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## Microfluidic tectonics platform: A colorimetric, disposable botulinum toxin enzyme-linked immunosorbent assay system

A fabrication platform for realizing integrated microfluidic devices is discussed. The platform allows for creating specific microsystems for multistep assays in an *ad hoc* manner as the components that perform the assay steps can be created at any location inside the device *via in situ* fabrication. The platform was utilized to create a prototype microsystem for detecting botulinum neurotoxin directly from whole blood. Process steps such as sample preparation by filtration, mixing and incubation with reagents was carried out on the device. Various microfluidic components such as channel network, valves and porous filter were fabricated from prepolymer mixture consisting of monomer, cross-linker and a photoinitiator. For detection of the toxoid, biotinylated antibodies were immobilized on streptavidin-functionalized agarose gel beads. The gel beads were introduced into the device and were used as readouts. Enzymatic reaction between alkaline phosphatase (on secondary antibody) and substrate produced an insoluble, colored precipitate that coated the beads thus making the readout visible to the naked eye. Clinically relevant amounts of the toxin can be detected from whole blood using the portable enzyme-linked immunosorbent assay (ELISA) system. Multiple layers can be realized for effective space utilization and creating a three-dimensional (3-D) chaotic mixer. In addition, external materials such as membranes can be incorporated into the device as components. Individual components that were necessary to perform these steps were characterized, and their mutual compatibility is also discussed.

**Keywords:** Botulinum neurotoxin / Enzyme-linked immunosorbent assay / Hydrogel / Miniaturization / Microfluidic tectonics / Photopolymerization  
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### 1 Introduction

Miniaturized laboratory-on-a-chip for chemical and biological assays has gained attention in recent years [1–7]. The potential capability of these systems to carry out assays in a cost-effective and time-efficient manner has made them popular platforms for the development of portable, personalized diagnostic kits and for performing high-throughput screening (e.g., drug testing). Such systems also referred to as microfluidic devices, typically consist of channels, reservoirs, pumps, and valves (microfluidic components) that allow for the manipulation of

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**Abbreviations:** **BCIP/NBT**, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium; **G-6-PDH**, glucose-6-phosphate dehydrogenase; **HEMA**, 2-hydroxyethyl methacrylate; **LP3**, liquid photopolymerization; **PDMS**, poly(dimethylsiloxane); **poly(IBA)**, cross-linked poly(isobornyl acrylate); **TAP**, thumb-actuated pump; **μFT**, microfluidic tectonics

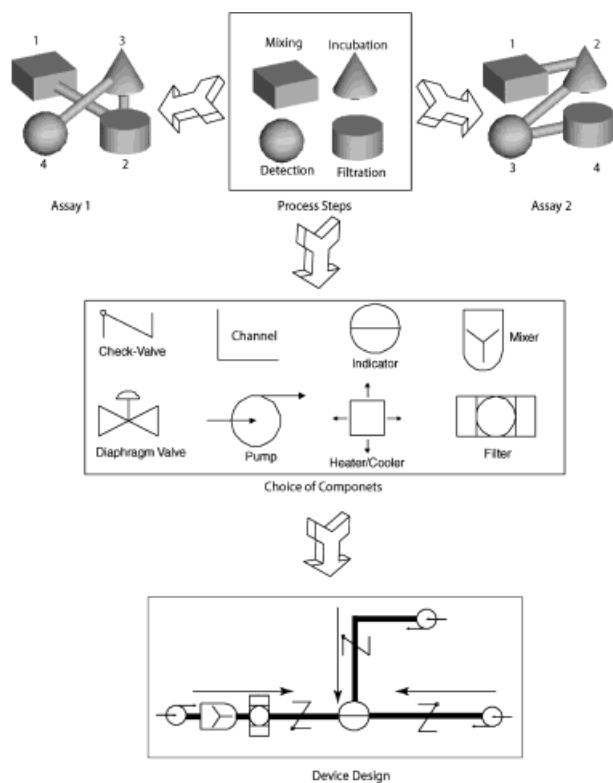
small volumes of fluid and performing chemical and biological assay steps. One approach to designing such an integrated system is to fabricate individual components and assemble them to achieve the required system level functionality. This approach is useful when the arrangement and connectivity of components is well defined. In biological or chemical assays, the protocol varies with the type of analysis and analyte requiring different arrangement and connectivity of microfluidic components. For example, in certain reactions a separation step is performed before a detection step to remove contaminants, while in others the separation step is performed after detection to remove by-products. Thus, there is a need for approaches that enable rapid design and fabrication of microfluidic circuits that can address the inherent specificity of analytical processes and yet be versatile enough to be restructured in a simple and efficient manner for use in more than one type of analysis.

In this paper, we describe the use of a design-fabrication platform referred to as microfluidic tectonics (μFT) [8], in creating an integrated microsystem for specific assay

needs.  $\mu$ FT allows for creating various components in an *in situ* manner, thus giving the flexibility to place components in any order or geometric location. The design process on this platform begins by regarding the process steps in an assay as modules and connecting them in any desired order (Fig. 1). These process steps are then replaced by microfluidic components (e.g., mixing step replaced by a mixer). Based on specific functionality, the design and appropriate materials are chosen to create the component [9]. Next, the appropriate order of the fabrication steps is determined based on the compatibility of the materials and limitations imposed by fabricated components. Finally, the device is realized (typically in less than 1 h) by utilizing the liquid-phase photopolymerization methods of  $\mu$ FT [10].

As a specific example, the design and fabrication of a microfluidic device for assaying botulinum toxoid (formalin-treated toxin) by sandwich ELISA (enzyme-linked immunosorbent assay) directly from whole blood will be described. Botulinum neurotoxin is the most poisonous substance known with an estimated human lethal dose

of approximately 1 ng/kg [11]. Although outbreaks due to contaminated food are rare, the infection can have a profound impact on areas in which the outbreaks occur [12]. Due to its high specific toxicity, botulinum toxin is also considered a potential agent for use in bioterrorism [13]. Cases of suspected botulism can be confirmed by detection of neurotoxin in fecal material, blood serum or food consumed by the ill person using the mouse bioassay [14]. Determination of neurotoxin activity and serotype (A–G) by neutralization of samples with type-specific antiserum can take up to four days. Currently, the only therapeutic treatment for botulinum poisoning is the administration of antitoxin as quickly as possible after exposure to the toxin, but this treatment is only effective prior to the toxin binding to nerves (ca. 24 h). A sandwich ELISA developed for detection of botulinum toxins [15] could help in accelerating the diagnosis of exposure of an individual to the neurotoxin. By transferring the ELISA protocols to a microfluidic device, the assay time may be further shortened allowing more rapid treatment of exposed individuals with antitoxin along with other advantages such as portability, and consumption of small sample and reagent volumes.



**Figure 1.** Biological and chemical assays often differ in process steps and in the order these steps are performed (illustrated by the 3-D shapes).  $\mu$ FT platform allows for designing the device for a specific need. Depending on a process step, one or more components (from the “toolbox”) may be required. After the device layout is ready, the channel networks and components are fabricated.

## 2 Materials and methods

The device is designed to perform all assay steps including sample preparation and sample detection using ELISA. Multiple functions such as diluting and mixing blood, separating whole blood to serum and cells, and detecting botulinum toxoid in the serum are performed in the device. The following sections describe the design and fabrication of the device, and the protocols for the assay.

### 2.1 Device design

ELISA is a multistep process requiring incubation with various reagents. The device is designed to store the reagents in reservoirs. The reservoir is prepared from compliant material so that pressing (with finger) provides the driving force to move fluid through the channels. Check valves are designed so that reagents do not contaminate each other during operation. Near the sample input port, a chaotic mixer [16] and a porous filter [17] are incorporated for preparing the sample before detection. Sample preparation includes dilution, mixing and separating the blood cells from whole blood. The sensing components consist of avidin-agarose beads held by a filter membrane. The avidin derivative on the agarose beads allow for immobilizing the primary antibody (biotin-labeled) *via* biotin-avidin interaction.

## 2.2 Fabrication

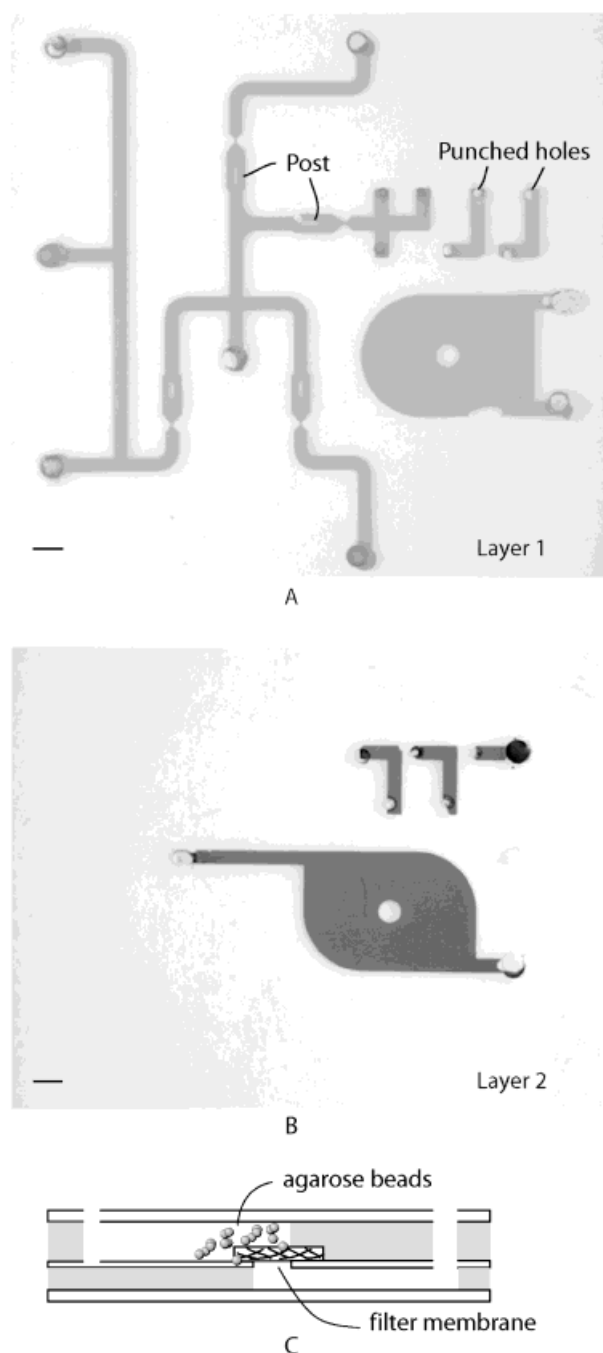
The channel network, filter, and valve are fabricated in the tectonics platform while the thumb-actuated pump (TAP) is molded from poly(dimethylsiloxane) (PDMS) and attached to outlets of the device (Table 1). In  $\mu$ FT, the channel walls and the microfluidic components are photopolymerized from monomer solutions. Liquid-phase photopolymerization (LP3) allows for fabrication of structures directly inside a cartridge, which is formed by bonding a polycarbonate film to a glass slide *via* an adhesive gasket (Grace Bio-labs, Bend, OR, USA). By stacking polycarbonate layers, effective utilization of space and fabrication of a three-dimensional (3-D) channel network is possible (Beebe *et al.*, submitted). Interconnection between the two layers is possible through holes punched into the first layer using an arbor press (1/32" punch and die set; Micro Mark, Berkeley Heights, NJ, USA) (Fig. 2A). The channel network and anchor posts are fabricated from a prepolymer mixture consisting of isobornyl acrylate (IBA) monomer, tetraethylene glycol dimethacrylate (TeGDMA) cross-linker and 2,2-dimethoxy-2-phenyl-acetophenone (DMPA) photoinitiator (1.9:0.1:0.06 weight ratio). After attaching a polycarbonate film to a microscope glass slide and filling with the prepolymer mixture, the layer 1 mask was placed on top. The device was exposed to light of wavelength 365 nm and intensity 12 mW/cm<sup>2</sup> (Acticure; EFOS, Quebec, Canada) for 12 s forming the first layer of the channel network (Fig. 2A). After removing the unpolymerized mixture, the channel was filled with water and further exposed to UV light (12 s, 12 mW/cm<sup>2</sup>) to obtain well-defined channel walls. At this stage, a polycarbonate filter membrane (GE Osmotics, Minnetonka, MN, USA) of 8  $\mu$ m pore size, was placed on top of layer 1 and was attached by applying and poly-

**Table 1.** List of microfluidic components incorporated in the ELISA system

Process	Component	Material	Fabrication
Storage	Reservoir	PDMS	Micromolding
Fluid actuation	Pump	PDMS	Micromolding
Mixing	Chaotic mixer	Poly(IBA)	LP3
Separation	Porous filter	Poly(HEMA)	LP3
Prevent contamination	Check valve	Poly(HEMA) piston with rigid Poly(IBA) post	LP3
Fixation	Filter membrane	Polycarbonate	Commercially available
Sensing/display	Gel beads	Agarose	Commercially available

IBA, isobornyl acrylate

HEMA, 2-hydroxyethyl methacrylate

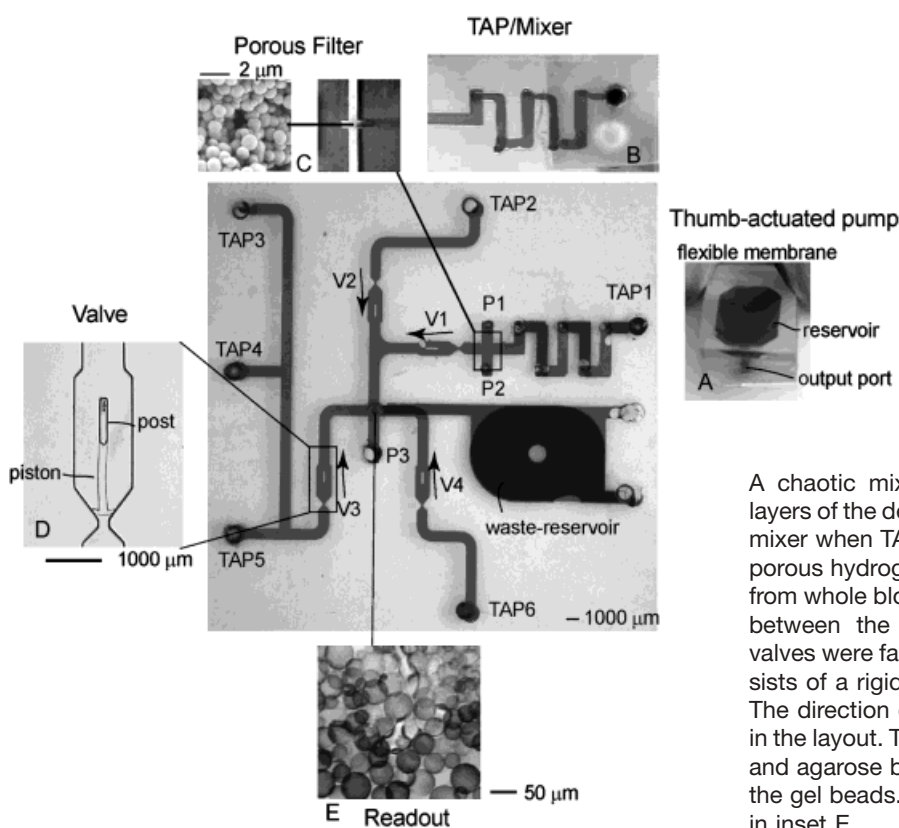


**Figure 2.** Top view of the (A) first and (B) second layer fabricated for the ELISA system. The fluidic paths are filled with food color for visualization. The channel width is 1000  $\mu$ m while the depth is 125  $\mu$ m. (C) Side view of the filter incorporated between the layers. Scale bar, 2 mm.

merizing a small amount of prepolymer mixture to the edges of the membrane. In the final device, the membrane forms a part of the readout by holding the agarose gel beads (Fig. 2C). When the second layer is fabricated on top of the first, the membrane is sealed into the walls of

the device. Before attaching the second cartridge, the channels were filled with a dyed glycerin solution. Because glycerin and the prepolymer are immiscible, the channel network in the first layer is “protected” during the polymerization of the second layer. The glycerin solution along with unpolymerized prepolymer mixture was removed when the final channel network is completed. A serpentine (chaotic) mixer was realized between the two-layer channels (Fig. 3B). Other components were fabricated or added to the device as described below. The valve is composed of a rigid post and a hydrogel piston (Fig. 3D) [18]. The rigid post is formed from cross-linked poly(isobornyl acrylate) (Poly(IBA)) and was created during fabrication of the first layer (Fig. 2A). The hydrogel part of the valve was fabricated from a mixture containing 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA) as cross-linker and photoinitiator DMPA (1:0.03:0.03 weight ratio). This mixture was introduced into the device and the mask was aligned using a microscope. The piston/head of the valves were sequentially formed at designated locations by irradiating UV light (from the mercury source of the microscope) through the mask. A porous filter whose function is to separate blood cells from whole blood was fabricated *in situ* via emulsion photopolymerization [17]. In this pro-

cess, a mixture containing HEMA, DMPA, EGDMA, and water (0.22:0.002:0.008:0.78 weight ratio) was introduced into channel P1-P2 (Fig. 3) of the device. The mixture was irradiated with UV light ( $15 \text{ mW/cm}^2$ ) through a mask for a period of 5 min to form a porous monolith. The different assay solutions are introduced into the device via the TAP. The TAP consists of a fluid reservoir, flexible top, outlet port, and adhesive bottom as shown in Fig. 3A. The fluid reservoir and flexible top are fabricated from PDMS (Sylgard 184; Dow Corning, Midland, MI, USA). For the flexible top, uncured PDMS was spin-coated on a silicon wafer coated with Teflon (AE Yale Enterprises, San Diego, CA, USA) at 1600 rpm for 20 s to obtain a  $75 \mu\text{m}$  thick membrane (after curing). The Teflon layer facilitates membrane removal from the wafer. The reservoir was fabricated through negative relief molding of plastic gems (The Beadery, Hope Valley, RI, USA) of desired reservoir volume. For this specific application, an emerald cut (rectangular; volume,  $150 \mu\text{L}$ ) and a marquise variation cut (largest TAP; volume,  $150 \mu\text{L}$ ) were utilized. After the silicone oligomer was poured over the negative mold master, an adhesive layer comprising of a double-sided adhesive (Grace Bio-labs) attached to a nitrocellulose filter (Millipore, Billerica, MA, USA) was floated on top of the mixture. The porosity of the nitrocellulose filter allows



**Figure 3.** Layout of the ELISA device. All fluidic paths are filled with food color for visualization. For protein storage and driving flow through the channel network, six TAPs were included. The TAP is micromolded from PDMS and consists of a reservoir, an outlet port and an adhesive layer at the bottom (inset A).

A chaotic mixer was fabricated between the two layers of the device. Fluid moving through the chaotic mixer when TAP is depressed is shown in inset B. A porous hydrogel was utilized to separate blood cells from whole blood (inset C). To prevent contamination between the reagents and backflow, four check valves were fabricated in the device. Each valve consists of a rigid post and a hydrogel piston (inset D). The direction of flow allowed is indicated as arrows in the layout. The readout consists of filter membrane and agarose beads. ELISA is performed on (and in) the gel beads. The beads after the assay are shown in inset E.

for PDMS to wick into the filter matrix to provide a strong mechanical bond between the adhesive and the bulk PDMS. After heating at 85°C for 100 min, the cured PDMS was removed from the mold master and access holes were cored with a 14-gauge needle. The reservoir (with adhesive bottom) and the spin-coated PDMS were irreversibly bonded after treating the surfaces in oxygen plasma (15 s at 70 W). The completed array of TAPs was sectioned into individual TAPs with a razor.

## 2.3 ELISA protocol

Avidin-agarose gel beads were functionalized (in a test tube) with antitoxin (serotype A) antibody (labeled with biotin) by incubating in a 10 µg/mL solution of the antibody for a period of 12 h at 4°C after washing the beads in the dilution buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4). To reduce nonspecific interactions and background noise, the beads were blocked with casein solution for 24 h at 4°C. Meanwhile, the filter membrane on the device was blocked with the casein solution for 1 h at room temperature in a high-humidity chamber so that the valves and the porous filter do not dry. The functionalized beads were introduced into the device through port P3 (Fig. 3) and seated in the filter membrane by applying vacuum at the outlet of the waste reservoir. The TAPs were filled with appropriate reagent solution using a 30-gauge needle, and adhered to the device outlet ports. A few drops of rabbit whole blood (with 10 mM EDTA as anticoagulant) was added into the toxoid solution and introduced through the sample TAP. The sample is transported to the agarose beads through the mixer (dilution step) and the porous filter (filtration step). After incubating for 1 h at room temperature, the sample was removed and replaced with wash buffer (10 mM Tris-HCl, 150 mM NaCl, 0.005% Tween 20, pH 7.4) by pressing one of the wash TAPs (3, 4 or 5). Secondary antibody (5 µg/mL) conjugated with alkaline phosphatase stored in TAP 2 on the device was brought to the readout by pressing the appropriate TAP. The beads were incubated in the antibody for 30 min at room temperature. After thoroughly washing the readout by pressing the wash TAP multiple times (to minimize background), the substrate solution 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT; Sigma, St. Louis, MO, USA) from TAP 6 was introduced and the color change of the agarose bead was monitored. To test the sensitivity of the agarose beads for the assay, a separate test was performed where the concentration of the botulinum toxoid was varied (serially diluted from 100 µg/mL to 0 µg/mL). The test device consisted of two straight channels (in two different layers) connected through a filter membrane. The in-

tensity of color change was recorded and analyzed using MetaMorph imaging software (Universal Imaging, Downingtown, PA, USA). Polyclonal antibodies to specific toxin types were purified from rabbit serum by protein A chromatography. Biotinylation and alkaline phosphatase conjugation of the antibodies were done according to standard methods [19].

### 2.3.1 Pressure and flow rate from TAP and valve

The pressure generated from the TAPs (reservoir volumes of 50, 150 and 250 µL) was measured by attaching the TAP to a microchannel with the other end of the channel connected to a pressure transducer (142PC30D; Omega Engineering, Stamford, CT, USA). Similarly, the opening pressure of a valve in a straight channel was measured. The flow rate from depressing the TAP was measured by connecting a long collection tube to the output port of the TAP and monitoring the displacement of the fluid front with respect to time. The distance was converted to a volume since the diameter of the collection tube is fixed and known. The TAP was tested under both high- and low-force actuation to simulate the range in human operation.

### 2.3.2 Protein adsorption to TAPs

In the ELISA system, along with the wash buffer and substrate, the secondary antibody is stored on the device. To test the efficiency of protein storage in the PDMS TAP, protein solutions (bovine serum albumin) of varying concentration (serially diluted from 200 µg/mL to 0 µg/mL) were introduced into the TAP. The TAPs were stored for 20 h at 4°C. To minimize evaporation, silicone glue was applied to the puncture holes on the TAPs. A high-humidity environment was maintained in the petri-dish (used for storing the TAP) by placing a small container with water and sealing the petri-dish with parafilm. The concentration was verified after storage by removing the protein solution and performing Coomassie assay (Coomassie Plus protein assay reagent; Pierce, Rockford, IL, USA) in a test tube. For this assay, a 100 µL volume of the protein solution was mixed with 1 mL of the assay solution. The absorption was measured at 595 nm after incubating for 10 min at room temperature.

### 2.3.3 Effect of TAP and mixer on cell lysing

The force exerted while pressing the TAP and the shear stress in the chaotic mixer could possibly lyse blood cells, whose contents might interfere with the assay. To check for cell lysing with these components, a simplified device containing chaotic mixer and TAP was utilized. Rabbit

whole blood diluted ( $10\times$ ) in hypotonic phosphate buffer was injected into the TAP. The TAP was pressed down (maximum pressure *ca.* 25 psi), and the blood sample was collected at the exit port. After centrifuging to remove blood cells, glucose-6-phosphate dehydrogenase (G-6-PDH) assay (Sigma) was performed on the supernatant (in a test tube). In this assay, 10  $\mu$ L of the supernatant was added to 1 mL of assay solution and the mixture was allowed to stand for 5 min at room temperature. To this mixture, 2 mL of substrate solution was added and the amount of NADPH, which is one of the products of the enzymatic reaction, was calculated by measuring the change in absorption at 340 nm.

### 3 Results and discussion

One of the requirements for a portable diagnostic device is that it must be able to perform the assay directly from a biological sample such as blood, saliva or urine. To realize this capability, a sample preparation step may be necessary. The presence of botulinum neurotoxin can be tested in a blood sample; thus, the device was designed to perform the assay using whole blood. Typically, blood cells are removed from whole blood and the serum is assayed for the presence of a specific marker (antigen). In the device, a porous filter [17] carries out this sample preparation step. The porosity of the filter depends on the prepolymer mixture. The composition was chosen such that the size of the pores ranges from 2  $\mu$ m to 4  $\mu$ m; thus making the filter efficient for removing blood cells that are about 7  $\mu$ m in diameter. The blood cells are compliant and can squeeze through the pores of the filter. To prevent the cells from flowing through the filter, the blood is mixed with a hypotonic solution that causes the cells to swell and become more rigid. The mixing step is performed in the chaotic mixer that is placed prior to the porous filter.

The reagents were delivered to the readout by pressing appropriate TAPs. When the TAP is released, the reagent solution tends to flow back into the TAP reservoir due to back-pressure. By incorporating check valves at appropriate locations (Fig. 3), the backflow was prevented. Another function of the valve is to prevent contamination between the reagents. To minimize false positives, it is imperative that the secondary antibody in solution does not mix with the substrate solution. Hence, the TAPs containing these two solutions were placed far away from each other (TAP2, TAP6) to minimize the chance of cross-contamination. Moreover, the channels connecting these TAPs to the readout have separate valves (V2 and V4 in Fig. 3). The wash buffer was required to remove the reagents (after incubation) and any nonspecific interactions. For simplicity, the wash buffer TAPs (3, 4, 5)

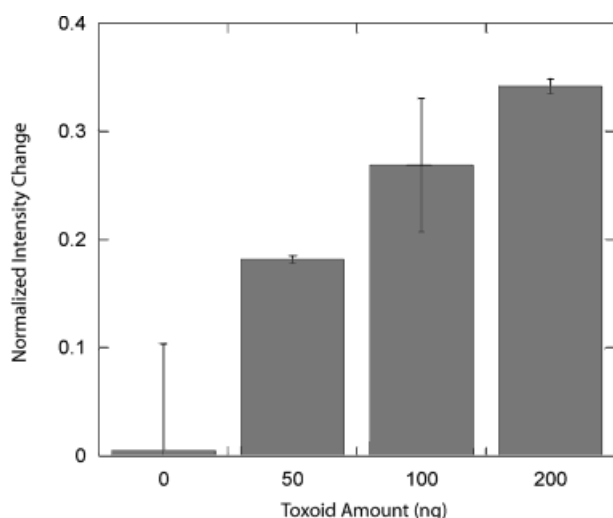
were placed on the same side of the device and protected from the rest of the device by one check valve (V3). These TAPs can be used to store other reagents and additional valves could be incorporated as needed for different assays.

In the sandwich ELISA used in this study, the antigen is first bound to a biotinylated primary antibody immobilized onto streptavidin-coated beads. This step was performed outside the device. An alkaline phosphatase toxoid-binding antibody is then added and the recognition is amplified by enzymatic reaction, which enhances the sensitivity. The sensing components consist of avidin-agarose beads held by a filter membrane. Advantages in keeping the sensor and the device separate include: (i) the fabrication procedures (UV exposure and solvents) do not affect the readout and the immobilized protein, (ii) the device can be pretreated (blocked) before introducing the sensor thus minimizing background, (iii) the sensor can be stored in appropriate conditions (buffer, temperature) independent of the device, and (iv) the device provides the framework for detecting any other antigen depending on the antibody immobilized on the sensor. Moreover, as bead-based ELISA is becoming increasingly popular [7, 20], the device can be utilized for different applications. The readout signal is from an insoluble product formed from the reaction between alkaline phosphatase and BCIP/NBT. This substrate is commonly used in immunoblotting studies. The advantage of insoluble compounds is that the color can be easily seen with an unaided eye, as opposed to spectroscopic measurements typically necessary for soluble products. However, the capability to quantify the amount of antigen is limited by availability of imaging and analysis tools.

#### 3.1 Characteristics of the individual components

##### 3.1.1 Sensitivity of readout

In a separate test, the ability of the agarose gel beads to discern various amounts of toxoid was tested. The volume in the sensing channel was 2  $\mu$ L, and the amount indicated in the graph was calculated using this volume (Fig. 4). Because the color of the agarose beads increases with time, the intensity of the readout was compared 12 min after introducing substrate solution. A concentration-dependent change in signal intensity was seen. The large error bars are mainly due to the difference in the amount of gel beads in the channel. Although the volume of mixed agarose solution was maintained constant for each concentration, a variation in the amount of beads and their arrangement in the channel was observed. Both these factors affect the intensity of the signal because the surface area of the sensor is affected. By



**Figure 4.** Quantitative ELISA was performed on the agarose beads in a separate device. The amount of botulinum toxoid was varied while keeping the concentration of the primary and secondary antibodies constant. The intensity of color was measured after 12 min of incubation with the substrate (BCIP/NBT) solution. The graph shows the normalized (averaged for three tests) intensity for each toxoid concentration.

improving the repeatability of the volume and placement of the agarose beads in a more controlled manufacturing environment, the sensitivity of the assay could be improved. Further optimization in utilizing the agarose solution would be necessary for quantitative assays. However, for the present qualitative assay the agarose bead is appropriate. The toxoid binding to the antibody is approximately 10 times lower than the native neurotoxin binding (compared in microtiter plates). Therefore, with the present format, detection is of the order of  $1.5 \times 10^{-3}$  human MLD\*/mL ( $1 \mu\text{g/mL} \sim 15 \times 10^{-3}$  MLD/mL [15]). By incorporating a sample concentration step [21] and further optimizing the sensor, it should be possible to detect lower amounts of botulinum toxin with a low chance of false positives (1 in 250) [15]. Thus, the system has the ability to rapidly screen clinically relevant amounts of the neurotoxin from whole blood in the field without the need of electronics.

### 3.1.2 Contamination prevention with valves

The check valves are fabricated from a hydrogel piston that is attached to a rigid post. The spring force generated by swelling the hydrogel piston holds the piston head in contact with the neck forming a functional check valve

\* Minimal lethal dose; this is a measure of the minimal dose of toxic or infectious agents that is lethal.

[18]. The operating pressure of the valve can be adjusted by altering the gap between the neck and head of the piston, which changes the spring force. Other factors that can be tuned to adjust the valve performance are the composition of the prepolymer mixture for forming the hydrogel piston and the polymerization conditions (intensity and time). In the ELISA device, a total of four check valves were located in channels leading to the readout (Fig. 3). The functional pressure of the valves was between 7 and 8 psi. The valves prevented contamination between reagents, as color change was not observed in any other location other than on the agarose beads.

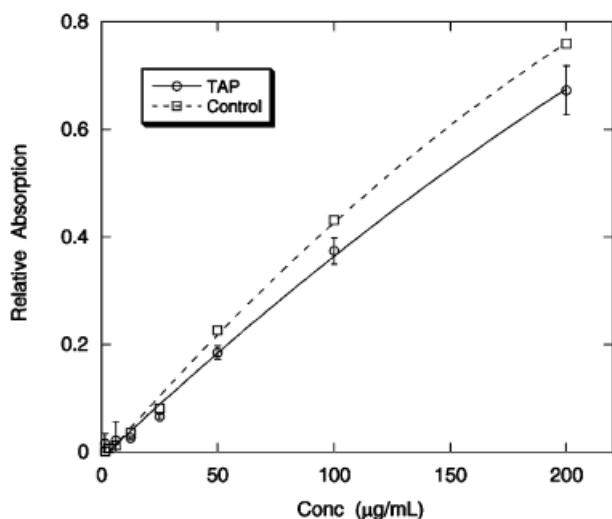
### 3.1.3 TAP

The pressure output of the TAP is directly correlated to the volume of the TAP. The morphology of the reservoir is dictated by the stencil used and can be altered to best fit the application. For the ELISA device, TAPs with 150  $\mu\text{L}$  and 250  $\mu\text{L}$  were used to deliver the assay reagents. The rate of depression of the flexible membrane had no effect on the maximum pressure obtained. Depending on the impulse (rate of depression), the flow rate in the channel can be adjusted between 0.7 and 14 mL/min. This range in flow rate was similar for the different TAPs (50, 150, and 250  $\mu\text{L}$ ) tested. The higher limit (14 mL/min) was achieved when the thumb was pressed into the TAP by the experimenter with maximal impulse (25 psi).

One concern for storing protein solution inside the TAPs is the loss of protein to adsorption to the PDMS surface (during storage), which could affect the efficiency of the assay. In comparison to storing protein in an Eppendorf tube, there was a marginal decrease in concentration when stored in the TAP (Fig. 5) for a period of 20 h. The loss was more significant at higher protein concentrations. In the present design, very low concentration (5  $\mu\text{g/mL}$ ) of the protein (secondary antibody) is stored in the TAPs, and therefore loss during storage is minimal. For applications where loss of protein might have a large affect in the outcome of the assay, adsorption can be minimized by treating the walls of TAPs such as coating with albumin (or casein).

### 3.1.4 Cell lysing

The ELISA system is designed so that whole blood sample is delivered by pressing the TAP. By performing G-6-PDH assay, the shear stress from pressing the TAP and flowing blood solution through the mixer on cell lysis was tested. The activity of the G-6-PDH enzyme, which is present only inside the cells, was measured by recording a



**Figure 5.** Protein solutions of varying concentration were stored for 20 h at 4°C in PDMS TAPs. After storage, the concentration was measured using Coomassie assay and the relative absorption at 595 nm was compared with solution stored in an Eppendorf tube ( $R > 0.99$ ).

change in absorption at 340 nm. Only a marginal change (3–5%) in absorption was recorded for the blood sample that was “pushed” through the chaotic mixer at maximal TAP pressure. By utilizing lower pressure, cell lysis can be prevented.

### 3.2 Fabrication platform

The fabrication platform allows for the creation of microfluidic components at any desired location physically making it possible to quickly reconfigure the process order to fit a specific assay. The properties of each component can be adjusted by varying the prepolymer mixture (porous filter, valve) or geometry (valve – piston-to-neck distance, TAP volume). Some of the components such as the chaotic mixer and part of the check valve (rigid post) are formed during fabrication of the channel network, while other components (or subcomponents) are added in subsequent polymerization steps. The fabrication platform allows for effective utilization of space by exploring the third dimension to create the 3-D chaotic mixer. In the ELISA device described in this paper, two layers were utilized to realize the device. However, based on the assay needs, any number of layers can be introduced; one on top of the next until the required space and geometry is realized for the assay. Unlike other micro-device fabrication, no bonding is required to connect the layers. The adhesive nature of the polymer (poly(IBA)) holds the layers during photopolymerization. When device failure occurred it was often due to delamination of

layers upon TAP operation. Because the TAPs are operated by utilizing the force of a human finger, the input pressure is uncontrolled and can be quite high. These problems can be overcome *via* improved bond strength and/or alternative fluid pumping schemes such as hydrogel pumps [22]. In addition, the platform allows for incorporating external parts such as the filter membrane between the layers (for readout). The fabrication procedures presented here requires little time (a few hours), no sophisticated bonding methods, or clean room facilities. And, by using PDMS TAPs, we demonstrate here that the strengths of liquid-phase photopolymerization and micro-molding can be combined to realize a stand-alone, non-electronic device for portable diagnostic systems.

### 4 Concluding remarks

Realizing microfluidic-based devices for chemical and biological assay can be challenging because of variation in protocol steps between different assays. Here, we have described a rapid fabrication approach whereby a device specific for a given assay can be designed and realized by the end user once the process steps and their connectivity are laid out and appropriate microfluidic components are chosen. The platform was utilized to create a portable microsystem for detecting botulinum neurotoxin directly from whole blood. Sample preparation steps and detection of the toxoid was performed on the device without the use of electronic parts. The system is capable of detecting clinically relevant amounts of the neurotoxin. By utilizing “add-on” parts such as agarose beads for readout and TAPs for storage, the device and reagents can be stored separately until just before the assay is performed. Moreover, by using different antibodies, the device can be used as is or easily reconfigured for other assays that require similar functional processes. In this design format, multiplexed detection schemes can be conceived which would be useful for reducing false positives.

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