Sustained and Localized In Vitro Release of Vancomycin and rhBMP-2 from an Elastomeric Osteoconductive Synthetic Bone Graft Substitute

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INTRODUCTION:
Current treatment of patients with significant volumetric bone loss or segmental bone defect involves either utilization of autograft (current standard) or allograft. These methods are associated with patient morbidity and multiple complications. Development of synthetic bone grafts that will mimic the structural compositions of human bone and provide elastomeric handling properties while exhibiting the ability to release growth factors and antibiotics is highly desired. We have recently developed a high mineral content poly(2-hydroxyethyl methacrylate)-hydroxyapatite (50% wt HA) composite bone graft substitute termed FlexBone that exhibit osteoconductive and elastomeric properties designed to facilitate press fitting into bony defects. Previous study from our lab has demonstrated that FlexBone exhibited excellent structural integration between the hydrogel matrix and the inorganic component, and was able to encapsulate and locally deliver therapeutic agents in vitro.

Vancomycin is an antibiotic frequently used in orthopedic surgery for the treatment of infections caused by gram positive organisms such as staphylococcus, streptococcus, and those unresponsive to other antibiotics (e.g. Methicillin Resistant Staphylococcus Aureus, MRSA). rhBMP-2 is an FDA-approved osteoinductive growth factor that promotes bone repair and bone graft healing. Here we report the strategy of encapsulating vancomycin and rhBMP-2 to enhance the repair of critical sized bone defects while minimizing infections. This study will determine the in vitro release kinetics of vancomycin and rhBMP-2 from FlexBone and the ability of the released rhBMP-2 to induce osteogenic differentiation of myoblast cells in culture.

METHODS:
Using a protocol we previously developed, FlexBone-vancomycin composite grafts were prepared by crosslinking 2-hydroxyethyl methacrylate with 2% crosslinker ethylene glycol dimethacrylate in the presence of 50wt% HA and 2.4, 4.8, 9.0 or 13.0 wt% vancomycin powder in a viscous solvent. The dynamic compressive behavior of FlexBone as a function of vancomycin content was analyzed in PBS at 37 °C with a Q800 Dynamic Mechanical Analyzer (DMA) (10 cycles, force-controlled mode) using parameters as previously described. After the repetitive compressive loading-unloadings, FlexBone-vancomycin constructs were bisected longitudinally for examination of the microstructures of the cross-section by scanning electron microscopy (SEM).

Vancomycin release kinetics from FlexBone was determined by incubating freshly prepared cylindrical specimens (3.8mm in diameter, 4.0mm in height) in MiliQ water at 37 °C for 1 week and quantifying the vancomycin released into the solution over time by UV-Vis spectroscopy (absorption @ 280 nm). A standard vancomycin concentration-absorption curve was generated for the quantification. BMP-2 release kinetics from FlexBone containing 2.4% or 4.8% vancomycin was determined using a BMP-2 Immunoaassay kit (R&D Systems). A sample size of 3 was applied to all release studies.

Osteogenic trans-differentiation of murine C2C12 myoblast cells induced by rhBMP-2 release from FlexBone-vancomycin graft was investigated in culture. Upon myoblast cell attachment to tissue culture plastic, a FlexBone-Vancomycin carrier loaded with rhBMP-2 (300ng/graft) was placed in the culture. In the first experiment, the FlexBone carrier was retrieved from the culture on day 4 and the cells were stained for alkaline phosphatase (ALP). The retrieved FlexBone-vancomycin carrier was then placed in a freshly seeded myoblast culture for 4 days before the cells were stained for ALP. Detailed culture conditions were described previously.

RESULTS:
DMA and SEM analysis showed that up to 4.8wt% vancomycin could be encapsulated in FlexBone without exerting detrimental effect to the desired compressive behavior and structural integration of FlexBone. Unlike the composites containing 9.0 wt% or more vancomycin, the composites containing 4.8 wt% or less vancomycin withstood repetitive compressive loading with excellent shape recovery (Fig. 1A) without developing any microcracks (Fig. 1B).

Sustained release of vancomycin was observed in all composites examined, with the 2.4% composite graft retaining 50% vancomycin and the 4.8% graft retaining ~30% vancomycin at day 7. By 2 weeks, both composites were still releasing the remaining 20-40% vancomycin (Fig. 2). Early release profile of BMP-2 showed that the 2.4% vancomycin graft released twice the amount of BMP-2 (12%) than the 4.8% graft (6%) within the first 48 h (Fig. 3).

Cell culture studies revealed localized differentiation of C2C12 myoblasts, as evidenced by the positive ALP stains, near where the FlexBone-vancomycin-BMP-2 graft was placed (Fig. 4A), supporting that the release of BMP-2 was accomplished in a localized manner and that the released BMP-2 remained bioactive for 8 days (Fig. 4B).

DISCUSSION:
In this work, we determined 1) suitable doses of vancomycin that can be encapsulated in FlexBone without compromising its structural and mechanical properties, 2) the kinetic release profiles of vancomycin and rhBMP-2 from FlexBone, and 3) the ability of FlexBone-vancomycin composite to release rhBMP-2 and induce osteogenic differentiation of C2C12 cells in culture. The demonstrated ability of FlexBone to release an FDA-approved osteogenic protein therapeutic and a potent antibiotic in a localized and sustained manner has significant clinical implications for the expedited repair of bony lesions with minimized infections.

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REFERENCES: