Fabrication of Hydrogel-Coated Gold Nanoshells as a Biosensor for Protein Biomarker Quantification

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Purpose: There is a growing interest in the use of protein biomarkers in saliva, tears, urine, or serum for minimally-invasive disease screening. However, in many cases, there is a lack of specific biomarkers for the target disease, necessitating the detection of multiple biomarkers in order to make a confident diagnosis. Differential sensor arrays can help address this challenge by exploiting semi-selective recognitive elements in order to screen for differences in multiple protein biomarkers in a single assay. Previous work from our group has developed a localized surface plasmon resonance (LSPR) biosensor based on gold nanoshells (AuNS) that uses hydrogel coatings on the surface to control protein affinity (Culver HR. ACS Nano 2018; 12, 9342−9354). However, the use of the precipitation polymerization of N-isopropylacrylamide (NIPAM) results in thick hydrogel coatings of hundreds of nanometers that can limit diffusion close to the nanoshell surface where the LSPR response is strongest. In this work we explore the use of inverse emulsion polymerization to form multifunctional hydrogel networks on the surface of gold nanoshells (AuNS) with both NIPAM and acrylamide (Am) to achieve thinner coatings that will enable more sensitive LSPR responses.

Methods: Hydrogel nanoparticles were formed by an inverse emulsion copolymerization of methacrylic acid (MAA) with Am or NIPAM, crosslinked with N,N'-methylenebis(acrylamide) in either a 30 mL or 50 mL reaction volume. The resulting nanoparticles were purified by dialysis and characterized by dynamic light scattering (DLS), potentiometric titration, and Fourier-transform infrared spectroscopy (FTIR). AuNS were prepared using the method of seeded growth of gold colloids on aminated silica nanoparticles followed by encapsulation with poly(maleic anhydride-alt-1-octadecene)-g-poly(ethylene glycol) methacrylate (PMAO-g-PEGMA) graft copolymer to improve particle stability. Hydrogel-coated AuNS were prepared using the same nanoparticle synthesis method with the addition of PMAO-g-PEGMA AuNS to the aqueous phase of the inverse emulsion. UV-Vis spectroscopy was used to measure the shifts in the wavelength of the localized surface plasmon resonance for the hydrogel-coated AuNS.

Results: Nanoparticles were first prepared without the AuNS and their composition and properties were analyzed for two different batch sizes. From FTIR results, the nanoparticles showed successful incorporation of both comonomers for each of the formulations, and no significant difference was detected between the different reaction volumes. From potentiometric titration studies, the pAm-co-MAA nanoparticles were found to have a higher acid content than pNIPAM-co-MAA nanoparticles; however, the acid content of the different reaction volumes was comparable as shown in Figure 1. From DLS analysis, all formulations were confirmed to be nanoscale and

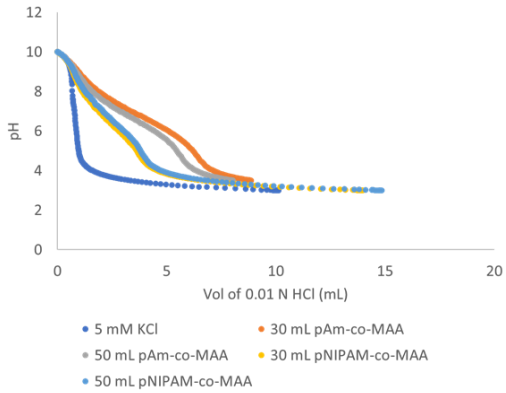

Figure 1: Potentiometric Titration Analysis of Polymer Nanoparticles

monodisperse with the pNIPAM-co-MAA formulations being larger than the pAm-co-MAA formulations as shown in Table 1. The zeta potentials were comparable for all formulations. Repeating the synthesis with AuNS in the aqueous phase of the inverse emulsion of pAm-co-MAA, DLS confirmed a hydrogel thickness of approximately 50 nm and total diameter of 296.6 ± 83.3 nm for the coated nanoshells; however, the resulting hydrogel-coated AuNS were found to be polydisperse (PDI = 0.25). UV-Vis spectroscopy confirmed the hydrogel-coated AuNS retained their absorbance profile following the synthesis.

Polymer Formulation	Hydrodynamic Diameter (nm)	Polydispersity Index	Zeta Potential (mV)
pAm-co-MAA 30 mL	51.7 ± 6.0	0.10 ± 0.02	-22.5 ± 2.5
pAm-co-MAA 50 mL	74.1 ± 20.7	0.06 ± 0.01	-28.7 ± 4.6
pNIPAM-co-MAA 30 mL	119.9 ± 10.7	0.16 ± 0.01	-23.7 ± 1.9
pNIPAM-co-MAA 50 mL	116.4 ± 11.3	0.15 ± 0.02	-23.2 ± 1.8

Table 1: Dynamic Light Scattering Analysis of Polymer Nanoparticles

Conclusions: Hydrogel-coated AuNS were successfully fabricated with thinner hydrogel coatings than previous work on the precipitation polymerization of NIPAM as well as allowing for the fabrication of coated nanoshells with Am which is not temperature responsive. The resulting hydrogel-coated AuNS retain their localized surface plasmon resonance, enabling their use as a sensitive biosensor for protein biomarkers.

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