



Augmenting Suture Tape Used in Rotator Cuff Surgery With Magnesium Increases In Vitro Cellular Adhesion of Human Subacromial Bursal Tissue

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Purpose: To evaluate the effect of magnesium on cellular adhesion and proliferation of human subacromial bursal tissue (SBT), osteoblasts, and tenocytes on nonabsorbable suture tape commonly used in rotator cuff surgery. **Methods:** Human SBT cells, primary human osteoblasts (HOBs), and primary human tenocytes were isolated from tissue samples and cultured in growth media. Commercially available collagen-coated nonabsorbable suture tape was cut into one-inch pieces, placed into 48-well culture dishes, sterilized under ultraviolet light, and treated with (+) or without (–) magnesium. For the (+) magnesium group, a one-time dose of 5 mM sterile magnesium chloride was added. Subsequently, cells were plated at a density of 20,000 cells/cm². For each cell source (SBT, HOBs, tenocytes) cellular proliferation and adhesion assays on suture tape treated (+) or (–) magnesium were performed. **Results:** SBT, HOBs, and tenocytes each demonstrated the ability to adhere and proliferate on suture tape. Augmenting suture tape with magnesium resulted in a significantly increased cellular adhesion of SBT compared with nonaugmented sutures ($P = .001$), whereas no significant differences were observed for HOBs ($P = .081$) and tenocytes ($P = .907$). Augmentation with magnesium demonstrated no significant difference in cellular proliferation of SBT ($P = .856$), HOBs ($P = .672$), and tenocytes ($P = .251$) compared with nonaugmented sutures. **Conclusions:** SBT, osteoblasts, and tenocytes each demonstrated the ability to adhere and proliferate on suture tape. In addition, augmenting the suture with magnesium resulted in a significantly increased cellular adhesion of SBT compared with nonaugmented sutures, whereas no significant differences were observed for osteoblasts and tenocytes. Further, magnesium did not impair the proliferative activity of SBT, osteoblasts, and tenocytes on suture tape used in rotator cuff surgery. **Clinical Relevance:** Modifying the surface of the suture used for repair with application of magnesium may be an inexpensive and technically feasible option to improve the use of SBT for biologic augmentation of rotator cuff repair.

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Despite advances in surgical techniques along with improved suture materials, high rates of retears of the repaired rotator cuff tendon remain a major concern, often correlating with poor postoperative outcomes.¹⁻³ Although the complexity of the healing process is not yet fully understood, both osteoblasts and tenocytes have been reported to be important for sufficient bone–entheses healing.^{4,5} Further, recent in vitro studies suggested human subacromial bursal tissue (SBT) as an easily accessible, inexpensive, and viable biologic augment for arthroscopic rotator cuff repair in an attempt to support the endogenous healing potential of the repaired tendon.⁶⁻¹³

Previous in vitro studies demonstrated that osteoblasts and tenocytes have the ability to adhere and proliferate on suture material used in rotator cuff surgery, which may aid in the tendon-to-bone incorporation process critical for rotator cuff healing.^{4,14}

However, the adhesive and proliferative capacity of SBT on suture material ubiquitously used in rotator cuff repair remains unknown. If suture material used for rotator cuff repair could be biologically augmented, it may enhance tendon healing along with providing biomechanical stability. By using the suture as a scaffold, this may facilitate retaining the cells being essential for rotator cuff healing at the targeted zone of repaired tissue.

Consequently, maximizing the amount of retained cells at the tear site may be of great clinical importance. In a rabbit model of meniscal repair, Zhang et al.¹⁵ recently observed that magnesium promoted the adhesion and migration of synovial fluid-derived mesenchymal stem cells (MSCs) at the repair site. Similarly, magnesium was found to enhance adherence of human synovial MSCs to osteochondral defects.¹⁶ Further, magnesium was reported to promote the proliferation of bone marrow-derived MSCs.¹⁷ While these previous studies indicate the good biocompatibility of magnesium and its potential impact on cellular activity of different cell types, its effect on the adhesive and proliferative capacity of cell sources being critical for rotator cuff healing remains largely unknown.

The purpose of the study was to evaluate the effect of magnesium on cellular adhesion and proliferation of human SBT, osteoblasts, and tenocytes on nonabsorbable suture tape commonly used in rotator cuff surgery. It was hypothesized that augmenting suture tape with magnesium would significantly increase cellular adhesion while maintaining similar proliferative potential.

Methods

Discarded tendon and bone specimens were collected at the University of Connecticut Health Center (institutional review board No. 10-204-2). Primary human osteoblasts (HOBs) were prepared from bone fragments obtained during total knee arthroplasty, whereas primary human tenocytes were isolated from biceps tendon samples. Human SBT was obtained during arthroscopic rotator cuff repair. Only samples of healthy male donors aged between 30 and 60 years were used.

For each cell source (SBT, HOBs, tenocytes) cellular proliferation and adhesion assays on suture tape treated with (+) or without (−) a one-time dose of magnesium were performed. Consequently, 4 pieces of suture per cell source (SBT, HOB, tenocytes) and treatment group ([+] or [−] administration of magnesium) were evaluated for each assay, resulting in a total of 24 suture pieces per assay in each patient.

Cell Isolation and Culture

Tendon cultures were prepared as previously described.^{4,14} Primary human tenocytes were obtained during biceps tenodesis procedures from 3 discarded long head of the biceps tendon samples (mean age:

40.3 ± 3.6 years) In brief, tendon samples were cut into small pieces, digested with 2 mg/mL collagenase P (Sigma-Aldrich, St Louis, MO) for 2.5 hours at 37°C, pelleted using a centrifuge, resuspended and plated in Primaria culture dishes (Fisher Scientific, Agawam, MA) with growth medium containing Dulbecco's Modified Eagle Medium, 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL penicillin streptomycin.^{4,14}

HOBs were prepared from 3 discarded human bone samples (mean age of patients 57.3 ± 1.5 years) obtained during total knee arthroplasty. In brief, bone specimens of the distal femur were minced and cultured in Primaria culture dishes with growth medium containing Dulbecco's Modified Eagle Medium/Ham's F-12 medium, 10% fetal bovine serum, and penicillin streptomycin for 2 weeks to allow for osteoblast outgrowth.^{4,14} After 2 weeks in culture, residual bone chips were removed from the culture dish and cells were grown until confluent.^{4,14}

SBT of 3 patients (mean age: 47.0 ± 4.5 years) was collected from over the rotator cuff tendon using an arthroscopic grasper device and placed into sterile 3-mL syringes, according to a previously published technique.^{10-12,18,19} Each sample was immediately transported from the operating room to a laminar flow hood for processing. The SBT sample of each patient was digested in 2 mg/mL collagenase P in alpha Minimum Essential Medium (α-MEM, Gibco by Life Technologies, Grand Island, NY) at 37°C. After 2.5 hours, the digested cells were filtered through a 70-µm cell strainer (Fisher Scientific, Pittsburgh, PA), the remaining tissue was discarded. Cells were pelleted using a centrifuge, resuspended and plated in Primaria culture dishes in growth culture medium, containing α MEM, 10% FBS, and 0.1% PS.¹⁰

Tenocytes, HOBs, and SBT were each cultured in growth media at 37°C with 5% CO₂ until reaching confluence.^{4,10,14}

Preparation of Suture Material

Commercially available collagen-coated nonabsorbable suture tape (FiberTape; Arthrex, Naples, FL) was used. Sutures were cut into one-inch pieces and placed into Primaria 48-well culture dishes (Fisher Scientific, Agawam, MA). According to a previous study, the sutures were held in place at the bottom of each well with a sterile inert aluminum metal mesh (1 cm × 1 cm) and were sterilized under ultraviolet light for 30 minutes before cell plating.⁴ For the (+) magnesium group, a one-time dose of 5 mM sterile magnesium chloride was added to each piece of suture and was allowed to absorb and dry onto the suture material at room temperature for approximately 20 minutes under the laminar flow hood.¹⁶ Magnesium is an endogenous trace element, which is approved by the Food and Drug

Administration (FDA) for human use. Further, it is ubiquitously available and can easily be sterilized for the use in the operating room.

Cell Plating

To define and ensure correct plating density, cells were trypsinized with 0.5 mL of 0.01% trypsin-0.04% ethylenediaminetetraacetic acid for 15 minutes at 37°C and counted using a Coulter counter (Coulter Electronics, Hialeah, FL).⁴ Subsequently, cells were plated at a density of 20,000 cells/cm² into 48-well Falcon plates containing the suture material.⁴ Cells were then cultured according to the required time for each assay.

Cellular Adhesion Assay

To measure the ability of the cells to adhere to the different substrates, adhesion assays were performed according to a previously published method.^{4,20} All sutures were subjected to the same adhesion protocol for analysis, where cells were added to the suture material for 24 hours before counting. At the end of this incubation period the mesh was discarded, and the sutures were transferred into empty clean 48-well plates. Sutures were then rinsed 3 times with 1X phosphate-buffered saline to remove nonadherent cells. This step was critical to ensure that only cells that actually adhered to the suture material were assayed. Cells attached to the suture were then released with 0.5 mL of 0.01% trypsin-0.04% ethylenediaminetetraacetic acid for 15 minutes at 37°C and counted in 9.5 mL of 0.9% saline solution, using a Coulter counter (Coulter Electronics, Hialeah, FL).^{4,20} Counting was performed three times for each sample to account for accuracy.²⁰ Suture pieces without any cells added were used as controls.⁴

Proliferation Assay

The ability of the cells to proliferate on the suture material was determined by an XTT (2,3-bis (2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) assay (Roche Diagnostics, Mannheim, Germany) as previously described.^{10,21} All sutures were subjected to the same proliferation protocol for analysis, where cells were added to the suture material for 48 hours before performing the proliferation assay. After 40 hours in culture, wells were aspirated, the mesh was discarded, and the suture was transferred into clean 48-well plates. Each new well was supplemented with 300 µL of growth media and 150 µL (0.3mg/mL) of XTT labeling mixture (0.1 mL electron coupling reagent/5 mL XTT labeling reagent), followed by incubation incubated at 37°C for 8 hours. At the end of the incubation period, a 100-µL aliquot was removed from each well and absorbance was measured using an automated plate

reader (BIO Tek, Bad Friedrichshall, Germany) at 450 nm with a reference wavelength of 650 nm.^{10,21} This assay is based on the cleavage of the tetrazolium salt XTT to a soluble formazan salt by mitochondrial dehydrogenases only being active in metabolically intact cells. As the increase in formazan formed is measured by the absorbance, this directly correlates to the number of viable cells within the sample. Suture pieces without any cells added were used as controls.⁴

Statistical Analysis

Data were summarized with mean and standard deviation. Differences in cellular adhesion and proliferation between treatment groups ([+] and [-] magnesium) were assessed using mixed effects linear regression. The distributions of the model residuals were examined to ensure large deviations from normality were not present. Pairwise comparisons of marginal mean values were carried out with adjustment for multiple comparisons using Bonferroni's method. An alpha level of 0.05 was set for all comparisons. All statistical analysis was performed using Stata 15.1 (StataCorp 2017, Stata Statistical Software: Release 15; StataCorp LP, College Station, TX).

Results

Cellular Adhesion

SBT, HOBs, and tenocytes each demonstrated the ability to adhere to suture tape. Augmenting suture tape with magnesium resulted in a significantly increased cellular adhesion of SBT compared with nonaugmented sutures ($P = .001$). However, cellular adhesion of HOBs ($P = .081$) and tenocytes ($P = .907$) was found to be similar when comparing sutures with and without augmentation of magnesium (Fig 1 and Table 1).

Cellular Proliferation

SBT, HOBs, and tenocytes each demonstrated the ability to proliferate on suture tape. Augmentation with magnesium demonstrated no significant difference in cellular proliferation of SBT ($P = 0.856$), HOBs ($P = 0.672$), and tenocytes ($P = 0.251$) on suture tape when compared with nonaugmented sutures (Fig 2 and Table 2).

Discussion

The most important finding of the present study was that SBT, HOBs, and tenocytes each demonstrated the ability to adhere and proliferate on suture tape. In addition, augmenting the suture with magnesium resulted in a significantly increased cellular adhesion of SBT compared with nonaugmented sutures, whereas no significant difference was observed for osteoblasts and tenocytes. Further, magnesium did not impair the

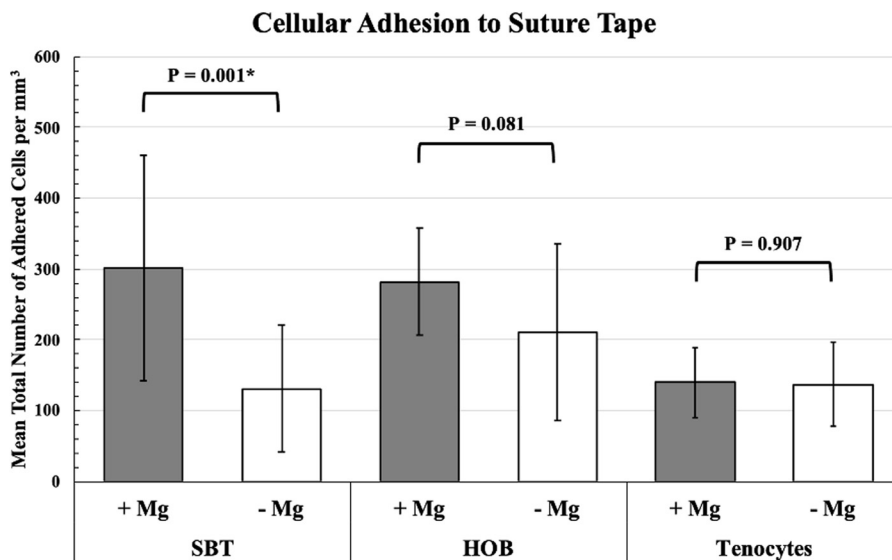


Fig 1. Cellular adhesion (total number of adhered cells per mm³) to suture material for SBT, HOB, and tenocytes treated with (+) and without (-) Mg. *Indicates statistical significance. (HOB, human osteoblast; Mg, magnesium, SBT; subacromial bursal tissue.)

proliferative activity of SBT, osteoblasts, and tenocytes on suture tape commonly used in rotator cuff surgery. As magnesium is an endogenous, ubiquitously available trace element being approved by the FDA for human use, it may be feasible to optimize the use of subacromial bursa for biologic augmentation of rotator cuff repair. Additionally, suture material represents a foreign body, which is placed directly into a vulnerable area of decreased vascularity during repair.²² Consequently, modifying the surface of the suture used for repair, to optimize adhesion and proliferation of cells involved in the healing process, may promote tissue recovery.

Although the complexity of the healing process is yet to be fully understood, both osteoblasts and tenocytes have been reported to be important for sufficient bone–enthesis healing.^{4,5} Further, previous in vitro studies demonstrated that osteoblasts and tenocytes

have the ability to adhere and proliferate on suture material used in rotator cuff surgery.^{4,14} Similarly, the present study found that osteoblasts and tenocytes were able to adhere to and proliferate on suture tape, which may aid in the tendon-to-bone incorporation process critical for rotator cuff healing. Further, maximizing the amount of retained cells directly at the tear site may be of great importance to create a sufficient healing environment, which may be achieved by improving the biologic acceptance of suture material.

In the present study, the surface of the suture tape was modified by augmenting it with magnesium, as previous studies indicated its good biocompatibility along with a beneficial impact on cellular activity of different cell types.^{15-17,23,24} In a rabbit model of meniscal repair, magnesium was found to promote the adhesion and migration of synovial fluid–derived MSCs at the repair site.¹⁵ Similarly, magnesium has been reported to enhance adherence of human synovial MSCs to osteochondral defects via integrins, which are cell surface receptors mediating cell adhesion to extracellular matrix, with collagen being recognized as one of the most abundant extracellular matrix proteins.^{4,16} Interestingly, the present study found that augmenting suture tape with magnesium only resulted in a significantly increased cellular adhesion of SBT compared with nonaugmented sutures, whereas no significant differences were observed for osteoblasts and tenocytes. Further, magnesium had no influence on the proliferative activity of SBT, osteoblasts, and tenocytes on suture tape, which is in contrast to previous findings that magnesium promoted the proliferative capacity of bone marrow–derived MSCs.¹⁷ However, the ability of SBT to adhere and proliferate on suture tape as well as the possibility of stimulating its cellular adhesion with

Table 1. Cellular Adhesion (Total Number of Adhered Cells per mm³) to Suture Tape for SBT, HOB, and Tenocytes Treated With (+) and Without (-) Mg

	Mean	SD	P Value
HOB			
(+) Mg	281.7	75.8	.081
(-) Mg	211.3	124.7	
SBT			
(+) Mg	301.0	158.6	.001*
(-) Mg	131.0	90.3	
Tenocytes			
(+) Mg	139.3	49.3	.907
(-) Mg	136.9	59.6	

HOB, human osteoblast; Mg, magnesium; SBT, subacromial bursal tissue; SD, standard deviation.

*Indicates statistical significance.

Cellular Proliferation on Suture Tape

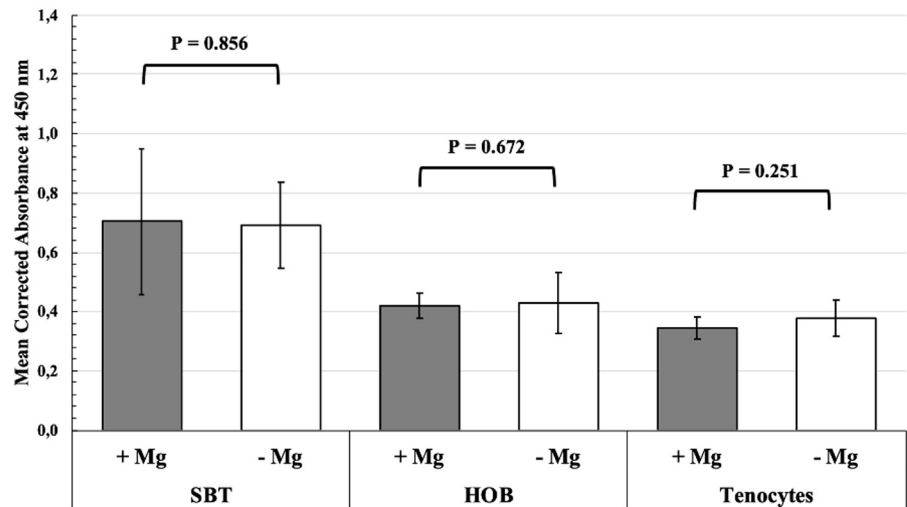


Fig 2. Cellular proliferation (corrected absorbance at 450 nm) on suture tape for SBT, HOB, and tenocytes treated with (+) and without (-) Mg. (HOB, human osteoblast; Mg, magnesium; SBT; subacromial bursal tissue.)

magnesium may be of clinical importance, in order to improve SBT-based biological augmentation techniques in the setting of rotator cuff repair.

Human SBT has recently been suggested as an easily accessible, inexpensive, and viable biologic augment for arthroscopic rotator cuff repair in an attempt to improve healing.⁶⁻¹³ SBT may be one of the main contributors to the complex healing process, as spontaneous healing of the rotator cuff has most noticeably been observed along the subacromial bursal wall, with the cells infiltrating the defects being continuous with the epitenon of the bursa along with minimal contribution from the healing enthesis.^{12,25-27} Further, it has been recommended to preserve SBT as a primary source of neovascularizing signals and fibroblastic cells necessary for biological repair of the torn tendon.²⁶ As these in vitro studies indicate that SBT is critical for rotator cuff tendon healing,^{6,8,9,25,27} the application of magnesium may be an easy, inexpensive, and practical

option to further enhance the cellular adhesion of SBT on suture tape used for repair, thus maximizing the number of SBT cells retained at the targeted zone of repaired tissue.

More importantly, the application of magnesium on suture material may also be transferable from translational basic science research to the clinical use in the real-time setting in the operating room, as magnesium is approved by the FDA and easily available. The suture is soaked in the sterilized magnesium solution directly in the operating room for a minute and is then allowed to dry at room temperature, while the rotator cuff for subsequent repair is being prepared. Especially for physicians without the experience or tools required for bone marrow acquisition and preparation, this may be clinically important to optimize the use of SBT as an alternative biological augment in rotator cuff repair. If greater cellular adhesion is attained with magnesium treatment, an improvement in healing may be achieved.

Table 2. Cellular Proliferation (Corrected Absorbance at 450 nm) on Suture Tape for SBT, HOB, and Tenocytes Treated With (+) and Without (-) Mg

	Mean	SD	P Value
HOB			
(+) Mg	0.419	0.043	.672
(-) Mg	0.427	0.103	
SBT			
(+) Mg	0.703	0.244	.856
(-) Mg	0.693	0.145	
Tenocytes			
(+) Mg	0.345	0.039	.251
(-) Mg	0.377	0.063	

HOB, human osteoblast; Mg, magnesium; SBT, subacromial bursal tissue; SD, standard deviation.

Limitations

There were several limitations to the study. As an in vitro study, these findings may not reflect the cellular adhesion and proliferation capacity of SBT, osteoblasts, and tenocytes in an in vivo shoulder environment. Thus, definitive conclusions regarding the impact of these in vitro findings on rotator cuff tendon healing cannot be drawn. Further, patient characteristics, including age and level of physical activity, may have influenced the results. Lastly, it is unknown to what extent a constant exposure to a wet environment during arthroscopic rotator cuff repair as well as repetitive suture shuttling may interfere with the amount of magnesium remaining bound to the suture.

Conclusions

SBT, osteoblasts, and tenocytes each demonstrated the ability to adhere and proliferate on suture tape. In addition, augmenting the suture with magnesium resulted in a significantly increased cellular adhesion of SBT compared with nonaugmented sutures, whereas no significant differences were observed for osteoblasts and tenocytes. Further, magnesium did not impair the proliferative activity of SBT, osteoblasts, and tenocytes on suture tape used in rotator cuff surgery.

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