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The Detection of Apolipoprotein E-epsilon 4 gene (APOE ε4) via Fluorescence Quenching of Quantum Dots Incorporated Magnetic Beads in Micro-fluidic Chip

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The APOE ε4 gene is a susceptibility gene or risk factor for Alzheimer's disease. The gene codes for a protein which limits the brain's ability to break down amyloid-beta, a protein that is toxic to the brain. In this study, we have prepared magnetic beads - CdSe/ZnS QDs-APOE probe DNA complexes for the detection of APOE DNA. QDs were conjugated to amine- functionalized magnetic beads by EDC/Sulfo-NHS coupling reaction. Thiol group modified single stranded probe oligonucleotides of APOE ε2 and APOE ε4 were immobilized on the surface of QDs to prepare magnetic bead-QDs-DNA complexes. APOE genes could be detected by fluorescence quenching of QDs by intercalation dye after hybridization. PDMS based micro-fluidic chips were prepared and the bead-QDs-DNA complexes were packed on the channel of the microfluidic chip. The APOE ε2 and APOE ε4 gene could be detected via fluorescence quenching of QDs on the channel.

Keywords Alzheimer's disease; APOE ε4 gene; QD; Microfluidic chip

1. Introduction

Recently, there have been several attempts to integrate the nanotechnology with biology and medicine, which lead to many noticeable advances in molecular diagnostics, therapeutics, molecular biology, and cell biology [1]. Quantum dots (QDs), or colloidal semiconductor nanocrystals, have been one of the most attractive and extensively studied nano-materials combining nanotechnology and biotechnology. QDs have many peculiar optical properties such as wide broad range of excitation wavelengths with a narrow emission spectrum, size tunable emission peak, and higher stability against photo-bleaching than conventional organic fluorescence dyes [2]. The application of QDs includes luminescent probes for biomedical imaging, biosensor, chemosensor, new materials for lasers and other photonic devices, and quantum computing [3].Various biosensors using FRET phenomena between QDs and fluorescence dye have been developed for the detection of specific DNA [4]. Several approaches have been made to Alzheimer's disease (AD) investigation via genetic

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and molecular engineering [5]. Alzheimer's disease is a heterogeneous genetic disorder which causes the degeneration of neurons in the cerebral vessels resulting in dementia paranoids [6]. Many kinds of devices have been developed to detect AD, including microarray, bio barcode, and microfluidic chip [7]. The apolipoprotein E (ApoE) is a plasma protein involved in cholesterol transport. The gene (*APOE*) is located on the long (q) arm of chromosome 19 and codes for a protein that exist in several different forms [8]. The *APOE* polymorphism is known as an important risk factor in the development of AD and cardiovascular disease. *APOE* genotype also influences susceptibility to atherosclerosis and vascular dementia. The *APOE* gene exists in three different forms, $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$. *APOE* $\varepsilon 2$ is relatively rare and known to protect AD. *APOE* $\varepsilon 3$ is believed to play a neutral role in the disease. Though the effect of *APOE* $\varepsilon 4$ allele as a risk factor for AD is influenced by age and ethnicity, people who develop AD are more likely to have an *APOE* $\varepsilon 4$ allele [9]. The *APOE* $\varepsilon 4$ carriers show poorer cognitive performance and executive functioning [10]. Recently various biosensors have been developed for rapid diagnosis and detection of viruses, bacteria, and screening for hereditary diseases. Various DNA sensors have been prepared which use acoustic, electrochemical, and optical responses to specific target genes. The authors recently developed a specific DNA detection method which is based on fluorescence quenching of QDs. Microbead-QDs complexes with probe DNA were prepared, and the p53 gene and K-ras oncogene could be detected in the solution and in the microfluidic chip[4, 11]. Very rapid detection of target DNA with low concentration was possible. Various methods of genotyping of APOE gene were used including single strand conformation polymorphism (SSCP) [12], hybridization with allele specific oligonucleotides (ASO) [13], direct sequencing [14]. But all these techniques requires time consuming amplification. We applied QD based DNA detection to *APOE* gene. In this paper we designed probe DNAs to distinguish APOE $\varepsilon 4$ from APOE $\varepsilon 2$ and $\varepsilon 3$. Among 264 bp DNA sequence [15], 30 bp DNA sequence was selected to represent APOE $\varepsilon 4$. We have prepared magnetic beads - CdSe/ZnS QDs-APOE probe DNA complexes for the detection of APOE DNA. QDs were conjugated to amine- functionalized magnetic beads by EDC/Sulfo-NHS coupling reaction. Thiol group modified single stranded probe oligonucleotides of APOE $\varepsilon 2$ and APOE $\varepsilon 4$ were immobilized on the surface of QDs to prepare magnetic bead-QDs-DNA complexes. The fluorescence from QDs could be quenched by intercalation dye (TOTO-3) after hybridization.

2. Experimental

2.1. Material and Instruments

Magnetic beads (SPHEROTM, diameter: 8.27μm) were purchased from Spherotech (IL, USA). Qdot®ITK™ quantum dots (QDs 525nm and 605nm) in borate buffer, TOTO-3, and PI intercalation dye were purchased from Invitrogen (NY). The phosphate buffer at pH 7 was purchased from Across Organic (USA) and borate buffer at pH 6.4 and Tris-HCl buffer at pH 7.4 were obtained from Bioneer (Daejeon, Korea). SU-8 2050 and SU-8 developer were purchased from MicroChem (Newton, MA). PDMS prepolymer (Sylgard 184) and a curing agent were purchased from Dow Corning (Midland, MI). 5′-C6-thiol-modified probe and target oligonucleotide were synthesized by Bioneer (Daejeon, Korea). The oligonucleotide sequences are shown in Table 1. Florescence image was obtained using IM-1 2005 Ratio Fluorescence Imaging System (PTI, USA), equipped with a xenon lamp and a fluorescent microscope (Olympus IX71). The excitation wavelength was kept

Table 1. Oligonucleotide sequences used in this study

	Use	Name	Sequence	T_m
APOE $\varepsilon 2$	Probe	Thiolated oligonucleotide	5′-HS-$(CH_2)_6$- CTG CAC CAG GCG GCC GCA CAC GTC CTC CAT -3′	81.7°C
	Target	Oligonucleotide	5′- ATG GAG GAC GTG TGC GGC CGC CTG GTG CAG -3′	81.7°C
APOE $\varepsilon 4$	Probe	Thiolated oligonucleotide	5′-HS-$(CH_2)_6$- CTG CAC CAG GCG GCC GCG CAC GTC CTC CAT -3′	83.9°C
	Target	Oligonucleotide	5′- ATG GAG GAC GTG CGC GGC CGC CTG GTG CAG -3′	83.9°C

at 400 nm via monochromator. Photoluminescence spectra were obtained using a Quanta Master spectrofluorometer (PTI, USA). Fluorescence intensity from QDs in microfluidic chip were obtained using Nikon SMZ1500 stereoscopic microscope with CCD camera and image software.

2.2. Preparation of Magnetic Bead-QDs-DNA Probe

The QDs with carboxyl group (QD@525 and QD@605) were conjugated to magnetic beads with amine functional group by EDC/Sulfo-NHS coupling reaction. The magnetic beads were incubated overnight in a mixture of 100/10 mM EDC/Sulfo-NHS (prepared in 0.1M borate buffer, pH 6.4), 8μM of QDs@525, and 0.4μM of QDs@605, kept in dark at room temperature. The magnetic beads-QDs complex was washed using phosphate buffer. Then, the magnetic beads-QDs were mixed with thiolated probe DNA in phosphate buffer and kept overnight at room temperature for conjugation with probe DNA. After this step, the magnetic beads-QDs-DNA probe was dissolved in 10mM of Tri-HCl buffer with 1M NaCl. In order to identify the conjugation of probe DNA and QDs, the conjugated products were stained with 1 μg/ml of ethidium bromide (EtBr) and subjected to electrophoresis on a 2% agarose gel.

2.3. Fabrication of Microfluidic Chip

The schematic of the microfluidic chip with magnetic beads-QDs-DNA is shown in Figure 1. PDMS microfluidic chip (25 mm × 12 mm) was fabricated using the protocols described in a previous paper [11]. SU-8 replica masters were fabricated on a silicon wafer using photolithography process. The channel width and height were 35 μm and 50 μm, respectively. The microfluidic chip was prepared from SU-8 mold through the thermal curing of PDMS. The surface of the PDMS replica was treated with oxygen plasma (10.5W, 60sec) and attached to the slide glass. The punched holes on the channel were used as inlet and outlet, and silicone tubes (OD = 1.6 mm, ID = 0.8 mm) were inserted into these ports to introduce magnetic beads and sample solution.

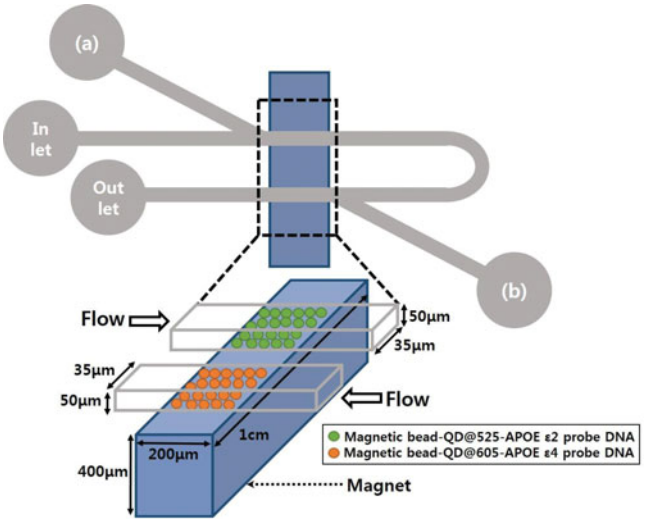

Figure 1. The schematic of the microfluidic chip with magnetic beads-QDs-DNA. The channel width and height were 35 μm and 50 μm, respectively. The magnet width, length, and height were 1cm, 200 μm, and 400 μm, respectively.

2.4. DNA Hybridization and Detection by Fluorescence Quenching

The QD conjugated probe were mixed with 100 μM of target oligonucleotide and kept for 1hr at room temperature for hybridization. The magnetic bead-QDs-DNA probes were packed into a channel defined by magnetic forces in the micro-channel of the PDMS chip. Before loading the target gene mixture, 0.5% BSA was injected and coated the surface of micro channel by incubating for 1hr to prevent the adsorption of nucleotides. The flow rate of buffer solution was kept at a constant value of 1μl/min in all experiments by using syringe pump. A 100 μM target gene mixed with PI and TOTO-3 was injected into the micro-channel.

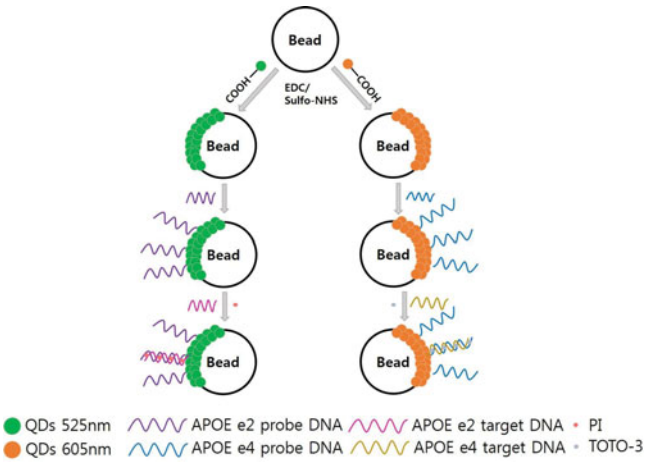

Figure 2. The detection scheme of APOE ε2 and APOE ε4 by fluorescence quenching of QDs.

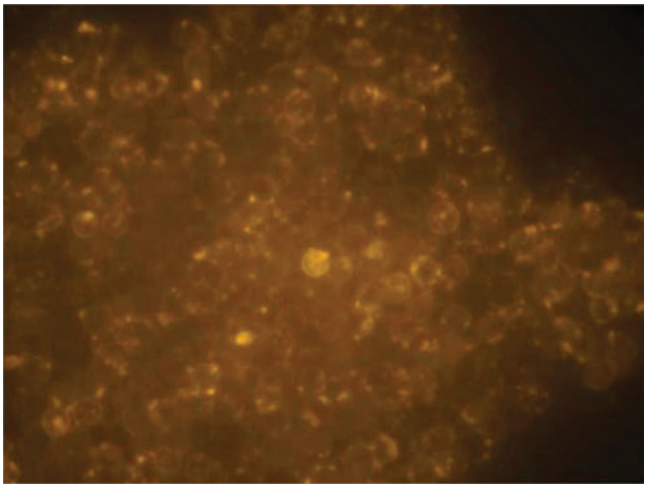

Figure 3. The fluorescence microscope image of magnetic bead-QDs on a slide glass under dark field.

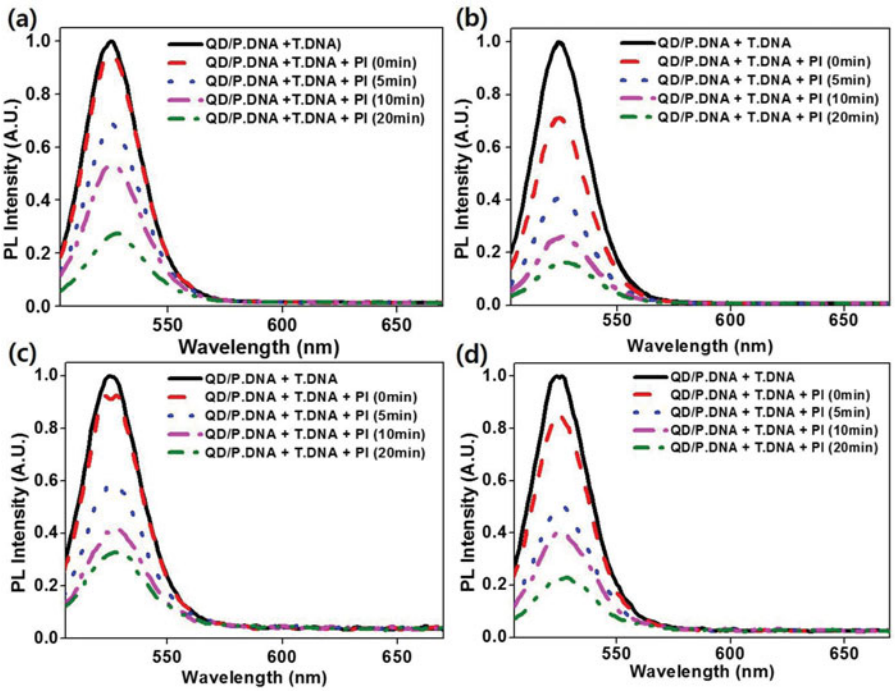

Figure 4. Fluorescence spectra obtained from QD@525 -probe DNA (APOE $\varepsilon 2$) after hybridization with target DNA and intercalation by various concentration of PI : (a) 0.2 μM (b) 0.4 μM (c) 0.8 μM (d) 1.6 μM. The excitation wavelength is 450 nm. The concentration QDs and DNA were 8 μM and 100 μM, respectively.

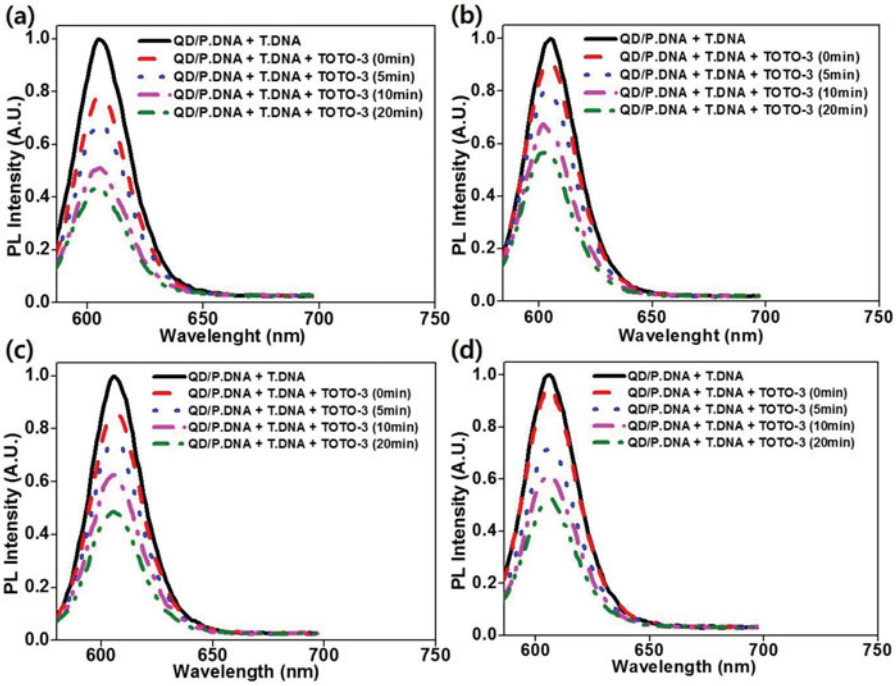

Figure 5. Fluorescence intensity from QD@605-probe DNA (APOE $\varepsilon 4$) gradually decreases by addition of TOTO-3: (a) 0.2 μM (b) 0.4 μM (c) 0.8 μM (d) 1.6 μM. The excitation wavelength is 400 nm. The concentration QDs and DNA were 0.4 μM and 100 μM, respectively.

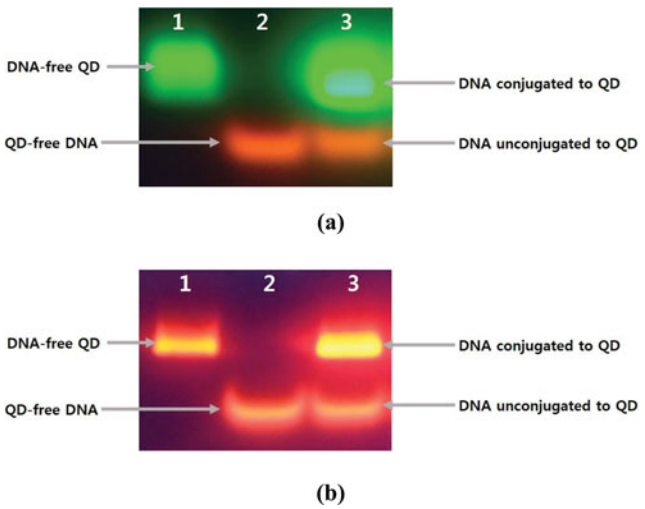

Figure 6. (a) The electrophoresis diagram of APOE $\varepsilon 2$ probe DNA conjugated with QD@525 and intercalation with EtBr; line 1 is QD@525, line 2 is APOE $\varepsilon 2$ Probe DNA and line 3 is a reaction mixture after conjugation. (b) The electrophoresis diagram of QD conjugated DNA; line 1 is QD@605, line 2 is APOE $\varepsilon 4$ Probe DNA and line 3 is a reaction mixture after conjugation.

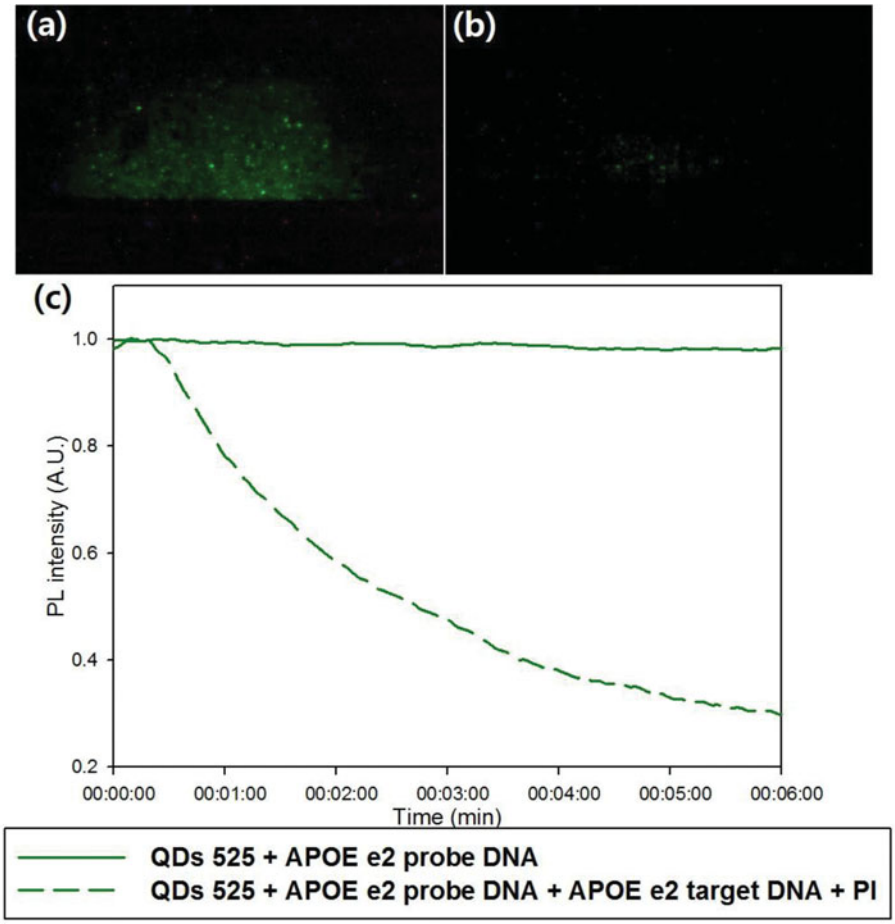

Figure 7. Fluorescence image of QD@525-Magnetic beads-APOE $\varepsilon 2$ probe DNA (a) before and (b) after injection of APOE $\varepsilon 2$ target DNA with PI dye. (c) The fluorescence intensity variation of QDs as a function of time.

3. Results and Discussion

In this study, the fluorescent dyes, TOTO-3 or PI, intercalated in double stranded APOE DNA were used as the quencher of QDs which are conjugated to probe DNA. The FRET between the dyes and QDs resulted in fluorescence quenching of QDs. Figure 2 shows the detection scheme of APOE $\varepsilon 2$ and APOE $\varepsilon 4$ by fluorescence quenching of QDs. The QDs with carboxyl group was conjugated to magnetic bead with amine functional group by EDC/Sulfo-NHS coupling reaction. Figure 3 shows the fluorescence microscope image of magnetic bead-QDs on a slide glass under dark field. The figure shows the magnetic beads were conjugated with QDs at a high rate. Figure 4 shows fluorescence spectra obtained from QD@525 -probe DNA (APOE $\varepsilon 2$) after hybridization with target DNA and intercalation by various concentration of PI. The concentration of hybridized QDs-DNA was kept constant and the concentration of PI was varied from 0.2 μM to 1.6 μM. The figure shows that the fluorescence intensity from QDs gradually decreases by addition of intercalation dye. The figure also shows that the fluorescence intensity becomes the lowest with 0.4μM of

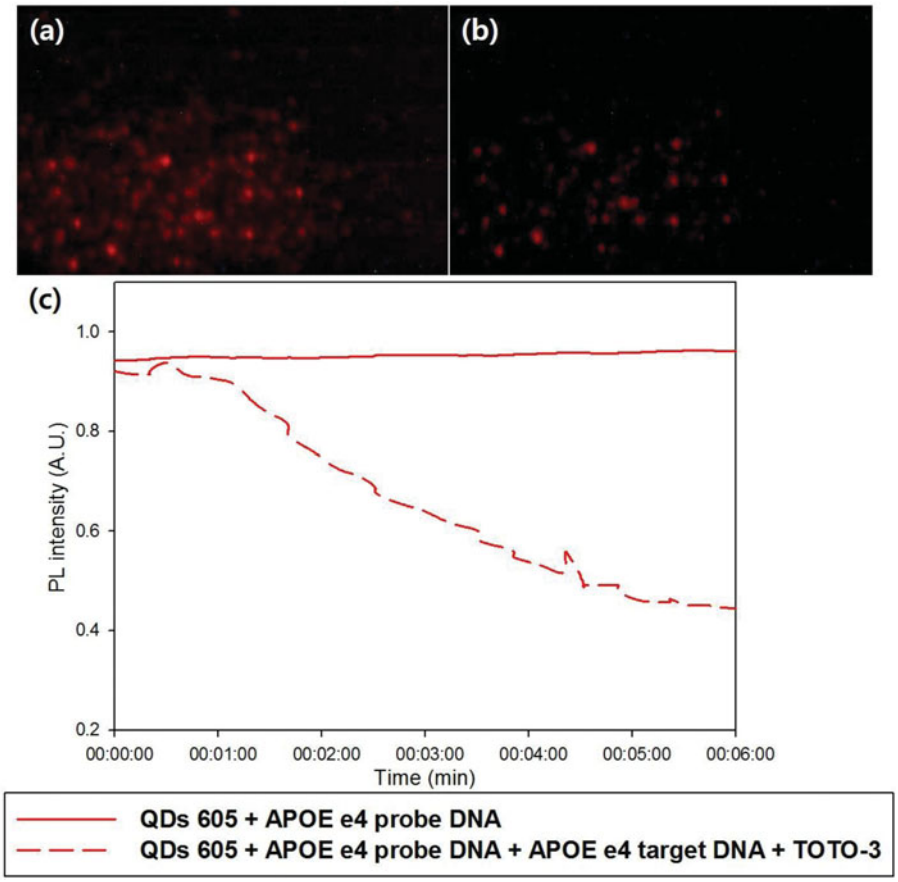

Figure 8. Fluorescence image of QD@605-Magnetic beads-APOE ε4 probe DNA (a) before and (b) after injection of APOE ε4 target DNA with TOTO-3 dye. (c) The fluorescence intensity variation of QDs as a function of time.

PI after 20 min. But in the micro channel, the fluorescence intensity from QDs should be reduced as fast as possible. After injection of 1.6 μM of PI, the fluorescence intensity decreases at highest rate for the first 5 min. Figure 5 also shows that fluorescence intensity from QD@605-probe DNA (APOE ε4) gradually decreases by addition of TOTO-3. The figure also shows that the fluorescence intensity decreases at highest rate for first 5 min with 1.6 μM of TOTO-3. The carboxyl QDs and thiolated oligonucleotide were conjugated by using a previously reported method [16], and the conjugated products were verified by gel electrophoresis using a 2% agarose gel. Figure 6 (a) shows the gel image of APOE ε2 probe DNA conjugated with QD@525 and intercalation with EtBr. In line 3, the upper band could be assigned to APOE ε2 probe DNA conjugated to QD@525 and the lower band to unconjugated APOE ε2 probe DNA, because QD@525-conjugated APOE ε2 probe DNA would move more slowly during the electrophoresis. QD@525 conjugated APOE ε2 probe DNA can be separated from unconjugated APOE ε2 probe DNA after gel electrophoresis. Figure 6 (b) shows the gel image of APOE ε4 probe DNA after QD@605 conjugation and intercalation with EtBr. In line 3, the upper band could be assigned to APOE ε4 probed DNA conjugated to QD@605 and the lower band to unconjugated APOE ε4 probe DNA.

The figure shows that Probe DNAs could be highly conjugated with QDs. The unconjugated free DNA s were washed away using buffer solution. The magnetic beads-QD-DNA probe was packed into the microfluidic channel using syringe pump and magnet. In Figure 1, magnetic bead conjugated with QD@525-APOE $\varepsilon 2$ probe DNAs were introduced through (a) hole. An external rectangular magnet was used to hold the probe complexes from outside of the channel. The magnetic bead conjugated with QD@605-APOE $\varepsilon 4$ probe DNAs were introduced through (b) hole. The probe complexes were held inside the channel. Figure 7 and 8 show the detection of APOE $\varepsilon 2$ and $\varepsilon 4$ by fluorescence quenching of QDs inside the channel. Figure 7 shows the fluorescence microscope image of magnetic beads- QD@525 -APOE $\varepsilon 2$ complexes (a) before and (b) after injection of APOE $\varepsilon 2$ target DNA with PI. The figure shows that the fluorescence from QDs was quenched due to the hybridization. Figure (c) shows the fluorescence intensity variation of QD@525 as a function of time after injection of APOE $\varepsilon 2$ target DNA and PI. When only intercalation dye was injected, no significant decrease of fluorescence intensity was observed (upper line), but when APOE $\varepsilon 2$ target DNAs and PI were injected together, the fluorescence intensity was decreased. Figure 8 shows the fluorescence microscope image of magnetic beads- QD@605 -APOE $\varepsilon 4$ complexes (a) before and (b) after injection of APOE $\varepsilon 4$ target DNA with TOTO-3. Figure (c) shows the fluorescence intensity variation of QD@605 as function of time after injection of APOE $\varepsilon 4$ target DNA and intercalating dyes. The figure also shows that APOE $\varepsilon 4$ can be detected through fluorescence quenching. The results show that both APOE $\varepsilon 2$ and $\varepsilon 4$ can be detected through fluorescence quenching of QDs, and different colors of QDs can be used to distinguish the alleles in PDMS chip.

4. Conclusion

Magnetic bead-QDs-DNA probes which have both magnetism and fluorescence were prepared by coupling reaction. The APOE $\varepsilon 2$ and $\varepsilon 4$ probe gene could be attached to the different color of QDs. The fluorescence of the QDs was quenched by intercalation dyes by the DNA hybridization both APOE $\varepsilon 2$ and $\varepsilon 4$ can be detected through fluorescence quenching of QDs, and different colors of QDs can be used to distinguish the alleles in PDMS chip.

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References

[1] Alivizatos, P. (2004). *Nat. Biotechnol*, *22*, 47.
[2] Barbacid, M., (1987). *Annu. REV. Biochem.*, *56*, 779.
[3] Bruchez, M., Morronne, M., Gin, P., Weiss, S., & Alivisatos, A. P., (1998). *Science*, *281*, 2013.
[4] Yoo, J. H. & Kim, J. S., (2011) *J. Nanosci. Nanotech.*, *11*, 4343.
[5] Giunta, S., Galeazzi, L., & Franceschi, C., (1998). *Arch. Geron. Geria.*, *27*, 49.
[6] Strittmatter, W. J. & Roses, A. D., (1996). *Neuroscience Letters*, *19*, 53.
[7] Lee, J. S., Ryu, J., & Park, C. B., (2009). *Anal. Chem.*, *81*, 2751.
[8] Mahley, R.W., (1988). *Science*, *240*, 622.
[9] Ghebranious, N., Ivacic, L., Mallum, J. & Dokken, C., (2005). *Nucleic Acids Res*., *33*, 17.
[10] Debette, S., Seshadri, S., Beiser, A., Au, R., Himali, J. J., Palumbo, C., Wolf, P. A., & DeCarli, C., (2011). *Neurology*, *77*, 461.

[11] Le, N. T. & Kim, J. S., (2013). *J. Nanosci. Nanotech.*, *13*, 1.
[12] Pathak, S., Choi, S. K., Arnheim, N., & Thompson, M. E. (2001). *J. Am. Chem. Soc. 123*, 4103.
[13] Elghanian, R., Storhoff, J. J., Mucic, R. C., & Letsinger, R. L., Mirkin, C. A. (1997). *Science*, *277*, 1078.
[14] Alivisatos, A. P., Jonhsson, K. P., Peng, X., Wilson, T. E., Loweth, C. J., Bruchez, M. P. & Schultz, P. G. (1996). *Nature (London)*, *382*, 609.
[15] Srinivasan, J. R., Kachman, M. T., Killeen, A. A., Akel, N., Siemieniak, D., & Lubman, D. M. (1998). *Rapid Commun, Mass Spectrom.*, *12*, 1045.
[16] Gao, X., Chan, W. C., & Nie, S., (2002). *J. Biomed. Opt.*, *7*, 532.