**Targeted Multifunctional Quantum dot™ Nanoparticles for *ex vivo* Cancer Diagnostics**

E. Haglund, L. Reece, C. Cooper, J. Leary.
Purdue University, West Lafayette, IN

**Statement of Purpose:** Conventional cancer therapies aim to cut out the diseased cells (surgery), burn them out (radiation therapy), or poison the diseased cells faster than the healthy cells (chemotherapy). Nanomedical therapies attempt to make smart decisions to target and diagnose or treat specific host cells (Leary and Prow, patent pending, 2005). The specific targeting of rare cancer cells *ex vivo* can be addressed with the use of Quantum dot™(Qdots) (Invitrogen Corp, Carlsbad, CA) nanoparticles. The uptake mechanism of these particles is being investigated with respect to cytotoxicity and targeting efficiency. The use of methods such as passive endocytosis, electroporation, optoinjection, and peptide-mediated endocytosis are being investigated. However, the cytotoxicity profile of these nanoparticles has not been fully evaluated. Previously observed effects involve creation of reactive oxygen species, elimination of biocoating on particle, and specific particle formulations (Lovric J.Chem Biol. 2005;12:1227-34.; Derfus A. Nano Lett 2004;4:11-18.). The future use of nanoparticle systems that have multiple layers will be utilized as systems that perform advanced diagnostic and therapeutic functions. The objective of this research is to identify the most safe and efficient nanoparticle uptake for specific cancer cells and the application of *ex vivo* diagnostics.

**Methods:** Amino-functionalized Quantum dot™ nanoparticles (Invitrogen Corp., Carlsbad, CA) were applied to MCF7 breast adenocarcinoma cell line to evaluate passive endocytotic uptake. ***Cell Culture:*** MCF7 breast adenocarcinoma cell lines (American Type Culture Collection, Manassas, VA) were cultured in media of Minimum Essential Eagle's Medium with L-glutamine, sodium bicarbonate, non-essential amino acids and sodium pyruvate, supplemented with insulin and 10% Fetalplex. The cell culture conditions are 5% CO2/ 95% humidity at 37°C. ***Quantum dot™ Endocytosis:*** The passive endocytosis process applied the nanoparticles in specific concentrations over 4 hours at incubation conditions. After this time period the cells were trypsinized and subjected to an apoptosis assay using protocols from the manufacturer. (http://probes.invitrogen.com/media/pis/mp13243.pdf). To determine if apoptosis is present in the cell populations, two dyes were utilized; YO-PRO®, which enters apoptotic cells, and propidium iodide (PI), which enters dead cells (Invitrogen Corp., Carlsbad, CA). ***Flow Cytometry:*** Cells with the Qdots were subjected to flow cytometry (Beckman Coulter, Cytomics™ FC 500). Forward scatter, side scatter, and fluorescence properties were evaluated to determine if the Qdots were present inside the cells and if the dyes, YOPRO™ and PI, were present. The control sample provided a comparison between the fluorescence and scatter properties of the labeled cells.

**Results/Discussion:** Nanoparticles were not efficiently uptaken into cells based on light scattering properties (data not shown). Passive endocytosis is not an effective uptake mechanism with the amino-functionalized Quantum dots™. While the nanoparticles were not uptaken, their presence did initiate apoptotic events and cell death. Their presence induced apoptosis and cell death at a concentration of 2nM (**Figure 1)**. In **Figure 1A and C,** the data illustrates the presence of apoptotic populations (green stain R2). This apoptotic population has increased with nanoparticles applied. In **Figure 1B and D**, dead cells are also observed with the presence of nanoparticles (green stain R2). The presence of the nanoparticles created these cytotoxic effects. Based on the cytotoxic effects observed, it is necessary to identify another method to increase the uptake efficiency and reduce cytotoxic effects.

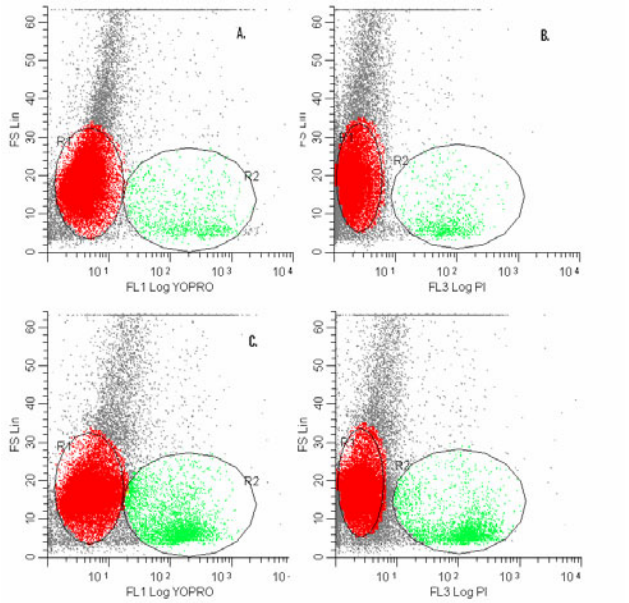


**Figure 1**. Fluorescent YO-PRO® and PI as a function of Quantum dot concentration, x-axis is Log YO-PRO or PI, y-axis is Forward Scatter, red: live cells, green: apoptotic or dead cells. A. YO-PRO® only, no Qdots, B. PI only, no Qdots, C. 2nM Qdots with YO-PRO®, D. 2nM Qdots with PI.

**Conclusions:** Passive endocytosis does not provide efficient uptake and does induce apoptotic and necrotic mechanisms in MCF7 cells. More specific uptake mechanisms are being explored. Further studies will involve evaluation of methodologies of electroporation, optoinjection, and peptide-mediated endocytosis to improve the targeting efficiency and minimize cytotoxic effects. More efficient targeting using multi-layered particles with Quantum dot™ cores will create a nanomedical system that will allow advanced targeting and diagnosis of rare cancer cells *ex vivo* and provide for minimal residual disease monitoring.