Sustained release of rhBMP-2 from microporous tricalciumphosphate using hydrogels as a carrier

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Abstract

BACKGROUND:

Tissue engineering and bone substitutes are subjects of intensive ongoing research. If the healing of bone fractures is delayed, osteoinductive materials that induce mesenchymal stem cells (MSCs) to form bone are necessary. The use of Bone Morphogenetic Protein - 2 is a common means to enhance effectiveness and accelerate the healing process. A delivery system that maintains and releases BMP biological activity in controlled fashion at the surgical site while preventing systemic diffusion (and thereby the risk of undesirable effects by controlling the amount of protein implanted) is essential. In this study, we aimed to test a cylindrical TCP-scaffold (porosity ~ 40 %, mean pore size 5 µm, high interconnectivity) in comparison to BMP-2. Recombinant human BMP-2 was dissolved in different hydrogels as a carrier, namely gelatin and alginate cross-linked with CaCl2-solution, or a solution of GDL and CaCO3. FITC-labeled Protein A was used as a model substance for rhBMP-2 in the pre-trials. For loading, the samples were put in a flow chamber and sealed with silicone rings. Using a directional vacuum, the samples were loaded with the alginate-BMP-2-mixture and the loading success monitored by observing changes in a fluorescent dye (FITC labeled Protein A) under a fluorescence microscope. A fluorescence reader and ELISA were employed to measure the release. Efficacy was determined in cell culture experiments (MG63 cells) via Live-Dead-Assay, FACS, WST-1-Assay, pNPP alkaline phosphatase assay and confocal microscopy. For statistical analysis, we calculated the mean and standard deviation and carried out an analysis of variance.

RESULTS:

Directional vacuum makes it possible to load nearly 100 % of the interconnected micropores with alginate mixed with rhBMP-2. Using alginate hardened with CaCl2 as a carrier, BMP-2's release can be decelerated significantly longer than with other hydrogels - eg, for over 28 days. The

effects on osteoblast-like cells were an increase of the growth rate and expression of alkaline phosphatase while triggering no toxic effect.

CONCLUSION:

The rhBMP-2-loaded microporous TCP scaffolds possess proliferative and osteoinductive potential. Alginate helps to lower the local growth factor dose below the cytotoxic limit, and allows the release period to be lengthened by at least 28 days.

keywords	Alginate; Biomaterials; Bone Morphogenetic Protein - 2 (BMP-2); Delayed drug release; Porous ceramics; β-Tricalciumphosphate
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