Musculoskeletal Research Center

Summer Research Program

2015

Department of Bioengineering

University of Pittsburgh

MSRC

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2015 Abstract Book Committee

Frances Anne Tosto

The work and findings presented in this abstract book represent the efforts of the class of 2015 summer interns at the Musculoskeletal Research Center. We were each assigned a research project and over the course of 12 weeks, dedicated our time to increasing our depth of knowledge in the subject matter and worked closely with our mentors to learn how to use the necessary equipment and complete testing for our projects. It has been an invaluable experience for us to have the chance to learn from the highly accomplished and knowledgeable faculty and graduate students of the MSRC. I think all the interns would agree that we each leave here better prepared for our futures, with greater understanding and new technical skills.

On behalf of all the interns, I would like to thank Dr. Woo for the opportunity to work in the incredible research facility and learning environment that he has worked so hard to build here at the MSRC. We will never forget our experience here and we thank him for all of his advice about research and about life. Nothing we did this summer would have been possible without him. I would also like to thank our mentors Katie Farraro and Jonquil Mau for sharing their research expertise and investing their time into helping us to grow as students. We will take the lessons that we learned this summer and carry them with us from this point forward.

- Frances Anne Tosto, Editor

2015 Summer Symposium Committee

Lindsay Hess

Lewis Gross

Jonathan Mahoney

Frances Anne Tosto

This year's MSRC Summer Research Symposium on July 30th demonstrated all of the participants' hard work during their time here. Each student received the opportunity to present the project they had worked on this summer. With each of us coming in with little to no knowledge of the specific field that we were asked to do research in, the presentations revealed the skills and understanding we gained through this opportunity. We have gained skills from this experience, not just in the lab room, but on the presentation floor and through our discussions as well. I know these lessons will follow each of us from this day forward.

On behalf of my fellow students, I would like to thank everyone who assisted for their help in making the symposium a success. I would especially like to thank Dr. Woo for his guidance and for granting us this opportunity to work in the MSRC. I would also like to thank Jonquil Mau and Katie Farraro for working with us and helping us every step of the way.

- Lindsay Hess, Symposium Committee Chairwoman

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I was born on June 30, 1994 in Baltimore, Maryland. After moving around the northeast several times in my early childhood, my family settled in Fanwood, New Jersey when I was four, and has lived there ever since. Ever since I can remember, sports have been a significant part of my life. I graduated from Union County Magnet High School in 2012 as a member of both the National Honor Society and French Honor Society. In high school, I played soccer and varsity lacrosse, and participated in our school's Student Movement Against Cancer.

Coming to the University of Pittsburgh, I knew that I wanted to study bioengineering. While I quickly ruled out going the pre-med track, I still really appreciated the numerous opportunities bioengineering would open for me in research and in industry. After some of my introductory classes, I decided to concentrate in cellular and tissue engineering. Outside of the classroom, I am an active member of several clubs, including Engineering Student Council, and the Pitt's undergraduate chapter BMES. I volunteered as an undergraduate teaching assistant for organic chemistry last fall, and will do so again this year. I will also be working as a TA in the sophomore bioengineering Cell Biology course. Additionally, I serve on the executive board of Delta Chi Fraternity as the recruitment chair. Any other free time I can find is usually spent playing soccer, basketball, or football or catching up with my favorite teams.

I have had the pleasure of working in the MSRC for almost a year, first as a volunteer during the school year and now as a member of the summer program. Katie Farraro has been an amazing mentor, guiding me in everything from project planning and proposals to abstract writing for the BMES conference. I would like to thank her, along with Cuiling Zhang for also teaching me lab techniques and procedures I had never seen before. Lastly, I would like to thank Dr. Woo, not only for giving me the opportunity to work in the MSRC, but also for all of the lessons and philosophies he has shared with me about research, and about everyday life. I will truly never forget his wise words, or my experience this summer in the MSRC.

EFFECT OF EXTRACTS FROM MG ALLOYS ON ACL FIBROBLASTS

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INTRODUCTION

Injuries to the anterior cruciate ligament (ACL) are amongst the most common in orthopaedic medicine, with approximately 200,000 cases of reported ACL injuries in the United States each year [1]. Since the ACL is typically not a self-healing tissue, major tears and injuries are often treated with complete ACL reconstruction. Reconstruction is a procedure in which the injured tissue is removed from the knee, and replaced with tissue grafts from elsewhere in the patient's body or from a cadaver donor. While this procedure is the standard, it also leads to several long-term complications, such as reinjury and osteoarthritis. [2] In order to address these complications, current research has shifted its focus to healing injured ACLs instead of replacing them.

In recent years, there has been a significant rise in the use of magnesium (Mg) and its alloys as a new class of biomaterials for orthopaedic and other surgical fields [3]. Mg is a favorable material for these applications due to its mechanical properties, biocompatibility, and biodegradability [4]. In the MSRC, we have explored the use of Mg devices for the healing of a transected goat ACL [5]. Some published studies have suggested that Mg may enhance proliferation and function of osteoblasts and endothelial cells [6, 7]. Thus, our research question was whether Mg could have similar positive effects on ACL fibroblasts.

OBJECTIVE

The objective of this study was thus to examine and compare the effects of extracts prepared from five Mg materials on the proliferation and collagen production by ACL fibroblasts.

MATERIALS AND METHODS

A primary cell line of goat ACL fibroblasts was used for this in vitro study. Extracts were prepared from 5 Mg materials in accordance with ISO 10993 [8]: single-crystal pure Mg (SC), a commercially available and widely tested Mg-Al-Zn alloy (AZ31), and three novel Mg alloys being explored for medical applications; Mg-Ca-Zr alloy (P13), and two Mg-Zn-Sr-Zr alloys (ZJ40 and ZJ41). SC magnesium was of the most interest, as it is the material currently used for the MSRC's Mg ring device.

Samples of each material were incubated in cell culture media at 37º C for 72 hours, using a sample-to-extraction media ratio of 0.2g ml^{-1}. Then, dilutions of the resulting extracts (100%) were made to produce concentrations of 50%, 25%, 12.5%, and 6%.

For cell proliferation experiments, 96-well plates were seeded with 5 x 10^3 cells in 200 µl α-MEM supplemented with 2% FBS, 1% penicillin/streptomycin, and for collagen production, 6-well plates were seeded with 4 x 10^5 cells in 2 ml of the same culture media. Following 24 hours for attachment, media was replaced with Mg extract dilutions, with cell culture media alone (0%) used as a control.

After incubation for 72 hours, the Click-It Edu cell proliferation assay (Molecular Probes, Inc., Eugene, OR) and Sircol collagen assay (Biocolor, Carrickfergus, UK) were performed according to manufacturer's protocol. For the proliferation assay, five samples of each extract were tested. For the collagen assay, a triplicate of samples for each extract was tested. Data was averaged and normalized to control values. They were compared using a 2-way analysis of variance with Bonferroni post-hoc testing, with significance set at $P \leq 0.05$.

RESULTS

Fibroblast proliferation in the SC extract group ranged from 89% to 137% of the control (Fig 1A). There were no statistical differences in proliferation between different Mg materials, nor with increasing extract concentration ($P \geq 0.05$).

In contrast, collagen production with SC extracts decreased significantly with increasing extract concentration (Fig 1B). The 12.5%, 25%, and 50% extract groups had significantly less collagen production compared to the control. In the 50% extract group, only 54% of the collagen of the control group was produced ($P \leq 0.05$). The other Mg materials showed similar trends in both proliferation and collagen production.

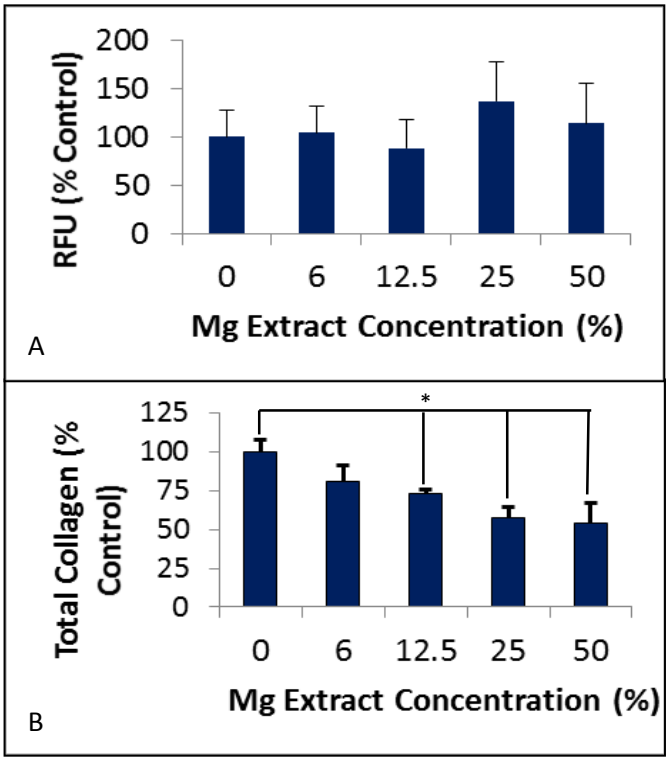

Figure 1. Proliferation (A) and collagen production (B) of goat ACL fibroblasts cultured in dilutions of SC magnesium. * $P \leq 0.05$

DISCUSSION

The results indicate that Mg did not influence proliferation of goat ACL fibroblasts, high Mg concentrations led to significantly reduced collagen production. This decreased collagen production could likely be attributed to several factors that had no impact on proliferation, including increased in pH and osmolality.

Our findings are in agreement with previous studies of in vitro cytotoxicity testing of Mg extracts [9]. Recent literature has suggested that a 10X extract dilution of Mg extract would be needed for cytotoxicity experiments to best approximate Mg exposure in vivo, as higher concentrations have been shown to lead to osmotic shock [9]. Indeed, at these lower extract concentrations, collagen was not significantly reduced in this study. Further studying the mechanisms by which Mg affects collagen production would provide better understanding of these results.

It should be noted that the cell culture methods used in these experiments do not accurately simulate the complex *in vivo* environment of ACL tissue. The extract concentrations tested represent a wide range of Mg concentrations, but not necessarily the ones to which fibroblasts would be exposed. Additionally, this study did not examine the effects of direct contact between Mg and fibroblasts, which would certainly be of interest to the application of the Mg ring device.

CONCLUSION

While Mg did not enhance cell proliferation or collagen production it did not appear to elicit a cytotoxic effect on goat ACL fibroblasts at concentrations relevant to *in vivo* applications.

ACKNOWLEDGEMENTS

I would like to thank Kathryn Farraro for guiding me on this project, and Cuiling Zhang for teaching me much of the technical skills I needed. I would also like to thank Andy Holmes and the rest of the machine shop for their help in producing the Mg disks. The authors gratefully acknowledge an NSF research grant (#8012348) for financial support. Thank you to Dr. Woo and the rest of the MSRC for providing me with an amazing opportunity and experience this summer, and for teaching my many lessons that I will never forget.

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I was born on October 9, 1993 and grew up north of Pittsburgh in Butler, Pennsylvania. I attended St. Joseph High School in Natrona Heights. While in high school, I excelled in my academics and was also involved in several extracurricular activities including Student Government, Girl Scouts, the Science Research Club, and the Women's Varsity golf team. I developed a particular interest in Math and Science, which eventually led me to decide to major in Biomedical Engineering in college.

I currently attend the Catholic University of America in Washington, D.C. At CUA, I am a member of the University Honors Program, Phi Eta Sigma Honors Society, and was I recently inducted into Tau Beta Pi Engineering Honors Society. I am also active on campus as president of the student chapter of Engineers Without Borders (EWB), as a math tutor with the Center for Academic Success and as a member of the Biomedical Engineering Society. I am a member of the Washington, DC professional chapter of EWB as well, and have been a travel team member on two assessment trips to a small village in Panama, where we plan to build a library and computer center for the community there. Additionally, this past spring, I had the opportunity to study abroad at the Hong Kong Polytechnic University. Outside of school, I like to travel, cook, and play golf. As I approach the beginning of my senior year, I am planning on pursuing a career in biomedical research and will be applying to Ph. D. programs this fall.

While this summer internship at the MSRC was not my first research experience, it was my first exposure to the field of orthopedics. I gained so much knowledge this summer, including the problems and complications with ACL reconstruction, the promising alternative ACL healing offers and the usefulness of robotic technology for testing joint kinematics. Additionally, I learned procedures involved in biomechanical testing of animal joint specimens including how to dissect and pot a specimen and how the robotic/UFS testing system and materials testing machine work. Finally, I have learned about the research process in general, how to critically analyze the literature, and how to give an effective scientific presentation. I would like to thank Dr. Woo for giving me the opportunity to work at the MSRC this summer and for sharing his knowledge and many years of experience. I would also like to thank Dr. Katie Farraro for being an amazing mentor and for all of her help and guidance. Finally I would like to thank Jonquil, Diann, and the entire MSRC for all of their support and for a wonderful summer experience.

REGENERATION OF A TREATED ANTERIOR CRUCIATE LIGAMENT USING BIOLOGICAL AND MECHANICAL AUGMENTATION-BIOMECHANICAL EVALUATION IN GOATS

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INTRODUCTION

Rupture of the anterior cruciate ligament (ACL) is a very common knee injury, with 200,000 cases each year in the United States alone [2, 3]. Because a torn ACL does not heal well, the most common treatment for ACL tears is ACL reconstruction, where the ACL is surgically replaced with an autograft or allograft. While this treatment can successfully restore joint stability in the short term, many complications arise in the long term due to the inability of the ACL replacement graft to adequately replicate the complex form of the native ACL. Among these complications is a heightened risk of the patient developing osteoarthritis [11, 12].

However, with recent advances in functional tissue engineering, alternative treatment options that aim to heal the native ACL have been explored. In our research center, we used an extracellular matrix (ECM) bioscaffold to heal a surgically transected goat ACL [6]. In this study, the ends of the transected ACL were first reconnected using sutures. Then, an ECM sheet was wrapped around the injury site and an ECM hydrogel was injected into the wound. By 12 weeks of healing, continuous neo-tissue was observed with limited hypertrophy. Additionally, uniaxial tensile testing revealed improvements in the structural properties of the femur-ACL-tibia complex (FATC) compared to a control group that was treated by suture repair alone. Furthermore, this study showed that the biological augmentation provided by the ECM bioscaffold and hydrogel could provide a successful healing response of a fully transected ACL. But in a follow-up study at 26 weeks of healing, the mode of failure of the FATC during uniaxial tensile testing changed from the ACL midsubstance to its insertion sites. This suggested that during the slow ACL healing process, there is a risk of disuse atrophy of the ACL's insertion sites due to a lack of loading. For this reason, it became clear that mechanical augmentation of a healing ACL would also be needed.

To this end, a bioresorbable, ring-shaped device made of magnesium (Mg) was designed for mechanical augmentation of an injured ACL. This "Mg ring" device connects the two ends of a transected ACL, restoring stifle joint stability and loading the ACL [4]. In order to determine surgical feasibility of Mg ring repair, an *in vitro* study was performed using robotic testing to evaluate joint function in cadaveric goat stifle joints after surgery. It was found that Mg ring repair could restore anterior tibial translation to within 5 mm of the intact joint and the ACL in-situ force to near normal levels. Following these positive results, this device was then used *in vivo* alongside an ECM bioscaffold to heal a surgically transected goat ACL. Specimen from an initial group of animals (Phase I; N = 4) showed a robust healing response and improved structural properties of the FATC compared to ECM treatment alone. With these promising results, a second group of goats (Phase II; N = 4) was tested using the same methods.

OBJECTIVE

The objective of this study was to assess the effect of Mg ring repair of a surgically transected goat ACL combined with ECM treatment on stifle joint kinematics, the in-situ forces in the ACL, and the structural properties of the FATC at 12 weeks of healing.

MATERIALS AND METHODS

Skeletally mature, female Spanish goats were used for this study (N = 4). All the animals tested underwent surgery on their right hind limb, while the left served as a sham-operated control. The ACL was transected through its midsubstance and Mg ring repair was performed. An ECM sheet was then wrapped around the ring and an ECM hydrogel was injected into the wound. Post-surgery, animals were allowed free cage activity. They were humanely sacrificed at 12 weeks of healing and the hind limbs were disarticulated at the hip joint and stored at -20°C.

The day prior to testing, the stifle joints were thawed and prepared for testing as described previously [1]. The stability and function of the stifle joints were evaluated using a robotic/universal force-moment sensor (UFS) testing system developed in our research center over 20 years ago [7, 9, 10]. To provide a reference point, a passive path of flexion-extension was found. Then, force control mode was used to apply a 67-N anterior-posterior (A-P) tibial load at 30, 60, and 90 degrees of joint flexion to find the resulting 5 degree-of-freedom joint kinematics. Next, these kinematics were repeated using position control mode after the medial collateral ligament (MCL), soft tissue, medial meniscus (MM), lateral meniscus (LM) and bony contact were each removed in sequence and a new set of forces and moments were recorded. Using the principle of superposition, the in-situ forces of each stifle joint structure were determined. The remaining force in the ACL-only specimen represented the in-situ force in the ACL.

Once robotic testing was completed, a custom laser micrometer system was used to find the cross-sectional areas of the ACL [8]. Finally, uniaxial tensile testing was used to evaluate the structural properties of the FATC [1, 13]. The joint was first mounted in a position that aligned the ACL along its longitudinal axis of loading. A 3 N preload was applied, followed by cyclic preconditioning between 0 and 1 mm for 10 cycles at a rate of 50 mm/min. The pre-load was then reapplied and load-to-failure testing was performed at a displacement

rate of 10 mm/min. From the resulting load-elongation curve, the ultimate load (maximum load at failure) was found. The stiffness was defined as the slope of the linear region of the load-elongation curve and was calculated by regression analysis.

RESULTS

The anterior-posterior tibial translation (APTT) of the treated goat stifle joints in response to the 67 N A-P load is shown in Figure 1. For the specimen tested previously from Phase I of the *in vivo* study, average APTT values of 9.8 ± 2.3 mm, 11.5 ± 1.8 mm and 8.2 ± 1.1 mm were found at joint flexion angles of 30°, 60°, and 90°, respectively. Comparatively, for the specimen tested here from Phase II of the study, average APTT values of 14.6 ± 1.1 mm, 14.2 ± 1.6 mm and 11.4 ± 1.2 mm were found at the same joint flexion

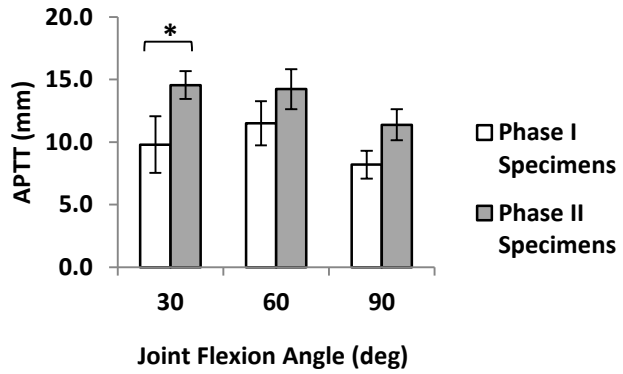

Figure 1. Anterior posterior tibial translation (in mm) at goat stifle joint flexion angles of 30°, 60°, and 90° in response to a 67 N anterior-posterior tibial load. (* $p < 0.05$)

angles. The average APTT values of the Phase II specimen were 48.5% higher at the 30° flexion angle, 23.7% higher at the 60° flexion angle and 38.7% higher at the 90° flexion angle than the Phase I specimen. At the 30° flexion angle, there was a significant difference between the average APTT value of the Phase II specimen compared to that of the Phase I specimen (P = 0.01).

The average in-situ forces of the ACL in the Phase I specimen were found to be 59 ± 16 N, 41 ± 29 N, and 19 ± 24 N at the joint flexion angles of 30°, 60°, and 90°, respectively. However, during position control testing of the Phase II specimen, the forces recorded by the robotic/UFS testing system became negligible, indicating that the ACL was not loaded and had not healed. For this reason, further testing was not performed on the specimen to find the cross-sectional area or structural properties of the FATC.

In terms of gross morphology, in the Phase I specimen a robust ACL healing response was seen with continuous neo-tissue with aligned collagen fibers (Figure 2A). Additionally, the Mg ring had completely degraded in all but one of the specimen. In comparison, in the Phase II specimen, there was very little neo-tissue seen (Figure 2B). All Phase II specimen showed signs of degeneration in the articular cartilage of the femur and patella. Most notably, distal patellar lesions were observed in three of the four Phase II specimen (Figure 2C), which were not observed in the Phase I specimen. Additionally, many large Mg ring fragments were found in all specimen, with a large ring fragment adhered to the femoral insertion site of one of the specimen (Figure 2D).

DISCUSSION

In Phase I of this study, the effect of Mg ring repair combined with ECM treatment on the 12-week healing response of a surgically transected goat ACL was demonstrated by evaluating the stifle joint kinematics, the in-situ forces in the ACL, and the structural properties of the FATC. This demonstrated that the Mg ring repair with ECM treatment could lead to a robust ACL healing response and

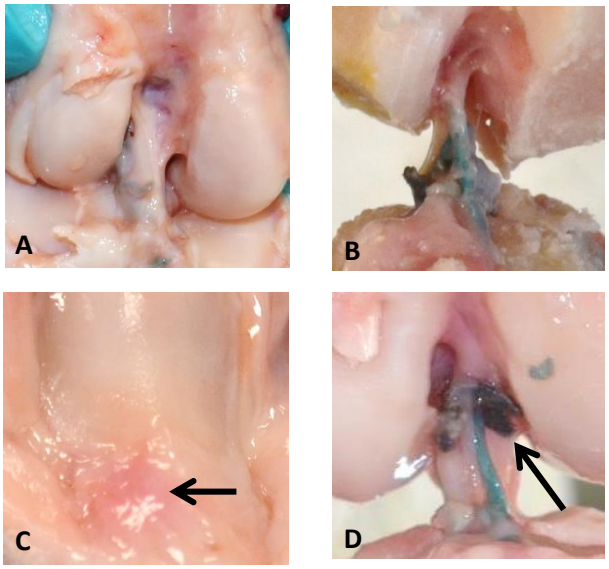

Figure 2. **(A)** Healing ACL of Phase I specimen at 12-weeks post-surgery. **(B)** Healing ACL of Phase II specimen at 12-weeks post-surgery. **(C)** Distal patellar lesion of Phase II specimen, as indicated by the arrow. **(D)** Large Mg-ring fragment adhered to the femoral insertion site of one Phase II specimen, as indicated by the arrow. (Note: Large tissue in the posterior is the PCL. The little ACL tissue that was present in this specimen was partially attached to the PCL)

improved structural properties of the FATC compared to ECM treatment alone. The results of the Phase II specimen, however, showed inferior results, with reduced joint stability evidenced by a higher APTT at all flexion angles compared to Phase I specimen, with a significant difference at 30° of joint flexion. In addition to reduced joint stability, the Phase II specimen showed a lack of ACL functionality, with negligible ACL in-situ forces and a poor healing response with little neo-tissue growth and distal patellar lesions that were not found in the Phase I specimen.

Based on these results, the question is raised as to what caused these notable differences between the specimen from Phases I and II. One factor that may have contributed to the differences in results is differences in the Mg ring devices used in the two phases of the study. Although intended to be homogeneous between batches, differences may have occurred in the Mg stock material

used to produce the devices, as well as the corrosion-resistant anodization coating. Indeed, the Mg ring appeared to have degraded almost entirely in Phase I of the study, while in Phase II, the Mg ring devices remained mostly intact. As such, future work will include an *in vitro* degradation study to compare the degradation rate of the rings used in Phase I to the rings used in Phase II.

Another factor that may have contributed to the differences in results is the tension of the Endobutton used for femoral fixation of the repair. Prior to Phase II, a correlation was seen between Endobutton fixation and the ACL healing response in the Phase I group. For this reason, in Phase II, the surgeon made an effort to make sure all the Endobuttons were tightly fixed, and thus may have over-corrected for this problem by over-tensioning the fixation sutures. The distal patellar lesions noted in the Phase II specimen are evidence of this. To investigate this, further investigation will be made on the effect of Endobutton tension on the function of the goat stifle joint and ACL using the robotic/UFS testing system.

In conclusion, while Phase I of this study showed that Mg ring repair combined with ECM treatment could lead to a positive ACL healing response, the results of the Phase II specimen suggest the degradation of the Mg ring device and fixation tension may be crucial factors in the healing response. Clearly, further study is warranted to understand the optimal conditions for Mg ring repair for ACL healing. Then, follow-up *in vivo* studies at multiple time points will be needed to confirm the efficacy of Mg-ring repair combined with ECM treatment on ACL healing. With these results, we will be closer towards our goal of addressing complications of ACL reconstruction and offering an alternative treatment option that leads to better patient outcomes.

LESSONS LEARNED

My summer research experience at the MSRC was a very fulfilling one. I gained so much knowledge, including the problems and complications with ACL reconstruction, the promising alternative ACL healing offers, and the usefulness of robotic technology for testing joint kinematics. Additionally, I learned procedures involved in biomechanical testing of animal joint specimens including how to dissect and pot a specimen and how the robotic/UFS testing system and materials testing machine work. Finally, I learned about the research process in general, how to critically analyze the literature, and how to give an effective scientific presentation.

ACKNOWLEDGEMENTS

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I was born on April 29, 1995 and grew up in the South Hills of Pittsburgh in Jefferson Hills, PA. I graduated from Thomas Jefferson High School. I participated in the French Club and the Science Club, I was a member of the National Honors Society, and I dual-enrolled at the Community College of Allegheny County during my senior year. Outside of school, I was a member of Peter's Creek Baptist Church youth group and Youth for Christ Metro Pittsburgh, and I volunteered at Jefferson Hills Regional Medical Center. I volunteered as a leader for Youth for Christ Metro Pittsburgh after graduating high school.

At Robert Morris University, I study biomedical engineering and mechanical engineering with a minor in mechatronics. I am also a part of the honors college. During my freshman year, I was elected as treasurer of the RMU chapter of the Society of Women Engineers, which I am still a member of as well as the Biomedical Engineering Society. Additionally, I am a founder and the vice-president of the Mechatronics club and a founder and treasurer of the RMU Lego Club. In my spare time, I have enjoyed attending several bioskills labs at UPMC involving practice orthopedic surgeries, and I am considering pursuing medicine.

The Summer Undergraduate Research Program at the MSRC was my first research experience. I have gained a great deal of knowledge during my time here about the research process in general and, more specifically, about rotator cuff repairs, biomechanical testing procedures, interpreting data, and critically analyzing literature. I would like to thank Dr. Woo for sharing his expertise and philosophy and for providing me with guidance. I would also like to thank my mentor, Jonquil Mau, for her support and for teaching me and guiding me throughout the summer, as well as Katie Farraro for her help and feedback.

BIOMECHANICAL EVALUATION OF ROTATOR CUFF REPAIR IN A GOAT MODEL USING MAGNESIUM-BASED SUTURE ANCHORS

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INTRODUCTION

The rotator cuff is a group of four muscles—the infraspinatus muscle, supraspinatus muscle, teres minor muscle, and subscapularis muscle— which surround the shoulder joint that allow movement and provide it stability. The tendons of the rotator cuff are commonly torn due to various causes such as an injury, overuse, poor blood supply, a fall, or a gradual weakening of the tendon.[7] Such tears often require surgical repair with over 250,000 rotator cuff repairs performed each year in the United States[1]. Typical procedures use two to four suture anchors to reattach the torn tendon to the humeral head.

Commercially available suture anchors are made of non-degradable metals, such as stainless steel or titanium alloys, or bioresorbable polymers. However, the materials of these current suture anchors pose several potential shortcomings. Titanium and stainless steel suture anchors can cause several complications including MRI interference, suture or tissue damage during insertion, and difficulties with removal during revision surgery.[8] Complications with polymer suture anchors include device breakage during and after implantation[9], inconsistent degradation[4], and poor osteointegration[11] which can leave voids in the bone and cause osseous cyst formation.[12] Therefore, an alternative to the current suture anchors are needed.

A potential solution is the use of Magnesium (Mg) alloys as a biomaterial for the development of suture anchors. Mg-based suture anchors could be a viable alternative because it has good biocompatibility[13], controllable degradation with the use of alloying or coating strategies[5, 14] the potential to promote bone formation, desirable mechanical properties, and eliminates MRI interference.[3]

The objective of this study is to evaluate rotator cuff repairs using Mg-based suture anchors (Figure 1), in an *in vitro* goat model. It is hypothesized that our results will show that Mg-based suture anchors are suitable for tendon fixation in rotator cuff repairs. Suture anchors are not the weakest link of the rotator cuff repair complex and are not expected to be the mode of failure. Thus, failure will occur in another component of the rotator cuff repair complex, such as the suture-tendon interface.

Figure 1: *Magnesium-based suture anchors rotator cuff repair.*

METHODS

The goats used in this study were female Spanish goats. Four fresh frozen goat were used for testing. The day before testing, they were thawed at room temperature. The specimen were dissected, removing all soft tissues except the infraspinatus tendon humeral bone complex.

A double row fixation technique was used for tendon repair by Dr. Patrick McMahon, M.D., an orthopedic surgeon. First, the infraspinatus tendon was detached from the humerus at the tendon footprint to mimic a full-thickness tear. Then two 6 mm medial holes were drilled on the humeral head 10 mm medial to the tendon footprint. The distance between the two medial holes was 10 mm. The suture anchors were loaded with sutures and screwed into the predrilled holes. The sutures were passed through the tendon medially to its lateral edge to return the tendon insertion to its original footprint and 6 knots were tied. Next, two 6 mm holes were drilled lateral to the tendon, one strand from each suture was loaded onto each anchor, and the suture anchors were inserted into the holes.

In order to prepare the specimen for testing, the muscle was cut from the tendon. The tendon was secured in a custom clamp 1.5 inches from the suture knots and mounted in a uniaxial materials testing machine 90° to the humerus (Figure 2). The tendon was preloaded at 5 N (10 mm/min). Cyclic loading was performed from 10 N to 30 N for 50 cycles at an extension rate of 50 mm/min to represent a short period of repetitive low-load application.[2] Gap formation was defined as the displacement between the tendon and its original footprint during cyclic testing and was measured between the peaks of the first and last cycles. Then, the specimen underwent load to failure testing at an extension rate of 50 mm/min. Stiffness and ultimate load were recorded for each specimen. The mode of failure was also noted.

Figure 2: *Photograph of a specimen before loading. Note that markers were not used for the analysis in this paper.*

RESULTS

The results of the uniaxial tensile testing are shown in Table 1. The cyclic loading tests showed a gap formation of 1 ± 0.1 mm. The ultimate load was defined as the peak load observed and was recorded as 88 ± 9 N. The stiffness, the linear slope on the load-elongation curve, was 29 ± 4 N/mm. All specimen failed as a result of the sutures pulling through the tendon.

Table 1: *Mean values of gap formation, ultimate load, and stiffness of the infraspinatus-humerus complexes*

Gap formation	1 ± 0.1 mm.
Ultimate load	88 ± 9 N
Stiffness	29 ± 4 N/mm

DISCUSSION

The objective of this study was to evaluate rotator cuff repairs using Mg-based suture anchors in an *in vitro* goat model. The small variation between the results of gap formation, ultimate load, and stiffness confirm that the surgical and testing procedures were consistent. Additionally, it was proven that the weakest link of the construct was the tendon-suture interface as hypothesized; therefore, it can be concluded that Mg-based suture anchors are suitable for rotator cuff repair.

Previous similar studies reported higher ultimate loads than observed in the current study. Spang et al. repaired the infraspinatus tendon to the humerus in sheep and reported ultimate loads of 421 ± 150 N[10]. Pauly et al. found an average ultimate load of 259.8 ± 21.1 N[6] in the same animal model. The lower values in this study may be due to the surgical or testing protocols, such as the distance from the edge of the tendon that the sutures were inserted or the extension rate. When compared to a study by Awwad et al. using corresponding surgical and testing protocols on 8-month-old female, skeletally immature Suffolk lambs[2], the results were similar. The mean ultimate load and gap formation of the study were 79.5 N and 1 mm, respectively. However, it is important to note that the failures in the three mentioned studies occurred as a result of the sutures pulling through the tendons and did not occur at the suture anchors. Because the suture anchors did not fail, the differences in ultimate load do not affect the outcome of the present study.

Further testing will evaluate additional infraspinatus-humerus complexes repaired with Mg-based suture anchors as well as repairs with polymer suture anchors as a control. This will confirm that the Mg-based suture anchors will be suitable in rotator cuff repairs for *in vivo* evaluations.

In conclusion, based on the results of this study the Mg-based suture anchors are suitable for rotator cuff repairs.

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Born and raised in Pittsburgh, I attended Mount Lebanon High School and graduated in 2014. While in High School, I took almost every AP and Honors science class available. I participated in Student Council, Model UN, the school Orchestra, Interfaith Alliance, and became a part of National Honor Society and Cum Laude Society.

After High School, I chose to study at the University of Wisconsin Madison, where I am currently a sophomore. Besides the engineering program, the school has much to offer in terms of athletics and student organizations. At UW-Madison, I work with the Undergraduate Learning Center and tutor calculus, chemistry, and differential equations. When I am not working or studying, I may be found at the gym, playing the piano or the viola, watching a Badger football, basketball, or hockey game, or relaxing with friends near the lake or inside during the winter.

I'd like to thank my mentor, Jonquil, for all I've learned this summer. She has fielded all my questions excellently, making the learning experience more tangible than just reading. Regarding finite element analysis, I now understand how to run a static structural analysis of a system. I'd also like to thank Dr. Woo for all his help this summer. Every presentation I gave in front of Dr. Woo was a learning experience. His careful critique of the content I displayed made me grow as a scientist and pay more attention to the small details and definitions in the field. Everyone at the MSRC has encouraged critical thinking, and as a result, I myself have learned more on how to think critically. Thanks for a great summer!


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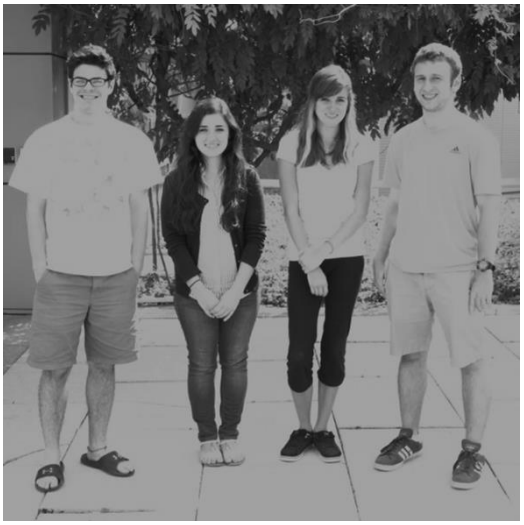

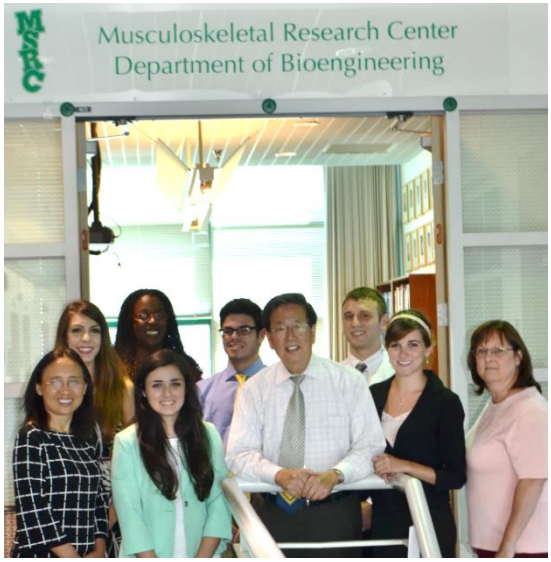
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