Stimuli-Responsive "Smart" Magnetic Nanoparticles for Point-of-Care Diagnostics

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Statement of Purpose: Point-of-care (POC) immunoassays (*i.e.*, lateral-flow tests) are rapid and cost-effective analytical tests, which can be performed outside of centralized laboratories with untrained users and minimum instrumentation. POC immunoassays exhibit significant potential for infectious disease diagnostics but have not yet been fully utilized because of the poor limit of detection (LOD). In order to improve the utility of these tests, there is a critical need for technologies to improve the LOD of POC immunoassays toward the range of diagnostics used in hospital laboratories[1]. To address this issue, we have developed a stimuli-responsive, "smart" polymer-coated magnetic nanoparticle (mNP) system[2,3] to provide one-step target isolation. The system retains the advantages of nanoparticles such as high surface to volume ratio and fast diffusive properties and also allows rapid and efficient magnetic separation by thermally aggregating the mNPs to achieve the high magnetophoretic mobility of larger mNPs. We have demonstrated the temperature-responsive mNP[2] capture/release and the separation of a model protein target using pH-responsive mNPs[3] in microfluidic devices. Recently gold-antibody conjugates, providing detection signal, were incorporated to construct a smart mNP malaria immunoassay.

Methods: Poly(*N*-isopropylacrylamide) (pNIPAAm) chains were synthesized by mixing appropriate amount of NIPAAm (monomer), DMP (trithiocarbonate-based CTA), and 4,4'-azobis(4-cyanovaleric acid) (initiator) in a flask charged with methanol (solvent). After being purged with nitrogen for 20 min, this solution was sealed and maintained at 60°C overnight. The polymer was purified by dissolving/precipitating in THF/pentane and then dried overnight in vacuo. pNIPAAm mNPs were synthesized by dissolving the pNIPAAm (surfactant) in tetraglyme, preheated to 100°C, at 3.6mM. After the $Fe(CO)_5$ addition, the solution was maintained at 190°C for 5 hours. The resulting mNPs were collected by precipitating in n-hexane and dried in vacuo. pNIPAAm mNPs were biotinylated via the carboxyl end group using carbodiimide chemistry with DCC/NHS in dioxane.

Results: Telechelic pNIPAAm polymer chains were synthesized with dodecyl tails at one end and a reactive carboxylate at the opposite end by the reversible addition fragmentation transfer (RAFT) technique. The dodecyl tails self-assemble to form nanoscale micelles with the $Fe(CO)_5$ concentrating in the hydrophobic core, where it is converted to γ-Fe_2O_3. The resulting superparamagnetic nanoparticles exhibit a γ-Fe_2O_3 core (~ 5nm diameter) with a layer of carboxylate-terminated pNIPAAm chains as a corona on the surface. The carboxylate was used to functionalize the mNPs with biotin and subsequently with streptavidin. The functionalized mNPs can be reversibly aggregated in solution as the temperature is cycled through the pNIPAAm lower critical solution temperature (LCST). While the magnetophoretic mobility of the individual nanoparticles below the LCST is negligible, the aggregates formed above the LCST are large enough to respond to an applied magnetic field.

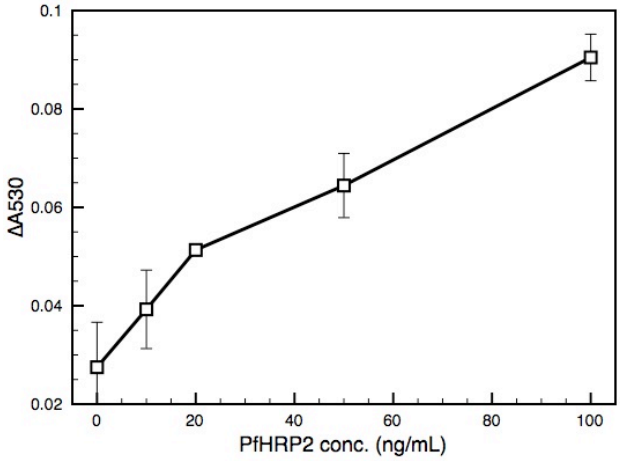

Figure 1. Response of smart mNP *Pf*HRP2 assay increases when the samples' PfHRP2 concentrations increase. The assay can detect 10 ng/mL PfHRP2.

The smart mNP system for biomolecule isolation was evaluated using *Plasmodium falciparum* histidine rich protein 2 (*Pf*HRP2), a malaria antigen, as the model target. The assay utilizes temperature-responsive anti-*Pf*HRP2 pNIPAAm mNP and anti-*Pf*HRP2 gold conjugate (detection). *Pf*HRP2 (target), forming sandwich full stacks with mNP conjugates and gold conjugates, are magnetically separated via pNIPAAm mNP aggregation at the temperature above LCST. The assay either measures gold precipitates generated by captured gold conjugates (direct) or free/unbound gold conjugates (indirect) to determine the concentration of *Pf*HRP2 in the spiked human plasma samples. The preliminary data show response of indirect smart mNP *Pf*HRP2 assay increases when the samples' PfHRP2 concentrations increase. Both direct and indirect methods can detect 10 ng/mL *Pf*HRP2, which is comparable to the detection limit of the clinically-utilized ELISA.

Conclusions: These results indicate that smart mNP technology may enable inexpensive and rapid POC immunoassays to achieve tests with hospital laboratory quality. Importantly this system is a platform technology and can be integrated into small portable diagnostic devices to perform highly sensitive immunoassays for infectious diseases (*i.e.*, HIV, malaria) in low resource settings (*e.g.*, developing countries).

References:

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