Cellular Delivery Modalities for Semiconductor Quantum Dots: A Comparative Analysis

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ABSTRACT

Semiconductor nanocrystals or quantum dots (QDs) have been demonstrated to be superior alternatives to traditional fluorophores (dyes and fluorescent proteins) due to their size-tunable photoluminescence, nanoscale size which produces a high surface area to volume ratio, broad excitation profiles coupled with narrow emission spectra and resistance to photobleaching. The development of delivery modalities to introduce these nanoscale probes to discrete cellular locations with control continues to be an active area of research. These methods can range from passive uptake to facilitated delivery (using bioactive ligands such as peptides) to active manipulation of the cell (microinjection). Each of these methods comes with inherent advantages and liabilities. Here, we have conducted a comparative study utilizing these various cellular delivery strategies to illustrate how a particular labeling/imaging application can be enabled and/or enhanced by choosing the optimal delivery strategy.

Keywords: quantum dots, microinjection, cell penetrating peptides

1. INTRODUCTION

Intracellular delivery of theranostic nanoparticles is a critical area of drug research, the study of which can increase understanding of intracellular process and can increase the efficacy and specificity of designed therapeutics. Current methods of examining these processes include appending fluorescent molecules onto the desired proteins for visualization. The effectiveness of these imaging moieties is hampered by the inherent optical properties of the fluorescent dyes used. Mainly, many fluorescent dyes have a high degree of photobleaching and long maturation times, which limits the time-scales that intracellular processes can be visualized over effectively [1]. An alternative method of labeling utilizes semiconductor nanocrystals, or quantum dots (QDs) which have unique photophysical properties. These include high quantum yields (QYs), broad excitation profiles and narrow emission spectra at wavelengths correlated to the size of the synthesized QDs [2]. Additionally, QDs are resistant to chemical degradation and due to the nanometer size, provide a large surface area-to-volume ratio that is ideal for attachment and display of localization peptides or drug cargo. Due to these ideal properties, use of QDs as delivery modalities is a growing field, with many diverse opportunities for study. Delivery can vary between modification with biological molecules that allow for cellular uptake or active administration via microinjection for more specified delivery.

1.1 Cellular-Penetrating Peptides

Cellular-penetrating peptides are a burgeoning field that have allowed for internalization and specified subcellular delivery of nanoparticles. The ease of synthesis, small size and vast variation in sequence makes CPPs ideal for appending to carriers [3]. QDs allow for visualization of the final location of the cargo that is facilitated by the designed CPP. One of the current issues with CPPs is endosomal sequestration, which occurs due to the internalization methods inherent in cellular function [4]. Release from the endo-lysosomal pathway is critical for cytosolic delivery, and is a critical area of research to determine new methods of escape. Some peptides have been developed that harness the physiological changes that occur as the particles move from early to late endosomes, mainly the lowering of the vesicular pH from 7 to around 5.5 [5]. A peptide sequence rich in glutamate−alanine−leucine repeats dubbed GALA was designed to mimic viral fusion proteins and has been shown to facilitate endosomal release with various carriers [6]. Future work in this area will be facilitated by attempting various peptide changes and determining the most effective sequences for cytosolic delivery.

1.1 Microinjection

In addition to their abilities as carrier molecules, QDs can be used as FRET donors due to their tunable optical properties and their ability to attach to target molecules intracellularly after delivery [2]. We have previously shown that the addition of a His6 tag allows for metal-affinity coordination between tagged proteins and the QD surface and can produce FRET-based sensors (Figure1A) [7]. Addition of a Caspase3 cleavage site between the His6 tag and the mCherry acceptor molecule provided a mechanism for elimination in FRET after protein administration. Due to the FRET potential between 550 nm emitting QDs and mCherry, it can be extrapolated that such a system can be used for intracellular sensors [8]. As mentioned previously, cytosolic delivery of QDs is a difficult process. This can be

mediated through the use of microinjection, which allows for specified delivery to desired cells in an individual manner (Figure1B). Using such a localized and precise delivery method can increase understanding of intracellular functions.

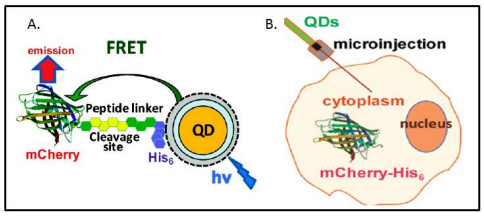

Figure 1A. Design for Caspase 3 specific sensor utilizing QDs as a donor and mCherry as an acceptor. The labeled His6 tag allows for the mCherry to be appended to the QD surface and a Caspase 3 cleavage site is localized to allow for mCherry to be released after Caspase 3 administration, and subsequently reducing FRET efficiency [8]. B. Schematic of microinjection process. Expressed mCherry containing a His6 residue is present intracellularly due to transfection and QDs are injected into the cytoplasm and allowed to complex and produce FRET. Adapted from ref. [7].

2. MATERIALS AND METHODS

2.1 QD synthesis and Peptides

QDs were synthesized as CdSe/ZnS (core/shell) with emission spectra centered around 550nm or 625nm. Various capping ligands were appended by exchanging the native hydrophobic capping shell with the desired ligand as previously described [9]. Peptides were synthesized and characterized as described previously [10]. Sequences used are described in Table 1.

Peptide	Focus	Sequence	Description	Mw / charge pH 7.4
JB577	Parent	WGDap$_d$VKIKKP$_9$GGH$_6$	Parent peptide	3034 / +3
JB729	Fatty acid	WGDap$_d$VKIKKP$_9$GGH$_6$	Palmitoyl → C_6F_{17}	3270 / +3
SI-09160	Fatty acid	WGDap$_e$VKIKKP$_9$GGH$_6$	Palmitoyl → cholesterol	3400 / +3
JB872	Fatty acid	WGC$_f$VKIKKP$_9$GGH$_6$	Palmitoyl → farnesyl	3017 / +3
JB865	Pro$_n$-linker	WGDap$_d$VKIKKP$_{12}$GGH$_6$	Pro$_9$ → Pro$_{12}$	3326 / +3
JB869	Charge	WGDap$_d$VRLP$_3$VRLP$_3$VRLP$_3$GGH$_6$	VKIKKP$_5$ → amphipathic-[VRLP$_3$]$_3$	3543 / +3
JB719	Control	KETWWETWWTEWSQPK$_6$RKVSGAAi-bA$_3$GGH$_6$	Chariot peptide	4297 / +4

Table1. Peptides studied for potential as CPPs. Parent peptide is JB577, with described sequence. All other peptides are modified versions with singular changes as labeled. Control peptide is commercially available Chariot peptide.

2.2 Cell Culture and QD delivery

Cell culture was performed using COS-1 cells maintained in DMEM (ATCC) supplemented with 10% FBS (ATCC) and 1% (v/v) antibiotic/antimycotic (Sigma). Cultures were grown in T25 flasks in 5%CO_2 at 37° C and passaged at 80% confluency. To perform QD deliveries, QD-peptide bioconjugates were produced by incubating the desired quantity of peptide with QDs in HEPES/DMEM and allowed to complex. Deliveries were performed on cells plated onto LabTek 8-well chambered cover glass (Nalge Nunc). Detailed description of delivery conditions is described here [10].

2.3 Microinjection and Imaging

COS-1 cells were plated and transfected using pmCherry-His6 N1 as previously described [7]. Injection was performed using an Eppendorf FemtoJet® Microinjector controlled by an InjectMan® NI 2 micromanipulator and 565nm ITK carboxyl/Ni2+ QDs. Images were taken with an Olympus IX-71 total internal reflection fluorescence microscope equipped with a 60X oil immersion lens, excitation was performed using a Xe lamp, and specific excitation and emission filters were used for all conditions.

3. RESULTS

3.1 Cellular-Penetrating Peptides

Efficient cellular uptake and endosomal release was described previously in relation to a peptide dubbed JB577. To determine the effect of certain moieties within the peptide sequence, variations on this peptide were synthesized and are described in Table 1. Of particular interest was the palmitoyl group in the parent peptide, and other changes were made within the sequence such as a lengthening of the proline chain. To determine the effectiveness against a commercially available control (Chariot), cells were administered QDs appended with the synthesized peptides and localization was viewed (Figure 2).

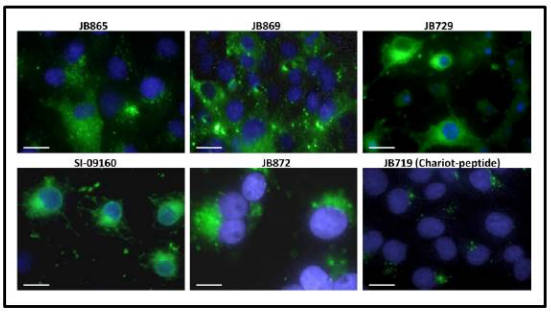

Figure 2. Modified peptides conjugated with 550 emitting QDs (Green) were visualized with DAPI as a co-stain (blue). Administration of the QD-peptide bioconjugates was performed at a QD concentration of 100nM with 75 peptides per QD. Cells were incubated with the constructs for 2 hours, after which the cells were washed and allowed to culture for 48 hours. Chariot peptide was used as a control. Image adapted from ref. [10].

All peptide variants increased the intracellular distribution of the QD-peptide bioconjugates over JB577

(not shown). Additionally, the synthesized peptide sequences are markedly better than the Chariot peptide at endosomal release, as can be seen by the disperse QD presence in all images except the bottom right, which shows punctate sequestration of the internalized QDs.

3.2 Microinjection

Cells transfected with mCherry containing a His6 segment were injected with 565 nm emitting ITK-COOH QDs. The cells were then imaged for both mCherry excitation and emission and FRET between the QD and the mCherry complex over a period of time to determine the effects of photobleaching (Figure 3). Addition of the QD allows for mCherry visualization for longer periods of time, reducing the photobleaching and producing efficient FRET over 60 seconds.

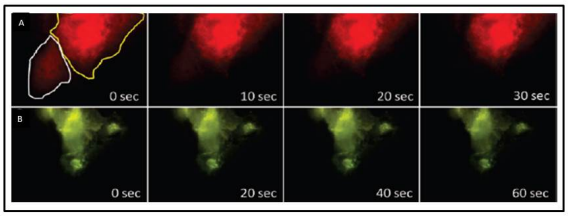

Figure 3. COS-1 cells transfected to express mCherry-His_6 (Red) and FRET between QDs and mCherry (Green). Images were taken at labeled time points during continuous excitation. Note the photobleaching of mCherry in an uninjected cell (white outline) vs. the photobleaching in an injected cell (yellow outline) [7].

4. CONCLUSION

Semiconductor QDs are a viable and effective method of visualizing administration methods, both through passive administration and internalization through conjugation with peptides and active administration using microinjection. Their photophysical properties allow for easy determination of intracellular localization after internalization and for FRET sensors to be designed and implemented effectively.

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