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BIOMATERIALS

Stratifying the mechanical performance of a decellularized xenogeneic tendon graft for anterior cruciate ligament reconstruction as a function of graft diameter

AN ANIMAL STUDY

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Objectives

This study investigated the biomechanical performance of decellularized porcine superflexor tendon (pSFT) grafts of varying diameters when utilized in conjunction with contemporary ACL graft fixation systems. This aimed to produce a range of 'off-the-shelf' products with predictable mechanical performance, depending on the individual requirements of the patient.

Methods

Decellularized pSFTs were prepared to create double-bundle grafts of 7 mm, 8 mm, and 9 mm diameter. Femoral and tibial fixation systems were simulated utilizing Arthrex suspension devices and interference screws in bovine bone, respectively. Dynamic stiffness and creep were measured, followed by ramp to failure from which linear stiffness and load at failure were measured. The mechanisms of failure were also recorded.

Results

Dynamic stiffness was found to increase with greater graft diameter, with significant differences between all groups. Conversely, dynamic creep reduced with increasing graft diameter with significant differences between the 7 mm and 9 mm groups and the 8 mm and 9 mm groups. Significant differences were also found between the 7 mm, 8 mm, and 9 mm groups for linear stiffness, but no significant differences were found between groups for load at failure. The distribution of failure mechanisms was found to change with graft diameter.

Conclusion

This study showed that decellularized pSFTs demonstrate comparable biomechanical properties to other ACL graft options and are a potentially viable option for ACL reconstruction. Although grafts can be stratified by their diameter to provide varying biomechanical properties, it may be more appropriate to alter the fixation technique to stratify for a greater diversity of biomechanical requirements.

Cite this article: *Bone Joint Res* **2019;8:518–525.**

Keywords: Anterior cruciate ligament, Reconstruction, Decellularized

Article focus

- To investigate an alternative option for anterior cruciate ligament (ACL) reconstruction that overcomes certain limitations of existing options.
- To identify the mechanical properties of varying diameters of decellularized porcine superflexor tendon (pSFT) graft.
- To stratify the mechanical profiles of varying diameters of pSFT graft to produce a range of options for differing patient requirements.

Key messages

- The required initial fixation stability can be successfully achieved with decellularized (pSFT) grafts using current market fixation devices.
- The mechanical properties of decellularized pSFT grafts can be significantly

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doi: 10.1302/2046-3758.811.BJR-2019-0065.R1

Bone Joint Res 2019;8:518–525.

altered by varying graft diameter, and potentially by fixation method.
- This study demonstrates that decellularized pSFT grafts present a potentially viable option for ACL reconstruction from a biomechanical standpoint.

Strengths and limitations

- This study demonstrated that different diameters of decellularized pSFT grafts could be reliably reproduced with predictable mechanical properties.
- The mechanical properties of the grafts were comparable to conventional graft options.
- Further research to explore the effect of differing fixation devices on graft properties is needed.

Introduction

Rupture of the anterior cruciate ligament (ACL) is a common injury affecting young and active people, in particular sports men and women.[1] Estimates of the annual incidence of ACL injuries range from 32 to 80 per 100 000,[2] and the number of ACL reconstruction procedures performed yearly has increased, rising from 32.9 to 43.5 per 100 000 in the United States between 1994 and 2006. This equates to nearly 130 000 procedures per year,[3] with recent estimates as high as 300 000 reconstructions per year.[4] In the United Kingdom, approximately 14 000 ACL reconstructions are performed each year.[5] Studies have shown an association between untreated ACL injuries and subsequent meniscal damage and development of degenerative changes within the knee,[6] all of which serve to highlight the growing burden facing orthopaedic surgeons and health services in general.

Graft options include hamstring tendon or bone-patella tendon-bone (BPTB) autograft, which are currently the most popular choice,[7] cadaveric allograft tendons, or synthetic grafts. Autograft tissues have potential inherent drawbacks related to donor-site morbidity,[8] limited donor-site options in revision cases, and potentially inadequate graft dimensions following harvest.[9] A recent meta-analysis suggests that overall outcomes in revision ACL reconstructions were superior with autografts compared with allografts.[10] Both autologous and allogeneic grafts cannot maintain cell vitality and suffer progressive structural degradation. This is associated with a reduction in mechanical properties,[11] as the rate of tissue degradation typically exceeds that of constructive remodelling.[12,13] Synthetic grafts are also a treatment option, but have fallen out of favour due to their wear characteristics, failure rate, and the resultant chronic synovitis that frequently occurs.[14,15] Synthetic options also often fail to provide the multiscale hierarchical structure present in natural tendons and ligaments, essential for directing appropriate cell behaviour.[16] While there is a wealth of preclinical research into methods of biologically augmenting ACL reconstruction, such as utilizing platelet-rich plasma,[17] a decellularized tendon/ligament graft has the potential to replace a ruptured native ACL with the possible advantage of improved integration and ligamentization processes when compared with current graft solutions.

Decellularization processes have been developed that remove the majority of cellular material from tissues to produce a sterile biological scaffold graft with well-preserved biomechanical properties.[18] The aim is that the scaffolds initially provide biomechanical function to replace the damaged tissue while providing an attractive regenerative environment for endogenous cells to repopulate the scaffold and recapitulate the natural tissue.[19] Previous investigations in our group have explored the use of porcine superflexor (flexor digitorum profundus) tendons (pSFTs), with a view to developing a decellularized biological scaffold for ACL reconstruction. These studies have extensively demonstrated that the scaffold possesses the appropriate mechanical properties for an ACL graft,[18,20-22] with significantly reduced DNA content (> 99%) and α-Gal epitope levels,[20] while also demonstrating retention of structural proteins, biocompatibility, and *in vivo* regenerative capacity.[20]

However, the mechanical performance of these biological scaffolds in conjunction with ACL graft fixation devices has not yet been investigated. Typically, a reconstructed ACL is a structural system consisting of three engineering subsystems performing in unison: the femoral fixation system, the ACL graft, and the tibial fixation system. Until biological incorporation has been achieved, failure within such a structural system is most commonly expected at either fixation system location, as failure of the ACL graft in isolation requires substantially more force.[23,24] In this study, we aimed to evaluate the mechanical performance of such a whole structural system *in vitro* while varying the ACL graft (in this case, the decellularized pSFT graft).

Moreover, we aimed to investigate whether decellularized pSFTs could be manufactured to create a portfolio of ACL grafts with different diameters that, when combined with ACL graft fixation devices, created structural systems that generate different mechanical properties (based on graft size) within a range suitable for ACL reconstruction. Specifically, the dynamic mechanical (dynamic stiffness and creep) and failure properties (failure load, linear stiffness, mechanisms of failure) were investigated. This could potentially stratify decellularized pSFTs into a range of graft sizes with predictable mechanical performance when used with fixation devices, providing a selection of 'off-the-shelf' ACL graft options. These could then be matched to each individual patient's requirements based on anthropometric measurements such as height and weight, femoral or tibial dimensions, or desired sport/activity level.

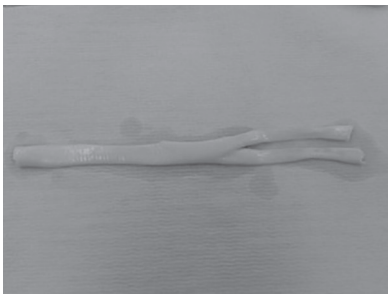

Fig. 1a

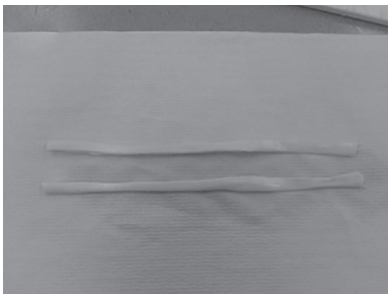

Fig. 1b

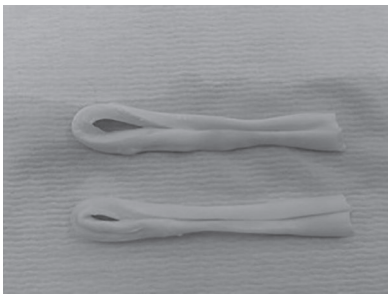

Fig. 1c

a) Porcine superflexor tendons are harvested and decellularized. b) They are split along their long axis. c) This yields two potential double bundle anterior cruciate ligament grafts, which are trimmed down appropriately to the desired graft diameter.

Materials and Methods

Tissue sourcing and decellularization. A total of 18 pSFTs were obtained from four-month-old, large, white, female pigs weighing between 70 kg and 80 kg from a local abattoir (J Penny, Leeds, United Kingdom) within 24 hours of slaughter. Following removal, pSFTs were stored at -20°C with phosphate buffered saline (PBS)-soaked filter paper prior to decellularization. Specimens were decellularized using a previously established procedure.[18,20] In brief, this consisted of multiple freeze-thaw cycles (× 3), antibiotic treatment (PBS containing vancomycin hydrochloride (Merck, Nottingham, United Kingdom), gentamycin sulphate (Merck), and polymyxin B sulphate (Merck)), acetone washes (VWR, Lutterworth, United Kingdom), low concentration detergent (sodium dodecyl sulphate (SDS; Sigma, Gillingham, United Kingdom), 0.1% w/v) washes and benzonase (Merck) treatment. The process also included protease inhibitor treatment (aprotinin; Nordic Pharma, Reading, United Kingdom) and a 0.1% peracetic acid (Sigma) sterilization step in the final stages of the process.

Graft preparation and fixation. Following decellularization, pSFTs were bisected with care along their long axis using a scalpel to produce two lengths of decellularized tendon, which were looped to form double bundle ACL grafts (Fig. 1). These were measured with digital calipers and cut to produce 7 mm, 8 mm, and 9 mm diameter grafts (n = 6 for each group). Graft diameters were confirmed using a graft sizing block (DePuy Synthes, Raynham, Massachusetts). The order of graft diameters produced was at random. Each graft was then looped through a Tightrope femoral fixation device (Arthrex, Naples, Florida) and the free ends were whip-stitched with #2 FiberWire (Arthrex).

Bone was sourced from the lateral femoral condyles of skeletally mature bovine femurs obtained from a local abattoir (J Penny). Blocks of bovine bone were cut to a uniform size (35 mm × 35 mm × 40 mm) using a custom-made jig. Tunnels were drilled in the bone blocks to a diameter equal to the intended tendon graft diameter, and grafts were fixed in place using an Arthrex bioabsorbable interference screw (Arthrex), representing the tibial fixation subsystem of the reconstruction. Screw diameters were downsized by 1 mm with respect to the graft/tunnel diameter, as use of equal screw sizes was found to cause fracture of either the bone block or screw itself in two pilot constructs. Each graft was then fixed via the Tightrope to a steel block representing the femoral fixation subsystem of the reconstruction (Fig. 2a). Preparation and assembly of the ACL replacement model was performed by a single surgically trained author to maintain consistency.

Test protocols. Biomechanical testing was performed using an Instron ElectroPuls E10,000 (Instron, High Wycombe, United Kingdom) incorporating a 1 kN load cell. ACL grafts and their fixation subsystems were attached in series to the testing machine using bespoke brackets (Fig. 2b). All grafts were preconditioned by loading them between 5 N and 50 N using a sinusoidal wave form for 30 cycles at 0.5 Hz. Immediately following this, dynamic testing was performed by sinusoidally loading grafts between 50 N and 250 N for 1000 cycles at 1 Hz to simulate dynamic physiological loading conditions. Similar dynamic loading protocols have been used previously by other authors.[25-30] During this phase of the testing, the dynamic stiffness (k* = Δ peak to peak applied load / Δ peak to peak induced extension) and maximum dynamic creep extension of all specimens within each graft diameter group were continuously recorded. Creep in this instance referred to whole system creep (including the fixation points and devices) with the point of graft entry into the bone tunnel marked with ink prior to testing to ensure the recorded extension was in fact due to creep rather than graft slippage.

Following dynamic testing, failure testing was performed by subjecting grafts and their fixation systems to an extension ramp to failure at 50 mm per minute, a rate consistent with other studies.[31,32] This phase of testing allowed for calculation of the linear stiffness (slope of the linear region of the load-extension curve) and failure load for all graft diameters investigated. Furthermore, it allowed for determination of the mechanisms of failure of the whole structural systems involved for the three groups. While it may have been logical to examine the

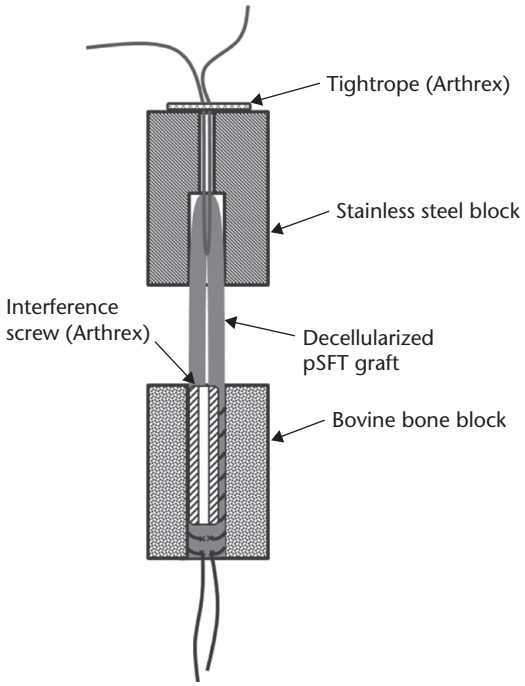

Fig. 2a

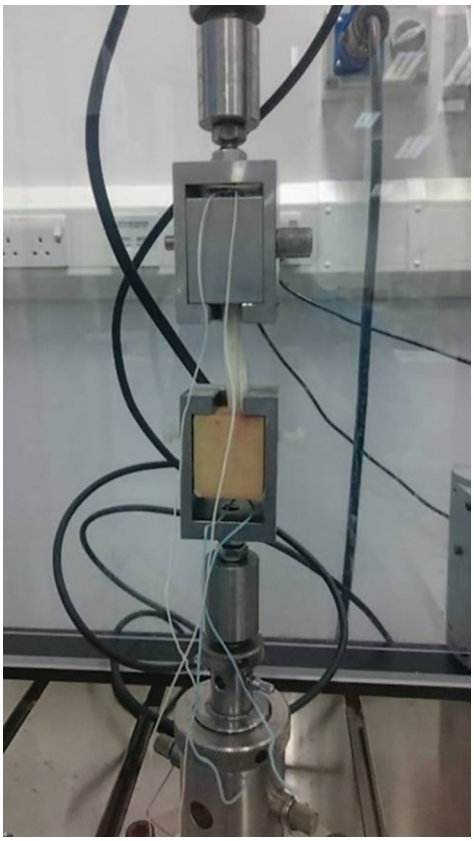

Fig. 2b

a) Schematic of fixation system used to model anterior cruciate ligament replacement with decellularized porcine superflexor tendon (pSFT) graft (Tightrope (Arthrex, Naples, Florida) femoral fixation, interference screw (Arthrex) tibial fixation). b) System when attached to Instron Electropuls E10,000 (Instron, High Wycombe, United Kingdom) for biomechanical testing.

failure mechanisms of each of the engineering subsystems in isolation, this approach has previously been performed extensively.[23-30] Examining the structural system as a whole would better inform on which of each engineering subsystems are more likely to fail having varied the graft diameter. For both dynamic and failure testing, data were captured at a frequency of 0.1 Hz.

Statistical analysis. For dynamic test parameters (dynamic stiffness and creep), variances between graft size groups were determined using a two-way analysis of variance (ANOVA). Tukey's significant difference test was used for *post hoc* evaluation. A p-value of < 0.05 was considered to be statistically significant.

For the failure test parameters calculated (linear stiffness and load at failure), a one-way ANOVA was performed. Again, Tukey's significant difference test was used for *post hoc* evaluation, with a p-value of < 0.05 considered to be statistically significant. The data associated with this paper (including raw data and results) are openly available from the University of Leeds Data Repository.[33]

Results

Dynamic testing. The dynamic stiffness responses for all three groups (7 mm, 8 mm, and 9 mm graft diameters) are presented in Figure 3a. Dynamic stiffness was found to increase significantly with cycles elapsed. Significant differences were found between all groups tested. Following 1000 cycles of loading, dynamic stiffness means of 143.4 N/mm (95% confidence interval (CI) 137.9 to 149.0), 157.7 N/mm (95% CI 144.9 to 170.4), and 175.2 N/mm (95% CI 166.5 to 183.9) were found for 7 mm, 8 mm, and 9 mm graft groups, respectively. No significant interaction was found between graft size and cycles elapsed.

The dynamic creep extension responses for all groups tested are presented in Figure 3b. Dynamic creep was found to significantly increase with cycles elapsed. Significant differences were found between 7 mm and 9 mm graft size groups, in addition to between 8 mm and 9 mm graft size groups. Dynamic creep means of 6.6 mm (95% CI 5.9 to 7.3), 6.2 mm (95% CI 5.6 to 6.9), and 5.8 mm (95% CI 5.3 to 6.3) were found following 1000 cycles of loading for 7 mm, 8 mm, and 9 mm graft groups, respectively. Again, no significant interaction was found between graft size and cycles elapsed.

Failure testing. Following failure testing, significant differences were found between all groups for linear stiffness with means of 167.8 N/mm (95% CI 162.8 to

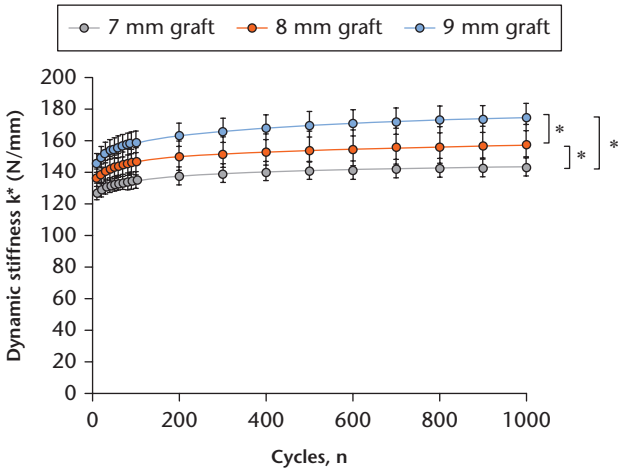

Fig. 3a

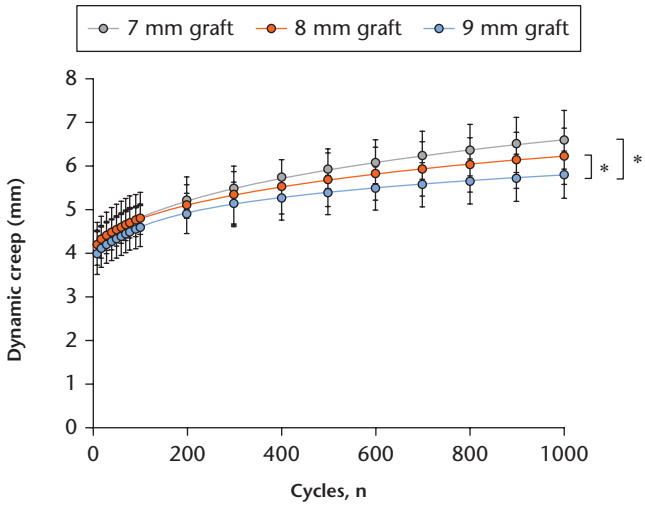

Fig. 3b

a) Dynamic stiffness and b) dynamic creep extension responses for 7 mm, 8 mm, and 9 mm diameter decellularized porcine superflexor tendon grafts with fixation systems following cyclic testing. Values presented as mean and 95% confidence intervals (n = 6). *Statistically significant difference between groups (two-way analysis of variance with Tukey *post hoc* analysis; $p < 0.05$).

172.8), 187.0 N/mm (95% CI 170.3 to 203.7), and 216.3 N/mm (95% CI 203.9 to 228.7) for 7 mm, 8 mm, and 9 mm graft groups, respectively (Fig. 4a). However, no significant differences were found for failure load between 7 mm, 8 mm, and 9 mm graft size groups (Fig. 4b), with means of 531.6 N (95% CI 472.7 to 590.5), 604.1 N (95% CI 420.8 to 787.5), and 628.0 N (95% CI 555.5 to 700.4), respectively.

Mechanisms of failure were observed at each of the three engineering subsystems: Tightrope failure, pull-out at the interference screw interface, and failure of the grafts itself. The number and distribution of these failures within graft size groups is presented in Table I. Two of the 7 mm grafts failed via 'cheese-wiring' of the Tightrope through the graft tissue, while all the remaining failures occurred at either of the fixation devices themselves: nine at the Tightrope, seven at the interference screw.

Discussion

The decellularization of xenogeneic tissues such as the pSFT offers a promising alternative solution for the reconstruction of ruptured ACLs, delivering immunologically safe biomaterials 'off the shelf' and in plentiful supply. It is important, however, that the biomechanical properties of these biological scaffolds are fully characterized to ensure satisfactory performance fit for clinical purpose. This study therefore aimed to assess decellularized pSFTs using an *in vitro* biomechanical model of ACL reconstruction simulating both femoral and tibial fixation subsystems. Furthermore, three different graft sizes (7 mm, 8 mm, and 9 mm diameters) were investigated with a view to creating whole structural systems of ACL reconstruction that result in mechanical performance distinct to each graft size, yet still within a range similar to native ACLs. This could allow the targeting of specific patient populations with grafts to match their requirements.

Mean stiffness values for native ACL tissue range from 130 N/mm to 300 N/mm,[34] depending on the age[35,36] and sex[37] of the donor.[34] Mean stiffness values for hamstring tendon and BPTB autografts are reported to be approximately 800 N/mm and 450 N/mm, respectively.[23,24] There is a deliberate overcompensation in bulk strength and stiffness with the use of these autografts to accommodate for their subsequent degradation after implantation due to necrosis. This overcompensation facilitates graft survival until remodelling and ligamentization is achieved. However, it may lead to a mismatch in mechanical properties and performance at the time of implantation, as the stiffness of these autografts far exceeds that of the native ACL. This presents an opportunity for decellularized biological scaffolds to better match native ACL properties due to potentially reduced necrosis and quicker incorporation, as non-viable cells have already been removed. When the decellularized pSFT grafts were utilized with conventional suspension devices and interference screws, both dynamic and linear mean stiffness values of these whole systems fell within the native ACL range regardless of graft diameter. This is a potential advantage over conventional autografts, as it is not clear what impact the implantation of a graft that is significantly stiffer than the native ACL has on the knee. This applies to both short-term recovery with respect to rehabilitation, and in the long term with potential development of osteoarthritis. In addition, creep and stiffness values varied significantly between graft diameters, and values were readily reproducible between different specimens of equal size. This confirms that the mechanical performance of decellularized pSFT grafts within a predetermined

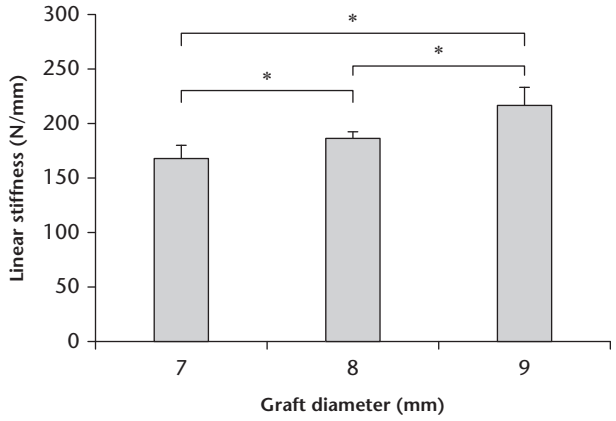

Fig. 4a

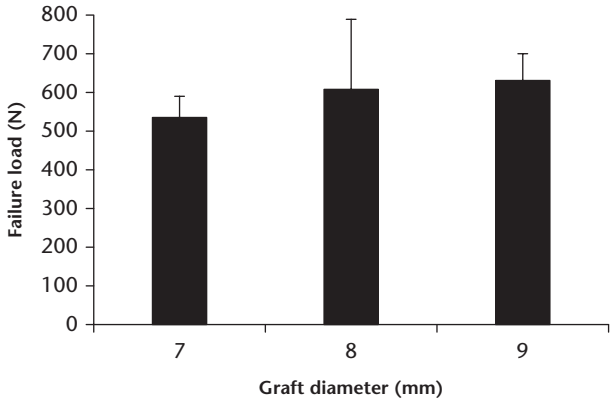

Fig. 4b

a) Linear stiffness and b) failure load values for 7 mm, 8 mm, and 9 mm diameter decellularized porcine superflexor tendon grafts with fixation systems following failure testing. Values presented as mean and 95% confidence intervals (n = 6). *Statistically significant difference between groups (one-way analysis of variance with Tukey *post hoc* analysis; $p < 0.05$).

fixation model could be modulated by altering the graft diameter.

A stratified approach such as this creates the potential for producing a range of graft options with variable properties once implanted, allowing customization for an individual patient. This stratification may be informed by anthropometric measurements or desired level of activity. Previous imaging-based studies have demonstrated significant variation between the native ACL insertion size and the cross-sectional area of potential graft donor tendons,[38] a mismatch issue that could be mitigated with a range of pSFT graft sizes available 'off the shelf'. Although significant differences were found between groups of different graft size, the dynamic stiffness in particular remained within the lower end (< 180 N/mm) of the native ACL stiffness range (130 N/mm to 300 N/mm).[34] Therefore, variation of the engineering systems of fixation may serve as a better approach in attempting to stratify for a broader variety of patient populations. Grafts utilizing transfemoral pins, for example, have previously demonstrated significantly less displacement than when using cortical suspension devices.[30] Utilizing interference screws at both femoral and tibial fixation locations may also achieve this as the effective graft length can be reduced, therefore increasing the stiffness.

Achieving adequate initial fixation stability at the femoral and tibial fixation locations is crucial to allow participation in modern rehabilitation protocols.[23] This is important as, at the time of implantation and until biological incorporation has been achieved, the forces required to cause failure at the fixation system locations are typically much lower than those required to cause failure of the graft itself.[23,24] *In vivo* simulations suggest normal daily activities such as level walking (during midstance in particular) or descending stairs exert a peak physiological load on the ACL of approximately 300 N during hyperextension.[34,39,40] It is therefore a requirement of any ACL graft under fixation to withstand load in excess of this peak physiological level at the point of implantation, to ensure fixation stability throughout the rehabilitation process. Quoted values for the minimum acceptable initial failure load for a given graft option range in the literature from 248 N[41] to 454 N.[35]

Table I. Failure mechanisms observed for the decellularized porcine superflexor tendon grafts with fixation systems. The distribution of failure mechanisms was found to vary between each graft size group

Failure mechanism	7 mm graft	8 mm graft	9 mm graft
Tightrope failure, n	1	3	5
Graft pull-out at screw interface, n	3	3	1
Graft failure, n	2	0	0

For existing autograft/allograft options combined with various fixation methods, mean ultimate failure loads ranged from 201 N to 930 N for quadrupled hamstring grafts, and from 215 N to 681 N for BPTB grafts.[23,24] These fixation methods included various combinations of suspensory devices, as well as metal and bioabsorbable interference screws. When compared with the maximum loads exerted on the ACL during physiological loading (taken as 300 N for reasons detailed above), the mean comparative failure loads of decellularized pSFT grafts under fixation were 532 N for 7 mm grafts, 604 N for 8 mm grafts, and 628 N for 9 mm grafts, all exceeding the 454 N quoted as the upper limit of the range of minimum acceptable initial ultimate failure load.[35] Therefore, the required initial fixation stability can also be successfully achieved with decellularized pSFT grafts using current market fixation devices.

It is possible that alternative fixation devices may produce a greater level of initial fixation stability, as most failures occurred at the fixation devices themselves rather than at the decellularized pSFT graft tissue. Only 2 of the 18 grafts tested failed directly (due to Tightrope

'cheese-wiring' through the grafts), both of which were 7 mm diameter specimens. This is in agreement with previous literature, which has shown that grafts measuring 8 mm or more are associated with reduced failure rates.[9] The distribution of mechanisms of failure was observed to change with decellularized pSFT graft size (Table I). For 7 mm diameter graft systems, failure of the suspension device occurred in only one case, with the remaining cases failing either due to pull-out at the screw interface (n = 3), or as mentioned previously, direct graft failure (n = 2). The 8 mm diameter graft systems failed equally at either the suspension device (n = 3) or screw interface (n = 3). The 9 mm graft systems failed predominately at the suspension device (n = 5), with only one screw interface failure. The interaction between the three engineering subsystems within this model of ACL reconstruction is evidently complex. However, it appears that as graft size increases, the graft itself and the screw interface become more resistant to failure causing a greater proportion of suspension device failures.

The pSFT tissue was selected as a potential graft choice as it can be routinely harvested at relatively low cost and the healthy tissue can be obtained from young donors of a predetermined age, species, and source. Such selection procedures may yield more consistent graft quality and therefore more predictable material properties than allograft tissues. The macroscopic dimensions of the pSFT grafts are similar to the human hamstring tendons commonly used as an autograft in ACL reconstruction, allowing for their use in conjunction with currently available fixation devices (in this instance, femoral suspension devices and interference screws).

There are a number of limitations to address in this study. First, test specimens were only implanted in bone on the tibial side of the construct, whereas the femoral side was represented with a stainless steel block. This may have affected the results, as any failure at the femoral side was inevitably due to device failure rather than at the graft-fixation interface. The use of bovine bone rather than cadaveric human bone could also have an impact due to the differences in density between the two tissues. Indeed, the decision to downsize the interference screw by 1 mm with respect to the graft/tunnel diameter was in direct response to two pilot constructs that failed during assembly, likely due to the higher density of the bovine bone block. However, there have been other studies examining ACL graft fixation methods that have similarly utilized bovine tissue to model the human knee.[42,43]

A common criticism of biomechanical studies of ACL grafts relates to the uniaxial tensile tests that are performed, which do not realistically reproduce the *in vivo* forces experienced by the ACL. In addition to enabling more direct comparisons between studies to be drawn, it is likely that the resultant ultimate failure loads recorded are an underestimate compared with those that would be produced *in vivo*, as the tensile force vector is in line with the femoral tunnel. This reduces the effect of shear forces that occur when the tunnel is oblique to the force vector, potentially altering the forces required to cause failure.[23]

Further research is required to understand the *in vivo* effects on implanted decellularized tissue in the context of ACL reconstruction. Such work would help ascertain the likely variation in biomechanical properties as the graft incorporates, as well as the effect of the host's immune response on both the implanted graft tissue and the overall healing response. While the utility of a potential tissue for ligament reconstruction initially hinges on the tissue's mechanical and material properties, the host tissue response and associated remodelling process will ultimately determine the success or failure of the implanted graft as a regenerative medicine solution.[44]

In conclusion, this study demonstrates that decellularized pSFT grafts present a potentially viable option for ACL reconstruction from a biomechanical point of view, exhibiting comparable initial fixation stability to existing graft options at the point of implantation. The ability to alter the mechanical performance of the graft by adjusting the graft diameter has also been demonstrated. Alteration of the fixation technique or device in tandem with graft diameter may provide opportunities to stratify for a greater diversity of biomechanical requirements.

Decellularized pSFT grafts present several potential benefits over existing graft options, in particular the elimination of donor site morbidity, low cost of production and storage, and a plentiful supply of base tissue. Further research is being conducted by our institution in the form of a large animal study to explore the *in vivo* response to decellularized pSFT tissue including local and systemic immunological effects, cell infiltration, neovascularization, integration, and *ex vivo* biomechanical properties compared with allograft controls and native ACLs.

References

1. **Gianotti SM, Marshall SW, Hume PA, Bunt L.** Incidence of anterior cruciate ligament injury and other knee ligament injuries: a national population-based study. *J Sci Med Sport* 2009;12:622-627.
2. **No authors listed.** Annual Report 2016. Swedish ACL Register. 2016. https://aclregister.nu/media/uploads/Annual%20reports/rapport2016en.pdf (date last accessed 11 October 2019).
3. **Mall NA, Chalmers PN, Moric M, et al.** Incidence and trends of anterior cruciate ligament reconstruction in the United States. *Am J Sports Med* 2014;42:2363-2370.
4. **Zaffagnini S, Grassi A, Marcheggiani Muccioli GM, et al.** Anterior cruciate ligament reconstruction with a novel porcine xenograft: the initial Italian experience. *Joints* 2015;3:85-90.
5. **Beard D, Cook J, Davies L, et al.** ACL SNNAP. Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences. https://www.ndorms.ox.ac.uk/clinical-trials/current-trials-and-studies/snnap (date last accessed 11 October 2019).
6. **Louboutin H, Debarge R, Richou J, et al.** Osteoarthritis in patients with anterior cruciate ligament rupture: a review of risk factors. *Knee* 2009;16:239-244.
7. **Holland T, Chitkara R, McNicholas M, Banks J.** Current trends in ACL reconstruction surgery in the United Kingdom. *J Orthop Trauma.* 2017;7:1-4.
8. **Kartus J, Movin T, Karlsson J.** Donor-site morbidity and anterior knee problems after anterior cruciate ligament reconstruction using autografts. *Arthroscopy* 2001;17:971-980.

9. **Conte EJ, Hyatt AE, Gatt CJ Jr, Dhawan A.** Hamstring autograft size can be predicted and is a potential risk factor for anterior cruciate ligament reconstruction failure. *Arthroscopy* 2014;30:882-890.
10. **Grassi A, Nitri M, Moulton SG, et al.** Does the type of graft affect the outcome of revision anterior cruciate ligament reconstruction? a meta-analysis of 32 studies. *Bone Joint J* 2017;99-B:714-723.
11. **Woods T, Gratzer PF.** Effectiveness of three extraction techniques in the development of a decellularized bone-anterior cruciate ligament-bone graft. *Biomaterials* 2005;26:7339-7349.
12. **Jackson DW, Corsetti J, Simon TM.** Biologic incorporation of allograft anterior cruciate ligament replacements. *Clin Orthop Relat Res* 1996;324:126-133.
13. **McFarland EG.** The biology of anterior cruciate ligament reconstructions. *Orthopedics* 1993;16:403-410.
14. **Satora W, Królikowska A, Czamara A, Reichert P.** Synthetic grafts in the treatment of ruptured anterior cruciate ligament of the knee joint. *Polim Med* 2017;47:55-59.
15. **Murray AW, Macnicol MF.** 10-16 year results of Leeds-Keio anterior cruciate ligament reconstruction. *Knee* 2004;11:9-14.
16. **Herbert A, Brown C, Rooney P, et al.** Bi-linear mechanical property determination of acellular human patellar tendon grafts for use in anterior cruciate ligament replacement. *J Biomech* 2016;49:1607-1612.
17. **Hexter AT, Thangarajah T, Blunn G, Haddad FS.** Biological augmentation of graft healing in anterior cruciate ligament reconstruction: a systematic review. *Bone Joint J* 2018;100-B:271-284.
18. **Herbert A, Jones GL, Ingham E, Fisher J.** A biomechanical characterisation of acellular porcine super flexor tendons for use in anterior cruciate ligament replacement: investigation into the effects of fat reduction and bioburden reduction bioprocesses. *J Biomech* 2015;48:22-29.
19. **Pridgen BC, Woon CY, Kim M, et al.** Flexor tendon tissue engineering: acellularization of human flexor tendons with preservation of biomechanical properties and biocompatibility. *Tissue Eng Part C Methods* 2011;17:819-828.
20. **Jones G, Herbert A, Berry H, et al.** Decellularization and characterization of porcine superflexor tendon: a potential anterior cruciate ligament replacement. *Tissue Eng Part A* 2017;23:124-134.
21. **Edwards JH, Herbert A, Jones GL, et al.** The effects of irradiation on the biological and biomechanical properties of an acellular porcine superflexor tendon graft for cruciate ligament repair. *J Biomed Mater Res B Appl Biomater* 2017;105:2477-2486.
22. **Edwards JH, Ingham E, Herbert A.** Decellularisation affects the strain rate dependent and dynamic mechanical properties of a xenogeneic tendon intended for anterior cruciate ligament replacement. *J Mech Behav Biomed Mater* 2019;91:18-23.
23. **Brand J Jr, Weiler A, Caborn DN, Brown CH Jr, Johnson DL.** Graft fixation in cruciate ligament reconstruction. *Am J Sports Med* 2000;28:761-774.
24. **Dargel J, Gotter M, Mader K, et al.** Biomechanics of the anterior cruciate ligament and implications for surgical reconstruction. *Strategies Trauma Limb Reconstr* 2007;2:1-12.
25. **Bohn MB, Vestergaard R, Dalstra M, et al.** Mechanical stability of the femoral fixation for single- and double-bundle ACL reconstruction in an in vitro experimental model. *Scand J Med Sci Sports* 2013;23:263-270.
26. **Kleweno CP, Jacir AM, Gardner TR, Ahmad CS, Levine WN.** Biomechanical evaluation of anterior cruciate ligament femoral fixation techniques. *Am J Sports Med* 2009;37:339-345.
27. **Mayr R, Heinrichs CH, Eichinger M, et al.** Biomechanical comparison of 2 anterior cruciate ligament graft preparation techniques for tibial fixation: adjustable-length loop cortical button or interference screw. *Am J Sports Med* 2015;43:1380-1385.
28. **Monaco E, Fabbri M, Lanzetti RM, et al.** Biomechanical comparison of four coupled fixation systems for ACL reconstruction with bone socket or full-tunnel on the tibial side. *Knee* 2017;24:705-710.
29. **Rylander L, Brunelli J, Taylor M, et al.** A biomechanical comparison of anterior cruciate ligament suspensory fixation devices in a porcine cadaver model. *Clin Biomech (Bristol, Avon)* 2014;29:230-234.
30. **Shen HC, Chang JH, Lee CH, et al.** Biomechanical comparison of Cross-pin and Endobutton-CL femoral fixation of a flexor tendon graft for anterior cruciate ligament reconstruction—a porcine femur-graft-tibia complex study. *J Surg Res* 2010;161:282-287.
31. **Petre BM, Smith SD, Jansson KS, et al.** Femoral cortical suspension devices for soft tissue anterior cruciate ligament reconstruction: a comparative biomechanical study. *Am J Sports Med* 2013;41:416-422.
32. **Johnson JS, Smith SD, LaPrade CM, et al.** A biomechanical comparison of femoral cortical suspension devices for soft tissue anterior cruciate ligament reconstruction under high loads. *Am J Sports Med* 2015;43:154-160.
33. **Herbert A, Edwards J.** Stratification of decellularised porcine super flexor tendon for ACL replacement. The University of Leeds. 2019. http://archive.researchdata.leeds.ac.uk/584/ (date last accessed 25 October 2019).
34. **Marieswaran M, Jain I, Garg B, Sharma V, Kalyanasundaram D.** A review on biomechanics of anterior cruciate ligament and materials for reconstruction. *Appl Bionics Biomech* 2018;2018:4657824.
35. **Noyes FR, Butler DL, Grood ES, Zernicke RF, Hefzy MS.** Biomechanical analysis of human ligament grafts used in knee-ligament repairs and reconstructions. *J Bone Joint Surg [Am]* 1984;66-A:344-352.
36. **Woo SL, Hollis JM, Adams DJ, Lyon RM, Takai S.** Tensile properties of the human femur-anterior cruciate ligament-tibia complex. The effects of specimen age and orientation. *Am J Sports Med* 1991;19:217-225.
37. **Chandrashekar N, Slauterbeck J, Hashemi J.** Sex-based differences in the anthropometric characteristics of the anterior cruciate ligament and its relation to intercondylar notch geometry: a cadaveric study. *Am J Sports Med* 2005;33:1492-1498.
38. **Offerhaus C, Albers M, Nagai K, et al.** Individualized anterior cruciate ligament graft matching: in vivo comparison of cross-sectional areas of hamstring, patellar, and quadriceps tendon grafts and ACL insertion area. *Am J Sports Med* 2018;46:2646-2652.
39. **Kadaba MP, Ramakrishnan HK, Wootten ME.** Measurement of lower extremity kinematics during level walking. *J Orthop Res* 1990;8:383-392.
40. **Shelburne KB, Pandy MG, Anderson FC, Torry MR.** Pattern of anterior cruciate ligament force in normal walking. *J Biomech* 2004;37:797-805.
41. **Shelbourne KD, Gray T.** Anterior cruciate ligament reconstruction with autogenous patellar tendon graft followed by accelerated rehabilitation. A two- to nine-year followup. *Am J Sports Med* 1997;25:786-795.
42. **Piltz S, Strunk P, Meyer L, Plitz W, Lob G.** Fixation strength of a novel bioabsorbable expansion bolt for patellar tendon bone graft fixation: an experimental study in calf tibial bone. *Knee Surg Sports Traumatol Arthrosc* 2004;12:376-383.
43. **Willis-Owen CA, Hearn TC, Keene GC, Costi JJ.** Biomechanical testing of implant free wedge shaped bone block fixation for bone patellar tendon bone anterior cruciate ligament reconstruction in a bovine model. *J Orthop Surg Res* 2010;5:66.
44. **Brown BN, Ratner BD, Goodman SB, Amar S, Badylak SF.** Macrophage polarization: an opportunity for improved outcomes in biomaterials and regenerative medicine. *Biomaterials* 2012;33:3792-3802.

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- S. Whitaker: Designed the study, Surgically prepared and fixated the specimens, Interpreted the data, Wrote the manuscript.
- J. H. Edwards: Designed the study, Decellularized the specimens, Interpreted the data, Wrote the manuscript.
- S. Guy: Designed the study, Interpreted the data, Wrote the manuscript.
- E. Ingham: Designed the study, Interpreted the data, Wrote the manuscript.
- A. Herbert: Designed the study, Performed biomechanical testing, Collected, analyzed, and interpreted the data, Wrote the manuscript.

Conflict of interest statement

- S. Guy reports expenses payments from DePuy Synthes, not related to this study. E. Ingham reports consultancy fees and expert testimony fees from DePuy Synthes, not related to this study.

Funding statement

- This was supported by an EPSRC ETERM Landscape Fellowship (EP/I017801/1) and an EPSRC Programme Grant (Optimising Knee Therapies; EP/P001076/1).
- No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Acknowledgements

- The authors would like to acknowledge Phil Wood and the Institute of Medical and Biological Engineering (iMBE) technical team for their expertise.

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