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Disposable polydimethylsiloxane/silicon hybrid chips for protein detection

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Abstract

This paper presents disposable protein analysis chips with single- or four-chamber—constructed from poly(dimethylsiloxane) (PDMS) and silicon. The chips are composed of a multilayer stack of PDMS layers that sandwich a silicon microchip. This inner silicon chip features an etched array of microcavities hosting polymeric beads. The sample is introduced into the fluid network through the top PDMS layer, where it is directed to the bead chamber. After reaction of the analyte with the probe beads, the signal generated on the beads is captured with a CCD camera, digitally processed, and analyzed. An established bead-based fluorescent assay for C-reactive protein (CRP) was used here to characterize these hybrid chips. The detection limit of the single-chamber protein chip was found to be 1 ng/ml. Additionally, using a back pressure compensation method, the signals from each chamber of the four-chamber chip were found to fall within 10% of each other.

Keywords: Poly(dimethylsiloxane); Taste chip; Disposable; C-reactive protein

1. Introduction

Polymer-based miniaturized systems provide a powerful platform for biological assays due to numerous advantages over conventional bench-top systems that include low cost, smaller reagent volume, less power requirement, higher throughput and portability (Whitesides et al., 2001; Beebe et al., 2002; Sia and Whitesides, 2003). The development of miniaturized devices must take into account the type of material used to fabricate the device. The material should be compatible with the sensitive methods of detection as well as enable easy integration with other functional components. If the devices are aimed for mass production, the material should be inexpensive so the device can be disposable. Due to these requirements, silicon and glass are not as favorable as polymer substrates like polyimide, poly(dimethylsiloxane) (PDMS), poly(methyl methacrylate) (PMMA), and polycarbonate.

There have been many different polymer-based assay chips constructed from polyimide (Metz et al., 2001), PMMA (Christodoulides et al., 2002; Goodey et al., 2001; Qi et al., 2002; Wang et al., 2003; Ali et al., 2003), PDMS (Bernard et al., 2001; Marquette and Blum, 2004; Culha et al., 2004; Hosokawa et al., 2004) and polycarbonate (Noerholm et al., 2004; Yang et al., 2002) using micromolding or injection molding. Some polymer-based chips have been built using a combination of materials, such as PDMS/silicon (Wolf et al., 2004), PMMA/PDMS (Ko et al., 2003; Keramas et al., 2004), and PDMS/glass (Yamamoto et al., 2002; Xu and Fang, 2004). Of these widely used materials, PDMS is an especially well-suited material for use in miniaturized

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microfluidic bioanalysis systems due to its biocompatibility, cost-effectiveness and disposability, transparent nature to UV/visible light, and mature fabrication techniques. Additionally, it is easily sealed to different substrates. In parallel to the rise of interest in polymer materials for assay chips, the assay format was also extensively studied. Fluorescent-based assays have received increasing attention in the past few years, especially when coupled to a bead-based assay platform (Christodoulides et al., 2002; Goodey et al., 2001; Ali et al., 2003; Sato et al., 2000, 2001). The microbeads can yield better results for surface binding assays due to the larger surface-to-volume ratio as compared to that of a surface immobilized assay (Verpoorte, 2003).

This paper focuses on the development of disposable analysis chips with single- and multiple-chamber for protein detection. These chips were constructed from PDMS and silicon. Using an established microchip-based C-reactive protein (CRP) assay, we evaluated the performances of the microchips, in terms of assay characteristics and multiplex capabilities.

2. Methods

2.1. Materials

Silicon wafers (4 in.) were obtained from Nova Electronic Materials Inc. (Richardson, TX, USA). Transparent photomasks were printed using 2000 dpi laser plotter from CADTech company (Austin, TX, USA). SU-8 and developer were obtained from MicroChem (Newton, MA, USA). PDMS (Sylgard 184) kits were purchased from Dow Corning (Midland, MI, USA). The prepolymer was prepared by mixing a curing agent with PDMS prepolymer in a 1:10 weight ratio. Micrometering valves (P-445) were purchased from Upchurch (Oak Harbor, WA, USA). Agarose microbeads were obtained from XC Corporation (Lowell, MA). The CRP-specific antibodies were purchased from Accurate Chemical, Scientific Corporation (Westbury, NY) and Biogenesis (Kingston, NH). Alexafluor-488 fluorophore was conjugated to the detecting antibody using a commercially available kit from Molecular Probes (Eugene, Oregon). The CRP standards were obtained from Cortex Biochemical (San Leandro, CA). Anti *H. pylori* antibody was obtained from Accurate Chemical and Scientific Corporation. The CRP-specific and control (rabbit anti *H. pylori*) antibodies were then coupled to the beads by reductive amination.

2.2. Microchip–protein assay

The microchip-based assay has been described previously (Christodoulides et al., 2002; Goodey et al., 2001; Ali et al., 2003). An array of square-pyramidal microcavities were anisotropically etched in a silicon wafer. Agarose beads with a diameter of ∼300 μm were placed in the microcavities of a 3 × 4 square array. Each of the 12 wells serves as a microreactor and microanalysis chamber. Beads within the microcavities are exposed to analytes as solutions are pumped into the upper reservoir of the flow cell, forced down through the wells to the bottom reservoir and out through a drain. Due to the three-dimensional geometry of the microcavities, mixing occurs when analytes flow through the microbeads. The mixing can enhance the antigen and antibody reaction. Additionally, the space between the upper layer and the silicon chip was designed to be around 50 μm to restrict the chamber volume, while allowing the sample to flow to each cavity and preventing the beads from being displaced out of their wells.

2.3. Instrumentation

The instrumentation setup was similar to those described in previous papers (Christodoulides et al., 2002; Goodey et al., 2001; Ali et al., 2003). The individual beads were placed into silicon microcavities patterned in a 3 × 4 square array. The silicon chip was packaged between two PDMS layers. Polycarbonate holders were used to hold the three components together. This fluidic system was coupled to the PDMS chip positioned on the motorized stage of a modified compound BX2 Olympus microscope. The microscope was equipped with various objectives, optical filters, and an automatically operated charge-coupled device (CCD) camera.

A mercury lamp was used as the light source. Fluorescence images were obtained with a FITC filter cube (fluoroisothiocyanate, 480 nm excitation, 505 long-pass beam splitter dichroic mirror, and 535 ± 25 nm emission), and captured by a DVC 1312C (Digital Video Camera Company, Austin, TX) charge-coupled device (CCD) mounted on the microscope and interfaced to Image Pro Plus 4.0 software (Media Cybernetics). Areas of interest in the images of the array were selected in an automated fashion and used to extract light intensities.

3. Results and discussion

3.1. Chip design and microfabrication

The chips consist of an inner layer composed of a silicon microchip. This Si chip has an etched array of microcavities, and is sandwiched between a top and a bottom fluidic network fabricated using soft lithography techniques. The 3 × 4 array hosts agarose beads that are used in a sandwich format immunoassay. Single- (Fig. 1a) and four-chamber chips (Fig. 1b) were designed for single and multiple analyte assays, respectively. The expansion of the number of chamber from 1 to 4 may have the potential to provide perfectly identical illumination conditions for assays that otherwise would have to be run separately. Additionally, the multiple-chamber format increases multiplexing capabilities.

In order to seal the microchip and handle the relatively high flow rates required for the assay (0.07–2 ml/min), impor-

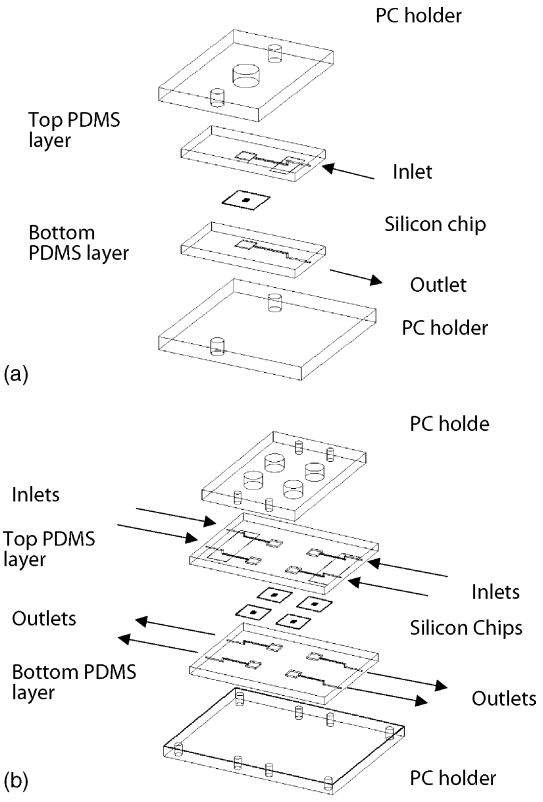

Fig. 1. Schematic diagram of (a) a single- and (b) a four-chamber PDMS chips.

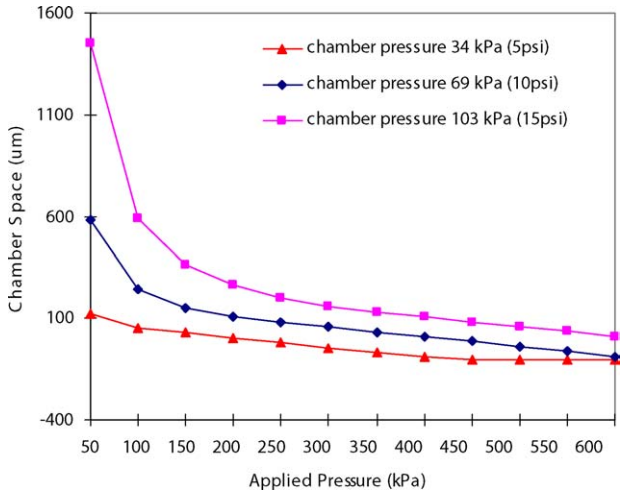

Fig. 2. Deformation of the PDMS chamber at different holding pressures and chamber pressures.

tant pressure considerations were taken into account. When the sample flows through the chamber, pressure builds up inside the chamber. This internal pressure along with an external holding pressure results in deformation of the chamber membrane. If the chamber volume defined by the space between the silicon chip and the upper PDMS membrane is too large, the beads may be displaced out of the microcavities because of the flow pressure. The maximum distance between the microchip and the ceiling of the chamber was found to be 200 μm. However, the external holding pressure had to be carefully controlled in order to prevent blockage of the microchannels inside the PDMS layers.

Critical dimensions and design parameters of the device were determined using ANSYS, finite element analysis software by considering factors such as chamber pressure, holding pressure, and chamber/channel deformation. The Young's modulus and the Poisson ratio of the PDMS membrane were assumed to be 3 MPa and 0.49, respectively (Chronis et al., 2003). The overall dimension of the microcavity array on the Si chip is around 3 mm × 2.7 mm, therefore the width of the PDMS chamber was chosen as 5 mm. Since the maximum chamber pressure was found to be less than 103 kPa (15 psi) from previous assays, a simulation was conducted with chamber pressures of 34 kPa (5 psi), 69 kPa (10 psi), and 103 kPa (15 psi). With the increase of the holding pressure, the chamber space was found to decrease. The simulation result suggested the use of a 100 μm space. The height and width of the channel were estimated to be 50 and 500 μm, respectively (Fig. 2).

The fabrication process of the PDMS layer structure is shown in Fig. 3. Transparencies printed with a 2000 dpi laser printer were used as the photomasks for creation of the silicon mold in soft lithography. Silicon wafers (4 in.) were cleaned using piranha solution (30% H_2O_2:96% H_2SO_4 = 825 ml:1650 ml). After the silicon wafers were dried with N_2, they were placed on hot plate at 120 °C for 5 min to remove water molecular. Hexamethyldisalizane (HMDS) was spun on the silicon wafer to enhance adhesion. Then, the negative photoresist SU-8 was coated on the silicon wafer at 3000 rpm for 30 s resulting in a thickness of around 50 μm. The coated silicon wafer was then placed in a 90 °C prebake oven for 1 h. The silicon wafer was relaxed for 10 min before exposure for 2.5 min using the aligner. The wafers relaxed again for 10 min and then placed back in the oven at 90 °C for 1 h post-exposure bake. After the post-exposure bake, the silicon wafer was developed for 6 min and rinsed using isopropyl alcohol (IPA). The 10-min post-baking aims to enhance SU-8 photoresist adhesion.

To prepare the PDMS layers, a curing agent and PDMS prepolymer were mixed in a 1:10 weight ratio. The prepolymer mixture was degassed in a 20–25 mmHg vacuum chamber for 1 h to remove air bubbles in the mixture and ensure complete mixing of the two components. A special two-step curing method was applied in this study to fabricate channels inside PDMS bulk material. Before the prepolymer was poured on the silicon mold, a thin carbon layer was deposited on the mold using reactive ion etching (RIE) to allow easier removal of the PDMS layer after curing. The parameters for RIE deposition are 100 W, 100 sccn CHF_3 for 1.5 min (Hosokawa and Maeda, 2000). In order to control the thickness of PDMS layers, a plastic ring of 100 mm diameter and 5 mm thickness was placed on the silicon mold. Into this plastic tube, 10 ml of the prepolymer mixture was poured. Due to the surface tension force, the prepolymer covers the

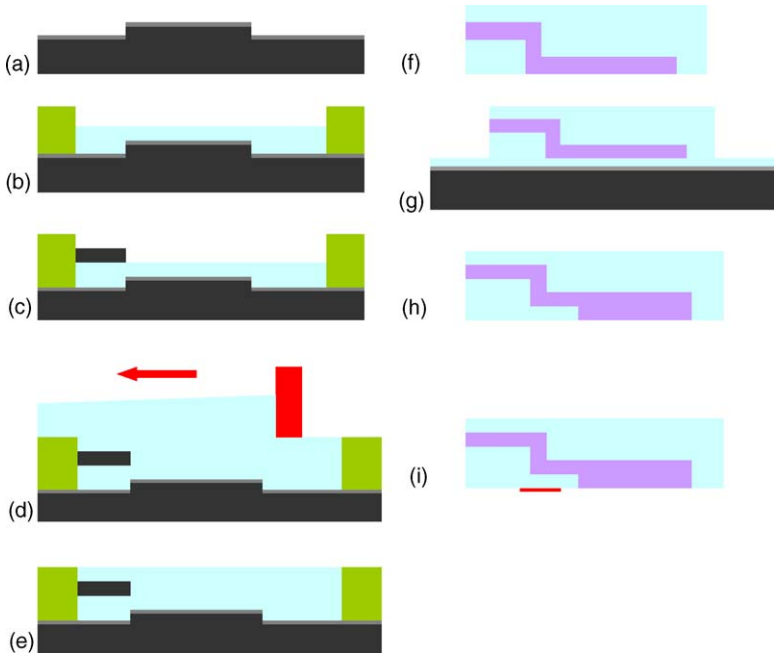

Fig. 3. The fabrication process flow of the PDMS fluid network: (a) carbon thin film deposition on a silicon wafer, (b) plastic ring position and the first PDMS prepolymer layer preparation, (c) pin positioning, (d) the second PDMS prepolymer layer injection, followed by removal of extra prepolymer, (e) 1 h curing at 80 °C, (f) PDMS layer removal and hole punching, (g) 50 μm (thickness) PDMS layer spin coating, curing and bonding to the thick PDMS layer, (h) PDMS chamber opening, and (i) 100 μm thick glass sheet bonding.

silicon wafer inside the plastic ring uniformly. The thickness of PDMS inside the ring was around 1–2 mm. After 5 min curing at 100 °C on a hot plate, metal pins of 800 μm in diameter were positioned on the first PDMS layer and then another 10–20 ml prepolymer mixture was poured to fill the whole ring. The extra prepolymer can be easily scraped from the plastic ring using a plastic blade. The PDMS was then cured for 1 h at 100 °C on a hot plate. Then, these two PDMS layers were cured into one PDMS layer and peeled off from the silicon mold. The thickness of the PDMS layer is approximately 5 mm. The PDMS layers were cut into 20 mm × 40 mm pieces, followed by the removal of metal pins inside the PDMS layer. Holes, 1.5 mm in diameter, were punched using 1.5 mm glass capillaries to connect the channels inside the PDMS layer with the open channels on the surface of the PDMS.

To seal the open channels, an additional 50 μm thick PDMS layer was prepared as following. Silicon wafers (4 in.) were cleaned using piranha solution. Using the aforementioned method, a thin carbon layer was deposited on the silicon wafer. Then 20 ml prepolymer was coated on the silicon wafer at 2000 rpm for 20 s. The coated silicon wafer was cured for 30 min at 100 °C on a hot plate. The final thickness of the thin PDMS layer was around 50 μm. Before bonding, the 5 mm thick PDMS layer was cleaned using IPA and deionized (DI) water for 5 min, respectively. After cleaning, PDMS layers were put into a 90 °C oven for 15 min to remove water. Then, both the thick PDMS layer (5 mm) and the thin PDMS layer (50 μm) were put into a reactive RIE chamber to activate the bonding surfaces using oxygen plasma. The parameters for oxygen plasma treatment were a power of 75 W, a pressure of 70 mTorr, for 15 s of exposure. After surface activation, both the thick and thin PDMS layers were put together immediately and heated at 100 °C for 30 min for permanent bonding. The bonded layers were then released from the silicon wafer. The detection chamber windows were opened using a razor blade. Since previous experiments showed that the membrane at the junction of the punched hole was easily disrupted at higher flow rates, a 100 μm thick glass sheet was bonded to strengthen the PDMS seal over the junction of the punched holes using oxygen plasma bonding. The glass and PDMS bonding was found to be resistant up to 483 kPa (70 psi) (Li and Chen, 2003). Fig. 4 shows the fabricated PDMS chips with a single- (Fig. 4a) and four-chamber (Fig. 4b).

3.2. Optimization of CRP assay protocol

PDMS was chosen as the device material for the assays for the following reasons: (1) it is an elastomer that can tightly seal different substrates, (2) it has hydrophobic surface properties, thus prevents protein adsorption from solution, (3) it gives a low background fluorescence signal, and (4) it is transparent over a wide range of wavelengths from UV to infrared (Wolf et al., 2004). However, PDMS is a soft material that easily deforms under pressure. From the finite element analysis and previous experiments, leakage occurred at high flow rates (>0.4 ml/min). The PDMS chip was found to function best at 0.1 ml/min. However, low flow rates make it harder to get rid of potential bubbles inside the chamber. The presence

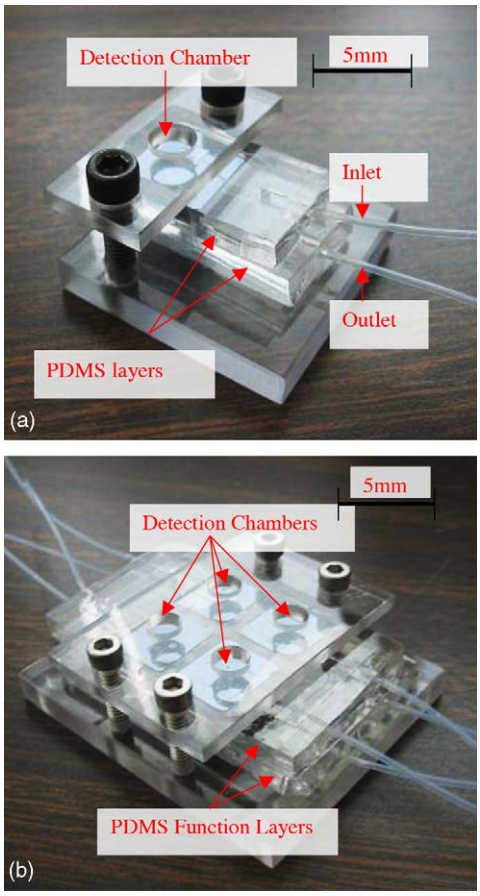

Fig. 4. An optical picture of a (a) single- and (b) four-chamber PDMS chips.

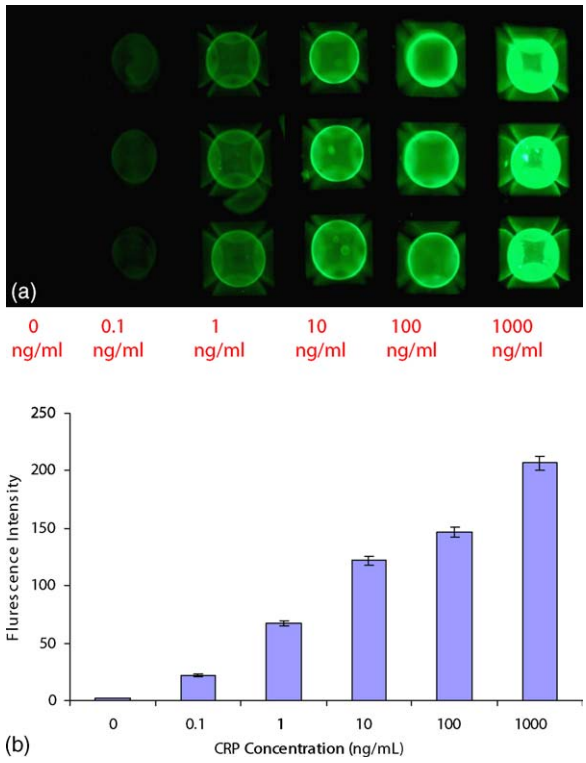

Fig. 5. (a) An optical micrograph of anti-CRP-coated beads at different CRP antigen concentrations and (b) measured intensity at different CRP antigen concentrations.

of bubble can be detrimental to signal acquisition or analysis. In order to eliminate bubbles inside the chamber, two methods were tested. First, we used continuous flow format instead of several priming steps between each step. Priming was found to introduce gas bubbles during the assay. Alternatively, we connected a micrometering valve with the drain tubing to regulate the backpressure. This was found to be effective in removing bubbles inside the flow cell chamber. The optimal CRP assay protocol was modified as following: prior to each assay, the inner walls of tubing used to introduce the reagent to the flow cell were primed. The CRP antigen was delivered to the flow cell at a flow rate of 0.1 ml/min for 30 min. Then, a wash with a phosphate-based saline (PBS) solution was delivered to the flow cell at 0.1 ml/min for 10 min. Following PBS washing, the detecting antibody conjugated with Alexa 488 fluorophore were delivered to the flow cell at 0.1 ml/min for 30-min incubation. A final PBS wash was achieved at 0.1 ml/min for 10 min.

3.3. Single-chamber PDMS chip CRP assay characterization

We studied CRP dose responses to characterize the performance of the single-chamber PDMS chip. Three of four columns of a 3×4 array were loaded with agarose beads coated with anti-CRP antibody. The fourth column was used to host the negative control microbeads. After the assay, the three columns coated with the anti-CRP beads gave a fluorescence signal. The negative control beads did not exhibit any detectable fluorescence, showing that non-specific binding is negligible. In order to further ensure the reduction is non-specific that would result in background fluctuation, the silicon chip was ultrasonically cleaned for 5 min subsequently and washed with DI water. Ethanol temporarily makes the PDMS surface hydrophilic, and enhances the wettability of the chip in the chamber.

An identical protocol was followed for all CRP concentrations used in the dose response curve. The CRP concentrations ranged from 0.1 to 1000 ng/ml (Fig. 5a). The detection limit was 1 ng/ml (Fig. 5b), comparable to that of our recently reported CRP assay system (Christodoulides et al., 2002).

3.4. Four-chamber PDMS chip CRP assay characterization

Associated with fluorescence measurements, and particularly with those using Hg arc lamps, is the risk of fluctuations in the excitation light from one experiment to the next. The ability to measure more samples under the same illumination conditions is appealing, because it would reduce the time needed for measurements, potentially improve

statistics, while subjecting all samples to the same conditions. Furthermore, multiple-chamber could allow the simultaneous use of multiple antibodies that would otherwise cross-react with each other in a single-chamber format. Simultaneous measurements in multiple-chamber generate new challenges, such as the assurance of equal fluid delivery to each chamber and placement of all silicon chip and beads in the same focal plane. There are many factors that can affect flow dynamics, such as chamber dimensions, relative position of microcavities inside the chamber, deformation of PDMS layers, applied pressure, connecting tubing and even the peristaltic pump function. In soft lithography, the critical dimension variation is much larger (>10 μm) than that typically encountered in photolithography, so the dimensions of the four-chamber can vary because of the fabrication process itself. The position of the microcavities inside the chambers can affect the pressure drop that drives the analyte flow through the beads. The deformation of the PDMS layers due to the external holding pressure and physical change of connecting tubing (for example, deformation or stress at tubing/connector interface) can alter flow resistance inside the channel and the chambers. Additionally, the peristaltic pump must deliver the solution at an equal rate in all chambers. To ensure identical flow dynamics in each chamber, a back pressure compensation method was proposed. In-line micrometering valves were connected to the outlet of each flow chamber to regulate the back pressure. The flow dynamics were mainly controlled by the back pressure inside the bottom PDMS chamber. This method was successful at providing identical flow dynamics characteristics throughout each step of the assay with optical micrographs of the four-chamber for a concentration of CRP of 1000 ng/ml.

Further analysis reveals a variation in signal between the four-chamber to be less than 10%. The extension of the single-chamber PDMS chip to a multiple-chamber format allows for higher throughput multiplexed experiments, in which each quarter of the flow cell handles fluids that may not be compatible if they were used together, therefore potentially simplifying reagent choices.

One of the major advantages of the PDMS chips is their disposability. Using micromolding techniques, PDMS chips can be easily and massively fabricated. The major cost of these chips comes from the micromachined silicon chip that can also be made of plastic. However, these silicon chips can be recycled as these hybrid chips can easily be re-separated into three components: PDMS top layer, silicon chip and PDMS bottom layer after the assays.

4. Conclusions

We have developed single- and four-chamber PDMS chips for protein detection. Using soft lithography techniques, a fluidic network was fabricated in the top and at the bottom PDMS layers, which sandwich an inner silicon chip hosting microspheres. Finite element analysis was conducted to determine the relationship between the deformation of the PDMS chamber, the PDMS channels, and the applied holding pressure. With consideration of the optimized parameters, the maximum assay flow rate was 0.4 ml/min. An immunoassay for CRP was used to characterize both the single- and multiple-chamber chips. For the single-chamber assay, the CRP detection limit was found to be 1 ng/ml comparable to that previously reported. The four-chamber PDMS chip showed great promise, since it was possible to generate the same signal within 10% in each chamber by applying the back pressure compensation method. These chips represent a step towards mass production and a significant reduction in costs because of the use of less expensive and easy-to-use material while conserving outstanding assay characteristics.

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