INTRODUCTION

Bone defects are a significant clinical problem caused by inflammation, trauma, congenital malformation, or oncolgical surgery, e.g. tumour resection and consecutive irradiation (1). Previous studies have been shown that bone defects can be a limiting factor in achievement of optimal orthodontic treatment (2). Recently, it was shown that exogenous bone grafts from bovine origin in combination with osteoinductive growth factors, e.g. bone morphogenic factors (BMF) and autogenous spongious bone can be used to create new bone structures (3). Furthermore, different synthetically produced biomaterials, in combination with growth factors and/or with cultured osteoblasts or precursor cells have been tested in several animal studies (4-6). These materials range from metals to polymers and ceramics. Two new synthetic biomaterials are BONITmatrix® which is delivered in granular form and a paste-like composite. Both materials consist of calcium phosphates embedded in a silica matrix. Silicic acid is a general name for a family of chemical compounds of the element silicon, hydrogen, and oxygen. Orthosilicic acid is the form predominantly absorbed by humans and is found in numerous tissues including bone, tendons, aorta, liver and kidney (7). Choline-stabilized orthosilicic acid is bioavailable nutritional supplement. It has been shown to stimulate collagen type 1 synthesis and osteoblastic differentiation in human osteoblast-like cells in vitro (8) and has potential beneficial effect on bone collagen formation in osteopenic females (9). BONITmatrix®, consists of up to 87% by weight of two calcium phosphates namely hydroxyapatite (HAP) and β-tricalcium phosphate (β-TCP) with a mass-ratio from HAP to β-TCP of 60/40. HPA and β-TCP are embedded in a nano-porous silicon dioxide matrix (13% of weight). Because of its overall high porosity and large inner surface of approx. 10^2 m^2/g this material serves also as a scaffold during bone formation. Ossification and biomaterial resorption are necessary for ideal ectopic bone formation with an increase in bone and a decrease of foreign material in a time-dependent manner. The aim of this study was to evaluate the healing of surgically created defects on the cranium of adult male WOK-W rats after application of the paste-like composite histologically compared to granular BONITmatrix®.

MATERIAL AND METHODS

In the design of biomaterials for therapeutic application the evaluation of cellular/tissue responses play a key role. In this study, the in vivo bone-regenerative capacity and resorption of granular BONITmatrix® and a paste-like bone substitution material on the basis of BONITmatrix® were investigated in a rat cranial defect model. The results obtained with both biomaterials were compared to each other. For these, the paste-like composite and the granular BONITmatrix® were implanted in adult male WOK-W rats, the skulls were harvested after eight weeks, and histopathological examined. The comparison of the both tested biomaterials showed that the paste-like composite is much better to handle, the resorption of the material and the ossification process is much faster than those of granular BONITmatrix®. The amount of newly formed bone was also measured and more bone formation was found in bone defects filled with the paste-like composite compared to those with granular BONITmatrix®. The present study showed that both biomaterials could stimulate bone regeneration, but the paste-like composite leads in comparison to granular BONITmatrix® to an accelerated more comprehensive bone regeneration.

Key words: bone defect, histology, calcium phosphate, silica, bone healing, alkaline phosphate, biomaterials

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MORPHOLOGICAL EVALUATION OF BONE DEFECT REGENERATION AFTER TREATMENT WITH TWO DIFFERENT FORMS OF BONE SUBSTITUTION MATERIALS ON THE BASIS OF BONITMATRIX®

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Experimental design and surgical procedure

Seventeen adult WOK-W rats (3 month old, body weight between 350 to 400 g) were divided into two groups. All surgical and experimental procedures were approved by the Animal Welfare Committee on the State Government (LALLF M-V/TSD/7221.3-2.4-027/08). For surgery, each rat was anesthetized with intraperitoneal injection of Ketamine (10%; CEVA Tiergesundheit, Dusseldorf, Germany) and Rompun (2%; Bayer HealthCare, Leverkusen, Germany) with a ratio from 3:2 and at an approximate dosage of 0.1 ml/100 g body weight. A midline skin incision was performed on the skull. Similar half-full-thickness bone defects with 7 x 5 mm size (using a pre-designed template) were created in each parietal region of the cranium with a trephine under constant irrigation. In group 1 (n=10) the left defect was filled with the paste-like bone substitution material on the basis of fragmented BONITmatrix® and the right one served as control. In group 2 (n=7) the left defect was filled with granular BONITmatrix® and the right one served as control. In both groups, the animals were sacrificed at eight weeks and the skull were harvested and subjected to histological examination. For this, each skull was placed in 4% PBS-buffered formalin, dehydrated in a graded series of alcohol, and embedded in methylmethacrylate (Technovit 9100 neu, Kulzer, Germany) as described previously (10, 11).

Histology

For staining serial cross sections (5 µm) were prepared from the centre of the implants using a microtome (Leica Reichert Jung, Nussloch, Germany), and either stained with haematoxylin/eosin, Masson-Goldner trichrome, and v. Kossa stain. A blind test was conducted at the same time using identical staff, equipment, and chemicals.

RESULTS

All animals recovered from the operation and healed uneventfully until the end of the experiments.

For ideal ectopic bone formation, ossification or mineralization and biomaterial resorption should happen both with an increase in bone and a decrease of foreign material over a certain period of time. The animals treated with both bone substitution materials don’t show any inflammation response or disturbed healing of the soft tissue. Using BONITmatrix® both, the resorption and the bone formation, seem to be incomplete, because leftover of the biomaterial was found after eight weeks and the bone defect is clearly locatable. In contrast, using the calcium silicate composite no foreign material was found after eight weeks and the bone defects was almost complete healed.

The harvested cranial samples were analysed histologically. Samples treated with the paste-like composite showed bone healing (Fig. 4). Osteoblasts had close contact to the paste-like composite. Cranial samples examined with BONITmatrix® showed histologically partial bone healing after eight weeks. In the modification area the amount of osteoclasts seems to be increased, whereas osteoblast are difficult to see in BONITmatrix® treated animals compared to the paste-like composite treated animals (Fig. 5).

DISCUSSION

In the design of biomaterials for therapeutic application the evaluation of cellular/tissue responses plays a key role. The efficiency of performed surgical treatment with use of biomaterials can be estimated in different ways: clinical, histopathological, molecular-biological and morphological examination. In our study we have focused on histopathological and morphological examination. In groups of seven to ten animals both bone substitute biomaterials, granular BONITmatrix® and a paste-like composite, was applied. The comparison of the both tested biomaterials showed that the paste-like composite is much better to handle, the resorption of
the material and the ossification process is much faster than those of granular BONITmatrix®.

The main difference between the two ascribed biomaterials was their topological appearance. BONITmatrix® is a nanoporous, granular scaffold composed of hydroxylapatite, tri-calcium phosphate embedded in SiO₂. Recently was shown that mesenchymal stem cells are able to differentiate within 14 days on this biomaterial (12). The cells showed an increase in the RNA expression of osteogenic genes and a higher alkaline phosphate activity as compared to the control. Furthermore, calcium phosphate stimulates the osteogenic differentiation of the mesenchymal stem cells to osteoblasts (12). The biocompatibility of BONITmatrix® was tested using endothelial cells. Microvascular endothelial cells predominantly spread on BONITmatrix® and maintained their typical morphology. Furthermore, the cells expressed endothelium-specific surface marker proteins (13). It is known that dense hydroxyl apatite/β-tricalcium phosphate particles (ratio 60%/40%) showed a beneficial effect on bone formation already after 1 and 2 weeks of implantation in non-critical-sized cranial defects in rats (14). The new bone formation was significantly increased in defects filled with hydroxyl apatite/β-tricalcium phosphate particles compared to empty defects. In our study, the size of cranial defects was the same used by Plachokova et al. (14) and it is known that it is a non-critical-sized defect in rats.

The second used biomaterial, the paste-like composite is a heavily fractionised BONITmatrix® material dispersed with a small amount of water so that the material can be placed via a syringe. It is known that the silica content of this material is beneficial for health, because its deficiency induces deformities in skull and peripheral bones, reduced contents of cartilage and collagen and poorly formed joints (7). Xu et al. (15) have tested the in vivo bone-regenerative capacity and resorption of a porous β-calcium silicate in rabbits. Furthermore, the results were compared to those of porous β-tricalcium phosphate. Using Micro-CT and histomorphometric analysis much higher resorption of β-calcium silicate was found compared to β-tricalcium phosphate. The amount of newly formed bone was statistically increased in calvarial defects filled with β-calcium silicate (15).

Recently it was shown, that the apatite formation ability of a series of β-calcium silicate/β-tricalcium phosphate composites was enhanced with increasing β-calcium silicate content in the composites. Composites with more than 50% β-calcium silicate content were completely covered by a layer of dense bone-like apatite in in vitro studies (16).
In summary, the resorption of the material and the ossification process is increased in bone defects filled with the paste-like bone substitution material compared to those filled with granular BONITmatrix®. Both variations of BONITmatrix® could stimulate bone regeneration, but the paste-like composite leads in comparison to granular material to an accelerated more comprehensive bone regeneration.

Results of this study were dedicated to the seventieth birthday of Prof. Dr. Hans-Georg Neumann.

Conflict of interests: None declared.

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Received: October 22, 2009
Accepted: December 18, 2009

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