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Growth factor-loaded scaffolds for bone engineering

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Abstract

The objective of the study presented here was to investigate the bone inductive properties as well as release kinetics of rhTGF-β1- and rhBMP-2-loaded Ti-fiber mesh and CaP cement scaffolds. Therefore, Ti-fiber mesh and porous CaP cement scaffolds were provided with these growth factors and inserted in subcutaneous and cranial implant locations in rats and rabbits.

In vitro, a rapid release of rhTGF-β_1 was observed during the first 2 h of the Ti-fiber mesh scaffolds. During this time, more than 50% of the total dose of rhTGF-β_1 was released. Following this initial peak, a decline in the level of rhTGF-β_1 occurred. After 1 week, the entire theoretical initial dose was observed to have been released. This in contrast to the rhTGF-β_1 and rhBMP-2 release of the porous CaP cement scaffolds. Here, no substantial initial burst release was observed. The scaffolds showed an initial release of about 1% after 1 day, followed by an additional marginal release after 1 week.

Histological analysis revealed excellent osteoconductive properties of non-loaded Ca-P material. Inside non-loaded Ti-mesh fiber scaffolds, also bone ingrowth occurred. Quantification of the bone ingrowth showed that bone formation was increased significantly in all scaffold materials by administration of rhTGF-β_1 and rhBMP-2.

Consequently, we conclude that the release kinetics of growth factors from porous CaP cement differs from other scaffold materials, like metals and polymers. Nevertheless, orthotopic bone formation in a rabbit cranial defect model was stimulated in rhTGF-β_1- and rhBMP-2-loaded CaP cement and Ti-fiber mesh scaffolds compared with non-loaded implants.

Keywords: Calcium phosphate cement; Transforming growth factor beta-1; Recombinant human BMP-2; Titanium fiber mesh; Osteoinduction; Bone engineering

1. Introduction

Osteoinductive factors, like Bone Morphogenetic Proteins (BMPs) or Transforming Growth Factor-βs (TGF-βs), have been used to improve the healing in

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bone defects as well as around medical and dental implants. BMPs can be isolated from bone [1]; TGF-βs can be isolated from both bone and platelets [2,3]. Alternatively, they can be produced by transfected cells using recombinant DNA techniques, the so-called recombinant (human) BMPS or TGFβs [4,5]. BMPs have been described to have both stimulating and inhibiting effects on cellular differentiation and proliferation of various cell types. Their effects are dependent on cell type, differentiation stage, dose, and exposure time. Different BMPs can also show varying levels of activity [6,7], for example, BMP-2 and BMP-7 are known to induce bone formation in heterotopic as well as orthotopic sites. TGF-β plays a significant role in wound healing [8] by enhancing the repair of injured tissue like skin and bone [9]. TGF-β acts on osteoblasts, chondrocytes and cells of the osteoclastic lineage as well as on other cells [10]. With respect to bone cells, both stimulating and inhibiting effects have been described for cellular differentiation and proliferation. In view of this, TGF-β_1 and TGF-β_2 have been shown to stimulate osteogenesis in orthotopic sites [11].

A prerequisite for the use of osteoinductive growth factors to regenerate bone tissue is a suitable scaffold. Scaffold materials currently used to support bone healing are polymers or co-polymers, mainly poly(α-hydroxy acid) and collagen. Other candidate materials for bone stimulating-factors are sintered titanium (Ti) fiber mesh material and porous calcium phosphate (CaP) ceramic [12]. The advantage of titanium fiber mesh is that it can be applied directly on, for example, titanium implants. This allows its use even in load-bearing situations. Titanium is currently being applied in surgical practice, because of its excellent mechanical characteristics in terms of stiffness and elasticity and bone compatibility. Calcium phosphate materials are widely used as bone substitutes in orthopaedic, reconstructive and oral surgery because of their beneficial effect on bone healing. These materials can be delivered as granules or prefabricated porous blocks, but they can also be fabricated as an injectable calcium-phosphate cement that can be shaped according to the bone defect dimensions [13]. Macro- and microporosity can be added to the cements, which increase their degradation rate [14]. Growth factors can also be added to such injectable ceramics either before or after complete setting of the cement.

The objective of the studies presented here was to investigate the bone inductive properties as well as release kinetics of rhTGF-β1- and rhBMP-2-loaded Ti-fiber mesh and CaP cement scaffolds.

2. Materials and methods

2.1. Scaffold materials

Sintered porous titanium (Ti) fiber mesh (Bekaert, Zwevegem, Belgium) with a volumetric porosity of 86%, density of 600 g/m^2 and fiber diameter of 50 μm was used as scaffold material. The average pore size of the mesh was approximately 250 μm. The prepared implants were disc-shaped with a diameter of 6 mm, thickness of 0.8 mm, and weight of approximately 15 mg. In addition, hollow, cylindrically shaped Ti-fiber mesh tubes were obtained with the same fiber diameter and porosity. These tubes with an inner diameter of 4 mm and height of 2 mm were sintered on the outer surface of solid titanium rods.

Disc-shaped porous calcium phosphate (Ca-P) cement implants were prepared by a CO_2 induction technique as previously described [14]. The discs were made out of Calcibon® (Biomet Merck, Darmstadt, Germany). This cement consists of a mixture of 62.5 wt.% tricalcium phosphate (α-TCP), 26.8 wt.% dicalcium phosphate anhydrous (DCPA), 8.9 wt.% calcium carbonate ($CaCO_3$) and 1.8 wt.% hydroxyapatite (Hap). An aqueous solution of Na_2HPO_4 (2 wt.%) was used as the liquid component. To generate macroporosity of the scaffold, $NaHCO_3$ was added for production of CO_2 gas in the cement. After mixing, the cement was immediately injected in a cylindrical mould of 2.7-mm height and 8.0-mm diameter. The discs were air-dried in a furnace at 50 °C for 1 h. Before use, all implants were sterilized by autoclaving for 15 min at 121 °C.

2.2. Bone morphogenetic proteins and transforming growth factor-β1

The growth factors used in the present study were recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) (supplied by Yamanouchi Europe, Leiderdorp, The Netherlands), S-300 BMP cocktail (isolated form powdered bovine metatarsal bone