SEPARATION AND DETECTION OF BIOCONJUGATED QUANTUM DOTS USING ON A CHIP ELECTROPHORESIS

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Abstract: Semiconductor nanocrystals, quantum dots (QDs), are nanoscale particles that have been attract a lot of attention due to their unique optic and electronic properties. Due to very diverse and numerous applications, it is really important to make a tool for precise and controlled detection of QDs. In this study, we investigate on a chip detection and separation of QDs bioconjugated with BSA (bovine serum albumin) using homemade equipment based on principle of capillary electrophoresis (CE) and optical detection. Quenching effect of different concentration of BSA on fluorescence intensity of QDs was monitored. It was found that with increasing concentration of BSA fluorescence intensity of QDs is decreasing. This research can lead to a better understanding of interaction between different size QDs and biomolecules.

Keywords: Quantum dots, bovine serum albumin, capillary electrophoresis, bioconjugation

1. INTRODUCTION

QDs belong to family of inorganic nanoparticles and they are defined as semiconductors nanocrystals [1]. Their unique optical and electronic properties can be placed between those of bulk materials and isolated molecules of atoms [2]. QDs are nanoscale particles that consist of semiconductor core, such as CdTe and have tunable emission spectrum, from ultraviolet to infrared, by simple changing of the size of cores [3].

CE with high separation efficiency based on the size-to-charge ratio of analytes, holds promise for efficient size determination of nanoparticles (NPs) in aqueous samples [4]. In recent 10 years, CE has emerged as a powerful tool for characterizing and separating QDs and other NPs [5]. On a chip electrophoresis has some advantages over conventional one since it requires low sample volume, fast separation time, high separation efficiencies and additionally microchip platform provides the possibility to integrate sampling, separation, and detection on a chip [6].

Due to their unique optical properties, photostability and resistance to chemical degradation in last decade QDs find great application in medicine and biology usage, for luminescence tagging and molecular imagining, drug delivery, as tumor biomarkers and other [7]. QDs modified by BSA have been applied as ion sensors, fluorescence resonance energy transfer and chemiluminescence resonance energy transfer [8]. Capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) has been used for characterization of the surface modifications of QDs and evolution of the particle charge [9], [3], [10]**.** Hung et al. demonstrated how simple optimization of pH of buffer can substantially improve the separation of CdTe QDs and their conjugates with an antibody [9]. Application of QDs as fluorescence label in immunoassay was reported by Feng et al, where QDs were first conjugate with antibody then separated by CE form free antibody and antibody-antigen complex [3]. However, the core of QDs created with inorganic elements is toxic for living systems and cells. Nowadays, concerning environment issues and toxic of QDs lot of improve environment-friendly technologies are used for synthesis of QDs as well as modification of their surface to make

it less toxic for usage in medicine [11]. Even, these modified QDs bring doubts [12], and more research need to be done to investigate QDs and their bioconjugation.

Taking in consideration everything above, aim of this work is to use fast and simple method for investigate bonding between QDs and biomolecules. In this work conjugation of QDs with bovine serum albumin (BSA) was achieved via covalent coupling using different crosslinkers: carbolyldiimidazole (CDI), 1-ethyl-3-(3-dimethylaminopropyl), carboiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (NHS/EDC). QDs and QDs-protein conjugates were successfully separated using on a chip CE. Fluorescence detection was used to study quenching effect of protein and QDs. It was found out that higher concentration of BSA led to a stronger quenching of fluorescence emission, which could be explained by the covalent interaction between the protein and QDs Drbohlavova, Chomoucka [13]. In this work detection and separation of bioconjugated QDs was done in microfluidic system, using on chip electrophoresis and optical detection. By investigating interaction between different sized QDs and biomolecules this research can lead to a better understanding of how QDs behave in cell environment, which is extremely important for their biological application.

2. MATERIALS AND METHODS

2.1. REAGENTS AND CHEMICALS

The following chemicals were used for preparation of QDs and their conjugates with BSA: Sodium borohydride ($NaBH_4$, 99%), BSA (96%), CDI (97%), Sodium citrate dihydrate (99%), cadmium chloride ($CdCl_2$, 99%), sodium tellurite (Na_2TeO_3, 99%) were purchased from Sigma Aldrich. Sodium hydroxide (NaOH, 98%) were purchased from Penta. MPA (98%) were purchased from Merck. Isopropyl alcohol (99.7%) was purchased from Lach-Ner. The following chemicals were used for fabrication of silica mold and polydimethylsiloxane (PDMS) chip: SU-8 developer were purchased from MicroChem (Newton, MA,USA). The poly dimethylsiloxane prepolymer (Sylgard 184) and a curing agent were purchased from Dow Corning.

2.2. SYNTHESIS OF CDTE QDS CAPPED WITH MPA

To obtain CdTe-MPA QDs, $CdCl_2$ solution (91.6 mg) was diluted to 50 ml in one-necked flask and sodium citrate dihydrate (200 mg) was added followed by addition of MPA (52 µl). The pH of the solution was adjusted to 10.5 using NaOH (1 mol/l), followed by addition of Na_2TeO_3 (22.15 mg) and $NaBH_4$ (50 mg) under vigorous stirring. Solution was then refluxed at 95°C for 4 h [14].

2.3. PREPARATION OF QDS-BSA CONJUGATES VIA CDI, EDC, EDC/NHS

Briefly, to the solution of CdTe QDs (1 mg/ml), CDI (10 µl, 10 mmol/l) containing PBS buffer (100 mmol/l, pH=7.4) was added and the solution was then incubated at room temperature for 30 min in order to activate carboxyl groups [14]. Then, BSA (200 µl; 0, 0.002, 0.005, 0.01, 0.05, 0.075, 0.1 mg/ml) was added to the solution and incubated at 32°C for 2 h while shaking [14].

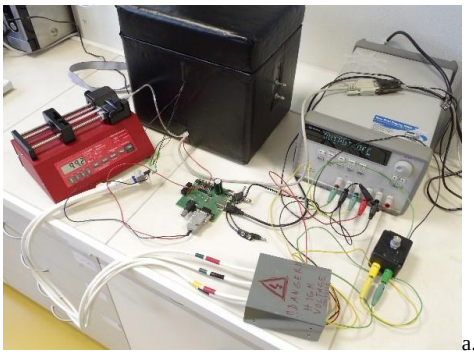
Briefly, to the solution of CdTe QDs (200 µl, 0.1 mg/ml) EDC (200 µl, 50 mmol/l) and NHS (200 µl, 5 mmol/l) were added and solution was then incubated at 32°C for 30 min. Then, BSA (200 µl; 0, 0.002, 0.005, 0.01, 0.05, 0.075, 0.1 mg/ml) was added to the solution and incubated at 32°C for 2 h while shaking [14].

Briefly, to the solution of CdTe QDs (250 µl, 0.1 mg/ml) BSA (250 µl; 0, 0.05, 0.5, 1 a 1.5 mg/ml) and EDC (57 µl, 10 mg/ml) were added. The solution was then incubated at room temperature for 2 h [14].

2.4. APPARATUS

Capillary electrophoresis of QDs and QDs-protein conjugates was carried out using home built system “black box” (**Figure 1**.). The black box consists of the three parts: light source, sample

holder and light detector. Excitation light is generated by ultraviolet light-emitting diode (UV LEDs) and filtered by optical 380 nm low pass filter. This light excites fluorescence of the sample and emitted light is selected by 560 nm high pass optical filter. This emitted light is detected by photodetector including photomultiplier.


a.

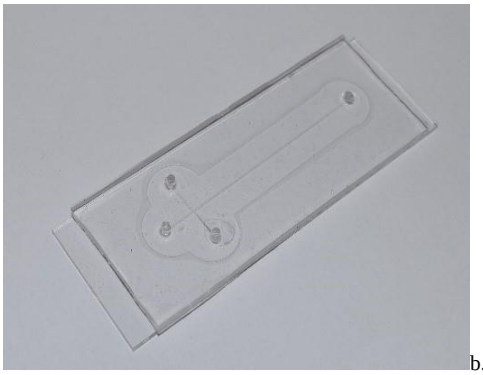

b.

Figure 1a: Experimental set up: "black box", syringe pump and power supplies, **b:** PDMS chip

2.5. FABRICATION OF A CHIP

Rapid prototyping of chip starts with creating design for a device in a computer/aided design program. A high resolution printing is used to print design on a chrome transparency mask. This transparency serves as a photomask in photolithography to produce a positive relief of photoresist on silica wafer. Silica mold with channels of 50μm width was made and used for rapid molding of PDMS. A mixture of the PDMS, prepolymer and curing agent at a ratio of 10:1 (w/w) was completely degassed and poured onto the silica mold, which was cured for 2 h at 65 ^{O}C, and peel off. After air drying of channels PDMS is punched to make holes (2 mm) as fluid reservoirs. The patterned side of the PDMS was treated with oxygen plasma (200 W, 15 min) and bonded permanently with a plasma-treated glass substrate to form a closed fluidic system (Figure 1b.).

2.6. CE PROCEDURE

After making the chip, microchannels are first filled with buffer using syringe pump. After filing the sample reservoir, platinum electrodes are placed in each fluid reservoir and contacted to power supplies. The applied voltage for the electrophoretic separation was 3 kV. Initially a new chip was flushed with 0.1M NaOH (10 min), water (10 min), and running buffer (10 min). A 10mM Borate Buffered Saline (BBS) aqueous solution pH 9.14 was used as a BGE. All solutions were filtrated through a 0.45μm membrane filter before use. In the slight alkaline solution, CdTe possess negative charges due to dissociation of carboxylic groups on their surface and therefore migrate to the anode in the electric field.

3. RESULTS

3.1. SEPARATION OF CONJUGATED CDTE- MPA QDS BY ON A CHIP CE

Measuring the changes in the electrophoretic mobility overtime of bioconjugated QDs by CE can determined binding of QDs with biomolecules, since interaction with these molecules will result in changes in the hydrodynamic size of QDs. The QDs used in this studies were conjugated to BSA (bovine serum albumin). First, CdTe QDs capped with MPA (CdTe-MPA) were prepared in aqueous solution phase. Crosslinkers CDI, was used to bioconjugate QDs with BSA. **Figure 2** shows electropherogram of free CdTe-MPA QDs, QDs modified by CDI, and QDs bioconjugated with BSA (QD-BSA). From results we can see that free QDs are well separated from bioconjugates by CE on a chip within 10 minutes.

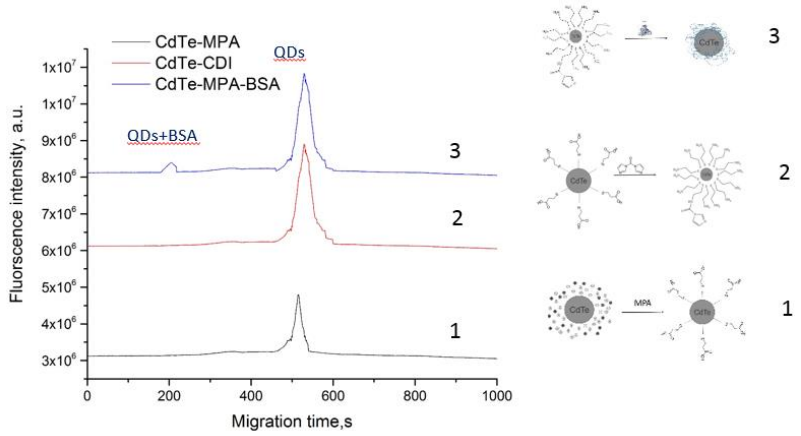


Figure 2: Electropherogram of the QDs CdTe-MPA bioconjugated with BSA

3.2. STUDDING OF FLUORESCENCE QUENCHING OF QDS EFFECT CAUSED BY COVALENT CONJUGATION WITH BSA

QDs and BSA were conjugated via covalent coupling using carboxyl groups of crosslinkers (CDI, EDC, and EDC/NHS). With increasing of concentration of BSA, fluorescence intensity of QDs is decreasing. The fluorescence quenching of QDs by BSA can be described by the linear Stern-Volmer equation:

$$I_0/I = 1 + K_{sv}\,[Q] \qquad (1)$$

where I_0 and I are the steady-state fluorescence intensities of QDs in the absence and presence of BSA, respectively. K_{SV} is the Stern-Volmer quenching constant, and $[Q]$ is the concentration of BSA. The I_0/I were calculated and plotted against BSA concentration as described in (1).

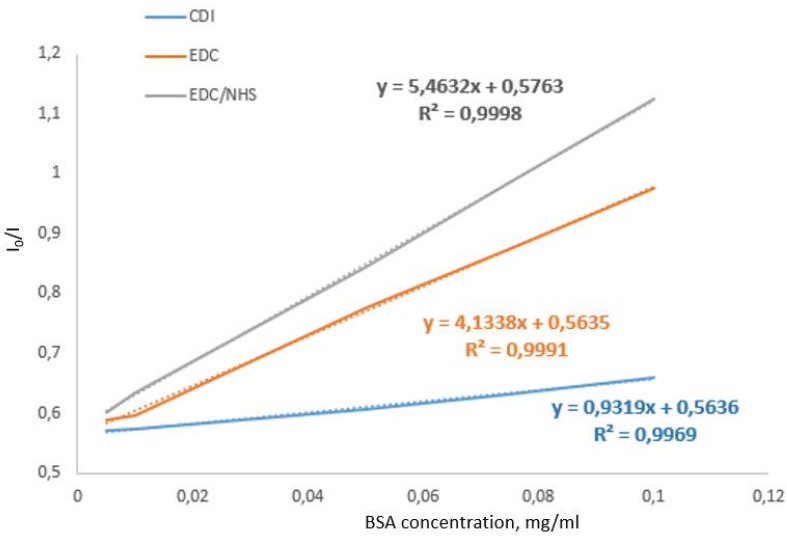


Figure 3: Stern- Volmer plot of QDs quenching effect caused by CdTe QDs electrostatic conjugation with BSA

The slope of the curve represents the Stren-Volmer quenching constant (K_{sv}) and the higher K_{sv}, the higher the quenching effect is. Stern- Volmer plot of all three crosslinkers quenching properties is shown exhibiting linear trend. The results show that the quenching constant is different for different types of bioconjugated QDs. From Figure 3 it is obvious that in case where EDC/NHS is

used as a crosslinker the fluorescence intensity is decreasing significantly, so the highest quenching effect was notable.

4. CONCLUSIONS

Water soluble CdTe QDs were prepared using a simple one step method. In this study, we successfully separated CdTe QDs bioconjugated with BSA using on a chip electrophoresis. What characterize this method are simplicity, short analysis time, small sample and reagent requirements, high separation efficient. The present of BSA is leading to a strong quenching effect of fluorescence emission of QDs. Quenching effect depends of a cross linker we are using to conjugate protein and it was shown to be the highest one when using EDC/NHS. Overall, this simple, fast and not expensive method can be used as powerful tool to better understand interaction between biomolecules and QDs.

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