CeNP Surface Properties and their Binding Kinetics to Immunoglobins in Mouse Plasma

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Abstract

Cerium Oxide is a nanoparticle and is reported to have simultaneous effects on curing brain trauma. When the nanoparticles are introduced intravenously, however, they immediately bind proteins in the plasma, which consequently changes the size, shape, and surface charge. Of particular interest, is the binding of immunoglobins (Ig), which results in rapid clearance by resident immune cells in the liver and spleen. Using the Fiber Optic-Particle Plasmon Resonance (FOPPR), the researchers have been able to test the surface plasmon resonance (SPR) to determine Ig binding to cerium oxide nanoparticles (CeNP). Using this approach, the suggested models of Ig binding to cerium oxide is evaluated *in silico.* The different stabilizing strategies presenting on CeO_2 nanoparticles will result in in different SPR signatures, and will correlate with the liver deposition in the previous studies. Meanwhile, the economic benefits of using FOPPR on the experimental applications are,

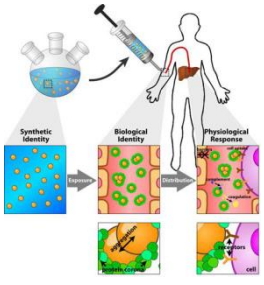
1. **Time saving** – only takes one or two days to complete data collection in which it normally spends a whole month to study in both animal and mass spectrometry aspects.
2. **Cost saving** – only spends couple hundreds dollars instead of taking $2000-3000 for a run of experiments, including animal study and MS.
3. **Life saving** – efficient FOPPR initial screenings to find potential NPs with good surface properties *in vitro* to avoid consequently more serious and costly animal study.

Background

•Surface plasmon resonance: at a specific wavelength of light, collective oscillation of electrons on the gold nanoparticle surface cause a phenomenon resulting in extinction of light (absorption and scattering)

•Protein corona consequently changes the size, shape, and surface charge

•Previous *in vivo* studies showed decreased liver deposition of stabilized CeNP compared to unstabilized CeNP



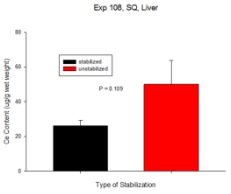


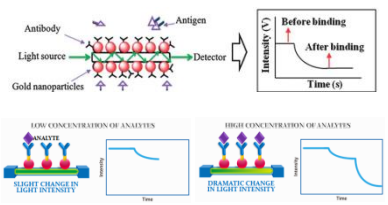
Figure 1. Decreased liver deposition of stabilized NP compared to unstabilized NP *in vivo.* Nine animals were used for each treatment.

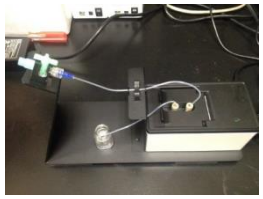
Methods

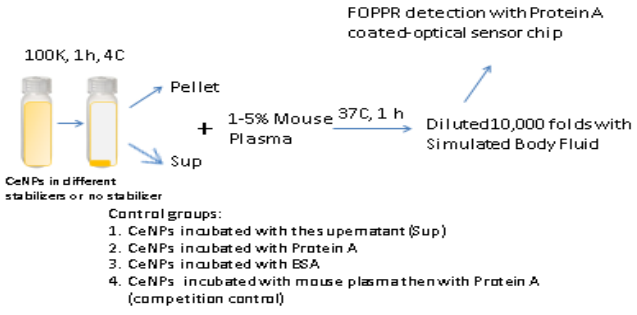
• Immobilization of protein A, a protein that binds IgG, on the optical fiber. CeNPs that bind IgG will subsequently bind to protein A on the optical fiber & increase the resonance energy generating a signal we can measure

• Determine interaction of Protein A with Human IgG, Mouse plasma, & BSA

• Determine binding of CeNPs onto immunoglobulins in mouse plasma







Results

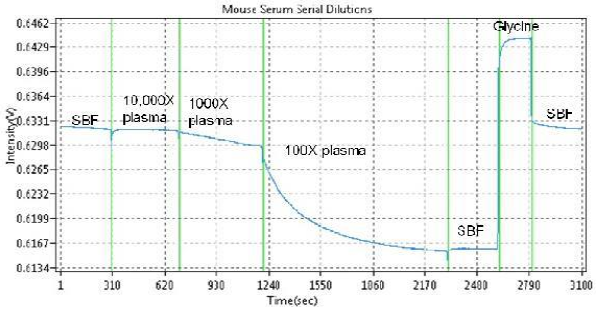


Figure 2. Interaction of Mouse Plasma with Protein A immobilized sensor at different dilutions. The 100 fold dilution had the strongest association curve, demonstrating a dose dependent response of the binding of mouse serum to protein A. There was little signal change at the 10,000 dilution so this dilution was used for the experiments with NP.

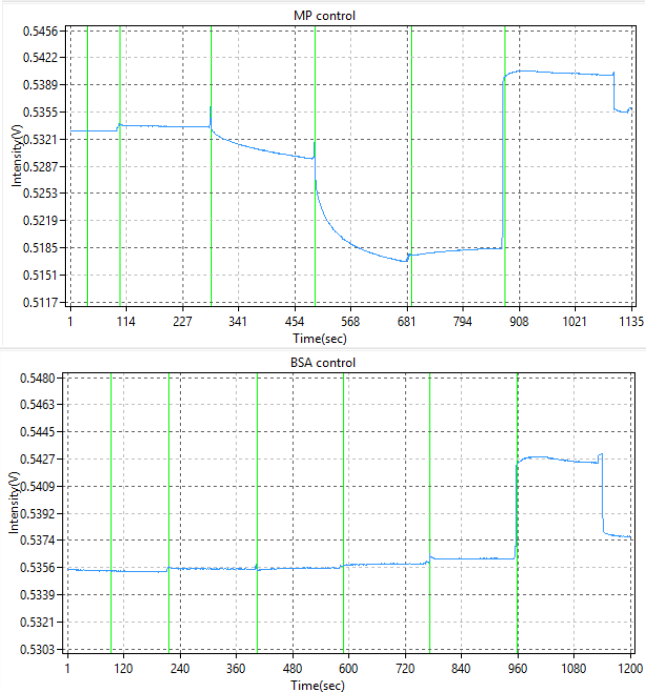


Figure 3. No interaction of IgG free BSA with Protein A immobilized on the optical fiber. BSA concentrations were calculated by the assumption that albumin occupies roughly 55% of total proteins in blood plasma.

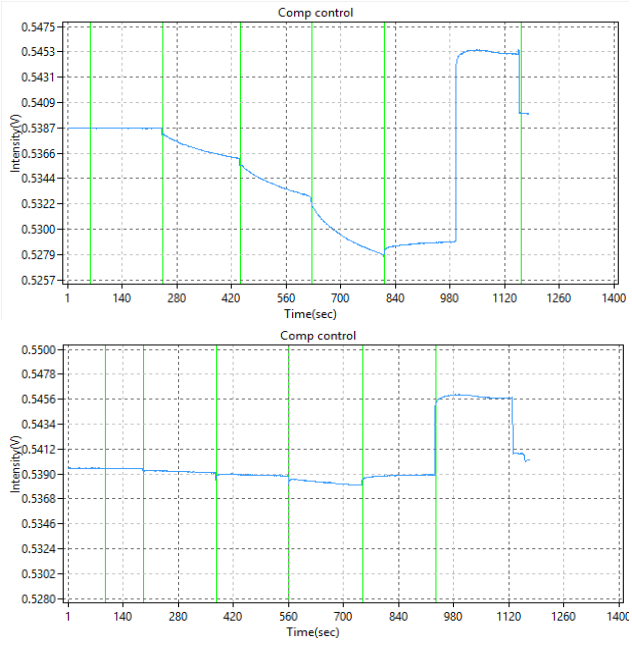


Figure 4. Competition assay with 10 μg/ml of free Protein A in solution containing the mouse plasma in different dilutions from the original stock. The results clearly showed a decreased SPR signals in the presence of free Protein A, suggesting that free Protein A competed with immobilized protein A on the optical fiber for IgG binding sites.

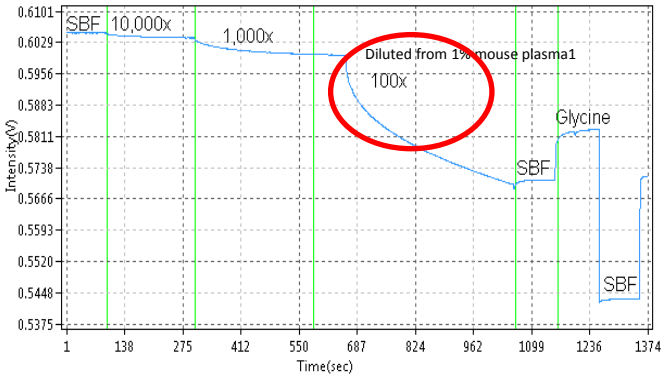


Figure 5. A 100 fold dilution of 1% mouse plasma that was incubated with the nanoparticles at 37° C for 1h shows a strong SPR signal with Protein A immobilized on the sensor. One hundred fold dilution of 1% mouse plasma is the same as 10,000 fold dilution from the original mouse plasma .

Conclusions

- Expected results: Because the stabilized CeO_2 NP had lower liver deposition *in vivo*, we expected to see weaker FOPPR signal from the interaction of plasma-treated stabilized CeO_2 and protein A due to a lower binding of IgG to the surface of stabilized CeO_2 NP
- Contrary to our hypothesis we found that stabilized CeNP had a stronger SPR signal than unstabilized CeNP, possibly due to larger mass of stabilized CeNP
- Future studies: perform more replicates to confirm results
- FOPPR detection system carries economic features such as time saving, cost saving, and life saving on approaching the characterization of CeO_2 NP towards blood plasma proteins in this research project.

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