Acta Biomaterialia 6 (2010) 1861–1868

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Magnesium hydroxide temporarily enhancing osteoblast activity and decreasing the osteoclast number in peri-implant bone remodelling ☆

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ARTICLE INFO

Article history:
Received 13 July 2009
Received in revised form 11 December 2009
Accepted 15 December 2009
Available online 24 December 2009

Keywords:
Magnesium
Corrosion product
Microtomography
Bone remodelling
Animal model

ABSTRACT

Repeated observations of enhanced bone growth around various degradable magnesium alloys in vivo raise the question: what is the major mutual origin of this biological stimulus? Several possible origins, e.g. the metal surface properties, electrochemical interactions and biological effects of alloying elements, can be excluded by investigating the sole bone response to the purified major corrosion product of all magnesium alloys, magnesium hydroxide ($Mg(OH)_2$). Isostatically compressed cylinders of pure $Mg(OH)_2$ were implanted into rabbit femur condyles for 2–6 weeks. We observed a temporarily increased bone volume (BV/TV) in the vicinity of $Mg(OH)_2$ at 4 weeks that returned to a level that was equal to the control at 6 weeks. The osteoclast surface (OcS/BS) was significantly reduced during the first four weeks around the $Mg(OH)_2$ cylinder, while an increase in osteoid surface (OS/BS) was observed at the same time. At 6 weeks, the OcS/BS adjacent to the $Mg(OH)_2$ cylinder was back within the same range of the control. The mineral apposition rate (MAR) was extensively enhanced until 4 weeks in the $Mg(OH)_2$ group before matching the control. Thus, the enhanced bone formation and temporarily decreased bone resorption resulted in a higher bone mass around the slowly dissolving $Mg(OH)_2$ cylinder. These data support the hypothesis that the major corrosion product $Mg(OH)_2$ from any magnesium alloy is the major origin of the observed enhanced bone growth in vivo. Further studies have to evaluate if the enhanced bone growth is mainly due to the local magnesium ion concentration or the local alkalosis accompanying the $Mg(OH)_2$ dissolution.

1. Introduction

In the field of biodegradable magnesium implants, a common in vivo observation of various authors has been the enhanced bone growth adjacent to degradable magnesium alloys regardless its alloy composition [1–7]. These findings lead to the question, what is the underlying explanation or mutual origin for the enhanced bone growth? One hypothesis is that electrical stimulation from the corroding magnesium alloys could enhance the local bone growth by stimulating osteoblast activity [7–11]. Although an enhanced number of osteoclasts could be shown around direct-current electrodes in tibia shafts of chicken bone [12], there is only little knowledge on the electrochemical effects on this cell type in the literature. Another hypothesis is based on the common knowledge that commercially pure magnesium needs to be alloyed with various elements to reduce the initial burst of hydrogen evolution which is associated with magnesium's initially high corrosion rate [13]. The initial corrosion rate can be reduced by various mechanical, electrochemical or coating procedures [13]. As a further hypothesis, different magnesium surface properties such as surface roughness, energy and charge could be created by various post-casting treatments. These implant surface properties are reported in the literature to enhance bone bonding to implant surfaces and stimulate adjacent bone growth [14–16]. The released alloying elements or elements from the coating on magnesium implants could be another source to influence local bone cell response [13,17,18]. Furthermore, magnesium ions from the corroding magnesium implant and the accompanying local alkalosis could also contribute to the enhanced bone growth [19,20]. It could be shown that unphysiologically high extracellular magnesium concentrations support chondrocyte proliferation and redifferentiation [21]. The most interesting finding from the literature is that all authors are reporting the same positive bone response regardless the elemen-

☆ Part of the Thermec'2009 Biodegradable Metals Special Issue, edited by Professor Diego Mantovani and Professor Frank Witte.

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doi:10.1016/j.actbio.2009.12.037

tal composition of the magnesium alloy. Thus, it is important to have a closer look at the used alloy systems. However, the major component in any magnesium alloy is the element magnesium. The major source of locally released magnesium ions is the main corrosion product, magnesium hydroxide [22,23]. Magnesium hydroxide is formed as a corrosion product in aqueous solutions and reacts to more water-soluble $MgCl_2$ in aqueous environments exceeding a chloride content higher than 30 mmol l^{-1} [24]. Since magnesium hydroxide is not stable in biological environments with a normal chloride content of 150 mmol l^{-1} [13], we hypothesize that the biological effects observed adjacent to the corroding magnesium implants will be mainly caused by the dissolving magnesium hydroxide and its locally enhanced magnesium ion concentration, as well as the accompanying local alkalosis. To compare the effects of magnesium hydroxide on bone with the results previously published on magnesium alloys, we implanted commercially pure, isostatically compressed magnesium hydroxide powder in a rabbit femur condyle and compared the surrounding postoperative changes in bone histomorphology to an empty bone defect.

2. Materials and methods

2.1. $Mg(OH)_2$ sample production

Magnesium hydroxide powder ($Mg(OH)_2$, purum P.a. >95%, Fluka, Buchs, Switzerland) was formed to cylindrical implants of 3 mm diameter and 5 mm height by using a cold isostatic pressure technique (Department of Inorganic Chemistry, University of Essen, Germany, Fig. 1a). The implants were gamma-sterilized with 25–29 kGy of cobalt-60 radiation (BBF Sterilisations service, Kernen, Germany).

2.2. Animal study design

The animal experiment was conducted under an ethics committee approved protocol in accordance with German federal animal welfare legislation (Approval No. 33-42502-04/831) and in accordance with the National Institute of Health guidelines for the use of laboratory animals. Forty-two female rabbits (New Zealand White, Charles River Laboratories, Sulzfeld, Germany) older than 6 months (4.46 ± 0.4 kg) were randomly split into three time groups. Each time group of 14 rabbits contained 8 animals with an implant in both knee joints, while 6 rabbits obtained only drill holes in both knees, but the drill holes were left empty as a control. The use of empty bone defects as control is a widely accepted approach to investigate degradable biomaterials in bone [25–28]. Moreover, the use of empty bone defects provides reproducible histology for the natural bone healing rate and the response to the drilling process.

Surgery was performed under isofluran anesthesia on both knees. After access to the knee joint was obtained using an anteromedial transection, an 8 mm deep hole was drilled through the cartilage into the cancellous part of the medial femur condyle. The magnesium hydroxide cylinders were inserted into the drill holes of the knees by a press fit technique (Fig. 1a). The wounds were closed by a three-layer suture.

Animals were sacrificed at 2 ($n = 14$), 4 ($n = 14$) and 6 ($n = 14$) weeks after surgery. Nine animals in each time group were used for histomorphology ($n = 5$ with cylinders and $n = 4$ as control) and microtomography providing 10 specimens with $Mg(OH)_2$ cylinders and 8 control specimens. Five animals in each time group were used for micro X-ray fluorescence analysis (μXRF analysis; $n = 3$ with cylinders, $n = 2$ as control) providing 6 specimens with $Mg(OH)_2$ cylinders and 4 controls.

2.3. Post operative treatment and sample retrieval

The rabbits were examined for lameness, swellings, suture failure and general health condition every day. Before sample retrieval, the animals were euthanized by an overdose of pentobarbital after sedation. The medial condyles were dissected en bloc from the knee joint and immediately deep frozen in liquid nitrogen. The medial condyles were kept frozen until microtomography was performed. Afterwards the condyles were fixed in 3.7% commercial formalin for 7 days at 4 °C.

2.4. Volume measurements of the dissolving $Mg(OH)_2$ cylinder

The dissolution of the isostatically compressed $Mg(OH)_2$ cylinders during the implantation period was observed using non-

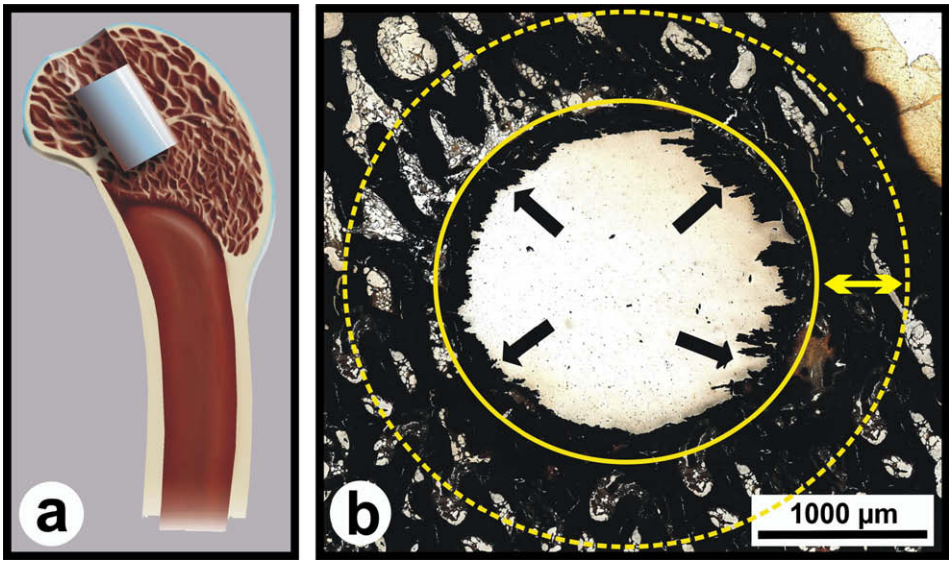

Fig. 1. Scheme showing the implantation site of the $Mg(OH)_2$ cylinder in the medial femur condyle of a rabbit (a). For the histomorphometrical analysis, a 500 μm broad seam (yellow arrow) around the drill hole was analysed in von Kossa stained serial sections (b). The original drill hole is marked by the yellow line (concomitantly the inner border) and the dashed yellow line marks the outer border of the analysed area. The black arrows in (b) point at residues of the $Mg(OH)_2$ cylinder inside the drill hole. Scale bar = 1000 μm.

ID	Title	Pages
1914	Magnesium hydroxide temporarily enhancing osteoblast activity and decreasing the osteoclast number in peri-implant bone remodelling ☆	8