Injectable, environmentally-responsive microspheres for growth factor delivery

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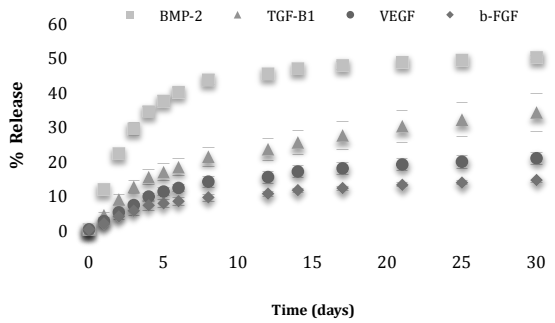
Purpose: Local administration of exogenous growth factors has shown significant advancement in tissue regeneration and remodeling after injury. Bolus injections have limited therapeutic benefits because of a short growth factor half-life and the large dose necessary to induce an in-vivo response often results in unwanted immune side effects. Sustained release of these factors via polymer delivery vehicles allows for cumulative therapeutic doses to be locally available over extended periods. In addition to providing sustained delivery, a bioresponsive vehicle will potentially enhance the therapeutic efficacy of the treatment as it will adapt to the local environment, creating pathology-triggered dosing.

Introduction: Our previous work demonstrates microspheres coated with hydroxyapatite mineral are able to serve as a delivery platform for a controlled release of acidic and basic proteins (Jongpaiboonkit). Comparing our hydroxyapatite coated microsphere system with the well-characterized delivery of encapsulated protein from PLG microspheres, we demonstrated 1) a higher cumulative dose released and 2) limited burst release kinetics. Because hydroxyapatite has shown advantage as a platform for controlled release, we propose to bind and release bioactive growth factors from our injectable, hydroxyapatite coated delivery system into in vivo-like conditions, supporting our ability to produce environmentally responsive release.

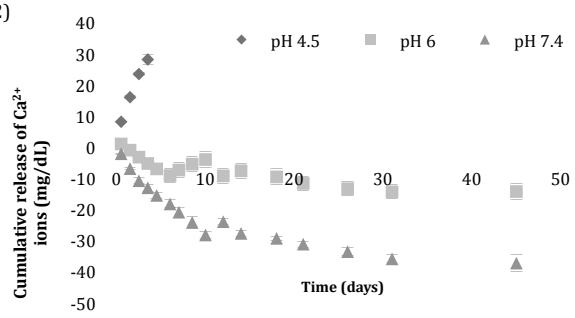
Methods: Poly(D,L-lactide-co-glycolide) 85:15 Sigma-Aldrich (St. Louis, MO) was fabricated into microspheres as described previously via water-in-oil-in-water (W/O/W) double emulsion technique with a PBS solution pH 7.4 in its aqueous core. Once fabricated, the resulting microspheres were collected, washed, resuspended, and purified via coarse filtration. After drying, the microspheres were incubated for 10 days in a modified Simulated Body Fluid, which contained ions in a similar concentration to that of blood plasma, and twice the blood concentration of calcium and phosphate ions. After 10 days incubation, the resulting mineral coated microspheres were collected, washed, and dried. Protein was loaded via a simple binding process in a PBS solution and protein-loaded microspheres were incubated in a Simulated Body Fluid at pH 7.4 and allowed to release into solution over time.

Results: Preliminary results illustrate sustained release of therapeutic growth factors from mineral-coated microspheres at physiologic pH (figure 1). By day 30, less than 50% of each bound protein was released. The four growth factors released have a myriad of affects on processes not limited to wound healing, angiogenesis, and cartilage matrix synthesis. Dissolution of the mineral layer from the microsphere surface was measured in Simulated Body Fluid buffered in three different pH environments, reflecting acidic conditions seen in vivo during tissue inflammation (figure 2).

(1)



(2)



Dissolution kinetics varied with pH environment. The pH=4.5 environment not only promoted more rapid dissolution, but also disrupted the integrity of the underlying polymer (not shown). Solutions closer to physiologic pH resulted in mineral re-precipitation on the microsphere surface, demonstrated by depletion of calcium ions from the surrounding solution.

Conclusions: Mineral-coated microspheres release bound proteins in a sustained manner at physiologic pH, and demonstrate mineral dissolution in a manner that is responsive to their surrounding environment. Understanding the relationship between mineral dissolution and protein release in a variety of pH environments may enable predictable release of bioactive growth factors in the acidic environments of inflamed or injured tissue, and perhaps encourage tissue remodeling.

References: Jongpaiboonkit L., Franklin-Ford, T., and Murphy, W.L. Advanced Materials 2009; 21:1960-1963.