Published in final edited form as:

Acta Biomater. 2014 May ; 10(5): 2323–2332. doi:10.1016/j.actbio.2013.12.040.

Magnesium Alloys as a Biomaterial for Degradable Craniofacial Screws

Sarah E. Henderson, B.S.¹, Konstantinos Verdelis, Ph.D.², Spandan Maiti, Ph.D.¹, Siladitya Pal, Ph.D.¹, William L. Chung, D.D.S., M.D.³, Da-Tren Chou, B.S.¹, Prashant N. Kumta, Ph.D.^{1,4,5}, and Alejandro J. Almarza, Ph.D.^{1,4,5,*}

¹Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

²Department of Restorative Dentistry/Comprehensive Care, University of Pittsburgh, Pittsburgh, PA

³Oral and Maxillofacial Surgery, University of Pittsburgh, Pittsburgh, PA

⁴Department of Oral Biology, University of Pittsburgh, Pittsburgh, PA

⁵McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Abstract

Recently, magnesium (Mg) alloys have received significant attention as a potential biomaterial for degradable implants, and this study was directed at evaluating the suitability of Mg for craniofacial bone screws. The objective was to implant screws fabricated from commercially available Mg-alloys (pure Mg and AZ31) in-vivo in a rabbit mandible. First, Mg-alloy screws were compared to stainless steel screws in an in-vitro pull-out test and determined to have a similar holding strength (~40N). A finite element model of the screw was created using the pull-out test data, and the model can be used for future Mg-alloy screw design. Then, Mg-alloy screws were implanted for 4, 8, and 12 weeks, with two controls of an osteotomy site (hole) with no implant and a stainless steel screw implanted for 12 weeks. MicroCT (computed tomography) was used to assess bone remodeling and Mg-alloy degradation, both visually and qualitatively through volume fraction measurements for all time points. Histologic analysis was also completed for the Mg-alloys at 12 weeks. The results showed that craniofacial bone remodeling occurred around both Mg-alloy screw types. Pure Mg had a different degradation profile than AZ31, however bone growth occurred around both screw types. The degradation rate of both Mg-alloy screw types in the bone marrow space and the muscle were faster than in the cortical bone space at 12 weeks. Furthermore, it was shown that by alloying Mg, the degradation profile could be changed. These results indicate the promise of using Mg-alloys for craniofacial applications.

Keywords

biodegradable metal; magnesium; craniofacial implants; finite element modeling

© 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

*Corresponding Author: Department of Bioengineering, University of Pittsburgh, 566 Salk Hall, 3501 Terrace Street, Pittsburgh, PA, 15261, USA, Telephone: 412-648-8500, Fax: 412-624-6685, aja19@pitt.edu.

Disclosures

The authors have no conflicts of interest to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. INTRODUCTION

Magnesium (Mg) alloys have recently been a focus of degradable implant research. Results to date are demonstrating great promise for Mg-alloys to regenerate both hard and soft musculoskeletal tissues [1–30], which is valuable due to the necessity for engineering degradable craniofacial implants. Craniofacial implants, such as plates and screws, are used in procedures such as osteotomies, bone graft stabilization during reconstructions, and for trauma reconstruction [31]. Previously, craniofacial bone plates and screws have been fabricated from stainless steel, vitallium, chromium-cobalt, and other metal alloys [31]. Titanium has become the preferred permanent metal of choice due to its ability to osteointegrate [32]. However, it is estimated that 10–12% of craniofacial implants are removed due to infection, exposure, pain, and discomfort [32]. Resorbable polymer plates and screws are becoming more popular to use for craniofacial implants because they allow for fixation and stabilization but are not permanent [32]. However, biodegradable polymers, such as poly-L-lactide, are biomechanically inferior to their metal counterparts [33]. Two other shortcomings of the polymer implants include the need for a heating device to provide implant malleability and the need to tap the bone prior to screw placement [34]. Thus degradable metals have both the strength and the ability to degrade, unlike their polymer and permanent metal counterparts. In particular, much research has been done on the degradable metal Mg [1–30, 35–38].

Many previous studies have looked at the effect of Mg-alloys on long bones [1–30], but the effect of Mg-alloys on craniofacial bone has not been thoroughly studied. Mg-alloy rods and cylinders have been implanted into guinea pig femurs [21, 22], rat femurs [26, 29], and rabbit femurs [8, 11, 15–18, 20, 23–25, 27, 28, 30] and tibias [1, 5–7, 10, 12–14]. Mg-alloy screws have been tested in-vitro [35–38] and have also been implanted into rabbit femurs [18] and tibias [3, 4, 9], as well as sheep hip bones [2, 19]. Studies of inflammatory and immune response show that degrading Mg scaffolds show good biocompatibility and react in-vivo with an appropriate inflammatory host response [1, 4, 11, 20, 24–26, 29, 30]. It has been shown that degrading Mg implants promote bone formation [2–6, 8, 11–14, 16, 18, 19, 21–23, 26–30] and osteoblastic activity [11, 19, 21, 23, 26, 29]. In these previous studies several different types of Mg-alloys have been implanted, specifically the commercially available alloy AZ31 (2.5–3.5 wt% aluminum, 0.6–1.4 wt% zinc, 0.2–1.0 wt% manganese) has been previously tested in bone in-vivo as rods [21, 28] and as screws [2, 19]. All four studies revealed new bone formation around the AZ31 implants [2, 19, 21, 28]. One of the studies showed that the corrosion behavior of the AZ31 screw differed depending on the location of the AZ31 in the original tissue [19]. Bone formation was noted around the Mg rods, but not in the surrounding soft tissue [21]. There was also little change to the blood composition and no inflammation from the degrading implant [28].

Long bones and flat bones, such as the craniofacial bones, form differently during development resulting in differences in the organic and inorganic phases [39]. Long bones and craniofacial bones also undergo different loading. Long bones can undergo extensive loading, as can the mandible, but the skull normally undergoes minimal loading. The blood flow in various regions of the body is also different. All of these factors could affect the degradation rates of the Mg-alloys and also the bone regeneration in these areas. An investigation should be conducted to see if there are differences in how Mg behaves in the craniofacial region compared to the long bones.

As a first step towards improving degradable craniofacial plates and screws, this study aimed to evaluate the use of Mg as a degradable biomaterial. The objective of this study was therefore to implant screws fabricated from commercially available Mg-alloys (pure Mg and

AZ31) in-vivo in a rabbit mandible. First the Mg-alloy screws were compared to commercially available stainless steel screws in an in-vitro pull-out test to determine the holding strength. A custom finite element code was then developed to simulate these pull-out tests on a computer. Factors contributing towards the pull-out strength were determined using this computational model. Then, the two Mg-alloys were implanted for three time periods (4, 8, or 12 weeks). The two controls consisted of only osteotomies (holes) with no implant or a stainless steel screw implanted for 12 weeks. MicroCT (computed tomography) was used to assess bone remodeling and Mg degradation for all time points, and histologic analysis was also used for the Mg-alloys at 12 weeks.

2. METHODS

2.1 Screw Fabrication

Bone screws were designed for the rabbit mandible and fabricated from commercially available pure Mg and a Mg aluminum zinc alloy (AZ31) purchased from Goodfellow (Oakdale, PA). The pure Mg was 99.9% pure, and the AZ31 alloy contained 2.5–3.5 wt% aluminum, 0.6–1.4 wt% zinc, and 0.2–1.0 wt% manganese with the remainder being Mg. Similarly sized, commercially available stainless steel screws were purchased from Small Parts (Seattle, WA). The screws were approximately 1 mm in diameter with M0.25 threads and the shaft was approximately 2 mm in length (Figure 1A).

The Mg-alloy screws were fabricated by the University of Pittsburgh Swanson Center for Product Innovation (SCPI) using CNC (computerized numerical control) machining. After fabrication, the screws were sonicated in isopropanol to remove any residual debris. The screws then underwent a stress relief heat treatment at 205 °C for 90 minutes in an argon atmosphere. Next, the screws underwent 3 cycles of sonication in isopropanol for 3 minutes each following which they were allowed to air dry. All of the screws were stored in air tight containers until documentation and use in this study. Documentation included weighing and imaging each individual screw.

2.2 In-vitro Testing and Finite Element Modeling

2.2.1 Pull-Out Test—A mechanical test was designed to compare the holding strength of the pure Mg and AZ31 screws to stainless steel screws. A material testing system was set up for complete axial pull-out tests (Figure 2A) (MTS Insight, MTS Systems, Eden Prairie, MN). Synthetic bone made of solid rigid polyurethane foam (ASTM F-1839-08) from Sawbones (a division of Pacific Research Laboratories, Inc. Vashon, WA) was used as the control material for the pull-out tests. Screws were placed in the foam after the holes were predrilled and tapped. A testing rate of 5 mm/min was used according to ASTM standard F543-07. The maximum force needed to release the screw from the foam was recorded for each screw. A one-way ANOVA with Tukey's post hoc test was used to compare the results with a statistical significance set at $p < 0.05$.

2.2.2 Computational Techniques—Custom three-dimensional (3D) finite element (FE) software was developed to simulate experimental pull-out tests and study the effect of various mechanical properties on the observed pull-out strength. Synthetic bone was modeled as a cylinder with a diameter of 5 mm with the screw inserted along the longitudinal axis (Figure 2Bi). The screw and the cylindrical bone were discretized with 3731 and 14254 four-noded tetrahedral finite elements, respectively. Details of the finite element discretization are shown in Figure 2Bii. The outer surface of the bone was held rigidly in place with fixed boundary conditions. The diameter of the cylinder was chosen such that boundary effects on the stress field were minimal in the vicinity of the screw. All finite element nodes on the surface of the screw head were given a prescribed displacement

of 5 mm/min to mimic the experimental loading condition. Young's modulus of the synthetic bone was assumed to be 0.5 GPa while that for the screw was varied to simulate different materials. Interface of the screw and the synthetic bone was represented with novel zero thickness interfacial finite elements. A phenomenological interfacial constitutive law was developed to govern the mechanical response of the interfacial region (see Figure 2Biii). This law takes into account the initial adhesion and interlocking of the bone and screw threads (initial rising region), onset of failure in surrounding bone material and subsequent softening of the interface (intermediate falling region), and ultimate frictional sliding (final horizontal region) leading to complete pull-out of the screw.

2.2.3 Calibration of FE model parameters—Our interfacial model possesses three parameters; maximum interfacial strength σ_c , relative sliding distance δ_c at which this strength is achieved, and a constant frictional traction τ_f . These parameters were calibrated using experimental data from the pull-out tests for pure Mg screws. Modulus of the screw material was assigned to that of Mg (45 GPa). Parametric range for σ_c was taken to be lower than the yield strength of Mg (~21 MPa) so that the screw itself never failed, as observed in the experiments. The entire pull-out force versus applied displacement response was simulated and represented in Figure 2C. A least square optimization technique was utilized to extract model parameters that fit the experimental curves best. A sensitivity analysis was performed to assess the effect of these parameters on the pull-out strength, *i.e.*, the peak of the pull-out curve.

2.3 Animal Procedures

Fifteen skeletally mature, female, New Zealand White rabbits approximately 1 year in age weighing between 5–7 kg were purchased from Myrtle's Rabbitry, Inc. (Thompsons Station, TN) and Charles River Laboratories International, Inc. (Wilmington, MA). All rabbits were examined by a veterinarian before surgery and were found to be in good health. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh and in accordance with the National Institutes of Health guidelines for the use of laboratory animals.

Before the implantation surgery, all rabbits were sedated with ketamine and xylazine and intubated, and a surgical plane of anesthesia was maintained with isoflurane. The surgical site was shaved and scrubbed with betadine and sterilely draped. The incisure of facial vessels, located where the curve of the mandible and the posterior end of the molar region meet, was palpated and an incision was made slightly posterior to the incisure. An incision was made with a sterile scalpel, and the skin and muscle were reflected to reveal the bone. A sterile drill with a 0.85 mm drill bit was used to pre-drill holes and the holes were tapped. Two screws of the same material were placed on one side of the mandible (Figure 1B,C). After implantation, the muscle and skin were sutured with resorbing sutures. Two screws of another material were placed on the opposite side of the mandible using the same procedure. Screw types were not mixed on a per side basis to avoid galvanic corrosion.

Following the surgical procedure the rabbits were extubated and observed until they had recovered from the anesthesia. The rabbits were monitored daily for the first week after surgery. The rabbits were given the option of soft food for the first few days after surgery and normal hard pellet food was available throughout the recovery period. Antibiotic injections were given twice a day for five days post-op. Skin sutures were removed two weeks post-op. The Mg-alloy screws were not able to be visualized using X-rays, likely due to the size of the screws and the resolution of the X-rays, as well as the similarity in density between the bone and the Mg-alloy screws. Also, no hydrogen pockets were observed physically or in the X-rays. At the appropriate endpoint, the rabbits were sedated with

ketamine and xylazine and euthanized with an intravenous injection of pentobarbital sodium. After death, the mandibles were collected, and assessed grossly, then wrapped in PBS soaked gauze and frozen until further processing and analysis.

The pure Mg and AZ31 screws were implanted in at least three different rabbits for each time point of 4, 8, and 12 weeks. Then after analysis of the longitudinal data, the control rabbits were implanted and incubated for 12 weeks. The control groups included a group with stainless steel screws implanted, and a group where osteomies (holes) were drilled into the mandible but no screws were placed. Naïve control bone was also examined.

2.4 In-Vivo Analysis

2.4.1 Qualitative MicroCT Assessment—First, all samples of the study were scanned with a 44.5 μm voxel size (80 kV, 500 μA) on an in-vivo Inveon micro computed tomography (CT) system (Siemens AG, Munich, Germany), referred to as Scan Set A, were performed to obtain a global perspective of overall screw integrity. The entire left and right sides of the mandible were scanned in Set A, with only the condyles, incisors, and most of the molars removed, and at least three samples were scanned per type and time point. Three-dimensional (3D) volumes were generated from lateral projections for every scan using the Siemens reconstruction software, and exported as dicom files for analysis using Mimics 12 software (Materialise, Leuven, Belgium). Regions of interest were user-defined around the screws for further analysis by generation of 3D renderings using a threshold initially set at the default software value for CT bone. New bone formation and the amount of residual magnesium were visually assessed.

Higher resolution microCT imaging was performed on a smaller subset of samples, referred to as Scan Set B (two screws per type and time point), using an in-vivo VivaCT 40 (Scanco Medical, Basselsdorf, Switzerland) or an ex-vivo SkyScan 1172 (Bruker-Skyscan, Contich, Belgium) system with a 10.5 μm (70 kV, 110 μA) or 12.9 μm (59 kV, 167 μA) voxel size, respectively. The whole mandible samples were cut down to only include the region around the screw implants. The 4 and 8 week samples from set B were scanned on the VivaCT 40 system, while the 12 week set B samples and the control samples were scanned on the Skyscan 1172 system. 3D volumes were generated as the respective file type for each system format (isq files for Scanco VivaCT 40 and a stack of bmp files from Skyscan 1172). These higher resolution set B scans and control scans were imported into Mimics 12 software for analysis. Bone remodeling and magnesium degradation were visually inspected in both the two-dimensional (2D) slices and 3D reconstructions from Mimics.

The higher resolution Set B screw scans were also qualitatively described by individual screw parts (Head, Shaft, Shaft in Cortical Bone, Shaft in Marrow Space) by calculating screw volume fraction using the Skyscan DataViewer and CTAn software. First, the scans were reoriented to align the standard $\times$ plane with the screw of interest. Regions of interest were subsequently defined around the screws extending to the boundary with the adjacent bone. As the peak representing the screw voxels mineral densities was distinct from the respective peak of background and degradation product voxels in distribution of mineral densities within the regions of interest histograms, a threshold for segmentation of the residual Mg screw was set at the inflection point between the two peaks (Figure 3). The volume of the remaining Mg-alloy was calculated from the microCT and expressed as a bone volume fraction meaning the remaining screw volume fraction (referred to as volume fraction) of the theoretical initial screw volume of interest. First, the total volume fraction of the remaining screw alloy was determined. The theoretical total screw volume was determined by assuming the shaft of the screw was a solid cylinder 1 mm in diameter and 2 mm long, plus the volume of the screw head as described below. As a difference in the degradation rate of the screws in the different regions of the bone (cortical and bone

marrow) and muscle was preliminarily assessed visually, the screws were for the purposes of the volume fraction measurements first divided into two parts, head and shaft. The shafts in turn were divided into two parts, the one inside cortical bone and the one inside the bone marrow space. The theoretical volume in the shaft, cortical bone, and bone marrow space measurements for volume fraction were calculated by assuming a theoretical cylinder with a diameter of 1 mm and a height that was equal to the height of the stack of images being analyzed. The theoretical volume of the head of the screw for volume fraction was calculated as the volume of a cylinder the size of the head (2 mm in diameter, 1 mm long) minus the volume of a rectangle from the drive slot in the screw.

2.4.2 Histology—The 12 week samples from the pure Mg and AZ31 screws were formalin fixed and sent to Alizeé Pathology (Thurmont, MD) for processing by embedding in 70/30 methylmethacrylate (MMA). Sections were ground and polished to a thickness of approximately $65 \pm 15 \mu\text{m}$. The slides were stained with hematoxylin and eosin and evaluated with light microscopy.

3. RESULTS

3.1 Pull-Out Test and FE Modeling

Pure Mg and AZ31 screws exhibited pull-out forces similar to that for the stainless steel screws when pulled out of a synthetic bone material. The pull-out strength for all of these screw materials was approximately 40 N (Figure 4A) with no statistical differences between the groups. This observation led us to believe that the threads of the Mg-alloy screws gripped the synthetic bone in the same manner as the stainless steel screws. A systematic computational study was then undertaken to gain further understanding of the mechanical parameters governing the pull-out strength. First the modulus of the individual screw material was taken as 1 GPa, 43.25 GPa (AZ31 alloy), 45 GPa (pure Mg), and 210 GPa (stainless steel), respectively, while all other model parameters remained constant. Simulated pull-out force profiles for all these cases have been plotted, see Figure 4B. These curves signify that for a constant diameter of the screw with similar interfacial conditions, *e.g.*, thread profile and depth of penetration, the pull-out strength remains essentially constant. Next the effect of the interfacial strength σ_c on the pull-out strength was examined keeping all other model parameters constant. The mean value of this parameter was found to be 4.5 MPa when calibrated against experimental pull-out forces for pure Mg screws. Figure 4C presents simulated pull-out curves for $\sigma_c = 2.25$ MPa, 4.5 MPa, and 6.75 MPa. Observe from Figure 4C that this variation of the interfacial strength resulted in more than a three-fold increase in the predicted pull-out strength. Thus it was concluded that pull-out strength is highly sensitive on the interfacial strength between the screw and the bone. Similar parametric studies were performed to examine the effect of remaining two interfacial parameters, δ_c and τ_f , on the pull-out strength (not reported here). However, we found that the pull-out strength did not exhibit any appreciable sensitivity to these parameters.

3.2 Qualitative MicroCT Analysis

Signs of degradation were observed for the pure Mg screws at 4 weeks in scans from both sets A and B, Set B is shown in Figure 5, as evidenced by the reduced brightness in certain regions of these screws. Bone remodeling occurred in the area surrounding the screws. Any bone noted around and over the screw heads was considered new bone growth, as the heads of the screws were completely above of the native bone at the time of implantation, while the bottom of the screw heads were placed flush with the bone. At 4 weeks, the pure Mg screws were in contact with the bone. Then at 8 weeks the shafts of the pure Mg screws appeared to be mostly degraded, as seen by the presence of holes within the screw bulk in the images, as well as major bone resorption with little new bone formation around the

screws (Figure 5, Set B Scans). By 12 weeks, the bone resorption seemed to subside, and new bone appeared to be growing over the pure Mg screw in 71% of the screws imaged from both sets of scans, while at the same time bone resorption under the head of the screw was still noted in approximately 85% of the pure Mg screws.

The AZ31 screws, in both scan sets A and B, showed little sign of degradation at 4 weeks (Figure 5, Set B Scans), and the surrounding tissue seemed to remain intact. The AZ31 set A scans at 8 and 12 weeks continued to show little degradation; however, differences in degradation products were only visible in the higher resolution set B scans and not the set A scans. At 8 weeks, in set B, the AZ31 screws began to show signs of degradation, with regions of reduced brightness appearing in the shaft region of the screws (Figure 5, Set B Scans). The adjacent tissue continued to remodel around the screw, as seen by some new bone growth around the screws, and little bone resorption. At 12 weeks, the set B AZ31 screws continued to show signs of degradation with a larger area of decreased brightness in the shaft of the screw (Figure 5, Set B Scans). The surrounding tissue continued to remodel and grow around the screws. From both sets of scans, there were signs of bone resorption under the head of the screw in approximately 71% of the cases at 12 weeks. Bone grew around and over the head of the AZ31 screws in approximately 57% of the cases for the AZ31 screws at 12 weeks. An example of the bone overgrowth and the approximate location of the original bone line is shown in Figure 6 clearly showing the overgrowth of bone over the head of the AZ31 screw at 12 weeks.

Based on the results from the longitudinal portion of the study, the control samples included the empty holes (negative control), and stainless steel screws (external control) implanted in the rabbits for 12 weeks, scanned as part of the high resolution Set B Scans. On a qualitative basis, after 12 weeks, the mandibles with holes and without screws showed many signs of remodeling. The original holes were not apparent (Figure 7), and new bone growth was seen throughout the region where the holes existed. When compared to naïve control bone, the remodeled bone appeared to be rougher and thicker (Figure 7). After 12 weeks, the stainless steel screws were fully intact (Figure 7). Bone growth occurred around the stainless steel screws, but growth over the screws and bone resorption under the screws were not observed.

For the longitudinal portion of the study, from the set B scans, the total volume fraction for both screw types seemed to decrease over time (Figure 8A). The head of the screw remained fairly intact between 4 and 8 weeks, but not at 12 weeks (Figure 8B). The volume fraction of the head at 12 weeks for the pure Mg was 31.3% and the AZ31 was 61.5%. In the Set B Scans, the screw shaft volume fraction seemed to decrease with time for both screw types (Figure 8C). The degradation rate varied depending on the surrounding tissues. Separating the shaft into the cortical bone region and the bone marrow region, the volume fraction in the cortical space seemed to remain fairly constant over time (Figure 8D), while the remaining volume fraction in the bone marrow space seemed to decrease with time for both screw types (Figure 8E). The bone marrow space volume fraction for the AZ31 screws at 4 weeks was not calculated because the implanted screws did not penetrate the bone marrow space. By 8 weeks most of the pure Mg screw shaft in the bone marrow space seemed to be degraded with a volume fraction of approximately 12.0% (Figure 8E), while the volume fraction in the cortical space was 43.4% (Figure 8D). By 12 weeks, more material seemed to be degraded for both screws types in the bone marrow space, with a mean volume fraction value of 9.6% in the pure Mg and 20.0% in the AZ31 group (Figure 8E), compared to 71.5% and 44.2% for the pure Mg and AZ31 groups, respectively, in the cortical bone (Figure 8D).

3.3 Histology

Histology confirmed the findings noted in the microCT images for pure Mg and AZ31 groups at 12 weeks, identifying the brighter areas around the screws on these images as

newly formed bone. The sections showed that in some locations the shafts of the pure Mg screws were completely degraded at 12 weeks (Figure 9A). New bone was seen growing up next to the pure Mg screws and there was also new bone growing over the head of the screws. The AZ31 screws showed continuous bone to metal interfaces, as the bone was integrated with the threading, with little overall degradation of the AZ31 screws. New bone was seen growing up next to and in contact with the metal for both screw types. It should be noted that significant bone overgrowth was also observed around the AZ31 screws.

4. DISCUSSION

Over time, the two Mg-alloys behaved differently in-vivo. The degradation products for the pure Mg screws appeared to be removed, while the degradation products for the AZ31 screws appeared to remain at the site. In the AZ31 screws, two density levels were observed in the screw shaft, while the Mg screws only had one density level. Comparing the denser regions of the remaining material for both screws types, the screws were similar in volume. However, when including the lower density region of the AZ31 screws, the volume was much larger. The lower density region in the AZ31 screws could be the remaining degradation product, while the pure Mg screws degradation product might have been resorbed and not observed in microCT.

Bone remodeling and growth was observed around both screw types after 12 weeks of implantation. The degradation rate of the screws was site-dependent, as the shafts degraded faster in the marrow space compared to the cortical bone. More studies need to be performed to see how well the screws can anchor a plate for up to at least 8 weeks in-vivo. Ideally, the Mg-alloy screws would allow for initial stabilization and adequate strength to permit bone healing during function, and then decrease in strength with time so that the physiological force is then transferred to the bone [31]. The amount of time for craniofacial bone to heal and varies, but one study reported that after 4 weeks a mandible fracture with screw and plate fixation was at an advanced stage of bony union demonstrating functional stability, and by 8 weeks the fracture sites had fully remodeled [40]. If the degradation rate is not ideal, alloying Mg with Al, Zn, and Mn as is the case with AZ31, the degradation profile could be controlled. Thus, these results indicate the promise of using Mg-alloys for craniofacial applications.

Comparing the in-vitro pull-out test results to previous studies [3, 35–38], similar pull-out forces were determined amongst screw types tested in each study, but the screws studied herein were much smaller than other screws tested previously, so the absolute force values were expectedly lower in this study. It is therefore difficult to do a direct comparison between the studies because of several factors including different screw size, design, and material, different bone or synthetic bone materials properties, and the screw insertion technique [3]. Nevertheless, our data showed that the design of our Mg-alloy screws resulted in a holding strength similar to stainless steel screws of a comparable size. The results agree with the previous studies showing similar holding strengths between screws of varying types of similar sizes [3, 35–38].

To gain a mechanistic insight into these pull-out experiments and the parameters that control the pull-out strength, this study resorted to a finite element based computational approach. The finite element algorithm, developed specifically for this purpose, allowed this study to examine separately the effects of mechanical properties of the screw material and the interface condition. The primary mechanical parameters investigated were the modulus of the screw material and the interface properties between screw and bone. Interface properties are in turn dependent on a number of factors such as thread profile, failure strength of the bone, coefficient of friction between the bone and the screw and the area of contact. The

model allowed this investigation to lump all these effects together in a novel interfacial constitutive law. The pull-out strength indeed was determined to be insensitive to the modulus of the screw material. It only influenced the initial slope of the pull-out curve when its magnitude was comparable to that of the synthetic bone. Peak pull-out force was related to the onset of weakening of the interface and exhibited a strong correlation with the interfacial strength. These simulations along with these pull-out experiments point to an important conclusion that the pull-out strength can be controlled by initiating a proper design of the interface, for example by choice of thread profiles, as long as failure strength of the screw material is higher than that of the surrounding bone.

These in-vivo Mg results were similar in the mandible as compared to the long bones. Bone formation occurred around the Mg-alloy screws. The Mg-alloy screws were also biocompatible, with no evident signs of inflammatory response and in general, a healthy profile of the surrounding tissues was observed on histological sections. However, the screws seemed to degrade much faster in the marrow space as compared to the cortical bone [16, 29]. The differences in the organic and inorganic phases of long bones compared to flat bones seemed to make little difference in the behavior of the Mg-alloy screws, at least in the mandible, which is loaded similar to long bones. Implanting Mg-alloys in the skull may however have different outcomes.

Comparing to the previous AZ31 screw studies, similar results were noted. New bone formation occurred around the implants [2, 19]. A difference in the degradation of the screws between the cortical bone and the marrow space or the head of the screw was seen, which agrees with the study that reported notable differences based on the location of the implant in the original tissue [19]. A limitation of this study was in the screw placement, some screws were placed in the region of the mandible with only cortical bone, so for some screws the marrow space could not be analyzed.

The Mg-alloys did not seem to inhibit bone remodeling around the holes created in the mandible. The bone with holes without screws was observed to remodel with excess bone growth. New bone was seen growing around the pure Mg, AZ31, and stainless steel screws; however, overgrowth was not observed with the stainless steel screws. It is possible that Mg helped the periosteum to promote bone growth over the heads of the screws. A previous study supported the hypothesis that the major corrosion products of Mg-alloys are the major origin of enhanced bone growth around the implant [8], the current study seemed to agree with this previous study's findings. However, more studies and longer term studies are warranted to understand the benefits and drawbacks of new bone growth in the craniofacial region.

Future studies will be needed to specifically determine if the degrading Mg or any of the alloying elements have any beneficial or detrimental effect on the surrounding craniofacial tissues, as well as overall systemic health. Novel alloys will need to be developed and tested incorporating suitable alloy and device designs exhibiting optimal degradation characteristics while promoting bone regeneration without inducing any undesired effects on the surrounding tissues. FE modeling can be used to compare future screw designs to determine the most appropriate design for the specific application. Now, as this system is in place, consideration for a bone fixation device can begin, such as plates and screws. Evaluation of shear strength, torque, and other mechanical properties, as well as analysis of bone stabilization from the fixation device needs to be completed. In addition to using Mg-alloys to improve craniofacial plates and screws, these results are also beneficial for conducting research for improving other craniofacial and long bone prosthetics.

5. CONCLUSIONS

Mg degradation and bone remodeling occurred with both Mg-alloy screw types. The pure Mg degraded with a different profile than the AZ31. Mg-alloy screws degraded at varying rates throughout the length of the screw depending on its location within the bone, subject to whether the screw was in the cortical bone, in the marrow space, or in the muscle. Alloying of Mg will allow for control of the degradation rates, which can then be adapted for site specific results. FE modeling can also be used to help design future screws for specific applications. The results of this study show promise for the future use of degradable Mg-alloys for craniofacial fixation applications.

Acknowledgments

We would like to acknowledge J. Andrew Holmes at the University of Pittsburgh SCPI for screw fabrication. We would also like to acknowledge funding from the National Science Foundation under grant number 0812348, as well as from the National Institutes of Health under grant number T32 EB003392-01, and the University Of Pittsburgh School Of Dental Medicine. PNK would also like to acknowledge support from the Edward R. Weildein Chair Professorship and the Center for Complex Engineered Multifunctional Materials (CEMM) at the University of Pittsburgh.

References

1. Bondarenko A, Hewicker-Trautwein M, Erdmann N, Angrisani N, Reifenrath J, Meyer-Lindenberg A. Comparison of morphological changes in efferent lymph nodes after implantation of resorbable and non-resorbable implants in rabbits. *BioMedical Engineering Online*. 2011;10. [PubMed: 21269508]
2. Duygulu O, Alper Kaya R, Oktay G, Arslan Kaya A. Investigation on the potential of magnesium alloy AZ31 as a bone implant. *Materials Science Forum*. 2007; 546–549:421–4.
3. Erdmann N, Angrisani N, Reifenrath J, Lucas A, Thorey F, Bormann D, et al. Biomechanical testing and degradation analysis of MgCa0.8 alloy screws: A comparative in vivo study in rabbits. *Acta Biomaterialia*. 2011; 7:1421–8. [PubMed: 21050898]
4. Erdmann N, Bondarenko A, Hewicker-Trautwein M, Angrisani N, Reifenrath J, Lucas A, et al. Evaluation of the soft tissue biocompatibility of MgCa0.8 and surgical steel 316L in vivo: A comparative study in rabbits. *BioMedical Engineering Online*. 2010;9. [PubMed: 20163724]
5. Hampp C, Ullmann B, Reifenrath J, Angrisani N, Dziuba D, Bormann D, et al. Research on the biocompatibility of the new magnesium alloy LANd442-An in vivo study in the rabbit tibia over 26 weeks. *Advanced Engineering Materials*. 2012; 14:B28–B37.
6. Hampp C, Angrisani N, Reifenrath J, Bormann D, Seitz JM, Meyer-Lindenberg A. Evaluation of the biocompatibility of two magnesium alloys as degradable implant materials in comparison to titanium as non-resorbable material in the rabbit. *Materials Science and Engineering C*. 2013; 33:317–26.
7. Huehnerschulte TA, Reifenrath J, von Rechenberg B, Dziuba D, Seitz JM, Bormann D, et al. In vivo assessment of the host reactions to the biodegradation of the two novel magnesium alloys ZEK100 and AX30 in an animal model. *BioMedical Engineering Online*. 2012;11. [PubMed: 22394477]
8. Janning C, Willbold E, Vogt C, Nellesen J, Meyer-Lindenberg A, Windhagen H, et al. Magnesium hydroxide temporarily enhancing osteoblast activity and decreasing the osteoclast number in peri-implant bone remodelling. *Acta Biomaterialia*. 2010; 6:1861–8. [PubMed: 20035905]
9. Kim S-M, Jo J-H, Lee S-M, Kang M-H, Kim H-E, Estrin Y, et al. Hydroxyapatite-coated magnesium implants with improved in vitro and in vivo biocorrosion, biocompatibility, and bone response. *Journal of Biomedical Materials Research Part A*. 2013; 0:000.
10. Krause A, Von Der Höh N, Bormann D, Krause C, Bach FW, Windhagen H, et al. Degradation behaviour and mechanical properties of magnesium implants in rabbit tibiae. *Journal of Materials Science*. 2010; 45:624–32.
11. Li Z, Gu X, Lou S, Zheng Y. The development of binary Mg–Ca alloys for use as biodegradable materials within bone. *Biomaterials*. 2008; 29:1329–44. [PubMed: 18191191]

12. Meyer-Lindenberg A, Thomann M, Krause A, Bormann D, Von Rechenberg B, Windhagen H. Investigation on the use of a magnesium alloy as a new resorbable implant material for orthopaedic surgery. *Kleintierpraxis*. 2010; 55:349–63.
13. Thomann M, Krause C, Bormann D, Von Der Höh N, Windhagen H, Meyer-Lindenberg A. Comparison of the resorbable magnesium alloys LAE442 und MgCa0. 8 concerning their mechanical properties, their progress of degradation and the bone-implant-contact after 12 months implantation duration in a rabbit model. *Materialwissenschaft und Werkstofftechnik*. 2009; 40:82–7.
14. Thomann M, Krause C, Angrisani N, Bormann D, Hassel T, Windhagen H, et al. Influence of a magnesium-fluoride coating of magnesium-based implants (MgCa0. 8) on degradation in a rabbit model. *Journal of Biomedical Materials Research - Part A*. 2010; 93:1609–19. [PubMed: 20073078]
15. Von Der Höh N, Krause A, Hackenbroich C, Bormann D, Lucas A, Meyer-Lindenberg A. Influence of different surface machining treatments of resorbable implants of different magnesium alloys - A primary study in rabbits. *Deutsche Tierärztliche Wochenschrift*. 2006; 113:439–46. [PubMed: 17233279]
16. Von Der Höh N, Bormann D, Lucas A, Denkena B, Hackenbroich C, Meyer-Lindenberg A. Influence of different surface machining treatments of magnesium-based resorbable implants on the degradation behavior in rabbits. *Advanced Engineering Materials*. 2009; 11:B47–B54.
17. Von Der Höh N, Von Rechenberg B, Bormann D, Lucas A, Meyer-Lindenberg A. Influence of different surface machining treatments of resorbable magnesium alloy implants on degradation – EDX-analysis and histology results. *Materialwissenschaft und Werkstofftechnik*. 2009; 40:88–93.
18. Von Der Höh N, Bormann D, Lucas A, Thorey F, Meyer-Lindenberg A. Comparison of the in vivo degradation progress of solid magnesium alloy cylinders and screw-shaped magnesium alloy cylinders in a rabbit model. *Materials Science Forum*. 2010; 638–642:742–7.
19. Willbold E, Kaya AA, Kaya RA, Beckmann F, Witte F. Corrosion of magnesium alloy AZ31 screws is dependent on the implantation site. *Materials Science and Engineering: B*. 2011; 176:1835–40.
20. Willbold E, Kalla K, Bartsch I, Bobe K, Brauneis M, Remennik S, et al. Biocompatibility of rapidly solidified magnesium alloy RS66 as a temporary biodegradable metal. *Acta Biomaterialia*. 2013
21. Witte F, Kaese V, Haferkamp H, Switzer E, Meyer-Lindenberg A, Wirth CJ, et al. In vivo corrosion of four magnesium alloys and the associated bone response. *Biomaterials*. 2005; 26:3557–63. [PubMed: 15621246]
22. Witte F, Fischer J, Nellesen J, Crostack H-A, Kaese V, Pisch A, et al. In vitro and in vivo corrosion measurements of magnesium alloys. *Biomaterials*. 2006; 27:1013–8. [PubMed: 16122786]
23. Witte F, Ulrich H, Palm C, Willbold E. Biodegradable magnesium scaffolds: Part II: Peri-implant bone remodeling. *Journal of Biomedical Materials Research - Part A*. 2007; 81:757–65. [PubMed: 17390322]
24. Witte F, Ulrich H, Rudert M, Willbold E. Biodegradable magnesium scaffolds: Part I: Appropriate inflammatory response. *Journal of Biomedical Materials Research - Part A*. 2007; 81:748–56. [PubMed: 17390368]
25. Witte F, Fischer J, Nellesen J, Vogt C, Vogt J, Donath T, et al. In vivo corrosion and corrosion protection of magnesium alloy LAE442. *Acta Biomaterialia*. 2010; 6:1792–9. [PubMed: 19822226]
26. Xu L, Yu G, Zhang E, Pan F, Yang K. In vivo corrosion behavior of Mg-Mn-Zn alloy for bone implant application. *Journal of Biomedical Materials Research - Part A*. 2007; 83:703–11. [PubMed: 17549695]
27. Xu L, Pan F, Yu G, Yang L, Zhang E, Yang K. In vitro and in vivo evaluation of the surface bioactivity of a calcium phosphate coated magnesium alloy. *Biomaterials*. 2009; 30:1512–23. [PubMed: 19111896]
28. Yang JX, Cui FZ, Lee IS, Zhang Y, Yin QS, Xia H, et al. In vivo biocompatibility and degradation behavior of Mg alloy coated by calcium phosphate in a rabbit model. *Journal of Biomaterials Applications*. 2012; 27:153–64. [PubMed: 21363872]

29. Zhang E, Xu L, Yu G, Pan F, Yang K. In vivo evaluation of biodegradable magnesium alloy bone implant in the first 6 months implantation. *Journal of Biomedical Materials Research Part A*. 2009; 90A:882–93. [PubMed: 18618719]
30. Zhang S, Zhang X, Zhao C, Li J, Song Y, Xie C, et al. Research on an Mg–Zn alloy as a degradable biomaterial. *Acta Biomaterialia*. 2010; 6:626–40. [PubMed: 19545650]
31. Turvey TA, Proffit WP, Phillips C. Biodegradable fixation for craniomaxillofacial surgery: A 10-year experience involving 761 operations and 745 patients. *International Journal of Oral and Maxillofacial Surgery*. 2011; 40:244–9. [PubMed: 21185695]
32. Mesleman D, Kellman RM. Recent advances in fixation of the craniomaxillofacial skeleton. *Current Opinion in Otolaryngology and Head and Neck Surgery*. 2012; 20:304–9. [PubMed: 22614719]
33. Suuronen R, Pohjonen T, Tech L, Vasenius J, Vainionpaa S. Comparison of absorbable self-reinforced multilayer poly-L-lactide and metallic plates for the fixation of mandibular body osteotomies: An experimental study in sheep. *Journal of Oral and Maxillofacial Surgery*. 1992; 50:255–62. [PubMed: 1542067]
34. Bell RB, Kindsfater CS. The Use of Biodegradable Plates and Screws to Stabilize Facial Fractures. *Journal of Oral and Maxillofacial Surgery*. 2006; 64:31–9. [PubMed: 16360854]
35. Waizy H, Weizbauer A, Maibaum M, Witte F, Windhagen H, Lucas A, et al. Biomechanical characterisation of a degradable magnesium-based (MgCa0.8) screw. *J Mater Sci: Mater Med*. 2012; 23:649–55. [PubMed: 22210311]
36. Denkena B, Podolsky C, Lucas A, Hassel T, Witte F, Palm O. Processing of degradable surgical implants made of magnesium alloys. *Biomaterialien*. 2005; 6:171.
37. Denkena, B.; Witte, F.; Podolsky, C.; Lucas, A. Degradable implants made of magnesium alloys. *Proceedings of the 5th Euspen International Conference*; Montpellier, France. 2005;
38. Witte F, Podolsky C, Hassel T, Lucas A. Fertigung von magnesium-implantaten. *Werkstattstechnik*. 2004; 94:692–6.
39. van den Bos T, Speijer D, Bank RA, Brömme D, Everts V. Differences in matrix composition between calvaria and long bone in mice suggest differences in biomechanical properties and resorption: Special emphasis on collagen. *Bone*. 2008; 43:459–68. [PubMed: 18583211]
40. Poon CCH, Verco S. Evaluation of fracture healing and subimplant bone response following fixation with a locking miniplate and screw system for mandibular angle fractures in a sheep model. *International Journal of Oral and Maxillofacial Surgery*. 2013; 42:736–45. [PubMed: 23374732]

Figure 1.

Mg-alloy craniofacial bone screws. A) Picture of a screw. The screws were machined from commercially available pure Mg and AZ31 stock rods. B) Screw implantation location in the rabbit mandible. The screws were placed along the lower edge of the mandible just posterior to the molars. C) View of two Mg-alloy screws implanted during surgery.

Figure 2.

Pull-out testing. A) Pull-out tests were used to compare the pure Mg and AZ31 screws to stainless steel screws. Screws were placed in synthetic bone and the force required to remove the screw was recorded. B) (i) Pull-out experiment simulation domain. The bone is colored golden while the screw is blue. (ii) Details of the finite element discretization. (iii) Interfacial constitutive law used to simulate the pull-out tests with three model parameters indicated. C) Simulated pull-out force profile along with two representative experimental data for pure Mg. Experimental curves were utilized to extract the model parameters as shown in Figure 2Biii.

Figure 3. MicroCT processing. A) Reoriented slice of a 12 week AZ31 screw. B) Binarized image of the region of interest of the same slice. C) Histogram showing the threshold set at the inflection point in the curve.

Figure 4.

Experimental and simulated pull-out test results. A) Experimental pull-out test results. Both the pure Mg (Mg) and AZ31 (AZ) screws had a similar pull-out force to the stainless steel (SS) screws of approximately 40 N, with no statistical differences ($p < 0.05$). B) Simulated pull-out profiles for different screw moduli corresponding to pure Mg (45 GPa), AZ31 (43.25 GPa), stainless steel (210 GPa), and a model very weak material (1 GPa). Interfacial parameters and synthetic bone modulus were kept constant for all these simulations. Yield strength for all these materials were more than the maximum interfacial strength of 4.5 MPa. C) Effect of maximum interfacial strength on the pull-out force profile. Other two interfacial parameters were kept constant. Screw material was taken as pure Mg.

Figure 5. 2D slices and 3D reconstructions from the set B microCT scans. Pure Mg and AZ31 screws are shown at the full length of the screws at 4, 8 and 12 weeks. Scale bar =1 mm. Representative samples shown from n=2.

Figure 6.

Example of bone overgrowth. A) 3D microCT reconstruction showing major bone overgrowth of an AZ31 screw at 12 weeks, from Scan Set A. (Scale bar =1mm) B) 2D microCT image showing the new bone growth around an AZ31 screw at 12 weeks, from Scan Set B. (Scale bar =1mm) The dashed lines in B) and C) show approximately where the original bone line was, anything above the line is new bone growth. The head of the screw was above the bone when the screws were implanted. C) Histological picture showing the overgrowth of bone over the head of an AZ31 screw at 12 weeks. (Scale bar =500 μ m)

Figure 7. MicroCT control data at 12 weeks. 2D slices and 3D reconstructions from naïve control bone, bone with holes drilled but no screws placed after 12 weeks, and stainless steel screws placed for 12 weeks, from the higher resolution Set B Scans. (Scale bar =1 mm.) Note that the stainless steel screws were much more radiodense than the magnesium ones, resulting in increased beam hardening in the images. Representative samples shown from at least n=3.

Figure 8.

Volume fraction of the remaining Mg-alloys. Volume fractions were calculated from the set B scans for A) the whole screw, separating the screw into B) the head and C) the shaft, and for separating the shaft into D) the cortical space, and E) the bone marrow space calculated from the 10.5 and 12.9 μm voxel microCT scans, averaged for 2 screws per group. Note in E at 4 weeks the AZ31 screws were not included because the screws did not penetrate the bone marrow space.

Figure 9. H&E histology at 12 weeks. A) Pure Mg and B) AZ31. Scale bar =500 μ m. Note that the sectioning method resulted in sections of varying thickness, so the red colors vary between samples. Representative samples shown from at least n=3.