Integrated Labeling, Enrichment, and Detection of Protein Analytes from Human Plasma by "Smart" Magnetic/Gold Nanoparticles for Point-of-Care Diagnostic Testing

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Statement of Purpose: We present an integrated bioseparation and detection system using stimuli-responsive nanoparticle reagents for applications in point-of-care diagnostic immunoassay. The system allows for labeling of the diagnostic target (e.g. protein) with colloidal gold, followed by magnetic separation/enrichment, and detection by lateral flow immunoassay (LFIA). The system is composed of iron oxide and gold nanoparticles conjugated to thermally-responsive poly(N-isopropylacrylamide) (pNIPAAm). These polymers undergo a hydrophilic to hydrophobic phase transition upon heating above a lower critical solution temperature (LCST) of ~32 ºC. Upon raising the temperature, the two particle types co-aggregate, self-assembling into structures containing both magnetic (iron oxide) and non-magnetic (gold) constituents. Subsequent application of a magnetic field results in magnetophoresis and separation of the diagnostic target bound to the gold nanoparticles. Enrichment is achieved by re-dissolving the aggregates into a smaller volume of carrier fluid. Detection of the concentrated gold-labeled diagnostic target is then achieved using lateral flow immunochromatography. We demonstrate the utility of this magnetic separation/detection system by capturing and detecting protein analytes from 50% human plasma. These results demonstrate the advantages of protein pre-concentration/enrichment in enhancing the sensitivity of conventional point-of-care LFIA.

Methods: A cationic diblock copolymer of N,N'-(dimethylaminoethylacrylamide) and NIPAAm was synthesized and characterized using gel permeation chromatography and ^{1}H-NMR spectroscopy. Diblock pNIPAAm was conjugated to PEG_2-biotin, and bound to citrate-stabilized gold nanoparticles (AuNPs). Iron oxide nanoparticles (mNPs) with a pNIPAAm surface coating were synthesized as previously described.[1] Samples of 50% human plasma (HP) spiked with the diagnostic target were used to mimic clinical samples. AuNPs, mNPs, and free pNIPAAm were added to the samples, and the temperature was raised above the LCST. After 30 minutes of magnetic capture, the aggregates were re-dissolved into a 200-fold smaller volume. LFIA strips were pre-spotted with IgG antibodies specific to the diagnostic target. The concentrated particle mixtures containing the gold-labeled target protein were applied to the LFIA strip and flowed past the region with pre-spotted capture antibodies. The purple AuNP spots that developed upon antigen-antibody binding were measured using a flatbed scanner.

Results: The integrated bioseparation/detection system is based on a two-step approach. In the first step (1), the diagnostic target is labeled with a high-efficiency absorbing species (gold colloid), and magnetically separated from the sample matrix by polymer-induced co-aggregation and separation with pNIPAAm-coated mNPs. In the second step (2), the gold-labeled diagnostic target is enriched several hundred fold by re-dissolution of the particle aggregates into a smaller volume of carrier fluid below the LCST. The concentrated diagnostic target bound to the AuNPs is then flowed past a region of capture antibody immobilized onto the solid phase of an immunochromatography strip, resulting in spot development and visual detection of the diagnostic target by eye.

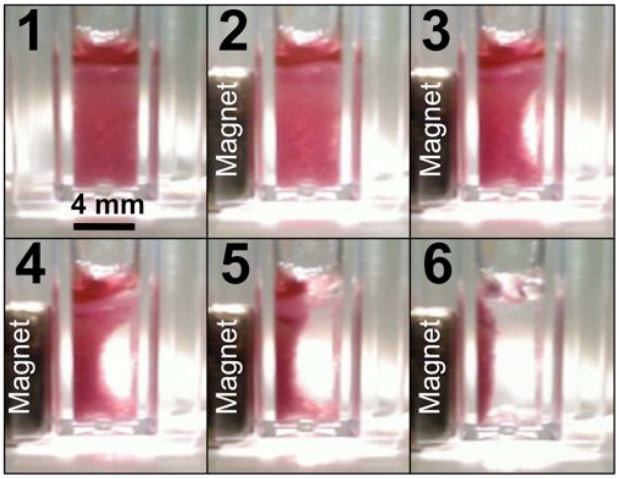

Figure 1. Time-lapse images demonstrating magnetic separation of AuNPs. (Image 1) AuNPs and mNPs are mixed and the temperature is raised above the LCST. (Images 2-6) A magnetic field is applied and the AuNPs are pulled to the side of the cuvette by polymer-induced cross-linking and co-separation with mNPs.

Figure 1 shows time-lapse images demonstrating co-aggregation and magnetophoresis of AuNPs via cross-linking with mNPs. The total separation time for the image sequence was 30 minutes. After magnetic separation, the supernatant was discarded and the particles concentrated 200-fold. The concentrated gold-labeled diagnostic target was detected with low background and high-sensitivity at 10 ng/mL using a LFIA. Larger starting sample volumes (2 mL vs. 0.5 mL) resulted in lower limits of detection for the immunoassay, demonstrating how sample pre-concentration/enrichment can be used to enhance the sensitivity of LFIAs. The total assay time was ~40 minutes.

Conclusions: This new integrated approach for labeling, enriching, and rapidly detecting protein analytes from human plasma has been shown to be advantageous for lowering the limits of detection of LFIA. Future work will focus on demonstrating multiplexed diagnostic target detection from a single sample.

References: [1](Lai JJ. Lab on a Chip. 2009;9:1997-2002.)

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