Histological and Biomechanical Evaluation of Biologic Adjuvants in a Murine Tendon-Bone Healing Model

J. Johnson¹, C. Kia¹, D. Morikawa¹,², J. Mehl¹,³, F.B. Imhoff¹,³,⁴, A. Otto¹,³, L. N. Muench¹,³, M. Wolf¹, J.B. Baldino¹, X. Xin¹, M. B. McCarthy¹, A.D. Mazzocca¹

¹ University of Connecticut Health Center, Farmington CT, USA
² Department of Orthopaedic Surgery, Juntendo University, Tokyo, Japan
³ Department of Orthopaedic Sports Surgery, Technical University of Munich, Munich, Germany
⁴ Department of Orthopedics, Balgrist University Hospital, Zurich, Switzerland.

SUMMARY
Background. The purpose of this study was to compare subacromial bursa to concentrated bone marrow aspirate (cBMA) in a murine, tendon-bone healing model. The authors hypothesized that subacromial bursa would show similar biomechanical, histological, and cell survivability to that of cBMA.

Methods. Thirty-nine NSG mice were included in the study. All mice underwent simulated patella tendon avulsion injuries and were divided into one of three treatment groups: subacromial bursa, cBMA, or fibrin clot only. Subacromial bursa and cBMA were obtained from a single patient during primary arthroscopic rotator cuff repair (male, age: 57 years). All mice were sacrificed at 2 weeks for biomechanical, histologic, and immunohistochemistry evaluation. Biomechanical analysis included load to failure and stiffness, with the contralateral knees used as controls. Human PCR analysis was performed to confirm survivability in the bursa and cBMA groups.

Results. With respect to the contralateral intact limb, treatment with subacromial bursa significantly increased load to failure (66% of intact) compared to fibrin only (47.87% of intact, P=0.018). cBMA was found to restore tendon strength to 49.9% to its intact side, however no significant difference was found when compared to bursa (P=0.06). No significant difference in stiffness was found between groups. Human nuclear antigen was positive in 75% of the bursa treated mice compared to only 25% of the cBMA specimens.

Conclusions. Subacromial bursa tissue demonstrated significant improvement in tendon strength compared to fibrin clot treatment (acellular control) at 2 weeks. Increased human cell survivability was also demonstrated using bursal tissue compared to cBMA.

KEY WORDS
Shoulder; rotator cuff; bone marrow aspirate; subacromial bursa

BACKGROUND
Pathology to the rotator cuff tendon has become one of the most common soft tissue injuries, affecting 30-50% of patients over the age of fifty (1). Recurrent tears after rotator cuff repair have remained an issue, with larger tears having re-tear rates as high as 94% (2-10). This lack of healing at the tendon-to-bone interface has been attributed to several predisposing factors, including chronicity of injury, muscle atrophy, and tendon quality at the time of repair (4). These risk factors have been shown to correlate with a decreasing number of mesenchymal stem cells (MSCs) present at the tendon-bone interface (11). This has given rise to augmenting rotator cuff repair using cell-based regenerative techniques to increase healing potential (12-14).
Pluripotent MSCs have garnered recent attention for their ability to differentiate into variable mesodermal tissue, allowing for an ideal environment for healing and tendon incorporation (15). Previous animal models examining autologous MSCs in tendon healing have demonstrated improved mechanical resistance, along with increased fibrocartilage formation at the repair site (16-18). Multiple sources for these MSCs have been described, including adipose tissue, muscle, and most commonly from bone marrow (18).

Bone marrow derived stromal cells are the current gold standard for MSCs, showing promising results in various in-vitro studies (19, 21, 22). Hernigou et al. compared bone marrow augment to rotator cuff repair alone, and found that patients with bone marrow augment had significantly higher rate of rotator cuff healing (12). This was also similarly shown by Gomes et al. in their case series of 14 patients, which found no re-tears after rotator cuff repair using bone marrow aspirate (23). Although traditionally obtained from the iliac crest (24), Mazzocca et al. has described a method for collection from the proximal humerus, to allow for a more suitable source during arthroscopic rotator cuff repair (25). This site has previously been shown to have similar number of MSCs to that of the distal femur (26).

Despite clinical evidence in support of bone marrow aspirate, other sources continue to be investigated. Recent attention has been placed on the subacromial bursa, which has been shown to be a potent source of MSCs, with the ability for tenocyte and osteocyte differentiation (27, 28). Although the bursa is commonly removed during arthroscopic repair, the regenerative and pluripotent properties could offer a potential source of MSCs that is easily accessible during surgery (29). Yoshida et al. identified bursa as the origin of progenitor cells involved in the healing response to a murine supraspinatus injury model (30). Dyrna et al. recently compared the use of subacromial bursa to concentrated bone marrow aspirate (cBMA) in an animal tendon defect model, and found that the implanted bursal cells displayed superior tissue engraftment and survival (31).

However, their study examined mid-substance tears instead of the tendon-bone interface, which is the problematic area in rotator cuff healing (31). The purpose of this study was to investigate cellular viability, survival, and biomechanical strength in a murine patellar avulsion defect model with either cBMA or subacromial bursa.

The authors hypothesized that subacromial bursal cells would show superior healing and tendon repair strength when compared to cBMA.

**MATERIALS AND METHODS**

**Experimental Design**

Subacromial bursa tissue and bone marrow aspirate obtained from the proximal humerus were from a single patient undergoing primary rotator cuff repair; a 57 years old male. Institutional review board (IE-07-224-2) was obtained prior to initiation of the study. Subacromial bursa and bone marrow aspirate were taken from the same donor during a single surgery to minimize genetic/epigenetic variation between patients and to provide direct comparisons between the two cell types. Subacromial bursal tissue and cBMA were placed in a fibrin clot and implanted in a mouse patellar avulsion injury the same day the cells were acquired from the subject. NOD scid gamma (NSG, NOD.Cg-Pkrdcsid Il2rgtm1Wjl/SzJ) mice (Jackson Laboratories, Bar Harbor), incapable of mounting an immune response to human tissue, were utilized in this study. In addition, a third control group was included and consisted of mice treated with a fibrin clot alone, which did not contain any human cells. Each mouse had a hind-leg that was randomly assigned to undergo a patellar tendon avulsion injury. The contralateral hindleg was utilized as an additional control to adjust for variations noted in tendon strength between mice based on preliminary testing. Mice were then randomly assigned to treatment with either cBMA, subacromial bursal tissue, or fibrin clot only. Thirty-nine NSG mice were initially obtained, to allow for 13 mice in each of the three treatment groups. Mice were sacrificed 2 weeks following surgery, and were randomly assigned to either immunohistochemistry (N=12, 4 per treatment group) or biomechanical testing (N=27, 9 per treatment group) (figure 1). Biomechanical testing was chosen to be performed at 2 weeks postoperatively, as pilot testing demonstrated earlier time-points did not allow for adequate healing time for the tendon to be tested. Further time-points were also not tested, as previous models have demonstrated no significant difference in biomechanical strength at 4 weeks (32, 33).

**Harvest Tissue Technique**

During primary arthroscopic rotator cuff repair, subacromial bursal tissue over the rotator cuff tendon was obtained using a grasper through a lateral portal. BMA was then collected from the proximal humeral head using a technique previously described by Mazzocca et al (25). It should be noted that the proximal humerus is an ideal location for harvesting bone mesenchymal stem cells during rotator cuff repair, as the epiphysis is made up of trabecular bone and is a source rich of hematopoietic cells (25). Briefly, a 14-gauge bone marrow aspiration trocar was fit with a 60-mL syringe...
containing 3 mL of Anticoagulant Citrate Dextrose Solution A (ACD-A) (Baxter Healthcare Corp). The trochar was then inserted to a depth of 2 to 3 cm into the medial aspect of the greater tuberosity (Bone Marrow Aspiration Kit; Arthrex) to create a tunnel for aspiration. This tunnel was later used for the medial suture anchor for rotator cuff repair. Finally, BMA was aspirated into two 60-mL syringes, for a total of 120 mL of aspirate (figure 2).
Cell Culture and Processing
Following collection of each cell tissue, samples were brought to the lab for further processing. BMA aspirate was concentrated using an automated centrifugation system for 25 minutes (Angel system, Arthrex) (31). Following centrifugation, the bone marrow concentrate was then ready to be placed in a fibrin clot. For all concentrated bone marrow samples, nucleated cell counts were recorded using a Z1 Coulter Particle Counter (Beckman Coulter Life Sciences, Indianapolis, IN, USA) to determine the approximate number of cells delivered to mice.

For the bursal tissue, total weight was first recorded and then the tissue was chopped using a small blunt-tip tenotomy scissors (Integra Life Science, Plainsboro NJ) onto a petri dish. Following chopping, the chopped bursa was then ready to be placed in a fibrin clot. In order to calculate the approximate number of bursa cells delivered to mice, chopped bursa (0.2g) was then plated in DMEM containing 10% FBS and 0.1% penicillin/streptomycin sulfate. Bursa cells were grown in a tri-gas incubator at 37°C with 5% O2 and 5% CO2. Nucleated cells counts were then performed using a Z1 Coulter Particle Counter (Beckman Coulter Life Sciences, Indianapolis, IN, USA) after cells were incubated for 2 weeks. Of note, this study used a mechanical method of isolating bursa cells instead of an enzymatic method which utilizes a collagenase. Mechanical isolations methods have been reported as an effective and faster method of isolating stem cells from adipose tissue (34). In addition, mechanical methods avoid the use of collagenase which may interfere with bone-tendon healing and therefore has a better clinical application.

Patellar Tendon Defect Model and Implantation
All animal experiments were performed under an approved Institutional Animal Care and Use Committee (IACUC) protocol at UConn Health (101111-0718). Thirty-nine male NSG mice (Jackson Laboratories, Inc, Bar Harbor ME) were used, all at 14 weeks of age. All mice underwent a single leg surgery under isoflurane anesthesia, with the surgeon blinded to the treatment group. Surgery was performed using an anterior midline incision over the proximal tibia, and the patella tendon insertion was identified and cut at the most distal point (figure 3). After dissection, any remaining tendon stump at the tibial tuberosity site was rasped away. The mice were then randomly allocated to treatment groups, allowing for 13 per group. One of three possible adjuvants were placed underneath the tendon, at the bone-tendon interface: (1) fibrin clot + BMCs (4μL), (2) fibrin clot + subacromial bursal cells (2 mg of chopped bursa), and (3) fibrin clot only. The fibrin clot was created as a cell or tissue carrier by combining 4-μl of fibrinogen...

Figure 3. Mouse patellar tendon avulsion injury. (A) An incision was made directly over the patellar tendon and the tendon was separated from the capsule. (B) The patellar tendon was incised at the tibia and any remaining tendon was rasped away at the tibia. (C) The injury was then treated with a fibrin clot comprised of human bone marrow cells, bursa tissue, or no human cells. (D) the wound was then closed with 7-0 prolene sutures.
(20 mg/ml, bovine fibrinogen, Sigma Aldrich) and 8-μl of thrombin (40 U/ml, bovine Thrombin, Sigma Aldrich) for 30 seconds, resulting in a cell-gel construct. Depending on the treatment group, 4 μl of BMCs, 2mg of chopped bursa or no cells were placed in the fibrin clot. The tendon stump was placed over the fibrin clot without being repaired. The decision to not repair the tendon was based on preliminary biomechanical data in our lab, which demonstrated no biomechanical difference in repaired versus non-repaired. The skin was then approximated and closed with 7-0 nylon suture (Ethicon). The mice were then sacrificed at two weeks postoperatively for either biomechanical or immunohistochemistry. Both surgical and nonsurgical hindlegs were harvested with the latter serving a normal control.

Biomechanical Testing
Mice allocated to biomechanical analysis had their hindlegs frozen in normal saline and were thawed to room temperature. The hindleg was dissected until only the quadriceps, patella, patella tendon and fibula remained (figure 4). The tibia and fibula were then potted in metal cylinders using bone cement (PMMA), with the tendon constantly kept moist. Next, the patella tendon was gripped with a custom-made fixture to a servohydraulic system (MTS, Eden Prairie MN) (31). Similar to the setup of Dyrna et al., the lower limb grip could be adjusted in the X-Y plane to ensure that the tendon was vertical at the time of testing (figure 5) (31).

Samples were preloaded to 1N for 20 seconds, and then loaded to failure at 0.05mm/second using the MTS machine. Load to failure (N, Newton) and stiffness (N/mm) of the tendon was recorded. Stiffness was measured as the slope of the force and displacement curve. The non-operative leg underwent biomechanical testing to serve as a control for variations in tendon strength between mice.

Histology and Immunohistochemistry
Four mice of each of the three different treatment groups were assigned for histological evaluation. Once the mice were sacrificed, the hindlegs were then fixed in formalin for five days and incubated in 30% sucrose solution overnight (31). The tendon was embedded in Cryomatrix (Thermo Fisher Scientific) and multiple serial sections (7-8 mm) were made along the distal stump of the tibial insertion. These sections were then glued onto a slide with chitosan and underwent Toludine blue staining and specific antibody staining was performed for human nuclear antigen, DAPI, collagen I and collagen III. The non-operative extremity was also assessed as a comparison.

Cell Survival
Twelve remaining mouse specimens, six from BMC and bursa individually, were further analyzed for the presence of human Col1A1 gene in order to confirm the human cells survived following implantation. In addition, two specimens treated with fibrin clot only, no human cells, were utilized as controls. Corresponding fixed samples, embedded for immuno-histochemistry analysis, were cryosectioned (50 µm) and the tissue pieces from the section were placed into 1.5 ml microfuge tubes. Tissues were lysed at 95°C for 30 min in 100 µl of Alkaline lysis buffer (25 mM NaOH and 0.2 mM EDTA), then neutralized with 100 µl of neutralization buffer (25 mM Tris HCL, pH6.8). Samples

Figure 4. Mouse hindleg dissection for biomechanical analysis. (A) An injured hindleg after hip/ankle disarticulation and skin removal. (B) The same hindleg after removal of muscle, leaving the tibia, fibula, patellar tendon, and patella. (C) The tibia and fibula were potted in a metal cylinder with cement leaving the patellar tendon exposed for biomechanical testing.
were centrifuged at 8000 gx for 10 min, and 2 µl of the supernatant from each sample were used for qPCR. 310 bp human COL1A1 gene sequences flanking the introns and exons around exon 22 and 23 were used to align with the mouse Col1a1 gene using the Nucleotide Blast search tool (nucleotide>nucleotide) published by the National Center for Biotechnology information (NCBI) for guiding the primer design. The mouse forward primer (5’ CCCACTCAAGGTGCTAC) contained 5 mismatches to the human corresponding sequences, with 2 mismatches in the 3’ end. The human forward primer (5’ CCTGCTGGAGAGGAAGGAAAG) contained 3 mismatches to the mouse sequence with 1 mismatch in the 3’ end. The reverse primer was designed to the sequences with 100% matches between human and mouse (5’ CCAGGGAAACCACGGCTAC). The size of the PCR amplicon for each mouse was 232 bp, while for human cells was 201 bp. The 31 bp of the size difference was distinguishable on the 2.5% agarose gel. The PCR annealing temperature was determined at 62°C by the temperature gradient test, which was the optimum temperature for both mouse and human PCR. Therefore, the multiplex qPCR reaction from mixing both mouse and human forward primers with mouse/human shared reverse primers in the same reaction tube, was designed. After 35 PCR cycles, two clear PCR products in the right corresponding sizes were observed on the 2.5% agarose gel. Within the 201 bp of human PCR product, a Xho1 restriction digest site was identified, which digested the PCR product into two fragments with sizes of 28 bp and 173 bp. The 28 bp fragment was undetectable on 2.5% agarose gel, however the 173 bp fragment was observed on 2.5% agarose gel, and the size difference of human PCR product digested by Xho1 was then distinguishable on 2.5% agarose gel and served as further indication of human COL1A1 gene in the tested samples.

Statistics
Pilot data from our laboratory was used to determine an adequate sample size. A sample size of 8 mouse per group provides 80% power to detect a 0.5 N difference in load to failure with a standard deviation 0.52 at an alpha level of 0.05. For both stiffness and load to failure, raw values were reported as a percentage of the normal contralateral limb. A general linear model with a logit link and the binomial family was used to examine differences in load to failure and stiffness between treatment groups. This approach is appropriate for the analysis of proportional data and produces predicted values that range between 0 and 1. Robust standard errors were obtained in each model. This An alpha level of 0.05 was set for all analyses. All statistical analysis was performed using Stata 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).
RESULTS
The mean weight of all mice was 30.67 (SD= ± 2.56), with no differences found between groups (P=0.0971). The number of human cells from the cBMA was approximately 8,400 cells for each mouse randomized to the cBMA treatment group, while approximately 1,265 bursa cells were used per mouse in the bursa treatment group.

Biomechanical Testing
Of the 27 mice allocated for biomechanical testing, one mouse from the cBMA and one from the subacromial bursa treatment group was excluded due to errors in initial biomechanical settings, leading to the specimen being destroyed before data could be obtained. This left 25 mice in total for complete testing (table I). Mean load to failure of the normal contralateral leg was 12.11 N (SD=±2.56) for all mice. For treatment groups, subacromial bursa was found to restore tendon strength to 62.2% (SD ±10.2%) of the normal contralateral tendon strength, compared to only 49.87% (SD ±16.4%) using cBMA (P=0.06). When compared to fibrin clot only (47.87%, SD ± 15.6%), treatment with subacromial bursal tissue demonstrated significantly higher tendon strength in relation to its contralateral tendon (P=0.018). The mechanism of failure occurred through the scar of the defect created in all treatment groups, while the normal tendon cohort all failed at the tibial insertion.

In relation to the normal contralateral limb, subacromial bursa restored tendon stiffness to 35.9% (SD ± 17.3%) at two weeks (table I). Similar findings were seen in the cBMA (38.12%, ± 12.3%) and fibrin clot only group (35.9% ± 17.3%) (P=0.657).

Immunohistochemistry and Histology
Four specimens from each treatment cohort were randomized to the histology and immunohistochemistry. These slices were taken through the midsection of the patella in order to analyze the bone-tendon healing at the patellar tendon-tibia interface and compared to normal patella tendon. Histologic evaluations of the tissue using a Leica Microscope (Leica Microsystems Inc., Buffalo Grove IL) at 10X magnification revealed increased cellularity and disorganized collagen formation at the tendon-bone interface. Healing was seen throughout the fibrocartilage tissue at the bone-tendon interface in all three cohorts (figure 6). Immunohistochemical staining showed that more type I collagen was seen in the normal mouse tendon, however, no significant differences were found between treatment groups (figure 7). Similarly, after staining for type III collagen, no significant differences in microscopic examination were found between treatment groups. The scar tissue present in all cohorts was comprised primarily of disorganized type III collagen. Of note, the transplanted bursa tissue was notable using immunohistochemistry and contained a substantial amount of collagen I and III and appeared to

<p>| Table I. Load to Failure and Stiffness Between Treatment Groups. |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|</p>
<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean Load to Failure (N) (SD)</th>
<th>Percent in Relationship to the Normal Limb (Control) (SD)</th>
<th>Mean Stiffness (N/mm) (SD)</th>
<th>Percent in Relationship to the Normal Limb (Control) (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subacromial Bursa (N=8)</td>
<td>7.43 (1.78)</td>
<td>62.2% (10.2)*</td>
<td>8.82 (1.9)</td>
<td>39.33 (13.2)</td>
</tr>
<tr>
<td>cBMA (n=8)</td>
<td>5.8 (1.49)</td>
<td>49.87% (16.4)</td>
<td>8.67 (2.6)</td>
<td>38.12 (12.3)</td>
</tr>
<tr>
<td>Fibrin Clot only (n=9)</td>
<td>5.78 (1.74)</td>
<td>47.87% (15.6)</td>
<td>7.57 (2.9)</td>
<td>35.9 (17.3)</td>
</tr>
</tbody>
</table>

*Subacromial bursa was found to restore ultimate tendon strength significantly higher than fibrin clot only (P=0.018).
serve as scaffold for mouse cells to infiltrate into the defect. Human nuclear antigen was positive in 3/4 (75%) of the bursa treated mice and 1/4 (25%) of the BMA treated mice. All fibrin treated mice served as a control and were negative for human nuclear antigen.

Cell Viability
Mice allocated to histology and immunohistochemistry were also assessed for human Col1A1 gene, in order to confirm the survivability of human cells after implantation. Seventy-five percent of specimens (3/4) that were augmented with subacromial bursa demonstrated positive human nuclear staining, compared to only 25% (1/4) of specimens treated with BMA (figure 8).

DISCUSSION
Using a murine tendon model, this study found that subacromial bursal tissue resulted in significant increased tendon strength compared to an acellular control (fibrin clot only) at the early healing phase. We also found that human survivability using subacromial burs was found in 75% of tested mice, compared to only 25% of mice treated with cBMA at 2 weeks. There were no significant biomechanical differences found between subacromial bursa or cBMA at the two week interval.

Healing at the tendon-bone interface remains a problem after rotator cuff repair, with evidence of a lack of progenitor cells found at the site of insertion in these patients (11). The current study demonstrated increased cell survivability using subacromial bursal tissue compared to bone marrow aspirate at 2 weeks. However, similar to Dyrna et al., this study did not find that cell augmentation restored the tendon to its native properties (31). However, they’re data suggested that implantation stimulated host murine cells to mount a more robust repair response, rather than through direct repair (31).

Khan et al. used a similar murine model to examine the use of MSCs on intra-synovial tendon healing, and found that the implanted progenitor cells did not improve healing, however they did show specific encroachment along specific sites of the tendon (35). These studies were limited in that the tendon defect model was mid-substance, instead of at the tendon-bone interface. Interestingly, the subacromial bursa tissue in this study appeared to have increased cellularity and infiltration of murine cells as seen on immunohistochemistry.

Figure 7. Immunohistological staining for type I and III collagen found under the microscope. All three treatment groups demonstrated majority of type III collagen with no significant differences found between groups.
Previous studies have examined the biomechanical affects of MSCs on soft-tissue healing in an animal model (31, 33). Lebaschi et al. examined the use of cBMA on a supraspinatus tendon repair murine model, and found that failure of the repaired tendon was only 36% in relation to its native tendon at two weeks (33). Similar results were found by Bell et al. who used a similar methodology, however did use biologic adjuvant (36). Their studies differ from the current study, both in the delivery of the biologic agent, as well as the location of the tear. In the current study, a fibrin clot was used to deliver MSCs and potentially allow for increased proliferation and migration of these cells (37, 38). Although significant biomechanical differences were not found between biologic augments, we did find tendon strength restored to roughly 50% or more compared to the native limb at 2 weeks, compared to only 25% as reported by Dyrna et al. for mid-substance tears (31).

Decreased cell survivability following implantation has been reported in several studies using an animal model (1, 31). This study was limited in its evaluation, as only one time point (2 weeks) were examined, however, we found that 75% of samples with subacromial bursa had identifiable human cells from implantation that remained, compared to only 25% from bone marrow aspirate. This is a similar finding as previously published (31), however, further time points are needed to determine if this holds true in later weeks. Cell survivability can be altered by attainment technique, preparation, and re-implantation methods. In addition, a fibrin clot’s affect on mice and healing is not yet fully understood, and may affect the long-term survivability in this model.

As tendon-bone healing remains a significant dilemma in rotator cuff repair, simulating animal models such as the current study is critical in developing an ideal healing environment in the clinical setting. Bone marrow aspirate has remained the most common source for biologic augmentation, showing promising results without the use of a fibrin clot (12). Hernigou et al. injected concentrated BMA directly into the site of rotator cuff repairs, and found a significantly decreased rate of re-tear when compared to control groups (12). Voss et al. first described the use of a fibrin clot as a scaffold to be used with BMA taken from the proximal humerus (14). This technique allows for ease of access during arthroscopic rotator cuff repair, and could potentially to be used in conjunction with subacromial bursa.

Figure 8. Human Nuclear Antibody (HuNu) staining of both bursa (A) and bone marrow aspirate (B) demonstrating live human cells still present within the mouse sample.
Despite the animal model, there were several strengths of the study that can add clinical value. For instance, the study supports the potential use of subacromial bursa as an augment for tendon-bone healing, which continues to be an issue during rotator cuff repair. It also demonstrates the importance of processing the bursa once harvested, and delivering it within a fibrin clot. This was shown to have continued presence in the mouse in up 2 weeks after implantation. Further studies into how to best process and deliver subacromial bursa in the operating room, in an efficient and realistic manner are warranted. Lastly, this study also supports future clinical studies examining subacromial bursal tissue on human patients undergoing rotator cuff repair for augmentation.

LIMITATIONS
There were several limitations to this study. One limitation was an animal model was used to mirror a tendon avulsion injury. The tendon was not repaired as the normal tendon would in the clinical setting. Similarly, there were postoperative limitations, including the inability to control range of motion and weight-bearing on the healing tendon, which differs from repair of a torn tendon that occurs in humans. Another limitation is that 2 weeks postoperatively was the only time-point examined. Further time-points would likely demonstrate differences in tendon strength and cell survivability. Finally, the murine surgical model involves an acute traumatic tear, unlike the degenerative tendon interface that rotator cuff pathology often involves.

CONCLUSION
Subacromial bursa tissue demonstrated significantly higher improvement in tendon strength compared to fibrin clot treatment (acellular control) at 2 weeks. Increased human cell survivability was also demonstrated using subacromial bursal tissue compared to cBMA.

ACKNOWLEDGEMENTS
Institutional review board was obtained prior to the study (IE-07-224-2).

CONFLICT OF INTERESTS
Dr. Mazzocca receives consulting fees as well as research support funding from Arthrex Inc. The company had no influence on study design, data collection or interpretation of the results or the final manuscript. No other author has disclosures.

DISCLAIMER
Dr. Mazzocca receives consulting fees as well as research support funding from Arthrex Inc. The company had no influence on study design, data collection or interpretation of the results or the final manuscript

FUNDING SOURCE
The study received direct funding from the OREF/UCONN Founders Endowment Grant.


