Conjugation of DNA to Streptavidin-coated Quantum Dots for the Real-time Imaging of Gene Transfer into Live Cells

J. K. Kim[*], S. H. Lim[*], Y. Lee[**], Y. S. Shin[**], C. Chung[*], J. Y. Yoo[**] and J. K. Chang[*,***]

[*]Digital Bio Technology, Co., Seoul 151-742, Korea
[**]School of Mechanical and Aerospace Engineering, [***]School of Electrical Engineering and Computer Science, Seoul National University, Seoul 151-742, Korea

ABSTRACT

We have developed the method for the conjugation of biotinylated DNA to streptavidin-coated quantum dots (QDs). QD-DNA conjugates and a highly sensitive fluorescence imaging technique are adopted to visualize gene transport across the membrane of the live cell in real time. Endocytotic cellular uptake of oligonucleotide is monitored by a real-time confocal imaging system. Long-term kinetic study enables us to reveal the unknown mechanisms and rate-limiting steps of extracellular and intracellular transport of DNA. Gel electrophoresis is used to verify the effect of incubation time and the molar ratio of QDs to DNA on the conjugation efficiency. It is possible to fractionate the QD-DNA conjugates according to the DNA concentration and obtain the purified conjugates by a gel extraction technique. QD-DNA conjugates have a potential to be nanoscale building blocks by self-assembly process as well as a versatile tool for fluorescence imaging and monitoring of biological systems.

Keywords: quantum dot, conjugation, gene transport, live cell imaging, gel electrophoresis

1 INTRODUCTION

Fluorescent semiconductor nanocrystals, quantum dots (QDs), have significant advantages over the classical organic dyes with their unique properties and they are expanding application fields in life sciences [1]. DNA labeling by conjugation of QDs to DNA is the first step for studying kinetics of DNA transfer from the extracellular to the intracellular space through live cell imaging techniques. Strategies for the conjugations of DNA to gold nanocrystals [2] or silanized semiconductor nanocrystals [3] have been developed by other groups. Jaiswal et al. [4] have developed procedures for using QDs to label live cells and applied them for long-term multiple color imaging. We have developed the method for the conjugation of biotinylated DNA to streptavidin-coated QDs. Optimized reaction condition is determined from an analogous scale-up conjugation model using micron-sized beads. QD-DNA conjugates and a high-sensitive fluorescence imaging technique are adopted to visualize gene transport across the membrane of the live cell in real time. Endocytotic cellular uptake of oligonucleotide, electrically-mediated plasmid DNA transfer into the live cell, and localization of DNA in the intracellular compartments are monitored by a real-time confocal imaging system. Elucidating the rate-limiting steps and deciphering the mechanism of gene delivery to the cell nucleus are crucial for the understanding of transfection as well as for the development of gene therapy tools.

2 MATERIALS AND METHODS

2.1 QD-DNA Conjugation

To obtain purified conjugates, careful considerations are required for attaching fluorescent nanocrystals to biological macromolecules covalently. We designed experimental protocols to conjugate the biotinylated oligonucleotide or the plasmid DNA to commercially available streptavidin-coated QDs (Qdot^{TM} 605 Streptavidin Conjugate, Quantum Dot Corp., CA, USA). Biotinylated oligonucleotides (ACE/F-biotin) were purchased from Bioneer (Daejeon, Korea). Each strand has a biotin group at the 5′ end. We used angiotensin-converting enzyme (ACE) gene. (5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′) which has 24 base pairs and its estimated total length is about 8 nm. Biotinylation of 4.7 kb pEGFP-N1 plasmid (Clontech, CA, USA) was realized by three different approaches; biotin labeling by nick translation with Klenow enzyme and random hexamer, by end-filling with Klenow enzyme, and by adding one or more deoxynucleotide on to the 3' terminus of a DNA molecule with terminal deoxynucleotidyl transferase (TDT). Schematic of QD-DNA conjugation is shown in Figure 1. Streptavidin-coated QDs diluted by 1/100 were mixed with biotinylated DNA and incubated at room temperature.

2.2 Electrophoretic Fractionation of QD-DNA Conjugates

Inappropriate reaction condition for QD-DNA conjugation resulted in complex formation by aggregation. Gel electrophoresis was used to verify the effect of incubation time and the molar ratio of QDs and DNA on the conjugation efficiency. The concentration ratio of QD to DNA solutions was varied from 1:20 to 1:0.025 resulting in 10 mixtures. The QD-DNA conjugates were diluted in loading buffer and were run in 0.5 × tris-borate-EDTA buffer on a 2% agarose gel at 10 V/cm. The gels were post

stained with ethidium bromide to illuminate the DNA. A polaroid camera was used to acquire the fluorescence images of QDs and DNA illuminated with an ultraviolet transilluminator. Fractionated conjugates were obtained by standard gel extraction technique.

2.3 Scale-up Model of Avidin-Biotin Conjugation

To avoid drying effects occurring in the use of transmission electron microscopy (TEM) for characterizing the nanoscale structures, we investigated DNA-mediated assemblies of 2-μm beads coupled with avidins and 0.5-μm beads conjugated with ACE/F-biotin using confocal microscopy. Carboxylate-modified FluoSpheres beads purchased from Molecular Probes (OR, USA) were diluted to final concentration of 0.5% with one ml PBS buffer. They were coated by incubation with 0.2 mg/ml EDC and 0.2 mg/ml NHS at room temperature to facilitate coupling with avidins. The molar ratios of avidins coupled with 2-μm beads to ACE/F-biotin conjugated with 0.5-μm beads were 1.67 μM:1.6 μM, 1.67 μM:16 nM, 16.7 nM:1.6 μM, and 16.7 nM: 16 nM. A control system was made by mixing of the bidisperse beads that were not functionalized.

2.4 Cell Preparation

SKOV and 293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Sigma), penicillin (100 units/ml), streptomycin (100 μg/ml) and L-glutamine (4 mM) at 37°C in a humidified 5% CO_2 incubator. Cells were dissociated from the 25-cm^2 tissue culture flask by using Trypsin-EDTA. The final cell suspension density was adjusted to 1×10^4 cells/ml. The cells were transferred to 8-well chambered coverglass (Lab-Tek II, Nalge NUNC International, IL, USA) and monitored by an imaging system after QD-DNA conjugates were added to each well.

2.5 Confocal Imaging System

Confocal fluorescence images of QD-DNA conjugates translocated into live cells were taken using CARV spinning-disk confocal system (Atto Bioscience, MD, USA) equipped with an Olympus IX71 fluorescence microscope and a Sensicam cooled CCD camera (Cooke, Mich, USA) as shown in Figure 2. Color images were acquired by a 3-CCD camera (AW-E300, Panasonic, CA, USA). The QDs fluoresce at any excitation wavelength in the visible range and emit at 605 nm. Excitation light from a 100 W mercury lamp was filtered by a 460-500 nm bandpass filter and then reflected by a dichroic mirror and focused through a 100×/1.4 NA objective into the sample. The fluorescence emission from the QDs was filtered with a longpass filter which transmits light above 510 nm. 640 × 480 pixel images were obtained by the cooled CCD camera in 2 × 2 binning mode with appropriate background rejection.

3 RESULTS AND DISCUSSION

Fig. 3 shows the gel electrophoresis migration pattern. We can observe the colocalized patterns of QDs and DNA by comparing the fluorescent gel images of QD and QD-DNA conjugates, which indicates that most DNA is specifically bound to the QDs. There was almost no existence of the complex formation even after overnight incubation. It is possible to fractionate the QD-DNA conjugates according to the DNA concentration and obtain the purified conjugates by a gel extraction technique.

Figure 4 represents confocal fluorescence images of assembly structures in suspensions comprised of 2.0 and 0.5-μm beads coupled with avidins and ACE/F-biotin, respectively. Self-assembled structures were compared with aggregates in the control system. The DNA-driven assemblies in the two-bead system were significantly affected by the concentration of avidins and highly aggregated patterns were observed at the control system.

Z-series confocal images of the cultured SKOV cells are shown in Figure 5. Lower right corner image is overlay of the bright-field and the confocal fluorescence images. Due to the high photo stability and high brightness of QDs we could acquire time-lapse fluorescent DNA images for a long time and analyze them quantitatively by appropriate image processing schemes. Figure 6 shows bright-field (upper row) and confocal fluorescence (lower row) images of the cultured cells uptaking QD-oligonucleotide conjugates by endocytosis. Highly localized patterns of QDs are observed predominantly in perinuclear region. It should be noted that the distribution of QD-DNA conjugates was asymmetric around the cell nucleus. Figure 7 shows z-series confocal fluorescence images of the 293 cell. The complicated cellular structures, such as a nucleus and vesicles, could be discriminated by quantum dot labeling and confocal imaging.

4 CONCLUSION

We have designed efficient protocols for the conjugation of biotinylated oligonucleotides or plasmid to streptavidin-coated QDs and have observed the localization patterns of DNA in live cells using real-time confocal fluorescence microscope system. Long-term kinetic study by monitoring multiple colored images of high spatial and temporal resolution will enable us to reveal the unknown mechanisms and rate-limiting steps in extracellular and intracellular gene delivery process.

REFERENCES

[1] W. J. Parak, D. Gerion, T. Pellegrino, D. Zanchet, C. Micheel, S. C. Williams, R. Boudreau, M. A. Le

Gros, C. A. Larabell and A. P. Alivisatos, Nanotechnology, 14, R15-R27, 2003.
[2] D. Zanchet, C. M. Micheel, W. J. Parak, D. Gerion and A. P. Alivisatos, Nano Letters, 1, 1, 32-35, 2001.
[3] W. J. Parak, D. Gerion, D. Zanchet, A. S. Woerz, T. Pellegrino, C. Micheel, S. C. Williams, M. Seitz, R. E. Bruehl, Z. Bryant, C. Bustamante, C. R. Bertozzi, and A. P. Alivisatos, Chem. Mater., 14, 2113-2119, 2002.
[4] J. K. Jaiswal, H. Mattoussi, J. M. Mauro and S. M. Simon, Nat. Biotech., 21, 47-51, 2003.

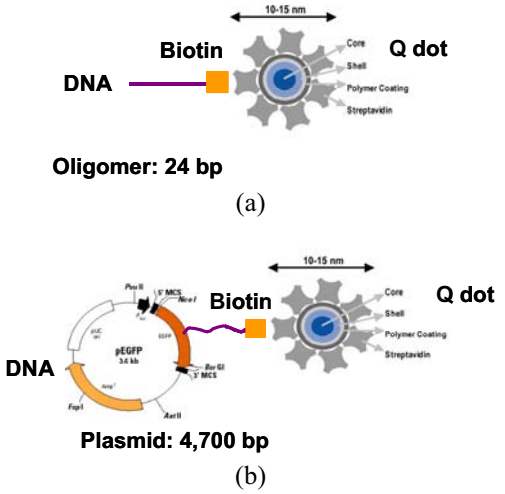

Figure 1: Schematic diagram of the conjugation of streptavidin-coated quantum dots to biotinylated DNA: (a) QD-oligonucleotide, (b) QD-plasmid.

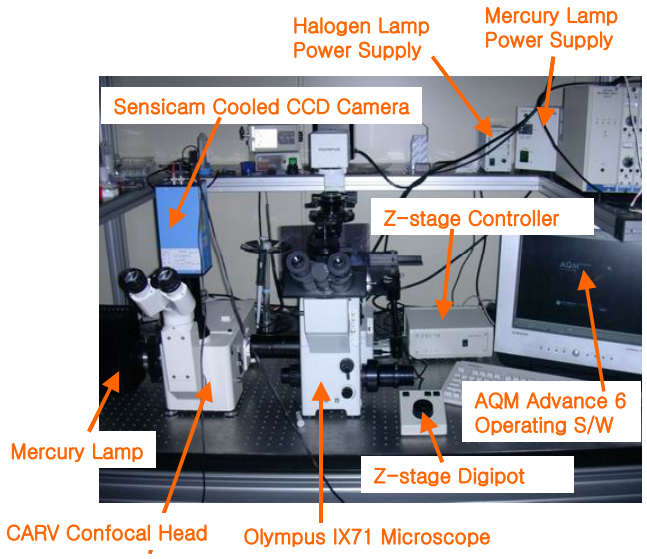

Figure 2: Spinning-disk type confocal microscope system for the real-time imaging of gene transfer into live cells.

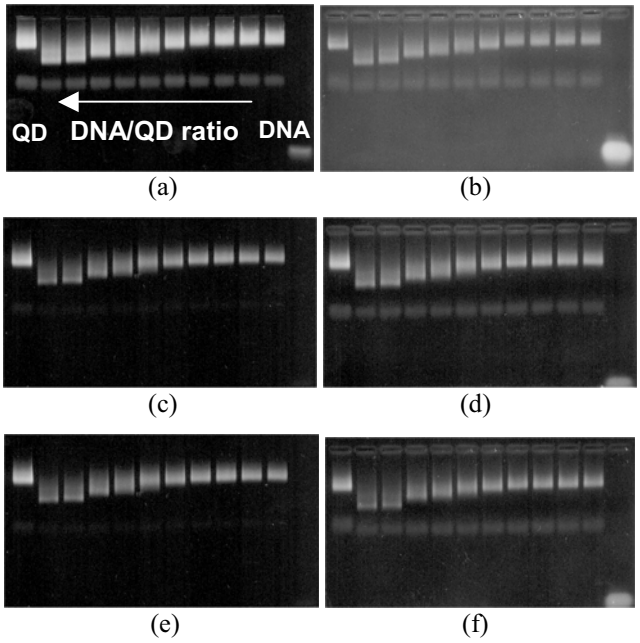

Figure 3: Gel electrophoresis of QD-oligonucleotide conjugates. (a) 1 h incubation, (b) 1 h incubation, EtBr stained, (c) 1 h incubation, (d) 2 h incubation, EtBr stained, (e) 24 h incubation, (f) 24 h incubation, EtBr stained.

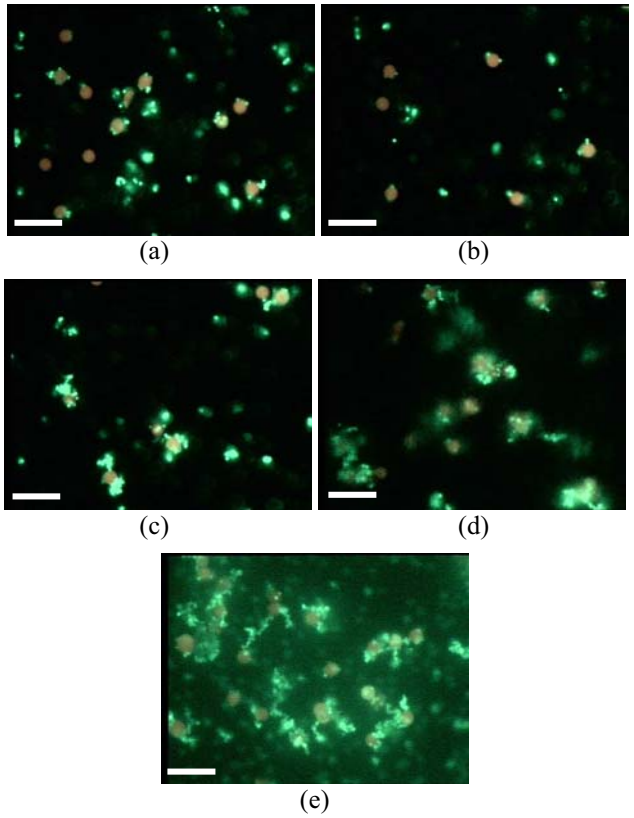

Figure 4: Confocal fluorescence images of assembly structures (a-d) in suspensions comprised of 2.0 and 0.5 μm beads coupled with avidins and ACE/F-biotin, respectively. (e) control system. Bar, 10 μm.

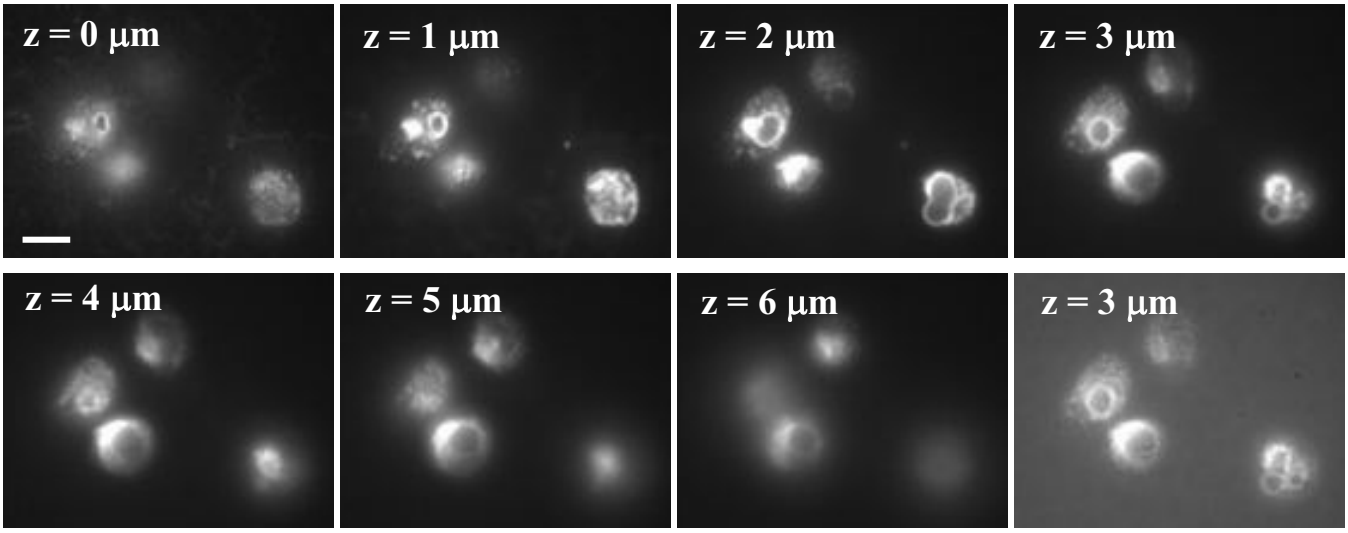

Figure 5: Z-series confocal images of the cultured SKOV cells. Lower right corner image is overlay of the bright-field and the confocal fluorescence images. Bar, 10 μm.

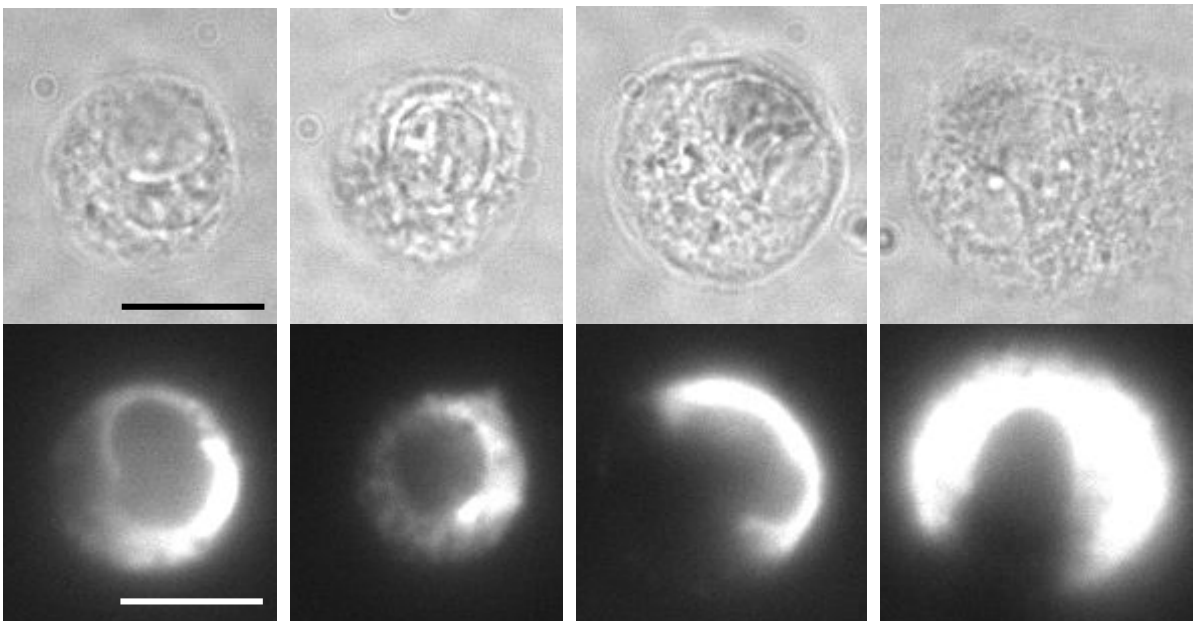

Figure 6: Bright-field (upper row) and confocal fluorescence (lower row) images of the cultured cells uptaking quantum dot-oligonucleotide conjugates by endocytosis. Highly localized patterns of quantum dots are observed in perinuclear region. Bar, 10 μm.

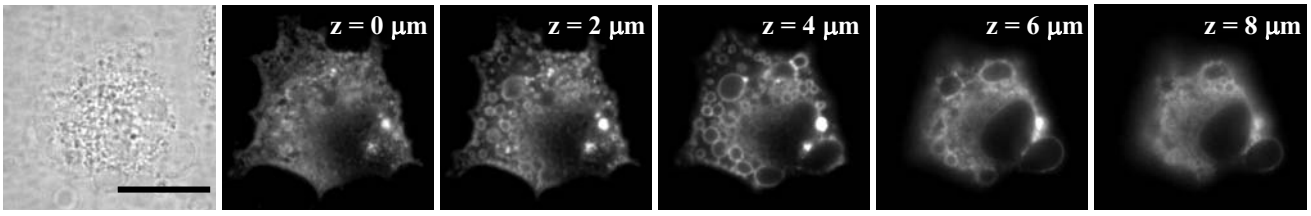

Figure 7: Bright-field (left) and z-series confocal fluorescence images of the 293 cell. The cellular structures, such as a nucleus and vesicles, are discriminated by quantum dot labeling. Bar, 10 μm.