The Effectiveness of Demineralised Cortical Bone Matrix in a Chronic Rotator Cuff Tear Model.

Conflict of Interest:

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.
Tendon Reattachment using Demineralised Bone Matrix

ABSTRACT

Background:

The purpose of this study was to assess the effect of demineralised bone matrix (DBM) on rotator cuff tendon-bone healing. The hypothesis was that compared to a commercially-available dermal matrix scaffold, DBM would result in a higher bone mineral density and regenerate a morphologically superior enthesis, in a rat model of chronic rotator cuff degeneration.

Methods:

Eighteen female Wistar rats underwent unilateral detachment of the supraspinatus tendon. Three weeks later, tendon repair was carried out in animals randomized into three groups: Group 1 were repaired with DBM (n = 6); Group 2 received augmentation with the dermal scaffold (n = 6); and Group 3 (controls) underwent non-augmented tendon-bone repair (n = 6). Specimens were retrieved at six weeks postoperatively for histological analysis and evaluation of bone mineral density.

Results:

No failures of tendon-bone healing were noted throughout the study. All groups demonstrated closure of the tendon-bone gap with a fibrocartilaginous interface. Dermal collagen specimens exhibited a disorganized structure with significantly more abnormal collagen fiber arrangement and cellularity than the DBM-based repairs.
Non-augmented repairs exhibited a significantly higher bone mineral density than DBM and the dermal collagen specimens, and were not significantly different to non-operated control limbs.

Conclusion:

The application of DBM to a rat model of chronic rotator cuff degeneration did not improve the composition of the healing enthesis compared to non-augmented controls and a commercially-available scaffold. However, perhaps the most important finding of this study was that the control group demonstrated a similar outcome to augmented repairs.

Level of evidence: Basic science study.

Keywords: animal model; enthesis healing; rotator cuff; scaffold; shoulder; tendon bone; tissue engineering
INTRODUCTION

Tendon-bone healing is an important factor affecting the outcome of rotator cuff repair. Anatomical reattachment of the rotator cuff to its bony insertion is crucial, but tendon degeneration and poor bone quality at the enthesis compromises the quality, healing capacity, and durability of the performed repair. In order to absorb the energy of loading between tendon and bone, the native enthesis comprises a natural gradation of four histological zones (tendon, demineralized fibrocartilage, mineralized fibrocartilage, and bone). Following injury, this is replaced by a weak fibrovascular bridge with inferior biomechanical properties. Osteopenia at the greater tuberosity often accompanies this change in structure, leading to a reduction in the pullout strength of suture anchors. Demineralised bone matrix (DBM) is an osteoinductive agent that consists of a collagen scaffold containing several growth factors. It has been demonstrated in vivo to regenerate a fibrocartilaginous enthesis capable of resisting physiological forces, but has not been investigated in a degenerative model of tendon-bone healing. The purpose of this study was to assess the effect of DBM on regeneration of an enthesis following repair of a degenerative rotator cuff tear. We compared DBM with another commercially available augmentation product with clinically-investigated profiles of activity (GraftJacket [Wright Medical Technology, Inc., Arlington, TN (Tennessee)]). GraftJacket (Wright Medical Technology, Inc., Arlington, TN) is obtained from donated human cadaveric dermal tissue processed to remove its cellular components whilst retaining its extra-cellular matrix. Its acellularity has the advantage of not causing a host inflammatory reaction and it has been safely used in rats to...
enhance healing of a large acute rotator cuff tear. The hypothesis was that augmentation with DBM will result in a higher bone mineral density in the recipient footprint and regenerate a morphologically superior enthesis characterized by greater fibrocartilage formation and improved collagen fiber organization in a rat model of chronic rotator cuff degeneration when compared to acellular human dermal matrix, after repair of a tendon tear.
MATERIALS AND METHODS

Study Design

In this basic science study, all animal work was conducted in accordance with a Project License protocol accepted under the UK Home Office Animals (Scientific Procedures) Act 1986. Eighteen female Wistar rats underwent unilateral detachment of the supraspinatus tendon. Previously published data was used to calculate the number of animals \( n = 6 \) required to generate a power of 0.8 with significance at the 0.05 level. Three weeks later, tendon repair was carried out in animals randomized into three groups: Group 1 received augmentation of the repair with cortical allogenic DBM \( (n = 6) \); Group 2 received augmentation with non-meshed, ultra-thick accellular human dermal matrix \( ((n = 6) \) (GraftJacket, Wright Medical Technology, Inc., Arlington, TN; average 1.4mm thickness); and Group 3 underwent direct tendon-bone repair without augmentation \( (n = 6) \). One surgeon carried out all procedures using a standard technique. Animals were allowed to mobilise freely after surgery. Specimens were retrieved at six weeks postoperatively for histological analysis and peripheral quantitative computer tomography (pQCT) to evaluate bone mineral density (BMD) at the reattachment footprint of the tendon, reversal of degenerative changes within the tendon, and histological remodeling of the implanted augmentation material.

DBM Manufacture
Tendon Reattachment using Demineralised Bone Matrix

7

DBM derived from cortical bone was manufactured according to Urist’s protocol, with modifications. 20 The tibiae of skeletally mature female Wistar rats were harvested immediately after euthanasia; all soft tissues and periosteum were stripped from the bone surface. Bones measuring approximately 30 mm length by 3 mm width were demineralized in 0.6 N HCL at room temperature. Demineralization was confirmed by taking radiographs (300 seconds, 30 kV, Faxitron Corporation, Illinois, USA). This was followed by washing in phosphate-buffered saline until the pH was 7.4 +/- 0.1. Samples were stored at -20°C for two hours and transferred to a lyophiliser (Edwards Girovac Ltd, Crawley, West Sussex, UK) for three days. Specimens were then sealed in individual plastic bags, sterilised by gamma irradiation at a dose of 25 kilograys (Isotron Limited, Reading, UK), and stored at -20°C. Samples were rehydrated at the time of surgery in normal saline for 30 minutes prior to use.

Surgical Technique

Two surgeries were performed on each animal: full-thickness supraspinatus tendon detachment and complete tendon reattachment. Anaesthesia was induced and maintained using 2% isoflurane mixed with pure oxygen via a facemask for both procedures. The right shoulder was operated on in all cases. A 1.5 cm skin incision was made directly over the anterolateral border of the acromion. The deltoid was detached from the acromion and split caudally for 0.5 cm, in order to identify the tendon of supraspinatus. The supraspinatus tendon was completely detached from its bony insertion on the humeral head, marked with a 5’0 prolene suture (Ethicon, Johnson & Johnson Medical Ltd., Berkshire, UK) at the musculotendinous junction.
Tendon Reattachment using Demineralised Bone Matrix

(TMJ), and allowed to retract medially. Deltoid muscle, superficial fascia, and skin were closed with 5’0 Vicryl suture (Ethicon, Johnson & Johnson Medical Ltd., Berkshire, UK). Animals were allowed unrestricted cage activity and received analgesia (subcutaneous buprenorphine) every 12 hours for three days. The second surgery to reattach the tendon was undertaken three weeks after the first procedure. Prior to making the skin incision, the DBM or GraftJacket (Wright Medical Technology, Inc., Arlington, TN) was rehydrated for 30 minutes in sterile normal saline at the operating table.

A 2 cm skin incision was made in line with the supraspinatus muscle belly, ending anterior to the lateral end of the clavicle. This approach was perpendicular to the incision used for tendon detachment in order to make use of a virgin anatomical plane devoid of scar tissue. The muscle belly of supraspinatus was identified and followed distally to reveal the tendon stump with the suture marker in the MTJ. Scar tissue between the tendon stump and its insertion was excised and the tendon was grasped with a double-armed 5’0 prolene using a modified Mason-Allen technique. Despite traction on the tendon stump, it could not be directly brought back to the humeral head in any of the cases. The bare tendon-bone insertion footprint was decorticated using a #11 surgical blade until bleeding was seen. A custom-made dental drill was used to drill a 1 mm hole from the neck of the humerus to the bony insertion of the detached supraspinatus.

The scaffold (DBM or GraftJacket) was cut into a strip 10 mm long and 3 mm wide. Each limb of the suture was passed through the scaffold to secure it in position. One suture limb was passed through the hole in the prepared tendon stump and the other
suture limb was passed through the hole on the neck of the humerus. The
supraspinatus tendon-scaffold complex was attached to the insertion site, with the
graft in contact with both the tendon stump proximally and decorticated bone surface
distally (Figure 1). In the control group the sutures were inserted directly into the drill
holes, leaving a 5 mm gap between the tendon and bone in all cases.

A layered wound closure was undertaken in a similar manner to the first surgery, and
the animals were permitted unrestricted cage activity. Postoperative analgesia (Intra-
muscular Buprenorphine 0.6 mg) was given every 12 hours for three days.

Histological Assessment

After euthanasia with carbon dioxide, the right shoulder was dissected and a specimen
comprising the humerus with its attached supraspinatus musculotendinous unit was
removed. Each sample was fixed in 10% formal saline and underwent decalcification
in EDTA, ascending graded alcohol dehydration, defatting in chloroform, and
embedding in paraffin. Multiple sections, 4μm thick, were cut in the coronal plane
through the humerus, enthesis, and supraspinatus musculotendinous unit using a
microtome. Sections were attached to glass slides and stained with hematoxylin and
eosin (H&E).

Two blinded observers evaluated all sections using an Olympus BH-2 light
microscope (Olympus, Glasgow, UK). Tendon degeneration was assessed according
to a modified Movin scale \(^3\) and included the following variables: (1) fiber structure,
(2) fiber arrangement, (3) rounding of the nuclei, (4) regional variations in cellularity,
Tendon Reattachment using Demineralised Bone Matrix

(5) increased vascularity, and (6) hyalinization. A four-point scoring system was used:

0 = normal appearance, 1 = slightly abnormal appearance, 2 = a moderately abnormal appearance, and 3 = a markedly abnormal appearance. Based on this, the total score for any given slide could range from 0 (normal tendon) to 18 (the greatest level of degeneration).

Maturation of the enthesis was assessed according to the scoring system developed by Ide et al: score 1 – the insertion had continuity without fibrous tissue or bone ingrowth, score 2 – the insertion had continuity with fibrous tissue ingrowth but no fibrocartilage cells, score 3 – the insertion had continuity with fibrous tissue ingrowth and fibrocartilage cells but no tidemark, and score 4 – the insertion had continuity with fibrous tissue ingrowth, fibrocartilage cells, and a tidemark.

Measurement of Bone Mineral Density

Changes in bone mineral density at the humeral head were assessed using pQCT scanning. One millimeter slices were obtained through the humeral head and supraspinatus musculotendinous unit using an XCT 2000 Bone Scanner (Stratec Medizintechnik GmbH, Germany) with Software version 6.20. Controls were obtained from the contralateral (non-operated) shoulder in six animals subjected to the same rehabilitation conditions as the study groups.

Statistical Analysis
Numerical data were analysed using SPSS software package, version 23 (SPSS Inc, an IBM Company, Chicago, Illinois). Mann Whitney U tests were used to compare data between groups. Results were considered significant at the p < 0.05 level.
RESULTS

All animals survived the duration of the study and none had post-operative infection. Limping was noted for the first three to five postoperative days but a normal gait pattern returned thereafter.

Macroscopic Findings

At the time of euthanasia there was continuity between the repaired tendon and the bone in all groups (Figure 2). No signs of infection were noted in any specimen, none of the repairs had failed, and all the sutures were intact. Remodeling of the graft material occurred to a greater extent in the DBM group, whereby the scaffold could not be discerned from other tissues in the regenerated tendon-bone interface. In contrast, accellular human dermal matrix was clearly visible at necropsy. Control group specimens demonstrated complete closure of the enthesis.

Quantitative Histology

Enthesis Maturation Score

No significant difference was observed in the enthesis maturation score between experimental groups. However, the dermal matrix specimens exhibited a more disorganized enthesis than control and DBM groups, which were characterized by a well organised, graded structure (Figures 3 and 4). The median enthesis maturation
Tendon Reattachment using Demineralised Bone Matrix

score was 2.5 (95% CI 1.88 to 3.46) in the controls, 3 (95% CI 2 to 4) in the DBM group, and 2.5 (95% CI 2.02 to 2.81) in the acellular human dermal matrix group.

Modified Movin Score

No significant difference in the modified Movin score (indicating degeneration) was demonstrated between experimental groups (Figure 5). The median modified Movin score was 7 (95% CI 5.04 to 10.62) in the controls, 6 (95% CI 3.37 to 10.47) in the DBM group, and 9.25 (95% CI 6.94 to 10.89) in the dermal matrix group.

Fiber Structure

All groups exhibited increased waviness and distance between collagen fibers (Figure 6). The median score was 1.75 (95% CI 1.07 to 2.10) in the controls, 1.75 (95% CI 0.66 to 2.83) in the DBM group, and 1.5 (95% CI 0.83 to 2.67) in the dermal matrix group. There were no significant inter-group differences.

Fiber Arrangement

All groups exhibited a loss of the parallel arrangement that typically characterises the fiber arrangement in tendons (Figure 6). The median score was 1.5 (95% CI 0.88 to 2.46) in the controls, 1.25 (95% CI 0.84 to 2.16) in the DBM group, and 2.25 (95% CI 1.90 to 2.76) in the acellular human dermal matrix group. Fiber arrangement was
significantly more abnormal in the dermal matrix group than in the DBM group \((p = 0.039)\). There were no other significant inter-group differences.

Tenocyte Nuclei

Rounding of nuclei (indicating persistent degeneration) was identified in all groups following tendon reattachment (Figure 7). The median score was 1.75 (95% CI 1.12 to 2.55) in the controls, 1.50 (95% CI 1.03 to 1.97) in the DBM group, and 2 (95% CI 1.56 to 2.10) in the dermal matrix group. There were no significant inter-group differences.

Cellularity

Specimens were evaluated for an increase in cellularity, indicating persistent degeneration. The median score was 1.50 (95% CI 0.97 to 2.20) in the controls, 1.25 (95% CI 0.70 to 1.97) in the DBM group, and 2 (95% CI 1.69 to 2.48) in the acellular human dermal matrix group. Cellularity was significantly less in the DBM group than in the dermal matrix group \((p = 0.037)\), but there were no other significant inter-group differences.
Tendon Reattachment using Demineralised Bone Matrix

285 Vascularity

Specimens were evaluated for an increase in vascularity, indicating persistent degeneration. The median score was 1.50 (95% CI 0.54 to 2.12) in the controls, 0.25 (95% CI -0.35 to 2.01) in the DBM group, and 1 (95% CI 0.30 to 1.53) in the acellular human dermal matrix group. There were no significant inter-group differences.

Hyalinisation

Hyalinisation was not observed in any of the specimens.

pQCT

For comparison with the operated side, control specimens were harvested from the contralateral non-operated shoulder of the animals not used in this study. In this group (n = 6), the median total bone mineral density at the supraspinatus tendon-bone insertion was 793.25 mg/ccm (95% CI 754.24 to 844.70) (Figure 8). This significantly decreased at six weeks following augmented tendon repair with DBM and acellular human dermal matrix to a median of 721.20 mg/ccm (95% CI 537.52 to 771.68) (p = 0.004) and 620.55 mg/ccm (95% CI 551.01 to 733.80) (p = 0.006) respectively. Following attempted direct repair of the supraspinatus tendon to bone without the addition of an augmentation strategy, median bone mineral density at the
enthesis was 756.30 mg/ccm (95% CI 648.01 to 818.46). This was not significantly different to controls (p = 0.078).
DISCUSSION

In this study, we hypothesised that: 1. DBM would regenerate a morphologically superior enthesis characterized by greater fibrocartilage formation and improved collagen fiber organisation compared to a commercially available acellular human dermal matrix scaffold (GraftJacket); 2. DBM would result in a higher bone mineral density at the footprint insertion site. However, our results do not support either hypothesis. Using a previously validated rat model of chronic rotator cuff degeneration, the supraspinatus tendon was reattached to its bony insertion with interposed DBM, dermal collagen, or no augmentation (controls) and analysed after six weeks. All groups demonstrated closure of the tendon-bone gap with a fibrocartilagenous interface, but the degenerative process at this time point could not be reversed (there was a persistently high Modified Movin score in all groups). Although there was no significant difference in enthesis maturation scores at the conclusion of the study, the dermal collagen specimens exhibited a disorganized structure with significantly more abnormal collagen fiber arrangement and cellularity than the DBM repairs (indicating more severe degeneration).

Bone mineral density at the enthesis was the second parameter examined because it has been shown to determine the quality and degree of tendon-bone healing, and the pullout strength of suture anchors in the clinical setting. Following tendon reattachment with DBM and dermal collagen, this did not recover to the baseline levels (bone mineral density at the non-operated tendon insertion site). In contrast, the non-augmented repairs exhibited a significantly higher bone mineral density than
DBM and dermal collagen specimens, and were not significantly different to control specimens.

Several *in vivo* studies have demonstrated the ability of DBM to enhance tendon-bone healing, but not in a degenerative rotator cuff model.\(^9\);\(^{12}\);\(^{16}\);\(^{17}\) In the first study to examine the use of DBM at the healing enthesis,\(^{16}\) created an acute tear in an ovine patellar tendon model and repaired it with the scaffold. Compared to controls at 12-weeks, the DBM group showed increased amounts of mineralised fibrocartilage and improved functional weight-bearing. To determine the effect of DBM on tendon healing within a bone tunnel, Kilicoglu et al\(^9\) developed a rabbit model and retrieved specimens three, six, and nine weeks after surgery. At three weeks, a higher number of Sharpey’s fibers, slightly increased fibrocartilage formation, and new bone formation was observed in the DBM group, but this difference was not significant at later time-points. In a further study examining the healing potential of a DBM paste in a tendon-bone tunnel, Lovric et al\(^{12}\) created a rat model of anterior cruciate ligament (ACL) reconstruction. No reconstitution of the fibrocartilage layer was observed in either the DBM or control groups. The main finding was a significantly greater amount of new bone formation in DBM-augmented animals associated with a significantly higher peak load to failure of the tendon-bone interface at six weeks. Similar findings were noted in an ovine model of severe tendon retraction, where DBM was used to enhance healing across a 1 cm defect. In this study, there was an improvement in functional weight bearing at successive time points and the development of a direct enthesis characterised by fibrocartilage.\(^{17}\)
The current study could not reproduce the results of DBM-induced tendon-bone healing observed in other animal models. Considering that the overall contact area between tendon and bone is a major determinant of healing, the limited tendon-bone surface area in a rat model does not present an environment that is as conducive to healing as large animal models and those that utilise a tendon-bone tunnel. Furthermore, the high shear forces from contraction of supraspinatus are distributed over a relatively small surface area and therefore may not have allowed adequate tendon healing to take place. Perhaps the most important finding of this study was that the non-augmented control group demonstrated a similar histological outcome to those tendons repaired with DBM and dermal collagen, and also resulted in a bone mineral density at the tendon insertion comparable to non-operated controls. This raises considerable doubt as to the suitability of a rat model to investigate rotator cuff tendon-bone healing, because in humans spontaneous healing is thought not to occur.

There are several limitations to this study. Previous work has shown that most control tendons in a rat model heal by eight weeks, making it challenging to detect differences between control and experimental groups because it is difficult to improve on a solid mass of healed scar tissue. Earlier time points in the healing phase (two and four weeks) may have highlighted differences that later became non-significant between groups. Similarly, later time points (nine and 12 weeks) may have allowed greater time for the scaffolds to remodel and exert their restorative effect on tendon-bone healing. Finally, biomechanical evaluation of the repair construct would have strengthened the results of this study, but due to the limited number of specimens this could not be performed.
CONCLUSION

This study has highlighted the difficulty of developing a scaffold to solve the problem of rotator cuff tendon-bone healing. Although the application of DBM to a chronic rotator cuff tear does result in an enthesis comprised of fibrocartilage, this was not significantly more mature than non-augmented controls or a commercially available alternative acellular human dermal matrix scaffold.
REFERENCES


6. Gulotta LV, Kovacevic D, Ehteshami JR, Dagher E, Packer JD, Rodeo SA. Application of bone marrow-derived mesenchymal stem cells in a rotator cuff repair
Tendon Reattachment using Demineralised Bone Matrix


Tendon Reattachment using Demineralised Bone Matrix


FIGURE AND TABLE LEGENDS

Figure 1: Supraspinatus tendon-bone fixation.

Figure 2: Supraspinatus tendon-bone fixation, post-mortem, with cortical DBM (A), GraftJacket (B), and direct repair of tendon to bone (Control) (C).

Figure 3: Photomicrograph of the enthesis at six weeks. Specimens stained with H&E. (a) Control: Direct tendon-bone repair characterized by a graded enthesis comprising tendon (T), fibrocartilage (FC), mineralised fibrocartilage, and bone (B). (b) DBM: DBM neo enthesis comprising a well organised, graded enthesis. (c) GraftJacket: GraftJacket neo enthesis with a disorganized structure.

Figure 4: Box and whiskers plot illustrating the enthesis maturation scores following tendon reattachment using no augmentation strategy (controls), DBM, and GraftJacket.

Figure 5: Box and whiskers plot illustrating the modified Movin scores following tendon reattachment using no augmentation strategy (controls), DBM, and GraftJacket.

Figure 6: Photomicrograph (under polarized light) showing collagen fiber structure. (a) Controls (direct tendon-bone repair). (b) DBM group. (c) GraftJacket group.
Figure 7: Photomicrograph illustrating rounded nuclei in control (direct tendon-bone repair) (a), DBM (b), and GraftJacket (c).

Figure 8: Box and whiskers plot showing total bone mineral density at the supraspinatus tendon-bone insertion 6 weeks following direct tendon to bone repair, repair with cortical DBM, and repair with GraftJacket.