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Reaction of bone nanostructure to a biodegrading Magnesium WZ21 implant – A scanning small-angle X-ray scattering time study

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A B S T R A C T

Understanding the implant–bone interaction is of prime interest for the development of novel biodegrading implants. Magnesium is a very promising material in the class of biodegrading metallic implants, owing to its mechanical properties and excellent immunologic response during healing. However, the influence of degrading Mg implants on the bone nanostructure is still an open question of crucial importance for the design of novel Mg implant alloys. This study investigates the changes in the nanostructure of bone following the application of a degrading WZ21 Mg implant (2 wt% Y, 1 wt% Zn, 0.25 wt% Ca and 0.15 wt% Mn) in a murine model system over the course of 15 months by small angle X-ray scattering. Our investigations showed a direct response of the bone nanostructure after as little as 1 month with a realignment of nano-sized bone mineral platelets along the bone–implant interface. The growth of new bone tissue after implant resorption is characterized by zones of lower mineral platelet thickness and slightly decreased order in the stacking of the platelets. The preferential orientation of the mineral platelets strongly deviates from the normal orientation along the shaft and still roughly follows the implant direction after 15 months. We explain our findings by considering geometrical, mechanical and chemical factors during the process of implant resorption.

Statement of significance

The advancement of surgical techniques and the increased life expectancy have caused a growing demand for improved bone implants. Ideally, they should be bio-resorbable, support bone as long as necessary and then be replaced by healthy bone tissue. Magnesium is a promising candidate for this purpose. Various studies have demonstrated its excellent mechanical performance, degradation behaviour and immunologic properties. The structural response of bone, however, is not well known. On the nanometer scale, the arrangement of collagen fibers and calcium mineral platelets is an important indicator of structural integrity. The present study provides insight into nanostructural changes in rat bone at different times after implant placement and different implant degradation states. The results are useful for further improved magnesium alloys.

1. Introduction

Novel technologies and materials development in the field of medical implants are increasingly focusing on bioresorbable tissue replacements. Bioresorbable implants are especially useful for surgical placement in children because, in contrast to adult bone, the pediatric bone is still growing and implants must be removed in a second surgery in order to avoid severe deformation of the growing bone. Biodegrading implants are designed to support the bone until new bone tissue has formed and built up sufficient integrity to support itself. Two material classes have been in the focus of biodegradable applications in recent years: polymers and metals. While polymeric implants show promising results [1], metallic materials are the emphasis of current research as they show greater mechanical strength, and metallic corrosion products

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provoke fewer inflammatory reactions. Such materials are highly favorable from a medical point of view and have therefore become a topic of intense research [2–4]. In general, magnesium-based implants are very promising candidates for this purpose, because they exhibit mechanical properties well matched to bone (Young's modulus 45 GPa [3] in comparison to ~20 GPa found for bone by other research groups [5,6] and our own nanoindentation measurements on similar samples with values ranging from 18.9 to 26.7 GPa indentation modulus [7]) and show a degradation behavior which supports the bone during the initial 8 weeks of callus formation and subsequent degradation, with ultimate disappearance after 12 to 15 months [8]. Other candidates such as iron implants also show promising results but their degradation is until now very slow [9].

Research on biodegradable Mg implants has focused on mechanical properties [10], medical aspects such as degradation kinetics [11,12], immunologic reaction of the implant and its residues [13], as well as the performance of the implant–bone interface [10,14] finally leading to improvement in the composition of the Mg alloy [8]. All these investigations showed the principal suitability of the implant material, the necessity of improved matching between bone and implant properties, and indicated changes in the bone morphology as a reaction to the implant.

Very sparse information exists on the structural response of bone to implant placement and subsequent degradation. Bone is a hierarchically structured composite material, macroscopically composed of three major components: (1) the cortical bone, forming the outside of the bone, (2) the trabecular bone and (3) the bone marrow which makes up the inner part. Bone consists of collagen tissue mineralized with hydroxyapatite mineral (HA). The HA mineral is deposited into the gap regions within the collagen fibers in the form of nano-sized platelets or needles which have a typical length of 50–100 nm and a thickness between 1 and 4 nm [15,16]. The lateral dimension is determined by the either needle- or platelet-like morphology. The form of deposition generates regularly spaced stacking within the collagen matrix. It is reported that collagen fibers and consequently also the HA nanocrystals show a strong preferential alignment along the direction of principal stress, a fundamental pattern that can be found also in other biomineralized tissues and is subject to adaptation throughout life [17–20].

Previous studies showed that the bone nanostructure also responds to implants. However, these studies were only carried out on static systems, such as Ti implants [21] or Zr implants [22] where no implant resorption takes place, and did not investigate the subsequent healing process of the bone. In the case of static implants, stress shielding affects the bone structure in the vicinity of the implant, which is a known problem for long-term bone quality and implant stability. The situation can be expected to be greatly improved in case of biodegradable implants, where the implant is replaced by bone tissue over time. Nevertheless, detailed studies are necessary to assess the influence and improve the design of novel biodegradable implants.

In the present paper we report on the characterization of the bone nanostructure in response to a slowly degrading Mg WZ21 implant starting one month after implantation over the time period of healing until the near complete resorption of the implant after 15 months. Small-angle X-ray scattering (SAXS) was used to determine structural parameters such as the thickness and shape of the mineral platelets, their degree and direction of orientation. This specific alloy type was chosen as it shows a good combination of degradation time (in the range of complete bone healing) and low production of gaseous by-products, and hence allows the study of an undisturbed bone–implant interface over the course of healing.

We show that a strong and rapid change of collagen fiber/mineral crystal orientation is visible close to the implant interface. The deviation from the normal orientation along the bone shaft is still prominent after 15 months and is mainly found in the area where the implant resided. We also show that in the regions of newly formed bone replacing the implant, the mineral platelet thickness is markedly lower. A growth in thickness could be observed throughout healing in the zones of newly formed bone. No great differences were observed in terms of particle spacing and order of the particle stacking in the collagen matrix. Only in small zones of newly formed bone did the order seem to decrease. From these observations we conclude that several factors play a role: locally elevated levels of Mg may influence the mineral crystal size [7], while geometric and mechanical factors are probably responsible for morphological changes and the preferential orientation of collagen and mineral.

Since Mg based bio-resorbable implant materials are highly promising candidates for future clinical applications, detailed information on the in vivo degradation process and its influence on the already existing bone and new bone formation is of major importance for their successful use. Furthermore, insights in this field will in the long run also help to tune the implant degradation behavior to match bone formation and remodeling in an optimized way.

2. Experimental method

2.1. Implant

The implant material used for this study is a crystalline Magnesium rare-earth-containing alloy WZ21, composed of 96.4 wt% Mg, 2 wt% Y, 1 wt% Zn, 0.25 wt% Ca and 0.15 wt% Mn. The average grain size was determined to be 7 μm. Detailed information can be found elsewhere [12]. This alloy type shows rather slow degradation kinetics. 1.6 mm diameter pins were produced from the alloys.

2.2. Bone samples

The samples for the investigation were obtained from male Sprague Dawley rat femur that had been implanted with WZ21 pins at an age of five weeks and characterized by micro-computed tomography (μ-CT) and histology [12] in addition to interfacial mechanical properties [10]. Details on the design of experiment and the sampling can be obtained from the reports mentioned. For our study we used six samples from different implant dwelling times (1, 3, 6, 9, 12 and 15 months). The Austrian Ministry of Science and Research authorized the animal experiments (accreditation number BMWF-66.010/0087-II/3b/2011). Longitudinal sections of the bone embedded in PMMA resin (Technovit 7100, Heraeus Kulzer, Werheim, Germany) were prepared and ground to a thickness of 200 μm using a "Struers Planopol-3" (Struers Ballerup, Denmark) polishing machine with a sanding paper grid size of 200. After excessive rinsing with distilled water the samples were conditioned and stored under vacuum.

Microscopic images were obtained using a "Wild-Heerbrugg M8" (Heerbrugg, Switzerland) stereo microscope with a "PixeLink PL-B686CF" (Ottawa, Canada) camera system to obtain images with 6x and 25x magnification.

Histological images were obtained from undecalcified thin-sections of ~30 μm thickness with Levai-Lazcko staining using an Olympus dotSlide Microscopic system (dotSlide – Virtual Slide System, Olympus, Japan).

2.3. Small-angle X-ray scattering (SAXS)

SAXS measurements were carried out using a "Rigaku S-Max 3000" (Rigaku Innovative Technologies, Auburn Hills, USA) using