

SYNTHETIC RHBMP-2 DELIVERY SYSTEMS: FROM SURFACE EROSION TO CELL TRIGGERED RHBMP RELEASE

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released from the foamspheres. When 12 μ g

INTRODUCTION: In 1988 the first cDNAs coding for bone morphogenetic proteins (BMPs) were cloned with the primary goal to use the bone inducing principle in the clinic for fracture healing especially for non-unions and the augmentation of bone. Meanwhile a lot of knowledge about the BMPs and their diverse roles has accumulated but the initial goal to use BMP routinely as inducer for bone formation in clinical situations has not been reached due to the lack of suitable carrier materials. Products, which came just recently on the market, use natural materials as delivery system. However, concerning their potential immunogenicity, batch variability, and complex purification procedures the clinical need for synthetic biomaterials is still large. Towards this end, we have designed slow release rhBMP delivery systems termed foamspheres. Foamspheres are based on poly (lactide-co-glycolide) acids, are mechanically more stable than hydrogels, and proved useful for the augmentation of bones. For the treatment of bone defects, we molecularly engineered synthetic hydrogels that contain a combination of adhesive and matrix-metallo-protease MMP-sensitive oligopeptides. Using these hydrogels in combination with rhBMP-2 to heal a critical size defect in the rat cranium, we demonstrate that they are an excellent delivery system for rhBMPs and can be applied in bone defects.

MATERIALS AND METHODS: PLGA based foamspheres were created by a double emulsion-solvent evaporation technique. Branched PEG's (Shearwater Polymers, USA; 4arm, Mw: 10, 15 and 20kD) were end-functionalized with vinylsulfone. Crosslinker peptides with different enzymatic activity (kcat/Km) were designed bearing a cysteine on both ends of a matrix metalloproteinase (MMP) substrate sequence, e.g. GCRD-GPQGIA-GQ-DRCG. Hydrogel disks were implanted in critical size calvarial defects in rats (N>3). After 3, and 5 wk, animals were sacrificed, and the defect regions explanted, radiographed, and histologically processed.

RESULTS AND DISCUSSION: In vitro assays showed that over a period of 10 days all rhBMP is

rhBMP-2 was administered via foamspheres the bone height of the calvarial bone increased significantly to 2.88 ± 0.39 mm ($P < 0.003$) compared to the control untreated calvarial bone (0.90 ± 0.1 mm). Application of 12 μ g rhBMP via a collagen based hydrogel increased bone height to 1.5 ± 0.8 mm, significantly less than achieved by the application of the same amount of rhBMP via foamspheres. Synthetic polyethylenglycol based hydrogels linked by MMP sensitive peptide designed for the application as rhBMP delivery systems in bone defects showed in vitro an initial rhBMP release of 10%. Following 60 h of continuous washing, 90% of the rhBMP was still entrapped in the gel compared to 60% entrapped in a collagen gel. An instant release of the rhBMP from the synthetic PEG gel could be achieved by the application of MMPs. In vivo at a dose of 5 μ g rhBMP-2, bone formation in the rat cranium defect was observed. By 5 wk, implant materials were fully resorbed, and new bone covered the defect area. The healing response was critically dependent on the proteolytic sensitivity and the hydrogel structure. Control materials made with an MMP-insensitive peptide, showed no cell infiltration and significantly less bone formation around the still intact gel implants. Therefore, entrapped rhBMP is biologically inactive and needs cell triggered release by MMPs for activation.

CONCLUSION: The clinical use of rhBMP demands for delivery systems optimized for specific applications. For bone augmentation, the foamspheres seem to be an appropriate slow rhBMP delivery system. For bone defects, a PEG based material, which imitates the natural ECM in its ability to undergo cell-triggered proteolytic degradation and rhBMP release proved to be a very suitable material. Due to the intrinsically non-adhesive/passive character of the major gel component PEG, cell-material interactions can be engineered de novo in an elegant and well-defined way. We believe that this new class of biomaterials is an alternative to existing natural biomaterials for the reconstruction of bone but also for various other tissues.