupture and merits further investigation.

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