4D Imaging of Soft Tissue and Implanted Biomaterial Mechanics; A Barbed-Suture Case Study for Tendon Repair

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**4D Imaging of Soft Tissue and Implanted Biomaterial
Mechanics; A Barbed-Suture Case Study for Tendon Repair**Shelley Dyan Rawson, Tom Shearer, Tristan Lowe, Marie
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suture, biomaterial, tendon, tissue engineering, tomography, medical devices

ABSTRACT

Timely, recent developments in X-ray micro-computed tomography (X μ CT) imaging such as increased resolution and improved sample preparation are enabling non-destructive time lapse imaging of polymeric biomaterials when implanted in soft tissue, which we demonstrate herein. Imaging the full 3D structure of an implanted biomaterial provides new opportunities to assess micromechanics of the interface between implant and tissues, and how this changes over time as force is applied in load bearing musculoskeletal applications. In this paper we present a case study demonstrating *in situ* X μ CT and FE analysis, using a dynamically loaded barbed suture repair for its novel

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3 use in tendon tissue. The aim of this study was to identify the distribution of
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6 stress in the suture and tendon as load is applied. The data gained
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9 demonstrates clear 3D visualization of micro-scale features in both the tissue
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12 and implant in wet conditions. X μ CT imaging has revealed, for the first time,
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15 pores around the suture, preventing full engagement of all the barbs with the
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18 tendon tissue. Subsequent finite element analysis reveals the localized stress
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21 and strain, which is not evenly distributed along the suture, or throughout the
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24 tissue. This case study demonstrates for the first time a powerful *in situ*
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27 mechanical imaging tool, which could be readily adapted by other laboratories
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30 to interrogate and optimize the interface between implanted biomaterials and
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55 1 INTRODUCTION

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Assessing new implantable medical devices in preclinical models is a resource heavy necessity which would benefit from advanced validation tools. The advancement of novel medical treatments necessitates the development of improved means with which to interrogate the new therapeutic devices and technologies. Recent developments across several areas of imaging such as X-ray micro-CT (X μ CT) have culminated in the timely ability to allow 4D imaging of soft tissue deformation. 3D studies of soft tissue can be achieved by confocal microscopy, serial sectioning or serial block face imaging; however, these methods are limited by a small field of view, can be technically challenging and can introduce imaging artefacts. Furthermore, serial sectioning and block face imaging are destructive, preventing the progressive application of force over time within the same sample ¹. 4D X μ CT studies of implanted devices in an aqueous environment permit validation of finite element (FE) modelling from which further prototypes may be developed *in silico*. Acquiring

validated soft tissue mechanics via X μ CT and *in silico* modelling permits a faster and more cost effective approach towards product development.

To enable soft tissue X μ CT imaging, previous literature has described sample staining using Osmium Tetroxide as a contrast agent ² and other less toxic stains have since been described, making sample staining more routine ³. Whilst these stains offer good tissue contrast, their effects on soft tissue mechanics limits their use to static imaging. Phase contrast imaging improves the visibility of edges between features of similar X-ray attenuation, and has been exploited on synchrotron systems for biological imaging since the mid 90's ⁴, and is now attainable using laboratory X-ray sources ⁵, making this technology more accessible. Phase contrast does not affect soft tissue mechanics; however, blurring of edges can make their exact position ambiguous, and imaging remains difficult at boundaries between materials of very similar X-ray attenuation such as water and tendon. X μ CT can now be performed at a spatial resolutions of <50nm ⁶, and when used in tandem with

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3 traditional techniques such as histology, EDX, SEM or TEM, a wealth of 3D
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6 structural and compositional data can be obtained ⁷. This is furthered by the
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9 potential of using X μ CT to inform Finite Element modelling, obtaining
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12 quantitative stress and strain data, which is becoming commonplace in
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15 osteoporotic bone density and strength studies ⁸⁻⁹ which can be validated using
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18 time lapse tomography ¹⁰. These advancements have culminated in the timely
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21 potential to concurrently interrogate the morphology, composition and
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24 mechanical response of soft tissue implants *in situ* using multi-modal techniques
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27 which we describe herein. It is with this recent advancement in CT resolution
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30 and contrast agent development that this case study has been performed in
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37 order to demonstrate capability in the biomaterial arena.
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44 Biomaterials and tissue engineering (TE) has become a diverse field seeking
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47 to mimic, grow and regenerate all tissues and organs of the body. Biomaterials
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50 for tissue scaffolds exhibit a diverse range of morphologies, but which typically
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54 have nanoscale features, produced by electrospinning, hot embossing or soft
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lithography for example, within a construct with dimensions typically on the centimetre length scale ¹¹⁻¹². The macro- and micro-mechanical environment of a given biomaterial *in situ* is becoming increasingly important. An implant must withstand mechanical forces throughout the lifetime of the device, which is particularly poignant in load bearing applications such as muscle, tendon, abdominal wall and skin. The implant may be required to transfer mechanical forces to the regenerating tissues in the case of degradable devices, or to maintain mechanical integrity for many years in the case of non-degradable devices. In cellularised scaffolds, localised mechanical forces can influence cell homeostasis, signalling, differentiation and lineage ¹⁴. An implantable device is subject to a challenging environment, affected by chemical, biological and mechanical damage, making prediction of this multi-factorial environment challenging. *In silico* modelling is therefore a vital development in designing reliable devices with a predictable *in situ* lifespan ¹⁴. For FE modelling, the 3D geometry of the implant and the surrounding environment must first be defined. This paper presents the novel combination of tailored sample preparation, *in*

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3 *situ* mechanical testing and X μ CT to both inform and validate FE analysis. The
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7 paper demonstrates this workflow using the case study of an incrementally
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10 loaded tendon with an implanted barbed suture made of a degradable
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13 polymeric biomaterial. This CT image-validated FE technique offers important
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17 localised stress distribution data sets on a loaded polymeric medical device
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20 implanted within soft tissues in an aqueous environment.
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27 1.1 Case Study; Barbed suture tendon repair

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33 We have selected the example of a sutured hand tendon repair since there is
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37 a clinical need for improved techniques within this field. Over 50,000 people
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40 are treated in the UK per annum for tendon injuries ¹⁴, over two thirds of which
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43 are hand tendon injuries ¹⁵. Zone 2 flexor tendon rupture poses a particular
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47 challenge since the tendon must glide through the flexor sheath within this
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50 region to enable flexion of the digit. Typical repairs involve re-approximating the
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54 severed ends using surgical suture, which was described in 1918 by Bunnell et
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3 al. ¹⁶. Over the decades, improvements have been made to the suture
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6 arrangement, to increase the strength of the repair and to minimise the
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9 formation of scar tissue (known as adhesions) which restricts tendon glide. In
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12 recent years, X-ray radiography has enabled detailed interrogation of failure in
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15 tendon suture arrangements ¹⁷. Many intricate suture arrangements can now be
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18 found in the literature, which have been reviewed in detail ¹⁸. Nevertheless, the
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21 procedure remains largely unchanged from that which was performed a century
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24 ago; severed tendon ends are still re-joined by non-degradable surgical suture.
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27 In recent years, several research groups internationally are investigating novel
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30 methods of tendon repair to improve prognoses, one of which is barbed suture
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33 repair ¹⁹. The research into barbed suture for tendon repair is still in its
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36 infancy.
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47 Barbed suture is said to distribute load evenly throughout the suture strand
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50 and tissue via periodic micro-scale barbs on its surface, providing superior
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53 tissue anchorage over conventional suture ²⁰. Whilst typically used in low load
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bearing tissues, barbed sutures are now being considered for high load bearing applications such as tendon repair ²¹. Their use may address the uneven stress distribution within conventionally sutured tendons ²² which is thought to incite acellular regions, observed within hours, and persisting for at least one year postoperatively ²³. A successful barbed tendon repair has the potential to benefit the 50,000 patients who undergo flexor tendon surgery in the UK per annum ¹⁴, over 25 % of whom attain poor mobility after 6 months postoperatively ²⁴. Unfortunately, however, published data to support the claims of evenly distributed stress from barbed suture is lacking. If barbed attachment is to be pursued for use in high load bearing applications such as tendon repair, load bearing characteristics must be assessed to consider how the barbs fail when overloaded, and how stress distributions may affect the healing environment of the tissue. Strength must be maintained during the initial 8 weeks of active mobilization rehabilitation ²⁵, after which sufficient collagen will have been replaced for normal activity to be resumed ²⁶. In the case of

degradable devices, the rate of degradation must allow necessary time for tissue healing.

Stress distribution within a tendon repair can be revealed using FE analysis²². One study by Ingle *et al.* performed FE analysis of individual suture barbs, considering how barb geometry can be tailored to attach to skin or tendon²⁷. Results showed that the ideal barb for skin attachment was different to the ideal geometry for tendon attachment. Furthermore, Ingle *et al.*, concluded that barbs fail by two mechanisms - bending, or peeling from the suture shaft²⁷; however, this study only considered stress distribution in individual barbs, rather than multiple barbs along a length of suture, and the study used an idealised geometry of the tendon and suture. Failure by bending or peeling may be in part due to the manufacturing technique of the suture used by Ingle *et al.*, whereby cuts were made in the suture to produce the barbs²⁸.

FE analysis can offer information on stress and deformation arising within an implanted device under force application, however, *in silico* modelling requires validation from experimental data. An FE model can be generated from X μ CT data, providing detailed 3D geometry and enabling accurate validation against experimental results of sample deformation; however, X μ CT poses significant challenges with respect to the imaging of polymeric biomaterials *in situ* within soft tissue. Sample hydration is a concern since X μ CT imaging typically takes several hours. The X-ray attenuation of a soft tissue, and polymeric implant and Good's buffer (a salt balanced solution to maintain sample hydration) is very similar, thus poor contrast would be achieved and the different structures would not be identifiable³. Heavy metal staining provides good X-ray contrast, allowing the discernment between different soft tissues, as demonstrated in a range of samples including embryos²⁻³, brain²⁹ and collagen based tissue engineering scaffolds³⁰. Iodine potassium iodide (I₂KI) is reported to provide good contrast in tendon, allowing observations of fascicles which form part of tendon hierarchical structure³². Staining does however cause tissue shrinkage,

exacerbated by longer staining duration or a higher concentration staining solution ³³. Tissue fixation is commonplace and intends to minimise the tissue shrinkage caused by staining; however, fixation affects mechanical properties due to cross-linking of the tissue ³⁴, which would affect the interaction between barbs and tendon, impacting on the results of tensile testing. Here we describe tendon surface staining without prior fixation, using a customised iodine stain designed to minimise the change in mechanical properties and tissue morphology.

This study is the first to perform 4D X μ CT imaging of an implanted biomaterial in soft tissue during *in situ* loading in an aqueous environment. The customised staining method developed for this case study has permitted X μ CT observation of a sutured tendon sample in the relaxed position, and subsequent X μ CT imaging of the same sample following suture withdrawal. X μ CT-informed FE analysis has provided details of local stresses and of tissue and biomaterial deformation. Feedback from the FE model allows validation against X μ CT

sample deformation data. Through the combination of these techniques we have investigated the viability of a novel soft tissue attachment method by identifying the deformation and stress distribution. The methods described can be readily adapted for the study of polymeric implants within soft tissue to provide 3D qualitative morphological and stress-strain data.

2 MATERIALS AND METHODS

2.1 Tendon Samples

Porcine fore-trotter *flexor digitorum profundus* (FDP) tendon is a suitable surrogate for human FDP. This is based on the robust study by Havulinna *et al.*, who conducted *ex vivo* tensile testing of sutured human cadaver FDP and porcine FDP from the central two digits, demonstrating no significant difference in failure force between the two groups ³⁵. Porcine fore-trotters from 12 month old pigs were sourced from a local abattoir (Staffordshire Meat Packers Ltd,

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4 Stoke-on-Trent) less than 24 hours after slaughter. The FDP tendons from the
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7 centre two digits of the trotters were excised (thus, 2 tendon samples were
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10 obtained from each trotter), and the central portion of the tendon was dissected
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13 down to 6cm in length (such that fibrocartilage regions were removed). Samples
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16 were submerged in Dulbecco's phosphate buffered saline (without Ca^{2+} or Mg^{2+} ;
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19 PBS; PAA Laboratories, Pasching, Austria), then immediately frozen and stored
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22 at -20°C . When required, tendons were defrosted at room temperature (RT)
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25 and immediately prepared for testing. Whilst freezing reportedly reduces tendon
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28 Young's modulus, it was required for practical purposes and is commonly
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31 performed ³¹.
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40 2.2 Staining Solutions

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47 A conventional iodine potassium iodide solution ($\text{I}_2\text{KI-dH}_2\text{O}$) was prepared as
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50 previously described ³²; 0.1% iodine metal and 0.2% potassium iodide were
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53 dissolved in distilled water at RT. I_2KI in Dulbecco's phosphate buffered saline
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(without Ca^{2+} or Mg^{2+} , PAA Laboratories, Pasching, Austria; I_2KI -PBS) was prepared by dissolving the same proportions of iodine metal and potassium iodide in PBS at RT.

2.3 Staining Time-course Study

A 6cm length section of tendon was incubated in I_2KI -PBS at 4°C with gentle tilting and rotation. At 2, 4, 6, 8 24 and 48 hours, a 2 mm length of tendon was cut from the end using a scalpel and discarded, then a 5 mm length of tendon was cut and frozen at -20°C in PBS until all time-point samples were harvested. Samples were then defrosted at RT and stacked vertically within a 1.5 ml eppendorf, secured together using cyanoacrylate adhesive (Loctite, Düsseldorf, Germany), then submerged in PBS before immediate $\text{X}\mu\text{CT}$ scanning. This arrangement was to permit simultaneous $\text{X}\mu\text{CT}$ scanning of all samples on a single scan.

Scanning was performed using the Nikon customised bay in the Manchester X-ray Imaging Facility, University of Manchester, which houses a Nikon Metris 225/320 kV system and employs a 2K x 2K Perkin Elmer 1621-16-bit amorphous silicon flat-panel detector with 200 micron pixel pitch (Nikon, Tokyo, Japan). Scanning was performed at a voltage of 60 kV and current of 175 μ A using a tungsten target. The source to sample and source to detector distances were 180.6 mm and 1400.2 mm respectively giving a voxel size of 15.4 μ m. The exposure time per projection was 1451 ms, and a total of 2001 projections were taken through 360° of rotation.

Following acquisition, data was reconstructed using Nikon Metris CTPro (Metris XT 1.6, version 2.1; Tokyo, Japan) with no beam hardening correction. Data was imported into Avizo (Version 7.0; Visualization Sciences Group, Bordeaux, France) and a non-local means filter was applied using default settings to remove noise from the data set. A virtual section from the centre of each time-point sample was exported as a .jpg for analysis using ImageJ

(version for Windows, open source software, available at <http://rsb.info.nih.gov/ij/download.html>). A 20 pixel-thick line was drawn across each tendon cross-section, intersecting the tendon centre, and greyscale values were obtained along the line (averaged over its 20 pixel thickness) and plotted against distance through the sample.

2.4 Observing Stained Tendon Morphology

Tendon samples were stained in I_2KI -dH₂O or I_2KI -PBS for 24 hours at 4°C with gentle tilting and rotation (6 replicates in each group). Two control groups were also prepared; one was incubated in PBS for 24 hours at 4°C with gentle tilting and rotation, and the other group was prepared for cryosectioning immediately, with no incubation (6 replicates in each control group). Ahead of cryosectioning, a 1 cm length of tendon was removed from the approximate centre of each sample using a scalpel. The 1 cm tendon samples were embedded longitudinally in optimal cutting temperature compound (OCT; Fisher

Scientific, Loughborough, UK) and immediately frozen using liquid nitrogen. Samples were then sectioned using a cryostat (CM3050S; Leica Biosystems, Newcastle upon Tyne, UK) at -20°C at a thickness of 50 µm. Samples were rinsed in water to remove any excess OCT. Control samples were then stained with hematoxylin and eosin to reveal tissue morphology (Fisher Scientific, Loughborough, UK). All sections were cover-slipped, then imaged using a light microscope (DMRB; Leica Biosystems, Newcastle upon Tyne, UK). It should be noted that the I₂KI-dH₂O and I₂KI-PBS sample groups have not been histologically stained.

2.5 Establishing the Tangential Modulus of Stained Tendons

Tendon samples, 6 cm in length, were stained in I₂KI-dH₂O or I₂KI-PBS for 24 hours at 4°C with gentle tilting and rotation, or left unstained as a control (8 replicates in each group). The unstained control was tested immediately with no

PBS incubation. (Preliminary data has demonstrated no significant difference in tendon tangential modulus when comparing no incubation with 24 hour PBS incubation (data not shown).)

Tendon width (h) and thickness (t) were measured at the tendon centre, and 1 cm either side, using digital vernier calipers and the mean cross sectional area (A) was calculated for each tendon using the equation of an ellipse ($A = \pi ht/4$)

Tensile testing was performed using an Instron 3344 load frame (Instron, High Wycombe, UK) and 2 kN load cell. Instron wedge-action grips (Instron, High Wycombe, UK) were positioned in the load frame at a grip-to-grip distance of 2 cm. The gripping surface was enhanced using 120 grit silicon carbide water proof electro-coated sandpaper (Asia Abrasives, Quezon City, Philippines) attached using double sided paper tape (RS, Corby, UK). Tendon samples were tightened into the grips, a 0.1 N pretension was applied, and samples

were then tested to failure in air at 0.5 mm s^{-1} . This is equivalent to a strain rate of $2.5\% \text{ sec}^{-1}$. Force and displacement were recorded at a sampling rate of 20 Hz. Tangential modulus was obtained from the approximately linear region of the stress-strain curve before calculation of the mean. Data was statistically analysed using ANOVA, with *post hoc* least significance difference test, using SPSS (version number 22) software. Data are presented as mean $\pm$ SD, a p-value less than 0.05 was considered to be significant.

2.6 The Effects of Staining on Barb Attachment

Size 2 polydioxanone barbed suture (Quill PDO-2; Angiotech Pharmaceuticals, Vancouver, Canada) was used throughout this study. This suture possesses unidirectional barbs, helically arranged around a monofilament polydioxanone suture. The suture was stained for 24 hours in $\text{I}_2\text{KI-dH}_2\text{O}$ at RT or left unstained as a control. A transverse cut was made in the centre of each

tendon and the distal end was discarded. The suture was inserted 10 mm into the tendon from the freshly cut end, exiting through the epitenon (4 replicates in each group). The excess suture was cut flush with the tendon surface.

Tensile testing was performed using an Instron 3344 load frame (Instron, High Wycombe, UK) and 100 N load cell. A Bose self-tightening tissue grip and Bose sandblasted tissue grip (Part of the Bose BioDynamic 5110 ElectroForce Instrument; Bose Corporation, Eden Prairie, Minnesota, USA) were attached to the top and bottom of the load frame respectively using custom made adapters and a PBS filled tank was positioned such that the whole sample would be submerged. The tendon was attached to the top grip, and suture was attached in the bottom grip. No pretension was applied due to the low expected failure load, in order to ensure acquisition of the full loading curve. Instead, this was accounted for during analysis by taking 0.1N as the start point of the test (thus a 0.1N pre-load was retrospectively applied). Samples were tested to failure at a rate of 0.5 mm s⁻¹. Force and displacement were recorded at a sampling

rate of 20 Hz. The maximum recorded force was taken as the failure force. The mean failure force of each experimental group was calculated. Significance was determined using the student t test and P values ≤ 0.05 were considered significant.

2.7 X μ CT Scans of Sutured Tendons

A transverse cut was made in the centre of the excised tendon sample and the distal end was discarded. The tendon was incubated in I₂KI-PBS for 24 hours at 4°C. Meanwhile, Quill PDO-2 barbed suture was incubated in I₂KI-dH₂O for 24 hours at RT. The suture was inserted 10 mm into the tendon from the freshly cut end, exiting through the epitenon. The excess suture was cut flush with the tendon surface. The sample was inserted into custom made tensioning apparatus which applies tension to the sample via rotation of a nut that withdraws a threaded shaft (Fig. 1). The apparatus permits submersion of

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3 the sample throughout testing to maintain hydration. The tendon end was
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7 secured in position using cyanoacrylate adhesive (Loctite, Düsseldorf, Germany)
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10 and the suture end was secured in position using rapid drying epoxy adhesive
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13 (Bostik, Milwaukee, Wisconsin, US). After 5 minutes of drying time, PBS was
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17 added to submerge the tendon.
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23 Two samples were prepared: sample 1 for the large displacement and sample
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27 2 for the small displacement X μ CT scan. Scanning was performed using the
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30 equipment and settings described in Section 2.3 with the following exceptions.
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33 For the large displacement study (sample 1) the source to sample and source
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36 to detector distances were 71.5 mm and 1407.0 mm respectively giving a voxel
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39 size of 10.2 μ m. For the small displacement study (sample 2) the source to
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42 sample and source to detector distances were 71.0 mm and 1400.2 mm
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45 respectively giving a voxel size of 10.0 μ m. After the initial scan with the
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48 sample in the relaxed position, tension was applied to withdraw the suture and
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51 a subsequent scan was performed. A 15 minute delay was imposed between
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the application of tension and the start of the next scan to allow for tissue relaxation, and thus prevent sample movement during scanning which would otherwise compromise the X μ CT scan. (15 minutes was determined sufficient in preliminary stress relaxation experiments, data not shown.) The large displacement sample was scanned at 0, 2 and 4 mm suture withdrawal. The small displacement sample was scanned at 0 and 0.5 mm suture withdrawal. The large and small displacement increments were selected to observe barb bending up until failure, and for validation of the FE model respectively.

Data was reconstructed using Nikon Metris CTPro (Metris XT 1.6, version 2.1; Tokyo, Japan) with no beam hardening correction. Data was imported into Avizo (Version 7.0; Visualization Sciences Group, Bordeaux, France), the non-local means filter was applied with default settings and segmentation was then performed manually. Data was resampled resulting in a voxel size of 40.8 μ m and 40.0 μ m for the large and small displacement samples respectively and surfaces were generated. The length and angle of each barb was measured

from each scan. Barb length was measured as the chord from the root of the barb to the tip of the barb (chord R-T; Fig. 2.b). A second chord was drawn from the root of the barb, to the tip of the barb cut-line on the suture shaft (chord R-C). Barb angle was measured as the angle between the chord R-T and chord R-C (Fig. 2.b).

Figure 1. (a) Schematic detailing the tension rig apparatus, and (b) flow diagram detailing how tension is applied to the sample using the rig.

2.8 FE Analysis; Small Displacement Study

The segmented data from the small displacement study (sample 2) scanned in the 0 mm suture withdrawal position was exported from Avizo as a stack of .tiff images and imported into ScanIP (Simpleware, Exeter, UK). Following a mesh convergence study (data not shown), the full data set was down-sampled to 30% of the original scan, which was required to produce a mesh of suitable compromise between accuracy and computational cost. All contact pairs were added between the tendon and suture, then a mesh of 10-node tetrahedral (Abaqus code: C3D10) elements was produced and exported from ScanIP as an Abaqus input file (version 6.14-2; Simulia, Providence, Rhode Island, USA). Two Abaqus meshes were produced via this method, one with and one without air filled pores (containing 331,659 and 303,088 elements respectively), which were observed within the tendon during X μ CT scans (Section 3.2).

Following data import into Abaqus, the tendon was described as transversely isotropic linear elastic using the engineering constants obtained from homogenisation of a microstructure model where fibril modulus was 1600 MPa, matrix modulus was 1 MPa, and Poisson's ratio was 0.3²². The values of the engineering constants were as follows: Young's modulus (E) values: $E_1 = E_2 = 4.751048729$ MPa, $E_3 = 962.99.9984$ MPa; Poisson's ratio (ν) values: $\nu_{12} = 0.253220532$, $\nu_{13} = \nu_{23} = 0.022529523$; Shear modulus (G) Values: $G_{12} = 1.181960429$ MPa, $G_{13} = G_{23} = 45.64068793$ MPa (10 significant figures are necessary to fully describe the material). The tendon orientation of anisotropy was defined such that stiffness was greater in the direction parallel with the long axis of the tendon. The suture was described as isotropic linear elastic with a Young's modulus of 1 GPa, a Poisson's ratio of 0.4, and the coefficient of friction was set to 0.005 as described previously²². Three boundary conditions were applied to withdraw the suture by 0.5 mm, to hold the tendon in position and to prevent free body movement (Fig. 2.a). This simulates the

loading conditions which were employed in the 0.5 mm suture pull X μ CT scan during the small displacement study (sample 2).

Once the resultant stress and deformation were obtained from the FE analysis, the angles of barbs were measured from the FE result of the deformed shape following 0.5 mm simulated suture withdrawal, along with the X μ CT data of sample 2 in the unloaded and 0.5 mm suture withdrawal position. These barb angle measurements were made as described in section 2.7 and are shown in Fig. 2.b.

Figure 2. (a) X μ CT-informed FE model, showing segmented data from the small displacement study (sample 2) in the unloaded position (used to inform the FE model geometry), and the boundary conditions applied to the FE model, noted in red (grey = tendon, blue = suture, green = air-filled pores. Scale bar = 4 mm). (b) Illustration showing the measurement of barb length and angle from FE results. (The same method was used for barb measurements of CT data.)

3 RESULTS

3.1 Development of the Staining Method

Uniaxial tensile testing of stained and unstained tendon demonstrated no significant difference in tendon Young's modulus between the two stained sample groups, and the unstained control tendon group which was tested immediately after excision (Fig. 3i).

Images of the tendon sections following 24 hours of staining revealed increased tissue damage from the I_2KI-dH_2O stain (Fig. 3d and 3h), compared with the $I_2KI-PBS$ stained sample (Fig. 3c and 3g).

No difference is observed when comparing tissue morphology of the unincubated controls (Fig. 3a and 3e) with those incubated in PBS for 24h (Fig. 3b and 3f) observed across all replicates. The characteristic crimp formation of the tendon collagen fibrils is also clearly visible in the samples incubated for 24h in $I_2KI-PBS$ (Fig. 3c and 3g) observed across all replicates. Samples

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3 incubated for 24h in I₂KI-dH₂O (Fig. 3d and 3h) present a different morphology
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6 compared with the un-incubated control; fibrils appear separated from each
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9 other, and the crimp appears disrupted. This was apparent in 4 out of the 6
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12 replicates. The I₂KI-PBS stain was selected for tendon staining going forward,
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15 in the interest of minimising tissue damage since the cryo-sections
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18 demonstrated that it had a lesser effect on the morphology of the tendon tissue
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21 compared with the conventional I₂KI-dH₂O stain.
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30 Results of the suture pull-out test demonstrated no significant difference in the
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33 force required to withdraw stained and unstained suture from tendon (Fig. 3j).
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36 This suggests that suture staining for 24 hours using I₂KI-dH₂O does not
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39 significantly affect the attachment of suture barbs to the tendon tissue. This
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42 staining protocol was therefore regarded to be suitable for subsequent X μ CT
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45 imaging of suture withdrawal.
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Results of the staining time course study are illustrated in Fig. 4. Similar staining penetration and intensity is observed from the 2, 4, 6 and 8 hour samples (Fig. 4a to 4d). From 8 to 24 hours staining duration there is an increase in staining intensity, resulting in higher greyscale values around the edge of the tendon in the X μ CT scan, and the staining penetration has approximately doubled from 1.5mm at 2-8 hours to 3 mm at 24-48 hours (Fig. 4a to 4f). Little increase in staining intensity or penetration is observed when comparing 24 and 48 hours staining duration (Fig. 4e and 4f). From the reconstructed virtual section of the samples, the tendon edge is clearly visible in the 2 hour stained sample (Fig. 4a); however, transmission is extremely low through the sample, and the tendon edge is not visible from the X-ray projection in the 2 hour sample (Fig. 4i). Lack of tendon visibility on the X-ray projection impairs centring of the sample and a larger field of view is then necessary to ensure the whole sample is in view, thus reducing achievable resolution. As such, a 24 hour staining duration was selected since the tendon edge is clearly visible on the X-ray projection at this time-point.

Figure 3. Effects of tendon and suture staining. (a to h) Tendon longitudinal sections (n=6) at 50 μ m thickness after undergoing the following treatments; immediately excised control samples with no incubation in solution (a & e), 24h incubation at 4°C in PBS, (b & f) 24h incubation at 4°C in IKI-PBS (c & g)

and 24h incubation in I₂KI-dH₂O (d & h). ((a to d) 2.5x magnification, (e to h) 10x magnification.) (i) Young's modulus of unstained (with no PBS incubation), unstained following 24 hour incubation in PBS at 4°C, I₂KI-PBS (24 hour incubation at 4°C) and I₂KI-dH₂O stained (24 hour incubation at 4°C) tendons (n = 8, error bars show standard deviation). (j) Failure force of tendons sutured with I₂KI-dH₂O stained, and unstained barbed suture, loaded under tension (n=4, error bars show standard deviation).

Figure 4. Optimisation of the iodine staining protocol. (a to f) Virtual sections from the reconstructed X μ CT time course study scan, through the approximate

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3 centre of each tendon sample following staining using I₂KI-PBS for (a) 2 hours
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7 (b) 4 hours (c) 6 hours (d) 8 hours (e) 24 hours (f) 48 hours. (g) Greyscale
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10 values from the edge to the centre of the tendon, obtained from the virtual
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13 sections (average over 20 pixels). A higher greyscale value signifies greater X-
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16 ray absorbance due to more uptake of the Iodine. (h to j) view of the I₂KI-PBS
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19 stained tendon samples stacked in the centrifuge tube during X μ CT imaging.
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23 (h) photograph. (i) X-ray radiograph. (j) X μ CT virtual section. (All scale bars 4
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26 mm)
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41 3.2 X μ CT Scans; large displacement study at 0, 2 and 4 mm Suture 42 43 44 45 Withdrawal 46 47 48 49 50

51 The X μ CT volumes from the large displacement study (sample 1) were
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55 reconstructed successfully and the segmented data is displayed in Fig. 5d to
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3 5f. Upon withdrawal of the suture by an initial 2 mm, the sample is first seen
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7 to straighten out Fig, 5d to 5e, whilst the suture remains in position within the
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10 tendon. When 4 mm suture withdrawal is reached, the suture can be seen to
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13 have displaced from its original position within the tendon and barb bending is
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16 observed (Fig. 5e to 5f, black arrow).
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23 Data obtained during staining optimisation shows the force-displacement
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26 curves as the barbed suture is withdrawn from the tendon (Fig. 6). All four
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29 samples withstood suture displacement of at least 4 mm before failure,
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32 providing confidence that the suture pull-out positions of 2 mm and 4 mm
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35 observed during the X μ CT study are likely to be prior to barbed suture
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38 attachment failure.
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47 Air-filled pores were identified within the tendon (Fig. 5a to 5c), recognised as
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50 air since they had the same greyscale value as other areas of the scan known
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3 to be regions of air. The air pores within the tendon were only located adjacent
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7 to the suture strand.
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13 Analysis of the barb angles as the suture is withdrawn demonstrated that only
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17 two barbs were continually increasing in angle throughout suture withdrawal
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20 (Fig. 7a). Five barbs present an initial angle increase from 0 to 2 mm suture
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23 withdrawal followed by a decrease in angle from 2 to 4 mm withdrawal (Fig.
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27 7b). Four barbs display the opposite profile, initially reducing in angle, followed
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30 by an increase (Fig. 7c). One barb continually decreases in angle throughout
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33 suture withdrawal (Fig. 7d). No correlation is observed between the location of
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37 the barbs and their bending profile. No significant difference in barb length was
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40 observed as the suture was withdrawn, demonstrating that none of the barbs
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43 peeled from the suture shaft.
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Figure 5. Large displacement study (Sample 1) X μ CT, unfiltered, virtual sections (a to c) and segmented data (d to f). (a and d) 0 mm suture withdrawal (unloaded) (b and e) 2 mm suture withdrawal. (c and f) 4 mm suture withdrawal. (Figures a to c; white = iodine stained suture outline, pale grey = iodine stained tendon outline, dark grey = unstained regions within tendon/suture, and PBS surrounding the sample, black = air.) (Figures d to f; pale grey = tendon, blue = suture, green = air-filled pores, grey arrow =

direction of suture pull, black arrow = barb number 4 exhibiting bending, grey dotted outline = unloaded position of suture and tendon, superimposed over figures e and f.) All scale bars = 4 mm.

Figure 6. Force-Displacement data obtained during suture pull-out testing of tendon samples sutured with I_2KI -dH₂O stained barbed suture.

Figure 7. Barb bending profiles during suture withdrawal, observed during the large displacement study (sample 1). (a) Increasing barb angle throughout, observed in barbs 4 and 6. (b) Initial increase, followed by a decrease in angle, observed in barbs 1, 2, 5, 7 and 12. (c) Initial decrease, followed by an increase in angle, observed in barbs 3, 8, 10 and 11. (d) Decreasing barb angle throughout, observed in barb 9. (e) Location of each barb within the tendon, shown on the unloaded sample position.

3.3 X μ CT Scans and Subsequent FE Analysis; Small Displacement Study at 0 and 0.5 mm Suture Withdrawal

The scanned volumes from the small displacement study (sample 2) were reconstructed and segmented successfully. The unloaded position X μ CT scan was converted into an FE mesh (as described in Section 2.8), and results of a 0.5mm suture pull FE simulation are shown in Fig. 8. The mean barb angle of the 12 barbs within the tendon in the 0 mm X μ CT scan, the 0.5 mm suture

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3 withdrawal X μ CT scan and the 0.5 mm suture withdrawal FE simulation results
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7 were 15.1° ($\pm$ 2.6°), 22.0° ($\pm$ 5.4°) and 18.9° ($\pm$ 5.7°) respectively (Fig. 9).
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13 The FE data highlights that stress is not evenly distributed throughout the
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17 tendon tissue. Instead, low stress is observed throughout the majority of the
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21 tendon tissue, with high stress regions concentrated close to the suture barbs.
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24 The highest stress is concentrated at the root of the suture barbs, and in
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27 particular, around the barbs closest to the cut end of the tendon. With respect
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30 to the suture, whilst all barbs do exhibit some level of stress, suggesting
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33 engagement between the barb and the tendon, stress is not evenly distributed
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37 throughout the strand. The greatest suture stress is observed towards the cut
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40 end of the tendon.
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47 Fig. 8c to 8e shows the stress distribution from FE results in the suture
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50 alone. Low stress is observed at the tip of barb 7 (Fig. 8d), which is
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54 embedded within an air filled pore; however, other barbs embedded within the
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tendon exhibit moderate to high stress at the tip. After the FE analysis was repeated with the air filled pores omitted from the model, barb 7 exhibited moderate stress at the tip (Fig. 8e). This demonstrates that the presence of the air filled pores impairs effective attachment between the barbs and tissue.

Figure 8. FE model results, simulating a 0.5 mm suture withdrawal. (a) Cut view through the centre of the tendon and suture (from the simulation which includes pores). (b) cut view through the centre of the tendon, showing the tendon alone with stress contour plot limits adjusted to more clearly show the

tendon stresses (from the simulation which includes pores). (c to e) View of the suture alone, with stress contour plot limits adjusted to more clearly show the suture stresses. (d) View of barb number 7 (black arrow) which lies within a pore, from the FE simulation which includes the pores. Note that low stress is seen at the barb tip (indicated by the dark blue colour). (e) View of barb number 7 (black arrow) from the FE simulation which omits the air filled pores. Note that moderate stress is seen at the barb tip (indicated by the green colour). (All scale bars 2 mm)

Figure 9. Mean barb angle measurements of the small displacement sample (sample 2), comparing barb positions from the 0 mm and 0.5 mm displacement

X μ CT scan, and the 0.5 mm displacement FE results. (error bars are standard deviation).

4 DISCUSSION

Through this case study we have demonstrated the effective sample preparation and time lapse X μ CT imaging of a polymeric biomaterial implanted within soft tissue to inform and validate an FE model. The sample preparation successfully enabled reliable reconstruction of the X μ CT data with sufficient contrast to allow 3D visualisation of the tendon and embedded barbed suture. The FE model could then be informed and validated by X μ CT data. *In silico* modelling offers a means of re-designing and optimising a device to better withstand the unique mechanical stresses arising in a given operating environment. Whilst FE analysis is commonplace in the engineering field, it is in relative infancy within the medical setting. This paper makes inroads towards

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3 wider *in silico* modelling of mechanical stress for clinical applications. This
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6 methodology could address the need to study the *in situ* micro- and macro-
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9 mechanics of tissue repair devices, wound closure techniques and implanted
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12 tissue constructs ¹⁴ in a range of soft tissues including tendon, ligament,
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17 muscle, abdominal tissues and skin.
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23 4.1 X μ CT of Implanted Biomaterials 24 25 26 27 28 29

30 Enabling the time lapse imaging of soft tissues in an aqueous environment
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33 hinges on effective sample preparation for enhanced X μ CT contrast. The
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36 predominant concerns are to maintain sample hydration during scanning and
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39 ensure that mechanical properties and tissue morphology are not altered. These
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42 challenges have been addressed through applying a heavy metal stain without
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47 prior tissue fixation, and development of a staining protocol which minimises
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50 change in tissue mechanical properties and morphology compared with the
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53 conventional fixing and staining protocol. For translation of this method to other
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materials and tissues, sample preparation will require tailoring to each given application. Staining uptake is likely to differ for different tissues and materials, and a time course study similar to that described here and elsewhere in previous literature ³⁶⁻³⁷ is advisable. Other tissues may also present a different degree of mechanical or morphological change during staining of the unfixed tissue, thus it cannot be assumed that it will behave the same as tendon in this study. Whilst some leaching of the stain from the tendon was observed over periods of several hours, the rate was not sufficient to adversely affect the X μ CT reconstruction following the 47 minute scans. This may be of concern for longer scanning times, and leaching rate may differ in other tissues, thus caution is advised. This study provides a framework for validating the sample preparation method for alternative tissues and materials, to ensure that the tissue is mechanically and morphologically preserved and to ensure that reliable X μ CT data can be obtained.

4.2 Sutured Tendon Case Study

Our finite element data provides insight into the distribution of stress through the suture and tissue upon suture withdrawal. One of the reported benefits of barbed suture is even distribution of stress along the suture length ²⁰. Contrary to this claim, our FE findings demonstrate that stress is not evenly distributed. Barbs closest to the cut end of the tendon withstand the greatest stress, with the deepest barbs withstanding less stress (Fig. 8c). Furthermore, the stress in the tissue is concentrated closest to the suture strand; very little stress is experienced throughout the majority of the tendon (Fig. 8b). Uneven stress distribution presents several detrimental consequences for the healing environment of the tendon. Abnormal stress can induce remodelling of the extracellular matrix and may lead to tendinopathies ³⁸⁻³⁹. High stress regions correlate with acellular regions ²²⁻²³ which is undesirable since tenocytes (tendon cells) maintain tissue homeostasis. High stress observed in our FE model adjacent to the barbed suture may be the mechanism for barbed suture loosening, which has been reported in clinical studies ⁴⁰. In the FE analysis of

the present study, only low loads have been applied as the tendon description is valid for low strains ²². It is possible that at higher loads the stress distribution may change and become more even across the barbs and throughout the tendon tissue.

The present study assumes a transversely isotropic, linear elastic behaviour of tendon tissue, which is appropriate for low strains ²². A constitutive material description of tendon valid for higher strains would require accounting for the hyperelastic and viscoelastic behaviour of tendon as opposed to using a linear elastic assumption. Such behaviour has been mathematically described in the literature ⁴¹⁻⁴² and could be implemented within the tendon FE model. Furthermore, including additional levels of tendon hierarchy in the multi-scale material model such as tendon fascicles, would provide a closer approximation to the behaviour of tendon tissue. This was beyond the scope of the present study; however, it is recommended for future development of this work, to

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3 consider whether stress is more evenly distributed throughout the suture and
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6 tendon at high loads.
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13 In previous studies, the failure of suture barbs was reported to occur either
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16 by barb bending or peeling from the suture shaft ²⁷. From X μ CT results of the
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19 large displacement study (sample 1) we observe only two barbs bending, and
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22 no barbs peeling (Section 3.2) and a pattern was not observed between barb
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25 position and bending profile (Fig. 7.). The differing suture geometry in the
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28 present, and previous studies may be responsible for the different barb failure
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31 mechanisms observed between this work and the work of Ingle *et al.* In the
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34 present study, a relatively large, polydioxanone (PDO) suture was used (size 2,
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37 0.6mm in diameter) to ensure that the barbs could be identified from the X μ CT
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40 reconstruction; however, the suture used by Ingle *et al.* was a smaller, (size 0,
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43 0.4mm in diameter) polypropylene (PP) suture ²⁷. Whilst the Young's modulus
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46 of PDO and PP is similar ⁴³, in the smaller suture the barbs are thinner, and
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49 therefore more flexible, making them more likely to bend or peel. In addition,
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3 despite the encouraging results of our tissue pull-out study (Fig. 3j), the
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6 staining procedure may have affected mechanical properties, and thus barb
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9 behaviour, particularly considering the fact that PDO is a degradable polymer.
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12 Future X μ CT scanning of barbed suture in tendon, using a variety of suture
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15 materials in different sizes would provide insight into the different barb failure
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18 mechanisms of different sutures. Such information would aid the surgeon in
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23 suture selection for a given surgical procedure.
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31 Whilst the FE analysis demonstrates that all barbs located within the tendon
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34 tissue undergo some stress as the suture is withdrawn (Section 3.3), indicating
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37 that they all engage with the tissue, it is curious that only 3 barbs exhibit
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40 bending or peeling. We therefore hypothesise that a third failure mechanism
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43 may be responsible for barb failure in the remaining 9 barbs; that the tendon
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46 tissue tears, resulting in dislodgement of the barb; which would be comparable
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49 to a pull-out failure in a conventional tendon repair. Development of the FE
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54 model to simulate application of higher loads across the barbed suture repair
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(as discussed previously) would allow investigation into whether tendon failure stress is reached in the tissue surrounding the barbs, which would indicate tissue tearing.

Whilst it is commonplace to use one replicate during X μ CT studies, we must take into consideration the potential for variability when interpreting the data from the X μ CT and FE study, since the FE model was developed from one sample. During tensile testing of barbed suture pull-out from tendon (Fig. 6), large variability between samples was observed in the force-displacement curves. This variability may have been caused by several potential reasons, such as variations between tissue samples or differing numbers of barbs engaging with the tissue. Furthermore, the mechanical response may differ *in vivo*, with the presence of cyclic loading and blood flow. Additionally, suture thickness and arrangement would differ from this study should barbed suture be used clinically for tendon repair. Suture pull-out tests (Fig. 6) demonstrate that a 2mm gap (considered clinically as a repair failure) would occur at low loads.

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3 Typically, 4-0 sutures would be used clinically, in a looping arrangement ¹⁷,
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7 which may indeed provide a more even distribution of stress throughout the
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10 tissues. A larger suture was appropriate in this study to enable X μ CT imaging
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13 of the barbs, and a simplified arrangement enabled consideration of stress
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16 distribution along a length of barbed suture. FE modelling of the looping
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19 arrangements with a thinner grade of suture is recommended for future work to
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22 identify stress distribution in barbed suture arrangements which are currently
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25 being considered for clinical use.
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33 Air-filled pores were observed from X μ CT scans of both samples, and were
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36 located only around the suture strand. These pores are not previously noted in
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39 the literature. We hypothesise that the pores would also arise in a surgical
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42 setting, and could potentially be minimized by performing the procedure in a
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45 more hydrated environment. FE analysis confirms that barbs which are located
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48 within a pore do not fully engage with the tendon tissue (Fig. 8d), yet if the
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51 pore is removed from analysis, a greater barb engagement is observed (Fig.
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8e). Minimising the formation of the pores is therefore desirable to improve barbed suture attachment to the tissue and increase the resultant repair strength. We hypothesise that the pores arise due to tissue damage during suture insertion. However, we are unable to determine why the pores occur, which could be due to the needle size or type, the suture diameter, material or type (barbed, monofilament, or braided). This could be studied using X μ CT imaging, comparing suture damage from various needles, sutures, and in different tissues. Results of such a study would have clear application in the development of barbed suture products for improved tissue attachment.

Whilst the displacement of the barbs in the 0.5 mm suture withdrawal X μ CT scan and FE simulation do not agree exactly (Fig. 9), the moderate agreement between barb bending in the X μ CT and FE results which we observed here was regarded as acceptable for verification of this FE model. This is since we were not concerned with absolute values from the model, such as exact values of barb bending, or exact values of stress. Rather, we were interested in more

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3 general, qualitative, information provided by the FE results, such as whether a
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6 given barb was exhibiting peeling or not, and which areas of the tendon
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10 exhibited higher or lower stress.
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20 5 CONCLUSIONS

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27 The use of an X μ CT informed and validated FE model in this case study has
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30 revealed the micro-mechanical stress response in a sutured tendon under force
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33 application. The technique was made possible due to the customised I₂KI-PBS
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36 staining method, which improved imaging contrast whilst minimising mechanical
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39 and morphological tissue changes. Several previously unknown findings have
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43 been revealed using this novel approach, including the uneven distribution of
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46 stress through the suture and tendon, and the observation and effect of pores
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49 surrounding the suture. This case study showcases the dramatic potential of
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53 X μ CT informed FE analysis on the study of implantable biomaterials for soft
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3 tissue applications. The technique can be readily translated for the study of
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7 other implantable devices and for other soft tissues, and we have discussed an
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10 approach towards adapting this method for other applications.
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34 Author Contributions

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38 The manuscript was written through contributions of all authors. All authors
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41 have given approval to the final version of the manuscript.
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50 ACKNOWLEDGMENT

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ABBREVIATIONS

X μ CT, X-ray micro computed tomography; FE, finite element; TE, tissue engineering; I₂KI, iodine potassium iodide; FDP, *flexor digitorum profundus*; RT, room temperature; I₂KI-dH₂O, iodine potassium iodide solution in distilled water; I₂KI-PBS iodine potassium iodide solution in Dulbecco's phosphate buffered saline (without Ca²⁺ or Mg²⁺); PDO, polydioxanone; PP, polypropylene.

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134x57mm (300 x 300 DPI)

Figure 1. (a) Schematic detailing the tension rig apparatus, and (b) flow diagram detailing how tension is applied to the sample using the rig.

108x83mm (300 x 300 DPI)

Figure 2. (a) X μ CT-informed FE model, showing segmented data from the small displacement study (sample 2) in the unloaded position (used to inform the FE model geometry), and the boundary conditions applied to the FE model, noted in red (grey = tendon, blue = suture, green = air-filled pores. Scale bar = 4 mm). (b) Illustration showing the measurement of barb length and angle from FE results. (The same method was used for barb measurements of CT data.)

180x91mm (300 x 300 DPI)

Figure 3. Effects of tendon and suture staining. (a to h) Tendon longitudinal sections (n=6) at 50 μm thickness after undergoing the following treatments; immediately excised control samples with no incubation in solution (a & e), 24h incubation at 4°C in PBS, (b & f) 24h incubation at 4°C in IKI-PBS (c & g) and 24h incubation in IKI-dH₂O (d & h). ((a to d) 2.5x magnification, (e to h) 10x magnification.) (i) Young's modulus of unstained (with no PBS incubation), unstained following 24 hour incubation in PBS at 4°C, I2KI-PBS (24 hour incubation at 4°C) and I2KI-dH₂O stained (24 hour incubation at 4°C) tendons (n = 8, error bars show standard deviation). (j) Failure force of tendons sutured with I2KI-dH₂O stained, and unstained barbed suture, loaded under tension (n=4, error bars show standard deviation).

468x413mm (96 x 96 DPI)

Figure 4. Optimisation of the iodine staining protocol. (a to f) Virtual sections from the reconstructed X μ CT time course study scan, through the approximate centre of each tendon sample following staining using I2KI-PBS for (a) 2 hours (b) 4 hours (c) 6 hours (d) 8 hours (e) 24 hours (f) 48 hours. (g) Greyscale values from the edge to the centre of the tendon, obtained from the virtual sections (average over 20 pixels). A higher greyscale value signifies greater X-ray absorbance due to more uptake of the Iodine. (h to j) view of the I2KI-PBS stained tendon samples stacked in the centrifuge tube during X μ CT imaging. (h) photograph. (i) X-ray radiograph. (j) X μ CT virtual section. (All scale bars 4 mm)

125x53mm (300 x 300 DPI)

Figure 5. Large displacement study (Sample 1) XpCT, unfiltered, virtual sections (a to c) and segmented data (d to f). (a and d) 0 mm suture withdrawal (unloaded) (b and e) 2 mm suture withdrawal. (c and f) 4 mm suture withdrawal. (Figures a to c; white = iodine stained suture outline, pale grey = iodine stained tendon outline, dark grey = unstained regions within tendon/suture, and PBS surrounding the sample, black = air.) (Figures d to f; pale grey = tendon, blue = suture, green = air-filled pores, grey arrow = direction of suture pull, black arrow = barb number 4 exhibiting bending, grey dotted outline = unloaded position of suture and tendon, superimposed over figures e and f.) All scale bars = 4 mm.

115x87mm (300 x 300 DPI)

Figure 6. Force-Displacement data obtained during suture pull-out testing of tendon samples sutured with I2KI-dH₂O stained barbed suture.

49x31mm (300 x 300 DPI)

Figure 7. Barb bending profiles during suture withdrawal, observed during the large displacement study (sample 1). (a) Increasing barb angle throughout, observed in barbs 4 and 6. (b) Initial increase, followed by a decrease in angle, observed in barbs 1, 2, 5, 7 and 12. (c) Initial decrease, followed by an increase in angle, observed in barbs 3, 8, 10 and 11. (d) Decreasing barb angle throughout, observed in barb 9. (e) Location of each barb within the tendon, shown on the unloaded sample position.

174x80mm (300 x 300 DPI)

Figure 8. Mean barb angle measurements of the small displacement sample (sample 2), comparing barb positions from the 0 mm and 0.5 mm displacement X μ CT scan, and the 0.5 mm displacement FE results. (error bars are standard deviation).

261x142mm (300 x 300 DPI)

Figure 9. FE model results, simulating a 0.5 mm suture withdrawal. (a) Cut view through the centre of the tendon and suture (from the simulation which includes pores). (b) cut view through the centre of the tendon, showing the tendon alone with stress contour plot limits adjusted to more clearly show the tendon stresses (from the simulation which includes pores). (c to e) View of the suture alone, with stress contour plot limits adjusted to more clearly show the suture stresses. (d) View of barb number 7 (black arrow) which lies within a pore, from the FE simulation which includes the pores. Note that low stress is seen at the barb tip (indicated by the dark blue colour). (e) View of barb number 7 (black arrow) from the FE simulation which omits the air filled pores. Note that moderate stress is seen at the barb tip (indicated by the green colour). (All scale bars 2 mm)

122x75mm (300 x 300 DPI)