Controlled Dual Drug Delivery with a Calcium Sulfate, Chitosan-Calcium Phosphate Scaffold

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Introduction

Over a million people each year require implants and grafting material to reconstruct bone defects from disease or trauma in the US alone. Additionally, it is estimated that nosocomial infections contribute or cause more than 77,000 deaths in the US annually. Thus, there is a need for a bone scaffold to have osteoinductive, biodegradable and drug delivery properties to aid in repair/regeneration, minimize healing time and prevent infections in bone infections. Sustained release of antibiotics is a desired property of a scaffold in order to prevent reinfections and/or resistant strains of bacteria. A combined antibiotic delivery strategy may provide a mechanism to eliminate bacteria, prevent reinfection and/or resistance. In this study, amikacin and vancomycin were used as model therapeutic agents since they are commonly used in the treatment of bone infections. Chitosan, calcium phosphate and calcium sulfate are promising materials for a bone scaffold due to their osteoconductivity, biodegradability, biocompatibility, drug delivery capabilities and ability to form 3D structures. The two goals of this study were to extend the drug release from chitosan-calcium phosphate composite scaffold material by coating the composite with calcium sulfate and to extend the release of a second drug from the pellet construct for over a month.

Materials and Methods

Composite Bead Fabrication: A co-precipitation method was employed for the fabrication of the composite chitosan-calcium phosphate beads. Solutions of 3.57 wt% chitosan (80% DDA, Prim-Ex), 0.1 M CaCl₂, 0.06 M NaH₂PO₄, (Ca:P ratio = 1.67) were prepared in 2 wt% acetic acid and mixed together. The mixture was slowly dripped into a continuously stirred solution of 20% NaOH, 30% methanol, 50% water (pH=13) to precipitate spherical beads. The beads were kept in the precipitating solution for 24 hours in order for crystalline hydroxyapatite to form. The beads were then washed with deionized (DI) water until a neutral pH was achieved (pH 7-8). The beads were frozen at -20°C for one hour and placed in a 2.5 liter Labconco freeze-dryer for 48 hours.

Amikacin and Vancomycin Loading: Approximately 80 mg of composite beads were loaded in 1.5 mL of a 1mg/mL solution of amikacin at room temperature (RT) for 24 hours. The amikacin solution was removed and the beads were washed two times with PBS. The beads were then placed in either clean vials with 2 mL of PBS at 37°C or mixed with calcium sulfate to form pellets. A vancomycin solution was added to calcium sulfate powder to yield 2% (w/w) of vancomycin in the pellet.

Pellet Fabrication and Elution: The pellets were made from 0.6g of α-hemihydrate calcium sulfate and 0.24mL dH₂O. Composite beads were mixed into some calcium sulfate samples prior to setting. Samples were allowed to dry over night and placed in 48 well plates containing 1.5mL PBS. Elution samples were taken at 1, 5, 12, 24, 48, 96, 168, 336, 480, 648 and 816 hrs. The PBS solution was completely refreshed after each elution time point. The samples (n=5) were analyzed via a fluorescent immunoassay with the TDx machine (Abbott Diagnostics, Abbott Park, IL).

Results

The elution profile showed that the pellets containing composite beads extended the release of both antibiotics compared to the elution from the composite beads or the calcium sulfate alone. The calcium sulfate coated composite beads released amikacin levels above 4µg/mL through day 27. The vancomycin release from the pellet containing composite beads was ~5 times greater at day 34, 16µg/mL, than the pellet without composite beads, 3µg/mL. The scaffolds degraded 47 ± 3% after six weeks.

Discussion

The combination of calcium sulfate and chitosan-calcium phosphate extended the release of both antibiotics. This is potentially due to an affinity between the drug and scaffold material for either drug and to the calcium sulfate coating dissolving over time and exposing the composite beads containing amikacin to the buffer. Extended release of vancomycin was seen throughout the entire study and future studies will examine lower therapeutic levels of vancomycin. Additionally, future research will address the ability of the eluents to kill and prevent bacterial biofilm formation, and evaluate affects on cells and healing processes.

References