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I was born on January 17th 1991 in Houston, Texas and lived there until I was five. After that, my family moved to Katy, Texas, a suburb of Houston, where I have lived ever since. I have one younger brother who is the brightest 9 year old I know, and I graduated from Cinco Ranch High School in 2008. While in high school I swam on varsity swim team all four years and participated in NHS, Academic Decathlon, and UIL Academics.

I chose Rice University for its small size, good undergraduate research opportunities and bioengineering department, closeness to home, and amazing residential college system. When I'm not studying for bioengineering classes at Rice, I enjoy watching horror movies, organizing social events for Rice Program Council such as campus-wide Assassins, and volunteering around the Houston area.

Coming into the MSRC, I had little to no knowledge about ACL reconstruction and no lab experience whatsoever. Coming out; however, I have not only gained knowledge about ACL reconstruction with interference screws as well as the biomaterials used to construct such screws, but I have also gained valuable cell culture techniques as well as the privilege of meeting some of the most experienced researchers in this field here at the University of Pittsburgh. I would like to thank Dan Perchy who has been kind and patient enough to teach me the basics of pipetting and cell culture while dealing with my clumsiness and lack of experience. I would also like to thank Dr. Liu for her instruction and guidance in teaching me how to perform a standard BrdU assay and giving me a fun and interesting project to do this summer. I truly learned a lot. Finally, I would like to thank Dr. Woo and the Pittsburgh Tissue Engineering Initiative for this rare and unforgettable opportunity in being able to conduct research in a lab as well as the MSRC for their support.

CELL PROLIFERATION ON PURE MAGNESIUM AND MAGNESIUM-YTTRIUM ALLOYS IN OXIDIZED AND POLISHED STATES

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INTRODUCTION

Interference screws have been used in ACL reconstruction surgery to fix tissue autografts or allografts with a bone block on one or both ends (e.g. bone-patellar tendon-bone) [4]. Two types of interference screws that have been commonly used are biodegradable polymer screws made of material such as Poly-L-lactic acid and metallic screws made of material such as titanium alloys [1]. However, both are subjected to several problems. Metallic screws, even though they have good strength and tensile properties, are not biodegradable and may induce a “stress shielding” effect, preventing the bone from healing properly on its own [1]. Polymeric screws, though biodegradable, may degrade too slowly, cause bone tunnel widening, and are more prone to infection [1]. For this reason, it is imperative to find a new biomaterial for interference screws that has a degradation rate similar to human bone growth rate as well as the ability to promote bone in-growth as it degrades. Magnesium, a promising biodegradable element, has the advantages of having a similar elastic modulus and density as human bone and is associated with the formation of bone apatite [2]. Although its degradation rate is faster than the human bone growth rate, alloying magnesium with elements such as yttrium has been hypothesized to slow magnesium’s degradation rate [3]. Because bone marrow derived cells (BMDCs) are associated with the regeneration of bone tissue, testing the cell proliferation of both magnesium and magnesium-yttrium alloy by culturing BMDCs on these samples as they degrade will allow us to determine whether magnesium is a suitable alternative to use in constructing ACL reconstruction interference screws.

OBJECTIVE

The objective of this study was to compare goat bone marrow derived cell (BMDC) proliferation on polished and oxidized magnesium-yttrium (Mg-Y) alloy as well as polished and oxidized pure magnesium (Mg) at 24, 48, and 72 hour time points using the BrdU assay in order to test these substrates’ potentials as biomaterials for interference screws.

MATERIALS AND METHODS

This experiment was repeated 3 times for the 3 time points tested: 24 hrs, 48 hrs, and 72 hrs. For each time point, six samples of size ~5.5 mm x 5.5 mm were cut from as-cast Mg-Y alloy imported from GKSS in Germany and six samples of the same size were cut from as-rolled pure Mg (purity 99.9%) imported from Goodfellow Cambridge Limited (Cat #MG000300/20). Three samples of the Mg-Y alloy and three samples of the pure Mg were left oxidized while the three other samples of the Mg-Y alloy and the three other samples of the pure Mg were polished. Goat BMDCs harvested from a

goat femur were cultured on the four types of samples for the predetermined length of time (24 hrs, 48 hrs, or 72 hrs) at an initial cell density of 10k cells. Goat BMDCs were also cultured in cell densities ranging from 0k to 20k as standards for the same time period. After culturing for the predetermined time period, bromodeoxyuridine (BrdU) was added to all above groups, and the BrdU assay was performed to determine BrdU incorporation of each group, proportional to the number of proliferating cells. Numbers of proliferating cells in the magnesium groups were interpolated from a standard curve.

RESULTS

Trends varied for all 3 time periods as can be seen in Figure 1. Actual cell proliferation counts are listed in Table 1. For the 24 hour BrdU assay, the oxidized Mg-Y alloy had the most cell proliferation, followed by the polished Mg-Y alloy, and by the oxidized pure Mg. Polished pure Mg had the least cell proliferation for the 24 hour period. For the 48 hour BrdU assay, polished Mg-Y had the most cell proliferation, followed by the oxidized pure Mg, and by the oxidized Mg-Y alloy. Again, polished pure Mg had the least cell proliferation. For the 72 hour BrdU assay, the trends reversed from the 24 hour BrdU assay, as far as metal type was concerned. Pure Mg oxidized had the most cell proliferation, followed by the pure Mg polished, and by the Mg-Y oxidized samples. The Mg-Y polished samples had the least number of proliferating cells. However, for both 24 hour and 72 hour periods, oxidized samples consistently had more cell proliferation than polished samples, regardless of metal type. The 48 hour period displayed no noticeable trend, with regards to metal type or surface treatment, but cell proliferation peaked at 48 hours for all magnesium samples.

A statistical 3-way ANOVA test ($p < 0.05$) was conducted to analyze the data with the 3 factors being type of metal (pure Mg vs. Mg-Y), surface treatment (oxidized vs. polished), and time period (24 hrs, 48 hrs, and 72 hrs). The results showed time as a significant factor in terms of cell proliferation with $p = 0.0001$. In addition, there seemed to be a metal-surface interaction with $p = 0.019$. This could signify that polishing Mg-Y alloy might increase cell proliferation while polishing pure Mg might decrease cell proliferation. However, these results could have been skewed by the 48 hour results which showed higher cell proliferation than either the 24 or 72 hour results. There was no main effect of surface treatment, which meant that polishing the substrate did not significantly change cell proliferation. Because the statistical 3-way ANOVA seemed inconclusive, a statistical 2-way ANOVA was also conducted for the 3 time points (24 hrs, 48 hrs, and 72 hrs) with the 2 factors being type of metal and surface treatment. This revealed that the pure Mg was significantly higher in cell proliferation than the Mg-Y alloy for the 72 hr

time point only ($p=0.014$). Further testing needs to be done with a larger sample size of data to confirm results and perform a better statistical analysis.

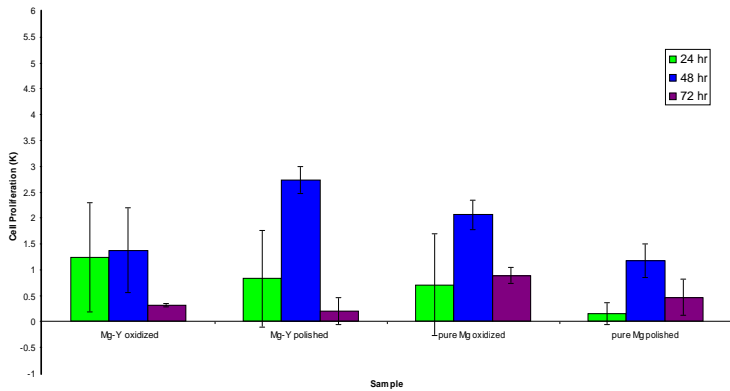


Figure 1. Cell proliferation on various magnesium samples

TABLE 1. Mean values in thousands (\pm standard deviation) of proliferating goat BMDCs on various magnesium samples for 24 hr, 48 hr, and 72 hr periods.

	24hrs	48hrs	72hrs
MgY_O	1.237 \pm 1.055	1.379 \pm 0.814	0.311 \pm 0.029
MgY_	0.829 \pm 0.937	2.736 \pm 0.264	0.207 \pm 0.262
P	0.712 \pm 0.982	2.062 \pm 0.287	0.886 \pm 0.153
Mg_O	0.150 \pm 0.212	1.176 \pm 0.326	0.467 \pm 0.347

DISCUSSION

Although there seemed to be a trend that Mg-Y did better than pure Mg in terms of cell proliferation in the short run (24 hrs) while pure Mg did better than Mg-Y in the long run (72 hrs), the exact cause or mechanism to explain these results is still unknown. This study was only a preliminary comparative study to compare all four types of substrates at the 3 different time points. Pictures of the metal substrates taken at 72 hours indicated that the Mg-Y samples degraded more than the pure Mg samples (in terms of weight difference). Pictures and weight measurements of the 24 hour and 48 hour samples were inconclusive. pH measurements were also taken, but other than higher pH values for the metal samples compared to the standards (possibly due to the hydroxide ions formed in degradation reactions) no conclusions could be drawn about the metal samples when comparing their pH values. For peak cell proliferation at 48 hours, it is possible that the initial increase on all samples was due to the precipitation product $Mg(OH)_2$ during degradation which has been shown to promote bone growth by temporarily enhancing osteoblast activity and decreasing osteoclast number [5]. The later decrease on all samples could possibly

have been due to the build-up of excess hydrogen gas or hydroxide ions, which would create a harmful environment to cells [6]. Because few studies have been done using the BrdU assay to determine cell proliferation on magnesium and magnesium alloys, it was difficult to compare experimental values to literature values.

In the future, repeated tests need to be done for better statistical analysis. Different alloys should also be tested, and in-vitro experiments should be conducted to evaluate the short-term and/or long-term effects of elements used in Mg alloys as well as Mg itself on cells. This will hopefully explain the mechanism of cell proliferation on the various samples. Finally, it will be advantageous to conduct in vivo testing of pure magnesium and various magnesium alloys in a small animal model such as a rabbit or guinea pig.

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I was born on September 26, 1985 in Akron, Ohio but grew up a few hours south in Columbus, Ohio. Like my older sister, Rachael, I attended St. Francis DeSales High School where I participated in football, track, student council, as well as many other activities. Upon graduation in 2004, I enrolled at Northwestern University where I was a 4-year letter-winner on the varsity football team while majoring in psychology. Thereafter, I completed a master's degree in exercise physiology at the University of Glasgow, Scotland. I am currently in my first year as an MD/PhD student in Pitt's Medical Scientist Training Program.

As a lifelong athlete, I have a deep appreciation for elite physical performance and the science that underlies such feats. However, I've also witnessed the prevalence of injury in sport and the chronic disability that can ensue. As an aspiring orthopaedic surgeon, I hope to develop a deep understanding of soft tissue biomechanics and tissue engineering in order to create novel ways to treat sports injuries, resulting in faster recovery and improved patient outcome. To achieve this end, I could not have come to a better place than the MSRC.

My summer experience at the MSRC has been both educational and enlightening. Not only have I further developed my lab skills while continuing to gain appreciation for conducting good research, but I have also grown surer of my future career path. I would like to thank Dr. Matt Fisher, Kwang Kim, and Dr. Huinan Liu for their intelligence and patience, as well as Dr. Woo for his wisdom and for allowing me to join the MSRC this summer. I had so much fun; I may be back for more next year.

DEVELOPMENT OF AN IN-VITRO BIOMECHANICAL PROTOCOL TO EVALUATE MAGNESIUM-BASED INTERFERENCE SCREWS FOR ACL RECONSTRUCTION

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INTRODUCTION

The anterior cruciate ligament (ACL) of the knee is frequently injured during sports and work related activities. In fact, over 100,000 ACL tears occur annually in the United States [1, 2]. However, the ACL has a limited healing capacity [3, 4] and its injury often results in knee instability, pain, and an increased risk of osteoarthritis [4]. Consequently ACL reconstruction surgery is frequently performed in order to restore knee stability and allow a return to pre-injury activities.

The bone-patellar tendon-bone (BPTB) autograft has been the gold standard for ACL reconstruction, and it is commonly fixed with metallic or polymeric interference screws [5]. While metallic screws possess high mechanical properties and provide good initial fixation of the graft, they can cause MRI distortion and often need to be removed for revision surgeries [6]. Likewise, bioabsorbable polymeric screws provide comparable fixation strength, but have been reported to fracture during implantation [7], induce foreign body reactions [8], and limit osseointegration due to excessively slow degradation rates [9].

The use of interference screws composed of porous magnesium (Mg) alloys may obviate several of these disadvantages. Mg alloys can be designed with controllable degradation rates and have been shown to be cytocompatible and osteoinductive [10, 11]. Furthermore, Mg alloys possess higher mechanical properties than polymers [11] and thus would be less likely to fracture during insertion. As a first step in determining the clinical viability of Mg-based interference screws, we will assess the in-vitro biomechanics of a BPTB graft secured with a Mg-based screw and compare these results with those obtained when using a commercially-available polymer screw (Milagro®, Johnson & Johnson). Since the initial fixation of the ACL replacement graft is largely dictated by the size and design of the screw and the mechanical properties of Mg are higher than those for polymers, we hypothesize that an Mg-based interference screw will provide similar initial fixation compared to polymer screws of similar size and design.

OBJECTIVE

To develop an in-vitro biomechanical protocol that will allow sufficient comparison of a BPTB graft secured with Mg-based vs. bioabsorbable polymer interference screws, in terms of fixation of the graft (i.e. graft slippage following cyclic loading) and its tensile properties.

METHODS

Five hind limbs were obtained from skeletally mature pigs and stored at -20°C. Prior to testing, the specimens were thawed overnight at room temperature. A 10-mm wide graft was harvested from the central third of the patella bone and

patella tendon. The tibial insertion site was left intact. An 11-mm diameter tunnel was drilled through the femoral footprint of the ACL and the bone block of the graft was secured from inside-out with a Titanium alloy interference screw (7x30 mm).

The femur-graft-tibia complex (FGTC) was dissected free of all remaining soft-tissue and then rigidly fixed in custom-made clamps of a materials testing machine (Instron, Model 4502, Canton, MA, USA). The clamp that held the femur allowed 6 degrees of freedom (DOF) motion for specimen orientation while the tibial clamp allowed 2 DOF. The FGTC was positioned so as to align the bone tunnel and collagen fibers of the graft along the axis of uniaxial tension.

A preload of 3N was applied to each FGTC and the gauge length was set to 0 mm (**Figure 1**). A series of three cyclic creep tests followed (C1-C3). Each test consisted of loading the FGTC between 20N and 70N for 50 cycles at 50mm/min. Upon completion of the test, the FGTC was completely unloaded, wrapped in saline-soaked gauze, and allowed a 60-min recovery period. Prior to the start of each cyclic test, the FGTC was preloaded to 3N and the gauge length was recorded (**Figure 1**). The recorded gauge length after each cyclic test was defined as the total residual elongation of the FGTC.

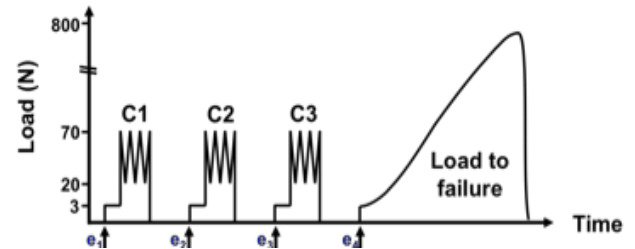


Figure 1. Tensile testing protocol. FGTC gauge length ($e_1 - e_4$) was recorded during a 3N preload applied prior to each test.

To determine the percent of residual elongation due to elongation at the femur-graft interface (slippage), patellar tendon midsubstance (creep), and tibia-graft insertion, a motion analysis system (DMAS6, Spica Tech., Maui, HI, USA) was used. Six reflective markers (1.5 mm diameter) were placed on the FGTC and fixed with cyanoacrylate, one each on the femur and tibia, three on the graft, and one on the tibial insertion site (**Figure 2**). The 2-dimensional positions of the



Figure 2. Reflective markers on FGTC

reflective markers prior to each cyclic test were recorded during the 3N preload, and changes in the length of each segment, as well the total FGTC, were calculated. The percent contribution of each segment to the total FGTC elongation was calculated according to the following equation:

$$\% \text{ of total elongation} = \frac{\text{elongation of segment}}{\text{elongation of total FGTC}} \times 100$$

Due to initial difficulties in accurately tracking changes in segment lengths of the FGTC, only three samples are reported in terms of total residual elongation and the relative contribution of each segment to this elongation (**Table 1**).

Finally, the FGTCs were loaded to failure at an elongation rate of 10 mm/min. The load-elongation behavior and the mode of failure were recorded. The maximal tensile force observed on the load-elongation curve was the ultimate load (N). The stiffness was taken as the slope of a linear trend line that was fitted to the linear region of the load-elongation curve ($R^2 = 0.995$).

RESULTS

As seen in **Table 1**, a mean residual elongation of 0.3 mm occurred over the series of three cyclic loading tests. Within the first 50 cycles (C1), 0.2 mm (67%) of the total elongation occurred, with an additional 0.1 mm (33%) of elongation occurring during the second 50 cycles (C2).

Sample	Total (mm)	C1 (mm)	C2 (mm)	C3 (mm)
1	0.3	0.3	0.1	-0.1
2	0.3	0.2	0.1	0.0
3	0.3	0.2	0.1	0.0
Mean ± S.D.	0.3 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.1

Table 1. Residual elongation of FGTC.

The percent of total residual elongation attributable to graft slippage, midsubstance creep, and elongation at the tibial insertion site was determined by use of the motion analysis system. Video analysis revealed that 100% of the total FGTC elongation over 150 cycles for all samples was due to slippage of the graft from the bone tunnel.

The structural properties of the FGTCs are shown in **Table 2**. Of particular importance, the average ultimate load was 809 ± 158 N and the average stiffness was 65 ± 13 N/mm. All samples failed by graft pullout from the femoral bone tunnel.

Sample	Ultimate Load (N)	Stiffness (N/mm)
1	773	60
2	828	81
3	608	46
4	789	69
5	1048	69
Mean ± S.D.	809 ± 158	65 ± 13

Table 2. Parameters describing the structural properties of the FGTC.

DISCUSSION

In this study, an in-vitro biomechanical protocol was developed to evaluate the initial fixation of a BPTB graft secured with an interference screw. Residual elongation of the FGTC following 150 cycles of cyclic loading was small (0.3 mm), but consistent across samples. No additional elongation occurred after 100 cycles. Video analysis revealed that the residual elongation was due to graft slippage, a finding congruent with previous studies [12]. In a follow-up test, one FGTC was cyclically loaded between 20N and 150N for the 150 cycles, but the total residual elongation increased only an additional 0.1 mm (data not shown). Taken together, these findings suggest that the surgical technique used in the study provides good initial graft stability. Furthermore, increasing the number of cycles performed during the cyclic loading test would not result in further residual elongation.

For these preliminary tests, the structural properties of the experimental FGTCs compare favorably with reported values for ultimate load (350N-945N) and stiffness (40N/mm-76N/mm) in the porcine model [6]. Likewise, all specimens failed by graft pullout, similar to previous laboratory studies examining the biomechanical properties of ACL reconstructions using BPTB grafts fixed with interference screws [12]. Therefore, we conclude that the surgical technique provided sufficient graft strength and stiffness and that the preceding cyclic loading did not adversely affect the failure properties of the FGTC to a great extent.

Using the values obtained in this study, a power analysis revealed that 10 subjects per group would be needed for future in-vitro studies comparing Mg-based and polymeric interference screws. Once it is determined that Mg-based interference screws can provide initial fixation of a BPTB graft to levels comparable of polymeric screws, we will conduct in-vivo studies in the goat model.

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