Bisphosphonate modified hydroxyethylchitosan as a bone scaffold for bone regeneration

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Statement of Purpose: As the population ages, the number of people affected by osteoporosis is increasing. In Europe and the United States it is estimated that >40% of women who are postmenopausal will suffer a bone fracture in their lifetime [1]. Costs incurred in the United States for fracture treatment during 2005 were >$19 billion with a continuous rise of >48% expected by 2025 [1]. Bisphosphonates have been used for clinical treatment of osteoporosis since the 1970's. They have also been shown to be effective in the treatment of other bone degenerative diseases such as Paget's disease, myeloma, and bone metastases [2]. Current methods for delivering bisphosphonate drugs include oral and intravenous administration ranging from a daily pill to once yearly intravenous injections.

We have coupled a bisphosphonate with a biodegradable hydroxyethlychitosan (HEC) substrate to increase hydroxyapatite binding and improve cellular response of the bone scaffold to enhance bone regeneration.

Methods: HEC (Sigma Aldrich) was dialyzed against deionized water for 48 hours prior to use. A series of bisphosphonate modifiers were synthesized through an efficient double Michael addition of aminoalcohols onto diethylvinylphosphonate, and this was followed by reaction of the hydroxy group with acryloyl chloride to afford ammonium bisphosphonate-functional acrylates. These bisphosphonates were then used to modify HEC through addition of the pendent amines across the acrylate double bond. Compositions have been investigated with 10% and 20% of the HEC amines modified with the bisphosphonates. The bisphosphonate-functional HEC was dissolved at a concentration of 2% w/w in phosphate buffered saline and crosslinked in the presence of sodium chloride with a poly(ethylene oxide) diacrylate oligomer having a M_n of ~700 g/mole. The sodium chloride was removed, and the resultant foams were frozen at -80 °C for 24 hours, then freeze dried. The crosslink density was varied to form a series of hydrogels. Degradation rates of the foams upon aging in 5xSBF, phosphate buffered saline and deionized water were studied by measuring weight loss with time. The capacities of the modified foams for stimulating in vitro mineralization of calcium phosphates was investigated as a function of their bisphosphonate concentration in a 5x simulated body fluid (5xSBF) environment and an alternate immersion method using 0.1M $CaCl_2$ and 0.1M NaH_2PO_4 solutions. Influences of mineralization on osteoblast proliferation were investigated.

Results: HEC was modified successfully via Michael addition of the bisphosphonate monomer in systematically varied compositions. Hydrogels were formed through crosslinking and a porous foam was created through lyophilization to be investigated as a material for novel bone scaffolds. The foams were examined by scanning electron microscopy (SEM) to view porosity and mineral formation. The images showed the foams were mineralized in both simulated body fluid and the alternate immersion method. By examining different sections of the foam (top, middle, bottom), it became clear that mineral deposits formed throughout the scaffolds. Energy-dispersive X-ray spectroscopy (EDS) coupled with the SEM confirmed that the minerals contained calcium-phosphate crystals. Figures 1 depicts representative SEM images of the unmodified (HEC) foam and modified foams. Weight loss studies upon aging the foams in various media at 37 °C were conducted to measure their degradation rates (Figure 2). The unmodified and modified foams also supported cell proliferation.

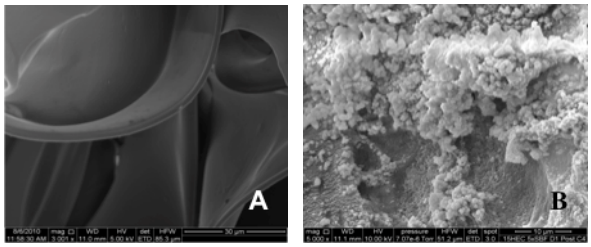

Figure 1. Representative SEM micrographs of A) unmodified HEC foam and B) modified HEC foam with mineral.

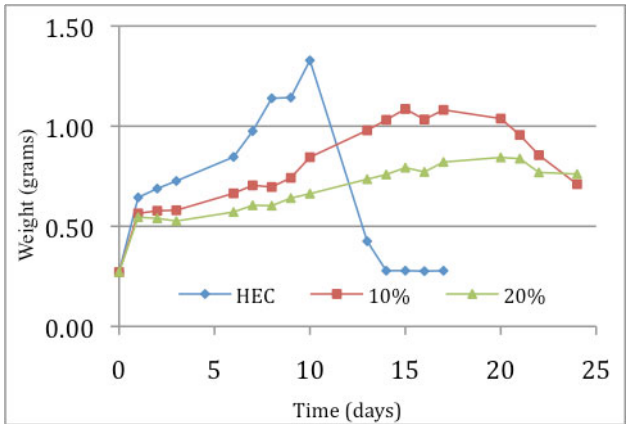

Figure 2. Degradation Profile in PBS

Conclusions: Modification of the HEC with the bisphosphonate clearly increases the affinity of the scaffold for mineralization. Future work will investigate the use of these scaffolds as a drug delivery device for these new bisphosphonates to assess their potential as a drug for osteoporosis or other bone degenerative diseases.

References: ([1]Boonen S. Int J Clin Prac. 2009;63:1792-1804) ([2]Graham R. Bone 2007;40:S21-S2)