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In vivo study of magnesium plate and screw degradation and bone fracture healing

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ABSTRACT

Each year, millions of Americans suffer bone fractures, often requiring internal fixation. Current devices, like plates and screws, are made with permanent metals or resorbable polymers. Permanent metals provide strength and biocompatibility, but cause long-term complications and may require removal. Resorbable polymers reduce long-term complications, but are unsuitable for many load-bearing applications. To mitigate complications, degradable magnesium (Mg) alloys are being developed for craniofacial and orthopedic applications. Their combination of strength and degradation make them ideal for bone fixation. Previously, we conducted a pilot study comparing Mg and titanium devices with a rabbit ulna fracture model. We observed Mg device degradation, with uninhibited healing. Interestingly, we observed bone formation around degrading Mg, but not titanium, devices. These results highlighted the potential for these fixation devices. To better assess their efficacy, we conducted a more thorough study assessing 99.9% Mg devices in a similar rabbit ulna fracture model. Device degradation, fracture healing, and bone formation were evaluated using microcomputed tomography, histology and biomechanical tests. We observed device degradation throughout, and calculated a corrosion rate of 0.40 ± 0.04 mm/year after 8 weeks. In addition, we observed fracture healing by 8 weeks, and maturation after 16 weeks. In accordance with our pilot study, we observed bone formation surrounding Mg devices, with complete overgrowth by 16 weeks. Bend tests revealed no difference in flexural load of healed ulnae with Mg devices compared to intact ulnae. These data suggest that Mg devices provide stabilization to facilitate healing, while degrading and stimulating new bone formation.

1. Introduction

Each year there are over 6 million bone fractures reported in the U.S. [1], approximately one third of which require internal fixation devices to help facilitate healing [2]. Currently, permanent and inert metals like titanium (Ti) alloys and stainless steel remain the gold standard for internal fixation devices; however, these materials are associated with various long-term complications such as interference with skeletal growth (particularly for pediatrics), tissue irritation, infection, interference with radiological imaging, and unfavorable esthetics (primarily for craniofacial implants) [3–7]. For these reasons, permanent fixation devices may necessitate invasive removal surgeries, increasing patient burden and risk, and draining valuable hospital resources [8,9]. To mitigate these concerns, resorbable polymer devices have been developed; however their mechanical properties often limit them as viable options for load-bearing applications [10]. Furthermore, studies have reported long-term foreign body reactions associated with polymeric device degradation, likely due to their acidic degradation products [3,11,12]. For these reasons, there remains a need to develop novel fracture fixation devices which mitigate long-term complications and eliminate the need for removal surgeries.

Unlike permanent metals and resorbable polymers, degradable magnesium (Mg) alloys provide a unique combination of strength and degradation. For these reasons, Mg alloys are being explored for various craniofacial and orthopedic applications. Interestingly,

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Mg alloys were first attempted as orthopedic devices over a century ago. Early Mg-based devices proved to be biocompatible, with low rates of infection; however, rapid degradation of Mg that is characteristic and to be expected with the current metallurgical knowledge known to date caused excessive hydrogen gas formation which ultimately prevented their clinical success [13,14]. Since these initial investigations, numerous advancements in alloying and corrosion control have been achieved. These advancements allow Mg and its alloys to be tailored to accommodate the desired mechanical properties and degradation behavior.

Numerous *in vitro* and *in vivo* studies have demonstrated the biocompatibility and osteoconductivity of these materials. Indirect assays, in which cells are exposed to media treated with Mg corrosion products, have shown cell viability with low concentrations of degradation product [3,13,15]. Similarly, direct assays, in which cells are cultured directly on Mg alloys, have shown cytocompatibility in the presence of ongoing Mg degradation [3,13,15]. Importantly, studies assessing Mg implants in endosseous sites, such as guinea pig [16], rat [17], and rabbit femora [18] have shown biocompatibility and normal foreign body response for various Mg alloys. In addition, several studies have shown high mineral apposition rates and increased bone mass and mineral density surrounding Mg implants in bone [10,16,19,20].

These data support the use of Mg implants as orthopedic devices; however there remains a lack of *in vivo* data assessing these materials as actual fixation plates and screws. For these reasons, we previously conducted a pilot study to evaluate Mg fixation plates and screws using a rabbit ulna fracture model [21]. In the pilot study, we compared 99.9% Mg plates and screws to clinically-used Ti devices, studying the effect of Mg degradation on fracture healing. Our pilot study results showed no significant difference in healing of fractures fixed with Mg or Ti devices. Interestingly, we also observed bone formation above the Mg, but not Ti, plates and screws.

In order to validate this pilot study data, we have conducted a more thorough follow-up assessment presented herein. In the present study, we aim to test our hypothesis that ongoing Mg device degradation will continue to stimulate osteogenic differentiation of local cell populations such as human bone marrow stromal cells (hBMSCs) and/or periosteal cells. In turn, this differentiation will result in local bone formation, providing additional fracture stabilization. Through this process, it is anticipated that Mg devices will be gradually replaced by bone growth, mitigating risk of long-term complications and device removal surgeries. To test this hypothesis, we have performed a thorough assessment of the degradation behavior and biological effect of Mg fixation devices. Specifically, we have designed and tested Mg fixation plates and screws in a rabbit ulna fracture model. Our results have confirmed our pilot study observations of new bone formation around Mg devices, especially above the devices where the periosteum and muscle tissue was present. In addition, we observed no inhibition of fracture healing. Mechanical testing demonstrated that healed fractures fixed with Mg devices responded similarly to healthy controls when subjected to three point bending. Taken together, these data demonstrate the efficacy of Mg fixation devices in a load bearing fracture site.

2. Materials and methods

2.1. Device design and development

Fixation devices (Fig. 1) were machined with 99.9% pure Mg (Goodfellow, Coraopolis, PA). All devices were designed to accommodate rabbit ulnar geometry. Plates were 20 × 4.5 mm with a thickness of 1–1.5 mm. Screws were 7 mm in length, with a shaft outer diameter of 1.75 mm and shaft inner diameter of 1 mm. Prior to implantation, devices were cleaned by sonicated washes in pure acetone and ethanol, followed by sterilization with gamma radiation (2×10^6 cGy, 23.5 Gy/min, cesium 137 source, Mark I 68, JL Shepherd and Associates, San Fernando, CA).

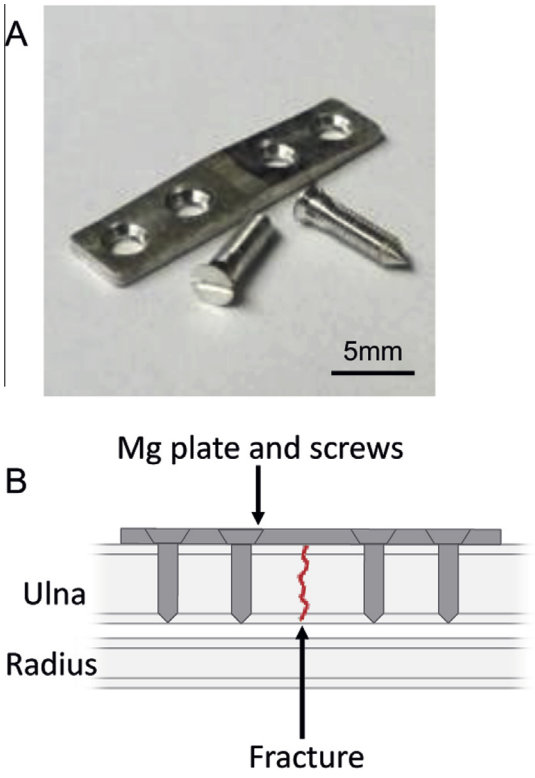

Fig. 1. Magnesium fixation plate and screws. Digital image showing devices prior to implantation (A). Schematic showing device placement with fractured ulna (B).

2.2. In vivo implantation

All animal experiments were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee. 12 New Zealand White rabbits (19 weeks of age, 3.5 ± 0.2 kg) were used in this study. Both ulnae (right and left) of all animals were used, providing a total of 24 surgical sites. Each time point consisted of 6 rabbits (12 surgical sites). Prior to surgery, animals were anesthetized and forearms were shaved and disinfected. A 2 cm incision was made over the ulna. Overlying skin and muscle was carefully retracted to expose the ulna. A complete ulnar osteotomy (0.5–1 mm thick) was created using a hand held drill. A fixation device consisting of one plate and four screws was then placed to stabilize the fracture. The incision was closed in layers with sutures and left un-casted. Animals were monitored daily for general behavior, movement, and food and water intake. In addition, forearms were checked thoroughly by visual inspection and gentle palpation for signs of infection or subcutaneous gas pocket formation. Based on consultation with the University of Pittsburgh's Division of Laboratory Animal Resources, observable gas pockets were removed with a sterile syringe. All gas pocket formations and removals were documented throughout the study.

2.3. X-ray imaging

X-ray imaging was used to monitor device placement and fracture healing throughout the study. All animals received X-rays immediately following surgery and every 2 weeks thereafter.

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