Site-directed Conjugation of Bioactive Molecules to Poly(lactic-co-glycolic) Acid Nanoparticles

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Statement of Purpose: Display of bioactive molecules, such as antibodies, on the surface of polymeric nanoparticles has applications in drug delivery and imaging. However, maintaining bioactivity and consistent, proper orientation of such molecules is not altogether trivial. Passive adsorption of bioactive species, while simple, can lead to an improper orientation of active groups and therefore concomitant reduced functionality. On the other hand, most covalent conjugation techniques are non-specific (e.g., via reaction with primary amines or carboxylates on the biomolecule of interest) and may lead to improper orientation or inactivation of bioactive moieties. To address the issue of orientation for preservation of bioactivity, we have recently applied a site-specific conjugation strategy to couple bioactive molecules to the surface of poly(lactic-co-glycolic) acid (PLGA) particles. This platform technology provides consistent biomolecule orientation and the possibility to site-specifically conjugate a wide-range of bioactive molecules. In this work, we describe the conjugation of IgG1 antibody Fab fragments to PLGA nanoparticles as a proof-of-principle.

Methods: PLGA polymer chains (75:25, MW 20k, Polysciences, Warrington, PA) were functionalized with alkoxyamine end groups (-ONH_2) using standard transformations. Modifications to PLGA polymer end groups were confirmed using nuclear magnetic resonance spectroscopy (NMR). PLGA-ONH_2 nanoparticles were prepared as previously described using oil-in-water emulsion[1]. Particle size and polydispersity were measured using dynamic light scattering on a Zetasizer 3000 (Malvern, Westborough, MA). Mouse IgG1 (R&D Systems, Minneapolis, MN) Fab fragments were generated using a Pierce Mouse IgG1 Fab and F(ab´)2 Micro Preparation Kit (Thermo Fisher Scientific, Rockford, IL). Generation of Fab fragments was confirmed by running digestion fractions on 12% Ready Gel Tris-HCl Gels (Bio-Rad, Hercules, CA) and staining with Biosafe Coomassie (Bio-Rad, Hercules, CA). Fab fragments were activated overnight by treatment with PLP and subsequently conjugated to PLGA-ONH_2 particles. Fluorescently-labeled antigens can be used to quantify the active antibody fragments at the PLGA surface.

Results: Dynamic Light Scattering readings of PLGA nanoparticles revealed an average diameter of 500 nm. (Figure 1). Coomassie staining of stock mouse IgG1 and Fab fragments purified from antibody digestion on reducing 12% SDS-PAGE gels revealed characteristic bands at 25 kD and 50 kD for IgG light and heavy chains, respectively, and prominent band at 25 kD representing purified Fab fragments (Figure 2). Purified Fab fragments activated overnight with PLP and subsequently conjugated to PLGA-ONH_2 nanoparticles are consistently oriented on the surface of the nanoparticle as envisioned in schematics in Figure 3.

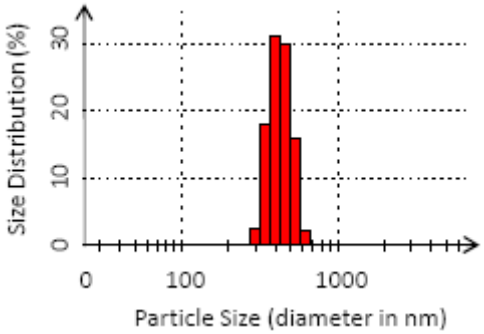

Figure 1. Dynamic Light Scattering Data. Average PLGA-ONH_2 nanoparticle size is 500 nm.

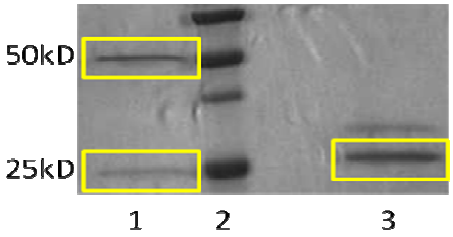

Figure 2. SDS-PAGE analysis of stock mouse IgG1 (lane 1) and Fab fragments purified from Pierce Mouse IgG1 Fab and F(ab´)2 Micro Preparation Kit. Upon Biosafe Coomassie staining, characteristic bands appear at 25 kD and 50 kD for IgG light and heavy chains, respectively, and a prominent band at 25 kD appears representing purified Fab fragments.

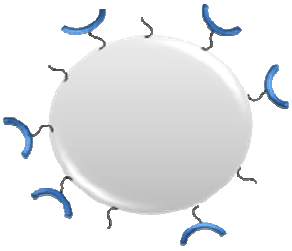

Figure 3. Diagram of Consistently-Oriented Biomolecules on PLGA Particle Surface

Conclusions: We have demonstrated a novel site-specific conjugation strategy for the display of bioactive molecules at the surface of degradable PLGA nanoparticles. This platform technology can be used to selectively conjugate a wide variety of bioactive molecules and provides a more consistent biomolecule presentation than adsorption or non-specific conjugation techniques. This method can be applied to increase the efficiency of existing imaging or targeting strategies using nanoparticles.

References: 1. Paramonov, SE. Bioconjugate Chem. 2008;19:911–919.