

The use of xenogeneic small intestinal submucosa as a biomaterial for Achilles's tendon repair in a dog model

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A study was conducted to evaluate the tissue response to a xenogeneic biomaterial when this material was used to repair an experimentally induced Achilles tendon defect in the dog. Twenty dogs had a 1.5 cm segmental defect of the Achilles tendon created surgically which was then repaired with acellular connective tissue derived from porcine small intestinal submucosa (SIS). The animals were sacrificed at 1, 2, 4, 8, 12, 16, 24, and 48 weeks and the neotendons examined for uniaxial longitudinal tensile strength, morphologic appearance, hydroxyproline (collagen) content, and disappearance of the originally implanted SIS material over time. The contralateral normal Achilles tendons served as controls as did four additional dogs that had a 1.5 cm segmental Achilles tendon defect created surgically

without subsequent surgical repair with SIS. Results showed the SIS remodeled neotendons to be stronger than the musculotendinous origin or the boney insertion (>1000 N) by 12 weeks after surgery and to consist of organized collagen-rich connective tissue similar to the contralateral normal tendons. The four dogs in which no SIS was implanted showed inferior strength at the comparable time points of 4, 8, 12, and 16 weeks. Immunohistochemical studies suggest that the SIS biomaterial becomes degraded within the first eight weeks and serves as a temporary scaffold around which the body deposits appropriate and organized connective tissue. SIS is a promising biomaterial worthy of further investigation for orthopedic soft tissue applications. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

A biomaterial derived from a specific trilaminar layer of the small intestine has been used in experimental animals as a replacement graft for large and small diameter arteries,¹⁻⁴ veins,⁵ urinary bladder defects,¹ and skin.¹ This xenogeneic biomaterial, referred to as SIS (small intestinal submucosa), has been shown to be biocompatible^{1,2,4} and infection resistant,⁶ possess predictable mechanical properties prior to implantation,^{7,8} and, perhaps most significantly, induce a host connective and epithelial tissue response that results in regeneration of specialized connective tissues rather than nonspecific scar tissue formation.

To date, the SIS material has not been evaluated for its usefulness in musculoskeletal applications. We conducted a study to determine the feasibility of us-

ing SIS for the repair of a localized orthopedic injury. Specifically, we evaluated the tissue response to SIS when this biomaterial was used as a replacement for a surgically created defect in the dog Achilles tendon.

There are obvious clinical implications for the present study. The surgical repair of ruptured tendons such as the Achilles tendon provides the best chance for restoration of strength, power and function of a muscle-tendon-bone complex with a lower risk of rupture.⁹⁻¹⁷ Potential complications of surgical repairs include infection, necrosis, nerve damage, persistent discomfort related to tenosynovial irritation, and scar tissue formation.^{10,11,18-20} Various prosthetic materials have been used to repair Achilles tendon ruptures including marlex mesh,²⁰ carbon fiber,²¹ and polymeric lactic acid.²² These materials alter the mechanical properties of the repaired tendon, lose their strength and integrity over time, have limited native tissue ingrowth and can cause abrasion of the surrounding tissues, modify the inflammatory response, and may cause an undesirable hyperplasia of the resulting scar tissue at the repair site.

The purpose of the present study was to evaluate

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the remodeling properties of SIS when placed in the Achilles tendon location in a dog model. We monitored the morphology, chemical composition, mechanical properties, and functional properties of the neotendon over a period of 48 weeks.

MATERIALS AND METHODS

Overview of experimental design

A 1.5-cm long segment of the Achilles tendon was surgically excised from 20 dogs (mean wt. = 17.9 ± 1.6 kg) and the resultant gap was filled with a hollow cylindrically shaped piece of SIS that was sutured into place with prolene suture material. The leg was immobilized with a brace for 5 weeks to prevent apposition of the cut ends of the tendon since the SIS material is very flexible. After removal of the brace, the dogs were allowed complete freedom of movement in a 13×9 ft kennel. Three animals were euthanized at each of the following times: 1, 4, 12, and 24 weeks post surgery. Two animals were euthanized at each of the following times: 2, 8, 16, and 48 weeks post surgery. Four additional dogs were entered into the study as controls. These four animals had the same segmental tendon excision performed but no SIS material was placed in the tendon defect. The postoperative care was identical to the SIS-implanted dogs. The monitored variables for this study included clinical, mechanical, biochemical, and morphologic parameters that are described in detail below. NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) were followed.

SIS graft preparation

The preparation of SIS graft material from small intestine has been described previously^{2,3} but will be reviewed here for the sake of clarity. Immediately following euthanasia of 200 to 250 kg sows at a local processing plant a 10 m long segment of jejunum was resected and placed in physiologic saline solution. All mesenteric tissues were removed. The superficial layers of the tunica mucosa, including the epithelium and lamina propria, were mechanically removed. Likewise the abluminal layers including the tunica serosa and tunica muscularis externa were mechanically removed. Care was taken to prevent perforation of the remaining trilaminar connective tissue layers. SIS consists of the remaining (approximately 80 μ m thick) trilaminar portion of the small intestine including the stratum compactum layer of the tunica mucosa,²³ the tunica muscularis mucosa (sparsely

present in some species including the pig), and the tunica submucosa (Fig. 1). The stratum compactum layer of SIS consists of more densely organized connective tissue than the underlying submucosal layer²³ but only 5 to 10% of the thickness of the material is occupied by the porcine stratum compactum. The bulk of the SIS material consists of extracellular connective tissue matrix and the cellular nuclei seen in Figure 1(b) are lysed and removed during the cleaning and sterilization procedures. The SIS graft is then

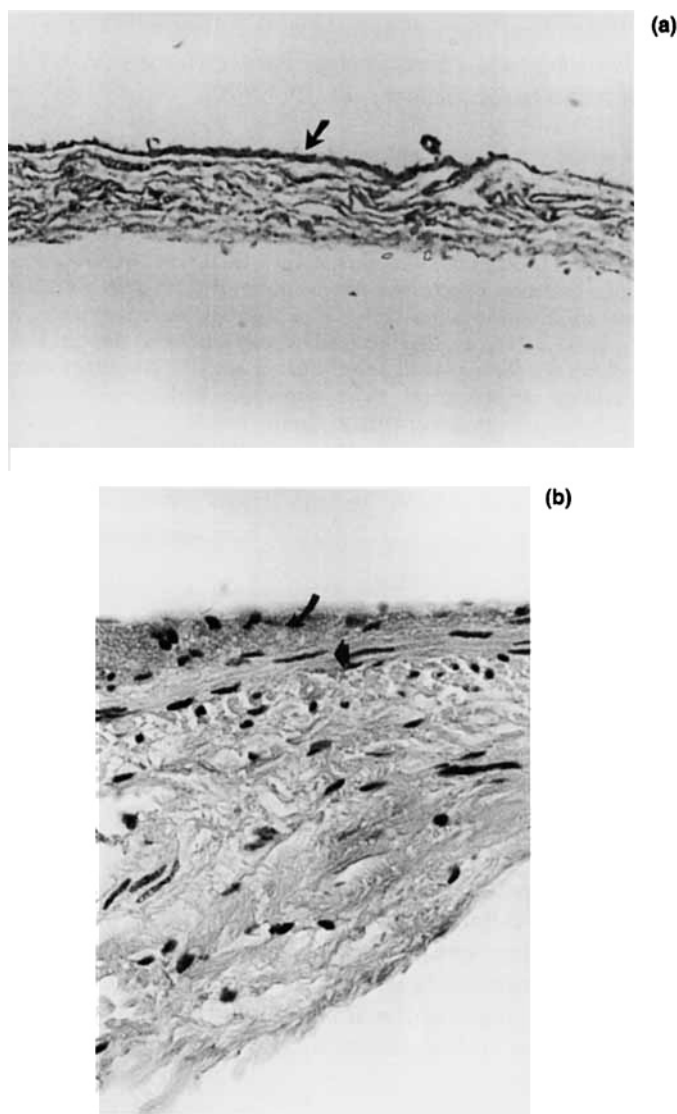


Figure 1. Small intestinal submucosa (SIS) from the pig consists of a thin, approximately 80 μ m, trilaminar connective tissue. (a) This photomicrograph shows the superficial stratum compactum (arrow) and the underlying submucosa. The intermediate muscularis mucosal layer cannot be seen at this magnification but is shown in Figure 1(b) ($\times 50$, H&E stain). (b) The stratum compactum layer of porcine SIS is identified by the curved arrow. The muscularis mucosa layer immediately beneath the stratum compactum is identified by the arrowhead. The remaining tissue represents the tunica submucosa layer of the trilaminar SIS material ($\times 375$, H&E stain).

rinsed with saline and placed in phosphate buffered saline containing gentamicin and protease inhibitors prior to sterilization with peracetic acid.^{24,25}

Surgical procedure

Each dog was anesthetized by intravenous administration of 2% sodium pentothal solution followed by intubation and inhalant isofluorane (1.5% in oxygen) to maintain a surgical plane of anesthesia. The skin which covered the right Achilles tendon was shaved and scrubbed for surgery. The entire Achilles tendon was exposed. The tendon was transected and a 1.5-cm long segment of the midsection of the tendon was excised. The SIS material was most easily handled by leaving the natural collapsible tube shape as a double layer elongate sheet (Fig. 2). A 2.0-cm-long segment of the flattened tube of sterile SIS material was then wrapped around the cut ends of the native tendon such that there was a 0.25-cm overlap of the SIS upon the external surface of both the remaining proximal and distal ends of the native tendon. Interrupted (4-0 prolene) sutures were used to appose the long sides of the SIS tube where they met after encircling the Achilles tendon defect. Since the SIS was used in its natural collapsed tube shape, there were two layers of SIS material in the walls of a newly formed hollow cylinder that bridged the 1.5-cm defect in the tendon. The adventitial tissue was approximated with a single polydioxanone (PDS) suture. A single suture was used to minimize the amount of suture material present at the defect site. Each animal received peri-operative Kefzol (500 mg, i.v. given during and immediately following surgery).

All dogs, including the four dogs which served as controls and in which no SIS had been implanted, had their limb immobilized with a brace to maintain

the 1.5-cm gap in the native tendon. The external brace kept the foot in dorsiflexion at an angle that assured a 1.5-cm defect size in the Achilles tendon. The brace was worn for five weeks after which time the animals were allowed ambulation without any external support. Each animal was observed daily for routine clinical parameters, including temperature, appetite, activity, and ability to bear weight upon the operated leg. In addition, a blood sample was collected weekly from each animal and a complete blood count performed.

The animals which had been implanted with the SIS material were euthanized (sodium pentothal, i.v., 90 mg/kg) at one of the following times: 1, 2, 4, 8, 12, 16, 24, or 48 weeks after surgery. The four control dogs in which no SIS was used to fill the defect were euthanized at either 4, 8, 12, or 16 weeks after surgery. At the time of sacrifice, tissue specimens were taken for: 1) mechanical properties testing that measured the uniaxial longitudinal tensile strength of the muscle-tendon unit including the distal gastrocnemius muscle, musculotendinous tissue, remodeled tendon at the surgical site, and the calcaneal insertion; 2) examination of the macroscopic and microscopic morphology of the neotendon tissue and the regional lymph nodes; 3) histochemical studies which examined for the presence of specialized connective tissues in the neotendon; 4) immunohistologic studies with monoclonal antibodies specific for porcine SIS that evaluated the chronology of the breakdown and disappearance of the implanted SIS biomaterial; and 5) biochemical studies that determined the change in collagen content of the neotendon over time.

Mechanical properties testing

Immediately following euthanasia, both Achilles tendons were removed from each animal. The entire gastrocnemius muscle and the calcaneal insertion were left intact in the excised musculotendinous unit. The calcaneus and attached tendon were embedded in Dynacastfast reproducing plastic (Kindt-Collins Co, Cleveland, OH). A 6-mm diameter rod was placed through the Dynacast block for attachment to the grips of the tensile test machine.

The gastrocnemius muscle and musculotendinous junction were reinforced with axial strands of 25 gage steel wire since this portion of the musculotendinous unit is inherently weaker than the normal Achilles tendon. The distal Dynacast block with rod attachment was inserted into the lower clamp of an MTS 810 material test system with 458.2 microconsole. The proximal gastrocnemius muscle and musculotendinous tissue were gripped with a custom manufactured 2 cycle, sinusoidal clamp that was constructed

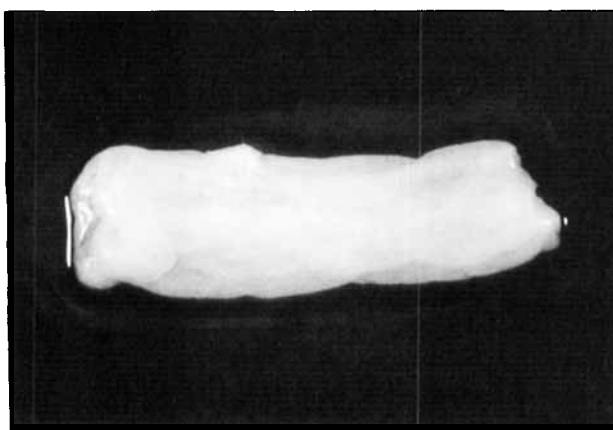


Figure 2. The SIS material is shown in its natural tube shape which tends to collapse into a double layer sheet during handling.

of titanium and had a 1.0-in-wide gripping surface. The gripping surface had a plasma sprayed and scintered surface. The clamps were manually tightened to avoid unnecessary damage to the gastrocnemius muscle. The tendon unit was then aligned, the cross-sectional area of the remodeled tendon measured, and the tendon pulled to failure in the longitudinal direction using a crosshead speed of 1 in min⁻¹. The results were recorded and the load to failure (N) was determined.

Microscopic examination

Following mechanical properties testing, nondamaged portions of the tendons were placed in 10% buffered zinc formalin solution (Z-Fix, Anatech, Battlecreek, MI) for fixation. Cross sections and longitudinal sections of the tendons were trimmed, embedded in paraffin, sectioned to 6 μ m thickness, and stained with hematoxylin and eosin (H&E), Mowry's stain for mucopolysaccharides²⁶ and Weigert's trichrome stain for elastic fibers.²⁶

Immunocytochemical examination

We have produced a monoclonal antibody (MoAB) against the extracellular matrix material of porcine SIS. This MoAB is specific for an unidentified epitope of the extracellular matrix of porcine SIS and does not cross react with SIS derived from any other species nor any other dog tissue including tendon, smooth and skeletal muscle, and skin. Immediately following mechanical testing, nonfixed portions of the excised SIS remodelled neotendons were quick frozen and cut to 6 μ m thick sections. After incubation of these sections with the mouse derived monoclonal antibody specific for porcine SIS, immunostaining of the sections was performed using the avidin-biotin complex (ABC) method of Hsu²⁷ and the Biogenex super-sensitive StrAviGen Kit (Vector Laboratories, Burlingame, CA). The slides were subsequently counterstained with Gill's hematoxylin (Sigma, St. Louis, MO).

Hydroxyproline (collagen) content determination

Following mechanical properties testing, portions of SIS grafted tendons and the contralateral normal tendons were removed, quick frozen, pulverized under liquid nitrogen, and lyophilized. Care was taken to harvest tissue only from the remodeled portion of

the tendon and not from the native tendon stump regions. Five separate 1-mg samples of each tendon specimen were then hydrolyzed in 6 N HCl at 100°C for 18 h. The hydrochloric acid was removed by evaporation under vacuum (Speed-Vac Concentrator, Savant). The residue was redissolved in water and assayed for hydroxyproline content according to the method of Stegemann and Stalder.²⁸ The hydroxyproline assay was performed in duplicate and the mean value $\pm$ SD for hydroxyproline content was determined for each normal and grafted tendon. Hydroxyproline values of the SIS grafted tendons were calculated as a percent of the contralateral control (normal) tendon for each animal. There was not enough connective tissue present in three of the four control (tendon resected but not implanted with SIS) dogs from which to conduct an hydroxyproline content assay.

Hydroxyproline closely parallels collagen content since this amino acid is unique to collagen in the tissues being assayed. However, it is not possible to accurately correlate hydroxyproline content with collagen content because the method of tissue preparation mixes all collagen types together and the specific amino acid sequence of these collagens is not known. However, hydroxyproline generally accounts for approximately 10% of the amino acids within collagen and this assay method is commonly used as an indicator of collagen content.²⁹

RESULTS

All animals recovered from anesthesia and the surgical procedure without complication. No animal had a fever, leucocytosis, or abnormal appetite. The activity of each animal was normal except for the hindered ambulation during the period of bracing. Following removal of the brace, the animals regained full use of the surgical leg over a period of four to eight weeks. Each animal varied in the amount of use and weight bearing but all animals were fully ambulatory without evidence of a limp by eight weeks after brace removal. No adhesions to surrounding adventitial structures were noted in the SIS implanted dogs. The length of the neotendon at the times of sacrifice ranged from 1.5 to 2.3 cm.

Mechanical properties testing

Determination of the ultimate load at failure for the dog Achilles tendon is problematic because of the inherent strength of the tendon compared to both the proximal musculotendinous portion and the distal in-

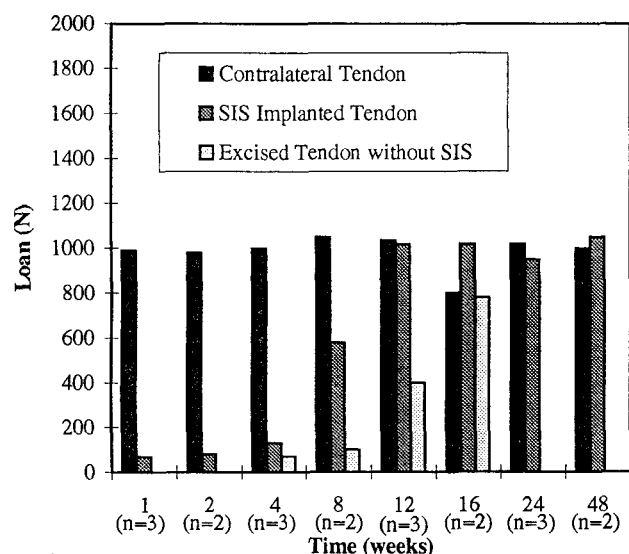


Figure 3. This histogram shows the comparative load bearing capacity of the SIS-repaired tendons over time versus the normal contralateral tendons and the control tendons in which the excised segment was not repaired with SIS material. By approximately 12 weeks, the SIS repaired tendons were stronger than either the musculotendinous origin or the calcaneal insertion which failed at approximately 1000 N.

sertion on the boney calcaneous. As described in the methods section, the musculotendinous tissue was reinforced with steel wire which was able to withstand a load up to approximately 1000 N. Likewise, the tendinous insertion on the calcaneous fails at a load of approximately 1000 N. Therefore, during the testing of the remodeled tendons and the contralateral control tendons from the dogs in this study, we obtained failure loads of approximately 1000 N whenever the tendon itself was stronger than either its origin or insertion. Stated differently, we were unable to measure the ultimate load bearing capability of the tendon itself for those tendon specimens in this study which were stronger than their muscular origin or boney insertion. However, because the experimental design accounted for tissue remodeling over time we could approximate the length of time required for remodelling process to provide a load bearing capacity which exceeded that of the gastrocnemius muscle and the calcaneal insertion.

As shown in Figure 3, all of the contralateral tendon units failed at either the musculotendinous junction or at the calcaneous with a load to failure of approximately 1000 N. All SIS implanted tendons that were tested from the 12-week timepoint and beyond also failed at approximately 1000 N in the same fashion as the contralateral tendons. The tendons harvested at 1, 2, 4, and 8 weeks failed at 64 N (mean value, range = 62–74 N), 80 N (range = 72–85 N), 130 N (range = 116–144 N), and 580 N (range = 543–612 N), respectively. All 10 of these tendons failed in the

area of the proximal juncture of the native tendon to the SIS material. None of the neotendons failed in midgauge region. The four non-SIS implanted tendons harvested at 4, 8, 12, and 16 weeks all failed in the region of the excised tissue at loads of 62 N, 82 N, 380 N, and 790 N, respectively. The tissue present at the site of the surgical excision in the four non-SIS implanted dogs consisted of disorganized scar tissue with adhesions to surrounding adventitia.

Microscopic examination

The control animals in which no SIS material was placed at the site of the excised tendon showed bands of disorganized collagenous connective tissue admixed with adipose connective tissue. The vascularity of these structures was minimal and there was only a small number of mononuclear inflammatory cells present in the tissue from the four week survivor. The 8, 12, and 16 week survivors showed increased amounts of the collagenous connective tissue but only minimal organization into bands which were oriented longitudinally along the line of weight bearing stress. There were no elastic fibers present and there was a large amount of mucopolysaccharide present based upon the staining affinity to Mowry's stain. No tenosynovium was present.

The SIS remodeled tendons which were examined after one and two weeks showed a large number of mononuclear cells, both round and spindle shaped, present in a richly vascular connective tissue matrix material (Fig. 4). The mononuclear cells often showed



Figure 4. The remodeling SIS material in the midsubstance region of the injured Achilles tendon site two weeks after surgery consists of sheets of mononuclear inflammatory cells and elongate spindle cells. These cells are embedded within a background matrix material which stains positively for mucopolysaccharides. Although not obvious in this photomicrograph, this connective tissue is very rich in capillary size blood vessels. ($\times 150$, H&E stain)

a spindle shape rather than a round shape. This matrix material stained positively for mucopolysaccharides with Mowry's stain but negatively for elastin. There was no evidence for necrosis or suppuration. The cellular and vascular components of the remodelling process extended to the anastomosis site and involved only the immediately adjacent normal tissue. The remainder of the musculotendinous unit showed normal morphology.

By four weeks, the mononuclear cell infiltrate was markedly diminished but the rich vascular component was still present. The eosinophilic staining connective tissue ground substance which was present appeared more homogeneous than at the two week time point and there was a clear organization into sheets that were aligned longitudinal to the weight bearing load (although the animals still wore a brace at this time).

The most significant change in microscopic appearance was seen at eight weeks when the mononuclear cell infiltrate was virtually gone but the connective tissue component now consisted of organized sheets of eosinophilic staining (H&E stain) material with spindle cells oriented in the longitudinal direction. The vascular component was diminished from the four week time point and there was a synovial lining cell population along the surface of the neotendons. There were no elastic fibers present based upon examination of the Weigert's stained tissues, but there was still a significant positive stain for mucopolysaccharides within the background matrix material. The histologic appearance of the regional lymph nodes showed normal node tissue with no evidence for hyperplasia or reactivity.

The histologic appearance of the 12, 16, 24, and 48



Figure 5. The histologic appearance of the remodelled SIS material was very similar to the adjacent normal Achilles tendon by 12 weeks after surgery. The arrow marks the distal juncture site and shows a smooth transition between the native tendon tissue (right of arrow) and the neotendon which developed at the site of the SIS material (left of arrow) ($\times 150$, H&E stain).



Figure 6. Macroscopic appearance of the neotendon from a dog which had a segmental resection of the Achilles tendon with subsequent SIS repair 12 weeks previously. Note the vascular mesotenon which has been left on the tendon for the purpose of illustration. The fingers show the approximate region of the tendon in which the SIS material was placed at the time of surgery.

week neotendons was very similar. All tissue showed organized sheets and bundles of connective tissue consistent with the appearance of collagen (Fig. 5). There was still a significant (but reduced from the eight week tissue specimens) amount of mucopolysaccharide present but no evidence for elastic fibers. The synovial lining cells were present in all of these specimens. It was not possible to identify the boundary (i.e., juncture) between the native tendon tissue and the neotendon except for the presence of small granulomas around the suture remnants. There was complete confluence of the connective tissue which now extended from the musculotendinous junction to the calcaneus. Macroscopically, the tendon had a uniform appearance with a mesotenon that was more vascular than the contralateral normal tissue (Fig. 6).

Immunocytochemical examination

The amount of material staining positive with the MoAB for SIS became progressively less over time. Examination of the remodelled tendons showed deposition of connective tissue matrix around all sides of the SIS material and between the bilayer of the flattened tube. By two weeks after implantation, the SIS material constituted less than 10% of the neotendon based upon the positive staining of the porcine origin biomaterial compared to the newly deposited matrix which stained negatively. The SIS material was positioned within the center of the cylindrical neotendon. By eight weeks after implantation, there were widely scattered areas of positively stained ma-

terial which were present within the central portion of the neotendon. At 12 weeks and at every sampling time thereafter, there was no longer any identifiable SIS material present within the neotendon by MoAB staining.

The contralateral tendons in all animals were negative for SIS MoAB staining. Likewise, the connective tissue that replaced the tendon defect in the four animals in which no SIS material was placed was negative for SIS MoAB staining.

Hydroxyproline content determination

The hydroxyproline content of the contralateral normal tendons from the experimental animals ranged from 7.15 to 10.89 $\mu\text{g}/\text{mg}$ dry weight. The hydroxyproline content of the sterilized SIS material used for replacement of the excised Achilles tendon was $3.80 \pm 0.78 \mu\text{g}/\text{mg}$ dry weight. Following SIS implantation there was a progressive increase in the hydroxyproline content of the neotendons over the 12-month study period (Fig. 7). Collagen biosynthesis within the SIS engrafted tendons was apparent as early as 1 to 2 weeks following implantation as indicated by slightly elevated hydroxyproline contents of 3.95–4.29 $\mu\text{g}/\text{mg}$ dry weight which represented 47–50% of the contralateral normal tendons. By eight weeks the hydroxyproline content of the neotendons

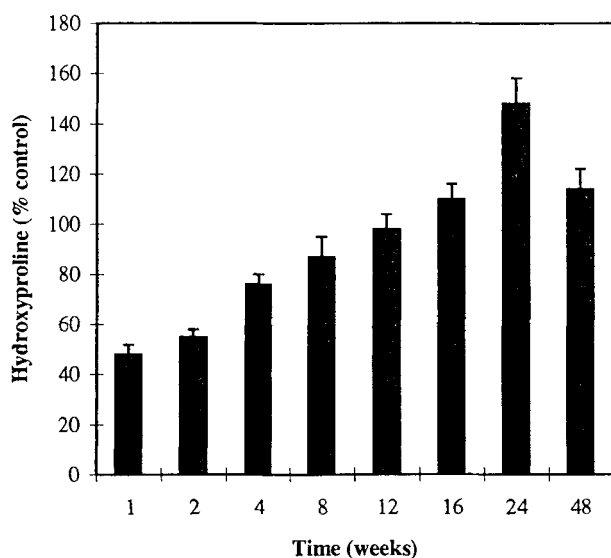


Figure 7. This histogram shows the hydroxyproline content of the SIS-remodeled tendons expressed as a percent of the hydroxyproline content of the contralateral normal tendon for each dog at each time point in the study. Note that there is a progressive increase in the amount of hydroxyproline present in the neotendons which eventually exceeds that present in the normal tendon at 24 weeks followed by a return toward 100% of the normal tendon by 48 weeks.

was 8.41 $\mu\text{g}/\text{mg}$ or 87% of the contralateral tendons. The hydroxyproline content of the neotendons was 148% of the contralateral tendons at 24 weeks and returned to 114% by week 48.

The hydroxyproline content of the connective tissue that formed within the tendon defect of the four dogs that had no SIS implanted could not be determined because of inadequate amounts of tissue present in the defect area for assay.

DISCUSSION

This study showed that the xenogeneic SIS material stimulates the rapid deposition of connective tissue in an Achilles tendon defect in this animal model. Furthermore, the newly deposited connective tissue becomes organized along the lines of stress and is comprised of hydroxyproline-rich connective tissue consistent with collagen and proteoglycans. The neotendon has mechanical strength in excess of the adjacent musculotendinous junction and the calcaneal insertion by 12 weeks after implantation. Finally, the findings of the present study suggest that the implanted SIS material becomes degraded and removed from the site of implantation leaving only a host derived replacement connective tissue that has the functional and morphologic characteristics of the original tendon. Since the findings are based upon an *n*-value of two or three at each time point, the conclusions must be considered preliminary. However, the uniformity of the morphologic and biomechanical results between animals at each time point, the vascular and connective tissue response, the type of cellular infiltrate, and the disappearance of SIS over time are consistent with the findings of earlier studies in which SIS was used to replace other types of tissues.¹⁻⁶

The morphologic appearance of the one and two week tendon defects in which SIS was placed showed a diffuse accumulation of mononuclear cells within a very vascular connective tissue matrix material. The population of cells and blood vessels was present within and around the remodeling SIS material and was indistinguishable from the tissue response observed when SIS is used as a vascular graft material²⁻⁵ and when reconstituted collagen was used as a meniscus.³⁰ The lack of any significant lymphoid and plasma cell population in this infiltrate and the regional lymph node was inconsistent with an immune rejection phenomenon to this xenogeneic material.

Acellular collagen based materials are reported to have minimal antigenicity³¹ and several collagen based medical devices are currently being tested as meniscal replacements and as augmentation devices for dermis and internal urinary sphincter in humans.

The cellular infiltrate which occurred during the first few weeks of the present study were likely part of the inflammatory response to the injury itself and to the presence of a devitalized collagen matrix material such as SIS. Alternatively or simultaneously, these cells may have represented the source of the immature connective tissue matrix material that was deposited in the first two weeks after SIS implantation. The rich vascularity seen at these early timepoints was consistent with the same early neovascularization observed when SIS was used as a vascular graft in earlier studies.²⁻⁵

By four weeks after SIS implantation, the mononuclear cell infiltrate had markedly diminished but the vascular component remained with the extensive extracellular hydroxyproline-rich matrix material that filled the defect area. The difference in the amount of matrix material present and the organization of the matrix material between the SIS-treated and the non-treated dogs appears to have been caused by the SIS material itself. Other variables that may have contributed to the amount and organization of this matrix material were controlled. The Mowry's stain and the Weigert's stains, in conjunction with the hydroxyproline assays, support the concept that this matrix material constituted an immature collagen rich extracellular material rich in mucopolysaccharides. The spindle cells present by two to four weeks were indistinguishable from fibroblasts and these cells likely served as the source of increasing amounts of the collagen matrix.

The most dramatic change which occurred in the remodeling SIS tendons after the initial mononuclear cells infiltrate diminished was the organization and amount of connective tissue which was evident between the four and eight week time points. This period coincided with the removal of the external braces and the resumption of unrestricted weight bearing and motion by the animals. The five week period for bracing was arbitrarily chosen since we did not know a priori the mechanical strength of these remodelling tendons over time, especially in the first few weeks. However, a five week immobilization period is not significantly different from reported human studies of Achilles tendon repair in which six to eight week immobilization periods were used.¹¹ It is known that the longitudinal mechanical strength of a single tube of SIS material such as was used in the present study is approximately 15 N;^{7,8} well below that needed to support the weight of these 18 kg dogs. Therefore, the change in longitudinal tensile load bearing capacity from 130 N to 580 N over a four week time period in which the brace was removed strongly supports the concept that external stressors are important signals in tissue remodeling along with whatever inherent remodeling properties the SIS material may possess.

It was not possible to determine the ultimate strength of the completely remodeled tendons in the present study. The neotendon, like the native tendon, is inherently stronger than its musculotendinous origin and its bony insertion on the calcaneus. Therefore, when a longitudinally oriented force is applied as in the testing apparatus used for this study, the failure point will obviously be at the weakest point in the muscle-tendon unit. However, it can be surmised that from a clinical standpoint the neotendon is strong enough to withstand the customary forces applied to the Achilles tendon. The results of this study were reported as ultimate load bearing capability of the muscle-tendon unit. It would be useful to report the ultimate stress (force per unit area) property of the remodeled tendons, but since the failure point was not within the remodeled tendon tissue itself this determination was not possible.

Although not part of the present study design, the stress strain characteristics (i.e., stiffness) of the neotendon versus the native tendon material would be useful information to consider for eventual clinical application. Our histochemical and biochemical analysis of the remodeled tendon material versus the contralateral native Achilles tendon suggests that the amounts of collagen, mucopolysaccharide, and elastin are very similar. The fact that the remodelled tendon contained a greater amount of hydroxyproline rich collagen like material than the native tendon at 24 weeks and a nearly equal amount at 48 weeks indicates that the repair process is directed by a control mechanism which maintains an optimal composition of matter in the functional tendon. The length of the remodelled section of tendon at the time of sacrifice (1.5-2.3 cm) showed that a small amount of elongation may have occurred during the remodeling period.

The immunohistochemical studies conducted upon the remodeled Achilles tendons showed that the SIS material degraded over time until only host origin connective tissue remained. The MoAB used in these studies was developed in our laboratory as the result of extensive efforts to raise an antibody in mice. The difficulty in development of this MoAB is consistent with the low antigenicity of the SIS biomaterial. The epitope against which the MoAB is directed has not been determined at this time. Therefore the conclusion regarding disappearance of the SIS material in the remodeling tendon cannot be made about the entirety of the SIS material. It is possible that certain portions of the SIS material remained within the neotendon. However, it is clear from the sheer mass of the remodeled tendon and the distribution of the newly deposited connective tissue that any remaining SIS material would be buried deeply within the central portions of the neotendon.

The present study showed that SIS caused a con-

nective tissue remodeling phenomenon to occur within the Achilles tendon location that was similar to that observed within other tissue locations in which SIS has been studied. The material served as a scaffold around which the body was stimulated to deposit appropriate connective tissues for the location in the body in which the material was placed and for the functional activity of the tissue. Furthermore, SIS did not appear to be associated with some of the complications that limit the use of other synthetic materials and biomaterials for this application such as infection, foreign body reaction, and disorganized scar tissue formation.

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