Rapid Isolation of Human Stem Cells (Connective Tissue Progenitor Cells) From the Proximal Humerus During Arthroscopic Rotator Cuff Surgery

Augustus D. Mazzocca,* MS, MD, Mary Beth R. McCarthy, BS, David M. Chowaniec, BS, Mark P. Cote, PT, DPT, Robert A. Arciero, MD, and Hicham Drissi, PhD
From the University of Connecticut Health Center, Department of Orthopaedic Surgery, MARB Building, Farmington, Connecticut

Background: Bone-to-tendon healing in the shoulder can be unpredictable. Biologic augmentation, through the implementation of adult mesenchymal stem cells, may improve this healing process.

Purpose: The purpose of this study was to (1) arthroscopically obtain bone marrow aspirates from the proximal humerus during rotator cuff repair, (2) purify and concentrate the connective tissue progenitor cells (CTPs) in the operating room efficiently, and (3) confirm these are stem cells through their ability to differentiate into bone cells. We hypothesize that CTPs can be quickly and efficiently isolated from bone marrow during arthroscopic surgery and that these cells are capable of osteogenesis.

Study Design: Cohort study; Level of evidence, 3; and Descriptive laboratory study.

Methods: Bone marrow aspirates were harvested through the anchor tunnel of the humeral head during arthroscopic rotator cuff repair in 23 patients. Twenty-three matched controls were selected from a clinical registry to evaluate for increased incidence of impingement. Connective tissue progenitor cells were isolated using 2 accepted methods and compared with a novel, rapid method designed for use in the operating room. Osteogenic potential was assessed by cytochemical and molecular analysis.

Results: Reverse transcription polymerase chain reaction analysis and cellular staining confirmed the osteogenic potential of these CTPs. There was no statistical significant difference in the Single Assessment Numeric Evaluation score (aspirate, 86.3 ± 10.5; control, 83.6 ± 15.1; P = .54), range of motion measures (postoperative external rotation: aspirate, 65.0° ± 20.4°; control, 62.5° ± 17.1°; P = .67; postoperative forward elevation: aspirate, 163.0° ± 30.5°; control, 145.7° ± 41.4°; P = .12), or postoperative strength measures between groups (median, 5; range, 4-5 in the aspirate group compared with median, 5; range, 4-5 in the control group; P > .05).

Conclusion: Connective tissue progenitor cells can be safely and efficiently aspirated from the proximal humerus using the anchor tunnel created during arthroscopic rotator cuff surgery. These cells may play an important role in cell-based therapies involving rotator cuff repair.

Clinical Relevance: We have established a reliable, reproducible protocol for isolating CTPs in the operating room. These cells may have the potential to enhance the healing process after rotator cuff repair.

Keywords: stem cells; shoulder; rotator cuff; arthroscopy

Rotator cuff tears are one of the most common causes of pain and disability in the upper extremity and often require operative intervention. Although surgery is widely considered the standard of care, the failure rate after repair of large and massive rotator cuff tears reported in the literature is variable, ranging from 38% to 94%. Studies have shown that bone may play an integral part in the tendon-to-bone reinsertion. Advances have been made to maximize the ability to mechanically repair the rotator cuff, but revision surgery often reveals that the tendon has retracted away from the bone with the suture anchor, corresponding suture material, and knot in place, raising suspicion of a biologic healing problem.


Bone marrow (BM) is a viable source of stem cells, commonly called connective tissue progenitor cells (CTPs), which can be differentiated into bone, tendon, cartilage, and ligament. Connective tissue progenitor cells are characterized by their ability to undergo self-renewal, multilineage differentiation, and form terminally differentiated cells. Bone marrow represents the most commonly used and studied source of CTPs, which may have the potential to aid in the progression of healing tissues because of their pluripotency.

To use CTPs for healing enhancement, an efficient and safe method of obtaining BM during shoulder surgery needs to be developed. Connective tissue progenitor cells have been successfully isolated from the iliac crest and used for bone regeneration therapy. Although this method is well documented, it is not ideal to use the iliac crest as a source of BM during arthroscopic rotator cuff repair (RCR) as it adds an additional procedure and increases patient morbidity. The epiphysis of the proximal humerus is made up of mostly trabecular bone and is thought to be a rich source of hematopoietic cells. The proximal humerus may be a suitable site for aspirating BM as there is adequate exposure of the humeral head and greater tuberosity during arthroscopic RCR surgery, eliminating the need for an additional procedure.

The purposes of this study were to determine (1) if BM could be safely aspirated from the proximal humerus efficiently without an increase in patient morbidity and with no compromise in sterility during arthroscopic RCR, (2) that BM could be quickly and safely purified in the operating room (OR) to isolate a fraction rich in CTPs, and (3) if this fraction contained CTPs capable of osteogenesis, confirming the presence of stem cells. Our hypothesis was that BM can be safely obtained from the proximal humerus at the time of arthroscopic RCR surgery in an efficient manner without any compromise in sterility. Further, BM can be quickly purified in the OR to isolate a fraction rich in CTPs that are capable of osteogenesis.

**MATERIALS AND METHODS**

**Inclusion and Exclusion Criteria**

All patients undergoing arthroscopic RCR from a single surgeon (A.D.M.) were considered eligible for this study (institutional review board No. 06-577-1). Exclusion criteria included patients who were pregnant, prisoners, and those with a history of diabetes, hepatitis, human immunodeficiency virus, or AIDS, or patients who had been subjected to radiation. All recruited patients were mature individuals, 41 to 76 years of age, who had elected to undergo arthroscopic RCR. The patients read and signed a consent form before enrollment.

**Clinical Outcome and Morbidity**

Arthroscopic RCRs were performed in 89 patients by a single surgeon (A.D.M.) from September 2006 through June 2008. Of these 89 patients, 23 consented to the harvesting of BM as part of an investigative study examining various characteristics of CTPs from human BM. From the remaining 66 patients who underwent arthroscopic RCR, 23 matched controls were selected to evaluate for increased incidence of complication (institutional review board No. 08-067-3). These controls were selected from the same surgeon’s practice and matched for age, sex, and follow-up time to allow for accurate comparisons. Controls were selected by an independent reviewer who was blinded to all outcome data. The remaining 43 patients (44 repairs) who underwent arthroscopic RCR in this period but who had not undergone aspiration and were not appropriate for selection as controls were also evaluated.

The medical records for this study and controls were reviewed to determine the presence of surgical or postoperative complications. Surgical complications included events or technical difficulties that occurred intraoperatively that were directly related to the harvesting of BM (e.g., needle breaking off in humeral head, fracture as a result of needle punch, suture hole enlargement, puncture of the neurovascular structures of the axilla secondary to the needle penetrating through the humeral head). Postoperative complications included infection, reflex sympathetic dystrophy (RSD), deep venous thrombosis (DVT), septic arthritis, stiffness, wound irregularities, hematoma, abnormal pain defined as continued postoperative pain of more than 3 months, and delayed healing or failure of the repair or reconstruction. The Single Assessment Numeric Evaluation (SANE) score, postoperative range of motion (external rotation and forward elevation), and measure of strength were collected and compared to evaluate whether a difference in postoperative outcome existed between these 2 groups.

Forward elevation was measured actively and externally, and external rotation was measured passively with the arm at the side. Visual estimation was used to quantify degrees of motion. Visual estimation measurements of shoulder range of motion have demonstrated good intra- and interrater reliability. As stiffness after surgery was a concern, all range of motion measures were assessed bilaterally. Postoperative stiffness was defined as a difference of 20° or greater in forward elevation and or 10° or greater in external rotation, and was graded as either being present or absent. Strength was measured in a position of scaption with manual muscle testing based on the Medical Research Council Scale for Muscle Strength. This measure was collected bilaterally, with the uninvolved side serving as the reference index for strength grading.

**Collection of Bone Marrow Aspirate**

Bone marrow aspirate (BMA) was collected during arthroscopic RCR. All repairs were performed using PEEK (polyetheretherketone) 5.5-mm suture anchors and high-strength, nonabsorbable No. 2 suture (Arthrex, Inc, Naples, Florida). Repair type and anchor configuration were determined based on type of tear and quality of the tissue. Before anchor placement, a 14-gauge aspiration...
Figure 1. Bone marrow aspiration from the proximal humerus during arthroscopic rotator cuff repair.

needle (Arthrex) fitted with a 60-mL syringe containing 1 mL of 1000 U of preservative-free sodium heparin as an anticoagulant in 9 mL of saline was placed 25 mm into the medullary cortex at the bone cartilage junction of the footprint where the first suture anchor would be placed (Figure 1). To standardize the method of aspiration, the surgeon pulled back on the syringe to maximize suction for 60 seconds, allowing BM to flow into the syringe. The needle was removed and the anchor was placed directly into the tunnel created by the needle. The needle gauge was chosen to accommodate the 5,5-mm diameter suture anchor used in the RCR procedures. Aspiration of peripheral blood and air bubbles was minimized by performing the procedure under 40 mm Hg constant fluid pressure maintained by an arthroscopic fluid pump (Arthrex). A surgical technician carefully overlaid the BM onto a 17.5% sucrose gradient in a 5Q-mL conical tube (Fisher Sm' Agawam, Massachusetts). The tube was capped, passed off the surgical field to the circulating OR nurse, and centrifuged for 5 minutes at 205 g.

The fractionated top yellow layer was easily distinguished from the pink middle layer and was drawn up by the surgical technician using the same aspiration needle used to obtain the BM and taken to the laboratory for contamination testing. The process took approximately 10 minutes and did not impair the work flow in the OR. This protocol was reviewed by the university's Office of Research Safety and was found compliant with Occupational Safety and Health Administration standards.

Isolation of CTPs From BMA

A standardized isolation protocol was used for all BMA (Figure 2). The BMA was filtered through a 70-micron mesh to remove blood clots. Before plating each sample, the total number of nucleated cells per 1.0 mL of BMA was counted and recorded using a Coulter counter (Beckman Coulter, Inc, Fullerton, California). Cell viability was assessed for each isolation procedure by trypan-blue exclusion using a hemocytometer.

In an attempt to maximize patient outcome and minimize anesthetic and surgery times, we sought to develop a rapid method of isolation of CTPs. Two commonly used methods were compared with this new method. Each BMA sample was divided into 3 groups:

- **Isolation 1 (OR):** A new method, designed for use in OR, consisting of one 5-minute centrifugation at 1500rpm in which BMA was overlaid onto a 17.5% sucrose gradient in a 60-mL conical tube followed by extraction of CTPs in the fractionated layer.

- **Isolation 2 (Laboratory):** A commonly used laboratory method to isolate CTPs in which a 30-minute centrifugation at 1500 rpm is followed by a fractionated layer extraction of CTPs using a Histopaque gradient (Sigma-Aldrich, St Louis, Missouri).

- **Isolation 3 (Laboratory):** A standard laboratory method for the isolation of CTPs that involves plating whole BM for 24 hours, aspirating nonadherent cells, and expanding the attached cells for surgical reimplantation.

Cell Culture Conditions

Nucleated cells harvested from the fractionated layer in isolations 1 and 2 were counted and plated on 100-mm Primaria dishes (BD Laboratories, Franklin Lakes, New Jersey) at a concentration of 0.5 × 10^6 cells/9.6 cm² into control media containing phenol red free α-minimum essential medium (Invitrogen, Carlsbad, California), 10% fetal bovine serum (Atlanta Biologicals, Atlanta, Georgia), and 0.1% penicillin/streptomycin (Invitrogen). For isolation 3 (laboratory), cells were counted to normalized for plating density and plated under the same conditions as in isolation 1 and 2. To remove nonadherent cells in isolation 3 (laboratory), media was aspirated after 24 hours of incubation.

Figure 2. Experimental outline of the isolation protocol.
To ensure that sterility was maintained, all cultures were checked daily for contamination. For all assays, six 100-mm dishes were plated per patient and for all 3 isolation methods for a total of 18. This was repeated for each patient, making a total of 414 dishes.

Flow Cytometric Analysis

Fluorescence-activated cell sorting (FACS) was used to ensure that cells obtained from the proximal humerus had surface markers characteristic of stem cells. Cells were grown in control media, trypsinized in 0.25% trypsin/EDTA at confluence, rinsed, and centrifuged. The pellet was resuspended in staining-buffer containing 1% human serum, 1% bovine serum albumin, and 1% fetal bovine serum in phosphate-buffered saline. Cells were incubated with either phycoerythrin or fluorescein isothiocyanate antibodies, washed with staining buffer, and analyzed using a FACS Calibur (BD Biosciences, San Jose, California).

Phycoerythrin-conjugated mouse monoclonal anti-CD73 immunoglobulin G (IgG), anti-CD90 IgG, and fluorescein isothiocyanate-conjugated antimouse CD45 monoclonal IgG were obtained from BD Biosciences. All antibodies were reactive against human antigens. Testing with negative and positive controls confirmed the specificity of these antibodies.

Quantification of Osteogenic CTP

Eight or more cells in a cluster were counted and defined as CFUs. Those staining positive for ALP (90% or more in a CFU) were defined as CTP-AP. The ALP-positive cells in all groups were counted on day 7 to assess the number of osteogenic CTPs. Cells were fixed for 20 minutes in a 37% formaldehyde solution containing sodium citrate and acetic acid and incubated for 30 minutes in 0.2M Tris Buffer, pH 8.3, with AS-MX phosphate as a substrate and Fast blue as a stain (Sigma). The ALP-positive cells stained blue. The CTP cell concentration and prevalence were calculated with respect to the volume of BMA and the total number of nucleated cells.

Alkaline Phosphatase Staining

Evaluation of the osteogenic nature of CTPs giving rise to CFUs was performed with ALP staining of treated and untreated cells from isolation procedures 1 through 3 and was assessed at days 7, 14, and 21. Colonies expressing 95% to 100% ALP-positive cells were considered positive. Cells were fixed with an acetone/citrate/formalin solution using an ALP staining kit (Sigma), washed, and stained using Naphthol AS-BI phosphate as substrate, and counterstained with neutral red.

RNA Isolation and Quantitative Polymerase Chain Reaction

Gene expression assays were performed by culturing cells for 7 and 14 days in OSCM. The RNA was isolated using Trizol reagent (Invitrogen). One microgram of RNA was reverse-transcribed using Reverse Transcriptase II kit and Oligo(dT) (Invitrogen). The cDNA was used in polymerase chain reactions (PCRs) containing a specific human primer (see online Appendix 1 for this article at http://ajs.sagepub.com/supplemental/) and SYBR-Green PCR master mix (Applied Biosystems, Foster City, California). The PCR reactions were performed in triplicate; the threshold cycles were obtained using Applied Biosystems software. Glyceraldehyde 3-phosphate dehydrogenase served as the endogenous control.

Statistical Analysis

Patient outcomes data were analyzed using 2-sample equal variance t test and Wilcoxon rank sum test to explore differences between the aspirate and control groups. Cellular data were analyzed using the Student t test and analysis of variance. The α-level for all statistics was set at .05.

RESULTS

Clinical Outcomes and Morbidity

There were 11 women and 12 men in both the aspirate and control groups. The mean ages in the aspirate and control groups were 56 ± 8.7 and 56 ± 8.9 years, respectively (see online Appendix 2 for this article at http://ajs.sagepub.com/supplemental/). The mean time to follow-up was 10.6 ± 6.7 months in the aspirate group and 10.0 ± 6.2 months in the control group. There were no surgical complications in any group. There was no incidence of RSD, DVT, wound irregularities, hematoma, or septic arthritis (0%). There was 1 superficial wound infection in the control group (4%) compared with zero in the aspirate group (0%). There was no incidence of abnormal pain in the aspirate group (0%) compared with 3 in the control group (13%). Two of these 3 patients had structural failures on postoperative MRI. There were 3 incidences of delayed or failed healing in the aspirate group (13%)
compared with 3 incidences in the control group (13%). Of the 3 incidences in the aspirate group, the first exhibited an avulsion fracture of the greater tuberosity on radiograph at postoperative day 9. Of note, there was a pre-existing tuberosity fracture seen on preoperative radiographs that appeared to heal. The second incidence exhibited a partial-thickness rotator cuff tear on the operative side on MRI at 9 months after surgery. The third incidence was considered a functional failure because MRI at 6 months after surgery revealed an intact rotator cuff. In the control group, all 3 incidences of delayed or failed healing exhibited structural failure of the RCR on postoperative MRI. There were 2 incidences of stiffness in the aspirate group (8%) compared with 1 incident in the control group (4%). All incidences of stiffness were attributed to postoperative adhesive capsulitis.

There were no statistically significant differences in the SANE score (aspirate, 86.3 ± 10.5; control, 89.6 ± 15.1; P = .54) or postoperative forward elevation (aspirate, 163.0° ± 30.6°; control, 146.7° ± 41.4°; P = .12) and external rotation between the 2 groups (P > .05). The mean postoperative external rotation at the time of follow-up was 65.0° ± 20.4° in the aspirate group and 62.5° ± 17.1° in the control group (P = .67). There was no significant difference in postoperative strength between groups (median, 5; range, 4-5 in the aspirate group compared with median, 5; range, 4-5 in the control group; P > .05).

The remaining 43 patients (44 RCRs) were also evaluated to ensure adequate examination of morbidity. There were 16 women and 27 men with a mean age of 52.0 ± 9.5 years. The mean time to follow-up was 4.6 ± 2.9 months. There was no incidence of RSD, DVT, wound irregularities, or septic arthritis (0%). There were 5 instances of abnormal pain (12%), 7 instances of delayed or failed healing (16%), 3 instances of stiffness (7%), all of which were exhibited by postoperative adhesive capsulitis, and 1 instance of hematoma (2%) and 5 instances of abnormal pain (9%), of which 4 patients had failed healing and 1 had adhesive capsulitis. The mean SANE score at the time of follow-up was 77.5 ± 11.0. The mean forward elevation and external rotation at the time of follow-up were 147.9° ± 48.1° and 57.1° ± 33.8°, respectively. The median strength grade was 4.5 of 5.

Collection of BMA

Bone marrow was successfully aspirated in all 23 cases. The average volume of BM after 60 seconds of aspiration was 23.5 ± 7.5 mL (see online Appendix 2 for this article at http://ajs.sagepub.com/supplemental/). Although it has been shown that a volume greater than 2 mL of BM contains peripheral blood, it has also been shown that no one volume is optimum for the achievement of all of the possible goals of aspiration.57 The intent of this study was to obtain a volume of BM for easier manipulation in the OR. The goal was to make the procedure easy for the staff to implement during arthroscopic surgery without any compromise in speed, sterility, or OR standards. No contamination was observed in any of the aspirates.

The intent of this study was to obtain a volume of BM for easier manipulation in the OR. The goal was to make the procedure easy for the staff to implement during arthroscopic surgery without any compromise in speed, sterility, or OR standards. No contamination was observed in any of the aspirates.

Figure 3. A, colony-forming units (indicated by arrows) after 48 hours cultured with control media. B, connective tissue progenitor cells grown for 7 days in control media align in a well-organized, parallel fashion. This observation is consistent with parental bone marrow stromal cells as described by Wang et al.48 C, connective tissue progenitor cells cultured for 21 to 28 days in osteogenic media resulted in morphologic transformation from long spindle-shaped cells to a cuboidal shape, indicative of an osteoblastic morphology (magnification ×10).

Cell Morphology

After 7 days the attached cells from all isolation procedures produced CFUs, which proliferated quickly (Figure 3A). By days 21 to 28 the colonies grown in control media had formed a uniform multicellular layer that had spindle-shaped cells, which are comparable with progenitor cells found throughout the literature (Figure 3B).58,30,39 Cells grown in OSCM resulted in a morphologic transformation from long spindle-shaped cells into a cuboidal-shaped cells, indicative of bone cells (Figure 3C).

FACS Analysis

The FACS analysis showed that CTPs grown in control media were positive for CD73 and CD90 and simultaneously negative for CD45 (Figure 4B-D), which is characteristic of adult mesenchymal stem cells.

Prevalence and Concentration of CTPs

The number of ALP-positive CFUs in BMA is determined by 2 variables: the number of nucleated cells and the prevalence of ALP-positive CFUs among the nucleated cells.57 Before plating the cells, each BMA was counted to obtain nucleated cell numbers and was normalized to a specific plating density (0.5 × 10⁶ cells per well). Cell viability as assessed by trypan blue exclusion for isolation procedures 1, 2, and 3 was 90 ± 1.2%, 94% ± 1.4%, and 89% ± 2.5%, respectively.
Figure 4. Flow cytometric analysis (FACS) of connective tissue progenitor cells. A, FACS analysis for a double-negative control: (-) phycoerythrin and (-) fluorescein isothiocyanate. B, the connective tissue progenitor cells (CTPs) were stained with fluorescein isothiocyanate-conjugated antimouse CD45 monoclonal immunoglobulin G, as a negative control. C and D, CTPs stained with CD73 and CD45 antibodies (C) and CD90 and CD45 antibodies (D). The average percentage sensitive cells for each marker in 6 CTP populations is listed in each panel.

determine the prevalence and concentration of CTPs capable of osteogenesis in the BMA, cells from each patient were plated into OSGM. On day 7 cells were stained for ALP expression, and colonies with 8 or more cells staining positive for ALP were counted (CTP-AP). The prevalence of CTPs was significantly increased for isolation 1 (OR) compared with isolations 2 and 3 (18.2 ± 0.95, 11.1 ± 1.4, and 7.2 ± 1.1 CTP-AP/million nucleated cells, respectively (P < .05) (Figure 5A). Furthermore, although variability with respect to prevalence of CTPs was noted for all patients, isolation 1 consistently showed a higher prevalence of CTPs compared with isolations 2 and 3 (Figure 6).

Differentiation of Isolated CTPs

Bone sialoprotein (BSP), ALP, and type I collagen are established bone markers. Cells cultured in osteogenic differentiation media exhibited up-regulation of BSP, ALP, and type I collagen transcription levels between 7 and 14 days in culture. Cells harvested from the 5- and 30-minute isolations expressed significantly higher transcription levels of ALP and type I collagen as compared with the 24-hour plated whole marrow after 7 and 14 days in culture (P < .05) (Figure 7A). At day 14, type I collagen transcription was significantly higher in the 5-minute isolation than in the 30-minute and whole marrow isolations (Figure 7B). The BSP transcription levels were significantly increased at day 14 in the 5-minute isolation compared with isolations 2 and 3 (Figure 7C).

Alkaline Phosphatase Staining

Gene expression profiles were complemented by ALP staining of the cells after 7, 14, and 21 days in culture. Alkaline phosphatase staining is similar to ALP gene expression, with increased positive colonies at days 14 and 21 (Figure 8). Interestingly, the 30-minute isolation method showed
Figure 6. Prevalence of connective tissue progenitor cells in individual patients. The CTP concentration/1.0 mL of BMA was also significantly increased in isolation 1 (OR) (222.6 ± 19.3) compared with the 30-minute fractionated cells (150 ± 20.0) and the 24-hour plated whole marrow samples (102.0 ± 15.0, P < .05) (Figure 5B). For isolations 1 and 2, the number of nucleated cells before plating was 12.1 ± 0.86 × 10⁶ cells/mL and 7.9 ± 0.80 × 10⁶ cells/mL of BMA (Figure 5C).

less ALP exhibiting cells than the 5-minute and 24-hour isolations.

DISCUSSION

In cases of massive RCR, bone-to-tendon healing is unpredictable. Biologic augmentation may improve this healing process. Early evidence indicates that adult stem cells are capable of regenerating diseased tissue. The first purpose of this study was to determine if BM could be aspirated from the proximal humerus efficiently and without an increase in patient morbidity. We found no significant increases in the incidence of postoperative complications. To ensure we had adequately examined morbidity, we evaluated all remaining patients who underwent arthroscopic RCR in the study period. These were patients who did not undergo aspiration and were not matched to the control cohort. The results of this comparison did not demonstrate any significant increase in complications, further supporting the hypothesis that aspiration from the proximal humerus does not increase morbidity.

For consistency and reproducible surgical technique, the surgeon pulled back on the syringe to maximize suction for 60 seconds. This method yielded a mean of 23.5 mL of BM. Although it has been well documented that a volume greater than 2 mL of BM contains peripheral blood, a sample this small is difficult to manipulate in the real-time setting of the OR. The intent of this study was to develop a protocol for purifying BM that was safe, simple, reproducible, and efficient (isolation 1, OR). To evaluate this purification method, we chose 2 standard methods (laboratory isolation 2 and 3) for comparison. Isolation 2 (laboratory) required 30 minutes of centrifugation whereas isolation 3 (laboratory) required plating of BM for a 24-hour period. While both of these methods have been shown to reproducibly isolate CTPs, the time involved in each is prohibitive to the surgeon. Nucleated cell counts from isolation 1 (OR) compared with isolation 2 (laboratory) were greater, indicating that 5 minutes of centrifugation was adequate to produce a fraction rich in cells capable of forming colonies. The number of nucleated cells was determined to be 12.1 ± 0.86 cells/mL, which is comparable with nucleated cell counts observed in the literature for the vertebral body and iliac crest. Further, untreated cells from all 3 isolation procedures produced spindle-shaped CFUs indicative of CTPs and were positive for cell surface markers characteristic of adult mesenchymal stem cells as shown by FACS analysis. By day 28, treated cells had formed a uniform multicellular layer that had a cobblestone morphologic appearance indicative of bone cells.

This study used a sucrose gradient based on its availability in the OR and its ease of sterility. The sucrose gradient was made to mimic the density of the
Stem Cells for Healing Enhancement of Rotator Cuff Surgery

**Figure 7.** Osteogenic potential of connective tissue progenitor cells isolated by various methods. Connective tissue progenitor cells cultured in osteogenic media exhibited significantly higher transcription levels of the osteogenic markers alkaline phosphatase (ALP), bone sialoprotein, and type I collagen after 14 days in culture. Five-minute fractionation provided a significantly higher expression of ALP mRNA levels than the 30-minute fractionated samples and the 24-hour plated marrow after 2 weeks in culture (A). After 2 weeks in culture, type I collagen and bone sialoprotein transcription levels were significantly higher in the 5-minute isolation method compared with the 30-minute and 24-hour isolation methods (B and C). Stars represent significance between isolation methods (P < .05).

Figure 8. Alkaline phosphatase (ALP) staining for the osteogenic marker ALP at 7, 14, and 21 days shows increased positive colonies after 14 and 21 days in culture. Interestingly, however, cells harvested from the 30-minute fractionation exhibited less ALP staining than the cells adhering for 24 hours after 14 days in culture.

exclusion demonstrated a mean cell viability of 95% after the use of a sucrose gradient, which was not statistically different from the other isolation procedures, further supporting its clinical applicability.

The third purpose of this study was to determine if the fractionated layer contained CTPs that could be purified and differentiated into bone. Bone was chosen for its involvement in the tendon-to-bone reinsertion during massive RCRs and the large amount of literature supporting the differentiation of CTPs into osteoblasts. The osteogenic potential of marrow-derived CTPs is determined by the occurrence of ALP-positive CFU among nucleated cells. Alkaline phosphatase is a glycoprotein that has a high activity in mineralizing bone, where it is mainly localized in the plasma membrane of osteoblastic cells. After 7 days of treatment in osteogenic media, cells demonstrated the formation of ALP-positive CFUs from each of the isolation procedures. The number of CFUs expressing ALP per one million nucleated cells after a 5-minute isolation was significantly higher than cells isolated from a 30-minute centrifugation, suggesting that the proximal humerus is a suitable site for the isolation of CTPs.

Gene expression was examined to further determine the osteogenic potential of these CTPs. Before gene expression analysis, equal amounts of RNA were used to normalize for cell density. Glycoproteins BSP, ALP, and type I collagen gene expression levels are up-regulated in an osteoblastic phenotype. At low concentrations, dexamethasone has been shown to induce mRNA levels of osteoblastic markers in BM-derived CTPs. Both 5- and 30-minute isolation methods produced higher transcription levels of ALP,

Figure 7.

**Figure 8.**
type I collagen, and BSP between 7 and 14 days. In addition, these 2 isolation techniques produced significantly higher transcription levels of ALP, type I collagen, and BSP at 14 days compared with the 24-hour isolation. This gene expression profile was complemented with positive ALP staining of these cells after 7 and 14 days in culture. These results show that CTPs isolated using a 5-minute centrifugation exhibit osteogenic potential similar to other accepted isolation procedures (isolations 2 and 3). A limitation of this study is that CTPs were only differentiated into an osteogenic lineage. Differentiation into other mesenchymal cells would have strengthened this study. Our intention was to develop a method of extracting and purifying BM to give the orthopaedic surgeon a simple, safe, and efficient way to isolate CTP during arthroscopic RCR surgery. The proposed method of aspiration did not result in an increase in complications. The BMA was successfully purified in the OR, with no resulting increase in operation time, and afractionated layer rich with CTP was successfully obtained. Furthermore, these cells demonstrated osteogenic potential evidenced by differentiation into osteoblasts. The results of this study may serve as an initial step toward enhancing the healing process in the surgical repair of massive rotator cuff tears.

REFERENCES


For reprints and permission queries, please visit SAGE's Web site at http://www.sagepub.com/journalsPermissions.nav
A Biomechanical Evaluation of Ulnar Collateral Ligament Reconstruction Using a Novel Technique for Ulnar-Sided Fixation

Robert J. Morgan,* † MD, James S. Starman,‡ MD, Nahir A. Habet,* MS, Richard D. Peindl,* PhD, Larry S. Bankston Jr,§ MD, Donald D. D’Alessandro,* MD, Patrick M. Connor,§ MD, and James E. Fleischli,* MD

From *Resurgens Orthopaedics, Atlanta, Georgia, ‡Carolinas Medical Center, Charlotte, North Carolina, §Baton Rouge Orthopaedic Clinic, Baton Rouge, Louisiana, and *OrthoCarolina, Charlotte, North Carolina

Background: Techniques for ulnar collateral ligament (UCL) reconstruction have evolved since its original description.

Hypothesis: Ulnar collateral ligament reconstruction using the ZipLoop for ulnar-sided fixation, as combined with the humeral docking technique supplemented with an interference screw, will restore valgus stability similar to that of the Jobe technique and the native ligament.

Study Design: Controlled laboratory study.

Methods: Kinematic testing was performed on 8 matched pairs of cadaver elbows with an electromagnetic tracking system through an arc of motion for the intact, disrupted, and reconstructed states of the UCL in an unloaded and loaded condition. From each pair, the docking technique using the ZipLoop for ulnar fixation and humeral docking technique supplemented with an interference screw and the traditional Jobe technique were performed with matched gracilis allograft tendons. After kinematic testing, both reconstruction groups were tested to failure at 70° of flexion.

Results: Kinematic results for the unloaded condition showed that both reconstruction techniques significantly overcorrected (less valgus angulation) the specimens between 40° and 120° of flexion when compared with the intact ligament (all P values < .027). Under loaded conditions, the ulnar trajectories for both reconstruction techniques exhibited significantly greater valgus angulation (undercorrection) at 20° of flexion (Jobe, P = .0084; ZipLoop, P = .0289) when compared with the intact ligament but were not significantly different over the remaining arc of motion. Failure testing resulted in no significant statistical difference between the 2 reconstruction groups. Failure testing demonstrated that humeral tunnel egress, midsubstance elongation, and ulnar tunnel egress of the ligament were similar between the reconstruction techniques.

Conclusion: The docking technique using the ZipLoop for ulnar-sided fixation is biomechanically equivalent to the Jobe technique for UCL reconstruction. Both reconstruction techniques restore valgus stability similar to that of the native UCL ligament.

Clinical Relevance: This modification in the docking technique restores elbow kinematics while eliminating the risk of ulnar bone bridge fracture, and it allows for retensioning of the graft after cortical fixation.

Keywords: ulnar collateral ligament; biomechanics; ulnar fixation; ulnar collateral ligament reconstruction

The ulnar collateral ligament (UCL) is an important primary valgus stabilizer in the elbow joint. Injury of the UCL is most commonly associated with overhead throwing activities in elite-level athletes, but it is seen in other activities and levels of competition. Tears of the UCL may result in valgus instability, chronic pain, and an inability to continue participation in high-level athletics. The native UCL is composed of 3 bands of tissue: anterior, transverse, and posterior. The anterior band originates on the anteroinferior surface of the medial epicondyle and inserts into the sublime tubercle of the ulna; it contributes the most to valgus stability. Biomechanical studies and clinical experience have demonstrated that anterior bundle reconstruction provides superior results when compared with those of the primary repair of the torn ligament. In 1986, Jobe et al described the first successful technique for reconstruction of the UCL, using 1 ulnar humeral tunnels with a figure-of-8 graft configuration.