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The potential of isotopically enriched magnesium to study bone implant degradation *in vivo*

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ABSTRACT

This pilot study highlights the substantial potential of using isotopically enriched (non-radioactive) metals to study the fate of biodegradable metal implants. It was possible to show that magnesium (Mg) release can be observed by combining isotopic mass spectrometry and isotopic pattern deconvolution for data reduction, even at low amounts of Mg released a from slowly degrading ^{26}Mg enriched (>99%) Mg metal. Following implantation into rats, structural *in vivo* changes were monitored by µCT. Results showed that the applied Mg had an average degradation rate of 16 ± 5 µm year^{-1}, which corresponds with the degradation rate of pure Mg. Bone and tissue extraction was performed 4, 24, and 52 weeks after implantation. Bone cross sections were analyzed by laser ablation inductively coupled plasma mass spectrometry (ICP-MS) to determine the lateral ^{26}Mg distribution. The ^{26}Mg/^{24}Mg ratios in digested tissue and excretion samples were analyzed by multi collector ICP-MS. Isotope pattern deconvolution in combination with ICP-MS enabled detection of Mg pin material in amounts as low as 200 ppm in bone tissues and 20 ppm in tissues up to two fold increased Mg levels with a contribution of pin-derived Mg of up to 75% (4 weeks) and 30% (24 weeks) were found adjacent to the implant. After complete degradation, no visual bone disturbance or residual pin-Mg could be detected in cortical bone. In organs, increased Δ^{26}Mg/^{24}Mg values up to 16‰ were determined compared to control samples. Increased Δ^{26}Mg/^{24}Mg values were detected in serum samples at a constant total Mg level. In contrast to urine, feces did not show a shift in the ^{26}Mg/^{24}Mg ratios. This investigation showed that the organism is capable of handling excess Mg well and that bones fully recover after degradation.

Statement of Significance

Magnesium alloys as bone implants have faced increasing attention over the past years. *In vivo* degradation and metabolism studies of these implant materials have shown the promising application in orthopaedic trauma surgery. With advance in Mg research it has become increasingly important to monitor the fate of the implant material in the organism. For the first time, the indispensible potential of isotopically enriched materials is documented by applying ^{26}Mg enriched Mg implants in an animal model. Therefore, the spatial distribution of pin-Mg in bone and the pin-Mg migration and excretion in the organism could be monitored to better understand metal degradation as well as Mg turn over and excretion.
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1. Introduction

Biodegradable magnesium (Mg) implants used for fracture fixation have attracted increasing attention over the past two decades [1–3]. Current research has shown promising results in alloy development [4–7] and in *in vivo* studies [1,8–11]. In particular, the

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adjustable degradation rate induced by different alloying elements and the ongoing progress towards 'custom made' implants are and advantage of these Mg-based materials.

A number of studies have focused on understanding degradation behavior [1,3,8] and the influence on bone remodeling [12–14]. The application of Mg-based implants leads to significant amounts of Mg being released into the surrounding (bone) tissue [15]. Serre et al. [16] investigated Mg levels in bone and reported decreased osteoblastic activity in sponges used for bone crafting, made from Mg substituted apatite. Anders [17] highlighted a positive effect of higher Mg content on tissue calcination. No reduced osteoblastic activity was reported. However, upon injection of high Mg levels, loss of crystalline structure was observed. Therefore, these studies have fostered the ambition of further investigating the distribution and potential local enrichment of Mg. Amerstorfer et al. [15] reported a significant increase of Mg content in cortical bone adjacent to a remaining implant. Wang et al. [18] further investigated *in vivo* degradation of Mg implants in the context of deteriorated kidney functions and concluded that the application of Mg based orthopaedic medical devices is free of biosafety concerns even for patients with chronic renal failure.

The use of isotopically enriched medications (e.g., ^{11}C or ^{18}F [19,20]) and the administration of stable isotopes (e.g. ^{41}Ca [21], ^{77}Se [22] or ^{70}Zn [23]) have proven to be important tools for studying elemental pathways in organisms. The application of stable (non-radioactive) and non-toxic isotopically enriched Mg implants therefore has the potential to elucidate the metabolic pathway of Mg in bone tissue and organs. Chemical imaging by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) allows for both by determining the elemental and isotopic distribution within hard tissues [15,24]. In order to detect minimum amounts of isotopically enriched material, isotope pattern deconvolution (IPD) has proven to be a powerful method [25]. The combination of bone structure analysis by microfocus computed tomography (μCT) and chemical imaging of the elemental distribution by LA-ICP-MS is therefore a successful approach to investigate both the *in vivo* degradation and the μm scale spatial distribution of elements originating from degrading implants [24].

This pilot study is the first to implant ^{26}Mg-enriched (>99%) cylindrical pins into rat femurs, in order to analyze the spatially resolved deposition of pin-derived Mg in bone (LA-ICP-MS), along with time resolved *in vivo* monitoring of pin degradation by μCT. The accumulation of pin-derived Mg in organs, blood serum and excreted material was studied over time by multi collector (MC) ICP-MS measurements. These tools were evaluated for their ability to reveal more details of Mg pathways in organisms.

2. Materials and methods

2.1. Reagents and materials

All laboratory work was performed in class 100000 (ISO 8) cleanrooms. Laboratory water type I (18.2 MΩ cm, TKA-GenPure (Thermo Electronics), Niederelbert, Germany) and nitric acid (p. a., Merck, Darmstadt, Germany) purified by (double) sub-boiling distillation (DST-1000, Savillex, Eden Prairie, MN, USA) were used for dilution and digestion. Single element standards were prepared gravimetrically from stock solutions (Mg, In; Inorganic Ventures, Christiansburg, VA, USA). A digest of the certified reference material (CRM) BCR185R (Bovine liver, IRMM, Geel, Belgium) was included in all solution-based ICP-MS measurements. Matrix matched LA-ICP-MS standards were prepared by co-precipitation of Mg in hydroxyapatite according to Draxler et al. [24]. Bone meal (SRM 1486, NIST, Gaithersburg, MD, USA) pressed into pellets without further binding material served as quality control standard for LA-ICP-MS measurements. All consumables were washed in 10% and 1% (*w*/*w*) HNO_3 each for 24 h, rinsed with purified water and dried in a laminar flow hood.

2.2. ^{26}Mg pin preparation

250 mg of isotopically enriched elemental Mg (electromagnetic enrichment, Trace Science International, Pilot Point, TX, USA) with a composition of 0.35 ± 0.02%, 0.08 ± 0.002% and 99.57 ± 0.02% for ^{24}Mg, ^{25}Mg and ^{26}Mg, respectively were embedded in epoxy resin (Epofix, Struers, Willich, Germany). Investigation of the elemental composition of the metal revealed contents of Cu, Ni, and Mn of <1 ng g^{-1} and Fe of about 5 μg g^{-1}, respectively. Six pins were cut out of the embedded block using an IsoMet low speed saw (Buehler, ITW Test & Measurement GmbH, Düsseldorf, Germany). The pins were manually burnished with a lapping film (120 μm) into cylindrical form, and further polished using a 30 μm fine-grained lapping film. Subsequently, the pins were cleaned in an ultrasonic bath in water for 5 min and in ethanol for 10 min and dried in a laminar flow hood. Pins with the dimensions of 1.3–1.5 × 5–7 mm and weights between 8 and 13 mg were produced. The resulting variable pin size was unavoidable due to the geometry of the available material and the manual in-house production which was optimized to minimize material loss. Moreover, only a limited number of pins could be produced from the available amount of expensive starting material. Prior to implantation, the pins were packed (Biegler GmbH, Mauerbach, Austria) and sterilized by gamma radiation (Mediscan GmbH & Co KG, Seibersdorf, Austria). Gamma rays passed through the plastic wrapping of the Mg pins and broke covalent bonds of bacterial DNA and by that sterilized the metal. No effect on the Mg content and the elemental composition is therefore expected. 9.36 mg of the original ^{26}Mg piece were dissolved in 1.5 mL of 30% (*w*/*w*) HNO_3 (open PFA vessel at ambient conditions) to verify the composition by ICP-MS prior to use.

2.3. Animal experiments

Animal experiments were performed according to Austrian regulations of ethical aspects under the project number BMWFW-66.010/0122-WF/V/3b/2014. Sprague–Dawley rats (140–160 g body weight) were housed at a maximum of 4 rats in clear plastic cages on standard bedding. Water and a standard pellet diet (Ssniff Spezialdiäten GmbH, Soest, Germany) were provided. Ten rats were used for this study. Six rats were subject to implantation of ^{26}Mg pins in their right femoral bones (their left femur remained untreated and was used as control). Two rats served as control animals for the organs, one for the untreated control femur and one for feces control. A complete list of samples is provided in Table 1.

For implantation, general anesthesia was administered and the surgical procedure as described in Kraus et al. [8] was followed. The rodents could move freely in their cages without external support. The well-being of the animals was checked daily throughout the entire study. μCT scans were performed one week after operation and continuously after 4, 8, 12 and 24 weeks until bone explantation.

Blood and feces were collected during the entire lifespan of all animals. Blood samples were collected under volatile anesthesia (Forane®, Abbot AG, Baar, Switzerland) by puncturing the tongue vein with a hollow needle. 1.2 mL blood was drawn into lithium heparin-coated tubes. 250 international units heparin were added to each sample to avoid blood coagulation. Blood was drawn immediately before pin implantation, weekly in the first four post-operative weeks and then every 4 weeks up to week 24 after implantation according to Pichler et al. [26].

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