A Prolonged Two-Phase Peptide Release Achieved Using Amino-Silane Chemistry Functionalization and Nanocrystalline Hydroxyapatite in a Degradable Polymer Composite

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Statement of Purpose: Pharmaceutical agents are often required to stimulate new bone formation for treating bone injuries or diseases. However, conventional systemic administrations of these agents can not effectively reach targeted sites and, even if intentionally injected locally to the damaged bone tissue, these agents tend to rapidly diffuse into adjacent tissues due to weak physical bonding to their drug carriers, which limits their potential to promote prolonged bone formation in targeted areas. Therefore, this study explored the chemical method for immobilizing the peptides derived from BMPs (bone morphogenetic proteins) to nano-HA (nanophase hydroxyapatite) to promote drug loading efficiency and to achieve prolonged release at local disease sites.

Methods: Nanocrystalline HA was synthesized using a wet chemistry precipitation method followed by a hydrothermal treatment. The peptide with a 12 amino-acid sequence of CKAISVLYFDDS was designed as the model peptide and termed as DIF-7c. Nano-HA was silanized in 3-aminopropyltriethoxysilane (APTES; Sigma) in hexane and coupled with N-succinimidyl-3-maleimido propionate (SMP; Sigma) in N,N-dimethylformamide (DMF; Sigma) [1]. The peptide DIF-7c was immobilized onto nano-HA in DMF through a reaction between the outer maleimide group with the thiol group of cysteine present in the terminal of DIF-7c. The resulted nano-HA and DIF-7c conjugates were termed as HA_Ps and were dispersed in PLGA (50/50 wt.% poly(DL-lactide/glycolide, Polysciences, Inc.) using sonication. The weight ratio of HA_Ps to PLGA was 30/70. A novel 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde (CBQCA, Molecular Probes) fluorescence technique was used to characterize the peptide loading. In vitro peptide release profiles were studied in PBS under standard cell culture conditions for 52 days. After prescribed days, the supernatants were collected and analyzed using a micro-BCA assay (Pierce).

Results: The results of the CBQCA assay demonstrated that nano-HA with chemically loaded peptide produced very good fluorescence, which indicated the successful attachment of the peptide onto nano-HA (Figure 1). The single phase drug carriers, including PLGA_P and HA_Ps, all demonstrated one-phase release, although the release happened at different time points for the HA carrier and the PLGA carrier (Figure 2). The total amount of peptide released by the HA_Ps was greater than the PLGA_P during 52 days. It indicated that the nano-HA had higher peptide loading efficiency compared to the PLGA. The composite drug carrier (HA_Ps_PLGA) demonstrated two-phase release. At phase I (from day 1 to 7), the HA_Ps_PGA demonstrated continuous peptide release at a gradually decreased amount. At phase II, the HA_Ps_PLGA demonstrated increased peptide release from day 30 to 52.

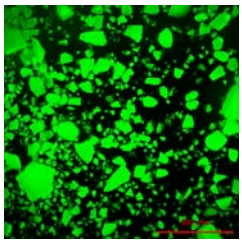

Figure 1: The CBQCA analysis of nano-HA loaded with the model peptide DIF-7c by the chemical bonding method. Scale bars are 500 μm.

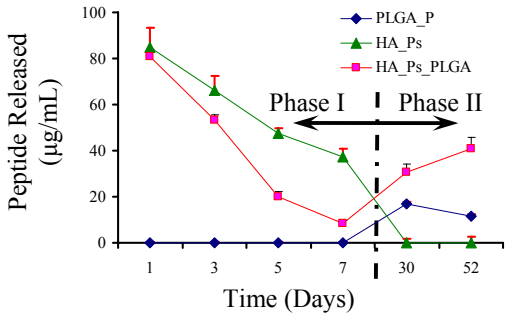

Figure 2: The amount of peptide DIF-7c released from the drug delivery systems. Values are mean ± SEM; N=3.

Conclusions: Three different drug release profiles were achieved by using various drug carriers. The drug loading efficiency are related to the drug carriers and the loading methods. The nanocomposite drug carrier (such as HA_Ps_PLGA) demonstrated a two-phase release profile. Importantly, a prolonged peptide release (up to 52 days) was achieved on the HA_Ps_PLGA drug delivery systems. The drug carriers and the drug loading methods are very important factors that should be considered when designing the next generation of drug-carrying orthopedic prostheses for various clinical applications. This study presented a useful guideline for designing more effective, controlled drug delivery systems according to the release requirements for specific clinical applications.

References: [1] Balasundaram G, Sato M, Webster TJ. Biomaterials 2006; 27(14): 2798-2805.