Quantum Dot Nanocrystals in Neuroscience

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ABSTRACT

Quantum dot nanocrystals are nanometer sized semiconductor particles which can be used for labeling biological systems. They are much brighter and more stable than conventional fluorophores. However, the potential for the use of quantum dots in biological sciences has yet to be realized because of the lack of reliable labeling methods using commercially available reagents. We have developed protocols for labeling neurons and glial cells with commercially available quantum dots, and have used these tools to label glial fibrillary acidic protein (GFAP) intermediate filament cellular bridges. These previously unidentifiable characteristics are now visible with quantum dots, which allow for much higher resolution and more specific labeling than conventional fluorophores.

Keywords: quantum dots, neurosciences, glial fibrillary acidic protein, beta-tubulin

1 INTRODUCTION

Quantum dots are nanocrystals, often made of a CdSe core with a ZnS cap. They can be used for studying biological systems in the same way as conventional fluorophores, but offer stability and resistance to photobleaching that organic fluorophores cannot provide, allowing long term studies in live cells. Other advantages of using QDots include tunable emission based on size and composition, broad excitation and narrow emission peaks, multicolor imaging, and resistance to chemical and metabolic degradation [1, 2].

Prior work in this area involves synthesizing and chemically conjugating quantum dots to molecules of interest in-house which is both time consuming and prone to human error [1, 3, 4]. Additionally, non-specific binding and nanoparticle aggregation currently prevent researchers from utilizing this system to its fullest capacity.

In this study, we developed protocols for labeling proteins of interest in neurons and glial cells using commercially available reagents [5]. With this refined protocol, scientists should be able to more easily label and track biological molecules of interest.

2 METHODS AND MATERIALS

Quantum dots conjugated to streptavidin were obtained from Quantum Dot Corporation and were used with biotinylated antibodies to label proteins of interest in neurons and glial cells. Briefly, glass cover slips were coated with 0.1% wt/vol poly-D lysine in double distilled water overnight. They were subsequently washed 3 times with phosphate buffer saline (PBS) and allowed to dry. Cells were seeded onto cover slips at a seeding density of 20,000 cells/well for a 24 well plate. They were incubated at 37º C for 24-48 hours to allow attachment to the substrate. Cell culture media was then removed from the cells and warmed PBS was added to remove excess media. Cells were fixed with 4% paraformaldehyde in PBS for 10 minutes at room temperature. The cells were then washed 3 times with PBS and permeabilized with 0.2% Triton X-100 in PBS for 5 minutes. They were washed again 3 times with PBS and incubated in 10% horse serum in PBS for 30 minutes at room temperature to allow for blocking of non-specific binding. After a final rinse with PBS, the biotin/streptavidin blocking kit was applied to block non-specific binding of the biotinylated antibody as well as the streptavidin conjugated quantum dots. All sites where biotin would non-specifically bind were quenched with the streptavidin added during this step. This was followed by another wash with PBS. Finally, we added our biotinylated molecule of interest. We used anti-GFAP and anti-β-tubulin at a dilution of 1:1000 and 1:100 respectively in PBS with 10% horse serum. As a control, we used each antibody without biotinylation in 10% horse serum and incubated 10% horse serum without a primary antibody in order to detect non-specific binding of quantum dots or streptavidin. These incubations were for 2 hours at room temperature. This was followed by washing 3 times with PBS. 605nm streptavidin conjugated quantum dots were added at a 1:100 dilution with 10% horse serum and incubated for 1 hour. An additional control of standard anti-mouse TRITC was added to compare results to quantum dot labeled cells. All samples were rinsed 3 times with PBS and mounted with 90% glycerol in PBS.

An alternate labeling method involves doing three steps instead of two steps but provides greater specificity and

higher resolution (Figs 1, 2, 3, & 4). Briefly, after the biotin/streptavidin blocking step, the primary antibody was added alone with 10% horse serum and incubated for 2 hours, followed by rinsing 3 times with PBS; a biotinylated secondary antibody was added with 10% horse serum and incubated for 1 hour, followed by rinsing 3 times with PBS; and finally, the streptavidin conjugated quantum dots were added and incubated for 30 minutes, followed by washing 3 times with PBS.

All images were obtained using an Olympus 1X81 inverted fluorescent confocal microscope. It was equipped with a Hamamatsu ORCA-ER digital camera and Image-Pro Plus data acquisition and morphometric software. The quantum dots were imaged using the XF304 Qdot605 filter set from Omega Optical.

3 RESULTS AND DISCUSSION

3.1 QDots vs. Standard Immunofluorescence

Quantum dots appear much brighter than conventional fluorophores such as TRITC as can be seen when comparing GFAP filament labeling in Muller cells and astrocytes (Figures 1-3). Additionally, the labeling is much more specific, as individual filaments can be resolved when visualized with quantum dots as opposed to TRITC. Finally, since quantum dots are more stable, photobleaching is not a problem and cells can be visualized weeks after fixation whereas TRITC labeled samples lost fluorescence in a few days. In Figure 4, β-tubulin is labeled in rat cortical neurons which appears much more brightly labeled than the TRITC control (not shown).

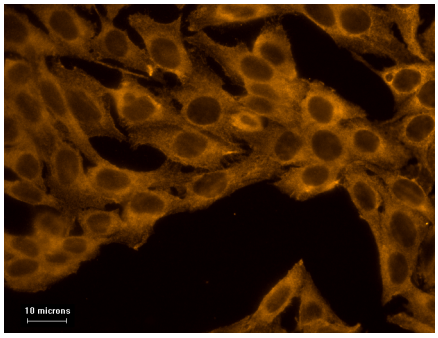

Figure 1: Rat Muller cells labeled for GFAP with 605nm emission quantum dots using the 3 step labeling method. Image obtained using 605 QDot filter for 5ms, 40X.

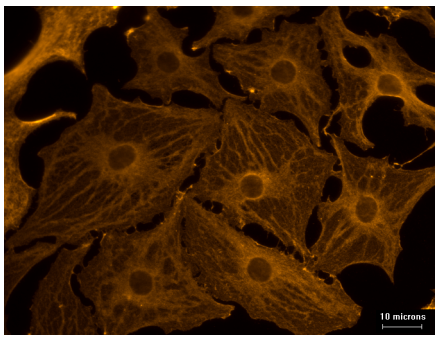

Figure 2: Rat Astrocytes labeled for GFAP with 605nm emission quantum dots using the 3 step labeling method. Image obtained using 605 QDot filter for 5 ms, 40X.

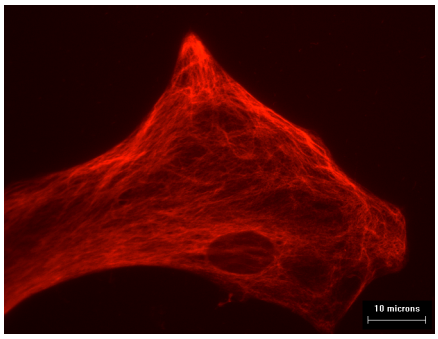

Figure 3: Rat Astrocytes labeled for GFAP with a TRITC conjugated secondary antibody. Image obtained using TRITC filter for 10ms, 60X.

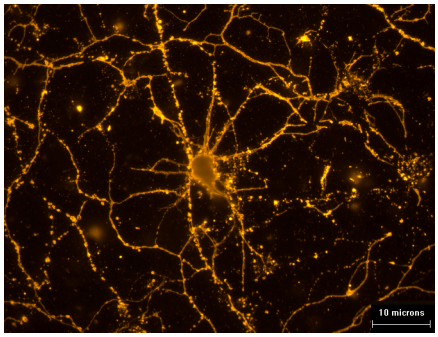

Figure 4: Rat Neuron labeled for β-tubulin with 605nm quantum dots. Image obtained using 605 QDot filter for 5ms, 60X.

3.2 Quantum Dots: 2 Step vs. 3 Step Labeling Method

The additional step in the three step labeling method adds another degree of specificity to the system. Since the biotin-streptavidin system has a high degree of non-specific binding in biological systems due to endogenous biotin or due to non-specific interactions, the primary-secondary antibody system can be used to add increased specificity to

detect the molecule of interest. Using the primary antibody without biotin in the first step allows more specific interactions to take place between the protein of interest and its antibody. In the second step, the secondary antibody binds to the primary conferring another degree of specificity. After this step, the biotin streptavidin system is used to detect the secondary antibody using the commercially available streptavidin conjugated quantum dots. It should be noted that an effort was made to use kits to bind the antibody directly to the quantum dot but this resulted in a high amount of unconjugated quantum dots aggregating in the nucleus of cells. Since it is difficult to quantify or detect the antibodies bound to each quantum dot, it is much simpler to use the biotin-streptavidin interaction where known molecules are added to the system.

Figure 5 offers an example of the two step labeling method which, when compared to the 3 step method for astrocytes (Fig 2), shows a much lower resolution image. This can be attributed to the lower specificity of binding due to the lack of a secondary antibody.

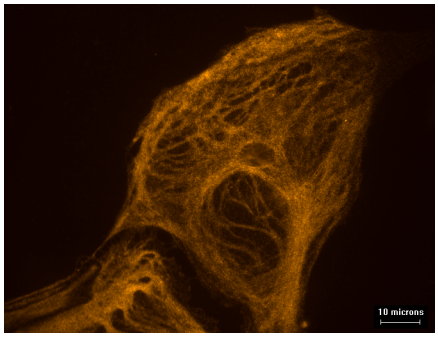

Figure 5: Rat Astrocytes labeled for GFAP with 605nm emission quantum dots using the 2 step labeling method. Note the reduced specificity that results from relying only on the biotin-streptavidin interaction instead utilizing the more specific primary-secondary antibody interaction. Image obtained using 605 QDot filter for 5 ms, 40X.

3.3 Other blocking agents

Other blocking agents were used to attempt to block non-specific binding of quantum dots to intracellular components. BSA, FBS and combinations of HS with these blocking agents were all used with varying degrees of success. It should be noted that BSA caused aggregation of quantum dots into bright clusters that did not specifically label anything in the cell (data not shown). The protocol using horse serum was the most successful for our cell types and when combined with the biotin-streptavidin blocking kit provides an appropriate amount of blocking for this system. All of the controls had either no signal or very dim signal (visible only at 100ms with QDot filter) with quantum dots spread out through the entire cover slip. When compared to the bright signal obtained at 5ms for the samples, this amount of non-specific binding was considered negligible.

3.4 Intermediate Filament Bridges

Previously unidentifiable bridges were observed with the quantum dot labeled cells due to the extremely brightly labeled filaments that resulted. In Figure 6, GFAP labeled astrocytes show a high amount of interconnectivity which was not visible using the fluorescent dye. These filaments are currently being investigated for cell signaling and communication between astrocytes.

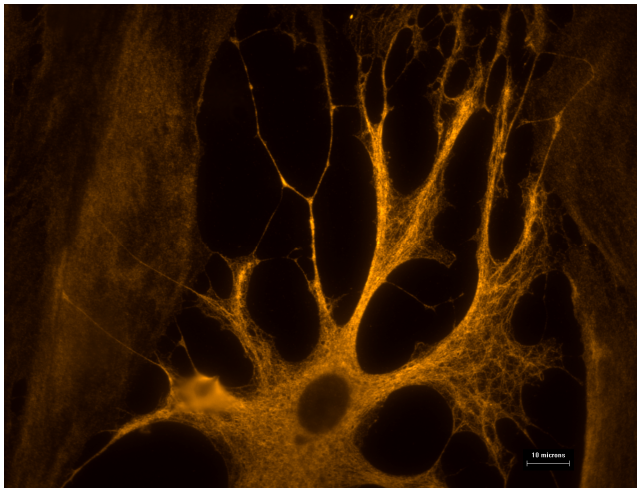

Figure 6: Rat Astrocytes labeled for GFAP with 605 quantum dots. Note the intermediate filament connections that appear between the center cell and the surrounding astrocytes. Image obtained using 605 QDot filter for 5ms, 60X, 1 X 1 bin.

4 CONCLUSION

We have developed ready to use protocols using commercially available reagents to label biological systems with quantum dot technology. Researchers will be able to label their systems and visualize previously unidentifiable interactions.

The advantages of using quantum dots over conventional dyes are clear but use of quantum dots with live cells is still limited. Developing protocols for use with live cells with commercially available reagents is the next step in the advancement of this powerful new technology.

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