

In Vitro Studies of Functionalized Magnetic Nanospheres for Selective Removal of a Simulant Biotoxin

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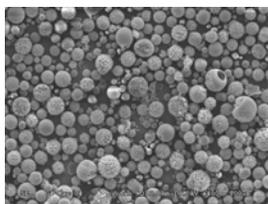
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Background

- **Goals:** Efficient and selective removal of biotoxins from biosystem
- Use of functionalized, magnetic nanoparticles
- Proof-of-concept employs highly selective, enzyme-antibody complex (biotin-streptavidin interaction of $K_a = 10^{15} M^{-1}$)
- **Simulant toxin (biotinylated horseradish peroxidase, bHRP)** removed from simple solutions and blood
- Sequestration of the toxin (bHRP) is realized with streptavidin-functionalized magnetic nanoparticles
- Quantitative removal of the toxin is achieved using an external magnetic field to trap the toxin-bound particles

Materials / Characterization

- Testing with (1) magnetic, latex nanoparticles and (2) biodegradable polymers spheres



Micrograph of biodegradable, streptavidin-coated, biotinylated poly(lactic acid)-poly(ethylene glycol) polymer microspheres.

Techniques employed for *in vitro* characterization of nano/ microparticles:

Dynamic Light Scattering for particle sizing of nanoparticles (to determine physiological circulation characteristics)

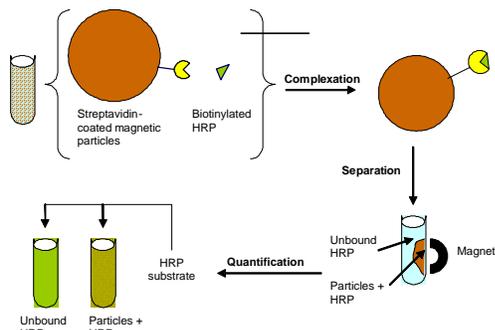
Scanning Electron Microscopy for particle sizing of microparticles (to determine physiological circulation characteristics)

Zeta Potential for surface charge properties (to determine physiological health characteristics)

Biotoxin Detection

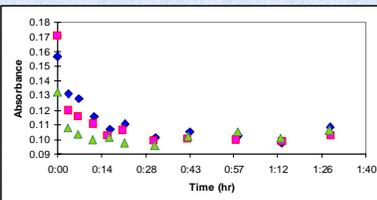
Enzyme Linked ImmunoSorbant Assay (ELISA)

- Assay developed to achieve optimal sensitivity level
- Robust and reproducible assay for samples tested
- Nonbound material is washed away from specific bound materials (strategy based on highly specific complex interaction)

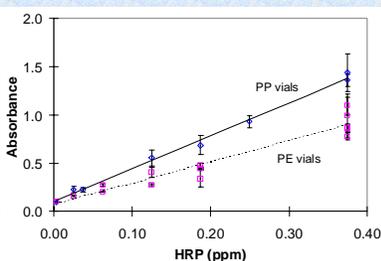


Optimization of Biotoxin Detection

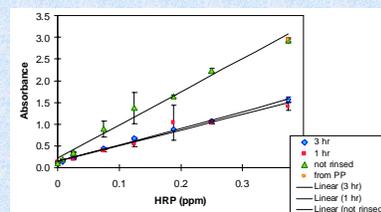
Initial, *in vitro* kinetic testing indicated a large difference between the predicted and measured absorbances of the samples vs the control (~ 80% lower than control)



- Differences in bHRP concentrations due to sorption
- ~30% greater sorption onto polyethylene (PE) than polypropylene (PP)



- Toxin (bHRP) contacted with streptavidin-coated microtiter wells; 50% of bHRP bound to streptavidin wells
- No difference between 1- and 3-hr incubation kinetics for binding



Toxin Detection in Blood

- Absorbance readings for blood, saline, and water as a function of number of well rinses at HRP substrate wavelength (405 nm)
- High background due to presence of blood in detection measurements eliminated by sufficient rinsings of wells or dilution of blood samples

| Sample | Absorbance @405 nm | Number of Well Rinses |
|---------------------|--------------------|-----------------------|
| Blood (undiluted) | 0.84 | 3 |
| Blood (diluted 1:2) | 0.78 | 3 |
| Blood (diluted 1:4) | 0.42 | 3 |
| Blood (diluted 1:6) | 0.11 | 3 |
| Saline | 0.09 | 3 |
| Water | 0.09 | 3 |
| Blood (undiluted) | 0.10 | 10 |
| Blood (undiluted) | 0.09 | 15 |

Magnetic Nanoparticle Loadings

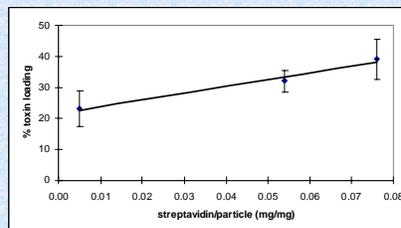
- Use of magnetic, latex particles (400 nm streptavidin-coated particles)
- Toxin loadings of 70% with single contact; incubation performed with gentle mixing
- Optimization of the tests is ongoing

| Media | Incubation Time | % Toxin Removal |
|--------|-----------------|-----------------|
| Saline | 15 min | 71.9 |
| Saline | 60 min | 72.8 |
| Blood | 15 min | 11.6 |
| Blood | 60 min | 38.9 |
| Blood | 60 min | 63.0 |
| Blood | 60 min* | 54.5 |
| Blood | 180 min | 71.9 |

*Denotes vortex mixing

Biodegradable Particle Loadings

- Use of biodegradable particles synthesized in our lab (streptavidin-biotinylated functionalized poly(lactic acid)-poly(ethylene glycol) polymer)
- Toxin loadings of up to 40% with single contact (1 hr incubation)
- Optimization of the biodegradable nanoparticle syntheses is ongoing



Summary

- *In vitro* nanoparticle testing demonstrated successful removal of simulant biotoxin (up to 70% with a single contact)
- Enzyme linked immunosorbant assay was developed for quantification of the enzyme-antibody magnetic nanoparticles
- The detection and quantitation of the biotoxin-bound particles developed from these *in vitro* studies will be extended to future *in vivo* studies
- Results from these *in vitro* studies support concept feasibility for detoxification of human subjects

COLLABORATIVE INVESTIGATORS FOR APPLIED NANOTECHNOLOGY IN MEDICINE



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