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Biocompatibility of rapidly solidified magnesium alloy RS66 as a temporary biodegradable metal ☆

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ABSTRACT

Biodegradable magnesium-based alloys are very promising materials for temporary implants. However, the clinical use of magnesium-based alloys is often limited by rapid corrosion and by insufficient mechanical stability. Here we investigated RS66, a magnesium-based alloy with extraordinary physicochemical properties of high tensile strength combined with a high ductility and a homogeneous grain size of ~1 μm which was obtained by rapid solidification processing and reciprocal extrusion. Using a series of in vitro and in vivo experiments, we analyzed the biodegradation behavior and the biocompatibility of this alloy. In vitro, RS66 had no cytotoxic effects in physiological concentrations on the viability and the proliferation of primary human osteoblasts. In vivo, RS66 cylinders were implanted into femur condyles, under the skin and in the muscle of adult rabbits and were monitored for 1, 2, 3, 4 and 8 weeks. After explantation, the RS66 cylinders were first analyzed by microtomography to determine the remaining RS66 alloy and calculate the corrosion rates. Then, the implantation sites were examined histologically for healing processes and foreign body reactions. We found that RS66 was corroded fastest subcutaneously followed by intramuscular and bony implantation of the samples.

No clinical harm with transient gas cavities during the first 6 weeks in subcutaneous and intramuscular implantation sites was observed. No gas cavities were formed around the implantation site in bone. The corrosion rates in the different anatomical locations correlated well with the local blood flow prior to implantation. A normal foreign body reaction occurred in all tissues. Interestingly, no enhanced bone formation could be observed around the corroding samples in the condyles. These data show that RS66 is biocompatible, and due to its interesting physicochemical properties, this magnesium alloy is a promising material for biodegradable implants.

1. Introduction

Since the last decades of the 19th century, several attempts have been made to use magnesium or magnesium-based alloys as materials for temporary cardiovascular or orthopaedic implants [1,2]. These materials offer some remarkable physicochemical properties such as high stiffness and specific strengths which resemble those of bone [3]. Moreover, these materials are biodegradable. They corrode and disintegrate gradually up to complete dissolution in physiological environments. All processes of wound healing, bone regeneration and functional tissue restoration can proceed inversely with the degradation of the implant, and thus harmful stress shielding effects are widely avoided [3–5]. Furthermore, increased bone growth has been repeatedly reported in the vicinity of corroding magnesium implants or their corrosion products [6–9]. The complete dissolution of biodegradable implants is highly beneficial both clinically and economically since it renders subsequent surgical interventions for implant removal unnecessary. Despite these promising benefits and properties, the practical and clinical utilization has been hampered in the past by various insurmountable obstacles and drawbacks such as the formation of extensive gas cavities and especially an insufficient mechanical stability of the implants due to rapid corrosion during extended implantation periods (for a comprehensive review see Ref. [1]).

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Even though several successful attempts have demonstrated magnesium-based materials which degrade very slow in vivo without clinical observation of gas cavity evolution [10,11], these magnesium-based materials have not been proven to dissolve completely [10,11]. Thus ideally, a magnesium alloy of high tensile strength and high ductility to allow intraoperative mechanical deformation (e.g. bending) of the implant combined with a complete dissolution within 1–2 years seems to be more important, since the initial corrosion rates can be reduced by additional surface treatments and coatings. Therefore, there is a great demand for new processing techniques and innovative approaches for improving both the physicochemical as well as the structural characteristics of magnesium alloys in loaded applications. One of these innovative approaches to refining and modifying the microstructure of alloys is so-called rapid solidification processing [12,13]. Alloys made by this technology are characterized by low segregation and high solubilty of the alloying elements. In combination with a process, called reciprocating extrusion, an innovative magnesium alloy (RS66) with a relatively high melting point of 505 °C has been produced which shows a homogeneous microstructure and interesting mechanical properties [14]. RS66 can reach a strength of almost 600 MPa, while at 300 MPa the same alloy has 27% ductility at room temperature. RS66 is superplastic at elevated temperatures and can be deformed to several hundred per cent at 250 °C. Although the mechanical properties of RS66 are promising, a thorough in vitro and in vivo investigation is required to evaluate its corrosion behavior and the reactions of the host tissues.

Here we investigated the corrosion behavior and the biocompatibility of RS66 in vitro and in vivo:

- In vitro, the viability and the proliferation rates of primary human osteoblasts were determined in extract tests according to ISO 10993.
- In vivo, cylindrical implants of RS66 were placed into the femur condyle, into muscle and under the skin of adult rabbits and were monitored for up to 8 weeks. Microtomography was used to analyze the in vivo corrosion behavior and the remaining metal volumes in all three implantation sites.
- To investigate the host tissue reactions on a cellular level, Technovit 9100 New-based histology was applied for intense structural and semiquantitive evaluations of important bone remodeling parameters.
- A special focus was the foreign body reaction (FBR) and the immunological response of the host tissues to corroding RS66 implants.

2. Materials and methods

2.1. RS66 sample production

RS66 is a magnesium alloy with a nominal composition of Mg–6.0% Zn–1.0% Y–0.6% Ce–0.6% Zr. The composition is based on the commercial alloy ZK60. Rapidly solidified ribbons were produced by a single-roller melt-spinning machine under a low-pressure argon atmosphere by remelting the cast material at 750 °C in a plain carbon steel crucible with an Al_2O_3 nozzle at its bottom. The width of the ribbon was 11 mm and the thickness was <80 μm. In a second manufacturing step, these ribbons were comminuted into ~2 mm × ~2 mm × 0.08 mm chips and stored under a protective argon atmosphere for further compaction and extrusion. Thirdly, the chips were hot compacted at 150 °C into a cylindrical billet of 200 mm diameter under vacuum and held 24 h for degassing. Then, the billet was forward extruded into cylindrical bars of 50 mm diameter at ~350 °C. Finally, the billets were extruded reciprocatively from 50 to 50 mm diameter through a 14 mm diameter die. The reciprocal extrusion temperatures were held between 320 and 338 °C, the ram speeds were selected between 3.22 and 8 mm min^{-1}, and the extrusion pressure was 9 MPa. For a detailed process description see Ref. [15] and for a detailed material characterization see Ref. [14]. The implants were gamma-sterilized with 25–29 kGy of ^{60}Co radiation (BBF Sterilisationsservice, Kernen, Germany).

2.2. Production of RS66 extracts

Medium supplemented with extracts of RS66 was prepared by incubating RS66 cylinders (3 mm diameter × 5 mm height) in 1.5 ml of osteoblast growth medium (HOB medium, Promocell, Heidelberg, Germany) supplemented with 1% CellCultureGuard (Applichem, Darmstadt, Germany) and supplement kit (Promocell) in a standard incubator at 37 °C and 5% CO_2 for 3 days. The osmolality was then measured with an osmometer (Semi-Micro Osmometer K-7400, Knauer, Berlin, Germany). The extract containing medium from 6 wells was pooled and diluted 1:2 and 1:11, respectively, with aged (i.e. without RS66 corrosion extracts) HOB medium which has been also incubated at 37 °C and 5% CO_2 for 3 days.

2.3. Viability and proliferation testing of primary human osteoblasts

Primary human osteoblasts (HOB, Promocell, Heidelberg, Germany) were precultivated for 24 h at 37 °C, 5% CO_2 and 92 ± 2% relative humidity in osteoblast growth medium. 200 μl of cell suspensions (3×10^4 cells ml^{-1}) were seeded into 96 well plates. After 24 h, the medium was removed and replaced by pure, 1:2 or 1:11 dilutions of RS66 extract containing medium and cultivated for a further 72 h. As a control, cells were incubated in aged HOB medium without RS66 extracts. The viability of the cells was tested using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) from Promega (Mannheim, Germany). Cell proliferation was determined with the Cell Proliferation ELISA BrdU (colorimetric) kit from Roche (Mannheim, Germany), both according to the manufacturer's instructions. RMA (10 mm diameter, 0.38 mm thick; a cytotoxic reference material according to ISO 10993; Hatano Research Institute, Hadano, Japan) was used as a positive control.

2.4. Inductively coupled plasma optical emission spectroscopy

The elemental contents of the RS66 extract media were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES).

2.5. Animal study design

The animal experiment was conducted under an ethics committee approved protocol in accordance with German federal animal welfare legislation (Approval No. 33.9-42502-04-08/1505) and in accordance with the National Institute of Health guidelines for the use of laboratory animals. Thirty female and adult rabbits (New Zealand White, Charles River Laboratories, Sulzfeld, Germany) with a body weight of 4.58 ± 0.48 kg were randomized into five groups ($n = 6$) with a study interval of 1, 2, 3, 4 and 8 weeks post-surgery. Each rabbit surgery was performed under general anesthetic. The same experienced surgeon conducted all implantations. After access to the knee joint by anteriomedial transsection, an 8 mm deep hole was drilled through the cartilage into the cancellous bone of the medial femur condyle. The RS66 cylinders (3 mm diameter and 5 mm height) were inserted into the drill holes by a press fit technique and the wounds were sutured in three layers. Additional RS66 cylinders were implanted into the lumbar musculature as well as subcutaneously on each

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