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Intramedullary Mg2Ag nails augment callus formation during fracture healing in mice

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ABSTRACT

Intramedullary stabilization is frequently used to treat long bone fractures. Implants usually remain unless complications arise. Since implant removal can become technically very challenging with the potential to cause further tissue damage, biodegradable materials are emerging as alternative options. Magnesium (Mg)-based biodegradable implants have a controllable degradation rate and good tissue compatibility, which makes them attractive for musculoskeletal research. Here we report for the first time the implantation of intramedullary nails made of an Mg alloy containing 2% silver (Mg2Ag) into intact and fractured femora of mice. Prior *in vitro* analyses revealed an inhibitory effect of Mg2Ag degradation products on osteoclast differentiation and function with no impair of osteoblast function. *In vivo*, Mg2Ag implants degraded under non-fracture and fracture conditions within 210 days and 133 days, respectively. During fracture repair, osteoblast function and subsequent bone formation were enhanced, while osteoclast activity and bone resorption were decreased, leading to an augmented callus formation. We observed a widening of the femoral shaft under steady state and regenerating conditions, which was at least in part due to an uncoupled bone remodeling. However, Mg2Ag implants did not cause any systemic adverse effects. These data suggest that Mg2Ag implants might be promising for intramedullary fixation of long bone fractures, a novel concept that has to be further investigated in future studies.

Statement of Significance

Biodegradable implants are promising alternatives to standard steel or titanium implants to avoid implant removal after fracture healing. We therefore developed an intramedullary nail using a novel biodegradable magnesium-silver-alloy (Mg2Ag) and investigated the *in vitro* and *in vivo* effects of the implants on bone remodeling under steady state and fracture healing conditions in mice. Our results demonstrate that intramedullary Mg2Ag nails degrade *in vivo* over time without causing adverse effects. Importantly, radiographs, μCT and bone histomorphometry revealed a significant increase in callus size due to an augmented bone formation rate and a reduced bone resorption in fractures supported by Mg2Ag nails, thereby improving bone healing. Thus, intramedullary Mg2Ag nails are promising biomaterials for fracture healing to circumvent implant removal.

1. Introduction

Fractures of the femoral and tibia shaft often occur in response to high energy trauma and are preferentially treated by an intramedullary nail that supports the fracture zone and facilitates bone healing [1–3]. The intramedullary stabilization usually remains

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since implant removal in general and extraction of a nail in particular can be very time-consuming, cumbersome and may cause further tissue damage, re-fracture and other subsequent problems [4–6]. Thus, the second surgery imposes a potential risk to the patient and causes additional costs. However, pain, dysesthesia, a broken nail, infection, pseudarthrosis or the need for implanting a hip or knee prosthesis later in life may require removal of the intramedullary nail [5,6]. Thus, biodegradable implants represent an attractive alternative to replace conventional implants. Among the various biodegradable materials, magnesium (Mg) and Mg-containing alloys have a long history in the musculoskeletal field [7]. Based on their high biocompatibility, biodegradability, and similar mechanical properties to bone, many studies investigated the use of Mg and its alloys for applications in orthopedic surgery [8–17]. In addition to its favorable mechanical properties, Mg has been shown to increase osteoblast differentiation *in vitro* [18,19] and to induce new bone formation *in vivo* [13], demonstrating a high osteogenic potential. Furthermore, as screws for hallux valgus surgery, Mg-based implants are already in use for clinical applications [10,20]. However, these implants are much smaller than intramedullary nails and are not entirely surrounded by bone marrow, an environment that might affect implant degradation and tissue response.

Among the challenges with Mg implants are the degradation rate and the biocompatibility [13–15]. For instance, Mg-Calcium alloys are biocompatible but degrade quite rapidly [21,22]. Mg-Fluoride coating has been reported to reduce the *in vivo* degradation rate of Mg implants and rare earth metals that are frequently used as alloy materials for Mg can cause cytotoxic effects [23,24]. Recently, members of our consortium reported a novel Mg alloy containing 2% silver (Mg2Ag), which was cast and treated by a solidification cooling process, resulting in appropriate mechanical properties and a rather low degradation rate. Furthermore, *in vitro* investigations of this alloy showed favorable antibacterial effects and no cytotoxicity to human osteoblasts [25].

To investigate the *in vivo* degradation of this novel material as well as its impact on bone remodeling and fracture healing, we implanted Mg2Ag intramedullary nails into mice with and without a femoral shaft fracture, followed by an analysis of *in vivo* degradation and tissue response under steady-state and bone healing conditions. Degradation of Mg2Ag alloys *in vivo* occurred without adverse effects but faster than *in vitro*. Mice were overall healthy and no adverse effects on body weight or kidney, liver, muscle, or spleen were observed. Radiographs and bone histomorphometry revealed that in comparison to steel implants, fractures supported by Mg2Ag intramedullary nails demonstrated a decreased bone resorption while bone formation was increased, leading to a significantly bigger callus during the first 21 days of fracture healing. These results demonstrate that fixation of long bone shaft fractures by intramedullary Mg2Ag nails might be a promising concept for further investigation.

2. Materials and methods

2.1. Mg2Ag implant preparation

Production of the Mg2Ag (2% Ag, wt/wt) alloy was performed by permanent mold casting using pure (99.99%) Mg and pure (99.99%) Ag granules. Briefly, molten Mg was maintained at 720 °C and preheated Ag (150 °C) was added under continuous stirring (200 rpm) for 15 min. The melt was poured into a mild steel mold pre-heated to 550 °C. For better separation of castings from the mold, hexagonal boron nitride was used as mold release agent. In one casting, 6 ingots with a diameter of 30 mm and a length of >170 mm were produced. During the casting process cover gas (Ar + 0.5% SF_6) was applied. Ingots were cut to a length of 80 mm and trimmed to a diameter of 28 mm. Next, the alloy was homogenized in Ar (6 h at 420 °C), followed by an extrusion at 370 °C with an extrusion ratio of 1/16 from a diameter of 28 mm to 3 mm. The extrusion speed was 4.5 mm/s. For wire production, the extruded rods were cut to 90 mm length and drawn by hand from a diameter of 3 mm to the final diameter of 0.8 mm in 0.05 mm increments. Prior to each drawing step, wires were heated to 300 °C for 45 min until a diameter of 1.6 mm was reached. At smaller diameters the heating time was reduced to 15 min. Standard drawing dies, pliers, and draw wax as lubricant were used on a drawing bench.

2.2. Implant characterization

Composition of the alloy was determined by energy-dispersive X-ray spectroscopy analysis (XRF) (Bruker Explorer S4, Bruker AXS GmbH, Karlsruhe, Germany).

2.3. In vitro degradation of Mg2Ag implants and quantification of osmolality and pH

Mg2Ag wires of a diameter of 0.8 mm and a length of 20 mm were incubated in 3 ml DMEM containing 10% fetal bovine serum (FBS) at cell culture conditions (37 °C, 5% CO_2, 95% humidity). Initial weight was determined and compared to the weight after 7 days of incubation. The average *in vitro* degradation rate was calculated using the weight differences of 6 independent samples. During the 7 days of incubation, changes in pH and osmolality of the supernatant were determined using a pH meter and a Gonotec 030D cryoscopic osmometer (Gonotec, Berlin, Germany), respectively.

2.4. Preparation of medium conditioned with Mg2Ag implant degradation products

Mg2Ag degradation products were prepared as described previously [26]. Briefly, Mg2Ag disks of 1 cm in diameter were immersed for 24 h in α-MEM containing 10% FBS and maintained under cell culture conditions (37 °C, 5% CO_2, 95% humidity). Conditioned medium (CM) enriched with Mg2Ag degradation products was added to osteoblast and osteoclast cultures at a final concentration of 20%, 10% or 3.3%. Mg concentration in the CM was determined by the xylidylblue-I chromogenic method according to the manufacturers instructions (Mg Assay Kit, Biomol GmbH, Hamburg, Germany). The effect of the CM on cell differentiation and function was compared to normal differentiation medium as control.

2.5. Osteoblast and osteoclast cultures

Long bone osteoblasts and bone marrow-derived osteoclast precursors were isolated from ten-week old C57Bl/6J wild type mice according to standard protocols [27,28]. Briefly, tibiae and femora were harvested and cleaned with a forceps and scalpel to remove adjacent soft tissues. Epiphyses were cut and bone marrow was flushed to obtain osteoclast precursor cells. Cells were then seeded into 10 cm^2 dishes with α-MEM containing 10% FBS and 1% Penicillin/Streptomycin (P/S). After incubation for 3 h, non-adherent osteoclast precursors were transferred into a new 10 cm^2 dish and cultured in α-MEM supplemented with 10% FBS and 1% P/S for 2 days in the presence of 100 ng/ml macrophage colony stimulating factor (M-CSF, PeproTech, INC, Rocky Hill, NJ, USA). Next, osteoclast precursors were transferred into 96-well plates at a density of 45.000 cells/cm^2 and were cultured in α-MEM with 10% FBS and 1% P/S containing 25 ng/ml M-CSF and 100 ng/ml recombinant