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Human Subacromial Bursal Cells Display Superior Engraftment Versus Bone Marrow Stromal Cells in Murine Tendon Repair

Felix Dyrna, MD^{*}, Philip Zakko, BSA[†], Leo Pauzenberger, MD[‡], Mary Beth McCarthy, BS[†], Augustus D. Mazzocca, MD[†], Nathaniel A. Dymant, PhD^{§,¶}, UConn Health, Farmington, Connecticut, USA, and University of Pennsylvania, Philadelphia, Pennsylvania, USA

^{*}Department of Orthopaedic Sports Medicine, Technical University, Munich, Germany.

[†]Department of Orthopaedic Surgery, UConn Health, Farmington, Connecticut, USA.

[‡]St Vincent Shoulder and Sports Clinic, Vienna, Austria.

[§]Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

Abstract

Background: Bone marrow aspirate is a primary source for cell-based therapies with increasing value in the world of orthopaedic surgery, especially in revision cases of tendon and ligament repairs. However, cells within peritendinous structures, such as the paratenon and surrounding bursa, contribute to the native tendon-healing response and offer promising cell populations for cell-based repair strategies. Therefore, the purpose of this study is to investigate the efficacy of cells derived from human subacromial bursa as compared with the current gold standard, bone marrow stromal cells (BMSCs), for tendon repairs in an established in vivo immunodeficient murine patellar tendon defect model.

Hypothesis: Subacromial bursal cells will show superior survival and engraftment into the host tissue as compared with BMSCs.

Study Design: Controlled laboratory study.

Methods: Human subacromial bursal and bone marrow aspirate were harvested from the same donor undergoing rotator cuff repair. Cells were transfected with a fluorescent lentiviral vector to permanently label the cells, encapsulated into fibrin gel, and implanted into bilateral full-length central-width patellar tendon defects of immunodeficient mice. Additional surgery was performed on control mice comparing fibrin without cells and natural healing. At the time of sacrifice, all limbs were scanned on a multiphoton microscope to monitor the engraftment of the human donor cells. Afterward, limbs were assigned to either immunohistochemical or biomechanical analysis.

Results: As compared with BMSCs, implanted subacromial bursal cells displayed superior tissue engraftment and survival. The main healing response in this defect model was the creation of new

[¶]Address correspondence to Nathaniel A. Dymant, PhD, McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, University of Pennsylvania, 109A Stemmler Hall, 3450 Hamilton Walk, Philadelphia, PA 19104-6081, USA (dymant@pennmedicine.upenn.edu).

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healing tissue over the anterior surface of the defect space. The implantation of cells significantly increased the thickness of the anterior healing tissue as compared with control limbs that did not receive cells. Cell proliferation was also increased in limbs that received implanted cells, suggesting that the donor cells stimulated a more robust healing response. Finally, these changes in the healing response did not lead to significant changes in mechanical properties.

Conclusion: The subacromial bursa, while often removed during rotator cuff repair, may harbor a more suitable cell source for tendon repair than BMSCs, as bursal cells display superior engraftment and survival in tendon tissue. In addition, the subacromial bursa may be a more accessible cell source than bone marrow aspirate.

Clinical Relevance: The subacromial bursa contains a cell population that responds to tendon injury and may provide a more optimal cell source for tendon repair and regeneration strategies. Therefore, cells could be harvested from this tissue in the future, as opposed to the current practice of bursectomy and debridement.

Keywords

subacromial bursa; bone marrow stromal cells; tendon repair; long-term cell engraftment

Given the limited healing potential of tendons, tissue-engineered augmentation of tendon repairs has evolved as a promising option, particularly cell-based repair/ regenerative strategies. Despite numerous preclinical cell-based tendon repair studies, the mechanism by which implanted cells contribute to the repair process is unclear because of the difficulty of tracking the long-term fate of the donor cells.^{1,14,15,18,20,27} For instance, do donor cells survive in a tendon repair environment? Do they actively participate in the repair through the synthesis and assembly of an appropriate extracellular matrix, or do they communicate with the surrounding host cells to augment the repair response? These are important questions that cannot be answered without stable labeling and long-term fate mapping of the implanted cell populations.

Multiple cell sources have been evaluated as possible candidates for cell-based repair augmentation' owing to their multipotency and/or ability to synthesize extracellular matrix.^{4,11,13,19,21,24,31} Bone marrow stromal cells (BMSCs) are the current gold standard cell source for cell-based tendon repair strategies, with promising in vitro and in vivo results.^{6-10,17} However, a resident cell population known as tendon-specific progenitor cells was recently suggested as a more appropriate cell population for tendon repair augmentation, as these progenitor cells are closer than BMSCs to the tenogenic lineage.^{3,22,24,27} Even with the extensive literature directed toward cell-based repair, there is still an open question regarding which cell source is the most appropriate to use for each tendon repair model and the mechanism by which implantation of these cells produces an improved repair outcome.

The cells that contribute to natural tendon healing originate from loose connective tissue surrounding the tendon fascicles (ie, endotenon) and tendon body (ie, epitendon and paratenon). In response to the injury, these cells proliferate and migrate toward the defect site where they form collagenous healing tissue.^{6,7,10} Lineage-tracing strategies from our laboratory demonstrated that peritendinous cell populations contribute to healing tissues of

patellar tendon and supraspinatus tendon injuries.^{7,32} Of particular interest is the expansion of cells within the subacromial space in the rotator cuff model that may originate from the sub-acromial bursa.

The importance of subacromial bursa was highlighted by Uhthoff and Sarkar,²⁸ who stated that the bursal expansion during rotator cuff tears was a reparative response rather than a degenerative change. However, bursectomy and debridement are generally performed to obtain visualization of the rotator cuff tear during repair, thereby removing this promising cell population. The regenerative potential of this tissue can be seen by a complete restoration after surgical resection within 6 months.²⁶ Furthermore, in vitro characterizations of cells derived from human subacromial bursa revealed surface antigen expression similar to mesenchymal stem cells in addition to multipotent differentiation and hence warrant further attention.²⁶ The efficacy of using this cell population as a therapeutic in a cell-based repair strategy has not been investigated, particularly in direct comparison with the gold standard, BMSCs.

The purpose of this study was to investigate the efficacy of cells derived from human subacromial bursa as compared with BMSCs for tendon repairs in an established in vivo immunodeficient¹⁵ murine patellar tendon defect model.¹⁴ The objectives were to (1) stably label the cells and image the entire healing tissue volume to track their long-term fate following implantation, (2) compare the engraftment and survival of bursa-derived cells and BMSCs after implantation, and (3) directly compare the outcomes of repairs augmented by these 2 cell types. It was hypothesized that subacromial bursal cells would show superior engraftment into host tissue as compared with BMSCs, yielding a superior repair outcome.

METHODS

Experimental Design

Subacromial bursa from human rotator cuffs and BMSCs from the proximal humeral head were harvested from the same human donor ($n = 5$ human donors; mean $\pm$ SD age, 57 ± 5.5 years) during primary arthroscopic rotator cuff repair. Cells were taken from the same donor during a single surgical procedure to minimize genetic/epigenetic variation among patients to provide direct comparisons between the cell types. The cells were expanded in culture and directly compared via bilateral implants into patellar tendon defects of NOD scid gamma (NSG) mice (72 mice total) (Figure 1). The mice were then assigned to either immunohistochemistry (IHC; 1, 2, 5, and 8 weeks after surgery; $n = 9$ per group) or biomechanics (2, 5, and 8 weeks after surgery; $n = 12$ per group). Cells from each donor were implanted into 14 mice, which were assigned to the 4 IHC and 3 biomechanics time points ($n = 2$ for each). All limbs were scanned on the multiphoton microscope before being prepared for IHC or biomechanics. Additional mice (40 total) served as controls, where 1 limb received fibrin without cells and the other limb received only the defect with no additional treatment (ie, natural healing control). These mice were scanned on the multiphoton microscope and assigned for IHC (1, 2, 5, and 8 weeks; $n = 4$ per group) or biomechanics (2, 5, and 8 weeks; $n = 8$ per group). Finally, age-matched intact controls were assigned to IHC ($n = 4$) or biomechanics ($n = 8$). Both male and female sexes were included in this study and were equally distributed across the treatment groups.

Cell Isolation, Expansion in Culture, and Lentiviral Transduction

The institutional review board approved the procedures for acquiring tissue samples during arthroscopic rotator cuff surgery (No. 06–577-2 for bone marrow aspirate [BMA] and No. 07–224-2 for collection of subacromial bursal tissue, which is classified as surgical waste). Subacromial bursal tissue was harvested with a grasper from human rotator cuffs, and BMA (at least 120 mL) was collected from the proximal humeral head from the same human donor during primary arthroscopic rotator cuff repair. The proximal humerus is an ideal location for harvesting BMSCs, as the epiphysis is made up of trabecular bone and is a source rich in hematopoietic cells. Mazzocca et al²³ showed the ability to safely and efficiently harvest bone marrow from the proximal humerus via anchor tunnels created during arthroscopic rotator cuff repair. Briefly, a 14-gauge bone marrow aspiration trocar, fit with a 60-mL syringe containing 3 mL of Anticoagulant Citrate Dextrose Solution A (Baxter Healthcare Corp), was inserted 2.5 to 3 cm into the medial aspect of the greater tuberosity (Bone Marrow Aspiration Kit; Arthrex). The tunnel created was later used to insert the first medial suture anchor. In a standardized method of aspiration, the surgeon pulled back on the syringe to maximize suction, allowing 20 mL of aspirate to flow into each of 6- to 60-mL syringes for a total of 120 mL of aspirate. The aspirate includes a mixture of blood, bone marrow, and arthroscopic fluid, which is concentrated to our final BMA sample.

Both tissue samples were taken immediately to the laboratory for further processing. Bursal tissue was digested with 2 mg/mL of Collagenase P (Sigma Aldrich) in 30 mL of DMEM (Life Technologies) at 37°C for 3 hours until no solid tissue could be seen. During this time, 120 mL of the BMA was concentrated by centrifugation for 25 minutes with the Arthrex Angel System (Arthrex). For all concentrated bone marrow samples, nucleated cell count (million per milliliter of BMA) and the number of colony-forming units (CFUs; per 1.0 mL of BMA) were obtained. For the bursa, total weight was recorded before collagenase digestion. After digestion, the total number of cells were obtained, and CFUs were counted after 7 days in culture. Both samples were plated in DMEM containing 10% fetal bovine serum and 0.1% penicillin/streptomycin sulfate. Cells were grown to confluence and then expanded to P1-P2 in a tri-gas incubator at 5% O₂, 5% CO₂, and 37°C, which increases cell proliferation and collagen production.⁵ During the first passage, cells were transfected with an ubiquitin-mCherry (Ub-mCherry) lentiviral vector to permanently label the cells and ensure long-term tracking of cell fate in the implant model.³⁰

Patellar Tendon Defect Implantation Model

All experiments were performed under an institutionally approved Institutional Animal Care and Use Committee protocol at UConn Health (100547–1015). Human bursal cells and BMSCs were implanted into defects created in NSG mice (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ), which allow for long-term engraftment of human cells because of their lack of B cells, T cells, and functional natural killer cells.^{25,30}

Bilateral full-length central-width patellar tendon defects were created as described by Dymont et al.^{9,10} Mice were anesthetized with isoflurane (1%–3%) and sterilely prepped. After a central 5-mm skin incision, the pre-patellar bursa was pushed aside to provide access to the patellar tendon. Two No. 11 blades clamped with needle holders were used to create

parallel full-length longitudinal incisions centered on the midline of the tendon. The center region was grasped and excised to create the defect (Figure 1).

Parallel to the surgical approach, implants were created by centrifuging 150,000 cells into a cell pellet, carefully aspirating as much media as possible, and resuspending the cells in 4 μL of fibrinogen (20 mg/mL, bovine fibrinogen; Sigma Aldrich) and 2 μL of thrombin (40 U/mL, bovine thrombin; Sigma Aldrich) for 30 seconds, resulting in a cell-gel construct (final concentrations: $\sim 15 \times 10^6$ cells/mL and ~ 8 mg/mL of fibrin). Immediately after the fibrin had gelled, the construct was gently compacted into the defect space. Bursal cells (group A) and BMSCs (group B) were implanted into contralateral limbs of the same mouse ($n = 72$). Control mice ($n = 40$) also received defects in both limbs; however, 1 limb received just the fibrin gel without cells (group C), and the other was left as a natural-healing control (group D). The fibrin in group C was a 50:50 mixture of bovine fibrinogen (same as groups A and B) and human fibrinogen Alexa Fluor 546 conjugate (Thermo Fisher Scientific) so that the fibrin gel could be better visualized during 3-dimensional (3D) and 2-dimensional microscopy. After implantation, the mouse skin was approximated and closed with 1 or 2 stitches of 5–0 nylon sutures. Mice moved freely in their cages until tissue harvest at designated time points.

Multiphoton Microscopy

Mice were sacrificed via CO_2 asphyxiation, and the limb was dissected down to the knee by removing the proximal femur and distal tibia. Samples that were assigned for histology were fixed in formalin with the knee flexed at 90° , whereas biomechanical samples were stored in OptiMEM to increase cell viability during the scan. All patellar tendons were scanned on the Prairie Ultima IV multiphoton microscope (Prairie Technologies), with a $20\times/0.95\text{W}$ Olympus water immersion objective (pixel size, $0.9 \mu\text{m}$). The samples were oriented such that the anterior surface of the patellar tendon was horizontal with custom clamps in a bath of OptiMEM. Three-dimensional scans over the entire healing tissue ($3000 \times 1000 \times 400 \mu\text{m}$) were conducted with 2 Ti:Sapphire lasers at excitation wavelengths of 890 nm and 1010 nm. Data were collected in 3 channels with the following bandpass filters: 435 to 485 nm (collagen second harmonic generation, 890-nm laser), 500 to 550 nm (background autofluorescence, 890-nm laser), 570 to 620 nm (Ub-mCherry fluorescence, 1010-nm laser). After the acquisition was finished, IHC samples were placed back into formalin and stored at 4°C for further processing, and biomechanical samples were put back into OptiMEM and stored at -20°C .

IHC and Cell Proliferation

One day before sacrifice, each mouse assigned for histology was injected with EdU (5-ethynyl-2'-deoxyuridine, 3 mg/kg) to label dividing cells. After mice were euthanized and scanned on the multiphoton microscope, the limbs were fixed in formalin for 1 to 2 days and incubated in 30% sucrose solution overnight. Before embedding the sample in Cryomatrix (Thermo Fisher Scientific), a fiducial marker (6.0 Prolene Suture; Ethicon) was inserted parallel to the medial tendon strut in line with the defect to help orient the tendon in proper sagittal plane before reaching the defect region (Appendix Figure A1, available in the online version of this article). Multiple serial sections ($7\text{--}8 \mu\text{m}$) were made at 5 levels from the

medial intact tendon strut, through the defect region, and into the lateral strut with Cryofilm type 2C.⁸ Selected sections of each level were glued onto a slide with chitosan, and specific antibody staining was performed. A set of 3 separate staining combinations for each animal represented a complete assessment. Therefore, slide I was costained with goat anti-collagen I (1:100, AB758; Millipore), rabbit anticollagen III (1:200, ab7778; Abcam), and mouse antihuman nuclear antigen (1:100, ab191181; Abcam) overnight at 40°C, followed by fluorescent secondary antibodies (Alexa Fluor 488, 647, and 555, respectively) as the primary matrix assessment. Slide II was costained with rat antite-nascin C (1:100, MAB2138; R&D Systems), mouse anti-human nuclear antigen, and EdU (C10269, Azide 647; Thermo Fisher Scientific) to analyze early matrix production and cell proliferation. Tenascin C was stained first and EdU afterward according to the manufacturer's protocol. Slide III was costained with rat anti-F4/80 (1:100, 123101; Biolegend), mouse antihuman nuclear antigen, and rabbit anticollagen IV (1:500, ab19808; Abcam) to assess macrophage response and neovascularization. Finally, the slides were counterstained with DAPI and imaged on the Axio Scan.Z1 (Zeiss) digital scanning epi-fluorescent microscope. Following fluorescent imaging, cover slips were removed, and the slides were stained again with toluidine blue O and reimaged on the Axio Scan.Z1.

Biomechanical Testing

Samples were thawed; remaining muscle was removed from the tibia and femur; and the tendon was trimmed to its central third with 2 No. 11 blades, similar to the surgical procedure. Next, the femur was disarticulated, leaving a tibia-tendon-patella unit. The tibia was potted with bone cement and secured with a long staple placed over the tibial plateau. The width and thickness were measured from digital images taken in the sagittal and coronal planes. Next, the patella was gripped with a custom-made fixture. The lower (patellar) grip could be adjusted in the X and Y plane to ensure that the tendon was vertical at the time of testing. Testing was performed in physiologic saline at 37°C on the Bose EnduraTec 3230. Samples were pre-loaded to 0.02 N, preconditioned (10 cycles, 0–0.03 mm, 0.03 mm/s), loaded for static stress relaxation (ramp to 0.15 mm at 0.15 mm/s, followed by 10-minute hold), and finally loaded to failure at a rate of 0.03 mm/s. Structural and material properties were computed.

Multiphoton Image Analysis

In Fiji image analysis software,²² the Stitching Plugin²⁴ was used to combine the TIF files generated by the multiphoton microscope into 3D image stacks.

Cell Engraftment and Survival.—The level of cell engraftment of the bursa or BMSC donor cells into the host tissue was quantified in 3 regions: (1) the anterior healing tissue (ie, bridge) that forms over the defect,^{6,7,10} (2) the remaining medial and lateral tendon struts that were not removed during the surgery, and (3) the defect space that included the cell-fibrin gel implant. In the transverse orientation, regions of interest were drawn per the collagen second harmonic generation signal (see Figure 2B) for several slices and then interpolated in between to create a 3D region of interest along the entire tendon length. An equivalent minimal threshold was defined for each sample, and the total number of positive pixels in the red channel was divided by the total number of pixels (ie, percentage positive

area) to quantify the relative level of Ub-mCherry cells within each tissue region. The mean value was computed for all slices within a given image stack, as reported in Figure 2 and used in downstream statistical analysis.

Thickness of Anterior Healing Tissue (Bridge).—The region of interest defined here for the anterior healing tissue bridge was then cropped to a 100-mm width centered on the middle of the defect in the transverse plane. The mean thickness across this 100 μ m was computed; then, the mean thickness across every slice along the tendon was computed and used in downstream statistical analysis.

IHC Image Analysis

See the Appendix (available online).

Statistics

Cell engraftment from multiphoton image stacks was compared between the bursa- and BMSC-implanted limbs at 1, 2, 5, and 8 weeks within 3 regions of interest: anterior bridge, adjacent struts, and remaining fibrin. As the data were not normally distributed, Mann-Whitney *U* tests were conducted at each time point ($P < .05$). F4/80 staining within the fibrin was compared among the bursa, BMSC, and fibrin-only groups at 1, 2, 5, and 8 weeks via 1-way analysis of variance (ANOVA) with Tamhane post hoc comparisons for unequal variance ($P < .05$). The thickness of the healing tissue bridge was compared between the cell implant groups (bursa and BMSC) and noncell groups (fibrin only and defect only) via 1-way ANOVA at 1, 2, 5, and 8 weeks ($P < .05$). Cell proliferation was compared between the cell implant and noncell groups via 1-way ANOVA at 1 and 2 weeks ($P < .05$). Structural properties (ultimate load and stiffness) and material properties (ultimate stress and modulus) were not normally distributed, so Kruskal-Wallis tests were used to determine if treatment (bursa, BMSC, fibrin control, natural healing, and native tendon) had a significant effect at each time point ($P < .05$). Mann-Whitney *U* tests were conducted at each time point between the treatment groups ($P < .005$ to adjust for multiple comparisons).

RESULTS

Bone Marrow and Bursa

After processing, the overall volume of concentrated bone marrow was 3.4 ± 0.5 mL (for 5 patients), including 33.2 ± 7.4 nucleated cells per 1 mL of concentrated BMA. After 7 days in culture, CFUs were counted to evaluate the number of BMSCs. Overall, 2309.0 ± 274.2 CFUs grew per 1 mL of BMA. For the bursa, 284.0 ± 179.5 mg of tissue was collected. The total number of cells after collagenase digestion was $0.43 \times 10^6 \pm 0.21 \times 10^6$ or 2309.0 ± 274.1 cells per 1 mg of bursa. Overall, 62.3 ± 15.5 CFUs were obtained per 1 mg of bursa.

Gross Observations

The overall success rate of the study was 71% (171 of 242 limbs), with failures defined as tendon ruptures or implant displacement from the defect site after surgery. None of the treatments had an effect on patellar tendon rupture rate (19%, 22%, 20%, and 18% for bursa, BMSC, fibrin only, and natural healing, respectively). The ruptures were also equally

distributed between male and female mice. Two mice died during surgery and were replaced. Cell implantation failure, where the implant was displaced from the defect site between surgery and tissue harvest, occurred in only 5.8% of limbs. In addition, cell survival was measured on a binary scale (ie, did or did not contain human cells) over the complete study period of 8 weeks. With this metric, implanted subacromial bursal cells displayed greater survival, with 82% of limbs still containing visible cells at 8 weeks versus only 50% of BMSC limbs at the same time point.

Bursa-Derived Cells Displayed Superior Engraftment Into the Host Tissue

Multiphoton 3D imaging of the entire defect space was used to measure cell engraftment of the donor cells. The bursal cells showed greater engraftment into the host tissue in all regions of interest (Figure 2, Appendix Figure 2A). Significantly more bursal cells were found in the anterior healing tissue at 2 weeks (174% increase) (Figure 2C) and adjacent tendon struts at 2 and 5 weeks (281% and 191% increase, respectively) (Figure 2D) as compared with BMSCs ($P < .05$). Additionally, the bursal cells survived longer than the BMSCs in the fibrin ($P < .05$) (Figure 2E). The engrafted cells also took on the 3D morphology of the surrounding tissue, as cells that infiltrated the adjacent tendon struts arranged in linear arrays similar to native tendon fibroblasts (Appendix Video and Figure A2, available online).

Macrophage Response Did Not Account for the Increased Survival of Bursal Cells

Since the bursal cells displayed increased survival with higher number of cells remaining in the fibrin at later time points (Figure 2E), sagittal sections of the repaired tendons were stained with F4/80 to label activated macrophages within the wound site. The volume of fibrin decreased with time in all groups. When the F4/80 staining was measured within regions of the fibrin implant (Figure 3, A–C), there was no significant difference in macrophage response between the bursal cells and BMSCs (Figure 3D). In addition, neither cell group was significantly different from the fibrin-only control (Figure 3D).

Implantation of Human Cells Resulted in a Thicker Healing Tissue Bridge With Increased Cell Proliferation

The healing tissue that forms over the anterior surface of the patellar tendon defect (ie, anterior bridge) is the main healing response to this injury.^{7,9,10} Therefore, the thickness of this healing tissue was measured with collagen second harmonic generation signal from the multiphoton microscope to examine the robustness of the repair response. There was no statistically significant difference among the individual groups, including bursa, BMSCs, fibrin only, and defect only ($P > .05$). However, when the cell implant groups (bursa and BMSC) were combined and compared with the control groups (fibrin only and defect only), there was a statistically significant increase in the thickness of the healing bridge at all time points ($P < .05$) (Figure 4). In fact, there was nearly a 40- μ m (216%) increase in bridge thickness at 1 week.

Since tissue can grow by producing more cells (ie, increased proliferation) or more matrix (ie, increased matrix synthesis), the number of proliferating cells and matrix composition of the healing bridge were measured via IHC. EdU was injected 1 day before sacrifice to label

proliferating cells. The number of EdU+ cells was significantly higher in the cell implant group as compared with the control at 1 week (Figure 5C). Therefore, cell proliferation may contribute to the increased bridge thickness measured by collagen second harmonic generation imaging (Figure 4). The matrix composition was also measured within the healing tissue through antibodies for type I collagen, type II collagen, type IV collagen, and tenascin C. There were no apparent differences in the overall staining of these matrix proteins between any of the treatment groups (data not shown).

Cell Augmentation Did Not Restore Repaired Tendons to Native Properties

The structural and material properties of the repair tissue were compared among the treatment groups at each time point to determine the efficacy of the repairs. While each group yielded stronger repairs with time, the tensile testing did not display significant differences in structural or material properties among the treatment groups ($P > .05$). The cross-sectional areas were not different among the groups at each time point ($P > .05$). None of the tested groups was able to restore native properties (Table 1 and Appendix Figure A3, available online), as the native tendon had significantly higher ultimate load, stiffness, ultimate stress, and modulus at each time point ($P < .005$).

DISCUSSION

Two potential mechanisms may lead to improved repair outcomes after cell-based tendon repair: (1) the implanted cells directly participate in the repair process by synthesizing and assembling an organized extracellular matrix while self-renewing to contribute to long-term cell maintenance of the tissue, or (2) the implanted cells indirectly participate in the repair process by stimulating resident cells through cell-to-cell communication to mount a more robust repair response. This study aimed to determine whether bursa-derived cells or BMSCs from human patients participated directly in the repair through long-term engraftment into the host tissue or indirectly through stimulation of resident murine cells. The patellar tendon defect model used in this study heals via synthesis of a new healing tissue that forms a bridge over the anterior surface of the defect.⁷ Therefore, multiphoton microscopy was used to measure the long-term cell fate (up to 8 weeks after implantation) to visualize the entire repair tissue along the full length of the tendon. As compared with BMSCs, bursal cells displayed superior engraftment (Figure 2), with a significantly higher number of cells infiltrating the healing bridge and adjacent tendon struts at 2 and 5 weeks after implantation ($P < .05$). Not only were the engrafted cells (ie, cells in the anterior healing tissue bridge and adjacent tendon struts) higher in the bursa group, but the quantity of cells within the fibrin was also higher (Figure 2E), indicating improved survival. However, the number of F4/80+ macrophages that invaded the cell implants (Figure 3) was similar in both cell groups and no different from that of acellular fibrin control, suggesting that neither cell type stimulated a phagocytic response that could explain differences seen in cell survival (Figure 2E). In addition, the implantation of human cells yielded a significant increase ($P < .05$) in the thickness of the healing tissue bridge at all 4 time points (Figure 4), which could be at least partly attributed to an increase in cell proliferation at early stages of healing (Figure 5). These data suggest that the implantation of human cells stimulates the host murine cells to mount a more robust repair response in this repair model.

Clinical strategies for cell-based augmentation of tendon repair typically involve injection of cells derived from the donor in the operating room with minimally manipulated techniques.^{12,16} Some groups incorporate these cells into patient-derived matrices within the operating room as well. The most common sources of cells for these therapies are BMA²⁹ and platelet-rich plasma. The efficacy of these strategies is still up for debate, as the results have been inconsistent and are likely affected by several factors, including severity of tear, formulation of cell source, delivery method of cells, genetics of the patient, epigenetics of the patient, and so on. Therefore, developing a platform where these cell sources can be tested in a consistent and high-throughput manner is critical to advance the clinical field.

Immunodeficient mouse models, such as the NSG mice used in this study, are one of the most receptive models for analyzing long-term engraftment of human cells. Determining the mechanisms by which these donor cell populations influence repair in murine models may predict the performance of these cells in patients. In addition, certain aspects of the response, such as long-term tracking of donor cells, cannot be performed in human patients; therefore, animal models are needed. The results in the current study, where the donor cells appeared to stimulate the host cells to mount a more robust repair response but did not survive long term in the tissue, suggests that a similar mechanism may occur in clinical cell-based augmentations.

While the bursal cells displayed superior engraftment and survival over the BMSCs, the number of engrafted bursal cells that survived to 5 weeks after implantation was only 16% of 2-week levels (Figure 2, C–E), indicating that long-term survival for both cell types is limited. This limitation is not unique to this study, as several other groups showed similar reductions in implanted cell numbers in a variety of tendon repair models.^{2,24,27,31} These studies suggest that new cell sources, cell preparation methods, and/or delivery techniques are needed if long-term engraftment is desired. Determining whether the limited survival is due to cell autonomous traits or influences from the surrounding environment is important. For instance, the cell sources used, while displaying multipotency in vitro, may be too differentiated/mature once implanted and therefore cannot self-renew to produce a long-term cell population. Yet, the biological or mechanical cues within the tendon-healing environment may prevent the donor cells from surviving. Future studies in our laboratory will begin to investigate these mechanisms.

This study was not without limitations. The fibrin gel used to deliver the cells, while being the most common scaffold used for cell augmentation in rodents, appeared to not provide the necessary cues to the cells. A high percentage of cells within the fibrin gel did not elongate after implantation and did not synthesize a connected collagenous network that would transmit tensile loads throughout the gel (see collagen second harmonic generation signal in Appendix Video). Our laboratory is working to develop scaffolds in addition to surgical procedures where the material will be anchored to the ends of the tendon in such a fashion that tensile loads can be transferred throughout the scaffold to drive the cells to synthesize additional matrix and survive longer within the repair. Another limitation in this study was the biomechanical assessment. Removing the adjacent tendon struts consistently from sample to sample is quite difficult. Instead of relying on gross dissection where the margins of the defect are determined by discoloration of the healing tissue as compared with the adjacent struts, the method chosen in this study was to re-create the surgical procedure in

each limb by creating the defect margins with 2 No. 11 blades and then remove the adjacent struts as opposed to the central third. This method was flawed in that the defect width of healing tendons varied such that the region tested in the biomechanical test contained different amounts of adjacent struts from sample to sample, which is a confounding variable.

There is a pressing need to develop novel therapies to improve tendon repair, particularly for rotator cuff tendons. Cell-based therapies will likely be needed to augment the inadequate resident cell response. The subacromial bursa, while often removed during rotator cuff repair, may harbor a more suitable cell source for tendon repair than BMSCs, as bursa cells display superior engraftment and survival in tendon tissue. In addition, the subacromial bursa may be a more accessible cell source than BMA. However, more study is warranted to characterize the cell populations within this tissue and their efficacy in cell-based repair strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A Experiment Design

B Cell-Based Repair Procedure

Figure 1.

Experimental design and cell-based repair procedure. (A) The first set of surgical procedures involved bilateral comparisons between bursal cell and bone marrow stromal cell (BMSC) implants. The second set involved controls in which 1 limb received fibrin without cells and the other healed naturally. Mice were assigned to 1-, 2-, 5-, or 8-week time points for immunohistochemical (IHC) or biomechanics (Mech) analysis. Each limb was imaged on the multiphoton microscope before being processed for IHC or biomechanics. (B) Subacromial bursa and bone marrow aspirate (BMA) were acquired from 5 patients undergoing primary rotator cuff repair. The cells were expanded in culture and transduced with ubiquitin-mCherry lentiviral vector. At surgery, the cells were encapsulated in fibrin gel and implanted into patellar tendon defects of NOD scid gamma immunodeficient mice.

Figure 2.

Bursa-derived cells display superior engraftment with host tissue and increased survival as compared with bone marrow stromal cells (BMSCs). To quantify the engraftment of the donor cells over time, the multiphoton scans were (A) reoriented into the axial plane and (B) segmented into 3 regions of interest: anterior healing tissue bridge (green outline), adjacent tendon struts (light blue outline), and fibrin within the defect space (red outline). These regions of interest were interpolated along the entire defect length such that the entire volume of the wound site was included in the analysis. The bursal cells displayed superior engraftment with significant increases in (C) the anterior bridge at 2 weeks and (D) the adjacent struts at 2 and 5 weeks. (E) They also displayed increased survival with higher levels in the defect region at 5 weeks. Error bars indicate SD. * $P < .05$.

Figure 3.

Mature macrophage response (ie, F4/80 staining) did not correlate with differences in cell survival seen between bursal cells and bone marrow stromal cells (BMSCs). Since the bursal cells displayed improved survival within the fibrin over time, we tested whether the BMSCs stimulated an influx of mature macrophages (indicated by F4/80 staining in green) to remove the fibrin-cell construct. There was no difference in F4/80 concentration within the fibrin regions among the BMSC, bursa, or fibrin-only groups, however. (A) Full-length sagittal section of fibrin-only control group at 1 week (green, F4/80; red, Alexa Fluor 546–conjugated fibrin; blue, DAPI nuclei). (B) Inset of panel A (white box) demonstrating F4/80 staining within the fibrin (white arrows), with (C) corresponding toluidine blue–stained section (black arrows). (D) F4/80 staining density within regions where the fibrin was located in the defect. Values are presented as median, interquartile range, 95% CI, and outliers.

Figure 4.

Implantation of cells produced thicker healing tissue (bridge) on the anterior surface of the tendon. (A) The thickness of the anterior bridge was calculated along (B) the entire defect length by taking the mean thickness across a 100-µm width centered on the midline of the defect (yellow box). (C) The bridge thickness was significantly greater in the cell implant groups as compared with the controls at all time points ($P < .05$). However, there was no significant difference between the bursa and BMSC groups. Error bars indicate SD. * $P < .05$.

Figure 5.

Implantation of cells yielded more proliferation in healing tissue (bridge) on anterior surface of tendon. Mice were injected with EdU the day before sacrifice to measure cell proliferation. The number of proliferating cells (EdU+) were quantified within the anterior healing tissue (ie, bridge; white dashed region in A3/B3). (A1-A3) Limbs receiving human cells displayed higher number of proliferating cells than (B1-B3) control limbs (C) at 1 week after surgery. Red arrows indicate EdU+ cells within healing tissue. Implanted fibrin (F in A1/B1; red in B2) contained human cells (red in A2) at these time points. Hum Cells = ubiquitinmCherry+ implanted cells. $*P < .05$. Scale bars = 100 μ m. Values are presented as median, interquartile range, 95% CI, and outliers.

Structural and Material Properties of the Cell Implants vs No-Cell Controls and Age-Matched Native Patellar Tendons (Mean $\pm$ SD)**TABLE 1**

	Cross-sectional Area, mm ²	Ultimate Load, N	Stiffness, N/mm	Ultimate Stress, MPa	Modulus, MPa
Cell implant					
2 wk	0.18 $\pm$ 0.10	0.83 $\pm$ 0.52	2.13 $\pm$ 1.44	6.19 $\pm$ 6.16	48.82 $\pm$ 51.25
5 wk	0.18 $\pm$ 0.10	1.37 $\pm$ 0.56	4.27 $\pm$ 2.00	9.46 $\pm$ 5.57	91.29 $\pm$ 58.30
8 wk	0.15 $\pm$ 0.05	1.7 $\pm$ 0.63	4.50 $\pm$ 1.71	12.59 $\pm$ 5.81	99.76 $\pm$ 41.05
No-cell controls					
2 wk	0.21 $\pm$ 0.08	0.77 $\pm$ 0.44	1.97 $\pm$ 1.25	3.60 $\pm$ 1.33	28.06 $\pm$ 11.98
5 wk	0.18 $\pm$ 0.13	1.47 $\pm$ 0.89	3.48 $\pm$ 1.94	10.15 $\pm$ 7.25	80.11 $\pm$ 77.57
8 wk	0.23 $\pm$ 0.18	1.66 $\pm$ 0.70	4.56 $\pm$ 1.83	10.35 $\pm$ 8.19	88.40 $\pm$ 77.76
Native tendon	0.16 $\pm$ 0.06	3.27 $\pm$ 0.94	9.32 $\pm$ 3.59	22.57 $\pm$ 10.46	185.35 $\pm$ 77.45