Nitrilotriacetic Acid-Nickel Affinity Protein Delivery via Polyketal Microparticles

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Statement of Purpose: Microparticles have widely been used as drug delivery devices for the sustained delivery of numerous therapeutics. Incorporating hydrophilic therapeutics, such as proteins and nucleic acids, has typically been accomplished by double emulsion techniques. While it is possible to encapsulate hydrophilic compounds this way, loading efficiencies are generally low. By adapting immobilized metal affinity chromatography (IMAC) techniques to microparticles, we are investigating using IMAC chemistries for the protein delivery and microparticle targeting. In this report, we characterize the binding capacity and release kinetics of His_6-green fluorescent protein (His-GFP) to polyketal particles loaded with nickel-nitrilotriacetic acid (Ni-NTA) on the surface.

Methods: Poly(cyclohexane-1,4-diyl acetone dimethylene ketone) (PCADK) was synthesized as previously described (1). Briefly, polymer was synthesized via the acetal exchange reaction between 1,4-cyclohexane dimethanol and 2,2-dimethoxy propane. NTA-functionalized microparticles were fabricated by the emulsion, solvent evaporation method. PCADK and 1,2-Dioleoyl-*sn*-glycero-3-{[N(5-amino-1-carbopentyl)iminodiacetic acid] succinyl} (DOGS-NTA, Avanti Polar Lipids) were dissolved in desired ratios (0, 1, 10 wt%) in methylene chloride. The polymer solution was homogenized in a PVA solution. The resulting emulsion was stirred for 4 h to allow the solvent to evaporate. Particles were then washed, frozen in liquid nitrogen, and lyophilized. Particles were loaded with Ni^{+2} by suspending the particles in 500 mM $NiCl_2$ solution for at least 2 h. Particles were then washed to remove unbound nickel. His_6-GFP (Millipore) was dissolved in PBS and incubated with the nickel-NTA loaded particles at 4°C overnight. Specifically bound GFP was both visualized with fluorescent microscopy and quantified using ELISA.

Results: In order to verify particle morphology, samples were mounted and sputtered coated with Au for examination under SEM. Analysis showed that particle size was polydisperse with a mean diameter measuring 8.2 ± 3.8 μm.

NTA (10%) particles were loaded with nickel then GFP and compared with identical particles that were suspended in PBS instead of $NiCl_2$ solution. Examination under fluorescence microscopy showed intense GFP fluorescence with the nickel loaded particles compared to the weak background fluorescence of the PBS loaded particles.

In order to quantify GFP binding to the particles, ELISA was run at several different loading concentrations (Figure 1). Particles containing 10% NTA appeared to saturate at 120 ng GFP/mg particle, while 1% NTA particle saturated at 80 ng GFP/mg particle. Particles specifically bound 40% of the protein at these concentrations.

In Vitro release studies were conducted in serum and serum-free conditions at 37°C using 10% NTA particles. Approximately 20 ng of GFP, or 50% of the total loaded, was released from the particles surface within 24 h in 10% fetal bovine serum conditions, while serum free conditions released only 5 ng over the same period of time. Treament with the Ni-NTA competitor imidazole (500 mM) lead to the rapid dissociation of protein, with 50% of the protein dissociating in less than 10 minutes.

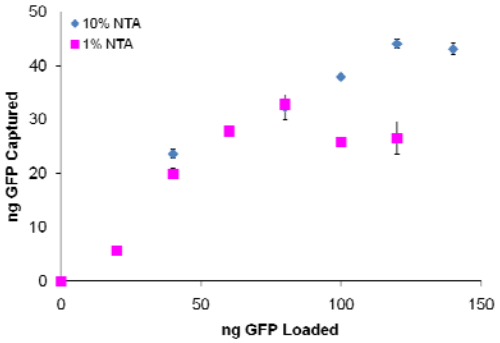

Figure 1. GFP Loading curve.

Conclusions: We demonstrate successful surface functionalization of microparticles with the NTA-Ni complex, using His-GFP as a model protein. IMAC chemistries for immobilization of proteins on the surface of microparticles may improve protein loading, as well as allow for specific binding of targeting proteins/peptides. Here we demonstrate that up to 40% of protein from dilute solutions can be specifically bound to the surface of polyketal particles. We are investigating possibilities for increasing this loading efficiency by applying this technique to double emulsion techniques, creating pockets of surface immobilized proteins.

The rapid release of proteins from the surface of our degradable polymer particle, along with the tunable hydrolysis rates of polyketal particles, makes this novel strategy ideal for the delivery of two substances with different time courses. Because many proteins are already expressed with a His_6-tag for purification, we are currently exploring the use of this technology for the treatment of heart failure following myocardial infarction by the dual delivery of pro-survival factors in the near term, coupled with the long term delivery of hydrophobic compounds that suppress inflammation.

References: (1) S Lee, et al. *Bioconj. Chem.* 18(1):4-7 (2007). (2) C-C Lin, AT Metters. *JBMR Part A* 83(4):954-64 (2007). (3) S Lauer, JP Nolan. *Cytometry* 48:136-145 (2002). (4) JD Patel, et al. *Pharm Res* 24(2):343-352 (2006).