Vascular Endothelial Growth Factor Release from Amino Acid Ester Polyphosphazene Scaffolds

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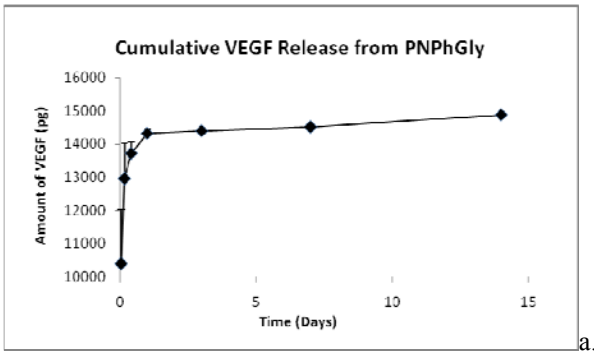
Statement of Purpose: In less than 20 years, the population of individuals aged 65 and over is projected to double [1], translating to an increased need for more efficient treatment of fall-related fractures. Additionally, statistics reveal that over 300,000 bone graft operations, with estimated costs of ~$2.5billion/year, were performed in 1998 in the United States [2,3]. Though several strategies exists for treatment of bone related injuries, commonly employed bone tissue substitutes often lack the potential for promoting and sustaining vascularization, which is needed to enhance oxygen and nutrient transport as well as provide a route for stem cells and osteogenic precursor cells to the site of damage. For this reason, our objective was to fabricate, using a novel sintering method for protein incorporation, and characterize vascular endothelial growth factor (VEGF)-loaded amino acid ester polyphosphazene (Pphos)-based scaffolds and their subsequent growth factor release kinetics [4]. We hypothesize that poly(ethyl phenyalanato-glycinato)phosphazene (PNPhGly) and composite poly(ethyl phenyalanato-glycinato)phosphazene-nanocrystalline hydroxyapatite (PNPhGly-HAp) microsphere scaffolds can be loaded with vascular endothelial growth factor (VEGF) using the solvent sintering technique and that this technique will allow for achieving sustained growth factor release.

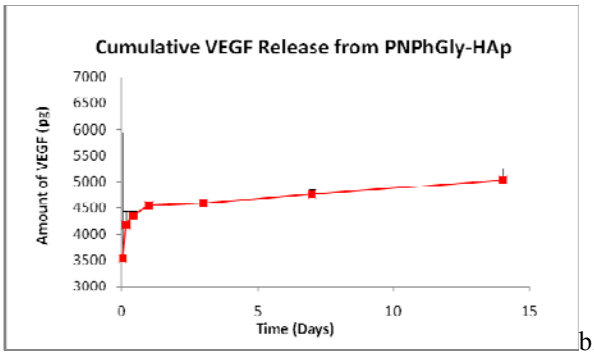
Methods: PNPhGly and composite PNPhGly-HAp microspheres were formed using the emulsion solvent evaporation method. Simultaneous VEGF loading and scaffold fabrication was performed using a solvent sintering solution consisting of 25% tetrahydrofuran (THF), 75% hexanes, in addition to VEGF in 10% bovine serum albumin. VEGF release from the respective scaffolds (n=4) was evaluated in a temperature controlled environment, 37^{o}C with 100RPM continuous agitation, and analyzed at the final time point using an enzyme-linked immunosorbant assay system (ELISA).

Results: The resulting VEGF-loaded microsphere based scaffolds were found to release VEGF over a 14 day time period. Release kinetics revealed an initial burst release followed by a sustained zero order release profile from day 3 to day 14. It is expected that the differences in VEGF release from the composite matrices compared to release from the pure PNPhGly matrices was due to the presence of HAp, which served to alter both the composite system pka as well as the release media pH.

Conclusions: Moreover, the results of this work provide great support for the use of the solvent/nonsolvent technique for VEGF loading into amino acid ester polyphosphazene microsphere matrices. It is apparent that factors such as the polymer pka, drug isolelectric point, and solubility would affect release parameters. Ultimately, future investigations will involve optimizing the system to release physiological levels of VEGF, levels needed to induce neovascularization events *in vitro* and *in vivo*.



a.



b.

Figure 1. (a) VEGF release from PNPhGly microsphere matrices, (b) VEGF release from composite PNPhGly-HAp microsphere matrices, n=4.

Sample	pKa
PNPhGly	9.285
PNPhGly-HAp	8.745

Table 1. Polymer acid dissociation constant measures.

References:

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