

Different cell populations are inducible by BMP-2 covalently covered Bioverit® II implants in rabbit subcutis and middle ear

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Abstract: To optimize the function of implants the formation of surrounding connective tissue should be adapted in dependence to the mechanical conditions. Therefore, with nanostructured silica coated Bioverit® II implants were only partly reacted with recombinant BMP-2. The histology was compared 28, 84 and 301 days after implantation in the rabbit middle ear and subcutis, respectively. The whole tissue blocks were embedded in Epon, sequentially grinded, stained with Toluidine Blue O and Eosin G.

The granulation tissue covering the implants varies related to cell types, cell amounts, extracellular matrix and vessels. Whereas the high cell density and the angiogenesis predominated in the subcutis, the formation of new bone could only be recognized in the scar around the implants in the middle ear.

Keywords: TORP, biomaterial, healing, ossification, connective tissue

Introduction

Bioverit® II, a glass-mica ceramic, is biocompatible, stable and easily machined [1]. For example, it has been successfully used as material for ossicular chain replacement (TORP) [2]. The histological analysis of the samples with a nanostructured silica layer showed good mucosal layer coverage, but a reduction in osseointegration [2]. To optimize the mechanical transfer of the acoustic waves to inner ear, the fixation of the TORP should be restricted only at the ends to the tympanic membrane and the footplate of the stapes.

Bone morphogenetic proteins (BMPs) have osteoinductive properties by regulating key steps in the osteogenic cascade [rev. by 3]. On the surface of nanoporous Bioverit® II functionalized aminogroups of trialkoxysilanes has been used for efficient immobilization of BMP-2 [4]. Therefore, we wanted to test, whether a local BMP-2 layer on the end of the TORPs could induce their osseointegration. For comparison also implants without any mechanical properties were inserted into the subcutis of the neck of the rabbits.

Methods

The Bioverit® II implants (3di GmbH, Jena, Germany) were coated with nanoporous silica layers using a dip procedure as described in details in [4]. The used freshly

prepared solution contained tetra-ethoxysilane (Sigma-Aldrich Chemie GmbH, Munich, Germany) : ethanol (Merck, Darmstadt, Germany) : water : hydrochloric acid : poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol) (all from Sigma-Aldrich Chemie) in a molar ratio of 1:48.9:26.9:0.06:0.0135. After drying at 60°C for 30 min the implants were dipped again until the coverage consisted of three layers of silica. After calcination at 415°C for 4 h the samples were incubated with 10 % 3-aminopropyl trimethoxysilane (Sigma-Aldrich Chemie) in water for 2 min, dried and shaking in a solution of 250 µg ml⁻¹ isolated recombinant human BMP-2 (see method in [5]) in 50 mM 2(N-morpholino)ethanesulfonic acid buffer, pH 5 (Sigma-Aldrich Chemie) overnight at 4°C under gentle shaking, washed with 0.066% sodium dodecyl sulphate in 0.125 M sodium tetraborate buffer, pH10.0 (Sigma-Aldrich Chemie), transferred in phosphate-buffered saline (Gibco tm, Invitrogen Corporation, Paisley, UK) and implanted immediately.

In an approved animal study 6 month old female New Zealand white rabbits (Charlez River, France) were sedated for implantation the prostheses into the right middle ear after partial removal of the ossicle and into the subcutis of the neck. After 28, 84 and 301 days it was taken out the tissue blocks of 5 animals including the implants without BMP-2, partly covered with BMP-2 and totally coated with BMP-2, respectively. After fixation in 4% glutardialdehyde (Merck) in phosphate-buffered saline the specimens were dehydrated in graded ethanol and embedded in epoxy resin (SpeziFix 20Kit®, Struers A/S, Rodovre, Denmark) under vacuum.

For histological evaluation several planes of the tissue were wet-sanded and polished with LaboPol-5 (Struers A/S) and stained with 0.5% Toluidine Blue O (Sigma-Aldrich Chemie) and 0.1% Eosin G (Merck) in 50% ethanol. The analysis was done with the Axioskop (Carl Zeiss Microscopy GmbH, Göttingen, Germany) illuminated by an external cold light source (Schott KL1500 LCD, Mainz, Germany) and documented with a digital camera system (Olympus Colorview, Soft Imaging Solutions GmbH, Germany). The micrographs were further processed with Adobe Photoshop CS6.

Results

A granulation tissue has been formed covering completely the implants. In regions, where BMP-2 has been fixed on the surface of the prosthesis, the cell density and the angiogenesis abruptly increased resulting in a thicker capsule (Fig. 1). This could be seen especially in the subcutis. The new formation of collagen fibrils was delayed.

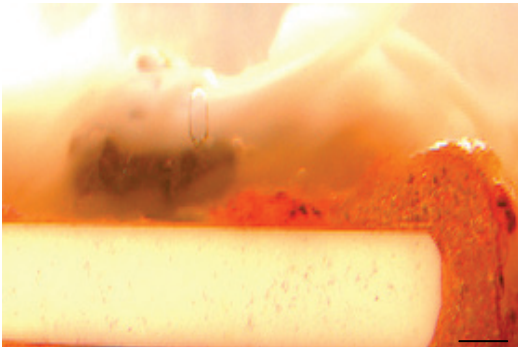


Figure 1: Transition region of the implant's surface with BMP-2 connecting only at the right part correlates with a local surrounding cell-rich capsule in the subcutis. Bar 0.2 mm.

Discussion

Covalently bound BMP-2 induced successfully bone formation after implantation in the mandible and tibia of dogs [6]. This can be confirmed also in the present study, where the osteogenesis could be increased after substitution of the ossicle by a TORP in the presence of this cytokine. Probably the necessary osteogenitor cells are missing in the subcutis, therefore preventing a comparable reaction. This is in agreement with a study of Hong and Mao [7]. Dermal fibroblasts between two collagen sponges loaded with BMP-2 were implanted into a cavity of a bone. After 4 weeks the highly perfused granulation tissue contained only fibroblasts in spite of some osteoblast-like cells near the neighbouring osseous rims.

Since the initiated biological reactions by BMP-2 remained visible over the whole 301 days, this procedure offers a possibility to favourite effectively and locally the healing process of the implants in the body.

Acknowledgement

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The capsule surrounding the TORP in the middle ear consisted of a distinct mucosal layer in addition to several bundles of collagen fibers. Bone formation was increased only in the scar around the BMP-2 containing implants (Fig. 2).

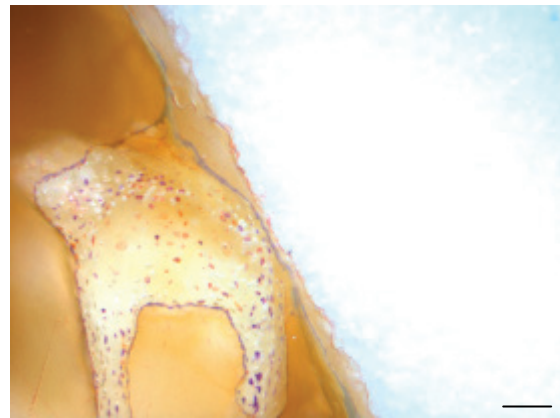


Figure 2: New formed bone near the BMP-2 covered TORP are present only in the middle ear. Bar 0.1 mm.

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