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The formation of an organic coat and the release of corrosion microparticles from metallic magnesium implants

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ABSTRACT

Magnesium alloys have been proposed as prospective degradable implant materials. To elucidate the complex interactions between the corroding implants and the tissue, magnesium implants were analyzed in a mouse model and the response was compared to that induced by Ti and by the resorbable polymer polyglactin, respectively. One month after implantation, distinct traces of corrosion were apparent but the magnesium implants were still intact, whereas resorbable polymeric wound suture implants were already fragmented. Analysis of magnesium implants 2 weeks after implantation by energy-dispersive X-ray spectroscopy indicated that magnesium, oxygen, calcium and phosphate were present at the implant surface. One month after implantation, the element composition of the outermost layer of the implant was indicative of tissue without detectable levels of magnesium, indicating a protective barrier function of this organic layer. In agreement with this notion, gene expression patterns in the surrounding tissue were highly similar for all implant materials investigated. However, high-resolution imaging using energy-filtered transmission electron microscopy revealed magnesium-containing microparticles in the tissue in the proximity of the implant. The release of such corrosion particles may contribute to the accumulation of calcium phosphate in the nearby tissue and to bone conductive activities of magnesium implants.

1. Introduction

The body responds to foreign implant materials by a specific series of events, including the almost immediate formation of a proteinaceous layer on the implant surface. Within the first hour, immune cells are attracted from the blood–most rapidly neutrophils, which are later followed by monocyte-derived macrophages. While the neutrophil accumulation is only transient, the macrophages remain in the granulation tissue and govern the subsequent inflammatory and wound healing responses, such as the formation of a fibrous capsule [1]. Implants that are no longer required are commonly removed after the healing process is completed. For temporary applications, degradable medical implants could overcome the requirement for revision surgery [2]. With respect to resorbable polymers, corrodible metal alloys appear to be less inflammatory and have superior mechanical properties in bone repair or as vascular stents [3–6]. Magnesium alloys are presently being investigated as promising biodegradable implant materials and could circumvent the major side effects of the currently used stents [7]. In bone repair, magnesium-based implants could help to reduce bone loss due to the stress-shielding effects that are associated with current permanent materials [4,8–10]. In addition, there are indications that magnesium acts osteoconductively and could promote bone repair [4,5,11–13]. Magnesium has even been shown to act antibacterially, and could therefore antagonize difficult-to-treat implant-associated infections [14–16]. After implantation, magnesium corrodes by reacting with water, whereby magnesium ions, hydrogen and hydroxide ions are generated [17,18]. These products have diverse biological properties. Cells contain up to 20 mM magnesium ions, and excess amounts can be excreted [19–23]. However, rapid and irregular corrosion and excess hydrogen production could lead to premature mechanical implant failure and to gas-filled cavities in the tissue, respectively [24]. If the degradation rate exceeds the buffering capacity of the tissue, hydroxide ions could lead to pH increases and cell death at implant–tissue interfaces [25]. Therefore, various strategies have been devised to improve the corrosion resistance, such as anti-corrosive implant coatings, optimization of alloy compositions or manufacturing processes such as for the generation of metallic glasses [14,26–35]. Such novel materials are conventionally tested first in vitro. Due to still incompletely understood interactions

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between corroding implants and the surrounding tissue, no standard in vitro testing protocols have been established that could reliably predict the behavior of degradable implant materials in vivo [26,36–38]. Consequently, animal models are essential to validate the in vitro results. Mouse models have specific advantages for evaluating tissue–implant material interactions, such as the availability of genetically highly defined mouse strains and strains that mimic human disease states, which facilitate the generation of reproducible results and the elucidation of molecular mechanisms [39–44].

In this study, tissue interactions with magnesium implants were evaluated in a simple mouse model in a side-by-side comparison with two diverse clinically established materials, titanium and the resorbable wound suture material polyglactin [45,46]. Histology and gene expression analyses were used to characterize implant–tissue interactions. Energy-dispersive X-ray spectroscopy (EDX) and energy-filtered transmission electron microscopy (EF-TEM) were used to analyze implant surfaces and the adjacent tissue, respectively. In agreement with previous findings, corroding magnesium implants induced the deposition of calcium phosphates in the corrosion layer [14,47–50]. Interestingly, magnesium-rich microparticles were detected in the tissue adjacent to magnesium implants.

2. Materials and methods

2.1. Implant preparation

Wires of pure magnesium (99.9% purity; Goodfellow Cambridge Ltd., Huntingdon, UK), 0.4 mm in diameter, were cut into 10 mm long pieces and incubated overnight in a aqueous solution of 1 M NaOH at 37 °C to obtain a partially protective magnesium hydroxide layer [51]. The pieces were briefly blotted dry on filter paper and then rinsed in sterile distilled water. The excess liquid was again removed with filter paper and the samples were air dried and stored dry until implantation. Titanium wire (ARA-T Advance GmbH, Dinslaken, Germany), 0.4 in mm diameter, was cleaned with ethanol and cut into 10 mm long pieces. Similarly, a 45 cm long, 0.4 mm diameter sterile resorbable surgical suture material Ethicon 6–0 coated Vicryl V489 (polyglactin 9.0) monofilament, a poly(glycolide lactide) copolymer (Aesculap Inc., Center Valley, PA), was cut into 10 mm long pieces for implantation into mouse tails.

2.2. Animal handling

A total of 42 female BALB/c mice (Harlan-Winkelmann, Borchen, Germany), 6–10 weeks old, were kept under specific pathogen-free conditions in groups of a maximum five animals per cage, each with individual aeration. All animals were fed with a standard diet without lipid or cholesterol supplements and with drinking water ad libitum. Animals were anesthetized by intraperitoneal injection of ketamine (10 mg kg^{-1}) and xylazine (4 mg kg^{-1}). Ten minutes later, the mice were laid on their back in a laminar flow hood and their tails disinfected with kodan (Schülke und Mayr, Norderstedt, Germany).The skin of the tail was pierced by a hypodermic needle (18 gauge) and then a piece of magnesium wire, titanium wire or polyglactin monofilament was inserted into the lower tail artery as previously described [52], except that no catheter was used. The implantation site, the appearance and the behavior of the animals were visually inspected daily in the first week after implantation and later at least twice a week (Fig. 1). For gene expression analysis, 36 mice in total were used (see below). Six mice in total were used for histology, each with three consecutive implants of titanium, magnesium or polyglactin. Implants were examined 2 and 4 weeks after implantation. The two proximal implants from each of three animals were used for histological analysis, while the implant that was located towards the distal tail tip was used for electron microscopic analysis. The experiments were approved by the local authorities (permission No. 33.42502/07-10.05).

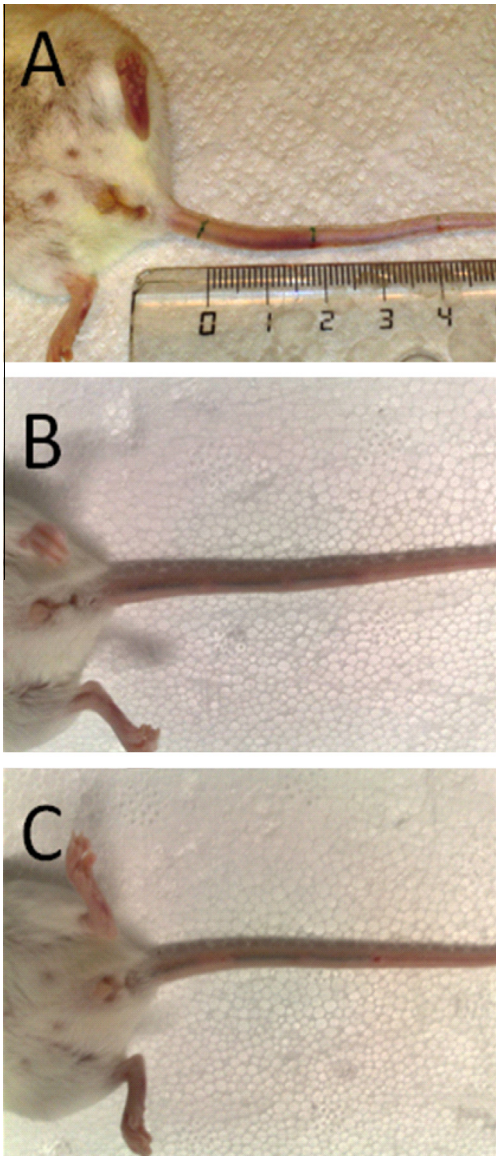

Fig. 1. Minor wounding and inconspicuous inflammatory reactions to magnesium implants in the mouse tail. Three magnesium pins with a diameter of 0.4 mm were inserted consecutively every 2 cm (demarcated with a felt pen) in the ventral tail vessel of BALB/c mice. The mice were anesthetized at the times indicated. Pictures were taken of the implantation site as follows: tail with magnesium implants immediately after implantation (A); 5 days after implantation (B); and 10 days after implantation (C).

2.3. Scanning reflection electron microscopic (REM) analysis

The samples were air dried and then fixed on the support with an electrically conducting glue foil and analyzed by EDX using a fully automated variable pressure Hitachi REM S-3400N. The acceleration voltage used was 10 keV and the distance was adjusted to 10 mm. Images were taken at a magnification of 200-fold at a working distance of 21–22 mm with an emission current of 80–110 μA. The quantifications were corrected according to the

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