# Surface characterization, in vitro and in vivo biocompatibility of Mg-0.3Sr-0.3Ca for temporary cardiovascular implant

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A B S T R A C T

Magnesium-based alloys are attractive candidate materials for medical applications. Our earlier work showed that the ternary Mg-0.3Sr-0.3Ca alloy exhibits slower degradation rates than both binary Mg-Sr and Mg-Ca alloys. The ternary alloy immersed in simulated body fluid (SBF) forms a compact surface layer of corrosion products that we hypothesized to be a Sr-substituted hydroxyapatite (HA). The main objectives of the current work are to understand the bio-degradation mechanism of Mg-0.3Sr-0.3Ca, to identify the exact nature of its protective layer and to evaluate the in vitro and in vivo biocompatibility of the alloy for cardiovascular applications. To better simulate the physiological environment, the alloy was immersed in SBF which was daily refreshed. Raman spectroscopy and X-Ray photoelectron spectroscopy (XPS) confirmed the formation of a thin, Sr-substituted HA layer at the interface between the alloy and the corrosion products. In vitro biocompatibility evaluated via indirect cytotoxicity assays using HUVECs showed no toxicity effect and ions extracted from Mg-0.3Sr-0.3Ca in fact increased the viability of HUVECs after one week. In vivo tests were performed by implanting a tubular Mg-0.3Sr-0.3Ca stent along with a WE43 control stent into the right and left femoral artery of a dog. Post implantation and histological analyses showed no thrombosis in the artery with Mg-0.3Sr-0.3Ca stent after 5 weeks of implantation while the artery implanted with WE43 stent was extensively occluded and thrombosed. Microscopic observation of the Mg-0.3Sr-0.3Ca implant-tissue interface confirmed the in situ formation of Sr-substituted HA on the surface during in vivo test. These results show that the interfacial layer protects the surface of the Mg-0.3Sr-0.3Ca alloy both in vitro and in vivo, and is the key factor in the bio-corrosion resistance of the alloy.

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## 1. Introduction

In recent years, magnesium (Mg) and its alloys have been recognized as a novel class of materials for biodegradable metallic implants in different medical applications due to the low density (~1.74 g/cm$^3$), good mechanical properties and biocompatibility [1–7]. One of the application areas is temporary cardiovascular stent. Currently, metallic stents are made of stainless steel, Nitinol (Ni-Ti) and cobalt-chromium alloys [1]. Mg has superior advantages over these permanent metallic stents which may release toxic ions and particles through corrosion or wear and cause immune response in the body [1,7]. Permanent metallic stents can also cause restenosis and hyperplasia due to the irritation of the endothelium [8]. They have much higher mechanical strength than natural tissues; the significant difference in the mechanical behavior of the stented and non-stented vessel areas can lead to growth restriction especially in the case of pediatric patients [4,9,10], necessitating a second surgery to remove the permanent metallic stent. This exposes patients to more surgical risks [11]. Other complications of permanent stents are thrombogenicity, permanent physical irritation, long-term endothelial dysfunction, and local chronic inflammatory reactions [9]. One of the advantages of Mg alloys over the traditional metals used for implants is that they are biodegradable [12–15]; their use as stents would thus eliminate the stimulus for hyperplasia and reduce the risk of restenosis [8,16]. Mg alloys also have better visibility with high dimensional accuracy in CT scan compared to stainless steel biomaterials. Some clinical trials with Mg alloys have been undertaken for cardiovascular stent applications [17,18].

Despite the first use of Mg in medical implants dates back to more than a century ago [4], its fast degradation rate has severely limited its applications [19]. During Mg degradation, high volumes of H$_2$ gas are generated, which cannot be tolerated by the host tissue [20,21]. Another complication is the loss of mechanical integrity before the healing process is complete [21]. This is a crucial problem for stent applications, since the main role of stents is to provide mechanical support for arterial walls and avoid early recoil while healing is in progress [22].

Thus, a considerable amount of research has focused on controlling the degradation rate of Mg by adding alloying elements [23–28]. Alloying elements should slow down the biodegradation without compromising the biocompatibility. Only a limited number of elements can be used in the body without causing toxicity or harmful effects on organs. Mg alloys containing rare earths (REs) such as WE43 (Mg-4Y-3RE) and LAE442 (Mg-4Li-4Al-2RE) have been evaluated for bio-implants [1]. Unless the alloys contain Al, substantial toxicity problems are not expected with REs; however, REs are not part of the human chemistry and they may cause undesired cell proliferation and high rate of neo-intima formation in stented vessels leading to potential risks of thrombosis [8,29]. Animal studies using WE43 as cardiovascular stent have shown that the alloy had good mechanical integrity but caused the formation of thick neo-intima and thrombosis [30].

Strontium (Sr) and calcium (Ca) are naturally found in the human body [28,31–35]. We previously reported that Mg-0.5Sr has a significantly lower degradation rate compared to pure Mg and other as-cast binary Mg-Sr alloys with Sr wt.% ranging from 0.3 to 2.5 [36]. We showed that Sr improves the corrosion resistance of Mg by altering its surface chemistry due to its surface active nature, and we detected a thin layer of Sr-modified hydroxyapatite on the surface of Mg-0.5Sr after immersion in SBF, which seemed to stabilize the surface and reduce the degradation rate [36]. Sr-containing HA coatings improved the bioactivity and bio-corrosion resistance of titanium alloys as well [37,38]. Sr-HA is known to have better biocompatibility, thermal stability and surface reactivity than pure HA [39,40].

In a subsequent study, we found that the combined addition of both Sr and Ca in small amounts increases the corrosion resistance of Mg in physiological conditions more than the single addition of Sr or Ca; this was related to the third element effect [41]. An alloy containing low levels of Sr and Ca, Mg-0.3Sr-0.3Ca had the lowest degradation rate (~0.8 mg/day/cm$^2$ in terms of mass loss and ~0.4 mg/day/cm$^2$ in terms of H$_2$ evolution) among all the ternary Mg-Sr-Ca and the binary Mg-Sr and Mg-Ca alloys in SBF; it exhibited 90% slower degradation rate than pure Mg. The alloy produced a slow change in the pH of SBF and the H$_2$ evolution rate obtained was ~0.01 ml cm$^{-2}$ h$^{-1}$, which is lower than that measured for Mg-Sr and Mg-Ca binary alloys (~0.04 ml cm$^{-2}$ h$^{-1}$) and significantly below that of pure Mg (~0.2 ml cm$^{-2}$ h$^{-1}$) [41].

Mg-0.3Sr-0.3Ca also showed the best mechanical and bending properties and thermal stability among any Mg-Sr and Mg-Ca binary alloys tested due to the formation of globular Ca/Sr-rich phases in the microstructure of Mg-0.3Sr-0.3Ca [41,42]. The microstructure of this ternary Mg alloy contains α-Mg matrix and (α-Mg + β) eutectic micro constituents. The existence of the finely dispersed new Sr/Ca phase in the matrix slows down the corrosion rate by reducing micro-galvanic corrosion and improves the mechanical properties of the alloy.

The surface of a medical implant is the first region that the natural tissue comes in contact with. Thus, understanding the surface chemistry of an implant and its interactions with the physiological environment is crucial for biomaterials. In our previous work, the XPS analysis was performed on the surface immediately after immersion of Mg-0.3Sr-0.3Ca in SBF to identify the corrosion products. We observed the formation of a fine and compact scale containing HA on the alloy surface (details of the microstructural observations, thickness of the layer and its electrochemical properties can be found elsewhere [41]). In our previous, the exact nature of the protective scale formed on the alloy surface and of the interface implant surface/corrosion product as well as its role in degradation were not studied. The present work is focused on the surface analysis of this scale and its effect on the mechanism of alloy degradation. The work was therefore conducted on samples that were cleaned off of corrosion residues and air dried immediately after the immersion tests. The investigation was the characterization of the surface of metallic sample after corrosion. Also in our present study, to better simulate the dynamic nature of the physiological environment with respect to the pH levels of the solution, the SBF immersion solution

was daily renewed. We elucidated the impact of time, the increase in pH and the formation of protective corrosion scale on the corrosion rate and degradation behavior of Mg-0.3Sr-0.3Ca. Finally, this work investigates for the first time the in vitro and in vivo biocompatibility of Mg-0.3Sr-0.3Ca, and its interactions with surrounding tissues for cardiovascular applications.

## 2. Experimental procedure

### 2.1. Materials

A thin plate of Mg-0.3Sr-0.3Ca was prepared by melting down pure Mg (99.9 wt.%,), pure Sr (99.99 wt.%) and Mg-30Ca master alloy all supplied by Applied Magnesium (Formerly Timminco) in a Lindberg/Blue M Crucible Furnace. CO$_2$ with 0.5% SF$_6$ was used at a flow rate of 1.1 l/min as protective gas to prevent burning during melting and casting. K-type (Chromel-Alumel) thermocouple was used with a digital thermometer to monitor the melt temperature. Steel die with a plate cavity coated with boron nitride release coating was heated to 400 °C before plates were cast. The chemical composition of alloy was analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) as listed in Table 1. Pure Mg and WE43 containing of 3.48 wt.% yttrium, 2 wt.% neodymium, 0.5 wt.% gadolinium and 0.15 wt.% praseodymium was also cast using the same procedure.

### 2.2. Immersion test

Interrupted corrosion experiments was conducted in SBF using Hank's solution (8.0 g/l NaCl, 0.4 g/l KCl, 0.14 g/l CaCl$_2$, 0.35 g/l NaHCO$_3$, 1.0 g/l C$_6$H$_6$O$_6$ (glucose), 0.1 g/l MgCl$_2$. 6H$_2$O, 0.06 g/l MgSO$_4$. 7H$_2$O, 0.06 g/l KH$_2$PO$_4$. H$_2$O, 0.06 g/l Na$_2$HPO$_4$ .7H$_2$O). Samples used in the experiment were cut from thin plates of 6 mm thickness into $2 \times 4$ cm$^2$ sections and were polished down to 800 grit with silicon-carbide paper. They were then cleaned, dried, and weighed. Interrupted immersion test was carried out in static conditions (without SBF renewal). In this test, the pH value of Hank's solution was adjusted to 7.4 at the beginning and the temperature was maintained at 37 °C using a hot plate during immersion testing. The samples were removed from SBF after a certain time, cleaned off of corrosion residues and dried. The variation of pH, the weight change and the volume of H$_2$ gas released were measured. The average value of corrosion rate in terms of both mass loss and H$_2$ evolution were calculated as average values of triplicate corrosion tests.

It should be mentioned that the corrosion rate calculated in term of mass loss is always higher than the one calculated in term of hydrogen evolution. This is due to two reasons: (i) the rate in terms of mass loss in always overestimated due to the physical detachment and separation of large chunks of the specimen during the experiment that is not due to the corrosion reaction and increases the corrosion rate value, and (ii) the corrosion rate in terms of hydrogen evolution might be slightly under estimated due to the difficulty on collecting all the gas bubbles released from the reaction.

Some of the samples were only rinsed with ethanol and dried without cleaning the corrosion product for further surface characterization. Another set of immersion test was carried out using fresh solution (daily SBF renewal) to investigate the effect of pH change on the degradation of the sample. The solution was replaced every day with fresh SBF and the variation of pH value and the amount of different ions

**Table 1**
The actual chemical composition of as-cast Mg-0.3Sr-0.3Ca obtained by ICP-AES.

| Alloy | Chemical composition (wt.%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sr | Ca | Al | Si | Fe | Mn | Mg |
| Mg-0.3Sr-0.3Ca | 0.31 | 0.39 | 0.011 | 0.005 | 0.044 | 0.003 | Balance |