Development of a Photo-Polymerizable Bone Graft Substitute as a Delivery System for Bone Morphogenetic Protein

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Statement of Purpose: Bone morphogenic proteins (BMP) are a subgroup of differentiation factors within the family of transforming growth factor-β supergene family (Einhorn 1998). Human recombinant bone morphogenetic proteins (rhBMP), such as rhBMP-2 have been shown to be potent differentiation factors capable of inducing bone formation (Seeherman et al 2006). In vitro studies have shown the ability of rhBMP-2 to induce differentiation of various cells into the osteoblast lineage (Katagiri et al 1994). However, there are several pitfalls when trying to deliver an optimal therapeutic amount of rhBMP-2. The lack of optimal matrices for controlled and sustained delivery may result in bone formation in unwanted locations (Burkus 2005). Due to suboptimal matrices, high doses of rhBMP-2 are used resulting in negative outcomes (Sciadini 2000). A delivery system for rhBMP-2 should maintain the protein in situ for sufficient time to interact with target cells and release the protein at effective concentrations during bone formation. An ideal delivery system should also result in the use of less rhBMP-2. This study aims to determine the release profile of rhBMP-2 from a novel photo-curable bone graft substitute (BGS).

Methods: To prepare the allograft BGS samples containing rhBMP-2 (R&D Systems), demineralized bone matrix (DBM), and the polyethylene glycol based hydrogel (macromer) carrier were combined and hydrated with a buffer solution containing the photo-initiator system. The DBM was first deproteinized in 4M guanidine hydrochloride (Gdn-HCl) to remove endogenous rhBMP-2. The loaded rhBMP-2 content of the photo-curable BGS paste was 1 µg. The components were thoroughly mixed and cylindrical samples (6mm diam x 12mm) were created with a mold, and the samples were photo-polymerized by exposure to high intensity visible light in the wavelength range of about 470-520nm. Six cylinders were created from one batch of BGS. In addition, three control cylinders with no rhBMP-2 were created. Each sample was immersed in 1mL of phosphate buffered saline (PBS) and release occurred at rest in a 37˚C incubator. Complete removal and replacement of PBS was performed every 0.5, 3, and 6 hours on day 1, and then every seven days up to 28 days. At 28 days, any remaining rhBMP-2 in the cylindrical plugs was extracted in 4.5mL of 4M Gdn-HCl for 7 days at 4˚C. The rhBMP-2 concentration in the PBS supernatant was determined using enzyme-linked immunosorbent assay (ELISA) with the Quanitikine ELISA Kit (R&D Systems).

Results: The recombinant human BMP-2 release showed an initial burst in the first 24 hours. On average the light cured DBM bone graft samples released about 23% of the loaded rhBMP-2 within 24 hours (Figure 1). After the initial burst, the release rate slowed significantly. On average only 38% of the loaded rhBMP-2 was released at the end of the experiment. By 28 days, the majority of the loaded rhBMP-2 remained in the cylindrical samples (Figure 2). On average, each of the six cylinders contained about 15 ng of rhBMP-2.

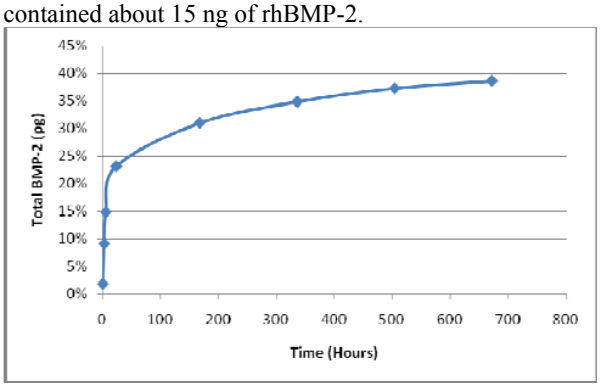

Figure 1. Average cumulative rhBMP-2 elution as a function of time

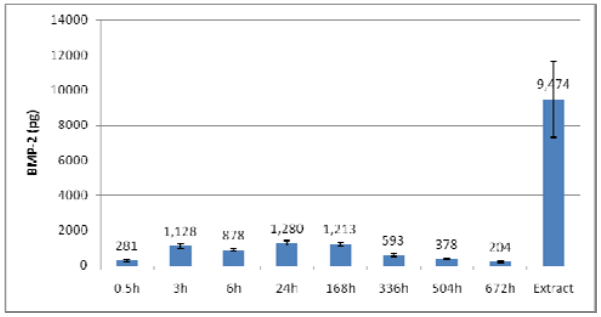

Figure 2. Recombinant human BMP-2 elution characteristics

Conclusions: Previous studies have demonstrated successful repair of defects with DBM and rhBMP-2 (Toriumi et al 1991). Preliminary data showed that basically no endogenous BMP-2 is eluted from DBM (data not shown). BMP may be bound and stabilized within the collagenous matrix of bone. DBM may therefore serve as a depot for exogenously administered rhBMP-2. Incorporation of this natural depot within a macromer carrier may provide enhanced containment of the growth factor within the application site. All of the loaded rhBMP-2 should be bound to the delivery system, but can diffuse out at a therapeutic amount and at an ideal rate over a desired period of time. The data from this study suggests that the light cured BGS may serve as an ideal delivery system for rhBMP-2. Further studies are needed to characterize this photo-curable BGS as a delivery system for rhBMP-2. In vitro and in vivo studies should be performed to evaluate the osteoinductive potential of the rhBMP-2 loaded light cured BGS.

References:

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