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Visualization of Gene Transfer into Live Cells Using Fluorescent Semiconductor Nanocrystals

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Abstract

We have developed the method for the conjugation of biotinylated DNA to streptavidin-coated QDs. 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 and electrically-mediated plasmid DNA transfer into the live cell are monitored by a quantitative microscopic imaging system. Long-term kinetic study enables us to reveal the unknown mechanisms and rate-limiting steps of extracellular and intracellular transport of biomolecules. We designed experimental protocols to conjugate the oligonucleotide or the plasmid DNA to commercially available streptavidin-coated QDs. Gel electrophoresis is used to verify the effect of incubation time and the molar ratio of QDs and 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.

Key Words : Live Cell Imaging(생체세포 가시화), Semiconductor Nanocrystal(반도체 나노결정), Quantum Dot(양자점), Conjugation(접합), Self-assembly(자가조립)

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]. We have developed the method for the conjugation of biotinylated DNA to streptavidin-coated QDs. QD-DNA conjugates and a high-sensitive fluorescence imaging technique[2] are adopted to visualize gene transport across the membrane of the live cell in real time. Endocytotic cellular uptake of oligonucleotide and electrically-mediated plasmid DNA transfer into the live cell are monitored by a quantitative microscopic imaging system. Long-term kinetic study enables us to reveal the unknown mechanisms and rate-limiting steps of extracellular and intracellular transport of biomolecules.

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2. Materials and Methods

To obtain purified conjugates, careful considerations are required for attaching fluorescent nanocrystals to biological macromolecules covalently. Strategies for the conjugations of DNA to gold nanocrystals[3] or silanized semiconductor nanocrystals[4] have been developed by other groups. We designed experimental protocols to conjugate the oligonucleotide or the plasmid DNA to commercially available streptavidin-coated QDs (Qdot™ 605 Streptavidin Conjugate, Quantum Dot Corp., CA). DNA biotinylation is 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).

3. Results and Discussion

Inappropriate reaction condition for QD-DNA conjugation resulted in complex formation by aggregation as shown in Fig. 1. Gel electrophoresis is used to verify the effect of incubation time and the molar

ratio of QDs and DNA on the conjugation efficiency. Fig. 2 shows the gel electrophoresis migration pattern. By comparing the fluorescent gel images of QD and QD-DNA conjugates we can observe the colocalized patterns of QDs and DNA, 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. Fig. 3 shows the bright-field and fluorescence images of cultured Vero cells incubated with QD-oligonucleotide conjugates and electroporated with the presence of QD-plasmid conjugates. We can visualize the translocation of DNA molecules with different lengths by long-term multiple color imaging of live cells. 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.

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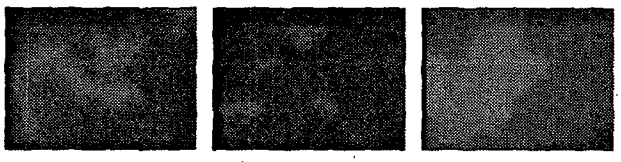
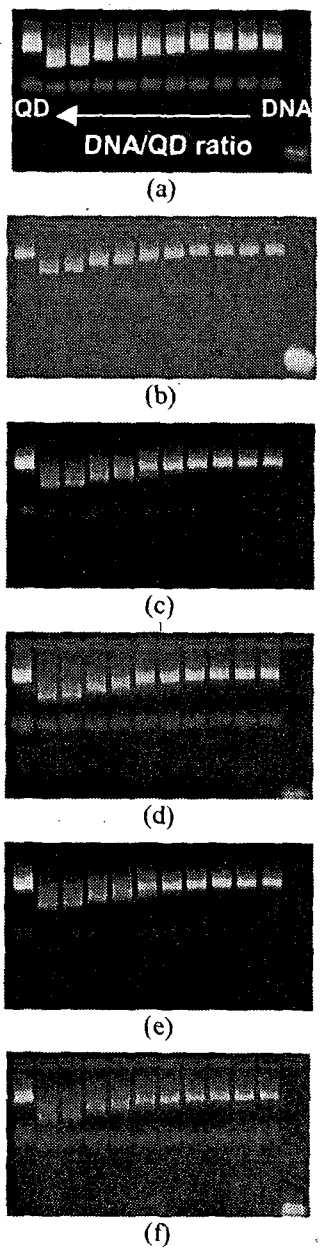


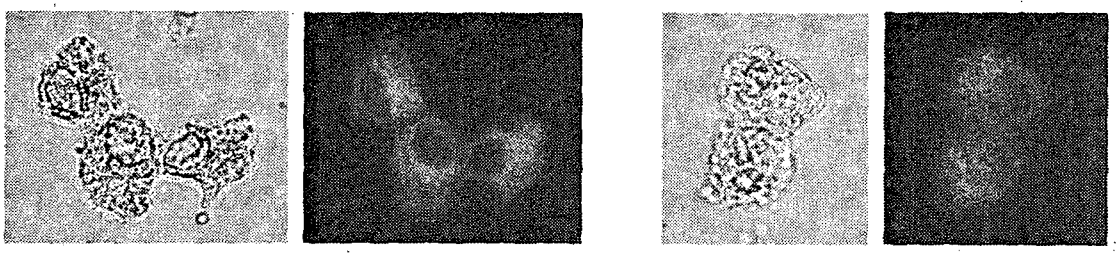
(a) (b) (c)

Fig. 1. Aggregated QD-DNA conjugates after overnight incubation in stirrer. (a) QD-oligonucleotide, (b) QD-plasmid (nick translation), (c) QD-plasmid (end filling).



(a) (b) (c) (d) (e) (f)

Fig. 2. 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.



(a) (b)

Fig. 3. Bright-field (left) and fluorescence (right) images of cultured Vero cells (a) incubated with QD-oligonucleotide conjugates and (b) electroporated with the presence of QD-plasmid conjugates.