Magnetic Nanoparticles as a Template for *In Vivo* Nanomedical Systems

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Statement of Purpose: Nanomedical approaches focus on specific cell targeting and intracellular delivery of information. Applications of nanomedicine emphasize therapeutic modification or delivery for diseased cells by targeted nanostructures.[1] Specifically, multifunctional layered nanoparticles are being created to target and deliver a therapeutic agent to cancer cells *in vivo*.[2] The ability to perform "theragnostics" to simultaneously use nanoparticles as contrast agent for *in vivo* magnetic resonance imaging diagnostics and to induce apoptosis in diseased cancer cells for therapeutics is desired. Therefore, it is detrimental to the nanomedical system to have nanoparticle materials that induce apoptosis in normal, healthy cells. Several apoptosis assays have indicated that the core magnetic nanoparticles do not markedly increase apoptosis events in human cells. Nanoparticle internalization by cells was also monitored by Prussian blue staining to provide evidence that apoptosis effects could be related to the physical presence of intracellular magnetic nanoparticles.

Methods: *Cytotoxicity and apoptosis assays.* Amino-functionalized iron oxide-dextran magnetic particles (Micromod, Warnemuende, Germany), 50 nm, were used as the starting core nanoparticle material. Human lymphoblastic leukemia (MOLT-4) and human breast cancer (MCF-7) cell lines (American Type Culture Collection, Manassas, VA) were utilized as the test cells for nanoparticle (NP) exposure. Control cultures were also initiated that saw no NP supplementation. The desired concentration of magnetic NPs was applied to the culture medium and was allowed to incubate with the cells for 24 hours at 37°C with 5% CO_2.

Cellular effects related to NP exposure were assessed by three assays: Annexin V fluorescence assay (Invitrogen, Carlsbad, CA), the Apo-BrdU TUNEL assay (Invitrogen, Carlsbad, CA), and the Vybrant Assay Kit #5 membrane permeability assay (Invitrogen, Carlsbad, CA). Briefly, the Annexin V assay was performed to detect extracellular phosphatidyl serine as a sign of early-stage apoptosis. After NP exposure, cells were rinsed in Annexin binding buffer and stained with Annexin V-Alexa 488 fluorophore and propidium iodide (for cellular viability). The TUNEL assay was performed to detect 200 base pair intranucleosomal cleavage DNA fragments, characteristic of late-stage apoptosis. The assay was performed per manufacturer protocol and involved cell fixation in Cytochex® (Streck, Omaha, NE), labeling the DNA break sites with BrdU, and detecting the BrdU by an Alexa 488 (Invitrogen, Carlsbad, CA) labeled anti-BrdU antibody. For the membrane permeability assay, live cells were stained with Hoechst 33342 (a membrane permeant DNA dye) and propidium iodide (PI) (a dye excluded by live or apoptotic cells with intact membranes) to distinguish between DNA condensation in late-stage apoptotic cells and necrotic cells, respectively. All cell samples were prepared at concentration of at least 10^6 cells/mL and fluorescence properties were recorded by a Beckman Coulter Cytomics FC500 flow cytometer.

Prussian blue detection of nanoparticle internalization in cells. MOLT-4 and MCF-7 cells were fixed in 4% glutaraldehyde (Sigma, St. Louis, MO) for 2 hours on ice. Fixed cells were washed and stained with 2% potassium ferrocyanate in 6% hydrochloric acid (Sigma, St. Louis, MO) for ferric iron. Images were obtained by light microscopy to visually compare differences.

Results/Discussion: Results of cytotoxicity and apoptosis assays indicate the magnetic NPs are well tolerated by cells at concentrations between 0.05 and 0.5 mg/mL NPs. Specifically, the Annexin V and TUNEL apoptosis assays show little difference in apoptosis rates between control and NP treated cells. Similarly, exposure to magnetic NPs did not indicate changes in membrane permeability. All NP treated samples were compared to untreated and hydrogen peroxide treated cells (for positive controls). Prussian blue staining indicated internalization of NPs by both MOLT-4 and MCF-7 cells at concentrations tested.

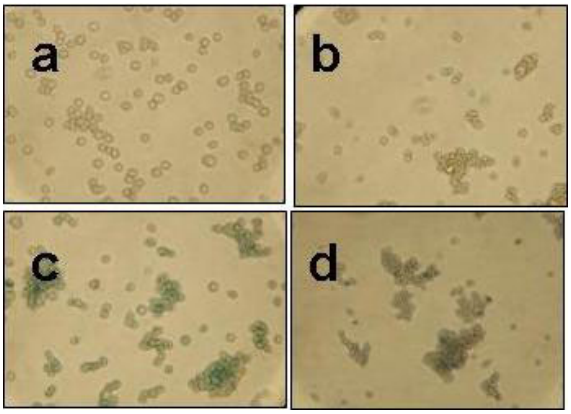

Figure 1. Fixed cells after Prussian blue staining at 200x magnification (a) MCF-7 cells not exposed to NPs, (b) MOLT-4 cells not exposed to NPs, (c) MCF-7 cells exposed to 0.5 mg/mL NPs for 24 hrs, (d) MOLT-4 cells exposed to 0.5 mg/mL NPs for 24 hrs.

Conclusions: Iron oxide-dextran magnetic nanoparticles provide show little inherent cytotoxicity or apoptosis effects as measured by cellular morphology and various fluorescence assays. These magnetic nanoparticles are also detectable *in vitro* by a simple Prussian blue staining method. These results indicate that these magnetic nanoparticles are a good template core particle to build multilayered nanomedical systems.

References:

(1) Farokhzad OC. *Adv Drug Deliv Rev*, 2006, in press.
(2) Prow TW. *J Mol Hist* **35**(6), 555-564, 2004.