Biosensors and Bioelectronics 31 (2012) 529–536

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Microfluidic bioassay system based on microarrays of hydrogel sensing elements entrapping quantum dot–enzyme conjugates

Eunji Jang[a], Sinyoung Kim[b], Won-Gun Koh[a,*]

[a] Department of Chemical and Biomolecular Engineering, Yonsei University, 134 Sinchon-Dong, Seodaemoon-Gu, Seoul 120-749, Republic of Korea
[b] Department of Laboratory Medicine, Yonsei University College of Medicine, 134 Sinchon-Dong, Seodaemoon-Gu, Seoul 120-749, Republic of Korea

ARTICLE INFO

Article history:
Received 26 August 2011
Received in revised form 3 November 2011
Accepted 21 November 2011
Available online 28 November 2011

Keywords:
Microfluidic biosensor
Hydrogel microstructure
Quantum dot–enzyme conjugates
Oxidase enzymes
Fluorescence quenching

ABSTRACT

This paper presents a simple method to fabricate a microfluidic biosensor that is able to detect substrates for H_2O_2-generating oxidase. The biosensor consists of three components (quantum dot–enzyme conjugates, hydrogel microstructures, and a set of microchannels) that were hierarchically integrated into a microfluidic device. The quantum dot (QD)–enzyme conjugates were entrapped within the poly(ethylene glycol) (PEG)-based hydrogel microstructures that were fabricated within the microchannels by a photopatterning process. Glucose oxidase (GOX) and alcohol oxidase (AOX) were chosen as the model oxidase enzymes, conjugated to carboxyl-terminated CdSe/ZnS QDs, and entrapped within the hydrogel microstructures, which resulted in a fluorescent hydrogel microarray that was responsive to glucose or alcohol. The hydrogel-entrapped GOX and AOX were able to perform enzyme-catalyzed oxidation of glucose and alcohol, respectively, to produce H_2O_2, which subsequently quenched the fluorescence of the conjugated QDs. The fluorescence intensity of the hydrogel microstructures decreased as the glucose and alcohol concentrations increased, and the detection limits of this system were found to be 50 μM of glucose and 70 μM of alcohol. Because each microchannel was able to carry out different assays independently, the simultaneous detection of glucose and alcohol was possible using our novel microfluidic device composed of multiple microchannels.

1. Introduction

Semiconductor quantum dots (QDs) are widely used in the field of optical biosensors as alternatives to organic fluorescence dyes due to their superior optical properties such as high quantum yield, long fluorescence lifetime, large extinction coefficient, and stability against photobleaching (Costa-Fernández et al., 2006; Frasco and Chaniotakis, 2010; Han and Li, 2010). QDs alone do not specifically recognize target molecules, and therefore they are coupled with different types of biomolecules (antibodies, nucleic acids, and enzymes) for the selective detection of various analytes (Gill et al., 2008b). Most QD-based biosensors still rely on the fluorescence resonance energy transfer (FRET) processes that occur during biorecognition events or biocatalytic processes (Mattoussi et al., 2005; Mauro et al., 2003; Ozkan et al., 2004; Rosenzweig et al., 2006), but electron transfer quenching has recently been proposed as an alternative detection method since Willner et al. reported the possibility of monitoring tyrosinase activity via the quinone-induced electron transfer quenching of QDs (Gill et al., 2006; Sandros et al., 2005). It is mandatory to include the quencher as a reporter unit in the analyzed sample when performing this assay. Because various enzyme–substrate reactions produce molecules that quench the photoluminescence of QDs, different analytes have been detected by QD–enzyme hybrid systems using different enzyme reactions. For example, Wang et al. reported that the photoluminescence quenching by quinone intermediates produced from an enzyme-catalyzed reaction could be used for the detection of phenolic compounds (Yuan et al., 2008b). Furthermore, different optical biosensors were developed using different H_2O_2-generating oxidases based on the fact that H_2O_2, a product of numerous oxidases, quenches the photoluminescence of QDs by the electron transfer reaction (Gill et al., 2008a). A representative application is the detection of glucose via QD–glucose oxidase (GOX) hybrid systems (Cao et al., 2008; Duong and Rhee, 2007; Hu et al., 2010; Huang et al., 2008; Wu et al., 2010; Yuan et al., 2008a). In most QD–enzyme hybrid systems, the enzymes are covalently conjugated to QDs rather than physically mixed with QDs for efficient electron transfer quenching. The sensing capability, enzyme kinetics, and stability of QD–enzyme conjugates have been thoroughly investigated in previous studies. However, most of the previous work on biosensors that are based on QD–enzyme conjugates has been restricted to solution-based sensing assays. The development of more useful sensing approaches requires the immobilization of the QD–enzyme conjugates in appropriate solid supports to

* Corresponding author. Tel.: +82 2 2123 5755; fax: +82 2 312 6401.
E-mail address: wongun@yonsei.ac.kr (W.-G. Koh).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.11.033

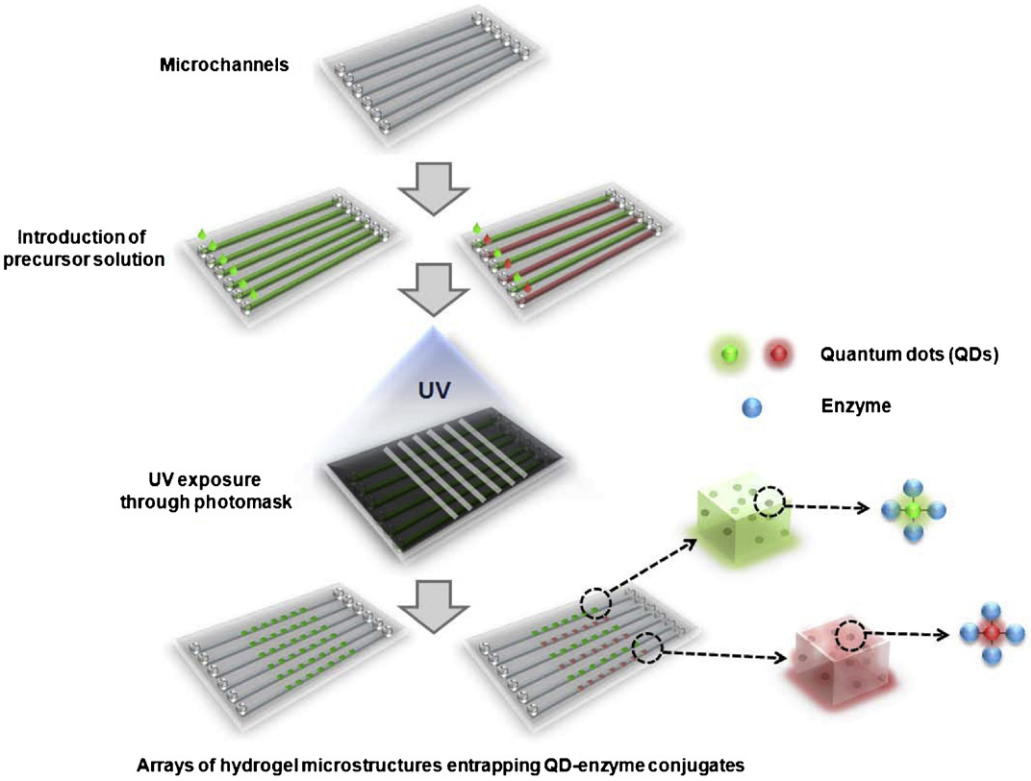

Fig. 1. Schematic illustration of fabricating arrays of hydrogel microstructures entrapping QD–enzyme conjugates within microfluidic devices.

fabricate the active solid phases for working in flowing conditions, which can eventually be used to realize chip-based sensors that are designed in array or microfluidic formats for rapid and high-throughput/content detection. Although multilayer films or sol–gel materials that immobilize QDs and enzymes have been prepared and successfully used for the detection of specific analytes (Bullen et al., 2004; Constantine et al., 2003; Li et al., 2009), to the best of our knowledge, the immobilization of QD–oxidase conjugates within a microarray format and their integration into microfluidic devices for the realization of a micro-total-analysis-system (μ-TAS) have not been reported.

Recently, the entrapment of enzymes within hydrogels has received a great deal of attention as an enzyme immobilization method. Hydrogels are crosslinked polymers with three-dimensional network arrangements that are able to retain large amounts of water. The soft, fluid environment of a fully hydrated hydrogel provides the enzymes with near-physiological conditions that minimize the denaturation that would be experienced under dry conditions and allows the enzymes to carry out their full biological functions (Ikeda et al., 2010; Kiyonaka et al., 2004; Zhang, 2004). Hydrogels can also provide protective environments for entrapped enzymes and can inhibit degradation and non-specific adsorption (Russell et al., 1999). Since Beebe et al. (2000) first proposed the fabrication of hydrogel microstructures inside microchannels for use as microactuators, several groups have tried to incorporate hydrogels that entrap biomolecules such as proteins, DNA, and cells into microfluidic devices (Choi et al., 2008; Heo et al., 2003; Ikami et al., 2010; Koh et al., 2002; Koh and Pishko, 2005; Olsen et al., 2002; Zhan et al., 2002).

In this study, we fabricated microfluidic devices that are composed of sets of microchannels that contain arrays of hydrogel microstructures entrapping QD–enzyme conjugates. Based on the fact that H_2O_2, a product of various oxidase-catalyzed reactions, can effectively quench the photoluminescence of QDs, the detection of glucose and alcohol was investigated using glucose oxidase or alcohol oxidase-conjugated QDs within the microfluidic devices.

2. Experimental

2.1. Materials

Poly(ethylene glycol) diacrylate (PEG-DA) (MW 575), photoinitiator 2-hydroxy-2-methylpropiophenone (HOMPP), hexane, hydrogen peroxide, perfluorooctane, 3-(trichlorosilyl) propyl methacrylate (TPM), D-glucose, ethanol, (1-ethyl-3(3-dimethylaminoprophyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), glucose oxidase (GOX, from *Aspergillus niger* type II, 50,000 unit/g solids), and alcohol oxidase (AOX, from *Pichia pastoris*, 10–40 units/mg protein) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). An 8 μM solution of carboxyl-terminated CdSe/ZnS quantum dots (QD) (Qdot® ITK™, emission wavelength 525 nm, 585 nm, and 625 nm) and phosphate buffered saline (PBS, 0.1 M, pH 7.4) were purchased from Invitrogen (Carlsbad, CA, USA). The Dow Corning Sylgard 184 poly(dimethylsiloxane) (PDMS) elastomer was from Dow Corning (Midland, MI, USA). The photomasks for photolithography were prepared using AUTO CAD and printed on transparencies using a standard laser jet printer (LaserWriter 16/600 PS, Apple Inc., Cupertino, CA, USA).

2.2. Preparation of QD–enzyme conjugates

The enzymes were conjugated with carboxyl-terminated QDs via an EDC/NHS-mediated reaction. First, 0.1 mL of QD solution was added to a 1 mL PBS solution containing 2 mM EDC and 5 mM NHS, and was incubated for 24 h at room temperature to convert the terminal carboxyl groups in the QDs to reactive intermediates

susceptible to attack by the amine groups on the enzymes. Enzyme (1 mg) was added to this mixture and reacted for 12 h at 4 °C. This resulted in the covalent attachment of the proteins to the QDs by amide bond formation. The QD–enzyme conjugates were separated from the solution by removing the free enzymes and other small molecules through ultrafiltration under centrifugation, as previously described (Cao et al., 2008). The concentrated QD–enzyme conjugates were diluted to 1.0 mL with PBS. Conjugation between the enzymes and the QDs was confirmed by measuring the sizes of QD–enzyme conjugates by dynamic light scattering (DLS, Zetasizer 3000HSA, Malvern Instruments Ltd., Worcestershire, UK) and by measuring the shifts of the fluorescence emission spectra of the QDs.

2.3. Fabrication of the microfluidic device

The microfluidic networks were formed from a 10:1 mixture of the PDMS prepolymer and the curing agent. The resulting mixture was poured onto a silicon master and cured at 60 °C for at least 2 h. All of the masters had negative patterns of six 100 μm microchannels defined with SU-8 50 negative photoresist. After curing, the PDMS replica was removed from the master and pierced from the backside of the network with syringe needles to open paths for incoming liquids. These PDMS microchannels were oxidized in oxygen plasma (Femto Science, Kyounggi, Korea) with glass slides for 1 min. Bringing the oxidized PDMS and glass slides into conformal contact resulted in irreversible seals and thus formed enclosed microchannels. The microchannels were then treated with a dilute TPM solution in perfluorooctane for 10 min immediately after sealing to enhance the adhesion of the hydrogel microstructures inside the microchannels (Koh and Pishko, 2005). Polyethylene tubes were inserted into the holes in the PDMS and then connected to a syringe pump (Harvard Apparatus, Holliston, MA, USA) to complete the microfluidic devices. The microfluidic devices were mounted onto the stage of a microscope for real-time fluorescence detection and imaging.

2.4. Arrays of hydrogel microstructures entrapping QD–enzyme conjugates

The hydrogel microstructures were fabricated from PEG-DA (MW 575) using photolithography. PEG-DA was dissolved in PBS to form a 50% (w/v) solution. The final hydrogel precursor solution was prepared by adding 200 μL of QD–enzyme conjugate solution and 10 μL of HOMPP to 1.0 mL of PEG solution. After the microchannels were filled with precursor solutions, a photomask with 12 lines (100 μm-lines and 100 μm-spaces) was placed on the other side of each glass slide, perpendicular to the PDMS microchannels. When the hydrogel precursor solution in the microchannels was exposed to a 365 nm, 300 mW/cm^2 light source (EXFO Omnicure UV spot lamp, Mississauga, Ontario, Canada) through the photomask for 1.0 s, only the exposed polymer regions underwent free radical cross-linking and became insoluble in common PEG solvents such as water. Finally, after the channels were flushed with PBS, the desired hydrogel microstructures were obtained inside microchannels. Fig. 1 illustrates the overall procedure that was used to fabricate the arrays of hydrogel microstructures entrapping QD–enzyme conjugates within a set of microchannels. Here, each microchannel can be filled with the same solution or with a different solution.

2.5. Detection of substrates

To investigate the feasibility of using hydrogel microstructures entrapping QD–enzyme conjugates to detect specific target substrates, the substrates (glucose or ethanol) were dissolved in 0.1 M PBS (pH 7.4, 37 °C) and were continuously supplied into the microchannels at 1.0 μL/min. Changes in the fluorescence intensity of the QDs due to the reactions between the substrates and enzymes were monitored and quantified using fluorescence microscopy and a fluorescence spectrometer. The fluorescence quenching data were compared with the data for the control samples that did not contain substrates and were expressed as the F/F_0 value (F and F_0 represent the fluorescence emission intensity of the QD in the presence and absence of substrate, respectively). A Zeiss Axiovert 200 microscope equipped with an integrated color CCD camera (Carl Zeiss Inc., Thornwood, NY, USA) was used to obtain the fluorescence images of the fluorescent hydrogel microstructures. Image analyses were performed using commercially available image analysis software (KS 300, Carl Zeiss Inc.). The fluorescence intensity of the QDs in solution was investigated using a QM-1 fluorescence spectrometer (Photon Technologies International, Monmouth, NJ, USA).

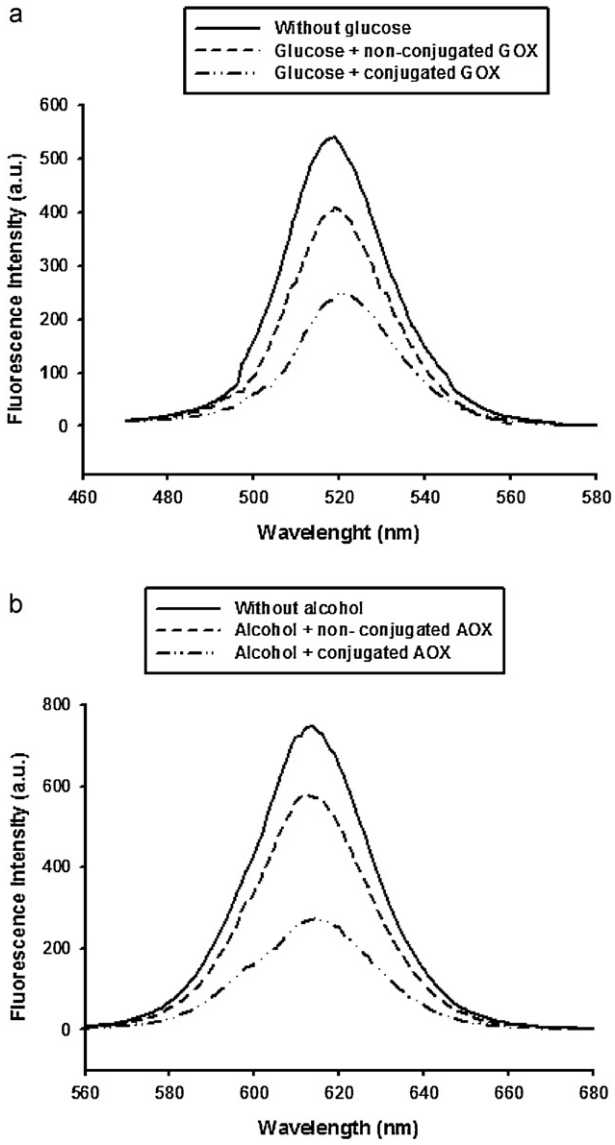

Fig. 2. Change of fluorescence emission spectra by H_2O_2 generated from oxidase-catalyzed reaction. (a) GOX-catalyzed reaction with glucose, (b) AOX-catalyzed reaction with alcohol (concentration of glucose and alcohol was 50 μM, reaction time was 5 min).

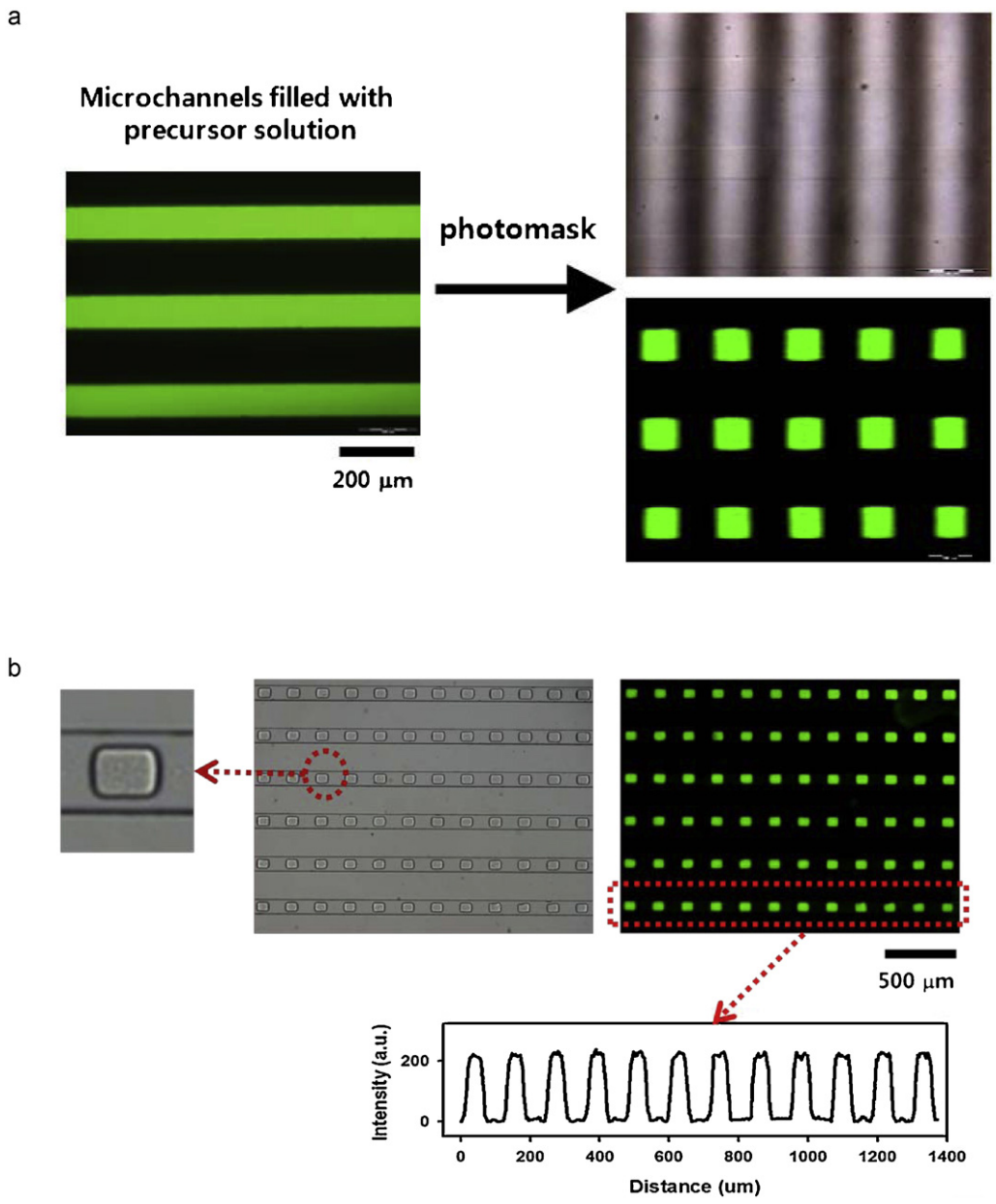

Fig. 3. Fabrication of hydrogel microarrays entrapping QD–enzyme conjugates within microchannels. (a) Optical and fluorescence images of a photomask that was placed perpendicularly to microchannels filled with precursor solutions, (b) arrays of fluorescent hydrogel microstructures fabricated in microchannels and fluorescence intensity profile of hydrogel microstructures in single microchannel.

2.6. Detection of glucose in blood sample

Five blood samples with known glucose concentrations were obtained from Severance Hospital (Seoul, Korea) and subjected to dilution with PBS solution. Each diluted blood sample was injected into the microchannels and reacted for 5 min with the QD–enzyme conjugates that were entrapped within hydrogel. The glucose concentrations were determined by the calibration curve and compared with the real values.

3. Results and discussion

3.1. Characterization of QD–enzyme conjugates

The QD–enzyme conjugates were prepared with the aid of coupling reagents (EDC and NHS) and purified. By the EDC/NHS-mediated reaction, the carboxyl groups in the QDs were converted into N-hydroxysuccinimide esters, which can react with the amine groups in enzymes to form a stable amide linkage between the QDs and the enzymes. The conjugation between the QDs (λ_{em}: 525 nm) and enzyme (GOX) was investigated by a DLS measurement, as shown in Fig. S1a (see supplementary material). Both the QDs and enzyme have relatively narrow size distributions (5.6–15.7 nm for the QDs and 10.1–21 nm for the enzyme), and the mean sizes of the QDs and the enzyme were 8.7 nm and 13.5 nm, respectively. However, the QD–enzyme conjugate had a broad size distribution ranging from 37.8 to 78.8 nm with average size of 50.7 nm. The increases in size confirmed the successful formation of the QD–enzyme conjugates, while the broad size distribution indicated that the enzymes can attach to the QD particles in many different ways. The observation of red shifts of the fluorescence emission peaks caused by increases of the

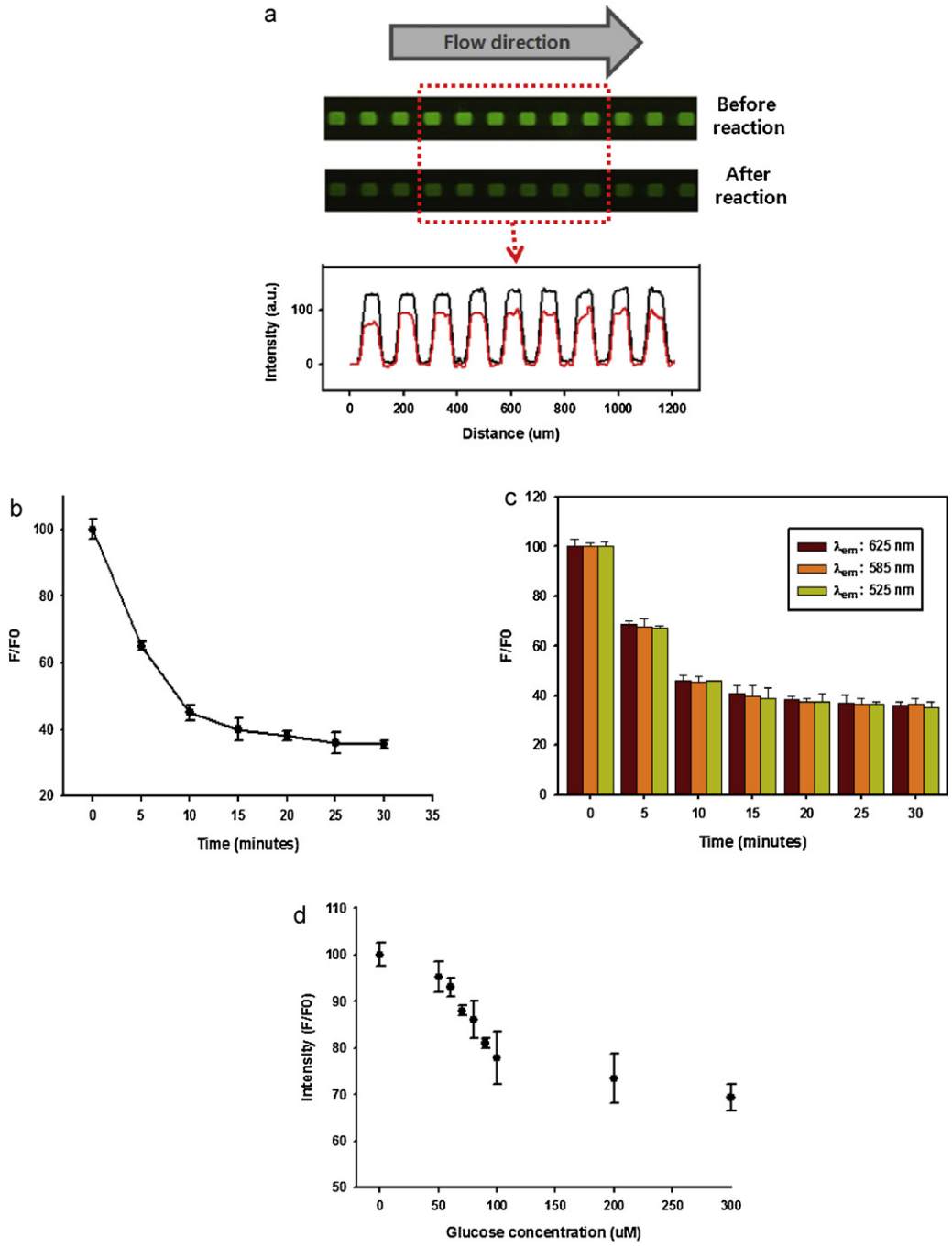

Fig. 4. Glucose assays using hydrogel-entrapped QD–GOX conjugates within microfluidic devices. (a) Change of fluorescence intensity by reaction between glucose and GOX (glucose concentration: 500 μM; reaction time: 5 min), (b) change of F/F_0 according to reaction time (glucose concentration: 500 μM), (c) effects of emission wavelength of QDs on the time-dependent quenching rates, (d) change of F/F_0 according to glucose concentration (reaction time: 5 min).

final size of the QD–enzyme complex is additional evidence of the successful conjugation between the QDs and the enzyme (see Fig. S1b in the supplementary material).

Reactions between GOX and glucose produce H_2O_2, which effectively quenches QDs such as CdSe/ZnS and CdTe by an electron transfer reaction as explained in the introduction. Prior to investigating the GOX-catalyzed reaction, we studied the quenching effect of H_2O_2 on the QDs. As shown in Fig. S2, H_2O_2 effectively quenched the fluorescence intensity of both naked QDs and enzyme-conjugated QDs at nearly same level, which indicates that the conjugation of the enzyme did not affect the hydrogen peroxide-induced quenching of the QDs (see supplementary material). Fig. 2a shows the changes in the fluorescence emission spectra that were caused by the quenching of the QDs via the GOX-catalyzed reaction. As expected, the fluorescence intensity decreased due to the production of H_2O_2 by the GOX–glucose reaction. More dramatic quenching was observed when the QD–GOX conjugate was used than when the non-conjugated QDs and GOX were used, which demonstrates that the conjugated system can facilitate electron transfer reactions. H_2O_2 is a product of many enzymatic processes, including those catalyzed by GOX, alcohol oxidase (AOX), lysine oxidase (LOX), and chlorine oxidase (COX). Therefore, coupling QDs with these enzymes yields a promising biosensing nano-complex for a series of oxidase enzyme substrates

without requiring extra quenching units for the QDs. In addition to GOX, we prepared QD–AOX conjugates and confirmed that AOX-catalyzed reactions with alcohol quenched the fluorescence of the QDs similarly to GOX-catalyzed reactions by H_2O_2 (Fig. 2b).

3.2. Fabrication of hydrogel microarrays entrapping QD–enzyme conjugates

The hydrogel microarrays were fabricated inside the microchannels by a simple photopatterning process. Fig. 3a shows the optical and fluorescence images of a photomask that was placed perpendicularly to the PDMS microchannels containing precursor solution composed of PEG-DA, photoinitiator, and QD–enzyme conjugates. The formation of the PEG hydrogels is based on UV-initiated free-radical cross-linking of acrylate end groups on PEG-DA, which results in the formation of polyacrylate networks that are highly cross-linked with PEG. To avoid the production of deformed structures, the introduced precursor solution was allowed to reach a stationary state and was then exposed to UV light. Because photopolymerization occurs only in the regions that are exposed to UV and that contain precursor solution, only the fluorescent area in Fig. 3a was polymerized, which resulted in the production of hydrogel microstructures. Fig. 3b shows hydrogel microarrays fabricated within different microchannels, which confirms that only the UV-illuminated regions underwent photopolymerization and gelled inside of the microchannels. Furthermore, fluorescence emissions were detected only from the hydrogel microarrays, which reveals that the unreacted precursor solution was completely removed by flushing the channels with PBS solution. Although a region 100 μm in width and 100 μm in length was exposed to UV, the width of the resultant hydrogel microstructures (approximately 80 μm) was smaller than that of the UV-exposed region, which left open-channel regions to both sides of the hydrogel microstructures that permitted solutions to flow through the microchannels. One possible reason for this decrease in hydrogel microstructure width is oxygen-aided inhibition near the PDMS surfaces. Oxygen can diffuse through the PDMS and react with the initiator species to form chain-terminating peroxide radicals and leave non-polymerized layers near the PDMS walls (Dendukuri et al., 2006). The fluorescence intensity profile shown in Fig. 3b indicates that all of the hydrogel microstructures showed nearly identical fluorescence intensity and that the fluorescence was almost homogeneous within a single hydrogel microstructure. These results imply that the amount of QD–enzyme conjugates within each hydrogel microstructure was similar and that the QD–enzyme conjugates were evenly distributed within the hydrogel microstructure. Due to covalent attachments to the glass substrates via TPM, the hydrogel microstructures remained in the same place in the microchannels throughout all of the experiments.

3.3. Detection of glucose and alcohol

To confirm that the hydrogel microstructures entrapping QD–enzyme conjugates were able to detect the substrates based on the same mechanism that was demonstrated in the solution state, a glucose solution was injected into the microchannels that contained arrays of hydrogel microstructures entrapping QD (λ_{em}: 525 nm)–GOX conjugates. As shown in Fig. 4a, the fluorescence intensity decreased after the glucose injection due to the production of H_2O_2, which demonstrates that the glucose was able to diffuse into the hydrogel matrix and react with the conjugated GOX that exhibited catalytic activity within the hydrogel microstructures after photopatterning. The fluorescence intensity profiles reveal that all of the hydrogel microstructures in the same microchannel had similar fluorescence intensity values after the reaction, which indicates that most of the H_2O_2 that

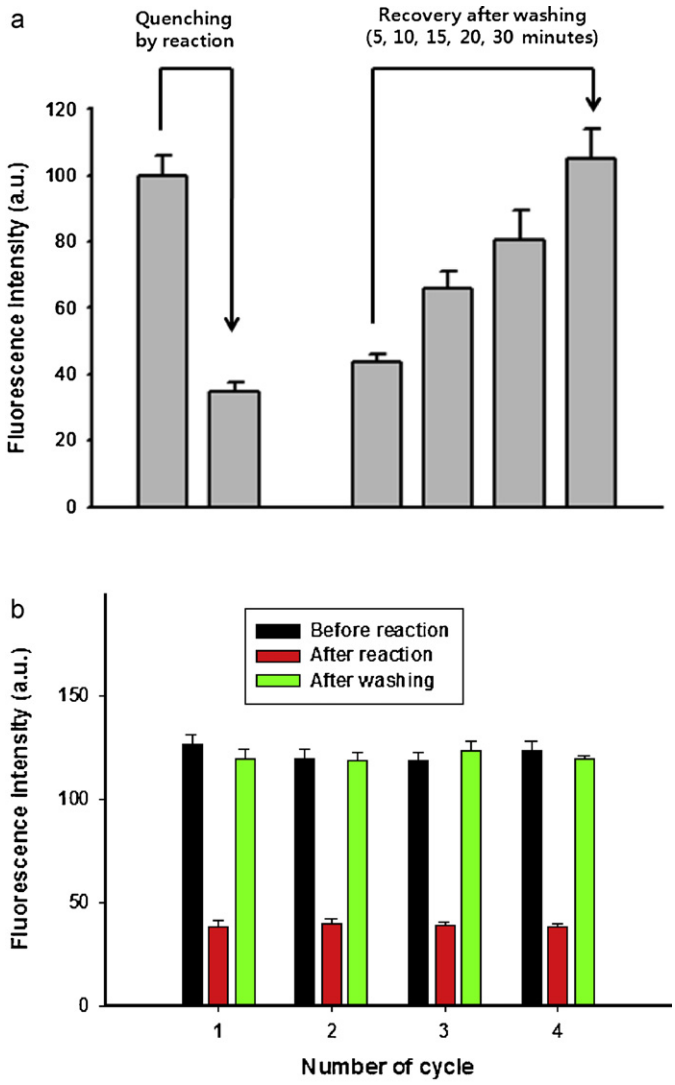

Fig. 5. (a) Recovery of quenched QD fluorescence by washing process, (b) change of fluorescence intensity of GOX-conjugated QDs by repeated cycles of reaction with glucose and washing with PBS.

was generated in the hydrogel microstructures quenched the QDs in the same hydrogel microstructures without affecting the QDs in the other hydrogel microstructures. The quantitative fluorescence intensity data were obtained by calculating the averages and the standard deviations of the fluorescence intensity from the individual hydrogel microstructures in the microchannels; these data demonstrate the time-dependent decrease of the fluorescence intensity (Fig. 4b). We also prepared QD–GOX conjugates with different QDs (λ_{em}: 585 nm and 625 nm) and observed the same time-dependent quenching rates as those shown in Fig. 4c, which demonstrates that the fluorescence quenching by H_2O_2 was not affected by the size or the emission wavelength of the QDs. When the F/F_0 value was plotted against the glucose concentration (Fig. 4d) after a fixed reaction time of 5 min, during which the most dramatic fluorescence quenching occurred, the F/F_0 value was observed to decrease almost linearly in the 50–100 μM glucose concentration range, and reached a plateau at the higher glucose concentrations, as expected for Michaelis–Menten kinetics. A plot of the Lineweaver–Burk equation yielded a Michaelis constant (K_m) of 0.24 mM. The detection limit of glucose was approximately 50 μM under these conditions. Finally, we investigated whether the quenched QD fluorescence recovered so that the hydrogel

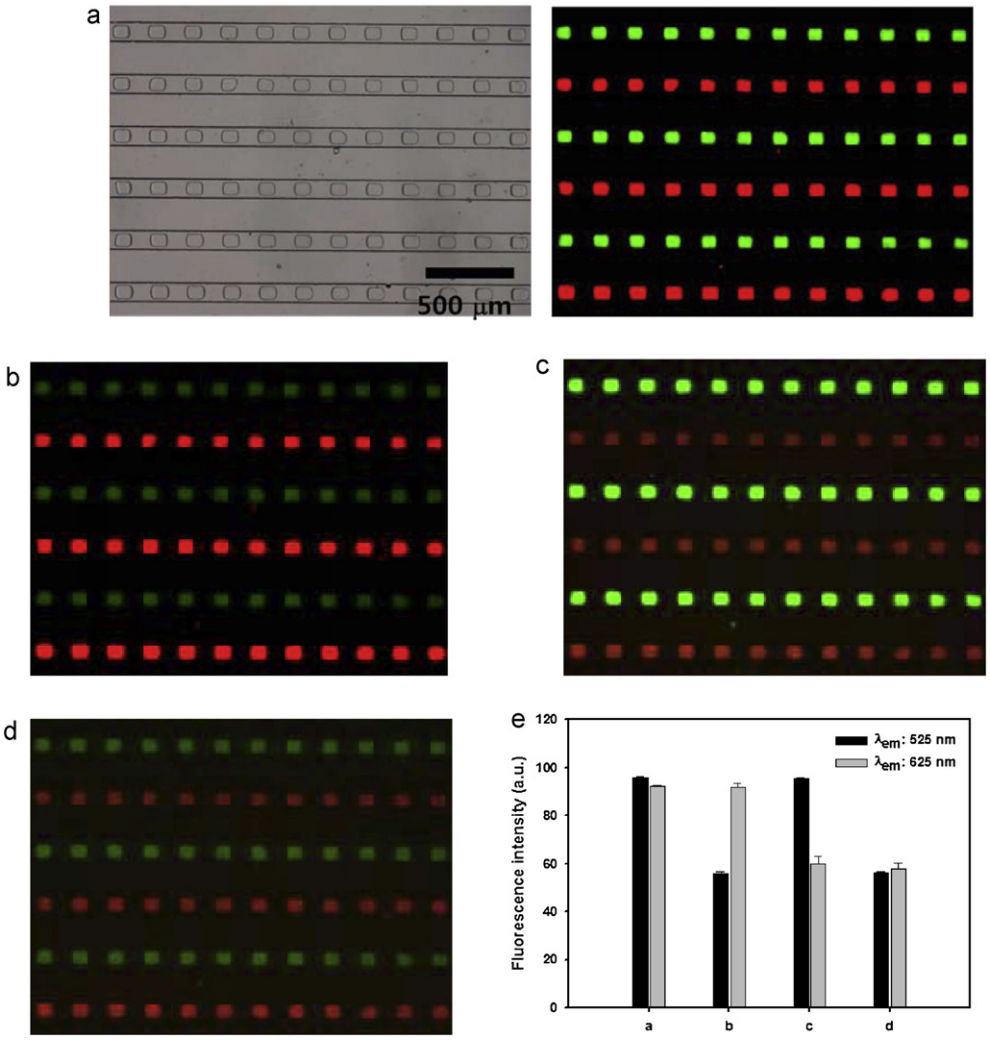

Fig. 6. Microfluidic system capable of simultaneous detection of glucose and alcohol. (a) Six microchannels incorporating hydrogel microarrays entrapping QD (λ_{em}: 525 nm)–GOX and QD (λ_{em}: 625 nm)–AOX conjugates alternatively. Fluorescence images obtained after microchannels were filled with (b) solution of glucose only, (c) alcohol only, and (d) solution containing both glucose and alcohol, (e) fluorescence intensity of hydrogel microstructures that were reacted with different solutions.

microstructures could be re-used for multiple assays. Fresh PBS solution was continuously injected into the used microchannels to remove the H_2O_2 from the hydrogel microstructures. Fig. 5a shows that the QD fluorescence within the hydrogel microstructures was fully recovered after 30 min of washing with PBS solution. Furthermore, the hydrogel microstructures maintained their sensing capabilities for glucose during repeated reaction and washing steps (Fig. 5b). Short-term stability tests revealed that the degree of quenching for the detection of 100 μM glucose within the hydrogel microstructures decreased by approximately 18% after one week when the hydrogel microstructures containing QD–GOX conjugates were stored at 4 °C. Because the maximum fluorescence intensity remained unchanged, we concluded that the decrease of quenching degree resulted mainly from the loss of enzyme activity. For practical applications, we propose to use five microchannels to obtain the standard curves for substrates and one microchannel to detect an unknown sample by utilizing the capability of the microfluidic systems to simultaneously carry out multiple reactions. A standard calibration curve was obtained using five different concentrations of glucose (60 μM, 70 μM, 80 μM, 90 μM, and 100 μM) and the sample solution (95 μM glucose solution) was injected into the detection microchannel. A glucose concentration of 95.75 μM was determined by interpolation, confirming the accuracy of our detection system. A similar glucose concentration (97.06 μM) was obtained after one week because the activity loss of the enzymes was reflected in both the calibration curves and the detection. As mentioned in the introduction, several glucose-detecting biosensors that are based on QDs have been reported and summarized in Table S1 (see supplementary material). Although some systems showed greater sensitivity and a better limit of detection, those previous works were restricted to solution-based sensing assays without a multiplex sensing capability and reusability. It should be emphasized that our system showed better performance than the other immobilized systems, and this is the first report regarding the immobilization of QD–enzyme conjugates within a microarray format and their integration into microfluidic devices.

After the successful demonstration of the feasibility of hydrogel microstructures entrapping QD–GOX conjugates for glucose detection within a microfluidic system, another enzyme–substrate reaction that produces H_2O_2 was also investigated within the same microfluidic system using an AOX-catalyzed reaction with alcohol; the results are shown in Fig. S3 (see supplementary material). Similar time- and concentration-dependent quenching data were observed, and the detection limit of alcohol was 70 μM. These results demonstrate that our miniaturized detection system can be applied for the detection of a variety of oxidase substrates.

Because of the relatively small mesh size of the PEG hydrogel (approximately 10 Å) (Mellott et al., 2001), no leaching of the QD–enzyme conjugates out of the hydrogel microstructures was observed in any of our experiments. Therefore, we concluded that the mesh size of the hydrogel microstructures was large enough to allow the diffusion of glucose and alcohol but small enough to prevent the leaching of the QD–enzyme conjugates.

3.4. Simultaneous detection

After confirming the successful detection of glucose and alcohol, we prepared a microfluidic system that was able to simultaneously detect both substrates. The simultaneous introduction of the independent gel chemistries into each microchannel was possible because the microchannels were fluidically isolated from each other. Hydrogel precursor solutions containing QD (λ_{em}: 525 nm)–GOX and QD (λ_{em}: 625 nm)–AOX conjugates were alternatively introduced into each microchannel. A subsequent single UV exposure and developing process produced a set of microchannels containing glucose and alcohol-detecting hydrogel microstructures, which were easily identified by the colors of the hydrogel microstructures as shown in Fig. 6a. When a 500-μM glucose solution was injected into all of the microchannels for 5 min, fluorescence quenching occurred only within the microchannels that contained green-emitting hydrogel microstructures without affecting the red-emitting hydrogel microstructures due to specific reactions between the enzymes and substrates (Fig. 6b); fluorescence quenching occurred only in the red-emitting hydrogel microstructures when a 500-μM alcohol solution was injected into the microchannels for 5 min (Fig. 6c). However, the introduction of solutions containing both glucose and alcohol resulted in fluorescence quenching in both the green- and red-emitting hydrogel microstructures, as shown in Fig. 6d. The quantitative fluorescence intensity data for these cases are shown in Fig. 6e.

3.5. Determination of glucose in blood samples

To investigate the feasibility of our sensing system for the analysis of biological fluids, the developed biosensor was applied to detect glucose in real blood samples. Prior to analysis, blood samples were diluted with PBS solution because the working range of our proposed system is at the micromolar level, while the normal glucose concentration in blood is at the millimolar level. No other pretreatment of the blood sample except dilution was carried out. It is expected that dilution can allow us to work with very small amounts of blood sample and can eliminate potential interferences by various small molecules within the blood. As shown in Table S2, glucose concentrations obtained using our proposed microfluidic system showed reasonable agreement with the real values (see supplementary material).

4. Conclusion

We have presented a simple method to fabricate arrays of PEG hydrogel microstructures that entrap QD–enzyme conjugates in microfluidic devices for potential applications as biosensors in μ-TAS. The enzymes were covalently attached to carboxyl-terminated CdSe/ZnS QDs via EDC/NHS chemistry, and a subsequent photopatterning process produced the hydrogel microstructures that entrapped the resultant QD–enzyme conjugates within microchannels. Based on the fluorescence quenching ability of H_2O_2 produced by oxidase-catalyzed reactions, we prepared GOX or AOX-conjugated QDs and demonstrated the abilities of conjugated QDs to detect glucose and alcohol inside the hydrogel microstructures in the microfluidic devices. Finally, we confirmed that the simultaneous detection of glucose and alcohol was possible by fabricating glucose and alcohol-sensitive hydrogel microstructures in different microchannels. The detection approach described in this paper can be extended to other biosensors based not only on H_2O_2-generating oxidase but also on any enzyme that generates QD-quenching products.

Acknowledgements

This work was supported by the National Research Foundation (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) (2010K001430 “Converging Research Center Program”, and R11-2007-050-03002-0 “Active Polymer Center for Pattern Integration at Yonsei University”).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.11.033.

References

Beebe, D.J., Moore, J.S., Bauer, J.M., Yu, Q., Liu, R.H., Devadoss, C., Jo, B., 2000. Nature 404, 588–590.
Bullen, C., Mulvaney, P., Sada, C., Ferrari, M., Alessandro, C.C., Martucci, A., 2004. J. Mater. Chem. 14, 1112–1116.
Cao, L., Ye, J., Tong, L., Tang, B., 2008. Chem.-Eur. J. 14, 9633–9640.
Choi, D., Jang, E., Park, J., Koh, W.G., 2008. Microfluid. Nanofluid. 5, 703–710.
Constantine, C.A., Gattas-Asfura, K.M., Mello, S.V., Crespo, G., Rastogi, V., Cheng, T.C., DeFrank, J.J., Leblanc, R.M., 2003. Langmuir 19, 9863–9867.
Costa-Fernández, J.M., Pereiro, R., Sanz-Medel, A., 2006. TrAC: Trends Anal. Chem. 25, 207–218.
Dendukuri, D., Pregibon, D.C., Collins, J., Hatton, T.A., Doyle, P.S., 2006. Nat. Mater. 5, 365–369.
Duong, H.D., Rhee, J.I., 2007. Talanta 73, 899–905.
Frasco, M., Chaniotakis, N., 2010. Anal. Bioanal. Chem. 396, 229–240.
Gill, R., Bahshi, L., Freeman, R., Willner, I., 2008a. Angew. Chem. Int. Ed. 120, 1700–1703.
Gill, R., Freeman, R., Xu, J.-P., Willner, I., Winograd, S., Shweky, I., Banin, U., 2006. J. Am. Chem. Soc. 128, 15376–15377.
Gill, R., Zayats, M., Willner, I., 2008b. Angew. Chem. Int. Ed. 47, 7602–7625.
Han, C., Li, H., 2010. Anal. Bioanal. Chem. 397, 1437–1444.
Heo, J., Thomas, K.J., Seong, G.H., Crooks, R.M., 2003. Anal. Chem. 75, 22–26.
Hu, M., Tian, J., Lu, H.T., Weng, L.X., Wang, L.H., 2010. Talanta 82, 997–1002.
Huang, C.-P., Liu, S.-W., Chen, T.-M., Li, Y.-K., 2008. Sens. Actuators B: Chem. 130, 338–342.
Ikami, M., Kawakami, A., Kakuta, M., Okamoto, Y., Kaji, N., Tokeshi, M., Baba, Y., 2010. Lab Chip 10, 3335–3340.
Ikeda, M., Ochi, R., Hamachi, I., 2010. Lab Chip 10, 3325–3334.
Kiyonaka, S., Sada, K., Yoshimura, I., Shinkai, S., Kato, N., Hamachi, I., 2004. Nat. Mater. 3, 58–64.
Koh, W., Revzin, A., Pishko, M.V., 2002. Langmuir 18, 2459–2462.
Koh, W.G., Pishko, M., 2005. Sens. Actuators B: Chem. 106, 335–342.
Li, X., Zhou, Y., Zheng, Z., Yue, X., Dai, Z., Liu, S., Tang, Z., 2009. Langmuir 25, 6580–6586.
Mattoussi, H., Clapp, A.R., Medintz, I.L., Uyeda, H.T., Fisher, B.R., Goldman, E.R., Bawendi, M.G., 2005. J. Am. Chem. Soc. 127, 18212–18221.
Mauro, J.M., Medintz, I.L., Clapp, A.R., Mattoussi, H., Goldman, E.R., Fisher, B., 2003. Nat. Mater. 2, 630–638.
Mellott, M.B., Searcy, K., Pishko, M.V., 2001. Biomaterials 22, 929–941.
Olsen, K.G., Ross, D.J., Tarlov, M.J., 2002. Anal. Chem. 74, 1436–1441.
Ozkan, M., Kim, J.H., Morikis, D., 2004. Sens. Actuators B: Chem. 102, 315–319.
Rosenzweig, Z., Shi, L.F., De Paoli, V., Rosenzweig, N., 2006. J. Am. Chem. Soc. 128, 10378–10379.
Russell, R.J., Simonian, A., Wild, J., Pishko, M.V., 1999. Anal. Chem. 71, 4909–4912.
Sandros, M.G., Gao, D., Benson, D.E., 2005. J. Am. Chem. Soc. 127, 12198–12199.
Wu, P., He, Y., Wang, H.F., Yan, X.P., 2010. Anal. Chem. 82, 1427–1433.
Yuan, J., Guo, W., Wang, E., 2008a. Biosens. Bioelectron. 23, 1567–1571.
Yuan, J., Guo, W., Wang, E., 2008b. Anal. Chem. 80, 1141–1145.
Zhan, W., Seong, G.H., Crooks, R.M., 2002. Anal. Chem. 74, 4647–4652.
Zhang, S., 2004. Nat. Mater. 3, 7–8.