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Blood compatibility of magnesium and its alloys

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ABSTRACT

Rationale: Blood compatibility analysis in the field of biomaterials is a highly controversial topic. Especially for degradable materials like magnesium and its alloys no established test methods are available.
Objective: The purpose of this study was to apply advanced test methodology for the analysis of degrading materials to get a mechanistic insight into the corrosion process in contact with human blood and plasma.
Methods and results: Pure magnesium and two magnesium alloys were analysed in a modified Chandler-Loop setup. Standard clinical parameters were determined, and a thorough analysis of the resulting implant surface chemistry was performed. The contact of the materials to blood evoked an accelerated inflammatory and cell-induced osteoconductive reaction. Corrosion products formed indicate a more realistic, *in vivo* like situation.
Conclusions: The active regulation of corrosion mechanisms of magnesium alloys by different cell types should be more in the focus of research to bridge the gap between *in vitro* and *in vivo* observations and to understand the mechanism of action. This in turn could lead to a better acceptance of these materials for implant applications.

Statement of Significance

The presented study deals with the first mechanistic insights during whole human blood contact and its influence on a degrading magnesium-based biomaterial. The combination of clinical parameters and corrosion layer analysis has been performed for the first time. It could be of interest due to the intended use of magnesium-based stents and for orthopaedic applications for clinical applications. An interest for the readers of Acta Biomaterialia may be given, as one of the first clinically approved magnesium-based devices is a wound-closure device, which is in direct contact with blood. Moreover, for orthopaedic applications also blood contact is of high interest. Although this is not the focus of the manuscript, it could help to rise awareness for potential future applications.

1. Introduction

The first contact of an implant material after implantation will be with blood, regardless of the implantation site. Therefore it is necessary to analyze the blood compatibility of novel materials. However, such tests are not easy to achieve and are lacking standardization [1]. Moreover, the corrosive nature of magnesium (Mg) materials and the accompanying change of the extraction medium in terms of pH, osmolality and change of the medium composition are adding further problems to such tests. To overcome these problems, a modified Chandler-Loop system can be used, which was initially developed for stent testing as an alternative to *in vivo* animal experiments [2]. As it was developed according to ISO 10993-4 also the standardization aspect was taken care of. Furthermore this system has the advantage, that clinically relevant parameters can be determined. The disadvantage is the relatively short immersion time due to clotting of the blood, which is limited to 6 h. In this study two time points were chosen (90 and 240 min) for whole blood contact. To achieve a longer immersion

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time (72 h) also plasma (the acellular part of blood, containing proteins, but no cells) was used.

Moreover, for the analysis of Mg degradation there is an ongoing discussion about the reliability and predictability of *in vitro* methods [3–5]. This has led to a systematic examination of the influence of different media [6,7], proteins and different environmental factors like temperature or gas supply [8]. Finally the term "physiological corrosion" was defined to describe corrosion under cell culture conditions and the relevance of the influencing factors was determined by neural network analysis [9]. From *in vivo* studies it is known that during the degradation of e.g. Mg stents the initial material is replaced by calcium phosphates over time, although no adverse effects for the vessel could be observed [10–12].

Furthermore, physiological conditions are characterized not only by the various solutions, but also by the major active mechanisms of cells. As an example, during platelet activation the formation of intra- and extracellular reactive oxygen species (ROS) has been reported, which is interwoven with uncoupled nitric oxide synthase (NOS). Additionally dense granules are released, which contain ADP, ATP and serotonin [13,14]. All such cell-triggered release of substances can change the local microenvironment and therefore also the corrosion rate and/or corrosion mechanism of Mg alloys.

The aims of this paper therefore are multiple: (1) to study the crystal chemistry of the corrosion layer in Mg-based implants exposed to blood and plasma in order to elucidate the role of blood cells in the corrosion processes; (2) to analyze the comparability of the corrosion products obtained in blood with those obtained in physiological solutions; and (3) to analyze the effect of rare-earth-element additives to the Mg implants on the corrosion mechanism by comparing the results obtained from pure Mg and Mg alloys exposed to the same corrosion medium.

2. Materials and methods

2.1. Material preparation

Three types of metals were used in this study: pure Mg (99.95 wt%, Magnesium Electron, UK), Mg4Y0.5Gd2Nd0.5Dy alloy abbreviated as WE43 (93 wt% Mg, 4 wt% Y (yttrium), 0.5 wt% Gd (gadolinium), 2 wt% Nd (neodymium), 0.5 wt% Dy (dysprosium)) [13], and Mg10Gd1Nd alloy abbreviated as E11 (89 wt% Mg, 10 wt% Gd, 1 wt% Dy). The pure elements (99.5%) were purchased from Grirem (Beijing, China).

Mg-based castings were prepared in a mild steel crucible under a cover gas mixture of Ar_2 and 0.3% SF_6. After mixing at 730 °C for 1.5 h, the alloy was cast to the mould preheated to 500 °C. The filled mould was held at 670 °C for 1 h under protective gas. The whole steel crucible with the melt was immersed into the continuous cooling water at 10 mm/s. When the bottom of steel crucible touched the water, it stopped for 1 s. As soon as the liquid level of inside melt was in alignment with the height of outside water, the solidification process was finished. The size of the ingot was 6 cm × 12 cm × 20 cm.

2.2. Sample preparation and sterilisation

Cylindrical samples (10 mm diameter, 1.5 mm height) were cut out of the ingots by electrical discharge machining. The samples were sonificated for 20 min in dry isopropanol, dried and gamma-sterilized at the In core irradiation (ICI) facility of the Geesthacht neutron facility with a total dosage of 29 kGy.

2.3. Blood donors

In total 10 healthy volunteers with an aPTT (activated partial thromboplastin time) in the normal range (age: >20 and <40 years) were chosen. The quality of the blood used for this experiment is of decisive importance. Therefore following exclusion criteria for the blood donators have to be strictly fulfilled: smokers, drug-taking in the last 2 weeks, especially hemostasis-affecting agents like acetylsalicylic acid, oral contraceptives, non-steroidal antiphlogistics and others. Blood was drawn without stasis, very carefully by venipuncture with butterfly needles (∅ 0.9 mm) directly and sterile in pre-anticoagulated containers. Anticoagulation was done by 2 IU/ml Heparin-Natrium 25.000 (Ratiopharm GmbH, Ulm, Germany) for the human whole blood contact.

For the analysis of various blood parameters the blood was sampled by different methods: (1) for the analysis of complement and blood cells 2.7 mL blood was sampled in syringes with 1.6 mg/mL potassium-ethylenediaminetetraacetic acid (potassium-EDTA); (2) to analyse PMN-elastase, Thrombin-Antithrombin-III complex (TAT) and hemolysis 10 mL blood was sampled in syringes containing 1.0 ml citrate solution (0.106 mol/L $C_6H_5Na_3O_7 \times 2H_2O$); (3) 4.5 mL blood was sampled in CTAD-medium (a mixture of citrate, theophylline, adenosine and dipyridamole; [15]) for the analysis of β-Thromboglobulin and (4) to check the amount of magnesium and calcium 2.7 mL lithium-heparin blood (50 IU/mL blood calcium-balanced lithium) was used.

Plasma was prepared by centrifugation (Hettich Rotanta 460 R, Hettich AG, Tuttlingen, Germany): CTAD-blood by 2× centrifugation with 2500*g* for 20 min at 4 °C; EDTA blood with 2500 g for 20 min at 4 °C; citrated blood with 1800 g for 18 min at 20 °C. The plasmas were then aliquoted in 100–400 µl samples, shock-frozen in liquid nitrogen and stored at −20 °C or −80 °C for subsequent biochemical analyses.

2.4. Chandler-Loop model

All experiments were performed in an *in vitro* closed loop model modified after [16] (see Fig. 1). The system consists of a water bath for temperature regulation (Hypothermic regulator Q102, Haake, Berlin, Germany) and a rotation unit. Samples were clamped in polyvinyl chloride (PVC) loops tubes (3/8 × 3/32″, length = 50 cm). The loops were filled with 20 mL blood or plasma from one donor and closed outside with a short piece of a silicon tube. The tubing loops were rotated at 30 rpm in the water bath, which was held at 37 °C. To perform baseline measurements (control) a blood sample was taken from each donor without loop contact. As further control blood was circulated in the loops without sample to measure the background activation ("tube control"). After 90 and 360 min of circulation the blood was collected with appropriate syringes. The contact to plasma was performed in the same way but for 72 h. This time difference is due to the clotting of blood, which will lead to artificial results when used for longer times. The plasma immersion time was chosen to be comparable to the typical immersion times in cell culture media.

2.5. Blood testing

Various clinically relevant blood parameters were analysed. To determine the influence on blood coagulation Thrombin-Antithrombin-III complex (TAT) was measured via an enzyme-linked immunosorbent assay (ELISA, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) according to the manufacturer's instructions. The number of platelets, white and red blood cells was determined by blood cell counting via a Micros 60 cell counter (ABX Hematology, Montpellier, France) and platelet activation was measured by an ELISA for