Protein Loading on Porous Hydroxyapatite/Poly(lactide-co-glycolide) Scaffolds for Bone Tissue Engineering

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Statement of Purpose: Tissue engineering offers an attractive alternative to traditional autografts and allografts, which are limited by supply deficiencies and disease transmission threats, respectively. A key element of bone graft substitute design is the method of loading growth factors onto the scaffold, as it largely dictates the mode of protein release. Carefully designed bone graft substitutes may be engineered to deliver growth factors in a controlled way, creating a chemical environment that induces osteogenesis and enhances healing. Previous work in our lab led to the development of a porous hydroxyapatite/poly(lactide-*co*-glycolide) (HA/PLAGA) scaffold for bone tissue engineering that has been shown to support osteoblasts *in vitro* [1] and mineralized tissue formation *in vivo* (unpublished data). In the current study, we have designed and characterized a procedure for loading protein onto the HA/PLAGA scaffolds.

Methods: HA/PLAGA microspheres were fabricated using a solution evaporization/*in situ* HA precipitation technique previously developed . Cylindrical scaffolds, 5mm X 5mm, were constructed by sintering microspheres [2] of diameters ranging from 350-600 µm. Scaffolds with two different HA/PLAGA compositions were studied, HIGH (83% PLAGA) and LOW (73% PLAGA), according to their polymer/ceramic ratio. Control scaffolds of pure polymer, called PLAGA, were constructed in the same way as the composite scaffolds, except for the omission of aqueous HA precursor solutions. Protein was loaded by soaking scaffolds in solutions of cytochrome c (Sigma-Aldrich, St. Louis, MO) in 5 mM glutamate buffer, pH 4.5 [3] for 24 hrs. Loaded protein not bound to the scaffold (FREE) was quantified using a series of centrifugation and weighing steps to calculate the volume of loading solution that occupied the pores of the scaffolds. Protein adsorbed to the scaffold (BOUND) was quantified via desorption with 0.5 M arginine buffer, pH 7.5 [2]. Protein in the loading and desorption buffers was measured using reverse phase high performance liquid chromatography (1100 series, Agilent Technologies, Wilmington, DE). A sample size of six was used for all loading experiments, and 2-way ANOVA with Tukey test was used for statistical analysis.

Results/Discussion: Results of the loading experiments, shown in Figure 1 for FREE (a) and BOUND (b) protein, illustrate an increased capacity of the composite scaffolds for protein loading, relative to those that are purely polymeric. Greater overall protein incorporation should allow the HA/PLAGA scaffolds to deliver more growth factor to local cells and expedite tissue regeneration. FREE protein, which is expected to be released quickly, was loaded in greater quantities on the composite scaffolds (Figure 1(a)) as a result of their increased porosity. LOW and HIGH scaffolds had greater void spaces than PLAGA scaffolds to accommodate larger volumes of protein solution, due to the porous surfaces and less uniform packing of the composite microspheres. BOUND protein, which is expected to be released upon scaffold degradation, was also greatest in the composite scaffolds. HIGH and LOW scaffolds had larger surface areas for protein-scaffold interaction than PLAGA scaffolds. Furthermore, the hydrophilicity introduced by the HA supplied more protein adsorption sites on the composites and facilitated wetting of the hydrophobic polymer by the aqueous loading solution. Finally, it is important to note that the composite scaffolds had an increased ratio of BOUND/FREE protein. Greater equity in the amounts of BOUND and FREE protein may give the composite scaffolds more steady release profiles. Additionally, if BOUND protein remains adsorbed to the scaffold after implantation, its gradual release may be controlled by engineering the scaffold degradation.

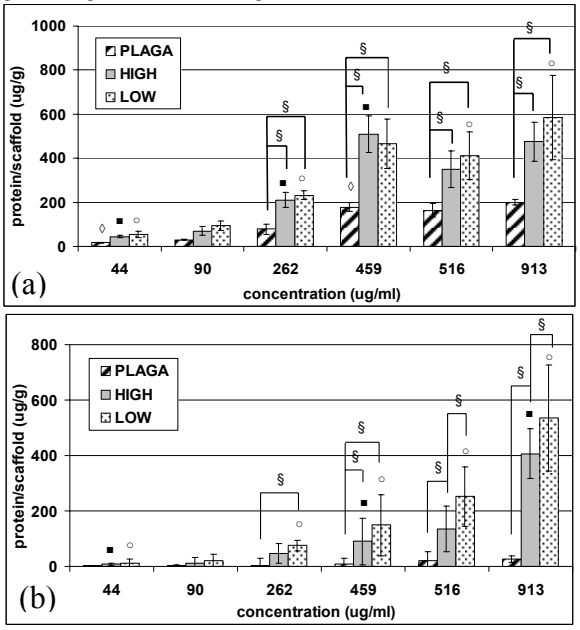

Figure 1. FREE (a) and BOUND (b) protein per scaffold weight (µg/g) versus loading solution concentration (µg/ml. Significance between scaffold types is shown with §, while significance between concentrations is shown with ◊ for PLAGA, ■ for HIGH, and ○ for LOW ($p<0.05$).

Conclusions: HA/PLAGA scaffolds were shown to load significantly more protein than purely polymeric scaffolds. The proportion of BOUND verses FREE protein was also greater with the composites, which may lend more control over osteogenic factor release. Additional studies are planned to investigate the *in vitro* release of protein from the HA/PLAGA scaffolds, as well as their effect on mesenchymal stem cell differentiation.

References: [1] Khan, J Bone Med 2004;728-737. [2] Khan, in Nano Mat Sci in Biol Med; Proc Mat Res Soc. 2005;63-68. [3] Duggirala, Phar Dev Tech 1996;11-19.