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Simultaneous in vivo comparison of bone substitutes in a guided bone regeneration model

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ABSTRACT

A direct, simultaneous comparison of bone substitutes is hampered by the limited number of samples that can be tested simultaneously. The goal of this study was to establish a preclinical model for guided bone regeneration that offers testing of different bone substitutes in a one-wall defect situation. We show here that up to eight titanium hemispheres can be placed on the calvaria of minipigs. To establish our model, titanium hemispheres were filled with and without Bio-Oss®, a deproteinized bovine bone mineral, Ostim®, an aqueous paste of synthetic nanoparticular hydroxyapatite, and Osteoinductal®, an oily calcium hydroxide suspension, before being positioned on the calvaria. After 6 and 12 weeks, titanium hemispheres were subjected to histological and histomorphometric analysis. We show here that bone filled approximately one-tenth of the area below the hemispheres which were left empty, indicating a critical size model for guided bone regeneration. In accordance with the documented osteoconductive properties of Bio-Oss® and Ostim®, titanium hemispheres were almost completely filled with bone. Moreover, the expected degradation profile of Bio-Oss® and Ostim® could be confirmed by histologic and histomorphometric analysis. Under the same conditions, Osteoinductal® failed to exert osteoconductive properties, rather a progressive resorption of the host bone was observed. These results demonstrate that the preclinical model presented here is suitable to simultaneously compare bone substitutes with different material properties. Our model based on the titanium hemispheres allows evaluation of graft consolidation under standardized conditions thereby avoiding intra-individual variations.

1. Introduction

Even though the biologic sequence of bone regeneration is rather conserved, the material properties of bone substitutes may affect the consolidation process [1–3]. Similar to tooth extraction sites and the periimplant space [4,5], bone substitutes are instantly entrapped by the developing blood clot that is later replaced by the highly vascularised granulation tissue [6]. Woven bone in turn sprouts into the vascularised space between the bone substitutes. Thus, by stabilizing the blood clot, bone substitutes guide new bone into the augmentation area. Bone substitutes also provide a surface for the lay down of new bone. Bone substitutes that fulfill these properties are considered to be "osteoconductive" [2]. Woven bone is later replaced by mature lamellar bone that further adapts to functional loading [7].

This complex process of graft consolidation can only partially be simulated in vitro. Preclinical models help to determine the osteoconductive properties of bone substitutes as reported for Bio-Oss® (Geistlich Biomaterials, Wolhusen, Switzerland), a deproteinized bovine bone mineral [8], Ostim® (Heraeus Kulzer GmbH, Hanau, Germany), an aqueous paste of synthetic nanoparticular hydroxyapatite [9], and Osteoinductal® (Dietz GmbH, Munich, Germany), a calcium hydroxide suspended in a formulation of carbohydrate chains, pedum and esterified vaselinum [10]. Clinical studies require biopsies to evaluate graft consolidation, e.g. for Bio-Oss® and Ostim® [11,12].

Because of the growing number of bone substitutes with different material properties, there is a need for standardized preclinical models that allow their direct, simultaneous comparison. The monocortical defect model of the minipig calvaria satisfies these requirements [13]. Monocortical defect represents three wall defects where bone formation originates form the bottom and the

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circumferential wall [13]. This model reflects clinical situations such as tooth extraction sites, three wall periodontal defects and cysts. Here we aim to develop a model that represents the clinical situation of a one-wall defect. In guided bone regeneration, bone substitutes being placed onto the bone surface are covered by a membrane [14]. Corresponding preclinical models where the membrane is substituted by hemispheres were developed in rat and rabbit [15–18]. The rabbit calvaria offer space for two hemispheres [16,18]. The studies described herein were designed to establish a preclinical model that allows a direct, simultaneous comparison of various bone substitutes in a one-wall defect situation.

We report here that the minipig model is suitable to secure up to eight titanium hemispheres that can be easily processed for histologic and histomorphometric analysis. Based on this model, we describe the status of progress of graft consolidation as exemplified with Bio-Oss® and Ostim® at two distinct time periods. Under these conditions, Osteoinductal® failed to show graft consolidation.

2. Material and methods

2.1. Animals and anesthesia

Ten adult minipigs (Ellegaard Göttinger Minipig, Solo Landevij 302, DK-4261 Dalmose) with a weight 41.5 ± 9.5 kg were fed a high-calorie diet and allowed to drink water *ad libitum*. The local Committee for Animal Experiments approved the study. General anesthesia was induced by application of Zoletilmixture with 6.25 ml xylazine (Rompun®, Bayer Austria, Vienna, Austria), 1.25 ml ketamin (Ketasol®, Schoeller Chemie Produkte GmbH, Vienna, Austria) and 2.5 ml butorphanol (Butomidor®, Richter Pharma AG, Wels, Austria). Rompun®, Ketasol®, and Butomidor® were mixed with Zoletil® (Virbac, Vienna, Austria) substance and given 1 ml/kg body weight by intramuscular injection. General anesthesia was maintained by inhalation of O_2, N_2O, and isoflurane (Forane®, Abbott GmbH, Vienna, Austria) under controlled respiration frequency and ECG monitoring.

2.2. Titanium hemispheres' placement and postoperative management

Through a crescent-shaped incision, the frontal bone of the calvaria was exposed by a flap technique. Eight circumferential bone defects of 10 mm in diameter and 1 mm in depth were trephined under saline irrigation. The result of this procedure was a 1 mm deep circular groove that surrounded an unaltered plateau of cortical bone (Fig. 1). In this circular area 11 drill holes 1 mm in diameter and 2 mm in depth were performed to facilitate bleeding into the experimental space (Fig. 1). Two titanium hemispheres per animal were filled with either of the grafting materials: Bio-Oss (0.25–1 mm particle size, Geistlich, Wolhusen, Switzerland), Ostim (Heraeus Kulzer GmbH, Hanau, Germany), Osteoinductal (Dietz GmbH, Munich, Germany) or left empty without any addition of saline or any other substance (Fig. 2). The titanium hemispheres were placed into the circular, groove-shaped bone defects. The dimensions of the hemisphere and the groove matched each other in such a way, that the rim of the domes snapped into the groove and a tight primary stabilization was established. The surgical site was closed in multiple layers using resorbable suture (Vicryl 3–0 and 2–0, Ethicon, Johnson & Johnson Medical, Vienna, Austria). Postoperatively, animals received a single i.m. dose of 1.5 mg amoxicillin (Duphamax®, Pfizer, Madrid, Spain). Carprofen (Rimadyl®, Pfizer, Vienna, Austria) at 4 mg/kg was administered for pain relief. After 6 and 12 weeks, animals were sacrificed by a lethal anesthetic dose of the barbiturate.

2.3. Histology and sample preparation

The relevant part of the skull was removed and fixed in neutral buffered 4% formalin. Block sections containing the whole, intact titanium hemisphere were prepared by cutting the cranial vault with a diamond saw (Exakt Apparatebau, Norderstedt, Germany). These tissue blocks were dehydrated in ascending grades of alcohol and embedded in light-curing resin (Technovit 7200 VLC+BPO, Kulzer & Co., Hanau, Germany). Further processing was done with the Exakt Cutting and Grinding Equipment (Exakt Apparatebau, Norderstedt, Germany) according to the sawing–grinding method [19]. Undecalcified ground sections with a thickness of about 30 μm were stained with the Levai–Laczko dye (Fig. 3). They were oriented perpendicular to the periosteal margin of the skull and cut the hemispheres exactly in half.

2.4. Histomorphometric analyses

Digital pictures at a resolution of 1 pixel equal to 2.21 μm were obtained with a microscope (Nikon Microphot-FXA, Nikon, Tokyo, Japan) and a Digital Camera (Nikon DXM 1200, Nikon, Tokyo, Japan) coupled with a motorized stage (Märzhäuser Wetzlar GmbH & Co KG, Wetzlar-Steindorf, Germany). Single pictures were assembled to generate large overview images (Lucia G 4.71, Laboratory Imaging Ltd., Brno, Czech Republic). Based on interactively drawn false color images (Adobe Photoshop®, Adobe, San Jose, CA) bone tissue and bone substitute materials were measured with the histomorphometry software Definiens Developer 7

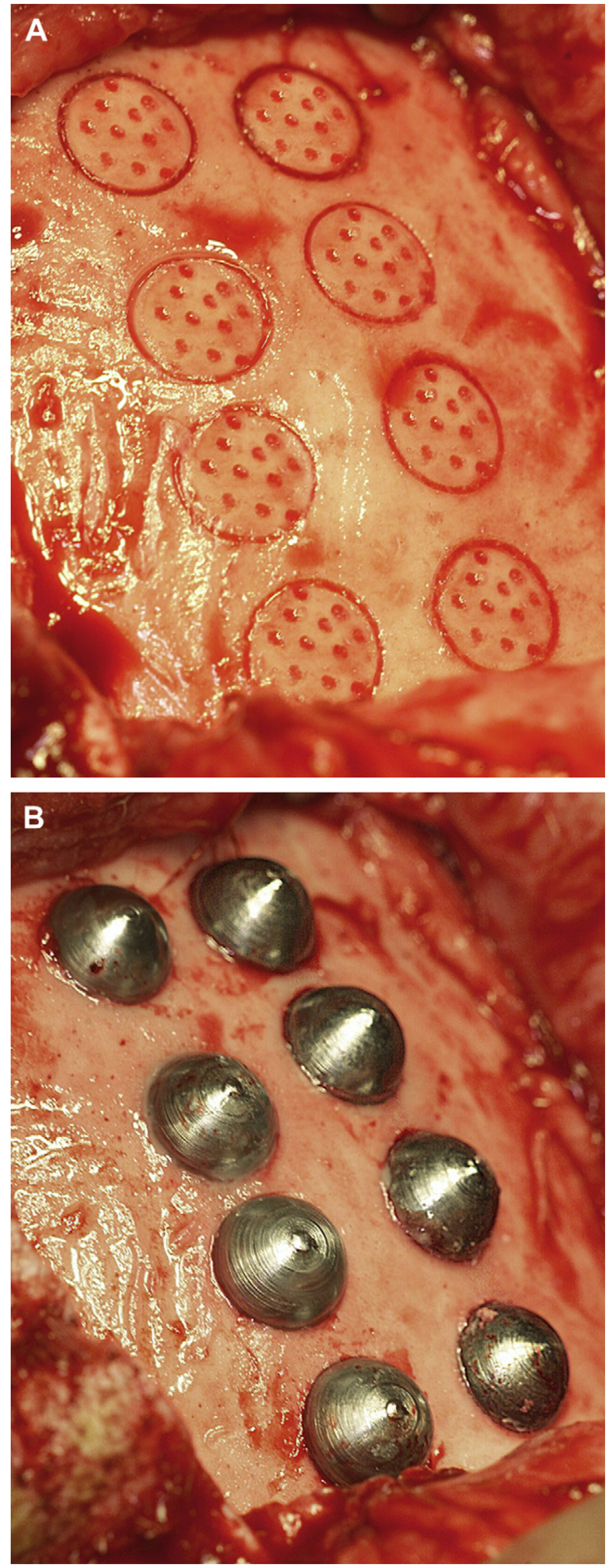

Fig. 1. Titanium hemispheres with and without bone substitutes, before fixation on the calvaria of minipig.