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Development of a large titanium bone chamber to study in vivo bone ingrowth

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Abstract

In the bone conduction chamber (BCC) various materials and factors have been tested for their effect on bone graft incorporation and bone healing. However, biomaterials often have to be crushed to fit in this small chamber. Since cellular responses to biomaterials are influenced by the size and shape of particles, research concerning the evaluation of biomaterials is limited by the dimensions of this bone chamber. We enlarged and modified the BCC in order to be able to investigate the in vivo influences of biomaterials, growth factors and bone graft processing on tissue and bone ingrowth. Seven goats received four bone chambers each, three modified models and a BCC. The first model (BCC+) had two ingrowth openings, similar to that of the BCC. The second model had two round ingrowth openings (ROU). The third model had a open bottom for bone ingrowth (BOT). After 12 weeks, bone ingrowth distances were measured on histological sections and using µCT. Bone ingrowth was significantly higher ($p = 0.009$ and 0.008) in the ROU compared to the BCC+ and the BOT, respectively. Similar results were found using µCT. The ROU model performed most similar to the BCC (gold standard) and is considered to be a promising new tool in biomaterials research.

Keywords: Bone ingrowth; Bone remodelling; Histomorphometry; Image analysis; In vivo test

1. Introduction

Calcium phosphate-based materials, such as tricalcium phosphate and hydroxy apatite are popular bone graft substitutes in a wide range of applications [1–3]. These materials have a proven osteocompatibility and can act as osteoconductive material. Promising is the combination of both biomaterials and bone inductive substances. There are in vivo models, e.g. critical size defects [4] and titanium chambers [5–7], in which various materials and factors have been tested for their capacity in graft incorporation and bone healing. In the evaluation of osteoconductive materials, the defect created should be large enough to challenge the adjacent bone with a space that can hardly fill spontaneously. The effects of growth factors on bone conductive materials are usually seen at an early stage during bone ingrowth. It is then difficult to find the right time to measure these effects, if new ingrown bone rapidly fills the defect or chamber.

Particularly, the 'bone conduction chamber' (BCC) of Aspenberg has appeared to be a useful tool for quantifying bone regeneration under the most variable conditions in both rats [8] and goats [9]. This bone chamber is unlikely to be completely filled with bone, so that the effects of growth factors, processing of bone and biomaterials within these chambers can be evaluated as differing final amounts of formed bone. However, biomaterials often have to be crushed to fit in this small bone chamber. Although these materials are identical in composition, this process creates irregularly shaped, sharp or jagged edged particles. When granules of these biomaterials are implanted into bone, bone growth behavior and cellular reactions are different due to the size and shape of the granules [10–14]. Biological responses such as inflammation, bone bonding and resorption of bioactive ceramics are very important in

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clinical applications [15]. Thus, the dimensions of this bone chamber limits research concerning the evaluation of biomaterials.

Therefore, we developed and tested three new large titanium bone chambers, based on the BCC, in order to be able to investigate the in vivo influences of biomaterials, growth factors and bone processing on tissue and bone ingrowth, biomaterial resorption and incorporation under reproducible, non-load bearing conditions.

2. Materials and methods

2.1. Bone conduction chamber

Our basic model is the BCC which is a model for membranous ossification [6] (Fig. 1a). The BCC consists of a titanium screw with a cylindrical interior space. It is made up of two threaded half cylinders held together by a hexagonal closed screw cap. One end of the implant is screwed into the bone. The interior of the chamber has a diameter of 2 mm, and a length of 7.5 mm. There are two ingrowth openings for bone ingrowth located at the bone end of the chamber. Thus, the ingrowing tissues enter the cylindrical space from the bone compartment. The chamber extends far out into the subcutaneous region and the ingrown bone-derived tissue can fill the chamber without competing with other tissues. Thus, the tissue ingrowth distance from the holes towards the other end of the chamber can be used to estimate tissue regeneration. The geometry of the chamber makes it easy to distinguish areas for histomorphometry [6]. Since the tibial cortex in rats is thinner than that in goats, we adjusted the BCC for use in goats. A 1 mm disk was placed in the cap of the BCC to provide for location of the ingrowth holes of the chamber to be deeper down, just at the level of the endosteum after the implant is screwed in [7].

2.2. New chambers

All three new chambers are modifications of the BCC. Similar to the BCC, all new models were made of two threaded half cylinders held together by a cylindrical closed screw cap and fabricated of commercially pure titanium. The interior of the new chambers has a diameter of 6 mm, and a length of 8.5 mm, so all chambers have the same large volume. The outer diameter is 8 mm, the overall length 13 mm. All ingrowth openings were located at the same height at endosteal level, only shape and size differed. The first model (BCC+) had two ingrowth openings, similar in shape and size to that of the BCC, which are located at the bottom of the chamber and are positioned subcortically when implanted into the bone (Fig. 1b). The second model (ROU) had two round ingrowth openings, each 1.5 mm in diameter (Fig. 1c). The third model (BOT) had no ingrowth openings present at the side of the cylinder but had an open bottom to allow bone/tissue ingrowth (Fig. 1d). All new chambers were designed for implantation in the proximal medial metaphysis of the goat tibia.

2.3. Surgical technique/ animals and operations

Seven mature Dutch milk goats (Capra Hircus Sana) (48–61 kg) were obtained from the Central Animal Laboratory, University of Nijmegen, The Netherlands. The goats received two empty bone chambers at each side in the cortical bone of the proximal medial tibia (Fig. 2). The position of implantation among the chambers and the side for each type of chamber were randomized. All procedures were approved by the Animal Ethics Committee of the University of Nijmegen, The Netherlands.

Anaesthesia was accomplished by intravenous administration of pentobarbital (CEVA Santé Animale, Maasluis, NL) (0.5 ml/kg) and maintained after intubation with nitrous oxide, oxygen and isoflurane (1.5–2%). Under aseptic conditions, a longitudinal incision was made in the skin and fascia over the medial side of the proximal tibia. After raising the periosteum, a hole was drilled through the medial cortex at approximately 4 cm from the joint cleft using a 3.1 mm drill for the BCC and a 7.3 mm watercooled hollow diamond tipped drill (Surgical Diamond Instruments®, Scientific Developments GmbH, Munich, Germany) for the modified chambers. The hole was tapped and bone debris from drilling was removed. The bone chamber was screwed in manually. The second bone chamber was placed at a distance of 10 mm from the first one. This was repeated for the other side. The skin was sutured in two layers. All animals were allowed unrestricted movement in their cages and had free access to water and food after the operation. After the implantation procedure the animals received subcutaneous ampicillin (Albipen LA, Intervet International BV, Boxmeer, The Netherlands) (15 mg/kg/48 h) three times. The goats received intravital fluochromes (calcein green) (20 mg/kg) at 8 days and 1 day before killing.

After 12 weeks all goats were killed with an overdose of pentobarbital. Tibiae were removed, and the bone chambers with surrounding cortex were fixed in 4% buffered formalin. After 1 day the content was removed from the chambers and fixed additionally.

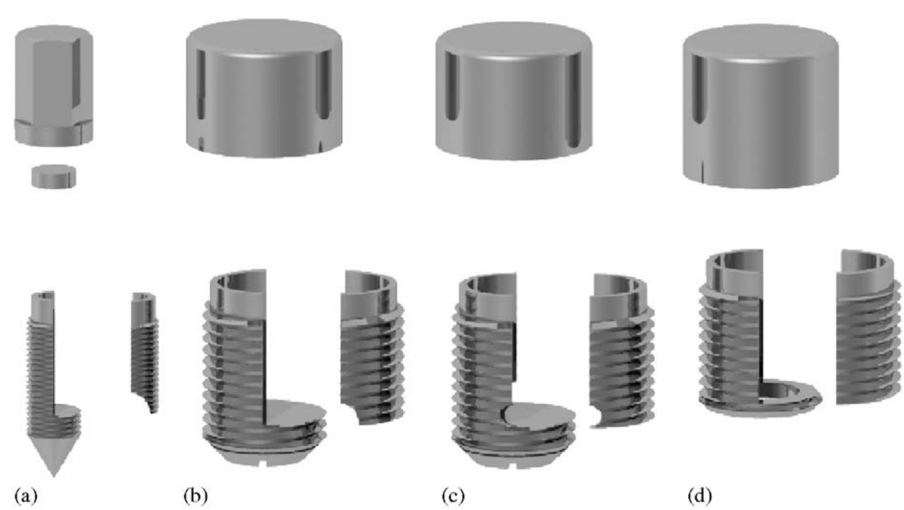

Fig. 1. The bone conduction chamber and the three modified models. All models are made of two threaded half cylinders held together by a closed screw cap and fabricated of commercially pure titanium. A disk is placed in the cap of the BCC to lower the ingrowth holes to the level of the endosteum. All ingrowth openings are located at the same level in the bone. From left to right: (a) BCC, (b) BCC+, (c) ROU and (d) BOT.