IN VITRO DEGRADATION TEST OF MAO COATED Mg IN SIMULATED BODY FLUID

Yajnesh Vedanaparti, Jonquil Mau, M.S., Savio L-Y. Woo, Ph.D., D. Sc., D. Eng.
Musculoskeletal Research Center, Department of Bioengineering, Swanson School of Engineering, University of Pittsburgh

INTRODUCTION
Anterior cruciate ligament (ACL) is one of the major knee stabilizers. The ACL is also the most commonly inured ligament of the knee, with over 200,000 injuries reported annually in the United States [1]. Currently, ACL reconstruction surgery is the primary method of repair. ACL reconstruction uses a tissue graft, usually derived from the patellar tendon, as a scaffold for the ACL to regrow on [2]. Although ACL reconstruction surgery allows for the patients to return to their daily activities in the short term, about 25% of the patients have reported complications in the long term [1].

To overcome these complications, our lab has developed a magnesium ring device that allows for the natural healing of the ACL. The Mg ring holds together the two transected ends of the ACL to re-stabilize the knee joint, load the injured site, and promote natural healing when coupled with an extracellular matrix (ECM) scaffold [1]. Aside from facilitating natural healing, the Mg ring allows for a less invasive procedure than ACL reconstruction surgery and is completely biodegradable and bioreabsorbable, thereby not requiring a second surgery to remove the device.

Due to Mg’s biodegradability, there has been an increasing interest in developing Mg based materials for clinical practices [3]. Mg has many advantages over other materials for clinical practices: its density is 1.6 to 4.5 times less than aluminum and steel, its elastic modulus and compressive yield strength are comparable to bone, and it is required for human metabolism of calcium [3]. However, Mg degrades relatively rapidly in the body, which causes a problem in that the device could potentially degrade before the ACL has a chance to heal enough to bear loads.

Micro-arc oxidation (MAO) coatings have been investigated as a potential solution to the biodegradability problem of Mg [4]. Gu et al. have reported that the corrosion resistance of Mg can be controlled using MAO coating time [4]. When a high applied voltage is coupled with an MAO coating solution, consisting of Na₃PO₄, Na₃SiO₃, and KF, an oxide layer is formed on the surface of the Mg disk [5]. This oxide layer can slow down the corrosion of Mg in the body and thereby allow the ring device to last longer at the site of repair. Due to the relative novelty of Mg based biomaterials, not enough research has been done on the effect of MAO coated Mg in the body.

OBJECTIVE
This study sought to study the effect of MAO coating time on the degradation/corrosion rate of Mg in the body, through an in-vitro degradation test.

SUCCESS CRITERIA
To accomplish the previously stated objective of the study, two success criteria were identified. The two parameters used to measure the degradation rate of Mg in the body were the percent change in mass of the Mg disk and the change in pH of the simulated body fluid. The first success criterion was to have a significant difference in the percent change in mass between the different coating times tested. The second criterion was to have a significant difference in the change in pH between the different coating times. A significant difference was defined as a p-value of less than 0.05 determined from an ANOVA test.

METHOD
Pure Mg specimens of 10 mm diameter, 1 mm thickness were used as samples for the MAO coating process. Three coating times were studied: 0 minutes (control), 2 minutes, and 5 minutes. Each time group had thirty samples of Mg disks. The Mg disks were first polished using 320, 600, and 1200 grit SiC abrasive papers. The samples were then stored in ethanol and dried in air prior to coating.

The MAO coating solution was prepared to contain 6M Na₃PO₄, 8M Na₃SiO₃, and 4M KF. The Mg disks were submerged in the MAO electrolytic solution as the anode, using a titanium wire as a connector as shown in Figure 1. An adjustable DC voltage source was used to apply coating under an applied voltage less than 400 V for the two coating times (2 min and 5 min). During the entire process, the container was kept cold using an ice bath.

Figure 1. The experimental setup for applying the MAO coatings: 1) pure polished Mg disk, 2) MAO coating solution, 3) container used to hold solution, 4) ice bath, and 5) DC power supply.

Once the coatings were applied, the samples were massed to determine their initial mass. Each disk was then individually submerged into a tube containing 50mL of Hank’s Balanced Salt Solution, whose pH was also initially measured, using fishing wire as shown in Figure 2. The Hank’s solution was prepared using one pack of H1387 Hank’s Balanced Salts and approximately 0.35g of sodium bicarbonate per 1L of deionized water and was used to simulate body fluid.
RESULTS

Figure 3 shows the percent change in mass of the Mg disks at each time point for each coating time, containing the corrosion products. All of the samples showed a positive change in mass until the 14-day time, after which a negative change can be seen. In general, the uncoated samples showed a greater percent change in mass than either the 2 minute and 5 minute coated samples, and the 5 minute coated samples generally showed a greater change in mass than the 2 minute samples.

Figure 4 shows the change in pH of the Hank’s solution for the uncoated, 2 min coated, and 5 min coated samples at each time point over the 4 weeks. Figure 4 shows the change in pH of the Hank’s solution at each time point for each coating time. In general, the change in pH showed a gradual increase over the 4 weeks. The uncoated samples showed the greatest change in pH at each time point while the 2 minute coated samples showed the smallest change in pH.

ANOVA statistical analysis resulted in a p-value of 0.224 for the percent change in mass between the different coating times and a p-value of 0.600 for the change in pH between the different coating times.

DISCUSSION

For significance to occur, the p-value had to be less than 0.05 as previously defined. For both the percent change in mass and the change in pH, the p-values were greater than 0.05, suggesting statistical insignificance. Therefore, the two defined success criteria failed, and the results were inconclusive. This study has shown that there is no statistical difference between the coating times.

These results were attributed to the many limitations inherently present in this study. First, the study was only four weeks long, which is not a long enough duration to study the degradation rate. As can be seen in Figure 3, the degradation did not start until the 14-day time point. Therefore, there were essentially only 3 data time points that contributed to the study’s objective. A second limitation occurs in that only 2 coating times were tested in this study, due to time constraints.

Figure 4 shows a greater change in pH for the uncoated samples than for the coated samples, suggesting a potential effect MAO coating has on the degradation rate. However, to establish a more concrete relationship between coating time and degradation rate, more coating times would be beneficial. Relating to the real-world applications of this study, a limitation occurs in that the simulated body fluid was not replaced during the 4 weeks. In-vivo, the body fluids are constantly replaced, potentially affecting the degradation rate of Mg.

To overcome these limitations, future studies should test more coating times for longer durations (preferably 6 months). In addition, the studies should focus on in-vivo testing to account for the various obstacles presented by the body. More research needs to be done regarding the slowing down of Mg degradation before clinically applying the ring to ACL tears.

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REFERENCES