Molecularly Imprinted Polymers on Surface-Modified Gold Nanoparticles for Biosensing Applications

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Statement of Purpose: The use of chemical sensors and biosensors has become increasingly prevalent as new demands and opportunities for their use have appeared in various areas such as clinical diagnostics, food analysis and detection of chemical warfare agents. The two key components necessary for biosensors are molecular recognition and signal transduction. To satisfy both of these requirements, molecular imprinting can be performed on gold nanoparticle cores (AuNPs). Molecularly imprinted polymers (MIPs) are crosslinked networks of polymers formed to recognize a chosen template molecule. The polymerization of the monomers to create the polymer occurs in the presence of the template molecule, which is later extracted. This extraction leaves behind three-dimensional cavities complementary to the functional groups of the template, thereby yielding a polymer with affinity for the template. This affinity created by molecular imprinting allows for specific recognition of the target analyte. Furthermore, synthesizing MIP shells on AuNPs allows us to take advantage of localized surface plasmon resonance (LSPR) spectroscopy as a method of signal transduction. Since LSPR is sensitive to the AuNPs' local dielectric environment, the addition and binding of the analyte to imprinted AuNPs causes a shift in the LSPR wavelength. Thus, through the use of imprinted AuNPs, we can achieve both the key characteristics necessary to design a biosensor for diagnostic applications.

Methods: *Core encapsulation*. Poly(ethylene glycol) methacrylate (PEGMA) was first grafted onto poly(maleic anhydride-*alt*-1-octadecene) (PMAO) via ring opening of the maleic anhydride. Citrate ions stabilizing AuNPs were displaced with 1-dodecanethiol and solvent displacement was then used to encapsulate the hydrophobically modified AuNPs in PMAO-g-PEGMA. This adds stability to the particles and provides vinyl groups for anchoring the MIP to the surface. Stability studies were conducted on the citrate capped AuNPs and the PMAO-g-PEGMA encapsulated AuNPs. Particle sizes before and after encapsulation were determined by transmission electron microscopy. *Molecular imprinting*. Acrylamide, methacrylic acid and 2-(dimethylamino)ethyl methacrylate were used as the functional monomers for molecular imprinting, and N,N'-methylenebisacrylamide was used as the crosslinker. These functional monomers provide neutral, negative and positive moieties, respectively, to compliment the residues on lysozyme, the template molecule. The monomers were then added to the AuNPs in Tris buffer. Lysozyme was added to the pre-polymer mix to make molecularly imprinted AuNPs (AuNP@MIP) but was not included in the synthesis of non-imprinted AuNPs (AuNP@NIP), which acted as the control. Following nitrogen purging, the monomers and template were allowed to pre-assemble for 30 minutes. Free radical polymerization was then initiated using APS/TEMED. After 22 hours, the particles were centrifuged and washed with acetic acid 10% (v/v) aqueous solution to remove lysozyme from the particles. *Rebinding studies*. Batch rebinding studies were performed to determine the binding capacity of the AuNP@MIPs. Known concentrations of AuNP@MIP and AuNP@NIP were combined with varying concentrations of lysozyme. After 1 hour, the absorbance spectra (450 – 800 nm) of the particles were measured to determine if a shift in the LSPR peak occurred.

Results: Stability studies were performed on citrate capped AuNPs and PMAO-g-PEGMA AuNPs in water and in various concentrations of phosphate buffered saline (PBS). The disappearance of the LSPR peak for citrate capped AuNPs in 1x and 10xPBS suggest decreased stability in higher ionic strength solutions. The PMAO-g-PEGMA encapsulated AuNPs showed a wider LSPR peak at higher salt concentrations but no precipitation was observed. This effect is likely due to the stabilizing effects of the PMAO-g-PEGMA on the AuNPs. Furthermore, the average size of the AuNPs did not change following the PMAO-g-PEGMA encapsulation. Absorbance spectra were also measured throughout the fabrication of the AuNP@MIPs. The LSPR peak occurred at 519 nm for citrate capped AuNPs, at 524 nm for PMAO-g-PEGMA AuNPs and at 533 nm for AuNPs@MIP with lysozyme, demonstrating a red-shift in response to changes in the surface dielectric (*Figure 1*).

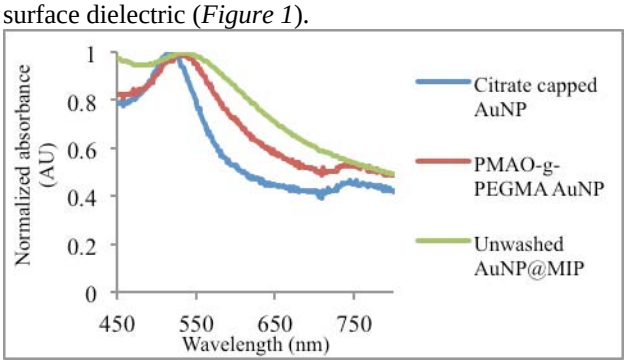

Figure 1. Absorbance spectra for AuNPs in 0.1x PBS.

Conclusions: AuNPs underwent successful surface modification with PMAO-g-PEGMA as a method to introduce vinyl groups for surface imprinting. These particles were then successfully imprinted for recognition of lysozyme and demonstrated an LSPR shift in the presence of lysozyme, thereby demonstrating a promising future as in the field of biosensors.

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