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Design And In Vivo Testing of Novel Single-Stage Tendon Graft Using Polyurethane Nanocomposite Polymer for Tendon Reconstruction

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LONDON'S GLOBAL UNIVERSITY

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To,
Editor-in-Chief

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I am enclosing herewith a manuscript entitled "***Design And In Vivo Testing of Novel Single-Stage Tendon Graft Using Polyurethane Nanocomposite Polymer for Tendon Reconstruction***" for publication in your esteemed journal. This has been transferred from JEPRAS Open.

Flexor tendon injuries are a particularly challenging sub-type of hand injuries, typically requiring a two-stage surgical invention and early mobilization rehabilitation. Despite fascinating promises of new surgical techniques, the development of a functional tendon tissue, and its' associated sheath with complete functionality is still a distant reality owing to numerous challenges. The choice of sheath substitute material, optimization of surgical protocols and their clinical translation, shortening of treatment time and costs, are few such major obstacles that remain unsolved.

Thus, in this manuscript, we propose:

1) The fabrication of bioinert nanocomposite polymer, *polyhedral oligomeric silsesquioxane poly(carbonate-urea) urethane*, as flexor tendon sheath substitute, made using a very **simple and cost-effective technology.**
2) By utilising a tubular POSS-PCU graft, we carried out a pilot study in sheep and successfully validated that using this material, **the two-stage flexor tendon reconstruction could be achieved in a single-stage surgical procedure**.
3) The **tendon graft retained functionality** (gliding movement, tendon healing and barrier to external soft tissue) in sheep model, confirming safety and efficacy of one-stage surgical procedure.

We would be grateful if you could consider this manuscript for publication in your esteemed journal.

Yours sincerely,

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Design And *In Vivo* Testing of Novel Single-Stage Tendon Graft Using Polyurethane Nanocomposite Polymer for Tendon Reconstruction

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ABSTRACT

Severe trauma, failure of prior surgical repair, delayed presentation and excessive scarring around the flexor tendon bed often necessitates a two-stage surgical reconstruction, where a silicone spacer is used in the first-stage to recreate the fibro-osseous tunnel through which the tendon graft can glide in the second-stage. This staged procedure involves great commitment on the part of both patient and surgeon, over the course of several months, involving a prolonged period of rehabilitation that can be quite disruptive to the patient's life and work. Reducing this from a two-stage into a single-stage procedure therefore has the potential to reduce rehabilitation time and cost, expedite return to work, and improve outcomes. To address this, we developed polyurethane nanocomposite (PU), as an engineered tendon sheath, for treatment of delayed flexor tendon division as a single-stage procedure. The clinically conformant tubular grafts were tested for their efficacy in the peroneus tertius tendon of 6 Mule sheep for 3 months. Semi-quantitative histological assessment was carried out by analysing four descriptive layers: tendon, tendon/polymer sheath interface, polymer sheath, and polymer sheath/surrounding tissue. 4 (out of 6) of the implanted PU nanocomposites showed moderate-to-substantial healing of the injured tendons, with minimal adhesion after repair, ensuring good gliding movement. No statistical differences were observed in tendon repair based on intra-regional variation in the explanted grafts, indicating homogeneity in tendon repair. Overall, the PU nanocomposite bears morphological stability and functionality for tendon repair, in single-stage surgical reconstruction, demonstrating promising evidence for clinical translation.

KEYWORDS: flexor, tendon reconstruction, PU nanocomposite, sheep, tendon sheath

1. Introduction

Hand and wrist injuries are extremely debilitating to patients, causing extended periods of disability and trauma-related distress,[1–4] accounting for 6.6% of all the patients at accident and emergency departments in the UK.[5] The cumulative effects of surgery time, out-patient visits, in-patient days, and hand surgery are of considerable costs to the National Health System with estimates varying between £100 to £1050 million annually.[6] Flexor tendon injuries are particularly challenging because the zone contains multiple annular pulleys, which increase the complexity of the surgery, as any increased bulk arising in the epitenon from surgical repair could impede tendon gliding.[7,8] In the context of severe trauma, failure of prior surgical repair, delayed presentation and other cases resulting in excessive scarring around the flexor tendon bed, a two-stage flexor tendon reconstruction may be necessary.[9–11]

This technique, first pioneered by Carrol in 1963, involves a prosthetic silicone rod, which is passed through the pulley system and sutured to the distal side of the distal phalanx, leaving the proximal end in the distal forearm free. The silicone rod is left *in situ* for at least 3 months to allow sufficient time for the formation of a tendon pseudo-sheath. This pseudo-sheath provides a tunnel through which the tendon graft can glide freely, mimicking the biomechanical role of the pulleys. Rehabilitation during this period is crucial to ensure mobility of the hand and fingers to optimise results of the second stage. The second stage of the procedure involves removing the silicone rod and interposing the defect between the two ends of the tendon with a tendon graft, usually harvested from the palmaris longus or plantaris tendons.[11,12] The tendon graft is passed through the pseudo-sheath and used to bridge the gap between the two cut ends of the tendon.[10,13] The patient undergoes early active mobilisation rehabilitation protocol to promote intrinsic tendon healing and minimise scar tissue formation, facilitating smooth gliding of the tendon inside the newly formed pseudo-sheath to enable a full range of motion.[14]

Functional outcomes from this two-stage procedure is not always predictable, and strict patient compliance is mandatory to ensure good post-operative results.[11,14] Moreover, having two procedures increases costs, exposes patients to higher complications and demands long durations off work due to prolonged rehabilitation time.[12] If the first stage can be 'bypassed', by way of an implantable device that could act as the 'pseudo-sheath', allowing for free gliding of the flexor tendon, it may then allow for a single-stage operation.

We propose a bioinert Polyurethane (PU) nanocomposite polymer, as an effective tendon sheath substitute in the flexor region. PU nanocomposite has been successfully explored in numerous applications, including auricular reconstruction, coronary arteries, and first-in-human synthetic lacrimal ducts and tracheas.[15–18] However, its' application in the form of a tubular graft for tendon sheath substitution, is being investigated for the first time. By utilising a tubular PU nanocomposite in a large animal model of tendon repair, we attempted to address

the following unanswered questions: (i) the *in vivo* efficacy of the PU tubular graft as flexor tendon sheath substitute, (ii) the inflammatory and other responses to the implant (local and systemic), and (iii) the ability to support tendon reconstruction in single-stage procedure.

2. MATERIALS AND METHODS

2.1 PU Nanocomposite Polymer Synthesis. The PU nanocomposite scaffolds were developed as before.[15] Briefly, polycarbonate polyol (MW2000), and *trans*-cyclohexanechloroydrin-isobutyl-silsesquioxane (Hybrid Plastics Inc., USA) were mixed with 4,4'-methylenebis (phenyl isocyanate) (MDI) flakes. Then ethylenediamine and diethylamine were added in DMAC (N,N-dimethylacetamide) to form a solution of POSS-modified polycarbonate urea-urethane. All chemicals were purchased from Aldrich Limited, UK, unless stated.

2.1.1 Polymer graft fabrication. Graft fabrication was performed using phase inversion coagulation extrusion of 18% polymer solutions on a purpose-built machine (**Supplementary Figure 1**), as before.[16,19]

2.1.2 Characterisation of PU nanocomposite Graft. X-ray microcomputed tomography (micro-CT) (Skyscan 1172, Bruker, Kontich, Belgium) and scanning electron microscopy at 11 kV operating voltage (SEM, JASP 5500; UK), were used for morphological analysis. A uniaxial load testing machine (Instron 5565, UK) was employed to obtain the stress-strain profiles of the grafts (n = 8), according to ISO 37, at displacement rate of 200 mm/minute until failure, and force-displacement data were obtained.

2.2 Animals Study Design. All live animal procedures were approved by the local Animal Welfare and Ethical Review Body and carried out under the Home office Licence no. PPL 70/8247 Protocol 11. Six, mature (average age, < 2 years), female Mule sheep were housed at our veterinary medical unit and identified by ear tags (SOP LIV 07). The sheep were given

individual identification numbers: 6630, 6631, 6633, 6634, 6635, and 6636. All six sheep were used to test the tubular PU nanocomposite grafts as pseudo-sheaths in flexor tendon grafts.

2.2.1 Surgical Procedure. The sheep were starved for 12 hours prior to surgery. Analgesic fentanyl patch was ic placed on a shaved region of the fore limb, this was changed two days after the surgery. Xylazine (0.2 mg/Kg, intravenous), was administered *via* intravenous catheter (20G) situated in the cephalic vein. Sodium thiopental (15 mg/Kg intravenous) was used to induce anaesthesia. 2% isoflurane carried by 100% oxygen with a flow of 2 L/min was administered *via* an endotracheal tube for anaesthetic maintenance. The sheep were placed in a right lateral recumbent position to reduce the risk of aspiration pneumonia during the surgery. The skin was aseptically prepared with an iodipovidone solution.

Tendon injury was carried out by a unilateral surgical incision transecting the peroneus tertius tendon of the left hind limb of all the 6 sheep. The peroneus tertius muscle was chosen for this procedure as its function is primarily involved in flexion of the hock in bipedal animals.[20,21] As this muscle is not involved in weight bearing, it mimics the flexor tendons of the human hand. A length of the tendon of peroneus longus muscle was harvested, leaving behind a portion of the tendon to ensure continuity and minimise functional deficit. A 10 cm long PU nanocomposite tubular implant was passed through the cut end of the tendon. Autologous tendon repair was then performed, with a donor tendon graft harvested from the adjacent peroneus longus and sutured to the peroneus tertius. The conduit was inserted into the tendon repair site prior to grafting, aiming to provide protection and gliding for the reconstructed tendon. The centre of the implant (approximately 5 cm long) was placed centrally over the initial injury site (**Figure 1**). The wound was closed in layers using 3-0 PDS monofilament absorbable core sutures and a 5-0 PDS peripheral running suture. The animals were allowed to recover gradually on discontinuation of isoflurane.

2.2.2 Force plate examination. Prior to surgery, the sheep were acclimatised to the assessment by being led over a walkway with two force plates using a halter and lead rope. Ground reaction forces (GRF) were measured for all four limbs pre- and post-operatively (months 1 and 3), using an in-ground piezoelectric forceplate (Kistler 9281CA; Kistler Intrumente, Winterthur, Switzerland) capturing at 300Hz. Each animal repeatedly crossed the region of the forceplate, allowing repeated measurements per leg per animal to be taken which was then averaged, as shown elsewhere.[22] All data recorded was normalised to body weight (BW) in Newtons.

2.2.3 Histopathological assessment. At 3 months post-surgery, animals were euthanized by an overdose of pentobarbitone (1 ml/kg body mass), and tissues were dissected for histology. 5 equidistant, transverse slices of 5 μm thickness were prepared for each specimen, spanning the length of the graft (**Supplementary Figure 2**), and stained with H&E. The sections were examined by a board certified/senior pathologist using semi-quantitative scoring system (**Table 1**) assessing:

1. quality of tendon repair
2. thickness and nature of the tissue between the PU sheath and the underlying tendon
3. integrity of the PU sheath
4. thickness and nature of the tissue between the PU sheath and the overlying tissue

The categories were scored from 0–3 (except for inflammatory cell type, suture material, bacterial infection), where 0 represents a normal state, and 3 refers to an abnormal tendon repair or pathological state.

Table 1. Histopathology scoring key for histological assessment of the PU nanocomposite implant.

		Grade 0	Grade 1	Grade 2	Grade 3
Tendon	Necrotic material	Nectrotic material absent	Small amounts of necrotic	Moderate amounts of necrotic	Large amounts of necrotic
	Residual tendon material	Substantial amounts of tendon material	Moderate amounts of tendon material	Minimal amounts of tendon material	Tendon material absent
	Fibrivascular repair tissue	No fibrous replacement	Small amounts of fibrous tissue	Moderate amounts of fibrous tissue	Large amounts of fibrous tissue
	Fibrouos tissue organisation	Diffuse well organized and aligned fibre bundles	Moderate discrete areas of fibre organisation within disarrayed fibres	Small discrete areas of fibre organisation within disarrayed fibres	Diffuse dissarrayed collegen fibres
	Vascularity	Absent/minimal	Small numbers of microvessels	Moderate numbers of microvessels	Large numbers of microvessels
	Inflammatory cell numbers	Absent/minimal	Small numbers of inflammatory cells	Moderate numbers of inflammatory cells	Large numbers of inflammatory cells
	Predominant inflammatory cell	Neutrophils (No), Mononuclear (Mono), Macrophages (Mo)			
	Haemoohage	Absent/minimal	Mild haemoohage	Moderate haemoohage	Marked haemoohage
	Suture material (Y/N)	Present Yes or No			
	Bacteria	Present Yes or No			
Tendon/ Sheath Interface	Fibrin	Absent/minimal	Thin layer of fibrin	Medium layer of fibrin	Thick layer of fibrin
	Inflammatory cell infiltrates	Absent/minimal	Small numbers of inflammatory cells	Moderate numbers of inflammatory cells	Large numbers of inflammatory cells
	Predominant inflammatory cell type	Neutrophils (No), Mononuclear (Mono), Macrophages (Mo)			
Polymer	Integrity	Intact continuous structure	Small cavities in sheath	Medium cavities in sheath	Large cavities in sheath
Sheath/ Surrounding interface	Thickness of connective tissue	No collagen	Small amounts of collagen	Moderate amounts of collagen	Large amounts of collagen
	Vascularity	None	Small numbers of microvessels	Moderate numbers of microvessels	Large numbers of microvessels
	Inflammatory cell infiltrates	Absent/minimal	Small numbers of inflammatory cells	Moderate numbers of inflammatory cells	Large numbers of inflammatory cells
	Predominant inflammatory cell type	Neutrophils (No), Mononuclear (Mono), Macrophages (Mo)			

2.3 Statistical Analysis. Analysis was performed using Graphpad prism version 7.0. For the GRF gait measurements, a Two-way ANOVA, followed by Sidak's multiple comparisons test, was performed. The histopathology data was analysed for regional variation in the healing process using a Two-way ANOVA, and Tukey's multiple comparisons test. Significance was stated in terms of (*), where $P < 0.05$ was significant, and the data expressed as mean $\pm$ the standard deviation of the mean.

3. RESULTS

3.1 Characterisation of POSS-PCU graft. The micrographs confirmed analogous morphology of the PU nanocomposite tubular graft to Hunter's silicone rod (2 to 6 mm in diameter, **Supplementary Figure 1**),[23] measuring 6 mm in diameter with wall thickness of 0.74 mm (**Figure 2A**). This is suitable for *in vivo* application in sheep (5.6 + 0.38 mm) and human tendons (5.1 + 0.47 mm) with similar medio-lateral diameters.[24–26] The cross-sectional images revealed the porous walls of the conduits, ranging from micro- to macro-scale level diameters **(Figure 2 B,C)**. The grafts failed at a mean load of 44.2 N ± 6 and mean strain at break of 334.7 ± 30 %.

3.2 Effect of Tendon Repair on Gait. For GFR measurements, 2 (out of 6) tendons were excluded after they failed to depict tendon repair. Operated animals **(Figure 3A)** resumed standing and walking, with some lameness during the first few days, after which they gradually recovered and showed normal gait. The plots of the vertical GRF of the right and left legs obtained 1 and 3-months post-surgery showed no significant changes ($p < 0.05$), when compared against pre-surgical data **(Figure 3B)**.

3.3 *In vivo* tendon repair. We checked for adhesions between the tendon and the implant, which poses a major clinical problem because as it impairs the gliding function. The surgeons assessed the integration of the tendon by pulling the tendon from both, distal as well as

proximal ends. The tendons, visibly intact, appeared to glide, indicating minimal adhesion of the tubular graft with the tendon (**supplementary videos 1 and 2)**.

3.4 Tendon Repair. Histopathological scoring was based on 4 descriptive regions: tendon, tendon/sheath interface, polymer sheath, and sheath/surrounding tissue interface. Using H & E staining, the tissues were scored either a function of the biological variation found within the animals (**Figure 4 & 5**), or regional variation within the harvested specimens (**Figure 6**).

In four of the six animals (6633, 6634, 6635, 6636), a moderate to substantial amount of viable tendon material remained. In these animals, the harvested tendon graft was relatively low in vascularity, contained no or only minimal haemorrhage and low numbers of inflammatory cells. In addition, their fibrovascular tissue was well ordered, with mature, collagen deposition in the form of bundles aligned parallelly along the axis of the tendon (**Figure 4**). These findings taken together indicate a good repair of the tendon injury. The marked haemorrhage in some sections (**Supplementary Table 1**) is surprising given the time post-injury, and in the absence of red cell breakdown products is likely to either be an ongoing process, perhaps related to tendon instability, or an agonal artefact of euthanasia. In terms of polymer sheath integrity, minimum disruption was observed in the animals (**Figure 4 & 5**). The interaction between the tendon and the polymer sheath, characterised by the thickness of the fibrin-containing layer containing variable numbers of inflammatory cells, was either absent or minimal, indicating good tolerance of the polymer sheath (**Figure 5**) in the 4 sheep. Generally, the sheath material appeared to be well tolerated by the surrounding tissue, which formed a variable thickness connective tissue capsule/layer around it. Judging the thickness of this connective tissue layer was difficult, as in some animals and sections, it appeared to be continuous with deeper connective tissue structures and even connecting to adjacent tendons or nerves. However, in animals where tendon repair was successful, the connective tissue layer was the least prominent

with minimal evidence of inflammatory cell infiltrates (**Supplementary Table 1**), whilst in animals with poorer tendon repair, it was noticeably thicker **(Supplementary Table 2)**.

Animals 6630 and 6631, showed little residual tendon material, accompanied with neutrophil cell infiltrates (**Supplementary Figure 3**). This suggested an ischaemic tendon injury and that further repair in these cases was unlikely (**Supplementary Table 3**). These two sheep were smaller in size, and were observed to have a very thin peroneus tertius tendon of (<4 mm diameter) at the time of tendon repair, which, in addition to the unprotected repair in an ambulant animal may have contributed to the eventual failure observed.

An important feature for an ideal polymer sheath, is the extent of homogeneity within the repaired tendon. To measure this, we analysed the tendon repair process based on the regional or zonal variation within each of the harvested specimens (**Figure 6**). Our data represented no statistically significant ($p > 0.05$) variation within the different sample zones.

4. DISCUSSION

Research on flexor tendon injures is one of the most frequently investigated subject in the orthoplastic field, still clinical outcomes are inconsistent, with high reoperation rates in complex trauma.[27] Further, adhesion of the tendon to surrounding granular tissue is a major problem in tendon repair, that curtails the motion of the flexor tendon impairing tendon glide and restricting range of movement in a quarter of cases.[28,29] Currently, in the context of two-stage tendon reconstruction, Hunter's silicone rods remain the gold standard.[30,31] The challenge of developing an implantable single-stage exogenous material capable of acting like a tendon pseudo-sheath remains unresolved. The success of any mechanical barrier depends upon the interplay between the degradation time and mechanical stability, while the duration of integrity must be only long enough to effectively prevent tissue infiltration during healing, and not provoke a fibro-inflammatory response. In addition, the product must be strong enough to

withstand the process of surgical application and post-operative mobilisation as part of the rehabilitation process. With regards to producing an anti-adhesive barrier to prevent ingrowth of surrounding granulation tissue, numerous biological and non-biological materials are being explored, but only as adjuncts to reduce adhesion formation in simple tendon repairs.[29,32–34]

Our pilot study provides the first *in vivo* evidence of utilising PU nanocomposite tubular graft for pseudo-sheath replacement, and converting an otherwise two-stage flexor tendon reconstruction, that is currently produced *via* the implantation of silicone rods, into a single-stage procedure. The tubular grafts implanted in the sheep retain the integrity of the polymer sheath, resulting in the synthesis of an intact and functional tendon sheath. With regards to the material we used in our study, it has demonstrated some clear advantages worthy of clinical merit: (i) the fabrication of clinically relevant polymer tendon sheath was done using a simple and cost-effective technology, (ii) the inert nature of PU graft, not only prevented cell invasion from surrounding tissue, but also inhibited exogenous fibroblasts from infiltrating into collagen matrix. Previous work carried out on the biological application of PU nanocomposite at the senior author's institution has shown that physico-chemical properties of the PU graft resulted in minimal platelet adhesion on the material, *in vitro*.[16] This data corroborates with our current histological findings indicating good gliding post-surgery, (iii) the intrinsic healing was promoted by nutrient diffusion through the sheath to the tendon injury, a phenomenon which was effectively regulated by the presence of interconnected pores in the PU nanocomposite graft (**Figure 2**).

Overall, we have demonstrated that PU nanocomposite graft could have potential application in single-stage tendon reconstruction. While the study is limited in size and scope, further studies are needed to conclusively validate and establish the procedure for clinical translation.

5. CONCLUSION

Research in producing a long-term synthetic pseudo-sheath implant has never been carried out before. This is the first *in vivo* evidence reporting successful application of tubular PU nanocomposite grafts as pseudo-sheaths for flexor tendon reflection. Future work should be carried out to identify whether this single-stage approach shows further clinical promise.

Declaration of Competing Interest. None.

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362

Graphical Abstract

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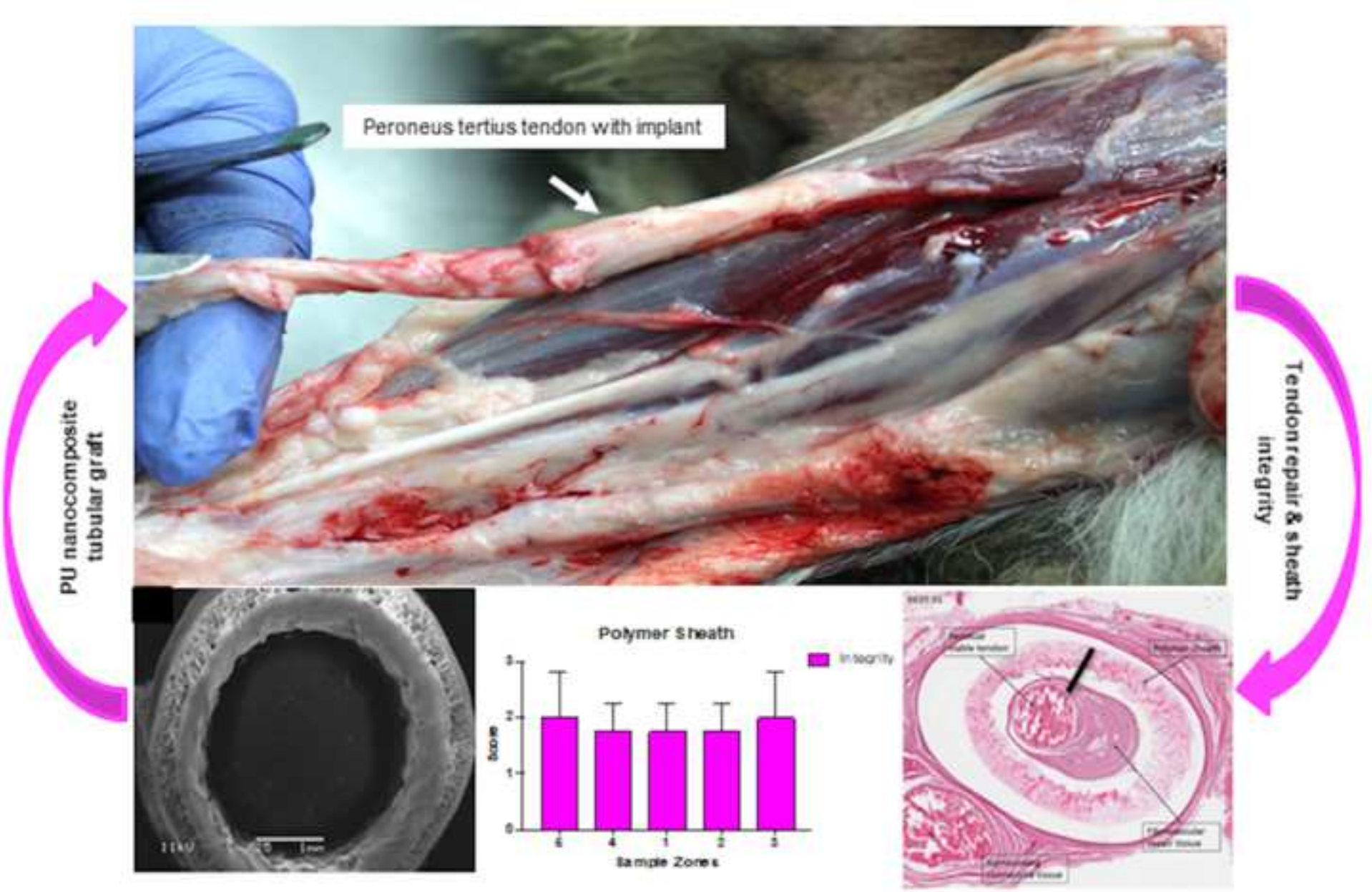

Figure 2

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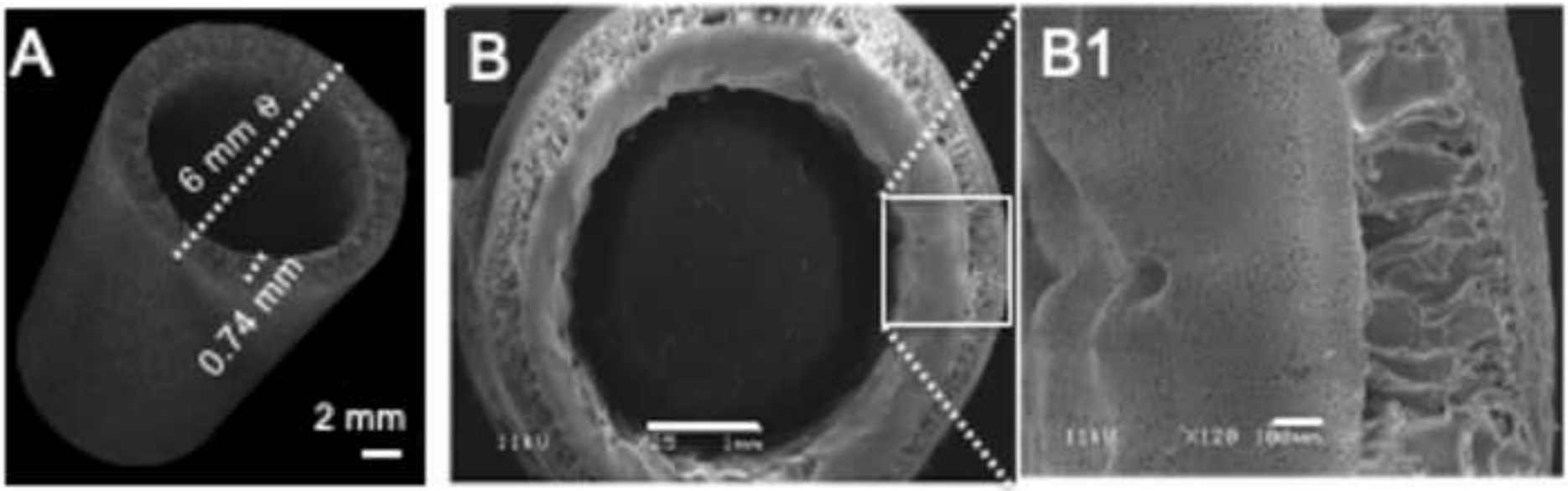

Figure 3

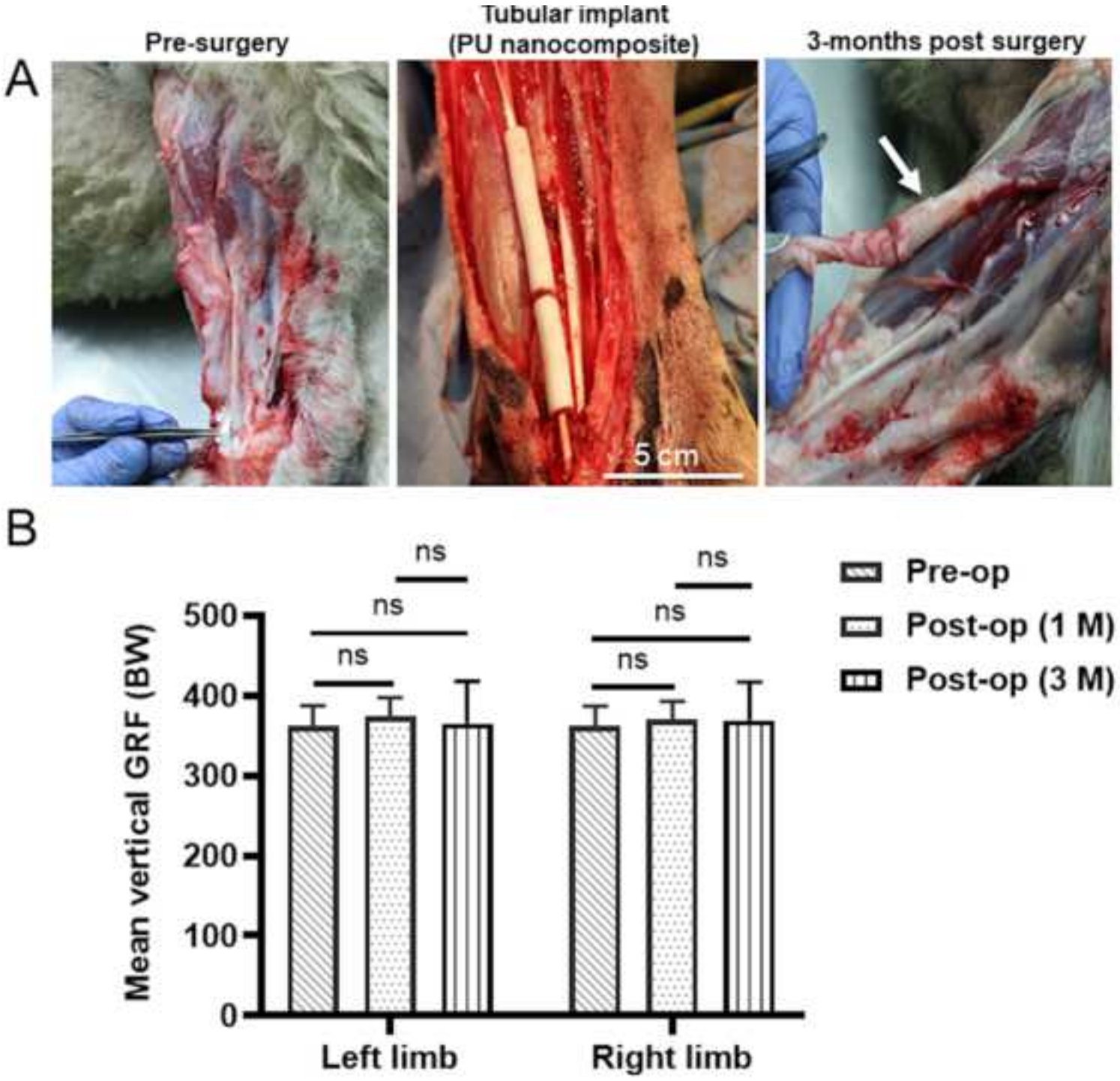

Figure 4

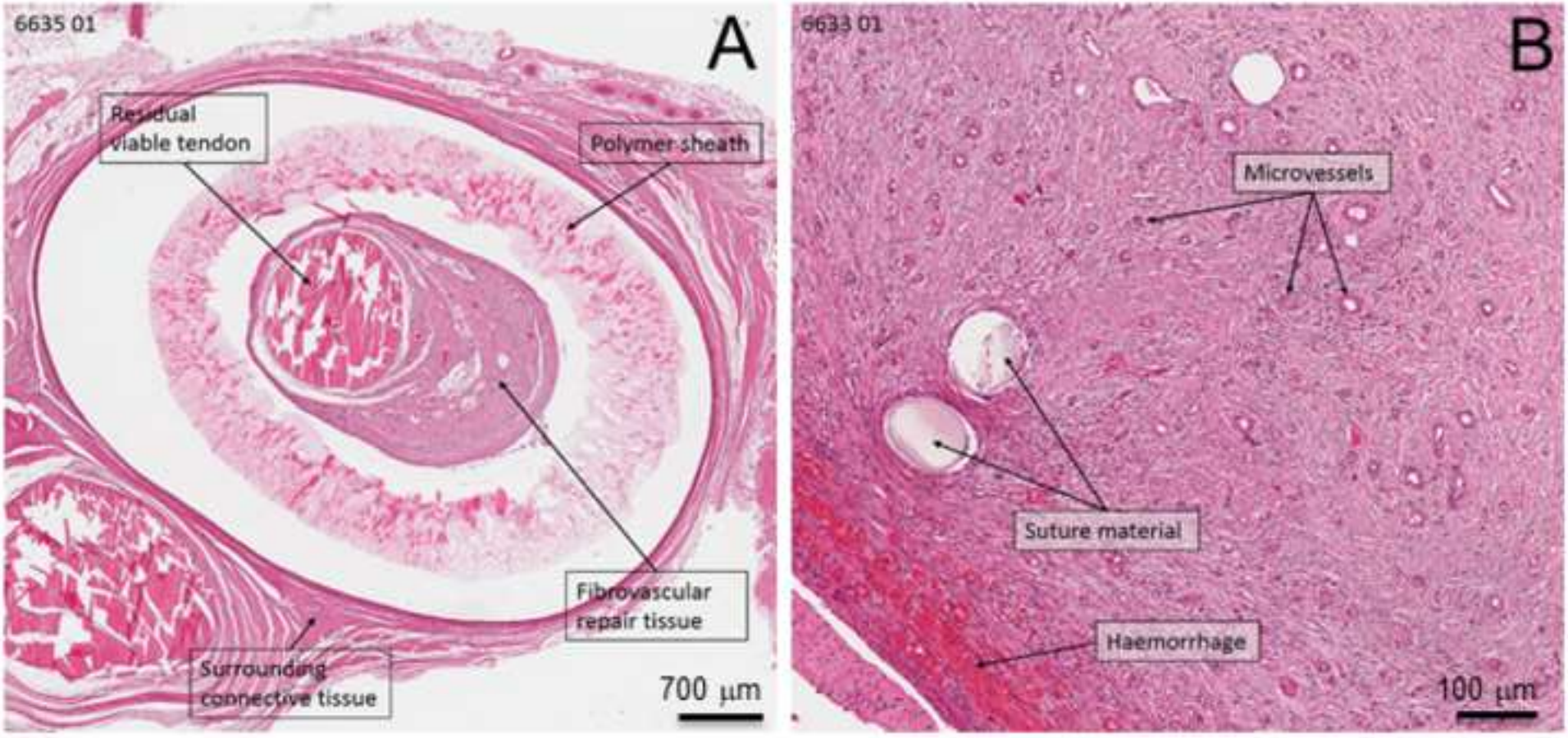

Figure 5

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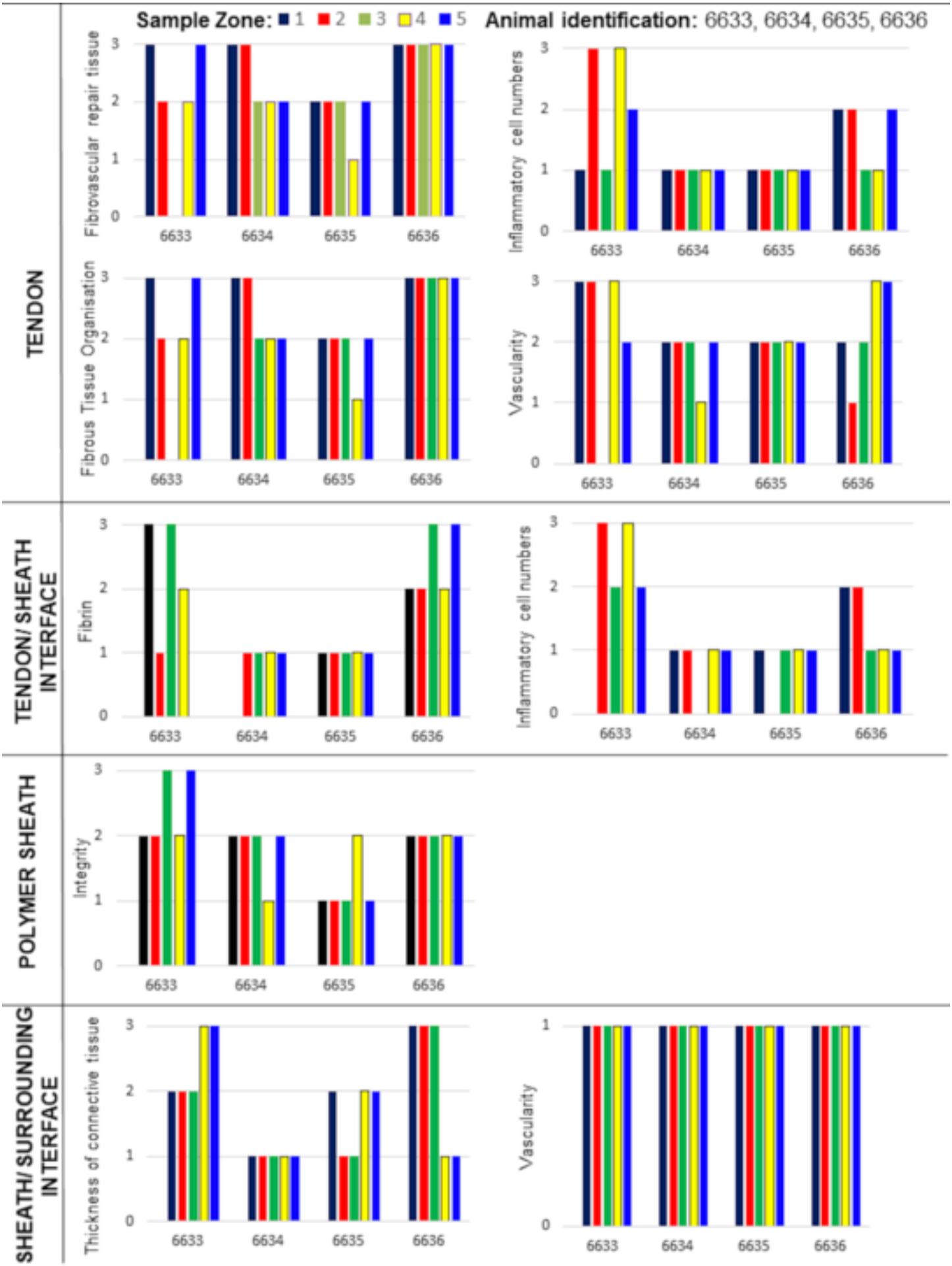

Figure Legends

Figure 1. Schematic of the surgical procedure for tendon repair of 6 (mature, female Mules) sheep. The tendon injury was induced unilaterally by surgical incision in the flexor tendon (peroneus tertius tendon of the left hind limb). The injury was repaired immediately with an overlying autogenous tendon graft (approximately 5 cm long) and the repair site was wrapped in tubular polymer implant (test implant) prior to wound closure. Then a locking core suture was placed with a running peripheral suture securing the donor tendon in place and the site covered with PU nanocomposite tubular polymer implant.

Figure 2. Fabrication and characterization of synthetic surgical grafts for tendon reconstruction. (A) Micro CT of a 6 mm PU nanocomposite tube suitable for single-stage tendon reconstruction. (B) SEM images of synthetic polymer conduits for tendon reconstruction: (B1) higher magnification cross-sectional view.

Figure 3. (A) Surgical implantation of the PU nanocomposite conduits into sheep model. (B) Graph depicting the ground reaction force (GRF) measured pre- and post-surgery (1 and 3 months). 'ns' corresponds to $p > 0.05$, indicating non-significant difference in the compared data sets. Abbreviations: GRF, ground reaction force; BW, body weight; ns, non-significant; pre-op, pre-operative; post-op, post-operative.

Figure 4. H&E stained Light micrographs of the trans-sectionally obtained *ex vivo* tendon grafts harvested 3 months post-surgery. The space between the polymer sheath and the surrounding tissue or the tendon is a processing artefact during histology.

Figure 5. Graphical representation of some of the key features of the extent of tendon repair from 4 descriptive regions: tendon, tendon/sheath interface, polymer sheath, and sheath/surrounding tissue interface, selected from the histopathological scoring sheet (Table 2). The X-axis represents the Animal identification number, and the Y-axis represents the score.

Figure 6. Regional variation in the repair process of the harvested tendon grafts, 3 months post-surgery. The X-axis shows the sample zones, with "1" representing the centre of the tendon, "4" and "2" immediately adjacent to the centre, and "5" and "3" along peripheral region. The P-values indicate the differences between the different sample zones were non-significant ($P>0.05$), in all the data.

Graphical Abstract. The longer treatment period and preserving are major obstacles preventing tendon sheath substitutes from reaching clinic. Here, we show that polyurethane nanocomposite (PU), as engineered tubular graft, implanted using single-stage surgical protocol instead of an otherwise two-stage flexor tendon reconstruction, in sheep model lead to substantial tendon repair in 3-months.

Declaration of interests forms

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Video 2 : After Surgery

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