The novel use of resorbable Vicryl mesh for *in vivo* tendon reconstruction to a metal prosthesis

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We examined the mechanical properties of Vicryl (polyglactin 910) mesh *in vitro* and assessed its use *in vivo* as a novel biomaterial to attach tendon to a hydroxyapatite-coated metal implant, the interface of which was augmented with autogenous bone and marrow graft. This was compared with tendon re-attachment using a compressive clamp device in an identical animal model. Two- and four-ply sleeves of Vicryl mesh tested to failure under tension reached 5.13% and 28.35% of the normal ovine patellar tendon, respectively. Four-ply sleeves supported gait in an ovine model with 67.05% weight-bearing through the operated limb at 12 weeks, without evidence of mechanical failure.

Mesh fibres were visible at six weeks but had been completely resorbed by 12 weeks, with no evidence of chronic inflammation. The tendon-implant neoentheses was predominantly an indirect type, with tendon attached to the bone-hydroxyapatite surface by perforating collagen fibres.

The replacement of large bony skeletal segments in tumour and revision arthroplasty requires the attachment of tendons to enable function. The ability to achieve sound fixation of soft tissues to metal would be a significant advance. The methods currently employed include direct mechanical clamping of the soft tissues,¹ fixation via loops on the prostheses² and non-absorbable augmentation materials.³⁻⁶ Experimental research in this field has been directed towards creating a neoentheses using combinations of implant surface characteristics, bone and marrow grafts and, recently, recombinant human osteogenic protein-1.⁷⁻¹¹ We have used tendon clamping devices with an interlocking spike mechanism to provide adequate mechanical stability for the construct.^{7,8}

Rempel and Abrahamsson¹² demonstrated that under hypoxic conditions tendon explants showed significantly reduced collagen synthesis. Tissue oxygen tensions of 20 mmHg to 30 mmHg have been shown to be required for the secretion of collagen by fibroblasts¹³ and are also necessary for energy-dependent metabolic processes, cell proliferation and epithelialisation.^{14,15} We believe that the current advances in interface biology might therefore be more successful if appropriate mechanical stability could be achieved without physical compression of the soft tissues.

Absorbable and non-absorbable meshes have been investigated and are currently being

used as augmentation materials in both orthopaedic and non-orthopaedic fields.¹⁶⁻²³ Vicryl (polyglactin 910) mesh (Johnson and Johnson Intl., St. Stevens-Woluwe, Belgium) is an uncoated, undyed synthetic copolymer that comprises 90% glycolide and 10% L-lactide, and is both inert and non-antigenic.²⁴⁻²⁶ It is reported to be totally resorbed after 60 to 90 days *in vivo*, and is supplied as a knitted mesh with a single layer tensile strength of 250 N, used as a scaffold during soft-tissue healing.

In this study we tested the hypothesis that a tendon can be attached to a metal implant using Vicryl mesh, which with biological augmentation will support the development of an interface having superior mechanical and biological properties to those of a compressive soft-tissue clamp.

Materials and Methods

***In vitro* investigations.** Single sheets of Vicryl mesh were folded into two-ply and four-ply sleeves, 40 mm x 25 mm in dimension. The ends of the sleeves were clamped between the plates of a Zwick (Zwick GmbH & Co., Ulm, Germany) (Fig. 1) mechanical testing machine and were tested to failure under tension at a rate of 200 mm per minute without preconditioning. Intact patella-patellar tendon-proximal tibia specimens were harvested from skeletally mature fresh cadaver Friesland ewes (65 kg to 85 kg) and were held in customised jigs for testing using the same procedure.

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©2006 British Editorial Society
of Bone and Joint Surgery
doi:10.1302/0301-620X.88B9.
17748 \$2.00

J Bone Joint Surg [Br]
2006;88-B:1245-51.
Received 7 February 2006;
Accepted 12 April 2006

Fig. 1

A four-ply mesh construct undergoing mechanical testing under tension to failure on a Zwick mechanical testing machine.

Fig. 2

Patellar tendon attached with a four-ply mesh sleeve.

In vivo study. The study was approved under the UK Home Office Animals (Scientific Procedures) Act 1986.

Implant. A customised titanium alloy (Ti-6Al-4V) base plate (30 mm in length) was produced to simulate the extramedullary surface of a proximal tibial replacement. It was plasma-sprayed with a 70 µm coating of CAPITAL Hydroxyapatite (HA) (Plasma Biotol Limited, Tideswell,

United Kingdom). The base plate had six press-fit spikes (1 mm in diameter and 4 mm in length) as anchor sites for the tendon in the mesh sleeve.

Study design. We used eight skeletally-mature Friesland ewes, weighing between 65 kg and 85 kg (mesh group). The patellar tendon was attached to the surface of a metal implant using a four-ply sleeve of Vicryl mesh (Fig. 2) supplied sterile (by ethylene oxide) from the distributor. The tendon-implant interface was augmented by an autogenous cancellous bone and marrow graft. Two animals were killed six weeks after the operation and the tendon-implant interfaces were examined histologically. The remaining six animals in the mesh group were killed at 12 weeks. The results were compared to those of a previously reported experimental group of six animals in which the patellar tendon was attached to a custom-made metal implant using a spiked compressive H-shaped clamp lid, with the interface biologically augmented with an autogenous bone and marrow graft of similar wet weight and consistency (autograft group).^{7,8} The mesh and autograft groups were compared histologically with intact patellar tendon insertions from the non-operated hindlimb of the autograft group. Data from the operated and un-operated hindlimbs was collected in a previous study.⁸

Surgery. The animals were given a subcutaneous premedication injection of xylazine hydrochloride (0.2 mg/kg; Bayer plc, Bury St. Edmunds, United Kingdom). Anaesthesia was induced using intravenous midazolam (2.5 mg stat dose; Roche Products Limited, Welwyn Garden City, United Kingdom) and ketamine hydrochloride (2 mg/kg; Fort Dodge Animal Health Ltd., Southampton, United Kingdom) and was maintained with halothane 3% (Merial Animal Health Ltd., Harlow, United Kingdom) and oxygen (4 l/min). The right patellar tendon was defined and elevated from the tibial tuberosity. An osteotomy was performed at the insertion site to create a flat bone bed to which the base plate was attached using two 2.7 mm self-tapping cortical bone screws (Synthes, Stratec Medical Ltd., Welwyn Garden City, United Kingdom). A single layer of Vicryl mesh was laid on the base plate, onto which a slurry of autogenous cancellous bone chips and marrow (1.5 g wet weight), harvested from the ipsilateral iliac crest, was packed along the whole length of the HA-coated surface of the implant. In the mesh group the patellar tendon was sutured using Vicryl between the middle layers of a four-ply sleeve of Vicryl mesh using size 0 Vicryl. The tendon-mesh construct was pressed on to the base plate spikes and held with a single screw through the distal extent of the mesh sleeve; the whole construct was sealed to retain the graft by suturing the outer extensions of the base layer of mesh together. The animals were given subcutaneous doses of ceftiofur antibiotic (1 mg/kg; Pharmacia & Upjohn, Stockholm, Sweden) intra-operatively and for five post-operative days, and intramuscular buprenorphine hydrochloride (10 µg/kg; Reckitt & Colman Products Ltd., Melton Mowbray, United Kingdom) for one post-operative

day. The animals were allowed to mobilise freely within a pen (3 m x 4 m).

Force plate. The animals underwent force plate assessment (Kistler Biomechanics Limited, Alton, United Kingdom) pre-operatively, and after six and 12 weeks. The data from 12 passages over the force plate were collected for each hindlimb for each animal. Using in-house custom-written software, the mean peak vertical (z coordinate) component of the ground reaction force (GRF), normalised for the weight of the animal ($F_{\max}/\text{weight}$), was obtained for both hindlimbs. We calculated the mean right hindlimb peak vertical (F_z) GRF as a percentage of the mean left hindlimb peak vertical (F_z) GRF (operated/non-operated as a percentage), and this was expressed as functional weight-bearing. Comparisons were made between the experimental groups at six and 12 weeks. The change in post-operative $F_{\max}/\text{weight}$ between six and 12 weeks was also determined.

Histological analysis. The operated patella-patellar tendon-implant specimens were harvested and fixed in 10% formalin for ten days. The samples underwent ascending graded alcohol dehydration, defatting in chloroform for five days, and embedding in LR White Hard Grade Resin (London Resin Company Limited, Reading, United Kingdom). The sections were cut, ground, and polished to 100 μm thickness, stained with toluidine blue and paragon, and underwent qualitative morphological assessment using an Olympus BH2 microscope (Olympus Optical Company Limited, Tokyo, Japan) linked to Zeiss KS300 3.0 image analysis software (Imaging Associates, Thame, United Kingdom). Three sections at one-third width intervals across the tendon-implant specimen were prepared for each animal. For each section, five fields of view along the length of the base plate were observed at a magnification of $\times 100$. Using Adobe Photoshop version 6.0.1 (Adobe Systems Inc., San Jose, California), a double-blind quantitative analysis of collagen fibre orientation at the implant surface was performed by measuring the angles of elevation of the collagen fibres per field of view, with respect to the whole length of the HA-coated base plate. The orientation of the collagen fibres in the normal patellar tendon-bone enthesis was similarly measured, using the tangent to the surface of the tibial tuberosity as a reference point. Comparisons were made between the mesh group, the autograft group, and the non-operated limbs of the autograft group (intact group). Morphological comparisons were also performed between the experimental and intact groups.

Statistical analysis. Numerical data were entered through SPSS version 12.0 for Windows (SPSS Inc., Chicago, Illinois) and analysed using non-parametric tests. The Mann-Whitney U test was used to examine differences between the *in vitro* mechanical testing data and the force plate functional weight-bearing and quantitative histological data. The Wilcoxon signed-rank test was used to examine differences in functional weight-bearing status between six

Fig. 3

Force-displacement graph for two- and four-ply mesh constructs and intact patellar tendon specimens.

and 12 weeks post-operation between groups. A result of $p < 0.05$ was considered to be significant.

Results

***In vitro* investigations.** Under loading, all of the mesh sleeves failed in their mid-substance. The median values (with 95% confidence intervals (CI)) of stress at failure were 129.12 N (68.32 to 212.10), and 713.32 N (681.68 to 744.95) for the two-ply and four-ply sleeves, respectively. The intact fresh cadaveric patella-patellar tendon-proximal tibia samples failed in their mid-substance at 2516.2 N (2272.32 to 2974.78), which was significantly higher than the four-ply mesh sleeve results ($p = 0.004$) (Fig. 3). Two- and four-ply sleeves of Vicryl mesh tested to failure under tension reached 5.13% and 28.35% of the normal ovine patellar tendon, respectively.

***In vivo* study.** All of the animals recovered from surgery well, and mobilised freely post-operatively. There was no infection or failure due to pull-out. At six weeks, the functional weight-bearing results (percentage operated/control limb – median with 95% CI) were 66.73% (51.40% to 79.40%) and 46.91% (35.22% to 54.11%) for the autograft and mesh groups, respectively ($p = 0.016$), while at 12 weeks the results were 90.55% (58.99% to 101.36%) and 67.05% (50.68% to 87.45%) for the autograft and mesh groups, respectively ($p = 0.201$).

The differences in functional weight-bearing status for the operated limb between six and 12 weeks ($F_{\max}/\text{weight}$) (median with 95% CI) were 4.61 (2.91 to 7.72) and 7.07 (3.93 to 10.13) for the autograft and mesh groups, respectively ($p = 0.345$) (Fig. 4). For the non-operated limb the values were -2.37 (-7.61 to 4.06) and -12.82 (-20.07 to -5.03) for the autograft and mesh groups, respectively ($p = 0.043$) (Fig. 4).

Fig. 4

Box-and-whiskers plot showing the difference in $F_{max}/weight$ between six and 12 weeks for the operated and non-operated hindlimbs (% of outlying data points indicating that 2 data points lie outside the interquartile range).

Histology. The normal patellar tendon insertion consisted of normal tendon attached via a layered direct-type enthesis.^{8,27} The median angle (with 95% CI) of collagen fibre orientation tangential to the insertion was 33.00° (29.58° to 40.24°). Macroscopically, the mesh group samples consisted of a dense collagenous attachment continuous from the patella to the implant base plate, with no evidence of failure due to pull-out. At six weeks, the tendon consisted of a dense collagenous connective tissue with parallel-orientated fibres displaying a crimp pattern similar to that of the normal tendon. At the periphery of the tendon the mesh fibres were observed clearly, with interweaving collagen fibres and a low-density inflammatory cell infiltrate of lymphocytes and macrophages (Fig. 5a). At the implant surface there was poor retention of the morcellised graft, which was seen both intimately associated with the HA surface and also within the tendon. The regions of tendon-bone interface were disorganised and it was impossible to make quantitative measures of fibre orientation (Fig. 5b). In areas where the morcellised graft had been poorly retained, the tendon-HA interface showed predominantly fibrous tissue encapsulation with a dense collagenous layer which was orientated parallel to the implant surface.

At 12 weeks no mesh fibres were observed and there was no evidence of a residual chronic inflammatory process. The tendon substance was normal and the tendon-bone interface regions had reorganised. The interface showed predominantly an indirect-type of enthesis, with Sharpey-like collagen fibres spanning between tendon and bone (Fig. 5c). Fibrocartilage-containing regions were also observed,

displaying characteristics more like those of a normal direct-type enthesis (Fig. 5d). The interface in the autograft group has been previously described at 12 weeks as a layered tendon-fibrocartilage-bone-HA attachment resembling an indirect enthesis.⁸ The angles (median with 95% CI) of collagen fibre orientation tangential to the implant surface were 17.80° (14.25° to 18.76°) and 28.30° (26.62° to 35.04°) for the autograft and mesh groups, respectively. The orientation angles were significantly greater for the mesh and intact groups than for the autograft group ($p = 0.000$). No significant difference was observed between the mesh and intact groups ($p = 0.087$).

Discussion

We have previously reported the initial investigations using an prototype tendon clamp and shown tissue necrosis *in vivo*, owing to the prevention of vascularisation because of the combination of mechanical pressure and inadequate exposure.⁷ Subsequent modifications to the lid of the clamp to an H shape with perforations allowed more access from overlying vascular tissue, while providing the same mechanical fixation. Augmentation of the tendon-implant interface with autogenous bone and marrow graft supported the development of a neoenthesis.⁷ This device provides adequate mechanical stability to allow early mobilisation during biological development of the neoenthesis.⁸ At 12 weeks the resulting enthesis contained regions of both direct and indirect-type insertions.⁷ We hypothesised that if non-compressive tendon fixation could be achieved, thus allowing an open environment for healing, a mechanically and biologically superior enthesis might develop compared with the closed clamp system.

Previous studies have reported the development of immature tendon-bone-implant entheses when compressive forces are applied with a clamp device.^{8,10,11,28} The detrimental effects of compression on tissue healing are well established and include ischaemia resulting from increased pressure, reduced microvascular flow, and limited delivery and removal of cellular substrates and metabolites, respectively,¹² and reduced oxygen tension limiting cell proliferation, proteoglycan and protein synthesis.^{13,14}

The mesh provided adequate mechanical support *in vivo* to allow gait, and matched the predicted values expected from the testing *in vitro*, where the construct failed in the region of 700 N. Using an implantable patellar tendon transducer, Korvick et al²⁹ demonstrated in a goat model that in normal walking, the tendon experiences a maximum force of about 800 N. We selected a four-ply sleeve, with the tendon between the middle layers, to minimise the volume of material between tendon and the graft retained by the basal mesh layer overlying the implant. Because we did not experience any *in vivo* failures we feel that this sleeve is a sufficient attachment; however, we appreciate that further *in vitro* testing is necessary, as well as similar mechanical studies of the tendon-clamp construct. The animals in the mesh group did compensate with increased weight-bearing

Fig. 5a

Fig. 5b

Fig. 5c

Fig. 5d

Photomicrographs showing a) collagen/mesh fibres interweaving in the peripheral tendon in the mesh group at six weeks (toluidine blue and paragon, x 50), b) the tendon-bone interface in the mesh group at six weeks (toluidine blue and paragon x 10), c) the indirect-type tendon-bone enthesis with Sharpey-like fibres spanning the interface in the mesh group at 12 weeks (toluidine blue and paragon, x 50), and d) the fibrocartilage-containing direct-type enthesis in the mesh group at 12 weeks (toluidine blue and paragon, x 50).

through the contralateral non-operated limb after six weeks *in vivo*. By 12 weeks, compensation by the non-operated limb was reduced but still observed in the mesh group, although despite this, no significant difference in functional weight-bearing was observed between the groups at this time point.

The orientation of the collagen fibres in a normal enthesis varies with the contour of the tibial tuberosity. Variation in fibre orientation with respect to the bone-implant surface has been shown to reflect the type and integrity of the resulting attachment.³⁰ We accept that the osteotomy angle may have contributed to changing the local mechanical environment, and that this may have influenced our

findings; however, the mean angle of insertion in the mesh group was not significantly different from that in the intact controls. The regions of less-organised indirect-type enthesis consisted of more perpendicularly orientated fibres perforating the bone, whereas in the more organised direct-type enthesis the collagen fibres approach the insertions at a more shallow angle. In addition, it has been shown that a tendon-HA attachment develops with collagen fibres orientated parallel to the implant surface, indicative of fibrous encapsulation.^{7,8} We observed that the mesh group contained regions of both indirect-type enthesis and of tissue encapsulation, and the resulting median collagen fibre orientation angle reflects this, as

opposed to being truly representative of the degree of maturation of the neointegration. The lack of significant difference between the mesh and intact group specimens could therefore be coincidental.

We postulate that the less compressive environment in the mesh group fixation was responsible for the lower degree of fibrocartilaginous tissue observed compared with the autograft group.⁸ Hence the predominantly tensile forces in the mesh group attachment led to the development of a more fibrous indirect enthesis. A longer term study is required to determine whether the mesh group interface would remodel into a direct-type enthesis, as observed in previous tendon-bone tunnel healing models.³¹ Lack of mechanical compression did result in poorer graft retention, and we believe that a further study using a bone block should overcome this problem.

The biocompatibility and applications of polylactic acid and polyglycolic acid copolymers have been previously reviewed by Athanasiou et al.²⁶ They emphasised that most studies have demonstrated no toxicity, minimal evidence for local tissue inflammation or host reaction, and indeed in some cases the copolymers have been implicated in accelerated fracture healing, making them attractive materials for 'delivery vehicles, grafts and scaffolds for neo-tissue growth'. More recent work has demonstrated that polylactide/glycolide polymers show better tenocyte adhesion strength when modified with extracellular matrix cell adhesion proteins, including fibronectin and type I collagen.³² However, a negative effect due to a weak inflammatory response and the acidic environment resulting from polymer degradation could prove detrimental to progenitor cells involved in the healing environment.³³ Roberts et al.²⁰ used polyglactin mesh experimentally in a model of acute Achilles tendon lacerations in a rabbit showing mild diffuse lymphocytic infiltrate and no discernible evidence of the biomaterial at 20 weeks, but equally no perceived advantage mechanically compared to direct suture repair. Cao et al.³⁴ used polyglycolic acid for tendon engineering in a hen model and observed a residual inflammatory reaction at eight weeks, with degradation of the biomaterial. The neotendon appeared normal by 14 weeks, with a breaking strength of 83% of normal. Sato et al.³⁵ used braided polylactic acid fibres woven as a neotendon in a rabbit model for Achilles tendon reconstruction, showing a mean strength compared with the intact side of 61% at 26 weeks, and good integration with type I and III collagen-containing fibrous tissue.

The Vicryl mesh used in this study provided a novel means of attaching a tendon to a HA-coated metal implant, with potential useful clinical implications. It provided sufficient initial mechanical fixation to enable early mobilisation and weight-bearing at 12 weeks, not significantly different from a mechanical clamp. Despite the potential detrimental effects of the mesh degradation products, an enthesis developed with regions of both direct and indirect types at 12 weeks. Poor retention of morcellised graft could

be reduced using a bone block. This requires further investigation.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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