Biosensors and Bioelectronics 26 (2011) 3050–3053

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Quantum dot based immunosensor using 3D circular microchannels fabricated in PDMS

Amit Morarka[a], Shailaja Agrawal[b], Sonia Kale[b], Anup Kale[c,d], Satish Ogale[d], Kishore Paknikar[b], Dhananjay Bodas[a,b,*]

[a] Center for Sensor Studies, University of Pune, Ganeshkhind road, Pune 411 007 India
[b] Center for Nanobioscience, Agharkar Research Institute, GG Agarkar road, Pune 411 004 India
[c] Center for Materials for Information Technology, University of Alabama, Tuscaloosa 35487 AL, USA
[d] Physical and Materials Chemistry Division, National Chemical Laboratory, Pashan, Pune 411 008 India

ARTICLE INFO

Article history:
Received 3 September 2010
Received in revised form 8 December 2010
Accepted 9 December 2010
Available online 16 December 2010

Keywords:
Circular microfluidics
Capillary driven flows
QDs
Immunosensor

ABSTRACT

Microchannel is basic functional component of microfluidic chip and every step-forward of its construction technique has been receiving concern all over the world. The present work describes a novel, rapid and simple fabrication technique for building 3D microchannels in poly(dimethyl siloxane) (PDMS) elastomer. These microchannels were used for rapid detection of antigens (*E. coli*) by quantum dot (QD) based approach. Luminescent QD (CdTe) were synthesized by aqueous method and characterized using high resolution transmission electron microscopy (HRTEM), fluorescence spectroscopy and X-ray diffraction (XRD). The QDs were functionalized with anti-*E. coli* antibodies for immuno-detection. The reported process allowed easier and faster method of fabrication of circular 3D micochannels and demonstrated their potential use in an immuno-biosensor device.

1. Introduction

Microfluidic chips are demonstrated to have significant potential applications in biological processing and chemical reactions (deMello and Wootton, 2009). Poly (dimethyl siloxane) (PDMS) chips are commonly used in microfluidic systems to achieve the goal of lab-on-a-chip. PDMS elastomer is the most widely and one of the most versatile material used in the construction of microfluidic devices, in particular for rapid prototyping. One of the reasons for popularity of PDMS is the particularly straightforward manufacturing methods. PDMS has essential characteristics such as elastomeric nature, optical transparency (230–700 nm), chemical inertness, biocompatibility, permeability to gases and amenability to fabrication via rapid prototyping. Unlike traditional microfabrication materials, such as silicon and glass, PDMS is a low-cost material. Micromolding processes are simple and rapid as compared to traditional etching and bonding approaches. In addition, the low curing temperature (<100 °C) makes it an excellent material for bonding polymer substrates since many polymer substrates cannot withstand a high bonding temperature (>200 °C) (Chow et al., 2006). Therefore, PDMS is particularly suitable for prototyping and testing of various microfluidic devices.

So far, methods for microchannel fabrication are mostly based on photolithography and soft lithography with few reports on direct fabrication techniques (Zhang et al., 2010; Song et al., 2010; Sia and Whitesides, 2003). Standard fabrication technology, viz. photolithography consists of the following steps: a) spin coating of photoresist on a substrate; b) photomask fabrication with targeted design/pattern; c) imprinting the design on the spin coated photoresist by exposing to UV; d) developing the substrate to transfer the printed pattern on to the substrate; e) etching of the substrate to realize the pattern transferred. The process is tedious, time consuming and requires expensive hardware for realizing the patterns/devices. Fabrication of 3D micochannels with circular geometry becomes a difficult task using standard method of fabrication i.e. photolithography. 2D and 3D structures fabricated in PDMS are used in many microfluidic applications such as micromixers, micro reactors, biosystem analysis, immunosensors etc. (Asthana et al., 2009; Friend and Yeo, 2010).

Recently fluorescent quantum dots (QDs) have attracted wide interest in immunoassays owing to their versatile optical and mechanical properties. Their size tuneable optical and electrical properties are unique, due to the quantum confinement of electronic states. Current thrust of the QD research is focused on optical, electrical, and mechanical properties as well as their applications ranging from electronic devices to biological sciences (Roco, 2003;

* Corresponding author at: Center for Nanobioscience, Agharkar Research Institute, GG Agarkar road, Pune 411 004 India. Tel.: +91 202 565 3680; fax: +91 202 565 1542.
E-mail address: dhananjay.bodas@gmail.com (D. Bodas).

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doi:10.1016/j.bios.2010.12.017

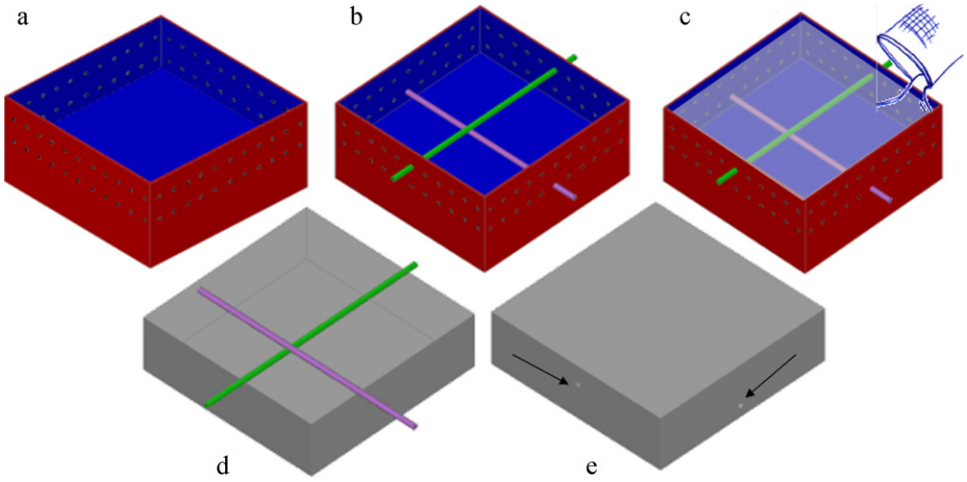

Fig. 1. Schematic of process for fabrication of 3D microchannels in PDMS (a) Device used for clamping micro-dimensional wires and molding PDMS; (b) Copper wires clamped in the device; (c) For microchannel fabrication liquid PDMS is poured; (d) Cured PDMS with wires is demolded form the device; (e) 3D microchannels fabricated after drawing the wires out from PDMS

Yamamoto and Sotobayashi, 2010). Detection and analysis of biological entities is vital in pathology, biosensor technology, clinical diagnosis, drug discovery, and environmental studies. Fluorescence detection is a technology which is sensitive and commonly used method to achieve this goal (Ache, 1989; Goldys, 2009; Haugland, 2002; Waggoner, 2006; Prasad, 2004).

Bacterial detection and identification is critical in many biological processes ranging from clinical diagnostics, monitoring of food borne pathogens, to detection of biological warfare agents etc. Conventional methods of bacterial detection and identification require sufficiently high cell densities, are usually time consuming and laborious. Hence there is a need for sensitive, small volume, low cost sensors that are capable of rapidly detecting and identifying bacteria from a variety of samples. Given the inherent advantages of microfluidics such as large surface to volume ratio, continuous fluid flow, requirement of small sample volumes for analysis and the possibility of multiplexing, microfluidic lab-on-a-chip presents an ideal solution for bacterial sensing and identification.

The present work deals with development of an immunosensor chip using circular 3D microchannels fabricated directly with microdimensional metal wires. The chip permits easy and visual detection of *Escherichia coli (E. coli)* using QDs. Briefly, this simple yet effective method comprises of following steps: immobilisation of anti-*E. coli* antibody in microchannels, allowing the target antigen (*E. coli*) to flow through, and detecting the captured antigen in the microchannel with the help of QD-antibody (anti-*E. coli)* conjugate. One of the most significant feature of the microfluidic device being reported in the present work is that the fluid flows were established by capillary force alone obviating the need of any external pumping.

2. Materials and methods

2.1. Fabrication of PDMS chip

Fig. 1(a–e) shows process for fabrication of 3D microchannels in PDMS. Fig. 1a shows the device used for fabrication of microchannels using microdimensional copper wires and PDMS. Copper wires of 150 μm diameter were inserted and clamped in the device (see Fig. 1b). Liquid PDMS (Sylgard 184 from Dow Corning) in the ratio 10:1 (base: curing agent) was poured, degassed for 20 min using a vacuum desiccator and allowed to cure in a convection oven for 3.5 h at 65 °C. Fig. 1c shows moulded PDMS with copper wires after curing. After PDMS was cured completely, PDMS mould with the wires was removed by de-clamping the wires from the device (Fig. 1d) and immersed in ethanol for 30 min. PDMS swelling in ethanol (swelling coefficient 1.04) (Jia et al., 2008) facilitated easy removal of wires. The wires were drawn out manually by applying a gentle force leading to formation of circular microchannels in PDMS as indicated by arrows in Fig. 1e. A reservoir was made at the centre of the chip by making a circular pit using a micropunch. The PDMS chip was activated in oxygen plasma (EMITECH: Model-K1050X) at RF power of 60 W for 1 min to create hydrophilic surface for liquid transport by capillary forces.

2.2. Synthesis of CdTe nanoparticles

All chemicals (analytical grade) like Cadmium chloride ($CdCl_2$, SD Fine chemicals, India), Mercaptopropionic acid (MPA, SRL chemicals, India), tellurium powder (Te), and sodium borohydride ($NaBH_4$) were used as received. Deionised water was acquired from Millipore Milli-Q system with resistivity ~18 MΩcm. MPA capped colloidal CdTe QDs were synthesized by organometallic route (Byrne et al., 2006; Kuo et al., 2009). All the preparations were carried out in inert atmosphere. NaHTe was used as the Te precursor and $CdCl_2$ as Cd precursor. Freshly prepared NaHTe was used for CdTe synthesis by reacting $NaBH_4$ and Te powder in molar ratio of 3:1. Tellurium powder (3 mM) was mixed with $NaBH_4$ (9 mM) in a closed round bottom flask containing 1.0 ml deionised water under N_2 atomsphere for 30 min. The reaction was stopped after the black Te powder disappeared and solution turned completely transparent pink in colour.

CdTe QDs were then prepared by reaction between $CdCl_2$ and NaHTe with MPA as a stabiliser. The molar ratio of Cd^{2+}:Te^{2-}:MPA was set as 1.0:0.2:2.5. In a 3-necked flask, 100 ml of N_2 saturated deionised water containing $CdCl_2$ (15 mM) and MPA (36 mM) were reacted with freshly prepared oxygen-free NaHTe with vigorous stirring. The pH of the solution was adjusted to 9.0 with 1 M NaOH prior to the reaction. The resulting solution mixture was heated and refluxed under nitrogen flow at 100 °C for 2 h. The growth solution was then cooled and CdTe QDs were precipitated out by 2-propanol. Further purification was performed by centrifugation and re-precipitation thrice from 2-propanol.

2.3. Preparation of QD–antibody conjugates

Antibodies (anti-*E. coli* rabbit polyclonal antibodies, Bangalore Genei, India) were conjugated with QDs using 1-ethyl-3-(3-

dimethylaminopropyl) carbodiimide (EDC) as a coupling reagent. 5 mM QD and 1% solution of EDC were prepared in PBS (Phosphate buffer saline pH 7.4). 100 μl of each solution were mixed and incubated at room temperature for 10 min in dark with gentle shaking. Equal volume of antibody (rabbit polyclonal anti- *E.coli* IgG) (2 mg/ml) was added, followed by incubation at room temperature for 2 h. Finally, the reaction was terminated by keeping the mixture at 4 °C overnight. The stock, ready-to use solution of the product was stored at 4 °C without luminescence intensity decrease for over a month.

2.4. QD based immunodetection

2.4.1. On nitrocellulose membrane

100 μl of bacterial culture (*E. coli* ATCC 117 culture, 0.1 OD), was spotted at the centre of the nitrocellulose membrane (Immobilon-NC, Millipore) of a flow through device. The latter comprised of a stack of 1.5 cm^2 filter papers with nitrocellulose membrane of the same size at top sandwitched in a plastic cartridge. The device was incubated at 37 °C for 30 min, followed by washing with wash buffer (phosphate buffer saline (PBS)-Triton X, pH 6). To avoid non-specific interaction, membrane was blocked with blocking buffer (5% BSA in PBS, pH 6) at 37 °C for 30 min, washed thrice with wash buffer and then QD- antibody conjugate (10 μl) was added. Washing was carried out with wash buffer to remove unbound QD-antibody conjugated and then membrane was visualized under UV trans-illuminator. The photograph was captured using Sony H50 digital camera.

2.4.2. In microchannel

E coli (ATCC 117) was grown in nutrient broth (Himedia) at 37 °C for 12 h. Absorption was monitored at 600 nm. 0.1 OD culture (~10^8 cells/μl) of *E. coli* was serially diluted. Four serial tenfold dilutions of bacterial suspensions were prepared in PBS. After preparation, a 100 μl sample from each dilution (corresponds to 10^6, 10^5, 10^4, 10^3, 10^2 cells/10 μl) were added to central reservoir of respective immunosensor chip and allowed to flow through the channels with capillary force. Incubation was carried out at 4 °C for 12 h for appropriate adsorption on to the oxygen plasma modified microchannels. The unbound surface of the channels was blocked with BSA (3% BSA in PBS, pH 7.4) followed by addition of QD-conjugated anti-*E. coli* antibody. Channels were washed with PBS by flushing with DI water by placing a cover slip on the central reservoir and applying finger pressure. Optical images of the flushed channel are given in the supplementary information. The channels were imaged using upright fluorescent microscope equipped with CCD (Nikon H600L, Japan). Images were analysed using MacBiophotonics ImageJ software. The fluorescent signal was recorded as absolute intensity from the whole channel.

3. Results and discussion

Using the present technique we were able to fabricate, 3D microchannels of the diameter ranging from 40 to 500 μm with length ranging from few mm to tens of mm. Fabrication of complex geometries as reported in (Song et al., 2010; Jia et al., 2008) was possible using our fabrication method. 3D circular microfluidic channel of diameter 150 μm with the length of 1 cm was fabricated for development of immuno-biosensor device. Due to the difference in depth of focus, only one channel was focused and the other two remained de-focused indicating channels at different heights. Water soluble ink viz. red, blue and green were injected into the channels using a syringe needle. This demonstrates that the channels are not intersecting with each other (Figure supplied as supplementary information). A central reservoir of 1 mm diameter is punched for liquid inlet. The liquid (blue ink) of fixed volume was placed in the reservoir and was driven by capillary to the ends of the microchannels where it can be collected by blotting paper. Scanning electron micrograph (SEM) images shown in Fig. 2 indicated a capillary formed in PDMS. A perfectly circular cross-section with smooth morphology of channel was obtained from the fabrication process as seen from the SEM image given in inset (Fig. 2).

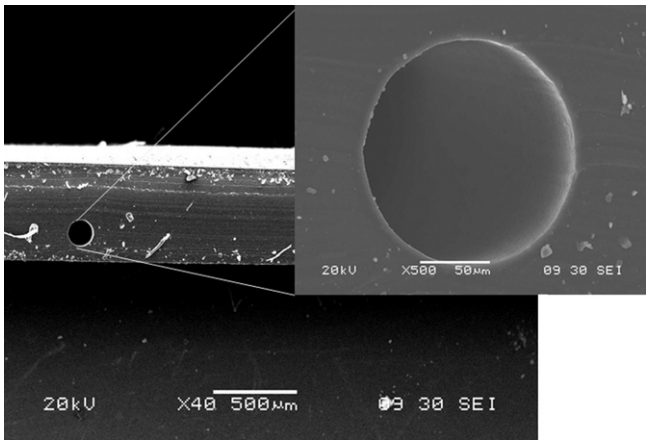

Fig. 2. SEM image of a circular microchannel fabricated in PDMS

Fabricating circular cross-section microchannel array in PDMS as described here is of immense practical significance. For example, it will be more convenient to analyse rheological parameters and their relationships in rounded channels than any other morphologies. We have demonstrated that a difficult-to-fabricate 3D channel orientation in PDMS could be quickly achieved using a simple procedure that could be mass replicated.

CdTe is a direct bandgap semiconductor, the bulk material is black with an absorption onset at 826 nm (E_g = 1.56 eV at 300 K). In this work, small colloidal nanoparticles of CdTe with diameter of 3.5 nm were prepared in aqueous solution. The size was determined from TEM images and from the broadening of the lines in X-ray diffractograms. The photoluminescence emission spectra of these CdTe QDs and CdTe antibody conjugates are presented in Fig. 3. CdTe QDs exhibited absorption peak at 479 nm (2.54 eV). The luminescence spectrum (λ_{ex} = 380 nm) of CdTe was observed with intense peak at 550 nm. The luminescence spectrum show excitonic emission near the respective absorption band edge of CdTe. A blue shift was observed for the peak of antibody conjugated CdTe with decrease in intensity. This is obvious due to change in size of the QD and attachment of antibody.

The powder X-ray diffraction (XRD) profile of the CdTe nanoparticles shows the diffraction pattern of (1 1 1), (2 2 0), and (3 1 1) reflections at 24.47°, 40.36°, and 46.75° 2θ angles respectively.

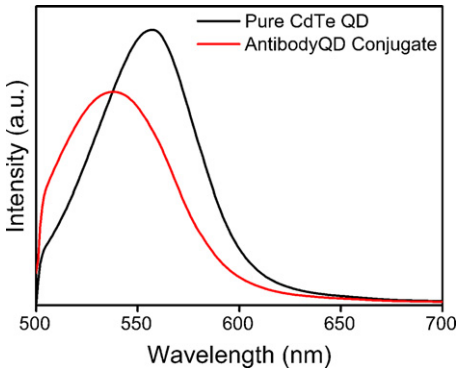

Fig. 3. Photoluminescence emission spectra of Pure and antibody conjugated CdTe quantum dots

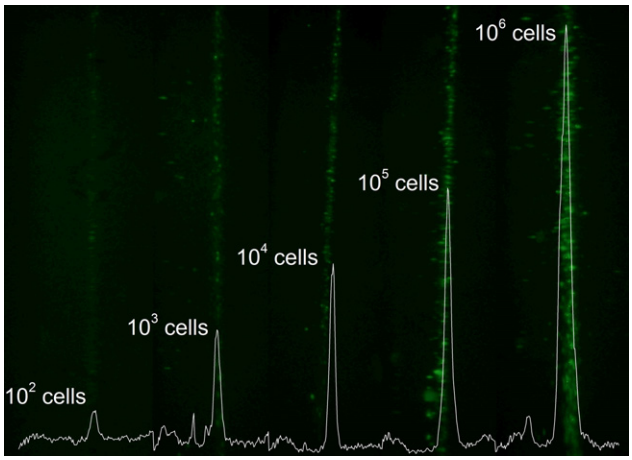

Fig. 4. Fluorescence image of different concentration of *E. coli* captured PDMS-IgG labeled with QD-antibody conjugates. The overlap graph shows increase in fluorescence intensity with increase in cell count.

Morphological analysis of CdTe nanoparticles was done using high resolution transmission electron microscopy (HRTEM): model FEI Technai 30 system operated at 300 kV. From HRTEM images it can be derived that CdTe nanoparticles with an average diameter of 3.5 ± 0.5 nm were formed and their size distribution is uniform. The lattice parameters derived from HRTEM and XRD measurements for MPA-stabilized CdTe nanoparticles fit to the cubic zinc blende structure of bulk CdTe crystal (Rogach et al., 1996).

The application potential of this microfluidic immunoassay platform was confirmed by bacterial detection (*E. coli.*). Fig. 4 shows the fluorescence of the QDs in microchannels. *E. coli* of different concentration ranging from 10^2 to 10^8 cells/μl were passed. Strong fluorescence signal in test indicating the presence of *E. coli* of greater concentration which can be seen from the overlayed graph of fluorescence intensity variation. Microchannels having captured *E. coli* shows fluorescence because of the binding of QD tagged detecting antibodies as seen in Fig. 4. No fluorescence was observed in control channels.

4. Conclusions

Microchannel fabrication process in PDMS without the use of large equipment and tedious process was demonstrated. This technique provides flexible, simple procedure to fabricate a range of multi-stack, 3D circular microchannels in various dimensions and geometries. Circular geometry presents no concerns with entrapment of bubbles hampering or manipulating the flows (entrapment at corners in square geometry). Moreover the present technique of fabrication is straightforward, easy, cost effective, fast and repeatable. CdTe nanoparticle QDs were synthesized, characterized and used for detection of *E.coli*. QDs are excellent material for detection due to their stable and high fluorescence signal. Development of an immunosensor is demonstrated using a novel fabrication process. The study opens up many possibilities of use of microchannels in biological detection and imaging and is likely to lead to inexpensive screening for multiple diseases which will open up new ways to simple on-the-chip diagnostics.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.12.017.

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