Fabrication of intelligent, protein-recognitive polymers on the surface of biodegradable nanoparticles

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Statement of Purpose: Molecularly imprinted polymers (MIPs) are polymers synthesized in such a way that they can selectively recognize and bind certain target molecules, similar to how an antibody would recognize its antigen. This recognitive property is achieved by incubating the target molecule, called the template, with a set of monomers that have functional groups complementary to those on the surface of the template, causing non-covalent interactions to form. The earliest imprinted polymers were for small molecule targets, but now many are being developed for larger biomolecules such as proteins. However, due to their large size, it is hard for proteins to diffuse through the pores and reach their binding sites in the bulk of the network. To overcome this diffusion limitation, the binding sites can be localized at the surface of a substrate. Nanomaterials are popular substrates for surface imprinting because of their high surface area to volume ratio and unique properties. This work focuses on the development of surface-imprinted, biodegradable polymer nanoparticles that can selectively detect lysozyme, an enzyme of the innate immune system that is overexpressed in a number of diseases including leukemia, meningitis, and renal diseases. The ability to encapsulate imaging agents and therapeutics in the nanoparticle cores makes them attractive for theranostic applications.

Methods: *Nanoparticle fabrication.* Poly(ε-caprolactone) (PCL) nanoparticles were synthesized using the nanoprecipitation technique. An amphiphilic graft copolymer, poly(maleic anhydride-*alt*-1-octadecene)-g-poly(ethylene glycol) methacrylate (PMAO-g-PEGMA), was synthesized and used to both stabilize PCL nanoparticles in aqueous solution and introduce polymerizable double bonds to the particle surfaces. The chemical identity of PMAO-g-PEGMA was confirmed by ^{1}H-NMR. Particle size was assessed with dynamic light scattering and transmission electron microscopy. Nile Red was encapsulated in the nanoparticles for imaging. *Molecular imprinting.* Methacrylic acid (MAA), acrylamide (AAm), and 2-(dimethylamino)ethyl methacrylate (DMAEMA) were used as the functional monomers with N,N'-methylenebisacrylamide (MBA) as the crosslinker. Monomers were mixed with the PCL nanoparticles in Tris-HCl (pH 7.6). Lysozyme was added for the MIP nanoparticles (MIPNPs) but was not included for non-imprinted nanoparticles (NIPNPs). After nitrogen purging, the lysozyme, monomers, and particles were allowed to pre-assemble for an hour before initiating polymerization with APS/TEMED. After 24 hours, particles were collected via centrifugation and washed extensively with 9:1 methanol:acetic acid solution to remove bound protein from the MIPNPs. *Rebinding studies*. Batch rebinding studies were performed to determine binding capacity. A known mass of particles was incubated with varying concentrations of lysozyme. After 60 minutes, particles with bound lysozyme were collected by centrifugation and the concentration of protein left in solution was determined from absorbance measurements at 280 nm. The amount of lysozyme bound to the particles was calculated, plotted as a function of equilibrium concentration, and fit to the Langmuir and Freundlich isotherms. To demonstrate specificity for lysozyme, binding capacities for lysozyme, cytochrome C, and bovine serum albumin were compared.

Results: PCL nanoparticles stabilized with PMAO-g-PEGMA had an average radius of 65 nm and a highly negative zeta potential (-68 mV) due to the pendant carboxyl groups present on the PMAO-g-PEGMA backbone. This is favorable for localizing the positively charged lysozyme (pI ~11) at the surface of the particles. After imprinting, the radius of the particles varied with pH, as was expected due to the presence of ionizable pendant groups of the imprinted polymer (MAA and DMAEMA). It was shown that MIPNPs had a binding capacity that was 165% higher than the NIPNP capacity. Furthermore, the binding was specific for lysozyme. ***Figure 1*** shows the binding capacities of the MIPNPs and NIPNPs for lysozyme, cytochrome C, and BSA. Cytochrome C is a structural analog for lysozyme, having a similar molecular weight and isoelectric point and BSA is known for non-specific adsoprtion. As expected, MIPNP binding capacity was highest for lysozyme, followed by cytochrome C, with low adsorption of BSA.

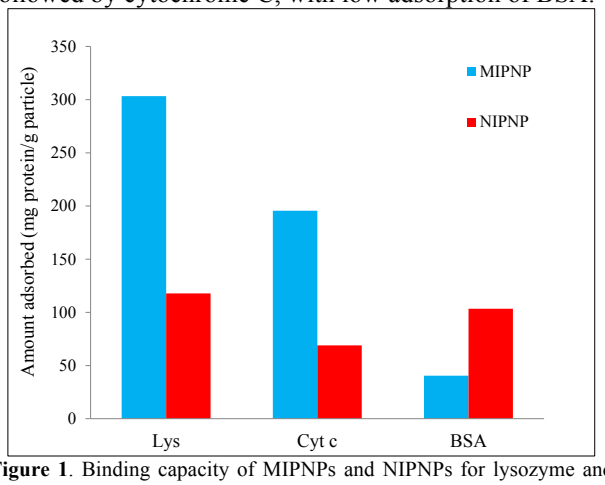

Figure 1. Binding capacity of MIPNPs and NIPNPs for lysozyme and two competitor proteins, cytochrome C and bovine serum albumin.

Conclusions: PCL nanoparticles were successfully imprinted for recognition of lysozyme. Quantifying MIPNP-lysozyme binding *in vitro* using fluorescence techniques, including flow cytometry and confocal microscopy, will demonstrate the applicability of MIPNPs as a diagnostic platform. In addition, the ease of loading therapeutic molecules into the biodegradable cores makes these nanoparticles excellent candidates for theranostics.

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