Acta Biomaterialia 9 (2013) 8714–8721

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Fast escape of hydrogen from gas cavities around corroding magnesium implants ☆

Julia Kuhlmann [a], Ivonne Bartsch [b,c], Elmar Willbold [b,c], Sven Schuchardt [d], Olaf Holz [d], Norbert Hort [e], Daniel Höche [e], William R. Heineman [a,*], Frank Witte [b,c,*]

[a] Department of Chemistry, University of Cincinnati, P.O. Box 210172, Cincinnati, OH 45221-0172, USA
[b] Laboratory for Biomechanics and Biomaterials, Orthopedic Clinic, Hannover Medical School, Anna-von-Borries Strasse 1-7, 30625 Hannover, Germany
[c] CrossBIT, Center for Biocompatibility and Implant Immunology, Hannover Medical School, Feodor-Lynen Strasse 31, 30625 Hannover, Germany
[d] Fraunhofer Institute for Toxicology and Experimental Medicine, Department for Bio- and Environmental Analysis, Nikolai-Fuchs Strasse 1, 30625 Hannover, Germany
[e] Helmholtz Zentrum Geesthacht, Max-Planck Strasse 1, 21502 Geesthacht, Germany

ARTICLE INFO

Article history:
Available online 13 October 2012

Keywords:
Magnesium alloys
Hydrogen
Biodegradable implants
Gas cavities
Amperometric hydrogen sensor

ABSTRACT

Magnesium materials are of increasing interest in the development of biodegradable implants as they exhibit properties that make them promising candidates. However, the formation of gas cavities after implantation of magnesium alloys has been widely reported in the literature. The composition of the gas and the concentration of its components in these cavities are not known as only a few studies using non-specific techniques were done about 60 years ago. Currently many researchers assume that these cavities contain primarily hydrogen because it is a product of magnesium corrosion in aqueous media. In order to clearly answer this question we implanted rare earth-containing magnesium alloy disks in mice and determined the concentration of hydrogen gas for up to 10 days using an amperometric hydrogen sensor and mass spectrometric measurements. We were able to directly monitor the hydrogen concentration over a period of 10 days and show that the gas cavities contained only a low concentration of hydrogen gas, even shortly after formation of the cavities. This means that hydrogen must be exchanged very quickly after implantation. To confirm these results hydrogen gas was directly injected subcutaneously. Most of the hydrogen gas was found to exchange within 1 h after injection. Overall, our results disprove the common misbelief that these cavities mainly contain hydrogen and show how quickly this gas is exchanged with the surrounding tissue.

© 2012 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Metals have been used as internal fixtures to aid the healing of fractured bones and tissue for more than 100 years [1]. Today commonly used metals for these types of implants are stainless steel, Ti and Co–Cr alloys [2]. While these permanent implants are invaluable and generally biocompatible, they can cause problems such as stress shielding and the release of toxic metal ions through corrosion over time [1,3]. Therefore, research groups are developing biodegradable (temporary) metallic implants, many of them focusing on Mg-based materials. Although Mg materials have traditionally been used for structural applications in the automotive and aerospace industry, they have gained attention in the orthopedic and biomedical engineering fields [4,5]. Their unique properties, which include physical and mechanical properties close to those of bone, make them promising candidates for biodegradable implants. Furthermore, these materials are generally non-toxic, light in weight and corrode rapidly in aqueous environments [6,7]. During corrosion Mg is oxidized to Mg^{2+} as water is reduced to H_2 and OH^-. While the human body buffering system can compensate for the release of OH^- and some increase in Mg^{2+} is non-toxic [8], little is known about the fate of H_2 in vivo. The evolution of H_2 gas after adding Mg and its alloys to aqueous solutions has been extensively observed [9,10], as has the formation of gas cavities in vivo [11–13]. Two studies conducted over 60 years ago used techniques available at that time to analyze the gas composition of these cavities. McBride [14] reported in 1938 that gas samples aspirated from a cavity 40 days after implanting a band of Mg alloy showed a gas composition of 5.6% CO_2, 6.5% O_2, 7.3% H_2 and 80.6% N_2. However, he did not state how the gas composition was determined. In 1942 McCord et al. [15] used an interferometer to analyze the composition of gas samples drawn from sites of gas gangrene formed in rats 5 days after Mg powder implantation. Their results showed a gas composition of 1.3% CO_2 and 15.2% O_2, and they calculated that the H_2 concentration must have been 2.2% using a method

☆ Part of the Biodegradable Metals Conference 2012 Special Issue, edited by Professor Frank Witte and Professor Diego Mantovani.

* Corresponding authors. Tel.: +1 513 556 9210; fax: +1 513 556 9239 (W.R. Heineman).

E-mail addresses: heinemwr@ucmail.uc.edu (W.R. Heineman), witte.frank@mh-hannover.de (F. Witte).

1742-7061/$ - see front matter © 2012 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2012.10.008

described by Edwards [16]. Additionally, their efforts to ignite the gas sample failed, which led to the conclusion that the sample must have contained less than the 4.1% H_2 required for ignition in air. More recently Witte et al. [17] also tried to ignite samples withdrawn from gas cavities, but no combustion was observed. While these studies suggested that H_2 might not be the major component in these gas cavities, they did not directly measure H_2 nor did they use current analytical methods to determine the concentration of H_2. More importantly, while these studies remain the only ones that have attempted to analyze the H_2 concentration, it is often still assumed that gas cavities formed during Mg material corrosion in vivo contain primarily H_2 [18–21]. Nevertheless, highly selective analytical techniques to measure H_2 are available. An electrochemical sensor for H_2, analogous to the commonly used Clark O_2 sensor, has previously been used in vivo to determine local blood flow using the H_2 washout technique [22]. This amperometric sensor detects H_2 by selectively oxidizing H_2 to H^+. Although this sensor is not implantable, it would enable measurements on the surface of the gas cavities. Mass spectrometry is another commonly used analytical technique that would allow direct analysis of H_2 and other gases in the cavity.

Here we report a method to directly analyze the H_2 concentration and the gas composition of cavities formed during in vivo degradation of subcutaneously implanted rare earth-containing Mg alloy disks. We used an amperometric H_2 sensor and mass spectrometry to analyze the gas in the cavities over the course of 10 days, thereby providing a time course for H_2 behavior in vivo. After the experiment we analyzed the response of the skin covering the alloy disks and the corrosion layer on the alloys. Our results are especially significant in that they alleviate concerns about H_2 gas accumulating in the bodies of implant patients.

2. Materials and methods

2.1. Mg alloy preparation

The Mg alloy Mg–4 wt.%Y–0.5 wt.%Gd–2 wt.%Nd–0.5 wt.%Dy was prepared using pure elements by direct chill permanent mold casting according to the standard procedure as describe by Elsayed et al. [23]. All casting operations were performed under a protective gas (Ar with 2% SF_6). Pure Mg was melted in a mild steel crucible. At a melting temperature of 730 °C the alloying elements Y, Nd, Gd and Dy were added. The alloy was stirred for 20 min at 150 rpm. Afterwards the melt was poured into a thin walled (3 mm thickness) mold made of mild steel. The mold was then kept in a holding furnace at 680 °C for 1 h to homogenize the melt. After holding, the mold was dipped into flowing water (15 °C) to solidify the material and to produce a casting. Mg alloy disks (8.0 mm diameter, 1.5 mm thick) were machined from the cast material, polished with SiC emery paper (up to 4000 grit), briefly etched and cleaned in 100% ethanol in an ultrasonic bath. All disks were γ-ray sterilized with 27 kGy of ^{60}Co radiation and had an average weight of 141.2 ± 1.3 mg before implantation.

2.2. Animal model

The animal experiment was conducted under an Ethics Committee approved protocol in accordance with German federal animal welfare legislation (Ref. No. 33.9-42502-04-08/1499) and in accordance with the *National Institutes of Health Guidelines for the Use of Laboratory Animals*. 10 female hairless mice from the Charles River Laboratories (Crl: SKH1-h) aged 12–24 weeks were used in this study. These mice are hairless but immunocompetent. Their fur is normal for up to 10 days, and then the hair is gradually lost, starting around the nose. Around day 20 the fur is lost completely. The average weight of the mice used was about 26 g. After implantation each mouse was housed individually and was fed a standard diet (Altromin1324) and water ad libitum. The animal husbandry rooms were illuminated by artificial light 14 h a day starting at 7 a.m. The mice were anaesthetized by intraperitoneal injection of 2% xylazine (10 mg kg body weight^{-1}, Rompun®, Bayer Health Care, Leverkusen, Germany) and 10% ketamine (100 mg kg body weight^{-1}, KetaminGräub®, Albrecht GmbH, Aulendorf, Germany). In order to avoid cooling of the body mice were placed on a custom made heating plate during surgery and measurements. The dorsal skin was cleaned according to surgical guidelines. Two longitudinal incisions (one in the shoulder region, one in the lumbar region) of 1 cm each were made in the median line through the full thickness of the skin. Subcutaneous pockets between the fascia of the dorsal muscles and the subcutaneous tissue were created by blunt dissection with scissors. The implants were placed in these pockets. The skin was closed with resorbable surgical suture material (Vicryl, Ethicon, Johnson & Johnson GmbH, Germany).

For the H_2 injection experiments (99.999%) was injected subcutaneously into hairless mice (three mice per time point). The gas was withdrawn with gastight syringes 1, 2, 4 and 12 h after injection and analyzed by mass spectrometry as described in Section 2.3.

2.3. H_2 measurements

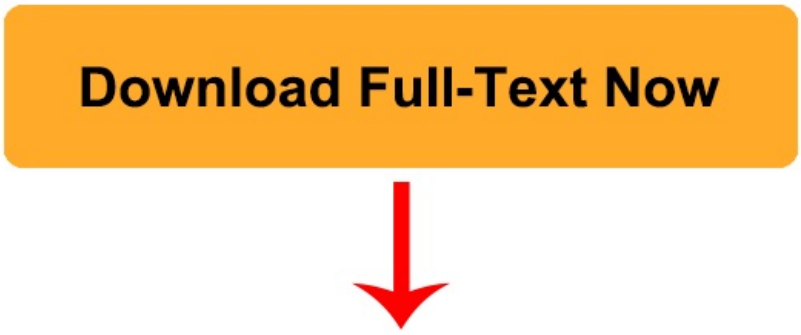
Amperometric H_2 measurements were performed using a H_2 microsensor (50 μm tip diameter) connected to a multimeter (both from Unisense, Aarhus, Denmark) polarized at +800 mV for at least 1 h [24]. After a stable current in the low picoampere range was established, the amperometric sensor was ready to be used. The sensor was calibrated by adding known amounts of H_2-saturated deionized H_2O to a known volume of deionized H_2O (according to the manufacturer's recommendations [24,25]). Measurements were taken for 3 min on the skin on top of the gas cavities and subcutaneously in an incision on top of the gas cavities. As a control, measurements were taken on top of the skin in an area without any gas cavities. Standard deviations (error bars in the graphs) were calculated from the averaged first 100 data points taken during each of the 3 min measurements. Microsensor readings were converted into vol.% for comparison with the mass spectrometry data. The manufacturer reported the limit of detection to be 0.02% (0.1 μM) in H_2O.

For mass spectrometry, the gas samples were withdrawn from the cavities using 2.5 ml gas tight syringes (Hamilton Messtechnik GmbH, Höchst, Germany). The needles were covered with a septum until the samples were analyzed using a SMart Nose® volatile organic compound analyzer system (former Smartnose SA, Switzerland), which was fitted with a special injection device and a capillary for gas phase transfer purposes. The analysis was performed with as-received gas samples, without any further preparation. About 1–2 ml of each sample were introduced into the injection port heated to 22 °C. Before injection, the N_2 flow was stopped for 20 s and acquisition was started simultaneously with opening of the gas outlet. Data were acquired at 70 eV, from 1 to 60 m/z, conducting four cycles within 150 s. Averaged cycles 2–4 were used for data analysis. After each injection the entire system was purged with N_2 at a flow rate of 80 ml min^{-1} for 2 min. The system was calibrated with pure gas samples of H_2 (99.999%), O_2 (99.5%), and CO_2 (99.995%) as well as wish by gas mixtures of H_2 and CO_2 injected in amounts from 0.1 to 1.0 ml under similar conditions.

2.4. Histology

The excised tissue samples were fixed in 3.7% commercial formalin (Otto Fischer, Saarbrücken, Germany), then embedded and

ID	Title	Pages
583	Fast escape of hydrogen from gas cavities around corroding magnesium implants ☆	8



http://fulltext.study/article/583

FullText.study

Categorized Journals
Thousands of scientific journals broken down into different categories to simplify your search

Full-Text Access
The full-text version of all the articles are available for you to purchase at the lowest price

Free Downloadable Articles
In each journal some of the articles are available to download for free

Free PDF Preview
A preview of the first 2 pages of each article is available for you to download for free

http://FullText.Study