Cellular Binding of Quantum Dots

Quachel Bazile
Chemistry, Elizabeth City State University

NNIN REU Site: Nanotechnology Research Center, Georgia Institute of Technology, Atlanta, GA
NNIN REU Principal Investigator: Professor Christine K. Payne, Chemistry, Georgia Institute of Technology
NNIN REU Mentor: Candace C. Fleischer, Chemistry, Georgia Institute of Technology
Contact: qbazile@gmail.com, christine.payne@chemistry.gatech.edu, ccthompson@gatech.edu

Introduction:

The need for new innovative treatments has led to the increase in nanoparticle (NP) research. NPs can improve treatment through drug delivery, cellular imaging and gene therapy. To properly use NPs for treatment, it is important to understand how NPs interact with the cellular environment. Recent research was done that compared cellular binding on cationic and anionic polystyrene NPs [1]. The results concluded that the cellular binding of cationic polystyrene NPs was enhanced in the presence of serum proteins and inhibited in the absence of serum proteins. The results also entailed that anionic polystyrene NPs cellular binding was inhibited in the presence of serum proteins and enhanced in the absence of serum proteins [1]. Serum proteins are a mixture of multiple proteins that are isolated from whole blood. Serum proteins may influence how NPs bind to cells because they have select sites on the cell where they bind [2]. The purpose of this research was to measure quantum dot (QD) binding to cells in the presence and absence of serum proteins and compare to prior work done with polystyrene NPs and cellular binding.

QDs are semiconductor nanocrystals that are fluorescent. QDs are fluorescent because they have a large band gap and when excited, energy is given off. QDs are used for variety of applications including imaging, solar cells, and LEDs [3]. To compare the difference between cationic and anionic QDs a coupling reaction was done to change the anionic QDs to cationic QDs.

Experimental Procedure:

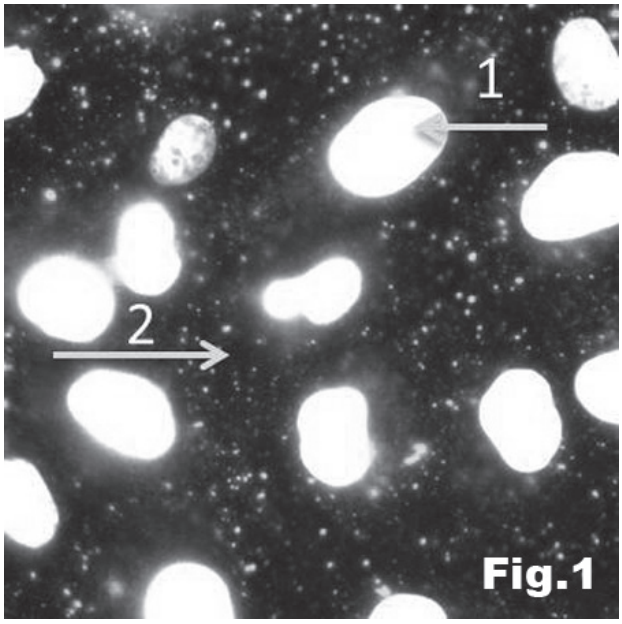
Characterization. The Nano-ZS Zetasizer (Malvern Instruments) was used to determine the hydrodynamic diameter and the zeta potential or effective surface charge of QDs. An 800 pM solution of QDs was used for both measurements, and all samples were measured in triplicate.

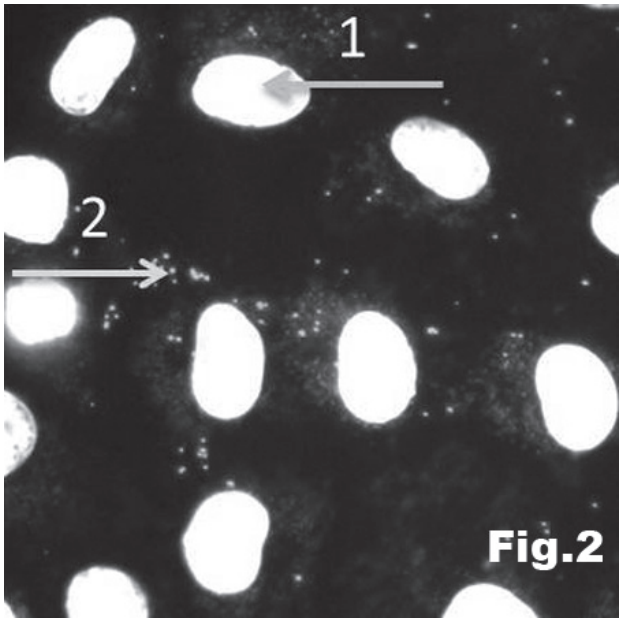
Cellular Binding. Carboxylate-modified QDs (Invitrogen, 525 nm emission) were used in cellular binding experiments in minimum essential medium (MEM), and MEM with 10% fetal bovine serum (FBS). Monkey kidney epithelial (BS-C-1) cells were cooled for 20 minutes at 4°C followed by a 20-minute incubation with QDs and 4',6-diamidino-2-phenylindole (DAPI). DAPI is a fluorescent stain that was used to see the cell nucleus while imaging. The cells were then rinsed twice with phosphate buffer saline (PBS) and imaged using epi-fluoresence microscopy.

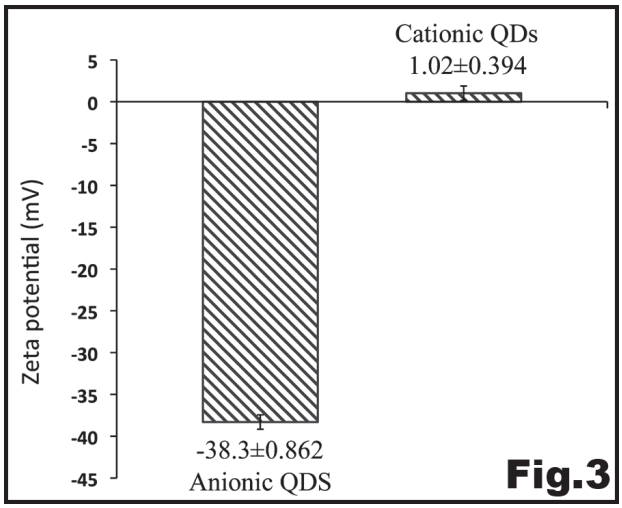
Cationic QD Formation. To modify the anionic QDs to cationic QDs, a coupling reaction was done. The reaction was done in a borate buffer (10 mM, pH 7.4). Amine groups were coupled to the carboxyl groups on the QDs using ethylene diamine (ED, Sigma Aldrich) as the coupling group. 1[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC, Thermo Scientific) was used as the coupling reagent. In a glass vial, QDs (80 nM), ED (135 mM), and EDC (1 M) were combined and incubated under stirring for five hours. After incubation, the solution was filtered using ultrafiltration (Millipore, 100K MWCO). To determine the final surface charge of the product, the zeta potential was measured.

Results and Conclusions:

We found that cellular binding is enhanced significantly in the absence of serum proteins (Figure 1). In comparison, cellular binding of anionic QDs in the presence of serum protein is inhibited (Figure 2). The results of cellular binding of the anionic QDs were similar to the results of anionic polystyrene NPs. For both types of NPs, cellular binding was inhibited in the presence of serum proteins and enhanced in the absence of serum protein. This suggests that the charge of the NP not the composition of the NP dictates cellular binding.







When amine groups were coupled to the carboxylate groups the QDs became more positive (Figure 3). The cellular binding of these cationic QDs were measured on BS-C-1 cells. No binding was observed. This may be due to the fact that the amine modified QDs were not as positive as expected.

Future Work:

In the future, the QD coupling reaction will be optimized to yield more positive QDs. Cellular binding studies with cationic QDs will also be completed. A comparison between the cationic QDs results and polystyrene NP results will also be done.

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References:

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Figure 1, top: Cellular binding of anionic QDs to live BS-C-1 cells in minimum essential medium (MEM). Epifluoresence microscopy was used to acquire images. Arrow 1 in the image is the nuclei, stained with DAPI, and Arrow 2 indicates the QDs. QDs bind to cells in the absence of serum proteins.

Figure 2, middle: Cellular binding of anionic QDs to live BS-C-1 cells in MEM + 10% fetal bovine serum (FBS). FBS is a mixture of serum proteins. Epi-flouresence microscopy was used to acquire images. Arrow 1 in the image is the nuclei, stained with DAPI, and Arrow 2 is the QDs. In the presence of serum proteins, QD cellular binding is inhibited.

Figure 3, bottom: The graph shows the surface charge or zeta potential of the QDs before and after the coupling reaction. The anionic QDs were measured in water. The cationic QDs were measured in a borate buffer solution (10 mM, pH 7.4). These samples were measured on the Zetasizer (Nano-ZS, Malvern Instruments). Each sample was measured in triplicate.