Devices for Alternating Release of Multiple Biomolecules

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Introduction

Controlled release technology is being used to deliver a variety of biomolecules. Most commonly, investigators seek to release one drug at constant concentrations for extended periods. However, such zero-order kinetics are often undesirable. For example, whereas intermittent administration of parathyroid hormone (PTH) enhances bone formation, continuous exposure has a catabolic effect (*J Bone Miner Res* 1992 7:65). Simvastatin (Sim) also has been reported to stimulate bone formation following daily injections (*Science* 1999 286:1946).

In other circumstances, multiple cytokines and growth factors may be needed for optimal biological effects. For example, tissue regeneration involves many biomolecules in a sequential cascade of events. Furthermore, if a defect site is compromised by microorganisms, they must be eradicated before repair can proceed. Cecropin B (CB) is a naturally occurring antimicrobial peptide that can kill or neturalize Gram-negative and Gram-positive bacteria, fungi, and parasites *(J Antimicrob Chemother* 1994 38:1498).

In previous studies, we developed a controlled release system based on blends of cellulose acetate phthalate (CAP) and Pluronic F-127 (PF-127) for intermittent release of small and large biomolecules. The objective of this research was to investigate use of this system for alternating release of different drugs.

Methods

CAP/PF-127 (7:3 blend ratio) microspheres containing PTH, Sim, or CB were prepared by an acetone-oil-water (W/A/O/W) triple emulsion process. Drugs were labeled with fluorophores to enable quantification of amounts in release supernatants. Devices were made using a layer-by-layer process in which the desired drug-loaded microspheres were alternately deposited followed by pressure-sintering at 20 Pa for 5 seconds. All but one surface of devices were coated three times with 10% PLGA solution to obtain directional release. For release studies, samples were immersed in PBS at 37°C, and supernatants were collected daily. Fluorescence was measured following acid-precipitation of CAP.

Results and Discussion

Figure 1 shows release profiles for devices designed to alternately release PTH and CB. When exposed to PBS, carboxyl groups on CAP were deprotonated and hydrogen bonds with ether groups in PF-127 were consequently lost. The PTH-containing top layer began to erode first and was followed by the CB-containing layer. This process continued for about 14 days. Overall, five discreet release peaks were observed each for PTH and CB. During the release period, CB showed uniform peak concentrations (0.8-0.94 µg/ml), and PTH also had relatively even maximum release, except for the last peak when integrity of the devices was lost.

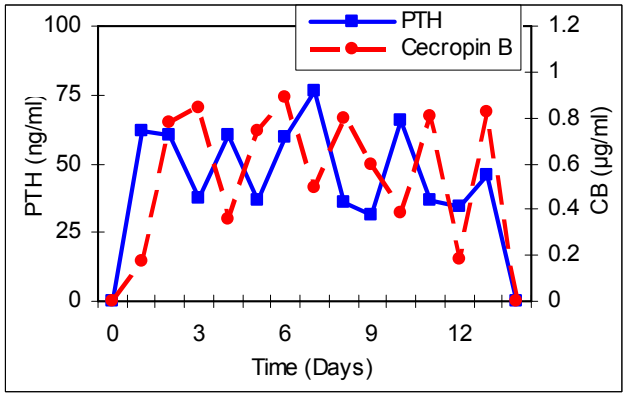

Figure 1. Alternating release profiles for 10-layer devices loaded with PTH and Cecropin B.

Figure 2 shows results for devices alternately loaded with PTH and Sim. Again, five discreet release peaks were observed for each component over a 15 day period. Peaks of PTH release were more distinct than those for Sim. Differences in behavior can be attributed, in part, to the smaller size and hydrophobicity of Sim compared to PTH. In addition, because of it's hydrophobicity, Sim-containing devices lasted one to two days longer than those without.

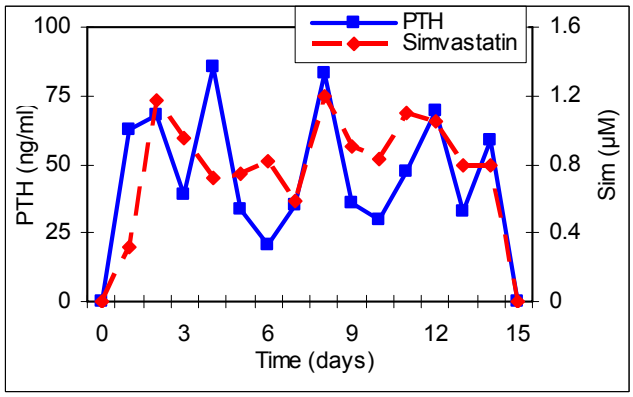

Figure 2. Alternating release profiles for 10-layer devices loaded with PTH and simvastatin.

Conclusion

Alternating release of bone anabolic molecules PTH and simvastatin and of the antimicrobial peptide cecropin B were achieved using the association polymer system of CAP and PF-127. Ongoing studies are determining the bioactivity of released peptides and simvastatin.

Acknowledgment

This work was supported by the NIH (AR048700).