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Long-term in vivo degradation behaviour and biocompatibility of the magnesium alloy ZEK100 for use as a biodegradable bone implant ☆

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ABSTRACT

Magnesium alloys are the focus of research as resorbable materials for osteosynthesis, as they provide sufficient stability and would make surgery to remove implants unnecessary. The new degradable magnesium alloy ZEK100 was developed to improve the stability and corrosion resistance by alloying with zinc, rare earth metals and zirconium. As the implants were degraded to only a limited extent after 6 months implantation in a previous in vivo study the present study was conducted to evaluate the long-term degradation behaviour and biocompatibility in the same animal model over 9 and 12 months. Five rabbits each with intramedullary tibia implants were examined over 9 and 12 months. Three legs were left without an implant to serve as negative controls. Numerous examinations were performed in the follow-up (clinical examinations, serum analysis, and radiographic and in vivo micro-CT investigations) and after death (ex vivo micro-CT, histology, and implant analysis) to assess the in vivo degradation and biocompatibility. It could be shown that favourable in vivo degradation behaviour is not necessarily associated with good biocompatibility. Although ZEK100 provided a very high initial stability and positive biodegradation, it must be excluded from further biomedical testing as it showed pathological effects on the host tissue following complete degradation.

1. Introduction

In recent years magnesium alloys have become a special focus of biomedical research [1–8] as they are considered to be suitable for fracture repair of weight-bearing bone [1,9,10]. Magnesium alloys are biodegradable and would therefore make surgery for the removal of implants unnecessary [11]. Furthermore, in contrast to conventional metals such as titanium, steel or cobalt–chromium alloys [12,13], their mechanical properties, which are similar to those of cortical bone [3,14], prevent stress shielding effects. The advantages of magnesium alloys over commercially available resorbable polymers are the higher mechanical stability [1,15,16] and the fact that their corrosion products do not induce as much inflammatory reaction [17].

As the degradation rate of pure magnesium is too high and often results in gas accumulation [18–21] and, additionally, pure magnesium lacks strength, the influence of alloying with various elements and of different surface treatments have been tested in vitro during recent years [6,14,22–27]. However, as it has been shown that results obtained in vitro do not necessarily predict the in vivo behaviour [28], a substantiated conclusion can only be drawn after in vivo evaluation.

A number of different promising magnesium alloys have been tested in vivo [16,29–35], but most of them still lack stability [29,30,36] or show a disadvantageous degradation behaviour [29,36]. Furthermore, most in vivo studies did not observe the complete degradation period, only the beginning of corrosion [16,32,33,37,38], so biocompatibility during the later stages of degradation have not been well researched. For these reasons the new degradable magnesium alloy ZEK100 was developed to improve the stability and corrosion resistance by alloying with zinc, rare earth metals and zirconium [3,39] and tested over 6 months in an in vivo study using an orthotopic implantation model [40]. As the implants were degraded to only a limited extent during the examination period and showed favourable degradation characteristics and suitable mechanical properties [40] the present study was conducted to evaluate the long-term degradation behaviour and biocompatibility in the same animal model over 9 and 12 months.

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2. Materials and methods

2.1. Implants

The magnesium alloy used in this study was designed and made as part of the research performed in the collaborative research center SFB599. It is not yet commercially available. The overall composition of ZEK100 was determined by means of inductively coupled plasma –optical emission spectroscopy (ICP-OES) (Spectro Ciros Vision EOP, SPECTRO Analytical Instruments GmbH, Kleve, Germany) resulting in 0.96 wt.% zinc, 0.21 wt.% zirconium, 0.3 wt.% rare earth elements (RE) and the remainder being magnesium. The nomenclature of this alloy is in accordance with the ASTM standard B275-90 [41].

Manufacture of the ZEK100 billets was by a gravity die casting process with subsequent direct extrusion, as described previously [40]. The final cylindrical implants were 2.5 mm in diameter and 25 mm in length and had a fine grained spherical microstructure with grain diameters of 3.75 μm (determined according to ISO 643:2003). Microstructural mapping showed that the ZEK cylinders are characterized by zirconium-rich precipitates along the grain boundaries (Fig. 1, marked by arrows) and RE- and zinc-rich spherical shaped phases inside the grains (Fig. 1, marked by circles), determined by means of an electron probe microanalyser using the compo-mode (JEOL GmbH, Eching, Germany).

As initial mechanical characterization values ZEK100 exhibit a yield strength of 227 MPa, a tensile strength of 271 MPa and an elongation of 15% (determined by tensile tests according to ISO 6892-1:2009), as well as a maximum force of 267 N (uncorroded state, diameter 2.5 mm, length: 25 mm, determined by three point bending tests using a universal testing machine Model Z 250, Zwick GmbH & Co. KG, Ulm, Germany).

All implants were washed in acetone and distilled water in an ultrasonic bath. They were sterilized by gamma irradiation at 25 kGy for 8 h by a commercial provider (BBF Sterilisationsservice, Kernen, Germany) according to DIN EN ISO 11137:2006 [1,3,40].

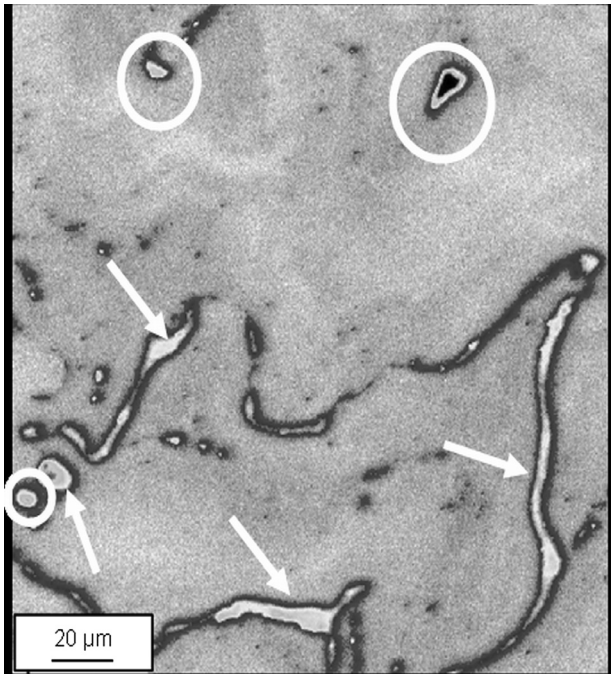

Fig. 1. Local allocations of alloying elements detected within the longitudinal specimen using EPMA analysis. Arrows point to zirconium-rich phases while circles mark RE- and zinc-rich phases.

2.2. Animal model

The animal experiments of this study were approved by the regional government and in accordance with Section 8 of the German Animal Welfare Act. They were approved by the Office for Consumer Protection and Food Safety under approval number 33.9-42502-04-07/1363.

For the animal experiment 10 adult female New Zealand White Rabbits (Charles River, Kisslegg, Germany) with a body weight of 3.0–3.5 kg were used.

The animals were randomized into two groups of five animals each with implantation times of 9 or 12 months. There were three animals (two in the 9 months group, one in the 12 months group) with just one leg with an implant. The other leg underwent the same surgical procedure but without implanting an intramedullary pin. This resulted in 17 implants in total and three negative controls, which served as the control group without intramedullary implants.

Anaesthesia was induced by intramuscular injection of 10 mg kg^{-1} s-ketamine hydrochloride (CP-Pharma, Burgdorf, Germany) and 0.125 mg kg^{-1} medetomidine (Domitor®, Pfizer GmbH, Berlin, Germany). Following endotracheal intubation, narcosis was maintained by inhalation anaesthesia using a mixture of isoflurane and oxygen (2–3 vol.% isoflurane, oxygen airflow 0.4–0.6 l min^{-1}) (Isoba®, Essex Pharma GmbH, Munich, Germany) under spontaneous or assisted respiration. Shortly before incision 10 μg kg^{-1} fentanyl dihydrogencitrate (Fentanyl-Janssen®, Janssen-Cilag GmbH, Neuss, Germany) was given intravenously. The rabbits also received an infusion of 10 ml kg^{-1} h^{-1} Paediafusin© (Baxter, Unterschleissheim, Germany) during surgery.

An incision was made on the medial side of the tibia, just medio-distal of the tibial tuberosity. A hole (2.5 mm) was drilled through the cortex so that the implant could be placed longitudinally in the middle third of the medullary cavity. In the control animals the operation procedure was performed as described above but no implant was inserted.

Before the operation procedure and for 10 days post-operatively the animals received antiphlogistic, 0.15 mg kg^{-1} day^{-1} meloxicam (Metacam®, Boehringer Ingelheim, Ingelheim, Germany), and antibiotic, 10 mg kg^{-1} day^{-1} enrofloxacin (Baytril® 2.5%, Bayer HealthCare, Leverkusen, Germany) medication.

2.3. Clinical examination

During the follow-up clinical orthopaedic examinations were performed on a daily basis. Special attention was paid to wound healing, swelling affecting the hind legs, subcutaneous accumulation of gas and the occurrence of lameness.

2.4. Serum magnesium

During the first 6 months after implantation blood samples were taken before implantation, bi-weekly until week 8 and every 4 weeks thereafter. The blood samples were centrifuged and the serum was analysed using a Roche Hitachi 911 Chemistry Analyzer (Roche Deutschland Holding GmbH, Grenzach-Wyhlen, Germany) using a special magnesium analysis reagent (Mg Cobas®, Roche Diagnostics GmbH, Mannheim, Germany).

2.5. Intravital fluorochrome staining

During the last 3 months of the implantation period fluorochrome solutions were applied subcutaneously according to a previously published protocol (Table 1). Based on Rahn et al. [42,43] the fluorochrome stains used were calcein green, xylenol orange, calcein blue and tetracycline.

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