Biomaterials 76 (2016) 250–260

Contents lists available at ScienceDirect

Biomaterials

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

Magnesium from bioresorbable implants: Distribution and impact on the nano- and mineral structure of bone

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ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form
16 October 2015
Accepted 21 October 2015
Available online 23 October 2015

Keywords:
Bioresorbable implant
Implant degradation
Magnesium
Bone nanostructure
Bone mineralization
X-ray microfocus techniques

ABSTRACT

Biocompatibility is a key issue in the development of new implant materials. In this context, a novel class of biodegrading Mg implants exhibits promising properties with regard to inflammatory response and mechanical properties. The interaction between Mg degradation products and the nanoscale structure and mineralization of bone, however, is not yet sufficiently understood. Investigations by synchrotron microbeam x-ray fluorescence (μXRF), small angle x-ray scattering (μSAXS) and x-ray diffraction (μXRD) have shown the impact of degradation speed on the sites of Mg accumulation in the bone, which are around blood vessels, lacunae and the bone marrow. Only at the highest degradation rates was Mg found at the implant–bone interface. The Mg inclusion into the bone matrix appeared to be non-permanent as the Mg-level decreased after completed implant degradation. μSAXS and μXRD showed that Mg influences the hydroxyl apatite (HAP) crystallite structure, because markedly shorter and thinner HAP crystallites were found in zones of high Mg concentration. These zones also exhibited a contraction of the HAP lattice and lower crystalline order.

1. Introduction

For several decades the development of biocompatible implant materials has focused on stationary implants involving minimum degradation in body fluid and, ideally, no release of material into the surrounding tissue. The advent of biodegradable implant materials changed this premise: degradation products are now generated in considerable amounts and the organism must be able to cope with them. Hence the biocompatibility of released substances and their impact on surrounding tissue and the whole organism have become important implant material design factors. The degradation of polymeric implant materials such as poly-lactic acid (PLA) or polyhydroxybutyrate (PHB) have been thoroughly investigated and are used in non-load bearing applications such as sutures or stents [1]. However, degradation products can exhibit adverse effects *in vivo* [2,3], and in most cases mechanical properties are too poor for orthopaedic, load-bearing purposes such as bone fractures. Here biodegradable metallic implants hold great promise because they not only exhibit mechanical properties which match those of bone but can be based on metals which occur naturally in the human body [4,5]. Here important materials classes are Fe-based [6] or Mg-based [7–9] alloys, which have been the subject of numerous attempts to understand implant degradation behaviour and histologic osseo-integration [9] as well as the impact of the degradation products on the bone forming processes [10]. The time-dependent distribution of degradation products of metallic implants and their impact on tissue structure and functionality, while of immense importance, has been considerably less investigated. This study therefore focuses on Mg-based bone implants, which have become very promising candidates for application in bioresorbable implants. We investigate in detail the distribution of Mg as the main degradation product and its impact on bone nanostructure and mineralization at micrometer resolution.

Mg is an element which occurs naturally in bone at a concentration of about 0.5–1%. In fact bone provides a reservoir for about

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http://dx.doi.org/10.1016/j.biomaterials.2015.10.054
0142-9612/

50% of the total Mg in the body [11]. While the importance of Mg for health is beyond doubt [12], its direct influence on bone growth and quality is a matter of debate. Mg deficiency has been shown to lead to bone loss along with a variety of factors, e.g. decreasing osteoblastic activity in cell cultures [11] while increasing osteoclast formation on the other hand [13], along with a thinning of the growth plate [14]. Much less is known about the effects of Mg supplementation. Some studies indicate that Mg supplementation may also inhibit osteoblastic activity, which leads to a decrease in bone mineral content as determined by ashing [15], as well as inhibiting periosteal bone formation [16]. A study by Serre et al. concerning the effect of locally elevated Mg levels in bone [17] indicated that sponges for bone grafting made from Mg substituted apatite showed decreased osteoblastic activity and even toxic effects at higher Mg levels. In contrast to this, Anders [18] reported positive effects of elevated Mg levels on tissue calcination. In that report on rabbits, no decreased osteoblastic activity was observed but a loss of crystalline structure was reported for high levels of Mg injection. The conclusion was that Mg treatment could be used to treat osteoporosis or osteomalacia.

Exposing bone to a degrading Mg implant makes locally high amounts of Mg available, which may influence bone formation and may also alter the structure of the bone mineral hydroxyl apatite (HAP). The mineral HAP is a major constituent of bone, the nano-sized HAP mineral platelets have a typical length of 50–100 nm and a thickness between 1 and 4 nm [19,20]. The lateral dimensions of the mineral particles depend on their either needle or platelet-like morphology. The HAP mineral is a hexagonal crystal with typical lattice dimensions of $a = b = 9.424$ Å and $c = 6.881$ Å [21]. It was shown that the lattice constants are very sensitive towards distortion by the inclusion of foreign ions [22]. Recent experiments on synthetic HAP showed the impact of Mg on the mineral structure [23,24]. While lower Mg contents (around 1 mol%) lead only to a decreased crystallinity and a shorter c-axis, elevated levels of Mg induced the formation of a β-tricalciumphosphate (TCP) phase. Another aspect is the local increase in pH due to Mg-implant degradation and the formation of $Mg(OH)_2$. Although the case of degrading Mg implants has not been directly investigated, the syndrome of elevated pH in the blood, called alkalosis, is known to slightly increase osteoblastic activity along with a reduction of osteoclastic function and lowering of the calcium efflux [25,26]. This effect may be underpinned by the finding that Mg-based implants show osteoinductivity compared to autologous bone grafts [27].

Only very few studies have been successful in tracking the influence of Mg on bone mineral structure *in-vivo*. Burnell et al. [16] described finding smaller mineral crystals via XRD analysis of Mg-supplemented rat bones. These findings were, however, obtained from a whole bone, so no spatial correlation was possible; and the observed XRD peak-broadening may be attributed to smaller crystallites or higher imperfections in the crystals (no specific analysis was performed). Bigi et al. [28] found a similar correlation between crystal size and Mg content in turkey leg tendon. However, the influence of HAP crystal maturation was the principal aim of the study and no controlled Mg supplementation was carried out. Tsuboi et al. tracked Mg locally in human bone in the vicinity of periosteal and endosteal tissue and in the boundary zone of haversian channels [29], and Wiesman found a hot-spot Mg cluster structure in rat incisors [30]. However, both of these studies dealt with naturally occurring Mg levels.

Potential influences of artificially elevated Mg levels on bone structure after implant placement are of immense scientific and clinical interest, because bone quality – and therefore also the long term success of surgery – is greatly influenced by its nanostructure and the integrity of the collagen-mineral composite. Here, the degree of mineralization and the size and arrangement of the mineral platelets are reported to have a particularly high impact [31]. Scattering techniques such as SAXS or XRD are especially useful to characterize the structure of biomineralized tissues as they provide insights into a representative sample volume [32–35], but since they do not allow direct examination of the sample in real space, data interpretation requires the use of models to extract meaningful information. An overview of data interpretation approaches is given by Pabisch et al. [36].

It is well known that bone serves as a reservoir for biologically relevant (trace) elements. In this context x-ray fluorescence (XRF) and quantitative backscattered electron imaging (qBEI) in particular have helped to understand both the accumulation of trace elements in biomineralized tissue [37–39] and near-surface mineral density. This study deployed μXRF measurements to determine the distribution of Mg at the bone-implant interface and at remote distances from it at micrometer and sub-micrometer spatial resolution.

The aim of this study was to track the distribution of Mg from degrading Mg implants in rat bone at different stages of *in-vivo* implant degradation and to elucidate the impact on Mg on bone nanostructure and mineral crystal structure. For this purpose we deployed synchrotron microbeam techniques such as micro x-ray fluorescence (μXRF) mapping, micro small-angle x-ray scattering (μSAXS) and micro x-ray diffraction (μXRD) to study Mg distribution and structural changes at the micron-length scale both in the close vicinity of the implant site and also at remote distances from it. We found that the preferred site of Mg accumulation was adjacent to the blood vessel system. The occurrence of Mg around lacunae also indicates a distribution of Mg throughout the lacunae network, which implies a reservoir with much more inner surface area than just the walls of the blood vessel system. Only the highest released doses of Mg generated enrichment at the bone-implant interface. In all other samples investigated the detectable Mg was found in the vicinity of blood vessels and bone cells. We also showed that Mg uptake is reversible around blood vessels and bone cells, and that Mg influences both the local nanostructure and the crystal structure. In zones of high Mg concentration a contraction of the HAP crystallite lattice was found. By comparing different ways to determine mineral platelet thickness, we show that high Mg levels also concur with lower crystalline order and decreased size of the HAP mineral platelets. This may be due to inclusion of Mg in HAP and hindered growth of mineral particles in the presence of Mg. The mechanical properties of the bone, as tested via nano-indentation, remained essentially unaffected by the Mg implant, and slight deviations were only recorded near the bone-implant interface.

2. Materials and methods

2.1. Bone samples

The distribution of Mg and local bone structure of Sprague–Dawley rats was tracked following *in-vivo* degradation of two types of bioresorbable Mg alloys: a rare-earth (RE) containing Mg-alloy WZ21, which degrades rather slowly, and a RE-free Mg-alloy ZX50, which degrades rather rapidly. A detailed description of the alloy production process can be found elsewhere [9,40]. The ZX50 alloy contains 5 wt.% Zn and 0.25 wt.% Ca, and WZ21 contains 1 wt.% Zn, 0.25 wt.% Ca, 0.15 wt.% Mn and 2 wt.% Y as alloying elements to Mg. The *in-vivo* studies were carried out using a Sprague–Dawley rat model. Cylindrical pins of 1.6 mm diameter were implanted in the femoral bones of 5-week-old male rats.

The degradation kinetics was monitored *in-vivo* by micro-computed tomography (μ-CT) and was reported by Kraus et al.