Novel Biodegradable Polyphosphazene-Nanohydroxyapatite Microsphere Scaffolds for Bone Tissue Engineering

Syam P Nukavarapu[a], Nicholas R. Krogman[b], Sangamesh G. Kumbar[a], Justin L. Brown[c], Lakshmi S. Nair[a], Harry R Allcock[b], Cato T Laurencin[a,c,d]

Department of Orthopaedic Surgery[a], Biomedical Engineering[c], and Chemical Engineering[d], University of Virginia

Department of Chemistry[b], Pennsylvania State University

Statement of Purpose: Biodegradable polyphosphazenes (PPN's) are a unique class of polymers with controllable physico-chemical and degradation properties. The non-toxic, neutral degradation products of PPN's make them potential candidates for *in vivo* orthopaedic applications as demonstrated in our laboratory[1]. Because bone is a composite, hydroxyapatite (HA)-polymer composites are known to better mimic the structure and composition of bone. Poly[bis(ethyl phenylalaninat-N-yl)phosphazene]-(PNPhA) was selected for composite preparation in the present study due to its excellent physical properties and biocompatibility. The aim of the present study was to develop three dimensional (3D), porous interconnected structures from PNPhA-HA composites using a novel solvent/non-solvent microsphere sintering method as scaffolds for bone tissue engineering applications.

Methods: PNPhA was synthesized by the ring-opening polymerization of hexachlorocyclotriphosphazene at 250°C to obtain poly(dichlorophosphazene) (PDCP) and the subsequent substitution of chlorine atoms on PDCP with phenylalanine-ethylester side group. PNPhA microspheres with different loadings of nano HA (10, 20 and 30 wt%) were prepared using emulsion/solvent evaporation method. Composite microspheres (350-500 μm) were sintered using a novel solvent/non-solvent method. Solvent/non-solvent systems used were as follows: Solvents- CH_2Cl_2, $CHCl_3$ and C_4H_8O (THF, T) and non-solvent- C_6H_{14} (Hexane, H). Solvent/non-solvent mixtures were added to microspheres (1:1 volume to weight) and vortexed for 10 s before packing into the mold of required shape. After lyophilization, the composition, morphology, mechanical strength and porosity of the 3D structures was determined by standard characterization techniques.

Results/Discussion: Figure 1 shows the successful incorporation of nano HA onto PNPhA microspheres. The presence of calcium (Ca) in the EDS spectra (Figure 1) confirm the presence of HA in the composite. The high phosphorous (P) content in the composite microsphere has been attributed to the presence of P in PNPhA backbone along with HA. HA loading was quantitatively determined using thermogravimetric analyses (not shown here) and observed that the loaded amount was consistent with the starting composition. Various solvent/non-solvent combinations (developed based on fractional solubility parameters) were evaluated for sintering microspheres (80wt% PNPhA-20wt% HA) and the THF-Hexane system has been found to be optimal. The effect of solvent/non-solvent composition on the scaffold sinterability was investigated. As shown in Figure 2, microsphere fusion is greatly enhanced by increasing THF from 15 to 22.5 vol% (with THF vol%+Hexane vol%=100). Increasing THF content has found to increase the compressive modules of the sintered matrices, however the pore size and porosity decreased with increase in THF vol%. Table 1 shows the properties for a scaffold sintered using a solvent/non-solvent composition of 20THF/80Hexane. The observed compressive modulus is in the range of human cancellous bone modulus.

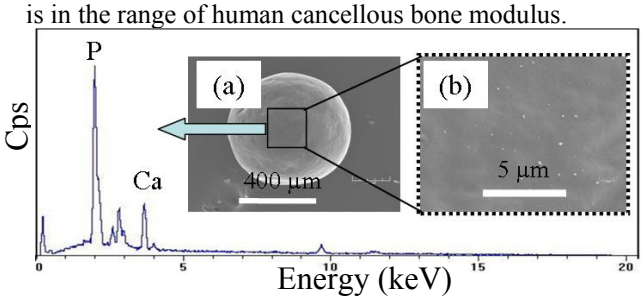

Figure 1. SEM micrographs recorded on a microsphere of composition 80PNPhA-20HA (a) a single microsphere and (b) high magnification image showing the HA nano particle dispersion and the corresponding Energy dispersive X-ray spectrum (EDS). Note the appearence of phosphorous (P) and calcium (Ca) peaks in the spectrum indicating the HA presence on the microsphere surface.

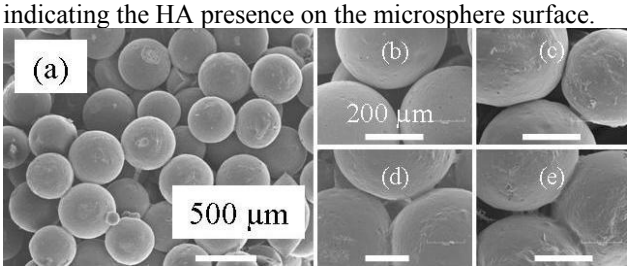

Figure 2. SEM micrographs showing the typical morphology of 80PNPhA-20HA scaffolds (a) sintered with the solvent mixture of 20THF+80Hexane and (b), (c), (d) and (e) are closeup images recorded on scaffolds that are sintered using solvent mixtures 15T+85H, 17.5T+82.5H, 20T+80H and 22.5T+77.5H, respectively.

Table 1. Solvent composition used, and the resulting 3D scaffold physical properties

Solvent Composition (vol%)	Fractional Solubility Parameters	Compressive Modulus	Pore diameter & Porosity
20 THF + 80 Hexane	*N=88 *D=5 *W=7	73±9 MPa	117±7 μm 20±1 %

*N is dispersion, D is polar and W is hydrogen bonding components

Conclusions: This is the first time a study demonstrated the feasibility of developing 3D composite biodegradable polyphosphazene scaffolds for tissue engineering applications. Work is in progress to improve mechanical properties of these scaffolds by varying material composition and process parameters.

References: 1).Laurencin et al., JBMR-A.2006;77:679-87

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