Novel Biodegradable Polyphosphazene Lighter than Water Microsphere Scaffolds for Bioreactor Based Bone Tissue Engineering

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Statement of Purpose: Bioreactors provide a means of culturing cells in a dynamic environment at near physiological levels of shear. In addition, cell culture in a bioreactor provides better nutrient transfer/waste removal from the scaffold allowing larger structures to be cultured than would be possible in a static environment, which is limited by diffusion in just one direction. Our laboratory has shown the advantages of buoyant poly(lactide-co-glycolide) (PLAGA) scaffolds for dynamic culture conditions (Figure 1).[1] Recently, our laboratory has demonstrated the unique advantages of biodegradable amino acid ester polyphosphazenes (PPhos) over PLAGA scaffolds for tissue engineering applications.[3] The objective of the present study is to investigate the feasibility of developing bouyant porous structures from biodegradable PPhos as potential scaffolds for bone tissue engineering applications under dynamic culture conditions. Two different types of PPhos will be used to fabricate three dimensional structures using a novel solvent/non-solvent sintering technique[2] recently developed in our laboratory.

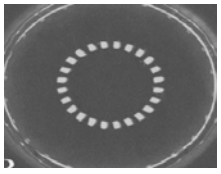


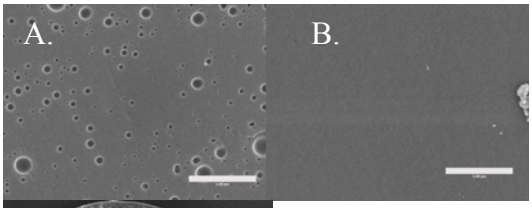
Figure 1: Time lapse illustration of buoyant scaffold motion in a bioreactor

Methods: PPhos were synthesized as reported earlier.[2] Heavier than water (HTW) poly[bis(ethyl alaninat-N-yl)phosphazene] (PNEA) microspheres were fabricated by dissolution in methylene chloride (MC) at 11% (wt/vol). The dissolved PNEA was then poured into a 1% (wt/vol) solution of polyvinyl alcohol (PVA) and water being stirred at 360rpm and maintained at 0^{oC}. After 24 hours the PNEA microspheres were removed and sieved into appropriate size ranges. Lighter than water (LTW) PNEA microspheres were obtained by creating a 9% (wt/vol) PNEA/MC solution and then adding 10% (vol/vol) water (W) and 10% (vol/vol) hexane (H). The resultant triple emulsion is vortexed and poured into a stirred solution of 1% (wt/vol) PVA maintained at room temperature. HTW poly[bis(ethyl phenylalaninat-N-yl)phosphazene] (PNEPhA) microspheres were made similarly using a 23% wt/vol solution of PNEPhA/MC poured into 0^{oC} PVA, LTW PNEPhA spheres were fabricated with a 23% wt/vol solution with 10% vol/vol H and W added and poured into room temperature PVA. Microsphere density was characterized using liquid displacement in H. PNEA and PNEPhA microspheres were sintered with an acetone (A) and H solution at varying concentrations using the solvent/non-solvent slurry sintering technique.[2]

Results/Discussion: Figure 2. A.&B. illustrate the surface of an HTW and LTW PNEPhA microsphere respectively, whereas Figure 2. C. illustrates the cross-section of the LTW PNEPhA sphere. The low density of the LTW micropsheres can be attributed to the highly porous structure of the microspheres (Figure 1.C.). Additionally the LTW sphere is fairly dense throughout the interior, which could be beneficial mechanically over the previously used poly(lactide-co-glycolide) hollow microspheres. The closed porosity has been verified by stirring the hollow microspheres in DI water for 24 hours, after which time there was no change in bouyancy. The feasibility of developing three dimensional bouyant porous structures having the target density of target 0.85 g/ml by the solvent sintering method is demonstrated (Figure 2) (Table 1).

Table 1: Microsphere density and mixing ratio

Polymer	LTW ρ g/ml	HTW ρ g/ml	LTW:HTW
PNEA	0.3±0.01	1.1±0.11	0.31:0.69
PNEPhA	0.74±0.03	1.25±0.06	0.78:0.22



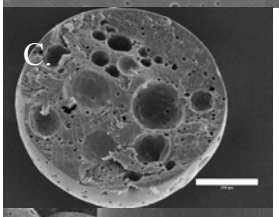


Figure 2: A. PNEPhA hollow sphere surface, B. PNEPhA solid sphere surface, and C. PNEPhA hollow sphere interior.

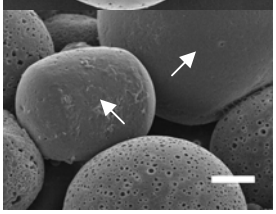


Figure 3: PNEA hollow/solid microsphere scaffold at 150x. White arrows represent solid microspheres.

Conclusions: Our study has demonstrated the feasibility of developing buoyant scaffolds from biodegradable polyphosphazenes by mixing LTW and HTW microspheres. Microsphere scaffolds were found to have a density of 0.85 g/ml. This optimized density will allow the scaffold to rotate in a bioreactor with a steady orbit at physiological shear as demonstrated previously.[1]

References:

1. Laurencin CT. J Biomed Mater Res A. 2004 1;69(2):205-15.
2. Laurencin CT. US patent pending (2006).
3. Laurencin CT. J Biomed Mater Res A. 2006 Jan;76(1):206-13.