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Original Research

Corrosion of magnesium and magnesium–calcium alloy in biologically-simulated environment

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Abstract

A study of biocompatibility and corrosion of both metallic magnesium (Mg) and a magnesium alloy containing 1% calcium (Mg–Ca) were investigated in *in vitro* culture conditions with and without the presence of bone marrow derived human mesenchymal stem cells (hMSCs). Chemical analysis of the degraded samples was performed using XRD and FEGSEM. The results from the XRD analysis strongly suggested that crystalline phase of magnesium carbonate was present on the surface of both the Mg and Mg–Ca samples. Flame absorption spectrometry was used to analyse the release of magnesium and calcium ions into the cell culture medium. Magnesium concentration was kept consistently at a level ranging from 40 to 80 mM for both Mg and Mg–Ca samples. No cell growth was observed when in direct contact with the metals apart from a few cells observed at the bottom of culture plate containing Mg–Ca alloy. In general, *in vitro* study of corrosion of Mg–Ca in a biologically-simulated environment using cell culture medium with the presence of hMSCs demonstrated close resemblances to *in vivo* corrosion. Although *in vitro* corrosion of Mg–Ca revealed slow corrosion rate and no immediate cytotoxicity effects to hMSCs, its corrosion rate was still too high to achieve normal stem cell growth when cells and alloys were cultured *in vitro* in direct contact.

Keywords: Magnesium; Biometal; Corrosion; Biocompatibility; Stem cells; *In vitro*

1. Introduction

Due to the limitations associated with the current methods better material for orthopaedic implantation is required and researchers have been studying the use of biodegradable magnesium alloy as a candidate for orthopaedic implantation. Of the metals currently employed as biomaterials, magnesium alloys thereof demonstrate an excellent load bearing profile with an elastic modulus of 41–45 GPa which is much closer to cortical bone tissue (20 GPa) than most common metal biomaterials [1–4]. This is further facilitated by increased osseointegration of the magnesium with bone tissue [5]. When combined, these factors reduce the potential for stress shielding and hence, the incidence of implant failure. Furthermore, magnesium structures readily degrade *in vivo* allowing the implant to act only as an aid to biological repair, becoming less important as the requirement for support lessens. In addition, magnesium ion also has a number of important biological functions, including taking part in bone and mineral homoeostasis [6–8] promoting DNA replication and transcription [7,9] and regulation of opening and closing of ion channels [7,8,10–12]. Magnesium normal physiological range is between 0.8 mM and 1.0 mM, approximately 60–65% is stored in the bone and homoeostasis is maintained by the kidneys and intestine [13].

During fracture healing the biomaterial implant should have the ability to sustain mechanical strength until the formation of new bone bridges the gap of the fracture. In order for this to happen the biodegradable material should degrade gradually to allow for tissue implant integration. Pure magnesium has a high corrosion rate in physiological environment; this is a major limitation as the implant corrodes faster than new bone

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formation [14–17]. When pure magnesium is placed in aqueous environment, magnesium reacts with water to form magnesium hydroxide and hydrogen gas. The hydroxide film provides some protection to further corrosion and exposure of magnesium to chloride ions results in pitting corrosion which in turn could lead to implant failure [18]. Furthermore cells are sensitive to the corrosion environment created by the corrosion of magnesium [19,20].

Research has shown that corrosion resistance of magnesium implants can be improved through alloying and surface coating. The use of hydroxyapatite for surface coating has been shown to reduce corrosion rate of magnesium and magnesium alloys [16,20,21], and to have osteo-conductive properties [22]. A number of alloying elements have been studied for magnesium-based implants; these include aluminium, zinc, calcium, yttrium and rare earths. Studies have shown that alloying results in increased implant strength, reduced corrosion rate [14,16,23,24], and slower release of magnesium ions into the medium [19]. Magnesium implants alloyed with calcium or zinc or both show improved cell viability, stability in physiological conditions and enhanced cell attachment and proliferation [14,24,25]. When examining the corrosion of a magnesium alloy for biological applications two broad factors, the corrosion behaviour of magnesium, and the interaction and toxicity of magnesium to local tissue, must be considered [2]. Hence, one of the major challenges faced in the development of magnesium based biomaterials is how to predict the corrosion process and the corresponding biological and physiological consequence of such corrosion in the context of implants and implantation sites. The biodegradability of magnesium through corrosion is a double-edged sword and it is just this particular nature that cause concerns on applying standard approach to *in vitro* biocompatibility and corrosion test.

In previous studies [24], a much faster corrosion rate of Mg–Ca alloy implant was observed in *in vitro* electrochemical test compared to those observed *in vivo*. Also high activity of osteoblast and circumferential osteogenesis were observed around the Mg–Ca alloy pin *in vivo*, but still an unfilled void was left at implantation site when the Mg pin was totally degraded at the end of the 3 month implantation. Thus, there is a lack of correlation of *in vitro* and *in vivo* corrosion study and, as well, of understanding the effects of Mg corrosion products on stem cells responsible for bone tissue regeneration [24]. Here we present study on the biocompatibility and corrosion of magnesium containing 1% calcium in the presence and absence of bone marrow derived human mesenchymal stem cells, aiming to mimic the *in vivo* environment at the implantation sites.

2. Materials and methods

2.1. Mg sample preparation

Commercial pure magnesium (99.9%) and magnesium–1% calcium were prepared in the form of cylindrical ingots, and treated and cleaned as described previously [24]. The magnesium ingots were then cut into disks and sterilised using ethanol and UV light. Mg disks had average measurements of 12.2 mm diameter and 4.75 mm depth. Mg–Ca disks had average measurements of 16.23 mm diameter and 3.37 mm depth. Average surface areas were 415.85 mm^2 per disk for Mg and 582.7 mm^2 for Mg–Ca. This allowed a correctional factor of 1.4 to be applied to the data to compensate for the lower surface area of the Mg disks.

2.2. Cell culture

hMSC (Lonza, UK) were used for experimental procedures. Cells were cultured in growth medium Dulbecco's Modified Eagle's Medium (Lonza, UK) supplemented with 10% (v/v) foetal calf serum (FCS) (Sigma-Aldrich, UK), L-glutamine final media concentration 2 mM (Sigma-Aldrich, UK), and 100 units/ml antibiotic–antimycotic (Sigma-Aldrich, UK).

2.3. In vitro corrosion with/without the cell presence

Samples were incubated for 11 days in cell culture medium at 37 °C 5% CO_2. Samples where either incubated in cell culture medium with or without the presence of cells to evaluate the effect of cells on magnesium alloy corrosion behaviour. Cells were seeded at a seeding density of 1.5×10^5 cells/well in a six well plate. 1 ml of conditioned medium was collected every 24 hrs and replaced with 1 ml of fresh medium. The conditioned medium collected was kept frozen for further analysis. Cells seeded in each well were fixed in 70% methanol in distilled water for 25 mins. These were then washed and stored in PBS (Sigma-Aldrich, UK). Assessment of cell presence was assessed using 1% toluidine blue (Sigma-Aldrich, UK) with distilled water. This was performed for one minute with three washes in distilled water to remove excess stain.

2.4. Flame absorption spectrometry (FAAS) analysis of Mg and Mg–Ca alloy corrosion

Samples of culture medium collected each day were analysed for calcium and magnesium using a SOLAAR S Series AA spectrometer (Thermo Fisher Scientific, USA). An air acetylene mix was used for combustion. Signal correction was performed using a deuterium lamp. Burner height was optimised and held constant at 13.4 mm for calcium and 11.8 mm for magnesium. Cumulative values were plotted by adding the calculated ion content value of each day to the sum of the days prior to it. This was performed for magnesium and calcium correspondingly.

2.5. Analysis of precipitates formed during corrosion

Analysis of the crystals formed on the surface of the samples was analysed by field emission gun scanning electron microscopy (FEGSEM). Precipitates on the surface of the samples were ground to fine powder and allowed to dry for five days. The powder was then mounted on carbon disk topped stubs and gold sputter coated using a Q150T ES turbo pumped sputter coater (Quorum Technologies Ltd., East Sussex, UK). Analysis was performed on a 1530 VP FEGSEM (Carl Zeiss [Leo], Cambridge, UK).