Novel Bilayered Polymeric Microspheres for Bone Tissue Engineering Applications: Effects of Alginate Coating on Release Kinetics

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Statement of Purpose: Fracture healing/ bone repair is a multidimensional event with overlapping timelines, but can be divided into three broad stages: inflammation, hard and soft callus formation, and remodeling [1]. These stages of healing are characterized by several biochemical events, including the release of several growth factors of varied dose, time, and functional interdependence [2]. In designing tissue engineered scaffolds for bone regeneration, the ability to deliver factors in a controlled manner is critical. Given the need for multiple growth factors for complete healing, it is equally critical to deliver multiple factors in temporally appropriate schemes. Factor delivery from biodegradable microspheres depends on the nature of factor incorporation, degradation rate of the micro-spheres, and material composition of the microspheres, but is generally limited by the material choice. The use of multiple polymers within one microsphere imparts the kinetics of each polymer and therefore increases the control of delivery. Toward this end, we have developed a bilayered, biodegradable microsphere to deliver factors for bone tissue engineering. Poly(hydroxybutyrate-co-valerate) (PHBV) microspheres have been coated with alginate (see figure 1), and this coating alters the release of a molecule encapsulated within the inner PHBV microsphere. The focus of the present work was to determine the effect of alginate coatings on the release of p-nitroalanine (PNA) from PHBV microspheres.

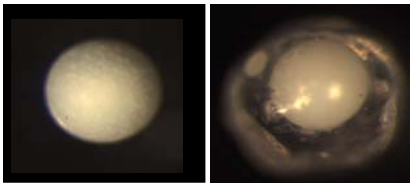

Figure 1. Uncoated (left) and coated (right) polymeric microsphere.

Methods: Alginate coated microsphere synthesis was based on previous work from this group. Briefly, p-nitroalanine (PNA) (1:250 (wt/wt) drug to polymer ratio) was added to a solution of PHBV (8% valerate) (Sigma Aldrich, Milwaukee WI) dissolved in methylene chloride. This solution was then added dropwise to a stirring solution of 1% (wt/vol) polyvinyl alcohol (PVA). The solution stirred overnight at 275 rpm at room temperature (RT). The resulting PHBV microspheres (MS) were rinsed with DH_2O and sieved to a size range of 425 – 600 µm. A 4% (wt/vol) alginate solution was made by dissolving alginate into DH_20. Calcium carbonate ($CaCO_3$), Span 80, and the PHBV microspheres were added to the alginate solution and subsequently added to a spinning W/O/W emulsion of soybean oil. Acetic acid was added to the oil mixture to cross link the alginate and fully encapsulate the microspheres. PNA release from both alginate coated and uncoated PHBV microspheres was evaluated by placing microspheres in a 1% phosphate buffered saline (PBS) solution, warmed to 37ºC and agitated slightly. PNA release was detected by evaluating optical density of PBS (at 405nm) for the following time points: 3, 6, 12 hours, 1, 3, 5, 7, 14, 21 and 28 days. Statistical significance of PNA release at each time point between coated and uncoated microspheres was determined using student's T-test ($p<0.05$).

Results/Discussion: PHBV microspheres appeared spherical and smooth while alginate-coated microspheres had a thick alginate layer completely encapsulating the PHBV inner sphere (see figure 1). Thickness of the coating was not entirely uniform around the inner sphere, and coating thickness varied marginally within a population of microspheres. Spectrophotometric analysis indicated that the alginate coating suppressed the release of PNA from the encapsulated MS after 6 hours, as seen in Figure 2. PNA release over 1 month was found to be elevated in uncoated microspheres, with statistically significant differences at 3, 5, 14, 21, and 28 day time points.

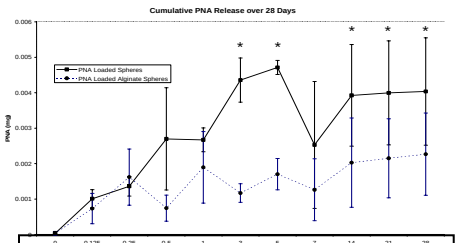

Figure 2. PNA release from coated (dashed line) and uncoated (solid line) microspheres shows suppressed release in coated spheres over 28 days.

The ability to regulate the release of proteins from biodegradable microspheres through the addition of an aqueous-based alginate layer provides an added layer of control when delivering growth factors for hard tissue repair. This study demonstrates this capability using loose microspheres but this technology can be easily applied to more complex scaffolds designed for large scale bone defect repair. In the complex multifaceted environment of bone repair, the ability to modulate factor release, and perhaps deliver more than one factor from the same source but in a temporally appropriate manner, would prove invaluable in the orthopaedic realm. Future studies will examine the ability of the alginate layer to act as both a factor release suppressor and as a delivery vehicle itself, allowing for more complex delivery schemes.

Conclusions: **C**oating degradable PHBV microspheres with a layer of alginate has been shown to delay the release of PNA from PHBV microspheres when compared to uncoated microspheres over a 28 day period. The addition of this layer to the microspheres brings an added level of control over release kinetics, and has implications for potential multi-factor delivery for the regeneration of bone via tissue engineering.

References: [1] Ostrum R. In Orthop Basic Science: Biology and Biomech. Muscul. System. Buckwalter. 2000. [2] Peng H, J Bone Min Res. 2005:20:2017-27