CdSe Quantum Dots in Human Models Derived from ALS Patients: Characterization, Nuclear Penetration Studies and Multiplexing

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INTRODUCTION

CdSe Quantum Dots (QDs) are useful probes that can be conjugated with antibodies offering a unique staining approach for multiplexing staining with excellent fluorescent properties including narrow emission spectra, a gain of photostability compared with traditional fluorophores, and a single excitation source[1,2]. In combination with pathology models derived from patients, they help to understand the molecular mechanism underlying pathological cellular processes and have a great potential to contribute to quantitative molecular profiling promoting personalized medicine[3]. However, there are limitations of these materials such as the commercial availability and the need to characterize QDs and their conjugates before use. Furthermore, it exists a lack of previous evidence about the behavior of these nanotechnology tools applied to primary human cells.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a progressive deterioration of motor neurons that presents protein inclusions in the cytosol, mainly composed of TDP-43, that have a role in the disease progression. However, the mechanism underlying selective motor neuron death remains an essential question[4].

OBJECTIVE

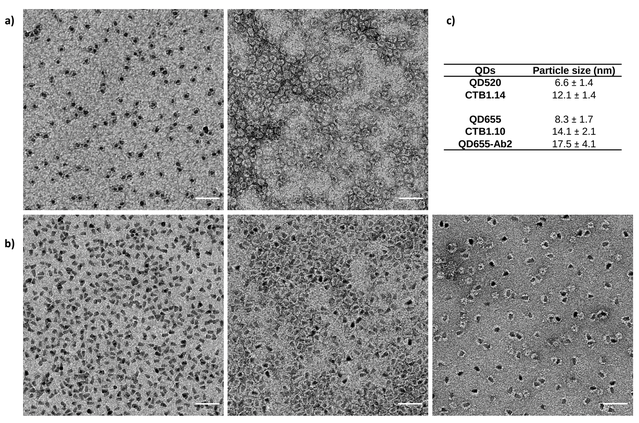
The objective of this work is to do a comparison between the characterization and labeling performance of several commercially available CdSe QDs and their conjugates in immunofluorescence (IF) in the neural cell line SH-SY5Y, primary human fibroblasts and immortalized lymphocytes derived from ALS patients.

METHODS

Two types of commercially available QDs have been used, including (1) secondary antibody conjugated QDs (QD-Ab2) and (2) amine-functionalized PEG-coated QDs that have been covalently conjugated in the laboratory to adaptor proteins A (SpA) and G (PG) before binding individually to primary antibodies to form QD-Ab probes. The emission peaks of the different QD-Abs were centered at 520, 605 and 655 nm and for QD-Ab2 at 565, 605 and 655 nm.

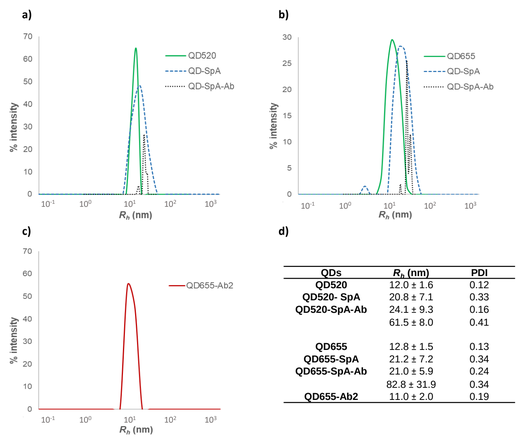
RESULTS

- **QDs characterization:** QD size, charge and its emission spectra were determined by several methodologies in order to help monitoring the lack of homogeneity and reproducibility between commercially available QDs batches.



QDs	Particle size (nm)
QD520	6.6 ± 1.4
CTB1.14	12.1 ± 1.4
QD655	8.3 ± 1.7
CTB1.10	14.1 ± 2.1
QD655-Ab2	17.5 ± 4.1

Figure 2. Transmission electron microscopy images of PEG-coated QDs, unconjugated, conjugated to SpA and commercially available QDs conjugated to secondary antibodies.



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QDs	R_h (nm)	PDI
QD520	12.0 ± 1.6	0.12
QD520- SpA	20.8 ± 7.1	0.33
QD520-SpA-Ab	24.1 ± 9.3	0.16
	61.5 ± 8.0	0.41
QD655	12.8 ± 1.5	0.13
QD655-SpA	21.2 ± 7.2	0.34
QD655-SpA-Ab	21.0 ± 5.9	0.24
	82.8 ± 31.9	0.34
QD655-Ab2	11.0 ± 2.0	0.19

Figure 3. Size distribution of QDs, QD bioconjugates and QD-Ab2 obtained by dynamic light scattering.

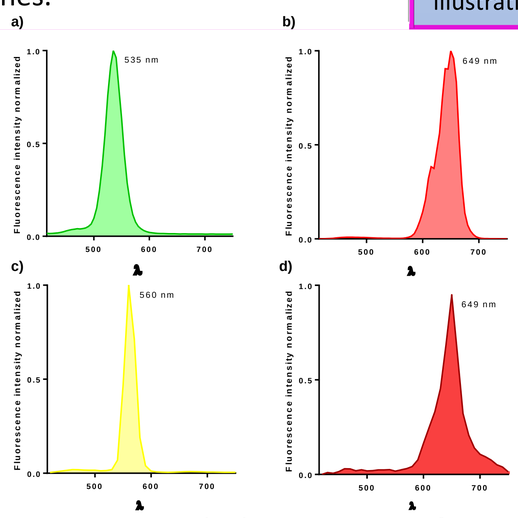

Figure 4. Spectral characterization of QDs. Emission spectrum of QDs was determined with a confocal microscope.

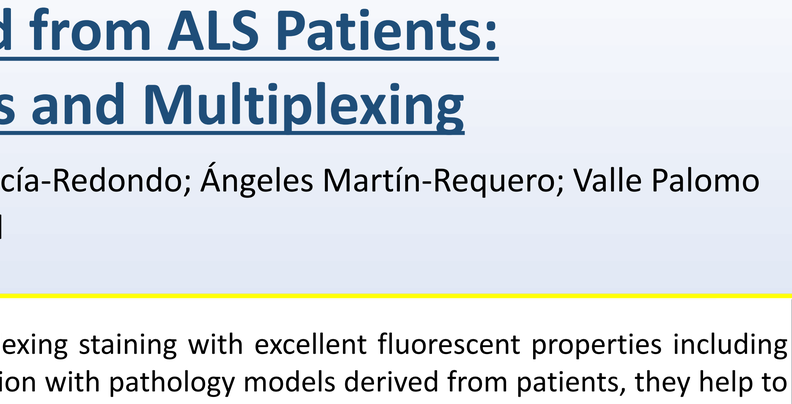

Figure 1. Bioconjugation strategy of QD-SpA or QD-PG and schematic illustration of the technology.

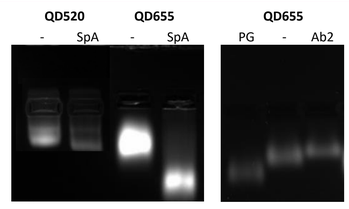

Figure 5. Electrophoretic mobility of unconjugated QDs, QD bioconjugates and commercially available QD-Ab2.

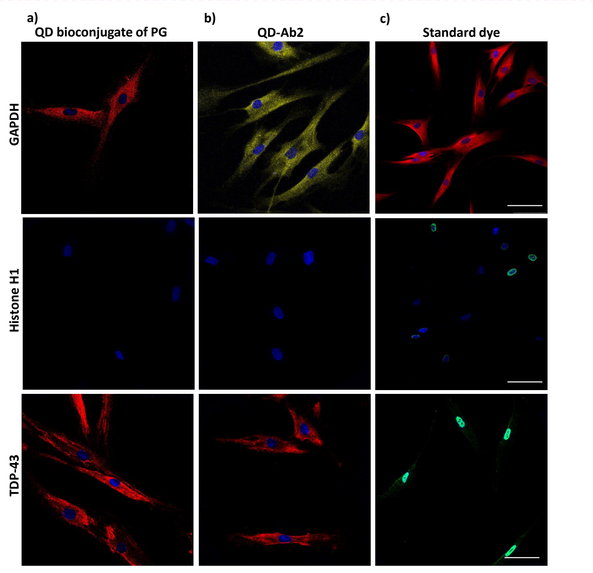

Figure 8. Labelling of different commercially available QD conjugates and classic IF in primary human fibroblasts from an ALS patient.

- **Immunoassay labelling:** After characterization, QD-immunofluorescence was performed to monitor differences between QDs probes and staining performance in cells derived from patients vs. commercial cell lines. The ability for nuclear penetration of QDs is one of the main discrepancies in the literature and was checked utilizing nuclear (Histone H1) and cytosolic (α –tubulin, GAPDH) proteins[5,6].

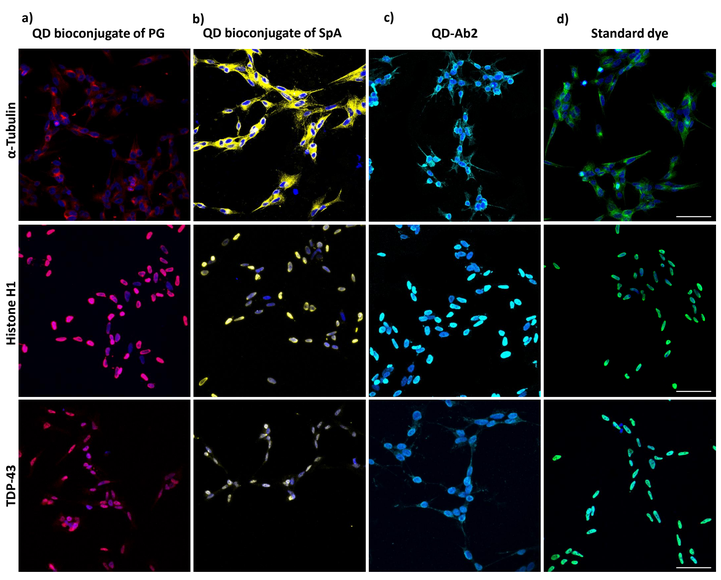

Figure 6. Staining of different commercially available QD conjugates and classic IF in SH-SY5Y cells.

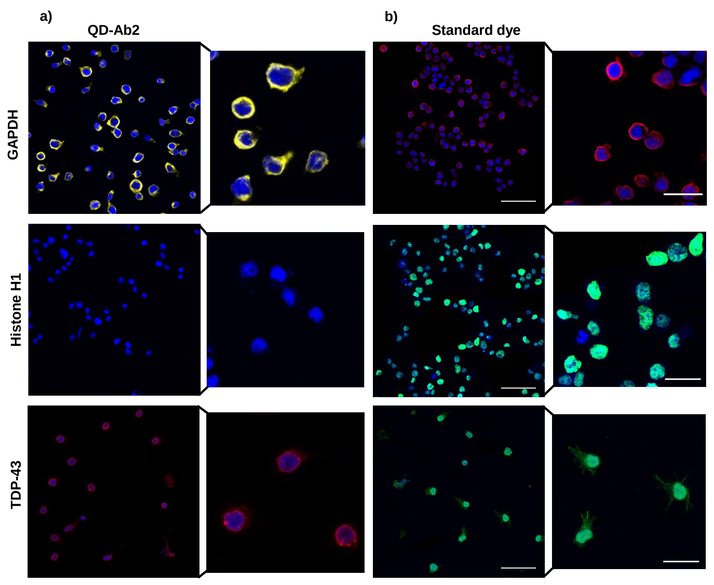

Figure 7. Staining of immortalized human lymphoblasts from ALS patient.

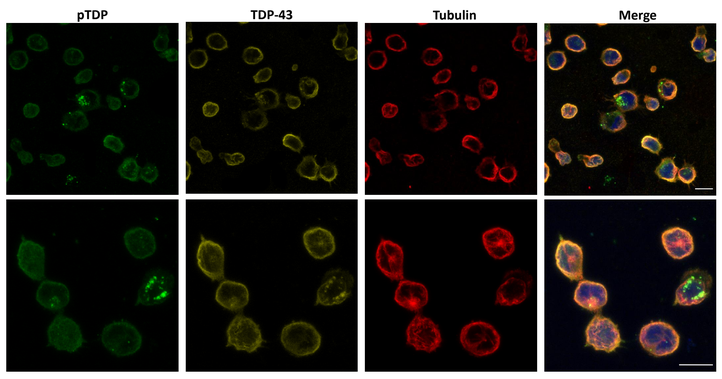

Figure 9. Multiplexing staining in a sporadic ALS patient.

CONCLUSIONS

- ✓ Cytosolic targets were successfully labelled with the different QDs achieving a similar staining to classic IF in the three types of cells.
- ✓ Nuclear target labelling was only achieved in the commercial line SH-SY5Y. This finding agrees with other studies in which it has been observed how nanoparticle permeability in live neural cell lines is significantly higher than in primary cells[7,8].
- ✓ The performance of extensive characterization techniques and protocols to achieve specific staining is needed to assure a proper function and reproducibility.
- ✓ We achieved a multiplexed staining of three targets on lymphoblasts derived from ALS patients.

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