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Comparison of two carbonated apatite ceramics in vivo

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ABSTRACT

Carbonated apatite ceramics, with a composition similar to that of bone mineral, are potentially interesting synthetic bone graft substitutes. In the present study, two porous carbonated apatite ceramics were developed, characterized and tested for their bone repair capacity and osteoinductive potential in a goat model. Although the two ceramics were prepared from a similar starting powder, their physico-chemical and structural characteristics differed as a consequence of different preparation methods. Both ceramics had an open and interconnected porous structure with a porosity of about 80%. The morphology of the surface of CA-A and CA-B at the submicron level differed significantly: CA-A consisted of irregular grains with a size of 5–20 μm, with 1–10 μm large micropores among the grains, whereas CA-B surface consisted of much smaller and regular shaped grains (0.05–0.5 μm), with most micropores smaller than 1 μm. The specific surface area of CA-B was about 10 times larger than that of CA-A due to its significantly smaller grain size. CA-A and CA-B ceramics contained 3 and 5 wt.% of B-type carbonated apatite, respectively. Although neither ceramic succeeded in completely bridging the 17 mm iliac wing defect with new bone after 12 weeks of implantation, CA-A showed significantly more bone formation in the pores of the implant than CA-B. The total area percentage of new bone in the total defect area was 12.7 ± 1.81 and 5.51 ± 1.37 (mean ± SEM) for CA-A and CA-B, respectively. Intramuscular implantation of the ceramics led to ectopic bone formation by CA-A in all three implanted specimens, in contrast to CA-B, where no new bone was observed in any of the 11 animals. CA-A showed a more pronounced degradation than CA-B both in vitro and in vivo at both implantation sites, which was unexpected based on the physico-chemical and structural properties of the two ceramics. Both physico-chemical and structural properties of the ceramics could, dependently or independently, have affected their in vivo behaviour, emphasizing the importance to control individual parameters for successful bone repair.

1. Introduction

The expanding number of bone reconstructive surgeries, accompanying the progressive growth of life expectancy in developed countries, makes research into and development of alternatives to natural bone grafts increasingly important. Natural bone grafts in general, and autografts in particular, are still the golden standard in bone repair and regeneration, but they are limited in quantity and require additional surgery. Various types of synthetic bone graft substitutes and tissue-engineered hybrids have been developed in the past few decades with the goal of taking over the role of natural bone grafts. Bone tissue engineering, based on both growth factors and cells, has shown some preclinical and clinical success [1], but there are issues with required dosages of growth factors, survival of cells and dependence on the carrier [2]. Furthermore, varying results with regard to performance of tissue-engineered constructs among species, among individual recipients as well as among implantation sites, impede the successful transfer from the laboratory to the clinic [3]. Synthetic bone graft substitutes are attractive because of their unlimited off-the-shelf availability and low price, as well as their excellent biocompatibility, in particular in the case of calcium phosphate-based substitutes [4]. Calcium phosphate ceramics, cements and thin coatings on metallic implants have shown the ability to heal bone defects, but their performance is often inferior to that of natural bone grafts as they lack angiogenic and osteogenic factors, they often possess poor mechanical properties due to the absence of organic compounds and their resorption is difficult to control [4,5]. The trend in synthetic bone graft substitute development is moving towards the design of "instructive" scaffolds which are able to trigger a certain biological function upon implantation [6]. Optimizing the geometry and ultrastructure of implants is a way of making customized implants fit the defect perfectly, and provide optimal

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nutrient- and oxygen supply and cell- and tissue infiltration into the defect [7,8]. The micro- and nanotopography of the surface is modified as a trigger to induce, directly or indirectly, and stimulate vascularization, osteogenesis and new bone apposition [9,10]. Altering the chemical composition of bone graft substitutes is another way of influencing their degradation behaviour in vivo and consequently their bone healing performance. From the physicochemical point of view, there exist a variety of calcium phosphate materials, hydroxyapatite and beta-tricalcium phosphate being the most widely used as bone fillers. A calcium phosphate phase that has gained attention is carbonated apatite, because of its close resemblance of bone mineral. Bone mineral of most mammals contains between about 2 and 8 wt.% carbonate [11–13], depending on the age of the individual [14]. Type B carbonated apatite (CO_3^{2-} for PO_4^{3-} substitution, coupled with Na^+ for Ca^{2+} substitution) prevails in biological apatites [13]. A small amount of CO_3^{2-} is believed to be a substituent for OH^- groups, known as type A substitution [15]. The ratio of type A to type B in biological apatites is between 0.7 and 0.9 [16]. Introducing carbonate groups into the structure of hydroxyapatite in general results in a decrease in crystallinity and an increase in solubility in vitro and in vivo [17]. Synthetic carbonated apatite type A has been successfully produced by using a time-consuming process of sintering hydroxyapatite powder under CO_2 supply or by soaking hydroxyapatite powder in an aqueous solution saturated with CO_2 [11,18]. Preparation of type B or type AB is more complex, and occurs predominantly via closely controlled aqueous precipitation reactions [19–23]. However, when carbonated apatite powders are sintered to produce highly crystalline, porous ceramics, excessive loss of carbonate may occur [22,24,25] due to the thermal instability of carbonated apatites [26]. Therefore, although a large number of publications exist on the application of carbonated apatites in the form of thin coatings on metallic implants or injectable cements in bone repair and regeneration [27–30], only a few studies have investigated the behaviour of sintered carbonated apatite ceramics in vivo [31,32]. In the present study, we have tested two carbonated apatite type B ceramics with regard to osteoconduction and osteoinduction by implantation in a critical-sized iliac wing defect and in paraspinal muscles of goats.

2. Materials and methods

2.1. Implants

Two types of porous CA ceramics were prepared within the EU "IntelliScaf" project (G5RD-CT-2002-00697) using an innovative technology based on slurry expansion. Both ceramic types were prepared from the same carbonated apatite powder with a starting carbonation level of 7–10%. CA-A "green bodies" were prepared using a proprietary method (under IPR protection) that involved shear dispersion of the powder into a polymer which releases a porogen during the crosslinking process. The samples were then heat treated in a controlled CO_2 atmosphere and sintered at 900 °C for 1 h. For the preparation of CA-B ceramic (Patent No. EP 1 411 035), the carbonated apatite powder was used to prepare a slurry, using water and a deflocculating agent. The slurry was homogenized using alumina balls, after which the surfactants were added to obtain the desired porosity. The expanded slip was poured into a mould for drying, which was carried out at room temperature. To consolidate and stabilize the structure of the "green" samples, a heat treatment was performed at 700 °C for 1 h in a CO_2 atmosphere.

The compositions and crystal structures of the as-prepared ceramics were determined by using Fourier transform infrared spectroscopy (FTIR; ABB Bomem, Quebec, Canada) and X-ray diffraction (XRD; Miniflex, Rigaku, Japan). XRD analysis of the ceramics post-implantation was performed on the resin-embedded samples that remained after sectioning of histological slides using the same apparatus. The amount of carbonate in the ceramics was determined by the thermogravimetry analysis (TGA; STA-449, Netzch, Geroeteban, Serb, Germany) in the temperature range from room temperature to 1400 °C with a heating rate of 10 °C min^{-1} in air. The ultrastructure of all ceramics was characterized by environmental scanning electron microscopy (ESEM; JEOL 840A, Japan). Total porosity and microporosity (pore diameter <50 μm) were determined using mercury porosimetry (MP; Micromeritics AutoPore IV 9500, Monchengladbach, Germany), and the specific surface area was determined using the Brunnauer–Emmet–Teller method (TriStar 3000, Micromeritics, ASAP 200, USA).

For orthotopic implantation, discs with a diameter of 17 mm and a thickness of 6 mm were produced, while for intramuscular implantation, cylinders with a diameter of 6.5 mm and a height of 10 mm were used. Prior to implantation, all implants were ultrasonically cleaned, dried and gamma-sterilized.

For the in vitro dissolution test, simulated physiological solution (SPS) at pH 7.3, containing sodium (137 mM), chloride (177 mM) and HEPES buffer (50 mM), was used. Under stirring, 0.5 cc ceramic was immersed in 100 ml of SPS at 37 °C over a period of 200 min. The calcium concentration of the solution was continuously measured using a calcium electrode (692 pH/ion meter Metrohm). The test was performed in triplicate for both CA-A and CA-B.

2.2. Animals and implantation

This study was approved by the Dutch Animal Care and Use Committee. In total, 12 adult Dutch milk goats were used. One animal was sacrificed before the end of the study after unsuccessful treatment of an inflammatory hoof disease (laminitis). Another animal was treated with a single dose of a non-steroidal anti-inflammatory drug (NSAID; Diclofenac®) for similar symptoms, after which her condition improved. Although NSAIDs are well known for disturbance of the prostaglandin pathway and associated bone formation, we decided not to exclude this goat from the results because this effect is only described for longer application periods [33]. Furthermore, as all comparisons were paired, a potential effect of the drugs would have been equal for all conditions.

The animals were housed in the Central Animal Laboratory Institute (GDL), Utrecht, The Netherlands, at least 4 weeks prior to surgery. Surgical procedures were performed under standard conditions. After shaving and disinfection of the dorsal thoracolumbar area, a central skin incision at T8–L5 was made to expose the muscle fascia. Both iliac wings were identified and cleared of muscle tissue. Central guide holes were drilled before ∅17 mm trephine holes were made, under constant saline cooling. CA-A and CA-B implants were press-fit inserted into the defects according to a randomized scheme, after which the muscles were tightly sutured to the remaining fascia on the wings. All 11 animals received both CA-A and CA-B implant.

For intramuscular implantation, separate fascia incisions were created in the paraspinal muscles (L1–L3). Using blunt dissection, intramuscular pockets were created and filled with an implant. Subsequently, fascias were closed with nonresorbable sutures to facilitate implant localization at explantation. The skin was closed in two layers. CA-B was implanted into all 11 animals, while CA-A was implanted into only three, because the other prepared implants were lost shortly before the implantation.

In addition to the ceramics presented here, other materials were investigated in the same group of animals using a different implantation model, the results of which are published separately. The tested materials could not influence their respective behaviour.