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# Hemocompatibility of plasma electrolytic oxidation (PEO) coated Mg-RE and Mg-Zn-Ca alloys for vascular scaffold applications

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ABSTRACT

Percutaneous transluminal coronary angioplasty and subsequent vascular scaffold implantation remains the prevalent invasive treatment of coronary heart disease. In-stent restenosis remained a problem with bare metal stents, until drug-eluting stents were introduced. The inhibition of the healing process by the antimitotic drug coating and the permanent metallic remnant can promote sub-acute and delayed stent thrombosis. Thus, the development of biodegradable stents emerged as a subject of research. Magnesium-based bioabsorbable devices can provide sufficient radial force in the acute phase of vessel-treatment and degrade thoroughly in aqueous environment, making them potential new candidates for vascular scaffold applications. Magnesium alloys tend to degrade very quickly due to their high electrochemical corrosion potential. Plasma Electrolytic Oxidation modification of magnesium alloys improves interface and degradadation properties and may therefore enhance the performance and suitability for vascular scaffold applications of these materials. Assuring the hemocompatibility and foremost assessing the thrombogenicity of new biomaterials prior to their use is essential in order to avoid adverse effects. The goal was to assess thrombocyte adhesion on coated Mg-RE and Mg-Zn-Ca alloys. Static experiments with human blood were carried out on the plasma-electrolytically treated or corresponding untreated Mg alloy in order to assess quantity and quality of thrombocyte adhesion via standardized SEM imaging. In a second step, a parallel plate flow chamber was designed in order to examine thrombocyte adhesion under dynamic flow conditions. During flow chamber experiments the test-materials were exposed to human thrombocyte concentrate and the number of adherent thrombocytes was assessed. The flow chamber was additionally perfused with human blood and thrombocyte adhesion was semiquantitatively and qualitatively assessed via SEM imaging and subsequent scoring. In conclusion, a new parallel plate flow chamber design simulating blood-circulation was successfully established, enabling the further assessment of platelet adhesion on bioabsorbable materials under dynamic flow conditions. Static and dynamic experiments showed, that plasma-electrolytically treated specimens showed low thrombocyte adhesion on both alloys, proposing their potential use in vascular scaffolds. The uncoated magnesium alloys showed rapid degradation along with gas formation due to the chemically active surface and therefore give concern regarding their safety and suitability for vascular applications.

## 1. Introduction

Arteriosclerotic alterations lead to progressive constriction of epicardial arteries and impaired circulation of the heart muscle, referred to as coronary heart disease [1]. Percutaneous transluminal coronary angioplasty (PTCA) remains the prevalent invasive treatment to reduce blockades and defiles in coronary arteries. Restenosis follows PTCA in 30–40% within six months [2]. Hence, PTCA is followed by the placement of vascular scaffolds (VS) in order to prevent post angioplasty arterial recoil (PAR) [3]. In-stent restenosis remained a problem with bare metal stents, until drug-eluting stents (DES) were introduced [4]. The pathophysiology of in-stent restenosis includes plaque

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redistribution, thrombosis, neointimal hyperplasia and remains a subject of active research [2]. DES release anti-proliferative drugs, which reduce neointimal hyperplasia [4]. The inhibition of the healing process by the antimitotic drug coating and the permanent metallic remnant can lead to sub-acute and delayed stent thrombosis, causing life-threatening conditions [4, 5]. Further approaches concerning the improvement of VS has led to the development of biodegradable non-metallic and metallic based stents (BDS). Potential advantages of BDS are the preservation of vessel geometry, the recovery of vasomotion and the remodeling of the vessel due to the disappearance of the BDS [6]. The possibility of repeated intervention in the same place or further downstream after disappearance of the BDS is also a potential advantage [7]. Non-metallic, Poly-L-Lactic acid (PLLA) based stents bare certain disadvantages in comparison to magnesium-based bioabsorbable scaffolds (MBS) which are currently under research. The inferiority concerning mechanical properties and strength is one [8]. MBS provide sufficient radial force in the acute phase of vessel-treatment preventing PAR. They are actively absorbed by the human body and therefore may counter long-term delayed stent thrombosis and avoid associated prolonged dual anti-platelet therapy [4]. Magnesium (Mg) corrodes in aqueous environment under formation of Mg ions and hydrogen gas. The degradation products of Mg can be regarded as nontoxic. Commercially pure Mg possesses poor mechanical strength and low corrosion resistance, especially in high chloride environments, such as blood [9, 10]. Therefore, Mg alloys are a subject of active research, as the addition of certain alloying elements improves corrosion resistances and enhances mechanical properties [11]. The addition of rare earths (RE) and zinc/calcium (Zn−Ca) as alloying elements form the two most prevalent groups of Mg alloys currently being translated into medical devices [12, 13]. In order to improve the electrochemical stability of Mg alloys, different surface modifications have been investigated. Plasma Electrolytic Oxidation (PEO), also referred to as micro-arc oxidation (MAO), is one of the most promising candidates, as the process markedly enhances surface properties and improves corrosion rates [14]. Since MBS do not yet outperform current DES, the modification of Mg alloys via PEO may upgrade the overall performance of future MBS [6, 15]. First of all, the hemocompatibility of these PEO-modified surfaces, especially in terms of thrombocyte adhesion and controlled degradation must be assured before endothelium growth occurs in vivo [9]. The presence of biomaterials in the circulatory system generally results in the activation of the coagulation system. While non-injured and non-altered intact endothelium secrets anti-thrombotic substances, e.g. prostacyclin and nitrogen monoxide, thrombocytes adhere, when vascular endothelium is altered, subendothelial matrix is exposed and contact to external materials occurs. This leads to microthrombi generation and thrombosis [16, 17]. These events become manifest especially during prolonged contact of blood with foreign materials, leading to an ongoing risk for thrombosis caused by VS by 1% per year [18]. Though it seems hard to find consensus as to which materials can be considered as blood compatible [19], the standard EN ISO 10993–4 serves as a European guideline, displaying methods for medical device testing in respect to interaction with blood and its components [20]. Following these suggestions, Mg-RE and Mg-Zn-Ca alloys were treated with PEO or left untreated and subsequently studied in a static, as well as in a customized dynamic test set-up in order to assess thrombogenicity under static and dynamic flow conditions.

## 2. Materials and methods

### 2.1. Material preparation

Mg-4%Y-3%RE (WE43) and Mg-1%Zn-1%Ca (ZX00) were fabricated following ASTM B80 by Meotec GmbH & Co. KG (Aachen, Germany) with compositions as depicted in Table 1.

After casting, samples of ø 6 mm and 2 mm in height for static test set-up and ø 18 mm and 1 mm in height for dynamic test set-up were cut-off grinded (Secotom-50, Struers, Willich, Germany) and ultrasonically degreased in pure ethanol for 10 min. The specimens were briefly etched in 15% hydrofluoric (HF) acid solution for purposes of surface cleaning and priming in order to enhance the initial PEO-coat buildup. They were then left either untreated or PEO-coated. For coating, a standard Kermasorb® electrolyte was used and a pulsed current of 1.35 A/dm$^2$ was applied in a cool jacketed steel container serving as the cathode. All specimens were washed with distilled water for 15 min and dried on a sterile blanket. As suggested in EN ISO 10993–4, high-density polyethylene (HDPE) was used as the negative control underlying sparse cell adhesion, whereas polypropylene (PP) was used as the positive control promoting abundant cell adhesion. The controls were punched from commercially available PP and HDPE sheets in the same dimensions as described above and equivalently cleaned without successive etching treatment.

**Table 1**
Alloy compositions in wt (%).

| Alloy | Zn | Ca | Y | Re | Mg | Impurities |
|---|---|---|---|---|---|---|
| WE43 | 0 | 0 | 3.77 | 2.91 | balance | 70 ppm |
| ZX00 | 0.73 | 0.53 | 0 | 0 | balance | 95 ppm |

### 2.2. Potentiodynamic polarization measurements

In order to determine the degradation rates of the specimens, potentiodynamic polarization measurements (PDP) were performed in a three-electrode cell. The experimental setup included a potentiostat (SP-150153, Bio-Logic Science Instruments, Claix, France), a glass corrosion cell equipped with minimum essential medium (MEM, Life Technologies, Carlsbad, USA), an Ag/AgCl, NaCl saturated electrode and a graphite rod as the counter electrode. Cell culture medium was chosen for ease of use due to its comparable chloride content to blood. In order to achieve a more reliable electrical contact of the working electrode (specimen) and improved conductive circuit, the PEO surface was locally removed from the site of contact. Degradation material was kept in a controlled environment of 37 °C and 5% $CO_2$. Open circuit potential (OCP) variations were recorded continuously during the immersion time of 1 h. Polarization curves were registered at a scan rate of 30 mV/min within a range of −500 mV to +500 mV, respectively to the OCP. Corresponding measurements were calculated according to Ascencio et al. [21].

### 2.3. Flow chamber design

In order to simulate arterial blood flow, an acrylic glass parallel plate flow chamber device was designed, Fig. 1. The device was composed of an inner sliding piston, supported by an underlying spring mechanism, allowing for different test specimen heights. The chamber dimensions were chosen to fit for evaluation under an upright-microscopic lens focusing on the inner flow region of interest. All parts in contact with either human thrombocyte concentrate (HTC) or human whole blood (HWB) were arranged from non-coagulant materials, such as polymethylmethacrylate (PMMA) for chamber and top cover, medical grade silicone for sealing, polytetrafluoroethylene (PTFE) for the inlet and outlet connectors and attached biocompatible PharMed BPT tubing (Saint-Gobain Performance Plastics SA, Solon, USA) [22–24]. The inner geometry further contained the flow region of 1.8 mm in height, 3 mm in width and 16 mm in length with the inlet and outlet region of the chamber being funnel shaped, enabling laminar blood flow and preventing turbulences [25]. For assembly, the 1 mm top cover plate and 0.5 mm silicone seal were placed between the specimen on the piston and the chamber. The flow region was ultimately sealed by a press fit metallic support plate and corresponding screws. The tubing leading to the chamber was then connected to a roll pump.