Characterisation of the Tensile Properties of Demineralised Cortical Bone used as an ACL Allograft

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Abstract

Background

Graft choice in anterior cruciate ligament (ACL) reconstruction remains controversial and some grafts fail due to inadequate osteointegration. Demineralised cortical bone (DCB) is an osteoinductive collagen-based scaffold. The aim of this study was to measure the tensile properties of DCB from different locations and from different ages, and determine its compatibility with current ACL fixation systems.

Methods

The tensile properties of DCB manufactured from femur and tibia of young (9 month) and old (2-3 years) sheep was measured to determine the most appropriate graft choice. The ultimate load and stiffness of DCB allograft using two fixation systems, interference screws and sutures tied around screw posts, was measured *ex vivo* in an ovine ACL reconstruction model. Comparison was made with superficial digital flexor tendon (SDFT) and ovine ACL.

Results

DCB derived from young tibia had the highest ultimate load and stiffness of 67.7 ± 10.6 N and 130.2 ± 64.3 N/mm respectively. No DCB fixation system reached the published peak *in vivo* force through the ovine ACL of 150 N. SDFT fixation with interference screws (308.2 ± 87.3 N) did reach the *in vivo* threshold but was significantly weaker than ovine ACL (871.0 ± 64.2 N).

Conclusion

The tensile properties of DCB were influenced by the donor age and bone. Owing to inferior tensile properties and incompatibility with suspensory fixation devices, this study indicates DCB is inferior to current tendon grafts options for ACL reconstruction.

Key words

Anterior Cruciate Ligament (ACL)

Demineralised Bone Matrix

Demineralised Cortical Bone

Graft Fixation

Tensile Properties

1. Introduction

The choice of graft material remains controversial in anterior cruciate ligament (ACL) reconstruction due to the limitations of current graft options (1). Autograft tendons are limited by donor site morbidity and unpredictable graft quality, whereas allogenic tendons are associated with delayed biological integration and increased rupture rates in younger patients (2). In terms of graft healing, current grafts regenerate a biomechanically inferior fibrous insertion compared to normal

ACL where the insertion is graded from the ligament to fibrocartilage to mineralised fibrocartilage and finally to bone (3). The search continues for an allograft that is widely available, avoids donor site morbidity and can achieve early osteointegration restoring the native insertion that permits earlier rehabilitation.

Demineralised cortical bone (DCB), also referred to as demineralised bone matrix (DBM), is a collagen-based matrix manufactured by removing the organic component of bone (4). Demineralised bone is widely used in orthopaedics and available in different forms including cortical strips (5). DCB has properties of the ideal ACL graft because it is widely available and contains an endogenous source of osteoinductive growth factors, such as bone morphogenetic proteins, which have potential to enhance osteointegration in the bone tunnels (6). An ovine study showed DCB can repair patella tendon defects by restoring a chondral enthesis (7) and a caprine study has successfully used DCB to replace an ACL (8). Mechanical analysis of bovine DCB indicate that DCB has mechanical properties similar to the ACL in terms of tensile strength, strain, stiffness and visco-elasticity (9). However DCB is not currently used clinically as a weight-bearing structure and therefore before DCB can be used as an ACL allograft it is essential to determine if it has adequate tensile strength required and compatibility with contemporary fixation devices. In addition, it is important to determine whether the bone from which DCB is manufactured and the age of the donor influences DCB's tensile properties in order to identify the optimal manufacturing technique. Sheep are a commonly used animal model in ACL reconstruction because the stifle joint is similar in size and structure to the human knee joint (10), and studies indicate that the peak force through an ovine ACL is 150 N (11).

The aim of this study is to measure the tensile properties of ovine DCB, and determine its compatibility with current ACL fixation systems. The study used an *ex vivo* ovine model to assess the ultimate load and stiffness of DCB grafts from different locations (femur and tibia) and donor ages and extrapolated the findings to the human clinical situation.

2. Materials and Methods

2.1 Study overview

This animal research was undertaken in accordance with a project license accepted under the UK Animals (Scientific Procedures) Act 1986. The animal specimens were provided by The Royal Veterinary College (Hatfield, Hertfordshire, UK). The tensile properties of ovine DCB manufactured from old and young sheep's femur and tibia was measured to determine the optimal source of DCB. The fixation strength of DCB graft fixation systems was measured *ex vivo* in an ovine ACL reconstruction model, considering both suspensory and interference fixation. Comparison was made with superficial digital flexor tendon (SDFT) allograft and ovine ACL.

2.2 DCB Manufacture

A peer-reviewed method was used which reflects the steps taken in clinical practice (12). Femur and tibia were harvested from cadaveric sheep from two different age categories, termed "old" and "young". The old category consisted of "full mouthed" non-pregnant female Mule sheep, aged 2 to 3 years old. The young category consisted of six female Mule lambs, aged nine months old. The femur and tibiae were harvested immediately after euthanasia. The soft tissues including the periosteum were stripped. The epiphyses were removed, leaving the diaphyseal bone region which was cut into longitudinal strips of cortical bone using a diamond edged band saw (Exact, Hamburg, Germany). The cortical bone strips were demineralised in 0.6 N hydrochloric acid (HCl) at room temperature for 5 days. Demineralization was confirmed with radiographs (300 seconds, 30 kV, Faxitron Corporation, Illinois, USA). The demineralized cortical bone (DCB) strips were washed in phosphate buffered saline (PBS) until pH 7.20. For storage, the DCB was lyophilised (BOC Edwards, Crawley, West Sussex, UK) and sterilized by gamma irradiation at a dose of 25 KGrays (Isotron, Reading, UK).

2.3 Tensile properties of DCB

The DCB was rehydrated in normal saline 90 minutes prior to testing. The DCB was cut into "dog-bone" shaped specimens of consistent dimensions to ensure the samples failed in the mid-section (Fig. 1). Due to differences in the thickness of cortical bone the thickness of the dog bone specimens was variable. The ultimate tensile stress, which describes the maximum stress a material can withstand before failure, was calculated to account for differences in thickness by dividing the ultimate load by the cross sectional area of the mid-section of the specimen. A custom-made jig in a material testing machine (Zwick/Roell Group, Ulm, Germany) was used to perform uniaxial tensile testing. Samples were mounted using custom-made clamps (Fig. 2). The samples were tested at 10 mm per minute until failure without preconditioning. A load-deformation curve was generated and the ultimate load determined. Stiffness was determined using the gradient of the maximum slope of the linear region of the load-deformation curve. Six samples were tested per category following a power analysis using pilot data.

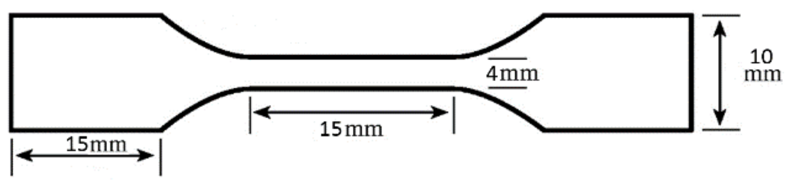

Figure 1. The dimensions of the DCB specimens used in the tensile testing of DCB

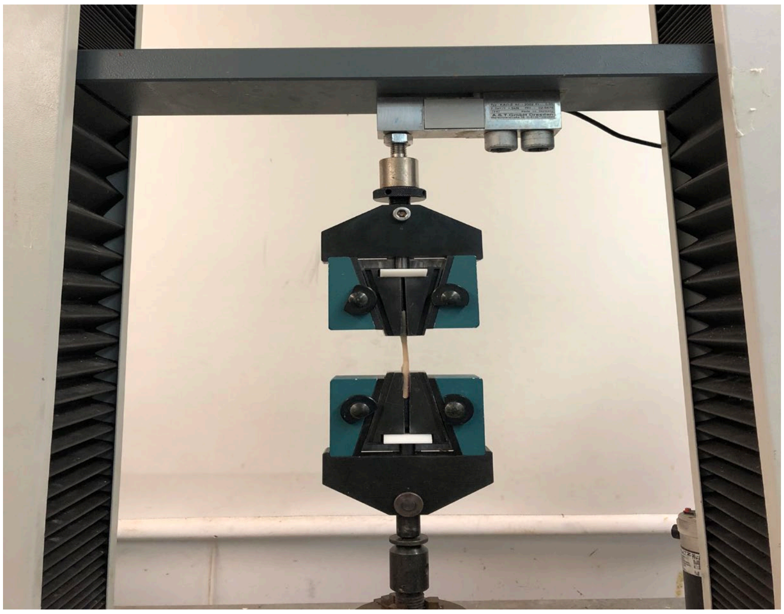

Figure 2. The jig used for tensile testing of DCB

2.4 Ex vivo tensile properties of DCB and SDFT graft fixation systems

The stifle joint in the hind limbs of female adult Mule sheep, aged 2-3 years old, were used to perform *ex vivo* ACL reconstruction. The stifle joint was exposed via a medial arthrotomy and the ACL was sharply excised. Osseous tunnels of 7 mm diameter were drilled in the femur and the tibia at the centres of the ACL footprints as this was the dimension of the bone tunnels previously used with DCB in a large animal (8). A whipstitch using No. 2 Ethibond was applied to both ends of the DCB graft (Fig. 3), which was passed through the bone tunnels from the tibia to the femur. The DCB graft was cut using a scalpel so that it occupied the entire length of the bone tunnels. The femoral fixation systems evaluated were Endobutton CL Fixation device (Smith & Nephew Endoscopy, Andover, MA), a 7mm x 25mm Biosure PK Interference Screws (Smith & Nephew Endoscopy, Andover, MA) and tying sutures around a screw post in the femur. The tibial fixation systems evaluated were a 7mm x 25mm Biosure PK Interference Screws (Smith & Nephew Endoscopy, Andover, MA) and tying sutures around a double spiked plate on the tibia (Smith & Nephew Endoscopy, Andover, MA). The graft was fixed at 40N tension. The femur and tibia were clamped independently using custom-made clamp with the stifle joint flexed at 45 degrees. Uniaxial tensile tested was undertaken at 10 mm per minute until failure without preconditioning. A load-deformation curve was generated and the ultimate load and stiffness determined..

In an ovine model the most commonly used soft tissue ACL graft is the SDFT because in sheep the semitendinosus is a fragile, fascia-like structure (13). As a comparison group representing tendon grafts, the SDFT was evaluated using the same fixation devices for DCB. The SDFT was harvested using a posterolateral skin incision, splitting the gastrocnemius in line with its fibres and harvested the underlying SDFT, yielding a graft with a typical length of 7-8 cm.

Figure 3. A photograph of SDFT (top) and DCB (bottom) grafts

2.5 Tensile properties of ovine ACL

The stifle joint of female adult Mule sheep, aged 2-3 years old, weighing 75 – 85 kg were used to measure the ultimate load of ovine ACL. Immediately after animal sacrifice the femur-ACL-tibia complex was harvested and stored at minus 20 degrees Celsius until the time of testing, at which point the specimens were thawed at room temperature overnight. All soft tissue structures were excised except from the ACL. The femur and the tibia was clamped independently and tensile testing was performed with the tibia flexed at 45 degrees to the femur. Uniaxial tensile tested was undertaken at 10mm per minute until failure without preconditioning. A load-deformation curve was generated and the ultimate load and stiffness determined. Six samples were tested.

2.6 Statistics

All statistical analysis was done using GraphPad Prism v6.0c. The Mann-Whitney U test was used to compare between groups as the data in a Kolmogorov–Smirnov test did not show a normal distribution. The cross sectional diameter, the ultimate load, ultimate tensile stress and stiffness were given as mean ± standard deviation. Statistical significance was considered at $p < 0.05$.

3. Results

3.1 Tensile properties of DCB

All specimens failed in the mid-substance. The tensile tests generated load-deformation curves with a non-linear toe region followed by a linear region until failure (Fig. 4). The ultimate load and ultimate tensile stress of DCB categories are shown in Table 1. Young tibia DCB had the highest ultimate load with a mean force of 67.7 ± 10.6 N, which corresponded to a mean ultimate tensile stress of 6.1 ± 1.9 N/mm^2. The second highest ultimate load was seen in the adult tibia (39.8 ± 6.7 N), corresponding ultimate tensile stress being 6.1 ± 1.9 N/mm^2, which was statistically greater than adult femur and young femur. The lowest two categories were adult femur with no statistical significant difference seen between these groups (Fig. 5-6). Young tibia DCB had the highest stiffness with a mean of 11.4 ± 2.2 N/mm, which statistically greater than adult tibia (p=0.009) with a mean of 7.2 ± 2.2 N/mm. In terms of stiffness, adult tibia was statistically greater than young femur (p=0.041), but not adult femur (p=0.132) (Fig. 7).

Table 1. Tensile properties of ovine DCB

DCB Category	Ultimate Load (N)	Specimen Cross sectional area (mm^2)	Ultimate Tensile Stress (N/mm^2)	Stiffness (N/mm)
Young Tibia	67.7 ± 10.6	11.1 ± 1.0	6.1 ± 1.0	11.4 ± 2.2
Adult Tibia	39.8 ± 6.7	11.6 ± 0.6	4.0 ± 1.1	7.2 ± 2.2
Young Femur	14.7 ± 6.2	10.3 ± 1.5	1.3 ± 0.5	4.2 ± 1.9
Adult Femur	19.6 ± 6.3	10.8 ± 1.2	1.9 ± 0.7	4.5 ± 2.0

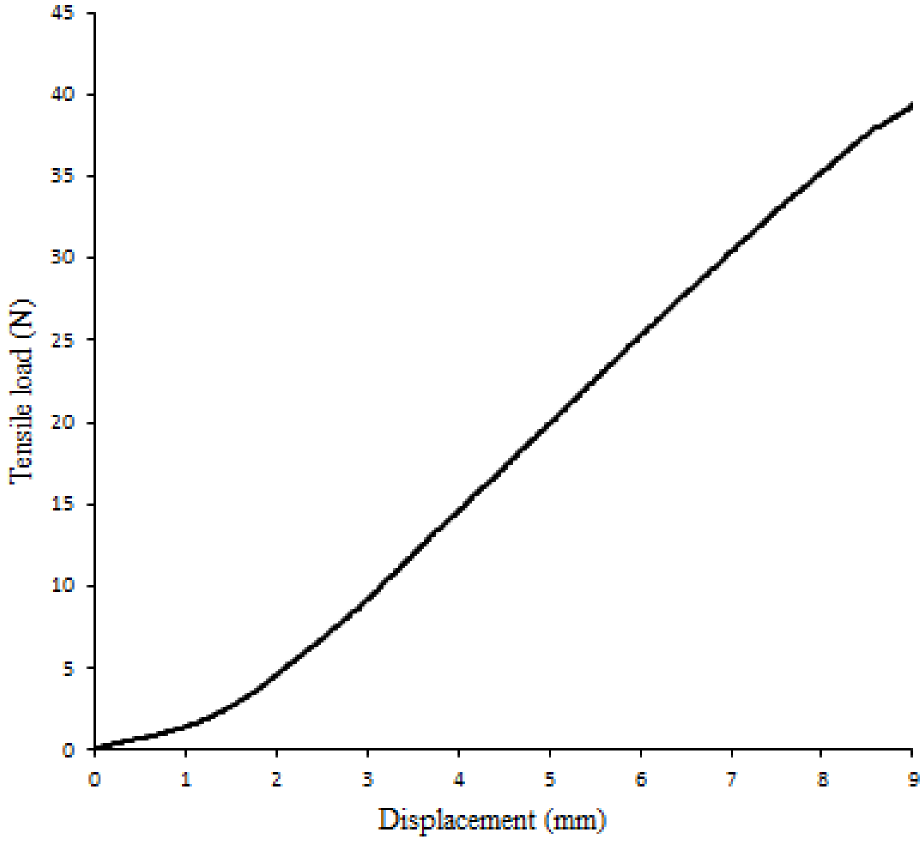

Figure 4. A typical tensile load-displacement curve for an ovine DCB specimen. The curves were characterised by a non-linear toe region followed by subsequent linear behaviour.

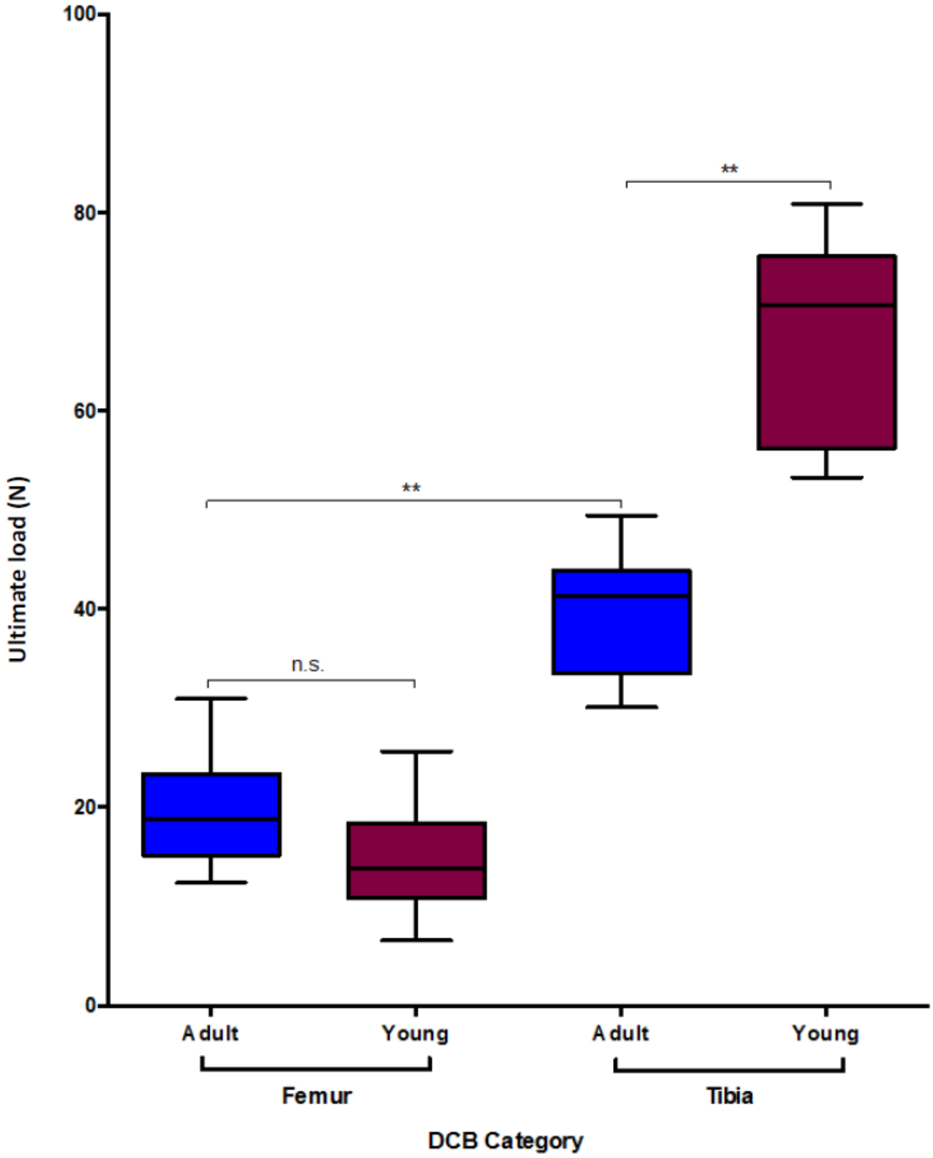

Figure 5. A box and whisker plot showing the ultimate load of ovine DCB. ***Mann-Whitney U test, ** indicates p<0.01, n.s. indicates non-significant difference***

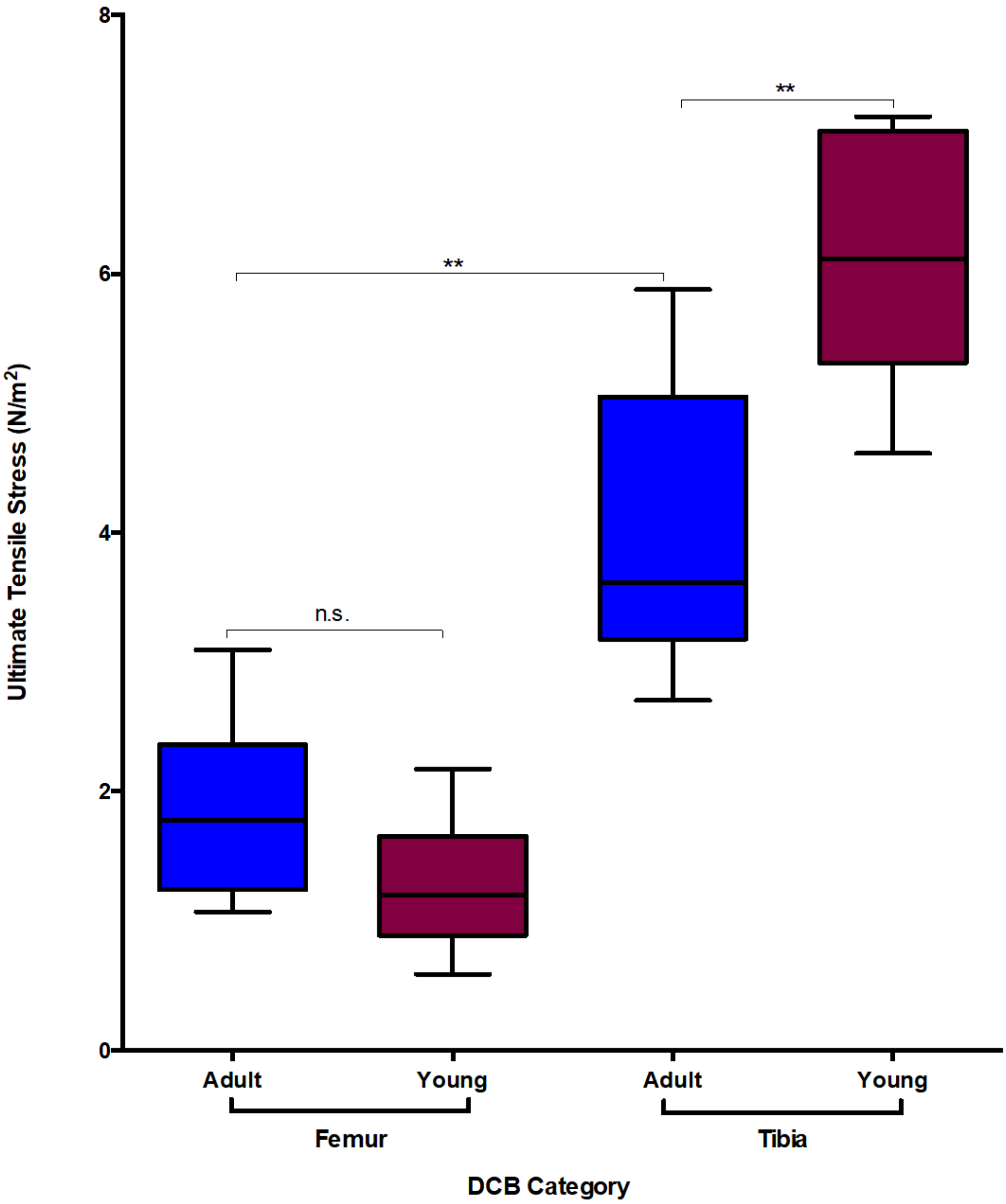

Figure 6. A box and whisker plot showing the ultimate tensile stress of ovine DCB.
*Mann-Whitney U test, ** indicates p<0.01, n.s. indicates non-significant difference*

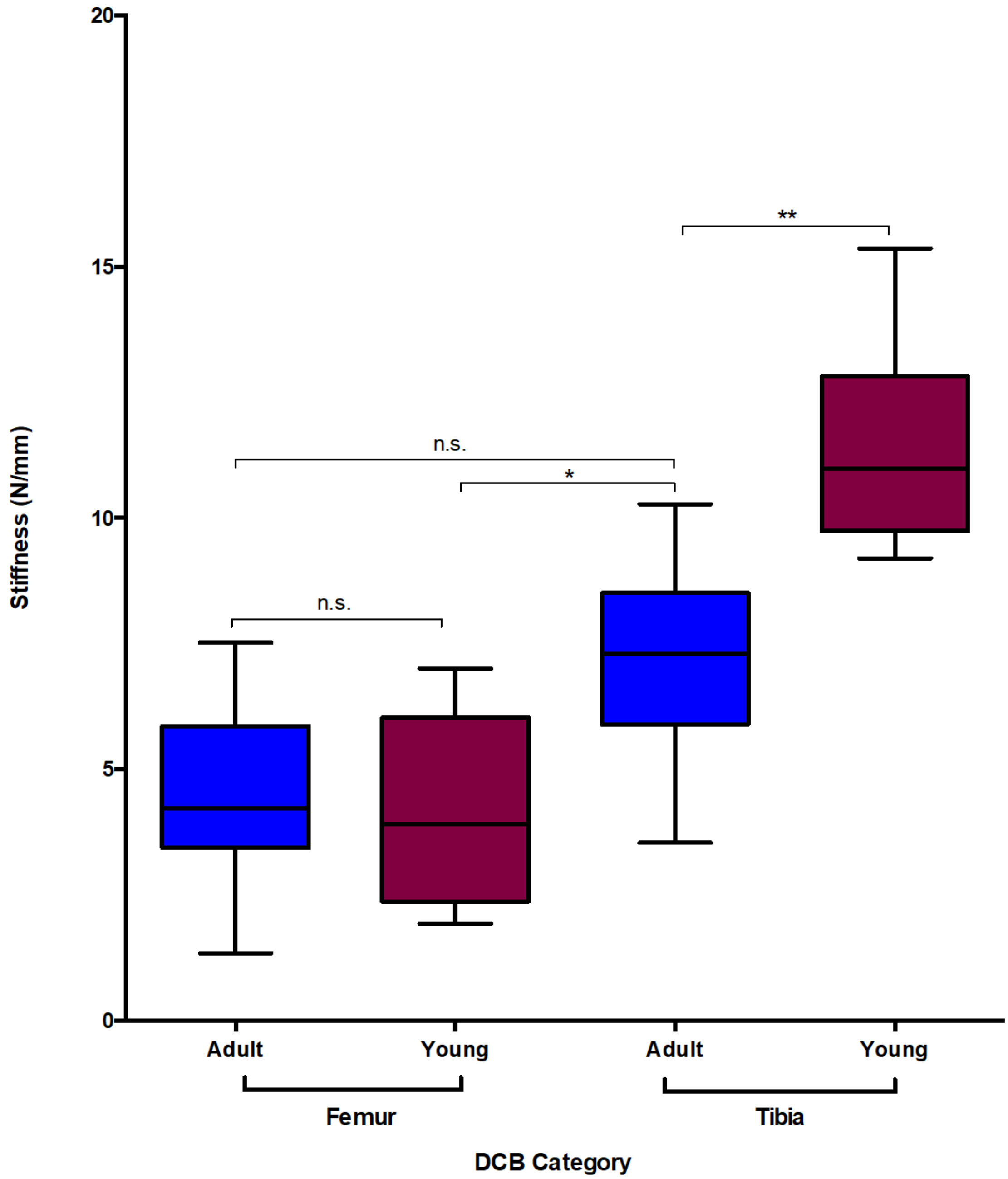

Figure 7. A box and whisker plot showing the stiffness of ovine DCB.
*Mann-Whitney U test, ** indicates p<0.01, n.s. indicates non-significant difference*

3.2 Ex vivo tensile properties of DCB and SDFT graft fixation systems

DCB derived from young tibia had the highest ultimate load and was used to represent DCB in this section of experiments. On palpation the DCB was a flexible, rubber-like material but DCB did not have the flexibility to bend back on itself and could not form a double-strand structure (Fig 8A.). When the DCB was flexed its structural integrity was compromised and the layers of DCB would detach. As a result DCB was not evaluated using the Endobutton CL suspensory femoral fixation device.

The highest mean ultimate load was seen using SDFT combined with interference screws at 308.2 ± 87.3 N (Table 2). This was the only system that consistently reached the *in vivo* requirement of 150 N (Fig. 9). The second highest strength was seen for SDFT fixed using sutures and screw posts at 146.7 ± 25.0 N, which was significantly less than SDFT combined with interference screws ($P<0.01$). The mean ultimate load for DCB fixed with interference screws was higher than fixed with sutures around post, although no statistically significant difference was seen. The ACL mean UTS was 871.0 ± 64.2 N and all failed by avulsion of the tibial attachment. When using suture and posts all failed by sutures ripping through the graft. A difference was seen in failure modes between DCB and SDFT grafts fixed with interference screws, with SDFT failing due to slippage in the tunnel and DCB grafts failing by graft rupture adjacent to the tibial screw (Fig. 8). The highest mean stiffness out of the four fixation systems was seen using SDFT combined with interference screws at 34.2 ± 13.0 N/mm, but this was considerably lower than the mean stiffness of the ACL which was 128.3 ± 16.6 ($p=0.009$).

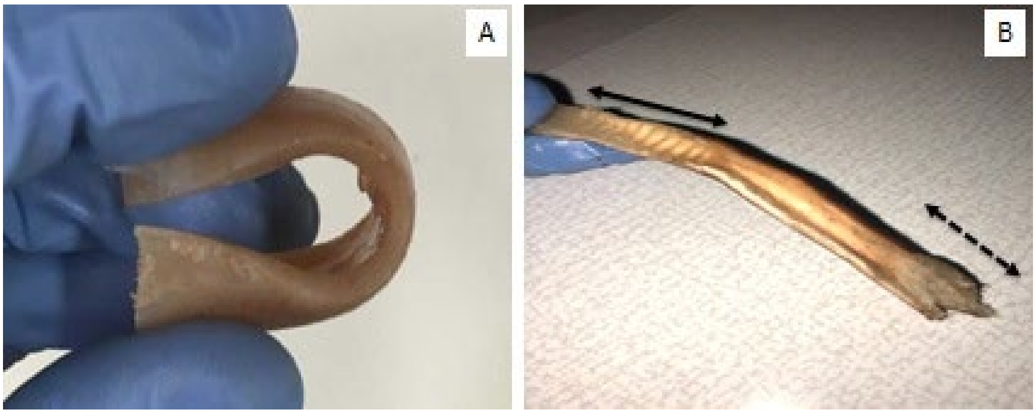

Figure 8. A. DCB under flexion unable to form a double strand. B, A strip of DCB retrieved after fixation with an interference screw showing imprinting of screw threads (arrow) and graft rupture (dashed arrow).

Table 2. The tensile properties of DCB and SDFT Fixation Systems

Graft	Fixation system	Graft thickness (mm)	Stiffness (N/mm)	Ultimate Load (N)	Mode of failure
DCB	Screws and Post	2.8 ± 0.3	2.4 ± 0.88	32.5 ± 9.7	Sutures tearing through graft
DCB	Interference screws	2.8 ± 0.3	9.0 ± 4.3	57.5 ± 9.7	Graft rupture adjacent to screw
SDFT	Screws and Post	5.8 ± 0.3	3.3 ± 0.72	146.7 ± 25.0	Sutures tearing through grafts
SDFT	Interference screws	5.8 ± 0.3	34.2 ± 13.0	308.2 ± 87.3	Graft slippage in tibial tunnel
Ovine ACL	n/a	5.5 ± 0.2	128.3 ± 16.6	871.0 ± 64.2	Avulsion of the tibial attachment

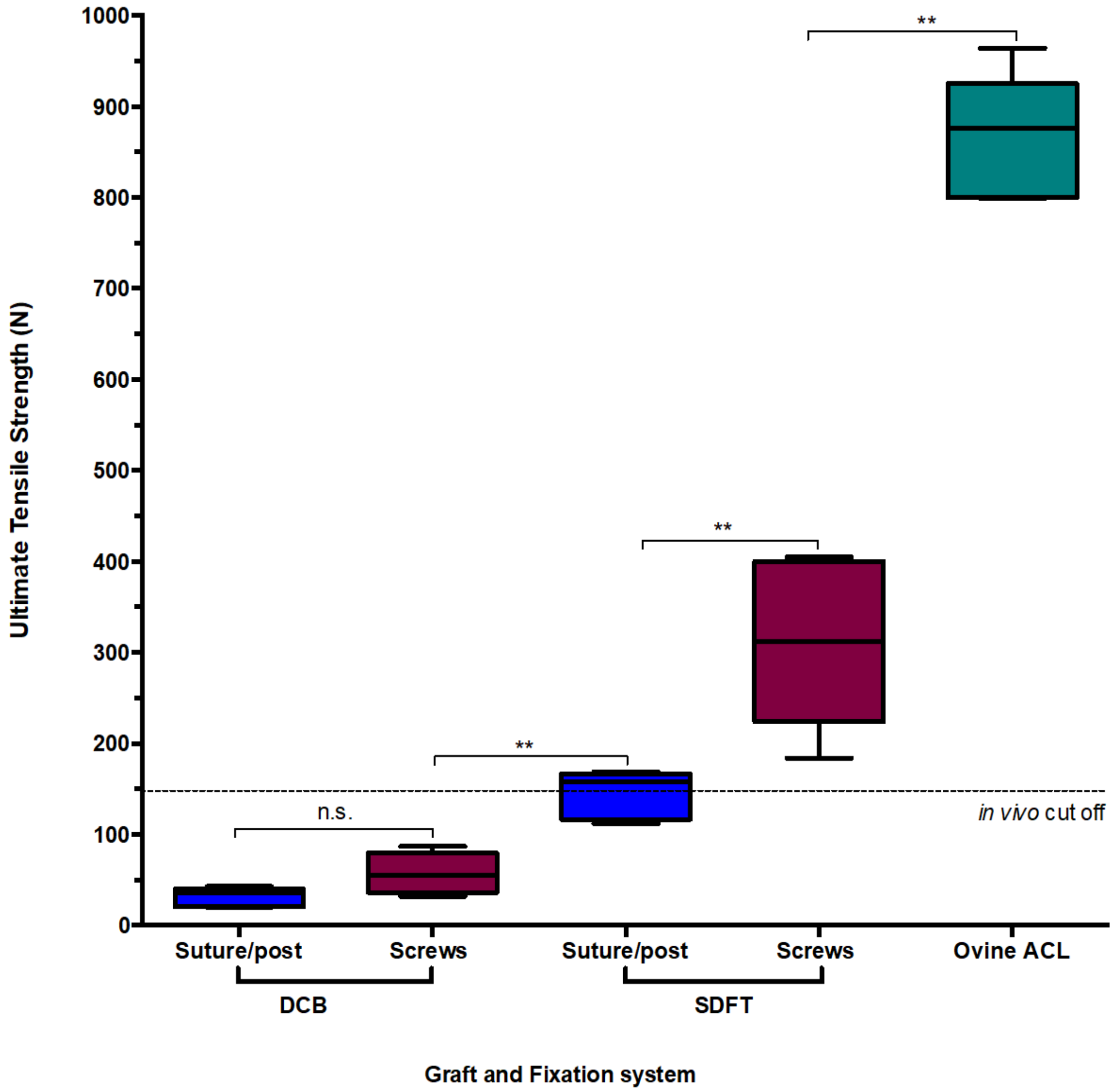

Figure 9. A box and whisker plot showing the ultimate loadof DCB and SDFT ACL fixation systems, and ovine ACL. *Mann-Whitney U test, ** indicates p<0.01, n.s. indicates non-significant difference*

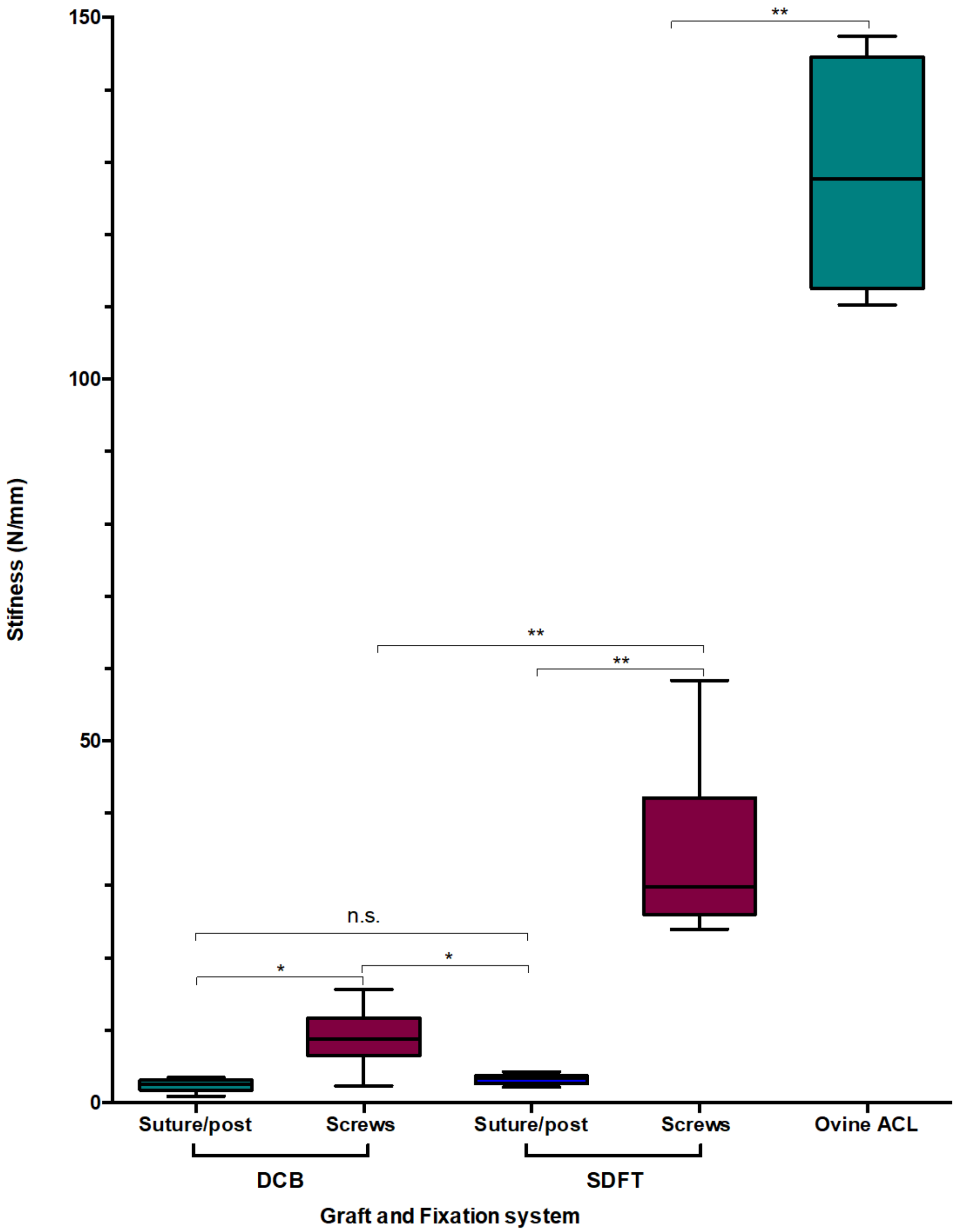

Figure 10. A box and whisker plot showing the stiffness of DCB and SDFT ACL fixation systems, and ovine ASCL. *Mann-Whitney U test, ** indicates $p<0.01$, * $p<0.05$, n.s. indicates non-significant difference*

4. Discussion

It is possible to extract DCB from cadaveric human patients for clinical use as evidenced by the increasing commercial availability of DCB in forms such as paste, putty and strips (5). The advantage of using DCB as an ACL allograft would be the use of a collagenous graft with inherent osteoinductive properties, due to endogenous growth factors (4), which would allow early graft integration in the bone tunnels and patient rehabilitation whilst avoiding the problem of donor site morbidity with allografts. Previous studies have indicated that DCB has the required mechanical properties to be used as an ACL graft, but the compatibility of DCB with human clinical ACL fixations systems has not previously been investigated. For the first time we have reported the tensile properties of ovine DCB, and this study is the first to report time-zero tensile properties of ACL reconstruction using DCB with the native ACL in the same species. This research is novel because few studies have considered the effect of donor age and harvest site on the tensile properties of DCB. Also this is the first study to evaluate the use of DCB with current ACL fixation systems. We found that DCB derived from young tibia had superior tensile properties when compared to other sources of DCB in terms of ultimate tensile stress and stiffness. The ultimate load of young tibia DCB combined with ACL fixation systems was insufficient for *in vivo* application because the value obtained is lower than the peak *in vivo* forces in sheep. In addition, the physical properties of DCB, specifically a lack of flexibility, prevented DCB being used in a double-strand format thus preventing use of the Endobutton CL femoral fixation device. In comparison, the tensile properties of SDFT grafts combined with interference fixation did reach *in vivo* threshold and therefore on the basis of this study DCB is inferior to free tendon grafts as an ACL graft.

Ovine DCB was a flexible, rubber-like material consistent with previous descriptions (8, 14). The load-deformation curve observed for all categories was similar to that reported for bovine DCB (9), consisting of a non-linear toe region and subsequent linear behaviour until failure. The toe region represents the straightening of crimped collagen fibres, whereas the linear region represents stretching of the collagen fibres themselves (14). All specimens failed in the mid-substance, which is important because failures at the end of grafts can be attributed to improper loading at the clamp. As a result we can be confident that the readings reflect the ultimate load of the ovine DCB.

The ultimate tensile stress of DCB shows a wide range of values in the literature (from 7 ± 2 to 40 ± 3 N/mm^2) and the values for ultimate stress in this study was smaller than values previously reported (Table 3). Summit et al. previously reported that DCB has mechanical properties similar to the ACL in terms of tensile strength and stiffness although raw values were not provided (9). There are a number of reasons why the ultimate tensile stress and stiffness in this study were lower than previous reports. First, acid saturations during demineralisation can negatively affect tensile properties and this study used a higher concentration than previous studies (9). The reason for the use of 0.6N HCl in this study is that this peer-reviewed protocol has been shown to yield biologically active grafts capable of remodelling when used to repair tendon injuries in sheep (12). Other studies have used different methods of demineralisation such as EDTA (16). Second, the differences might be related to differences in species, with previous studies reporting values for bovine and human DCB. When comparing to human bone, here are differences in the morphology of bone (23) with sheep exhibiting both plexiform and Haversian bone tissue, whereas human bone generally exhibiting Haversian bone tissue (25). Third, the age of bone can affect tensile properties (15), and differences might be due to different bone ages being analysed in previous studies. Fourth, gamma irradiation was used to sterilise the DCB, which might have further compromised mechanical strength, although previously it was not shown to affect the strength of bovine DCB (9). Finally, differences in tensile testing experimental conditions and specimens (size and shape) hinders comparison of results from different studies. In this study in part we looked at structural properties (when evaluating DCB fixation systems) and material properties (when looking at DCB specimens alone). It was necessary to look at the structural effects when looking at fixation systems and this may lead to inaccuracies when comparing literature. There are no comparable data for ovine DCB and but the testing conditions used in this study yielded similar results for ovine ACL when compared to the literature which shows are methods are reliable and consistent with the literature.. For instance, the average value for ovine ACL ultimate load and stiffness was 871 ± 64 N and 128.3 ± 16.6 N/mm respectively, which is similar to Hunt et al. who recorded a value of 888 ± 134 N and 143.9±16.1 N/mm (13). All ovine ACLs failed by avulsion of the tibial attachment, which in consistent with previous reports (16).

Table 3. Studies reporting the tensile stress of DCB

Study	Bone specimen	Demineralisation protocol	Ultimate Tensile Stress(N/mm^2)
This study	Tibia (young ovine) Femur (young ovine)	0.6N HCl	6.0 ± 1.0 1.4 ± 0.6
Mack (17)	Tibia (human)	HNO_3	7.0 ± 2
Sweeney et al. Sweeney, Byers (18)	Femur (human)	HCl	17.0 ± 4.1
Burstein, Zika (19)	Tibia (bovine)	0.5M HCl	40 ± 3
Wright, Vosburgh (20)	Femur (bovine)	0.2 M HCl	34 ± 7.5
Catanese, Iverson (21)	Tibia (bovine) Tibia (human)	0.5M EDTA	26 ± 4 18 ± 4

This study supports findings from previous studies that indicate the bone from which DCB strips are manufactured influences its mechanical properties. When considering bovine DCB, Summit and Reisinger (9) reported that the ultimate tensile stress and modulus was greatest in decreasing order from metatarsus to tibia to femur and humerus, and concluded that bones that are more aligned to the axis of loading have great mechanical properties. Cantanese et al. (21) examined human and bovine DCB and found that tibia had a higher ultimate stress and a higher elastic modulus than femur, although this did not reach statistical significance. The clinical significance of our finding is that if used commercially as an ACL allograft, the bone used to make DCB would influence the strength of the graft and would need to be considered.

This study supports other studies that indicate that increasing age is associated with a decrease in the mechanical strength of DCB. Leng et al. evaluated human femoral DCB and observed decreasing tensile strength with age when comparing young, middle aged and old donors

(15). Decreasing tensile strength with age has also been observed for rodent femoral DCB (22). Hence the literature suggests mechanical integrity of the collagen network of DCB decreases with increasing age. The effect of donor age could also be explained by age-related changes in cortical bone. Immature sheep cortical bone is different to mature sheep bone, with greater amount of plexiform bone and a small number of Haversian systems (23, 24). In younger sheep the Haversian Systems are likely to be of a primary nature and more aligned with the direction of the tensile force (25). As a result of remodelling, in mature animals the Haversian systems are likely to be more organised and not necessarily aligned with the direction of the tensile force, leading to a reduction in tensile strength. Our findings suggest the optimal source of bone to manufacture DCB would be from young bone. However in terms of commercial manufacturing process, cadaveric bone from young patients is in less supply than cadaveric bone from older patients.

The ideal ACL graft would have a time-zero ultimate load the same as the native ACL. After implantation tendon commences the process of “graft healing” both in the bone tunnels and the intra-articular graft (26). Tendon-bone healing in the bone tunnels restores an indirect-type insertion characterised by a fibrous interface with Sharpey-like fibres (3). In the joint space the graft remodels in a process termed “ligamentisation”, whereby the graft first becomes hypocellular and undergoes necrotic changes in the early phase but subsequently the graft revascularises and collagen fibres remodel thus regenerating a ligamentous ACL-like structure (27). For tendon grafts this correlates to improvement in mechanical strength over time but the ultimate load does not reach that of the native ACL (28). The only *in vivo* study that has evaluated DCB as an ACL allograft was a caprine study by Jackson et al. (8), which used DCB as a single-strand ACL allograft and fixed by tying whipstitch sutures to screw posts. It is unclear from the manuscript from where the DCB was derived, nor the age of the donor. The DCB graft underwent a similar process to tendon grafts, and the ultimate load rising from 73 ± 9 N by approximately 550% to 474 ± 146 N over a 12 month period. Whilst it is not necessary for the ultimate load for a graft and its fixation system at time zero to be equivalent to the native ACL, it is desirable for the ultimate load to exceed peak *in vivo* forces through the ACL (unless the graft is protected during rehabilitation) (29). The ultimate load of young tibia DCB combined with ACL fixation systems was insufficient and did not reach the *in vivo* threshold of 150N whereas SDFT did when combined with interference screws. In addition to inferior tensile strength, a major disadvantage of DCB appears to be that it lacks the flexibility required to generate a double-strand format required for femoral suspensions devices. DCB cannot be fixed in a double-strand format, which limits the possible graft diameter of DCB, a factor known to influence failure of ACL reconstruction (30). On the basis inferior mechanical strength and incompatibility with contemporary fixation devices, this study indicates that DCB is inferior option to free tendon grafts as an ACL graft. Small animal models of ACL reconstruction have shown that the application of DBM as paste around tendon grafts in the bone tunnels leads to superior graft integration associated with superior biomechanical properties (6, 7). We believe demineralised bone does have a role in ACL reconstruction but, due to inadequate tensile properties of DCB, future research should focus on how it can biologically modulate and enhance tendon grafts osteointegration.

This study has several limitations. First, a major limitation is that by performing experiments *ex vivo* the potential for *in vivo* biological remodelling was not considered. This study was a purely biomechanical study and did not consider biological considerations. Second this study did not evaluate human DCB and therefore its results cannot be directly extrapolated to humans. However as this is a comparative study in sheep one would anticipate that the results for DCB in humans would be equivalent. Nevertheless research is needed that rigorously examines the tensile properties of human DCB across a range of contemporary ACL fixation systems to verify our results. DCB is being considered for a number of clinical orthopaedic applications for both tendon and ligament reattachment to bone (7). This study highlights the importance of determining the optimal source of DCB for these applications, and establishing the strength of DCB with fixation systems in an environment that minics these applications. Second, preconditioning of samples was not performed, which could contribute to the appearance of a toe region. Third, consideration was not given to selection of the specific cortices that are loaded in tension during normal locomotion. This could be important because cortical bone has different loading modes at distinct anatomic sites during activity and thus collagen fibrils at the different sites might have different preferred orientations which may affect strength (15). Finally, for the ACL reconstruction systems the graft cross sectional area was not be measured and therefore tensile values were not normalised to cross sectional area (32).

5. Conclusion

The tensile properties of DCB were influenced by the donor age and site of origin, with young tibial DCB demonstrating the most appropriate tensile properties for use as an ACL allogrfaft. However owing to inferior tensile properties and incompatibility with suspensory femoral fixation devices, this study indicates DCB is mechanically inferior to current tendon grafts options for ACL reconstruction.

Conflicts of interest: None.

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References

1. Mehran N, Moutzouros VB, Bedi A. A Review of Current Graft Options for Anterior Cruciate Ligament Reconstruction. JBJS reviews. 2015;3(11).
2. Kaeding CC, Aros B, Pedroza A, Pifel E, Amendola A, Andrish JT, et al. Allograft Versus Autograft Anterior Cruciate Ligament Reconstruction: Predictors of Failure From a MOON Prospective Longitudinal Cohort. Sports health. 2011;3(1):73-81.
3. Rodeo SA, Arnoczky SP, Torzilli PA, Hidaka C, Warren RF. Tendon-healing in a bone tunnel. A biomechanical and histological study in the dog. JBJS. 1993;75(12):1795-803.
4. Urist MR. Bone: formation by autoinduction. Science (New York, NY). 1965;150(3698):893-9.
5. van der Stok J, Hartholt KA, Schoenmakers DAL, Arts JJC. The available evidence on demineralised bone matrix in trauma and orthopaedic surgery: A systematic review. Bone & joint research. 2017;6(7):423-32.
6. Lovric V, Russell N, Heuberer P, Goldberg J, Walsh W. Demineralized bone matrix augmented tendon-bone healing in transosseous-equivalent sheep rotator cuff model. Journal of Science and Medicine in Sport. 2012;15:S138-S9.
7. Hexter AT, Pendegrass C, Haddad F, Blunn G. Demineralized Bone Matrix to Augment Tendon-Bone Healing: A Systematic Review. Orthopaedic journal of sports medicine. 2017;5(10):2325967117734517.
8. Jackson DW, Simon TM, Lowery W, Gendler E. Biologic remodeling after anterior cruciate ligament reconstruction using a collagen matrix derived from demineralized bone. An experimental study in the goat model. American Journal of Sports Medicine. 1996;24(4):405-14.
9. Summitt MC, Reisinger KD. Characterization of the mechanical properties of demineralized bone. Journal of Biomedical Materials Research Part A. 2003;67(3):742-50.
10. Allen MJ, Houlton JE, Adams SB, Rushton N. The surgical anatomy of the stifle joint in sheep. Veterinary surgery : VS. 1998;27(6):596-605.
11. Holden JP, Grood ES, Korvick DL, Cummings JF, Butler DL, Bylski-Austrow DI. In vivo forces in the anterior cruciate ligament: direct measurements during walking and trotting in a quadruped. J Biomech. 1994;27(5):517-26.
12. Thangarajah T, Shahbazi S, Pendegrass CJ, Lambert S, Alexander S, Blunn GW. Tendon Reattachment to Bone in an Ovine Tendon Defect Model of Retraction Using Allogenic and Xenogenic Demineralised Bone Matrix Incorporated with Mesenchymal Stem Cells. PloS one. 2016;11(9):e0161473.
13. Hunt P, Scheffler SU, Unterhauser FN, Weiler A. A model of soft-tissue graft anterior cruciate ligament reconstruction in sheep. Archives of Orthopaedic and Trauma Surgery. 2005;125(4):238-48.
14. Bowman SM, Zeind J, Gibson LJ, Hayes WC, McMahon TA. The tensile behavior of demineralized bovine cortical bone. Journal of Biomechanics. 1996;29(11):1497-501.
15. Leng H, Reyes MJ, Dong XN, Wang X. Effect of age on mechanical properties of the collagen phase in different orientations of human cortical bone. Bone. 2013;55(2):288-91.
16. Yoshikawa T, Tohyama H, Katsura T, Kondo E, Kotani Y, Matsumoto H, et al. Effects of Local Administration of Vascular Endothelial Growth Factor on Mechanical Characteristics of the Semitendinosus Tendon Graft after Anterior Cruciate Ligament Reconstruction in Sheep. The American Journal of Sports Medicine. 2006;34(12):1918-25.
17. Mack RW. Bone, a Natural Two-phase Material: A Study of the Relative Strength and Elasticity of the Organic and Mineral Components of Bone: Biomechanics Laboratory, University of California; 1964.
18. Sweeney A, Byers RK, Kroon R, editors. Mechanical characteristics of bone and its constituents. MECHANICAL ENGINEERING; 1965: ASME-AMER SOC MECHANICAL ENG 345 E 47TH ST, NEW YORK, NY 10017.
19. Burstein AH, Zika JM, Heiple KG, Klein L. Contribution of collagen and mineral to the elastic-plastic properties of bone. The Journal of bone and joint surgery American volume. 1975;57(7):956-61.

20. Wright T, Vosburgh F, Burstein A. Permanent deformation of compact bone monitored by acoustic emission. Journal of biomechanics. 1981;14(6):405-9.
21. Catanese J, 3rd, Iverson EP, Ng RK, Keaveny TM. Heterogeneity of the mechanical properties of demineralized bone. J Biomech. 1999;32(12):1365-9.
22. Danielsen CC, Andreassen TT, Mosekilde L. Mechanical properties of collagen from decalcified rat femur in relation to age and in vitro maturation. Calcified tissue international. 1986;39(2):69-73.
23. Mulhern DM, Ubelaker DH. Differences in osteon banding between human and nonhuman bone. Journal of forensic sciences. 2001;46(2):220-2.
24. Foote JS. A contribution to the comparative histology of the femur: Smithsonian institution; 1916.
25. Hillier ML, Bell LS. Differentiating human bone from animal bone: a review of histological methods. Journal of forensic sciences. 2007;52(2):249-63.
26. 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(3):271-84.
27. Scheffler SU, Unterhauser FN, Weiler A. Graft remodeling and ligamentization after cruciate ligament reconstruction. Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA. 2008;16(9):834-42.
28. Janssen RP, Scheffler SU. Intra-articular remodelling of hamstring tendon grafts after anterior cruciate ligament reconstruction. Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA. 2014;22(9):2102-8.
29. Harvey A, Thomas N, Amis A. Fixation of the graft in reconstruction of the anterior cruciate ligament. The Journal of bone and joint surgery British volume. 2005;87(5):593-603.
30. Snaebjornsson T, Hamrin Senorski E, Ayeni OR, Alentorn-Geli E, Krupic F, Norberg F, et al. Graft Diameter as a Predictor for Revision Anterior Cruciate Ligament Reconstruction and KOOS and EQ-5D Values: A Cohort Study From the Swedish National Knee Ligament Register Based on 2240 Patients. The American journal of sports medicine. 2017;45(9):2092-7.
31. Datta A, Gheduzzi S, Miles AW. A comparison of the viscoelastic properties of bone grafts. Clinical biomechanics (Bristol, Avon). 2006;21(7):761-6.
32. Ellis DG. Cross-sectional area measurements for tendon specimens: a comparison of several methods. J Biomech. 1969;2(2):175-86.