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# In vitro and in vivo corrosion, cytocompatibility and mechanical properties of biodegradable Mg–Y–Ca–Zr alloys as implant materials ☆

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## ABSTRACT

This study introduces a class of biodegradable Mg–Y–Ca–Zr alloys novel to biological applications and presents evaluations for orthopedic and craniofacial implant applications. Mg–Y–Ca–Zr alloys were processed using conventional melting and casting techniques. The effects of increasing Y content from 1 to 4 wt.% as well as the effects of T4 solution treatment were assessed. Basic material phase characterization was conducted using X-ray diffraction, optical microscopy and scanning electron microscopy. Compressive and tensile tests allowed for the comparison of mechanical properties of the as-cast and T4-treated Mg–Y–Ca–Zr alloys to pure Mg and as-drawn AZ31. Potentiodynamic polarization tests and mass loss immersion tests were used to evaluate the corrosion behavior of the alloys. In vitro cytocompatibility tests on MC3T3-E1 pre-osteoblast cells were also conducted. Finally, alloy pellets were implanted into murine subcutaneous tissue to observe in vivo corrosion as well as local host response through H&E staining. SEM/EDS analysis showed that secondary phase intermetallics rich in yttrium were observed along the grain boundaries, with the T4 solution treatment diffusing the secondary phases into the matrix while increasing the grain size. The alloys demonstrated marked improvement in mechanical properties over pure Mg. Increasing the Y content contributed to improved corrosion resistance, while solution-treated alloys resulted in lower strength and compressive strain compared to as-cast alloys. The Mg–Y–Ca–Zr alloys demonstrated excellent in vitro cytocompatibility and normal in vivo host response. The mechanical, corrosion and biological evaluations performed in this study demonstrated that Mg–Y–Ca–Zr alloys, especially with the 4 wt.% Y content, would perform well as orthopedic and craniofacial implant biomaterials.

## 1. Introduction

Currently, biomaterials used for orthopedic and craniofacial applications are primarily chosen based on their ability to withstand cyclic load-bearing [1]. Metallic biomaterials, such as stainless steels, Ti and Co–Cr-based alloys, possess stiffness, rigidity and strength far exceeding those of natural bone. Their elastic moduli differ significantly from bone, causing stress-shielding effects that may lead to reduced loading of bone and decreased implant stability [2]. Current metallic biomaterials also suffer from the risk of releasing toxic metallic ions and particles through corrosion or wear [3–7], causing implant site immune response. They may also lead to hypersensitivity [8], growth restriction (most significantly for pediatric implants) [9], implant migration and imaging interferences [10]. Due to these complications, it is estimated that 10% of patients will require a second operation for the removal of permanent metallic plates and screws [11], exposing patients to additional risks, and increasing surgical time and resources.

In order to avoid complications associated with permanent bone fixation implants, degradable biomaterials have recently been developed. However, resorbable polymer fixation plates and screws are relatively weak and less rigid compared to metals [12], and have demonstrated local inflammatory reactions [13]. Magnesium alloys have recently emerged as a new class of biodegradable materials for orthopedic applications with more comparable properties to natural bone [14]. They retain a density

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similar to cortical bone, and much less than other implant metals. The elastic modulus of magnesium (41–45 GPa) [14] is much closer to natural bone (6–24 GPa) [15] compared to other commonly used metallic implants, thus reducing the risk of stress shielding. Most importantly, Mg degrades to produce a soluble, non-toxic corrosion hydroxide product which is harmlessly excreted through urine. Unfortunately, accelerated corrosion of Mg alloys may lead to accumulation of gas pockets around the implant as well as insufficient mechanical performance and implant stability throughout the degradation and tissue healing process [16,17].

Much research has therefore been focused on controlling the corrosion rate and improving mechanical properties of magnesium alloys through the introduction of alloying elements and use of different processing conditions. Magnesium corrosion and mechanical properties are strongly affected by alloying elements present in the solid solution [16,18]. In this study, the elements yttrium (Y), calcium (Ca) and zirconium (Zr) were alloyed with Mg to create a class of Mg alloys previously unexplored for biological applications. Mg–Y–Ca–Zr alloys have been pursued as ignition-proof alloys to improve oxidation resistance [19,20]. Y contributes to grain boundary strengthening of magnesium alloys [21] and also improves corrosion resistance when alloyed with Mg above 3 wt.% [22,23]. Ca is a major mineralized component in bone [24], and is known to improve corrosion resistance and mechanical properties of pure Mg up to 1 wt.% addition [25,26]. Density functional theory has shown that alloying with Ca and Y helps to form a stable and chemically less reactive hydroxide layer to impart greater corrosion resistance [27]. Zr serves as an effective grain-refining agent [28–31], imparting grain boundary strengthening [32] and corrosion resistance [33]. Addition of Ca to Zr-containing alloys promotes solutal undercooling, which in turn facilitates a greater number of suitable size Zr nuclei to become effective nucleation sites, thus further refining grain size [19,28–31]. The new alloys reported in this study, Mg–1Y–0.6Ca–0.4Zr (wt.%), denoted henceforth as WX11 (nomenclature according to ASTM B275-05) [34], and Mg–4Y–0.6Ca–0.4Zr (wt.%), denoted henceforth as WX41, were compared in their as-cast and T4 solution-treated conditions based on their cytocompatibility, corrosion behavior and mechanical properties with the objective of using in orthopedic medical implants. Cytocompatibility was determined in vitro using direct and indirect cell viability tests and in vivo by implanting the alloys into mouse subcutaneous tissue and analyzing the local host response. Corrosion behavior was also evaluated by electrochemical and mass loss tests in vitro and in vivo. Mechanical properties were measured under both compression and tension.

## 2. Materials and methods

### 2.1. Materials preparation and characterization

Elemental ingots of Mg (US Magnesium Inc., Salt Lake City, UT, 99.97%), Ca (Alfa-Aesar, Ward Hill, MA, 99.5%) and Y (Alfa-Aesar, 99.9%) were weighed according to the nominal composition and melted together in a graphite crucible inside an induction furnace (MTI Corporation, Richmond, CA) purged with ultrahigh-purity (UHP) Ar and vacuumed to avoid oxidation of the pure elements. The initial alloy produced by induction melting was cleaned thoroughly to remove residue and oxide scale and re-melted in a mild steel crucible using an electrical resistance furnace (Wenesco Inc., Chicago, IL) under the protection of Ar +1.5% $SF_6$ cover gas. The melting and pouring temperature was 780 °C, and once the temperature was reached, an equivalent amount of zirconium was added using Zirmax® (Mg–33.3% Zr) master alloy (Magnesium Elektron Ltd, Manchester, UK). After zirconium addition, the melt was stirred for 10 s at intervals of 1 and 5 min to dissolve and disperse the zirconium particles uniformly into the melt. The melt was held for 30 min and poured into a cylindrical mild steel mold preheated to 500 °C with dimensions of 44.5 mm diameter × 82.5 mm length. Appropriate stirring and holding times was essential to release Zr particles uniformly from Zirmax® master alloy in order to achieve high solubility of Zr in the melt and optimal grain refinement [35]. The as-cast samples were solution-treated (T4) at 525 °C for 6 h inside a tubular furnace covered under continuous UHP Ar flow and quenched in water. The alloy nominal compositions, determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, iCAP duo 6500 Thermo Fisher, Waltham, MA), are listed in Table 1. The WX11 and WX41 alloys were compared to as-cast Mg (US Magnesium Inc.) and as-drawn AZ31 (Goodfellow Corp., Coraopolis, PA) in reported tests.

In order to determine the phase formation, X-ray diffraction (XRD) was conducted using a Philips X'Pert PRO diffractometer employing Cu $K_\alpha$ ($\lambda$ = 1.54056 Å) radiation with a Si detector (X'celerator). The X-ray generator operated at 45 kV and 40 mA at a 2$\theta$ range of 10–80°.

### 2.2. Microstructure characterization

Square plate samples (10 mm × 10 mm × 1 mm) of the WX11 and WX41alloys were mounted in epoxy, mechanically polished (Tegramin-20, Struers, Ballerup, Denmark), and chemically etched in a solution of 5 ml acetic acid, 6 g picric acid, 10 ml water and 100 ml ethanol. The microstructure was observed using optical microscopy (Axiovert 40 MAT, Carl Zeiss, Jena, Germany) and scanning electron microscopy (SEM; JEOL JSM-6610, JEOL Ltd, Tokyo, Japan) with energy-dispersive X-ray (EDX; EDAX Genesis, Mahwah, NJ) to conduct elemental analysis. Average grain size was measured according to ASTM E112 [36] following the Abrams three-circle procedure.

### 2.3. Mechanical characterization

Samples were machined along the long axis of the Mg alloy ingots in dimensions in accordance with ASTM-E8-04 [37] for tensile testing and ASTM-E9-09 [38] for compressive testing. Tensile bar samples with a gage area of 3 × 3 mm were machined for tensile samples. Cylindrical samples of 10 mm diameter × 20 mm length were machined for compressive tests. Tensile and compressive stress–strain curves were obtained for each as-cast and T4 solution-treated alloy, and compared to as-cast pure Mg and as-drawn AZ31. The tensile and compressive tests were conducted by Ortho Kinetic Testing Technologies, LLC (Southport, NC) at room temperature using an MTS11 – 50 kN electro-mechanical load frame (MTS, Eden Prairie, MN) with laser extensometer. Tensile tests were carried out at a cross-head speed of 1.3 mm $min^{-1}$, while compression tests were carried out at a speed of 2 mm $min^{-1}$. Tensile and compressive yield strength, ultimate compressive and tensile strength, Young's modulus ($E$), percentage elongation and compression (%) were determined from the stress–strain curves. An average and standard deviation of at least three measurements was taken for each group, with the exception of T4-treated WX11, for which only one sample was available for testing.

### 2.4. Electrochemical corrosion test

To test corrosion of the WX alloys, the potentiodynamic polarization technique was used. Samples were connected to a copper wire using silver paste and mounted in epoxy resin. The mounted samples of dimensions 10 mm × 10 mm × 1 mm were mechanically polished, sonicated in isopropyl alcohol and dried in air. The potentiodynamic polarization was carried out with an electrochemical workstation (CH-604A, CH Instruments, Inc., Austin, TX) at a scanning rate of 1 mV $s^{-1}$ and potential window of 500 mV