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Hot embossed polyethylene through-hole chips for bead-based microfluidic devices

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Abstract

Over the past decade, there has been a growth of interest in the translation of microfluidic systems into real-world clinical practice, especially for use in point-of-care or near patient settings. While initial fabrication advances in microfluidics involved mainly the etching of silicon and glass, the economics of scaling of these materials is not amendable for point-of-care usage where single-test applications forces cost considerations to be kept low and throughput high. As such, a materials base more consistent with point-of-care needs is required. In this manuscript, the fabrication of a hot embossed, through-hole low-density polyethylene ensembles derived from an anisotropically etched silicon wafer is discussed. This semi-opaque polymer that can be easily sterilized and recycled provides low background noise for fluorescence measurements and yields more affordable cost than other thermoplastics commonly used for microfluidic applications such as cyclic olefin copolymer (COC). To fabrication through-hole microchips from this alternative material for microfluidics, a fabrication technique that uses a high-temperature, high-pressure resistant mold is described. This aluminum-based epoxy mold, serving as the positive master mold for embossing, is casted over etched arrays of pyramidal pits in a silicon wafer. Methods of surface treatment of the wafer prior to casting and PDMS casting of the epoxy are discussed to preserve the silicon wafer for future use. Changes in the thickness of polyethylene are observed for varying embossing temperatures. The methodology described herein can quickly fabricate 20 disposable, single use chips in less than 30 minutes with the ability to scale up 4x by using multiple molds simultaneously. When coupled as a platform supporting porous bead sensors, as in the recently developed Programmable Bio-Nano-Chip, this bead chip system can achieve limits of detection, for the cardiac biomarker C-reactive protein, of 0.3 ng/mL, thereby demonstrating the approach is compatible with high performance, real-world clinical measurements in the context of point-of-care testing.

Keywords

hot embossing; thermoplastics; point-of-care; beads; immunoassays; microfluidics

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1. Introduction

In the past few decades, microfluidic lab-on-a-chip (LOC) devices have shown potential for use in a wide range of clinical and bioagent detection applications.(Bange et al. 2005; Haeberle and Zengerle 2007) In contrast to existing detection approaches that are time-consuming, expensive, and confined to the laboratory, LOC devices offer potential to house tests that can be completed with low cost and low sample volume requirements, as well as rapid turnaround of results, often in multiplexed format as is desirable for use with near patient testing.(Chin et al. 2007; Vilkner et al. 2004; Weigl et al. 2008) Further, these sensitive early disease detection devices, have the potential to improve health and open up more effective therapeutic options for the management of the care of patients.(Gubala et al. 2011; Rivet et al. 2011)

Most LOC devices, traditionally composed of glass or silicon, are microfabricated using MEMS technologies, such as photolithography and anisotropic etching to produce simple, highly reproducible, passive channels and features.(Zhao and Bau 2008, 2009) However, long fabrication times for silicon and opaque optical properties of this semiconductor have limited its widespread use.(Chin et al. 2012) Moreover, while glass offers optical transparency possibilities for a wide spectral range, highly corrosive and toxic chemicals used in the processing of glass limit its widespread use by the research and in vitro diagnostics communities.(Lim and Zhang 2007) However, with the advent of soft lithography methodologies to produce LOC devices composed of polydimethylsiloxane (PDMS), active LOC components such as valves and pumps have been developed and yield enhanced LOC devices' mechanical functionality as well as allow for complex logic and scaled operations under low sample volumes.(Hashimoto et al. 2006; Walker and Beebe 2002a) While PDMS offers biocompatibility and the diffusion of gases, it is limited in its capacity to yield scalability due to slow fabrication times and high costs, and lack of mechanical properties of typical disposable medical device units.(Jrbe et al. 1988; Sramka et al. 1988; Sramkova and Kotrly 1988) Furthermore, although there are many advantages of these materials in research settings, they are limited in most cases to laboratory and research settings due to long production times and high manufacturability costs.(Arsenault et al. 2009; Kim et al. 2009a)

With these considerations in mind, a number of groups have started to develop biosensor devices that are amenable to distribution in point-of-care settings.(Arsenault et al. 2009; Liu et al. 2007) These important advances rely on a combination of low-cost raw materials that are integrated with detection modalities so as to yield disposable devices where each device ideally costs less than \$1.(Chin et al. 2007) For example, instrument-free devices using paper-based microfluidics have recently exploited the low costs of paper as well as capillary action due to the material's inherent porous structure with the aim to develop disposable, easy to use devices.(Lau and Liu 2007; Li et al. 2011; Ng et al. 2008; Phillips et al. 1988) With the ease of disposability through incineration, these colorimetric-based devices can be analyzed using mobile phones, which is increasingly beneficial in resource poor environments where distance and cost are barriers for diagnostic devices.(Zhang et al. 2006) To date these paper-based devices have focused on semi-quantitative results interpreted by human eyesight and in some cases evaluated by remote imaging systems. These devices have yet to be found suitable for the rigorous needs of traditional clinical diagnostics.(Prasad et al. 1988; Zhang et al. 2006)

With these considerations in mind, there has recently been a strong focus of the medical microdevice research sectors to explore thermoset polymers as potential materials for LOC devices for the use at the point-of-care.(Liu et al. 1987b; Wang et al. 1989) These polymer materials offer many appealing benefits including low cost, scalability, disposability, quick

production times, and strong optical performances.(Liu et al. 2006; Yuan et al. 1989) In the fabrication of such devices, a mold containing desired features is either embossed into or injected with thermoset polymers at a temperature above the polymer's glass transition temperature. Following a demolding phase to cool the mold to a temperature below the glass transition temperature, the part is removed from the mold.(Becker and Gartner 2000) Thermoplastic parts can achieve resolutions down to the sub-microns.(Kurzeja et al. 1986; Lin et al. 1987; Liu et al. 1984b) While these techniques are initially extremely expensive due to the high start up costs and long development times associated with the machine and mold, typically produced through micromilling, the low cost of thermoplastics and economics of scaling allow for a rapid decrease in cost per unit with increasing quantities produced.(Wang et al. 1989) To help with these barriers, several groups have developed low-cost alternatives for such fabrication tools.(Chung et al. 1987; Liu et al. 1984a)

Thermoset polymer-based devices have successfully been developed for the use in several microfluidic-based devices. For example, work by Sia and coworkers have developed integrated plastic devices that have moved into real world practice.(Sia et al. 2004; Sramkova and Kotrly 1988) Likewise, hot embossed devices, based on the thermoplastic cyclic polyolefin, have been developed for the extraction of DNA and for the detection of cardiac biomarkers in human sera.(Garstecki et al. 2006a; Gitlin et al. 2006a) Further, the adsorption properties of thermoplastics have been harnessed to immobilize antibodies onto polystyrene devices.(Liu et al. 2004) Similarly, surface treatment of thermoplastic can facilitate bonding and modify hydrophilic properties. Further, chemical treatment of non-adsorbing surfaces of thermoplastics allows for the functionalization of antibodies.(Gubala et al. 2011) (Lin et al. 1992; Ogle et al. 1988) These chemical treatments show promise for a wide range of plastic-based devices.

Further, PMMA has also been harnessed for the surface immobilization of antibodies in capillary flow-based devices. Application of a sol-gel technology allows for a surface coating that facilitates antibody bonding.(Chin et al. 2007) Additionally, thermoplastic-based devices, used to culture cells, reveal both strong cell attachment and growth to PMMA surfaces.(Liu et al. 1987a) Likewise, surface immobilization onto a plastics show the potential of plastic-based biochips as replacements for large equipments such as liquid chromatography for the detection of anesthetics.(Jokerst et al. 2011) Work completed by Klapperich and colleagues has demonstrated the application of plastic microfabrication to create devices with potential global applications for polymerase chain reactions. These developments reveal the capability of these plastics devices to service point-of-care DNA testing.(Cao et al. 2011; Cao et al. 2012)

Recently, there has been much interest in the use of beads as highly sensitive sensing elements in microfluidics. For example, David Walt and coworkers have utilized optical fibers with microarrays of polymer beads for the detection of vapors, representative of the olfactory glands. (Walt 2010a, b) Ng and coworkers have demonstrated a chip containing micropillars that hold a microarray of polymer beads for the detection of DNA. This device demonstrates the rapid detection of multiple bacterial species and nucleotides in under 10 minutes.(Ng et al. 2008) Furthermore, these sensors can be multiplexed to detect multiple DNA probes and proteins simultaneously.(Konry et al. 2009) Bau and coworkers have developed a model for the temporal and spatial distribution of captured analytes in porous beads. These models agree well with confocal imaging.(Thompson and Bau 2012) Similarly, our group has shown, through simulations and experimental evidence, the preponderance of porous beads as highly sensitive sensing elements due to the existence of internal convection inside porous beads.(Chou et al. 2012) These bead-based devices have shown potential as highly sensitive, robust sensing elements for rapid detection of biological agents.(Derveaux et al. 2008)

Over the last decade, our laboratory has sustained efforts to design, fabricate and validate in clinical settings for various health conditions a platform technology described as a programmable bio-nano-chip (p-BNC) system. (Kim et al. 2009b; Nguyen et al. 2009; Zhao and Bau 2010) This modular platform is **programmable** in the sense that it can move in an agile way from one biomarker to the next through the insertion of molecular level code. The **bio** element refers to the biological aspects of the various disease and health conditions that may be monitored with this system. The **nano** terminology refers to the miniaturization that is embodied with nanonets to capture with high efficiency the critical biomarkers as well as quantum particles that can be used to enhance signaling. Finally, the **chip** terminology refers to capacity to use microfabrication capabilities to produce the devices at scale. With this p-BNC system we have developed a flow through, pressure-driven ensemble consisting of an array of highly sensitive agarose beads resting in anisotropically etched silicon wells. This unique flow through design enables convection-driven internal transport within the situated porous beads. Versus lateral flow over designs, this design allows for shorter diffusion distances which allow for higher fractional capture efficiencies.

While silicon-based microcontainers offer high reproducibility, high fabrication costs and long fabrication time hinder their translation into real-world clinical practice. As such, a new material with low costs and fabrication times is needed. While the use of thermoplastics has been well explored in monolithic LOC test elements, no studies to our knowledge have examined the fabrication of hot embossed tapered, through-holes as microcontainers for flow-through channels that support agarose beads. This paper explores the hot embossing of the thermoplastics, low-density polyethylene, as potential material for microfabrication of through-hole microarrays. To retain the original flow through design, a mold is replicated from anisotropically etched silicon wafer prepared through standard photolithographic methods that include reactive ion etching and exposure of protective layers. These through-hole devices are of particular interest in pressure-driven, flow through microfluidic systems. These new structures are also demonstrated in their capacity to support the measurement of the essential inflammatory biomarker, cardiac reactive protein (CRP).

2. Materials and methods

2.1. Preparation of silicon mold

A P-type <100> silicon wafer with a diameter of 4", a thickness of 400 μ m, and pre-coated with a protective nitride layer, was prepared using standard photolithography and microfabrication techniques as described previously. (Liu et al. 2009) Briefly, S1813 was spun over the wafer for 3s at 1000rpm with a ramp up speed of 500rpm/s, followed by a 60s spin at 3000rpm with a ramp up speed of 500rpm/s, and soft baked on a hot plate at 115°C for 60s. A monochrome photomask film, designed with 12 arrays of 3 $\times$ 4 squares with dimensions 600 μ m $\times$ 600 μ m, was purchased from Fineline (Colorado Springs, Co). The photoresist-coated wafer was exposed under a MJB4 mask aligner (SUSS MicroTec; Garching, Germany) for 17s, and developed using MF-319 for approximately 10s. The exposed, protective nitride layer was removed using reactive ion etching (Oxford Plasma Lab 80 Plus; Concord, MA) using a gas mix of 45sccm CF₄ and 5sccm O₂, RIE forward power of 100W, and a ICP power of 60W at a pressure of 50mT for 80s. The wafer was then anisotropically etched in a double bath setup of KOH overnight (Figure 2A) until all wells were etched through the wafer.

2.2. Casting for Aluminum Epoxy Stamp

Prepared mold containing negative features was placed over a 150mm $\times$ 150mm scotch tape and surrounded by a plastic blocks (LEGO; Billund, Denmark) that served to shape the aluminum epoxy mold. The aluminum epoxy, PT4925 (PTM&W Industries; Santa Fe

Springs, CA) was chosen due to its resistance to high temperatures and pressures. A mixture weighing 75g was prepared on a weigh boat at a 100 to 9.5 ratio of PT4925A to PT4925B. To remove bubbles, the mixture was centrifuged for 2.5min at 2500rpm. Prior to pouring the epoxy over the wafer, Ease Release 200 (Smooth-On; Easton, PA) was applied to the wafer to improve the release of the epoxy from the wafer. After the aluminum epoxy was poured into the rubber mold (Figure 2B), the epoxy was warmed up at 70°C for 30s to reduce its viscosity and then degassed in a vacuum for 2 min. A manual wooden stick was used to ensure wells were filled with the epoxy. The epoxy was allowed to cure for 3 hours on a flat benchtop under continuous heating with a Stanley heating fan (#675945) set at 70°C. To increase the rigidity and mechanical strength of the epoxy mold, the mold was incrementally heated every hour at 66, 121, and 177°C.

2.3. Embossing

A 75mmx75mm layer of polyethylene with thicknesses of 375 μ m (#86255K61), purchased from McMaster-Carr (Atlanta, GA), was cut and placed over the epoxy mold with positive features touching the thermoset plastic. A layer of elastomer rubber (#86075K22) with thickness 1.3mm, placed on top of the polymer allowed reduced damage to the mold. This triplet was sandwiched between pairs of aluminum plates with thickness 2.5mm (#86075K22), elastomer rubber (#5787T31), and Kapton film (#2271K1), all purchased from McMaster-Carr (Figure 2C). These pairs served to provide mechanical support, equalized pressure over the arrays, and easy release from the machine.(Bhattacharyya and Klapperich 2005) This stack was placed inside an Autoseries AutoFour 15-NE Press (Carver; Wabash, IN). Two cycles, an embossing and demolding cycle, were used. The embossing cycle was set to 10min with applied pressure of the platens set to 680kg. The demolding step allowed the polymer to cool. This step was crucial to reduce warping and shrinkage, caused by a rapid descent in external temperature. Room temperature water was delivered to the hot press to cool it down. Upon achieving 100°C after approximately 5min for cooling, the mold and part were removed from the hot press.

2.4. Reagents and sensors

Porous beads, prepared using 2% agarose, using previously developed emulsion methods, were crosslinked and glyoxylated.(Christodoulides et al. 2007) Beads of approximately 280 μ m were selectively filtered using selective sieves with filter sizes between 280 μ m and 300 μ m. Agarose beads were conjugated overnight with 4mg/mL anti-CRP capturing antibodies (Fitzgerald; Concord, MA) per 500 μ L of beads using previously developed methods.(Christodoulides et al. 2005) Detecting anti-CRP antibodies (Fitzgerald; Concord, MA), were diluted 1:250 in phosphate buffered saline (PBS) and conjugated to Alexa-Fluor 488 (Invitrogen; Carlsbad, CA). Stock purified, native human CRP antigen was diluted to 50ng/mL in PBS. Calibration and negative control beads were conjugated to agarose beads overnight with 0.02mg/mL Alexa-Fluor 488 bound donkey anti-sheep IgG (Invitrogen, Carlsbad; CA) and 4mg/mL anti-TNF- α antibody (Cell Sciences; Canton, MA), respectively.

2.5. Device fabrication

Alternating layers of double sized adhesive (DSA), vinyl, and thermoplastic were sandwiched together to form the laminate-based test structure. Designs of channels and flow holes were constructed in AutoCAD 2011 (Autodesk, San Rafael, CA) and cut into layers using a plotter cutter. These alternating DSA, vinyl, and thermoplastic layers, consisting of a flow chamber and drain layer were sandwiched onto a base glass slide. Beads were manually loaded under a microscope with forceps and allowed to rest on each well before the coverslip layer was attached. The flow channel leading to the bead array, consisting of DSA, measured 3mm wide and 23mm long. Under the array contains a drain reservoir of

dimensions 4mm by 6.6mm that leads to a waste channel, measuring 12mm by 1.5mm. Modular, fluidic buses, used to connect channels to silicone tubing, were cut using a laser cutter (Universal Laser System; Scottsdale, AZ).

2.6. Assay delivery

Volumes of 100 μ L for sample and 10 μ L for detecting antibody were metered in a 10 port low pressure injector valve from Vici Valco Instruments (Houston, TX). A programmable NE-1000 syringe pump from New Era Pump Systems Inc. (Farmingdale, NY) filled with PBS flushed the injection loop at predefined flow rates to fully cover the beads with flowing fluids. The sample was delivered at 25 μ L/min for 4 min and detecting antibody was delivered at 10 μ L/min for 3 min, followed by a final PBS wash step at 500 μ L/min for 1 min. Samples with concentrations of 0.025, 0.25, 0.5, 1.0, 10, 25, and 50 ng/mL provided points to form a dose curve.

2.7. Data collection and image analysis

A DVC 1312 LVTE cooled camera (DVC; Austin, TX) connected to an Olympus BX-2 microscope (Center Valley, PA) with 4x objectives captured 8-bit TIFF images at 2s exposure and +15 gain. Beads in the images were analyzed using a custom written ImageJ (NIH; Bethesda, MD) macro. Each manually selected bead was radially scanned. The bead signal, taken as the highest signal in the collection of average circumference intensities, corresponds to the average signal on the ring of the bead. Values for points on dose response corresponded to the difference in signal intensities between the CRP bead and negative bead. Scanning electron microscopy images were acquired using a Hitachi TM3000 (Pleasanton, CA).

3. Results and Discussion

3.1. Device

Prior efforts to fabricate the p-BNC bead microcontainers have been completed using bulk micromachining methods based on anisotropic etching of silicon <100> wafers.(Liu et al. 2009) In addition to these core activities, we have completed some exploratory activities whereby the critical bead array holders have been fabricated in other materials including UV sensitive epoxy and stainless steel.(Arora et al. 2001; Srensen et al. 1988) While these materials have provided attractive alternatives to expensive silicon wafers, these approaches suffer from a lack of precision as well as challenges to scale large number of identical devices.

To move past these barriers, our recent efforts here described have focused on the development of hot embossing techniques that are compatible with producing flow through bead holder elements that are critical to the MEMS-based bead sensor ensembles. In order to maintain the same structure as the original silicon mold, the etched wafers is used as negative replication molds to produce polymer-based bead holders. An aluminum-based epoxy is used as an intermediate molding tool to replicate the features into a positive mold. After using the mold during hot embossing, the embossed thermoset polymer bead holder is then sequestered between layers of laminates and double sided adhesive. The top transparent cover, polycarbonate, is attached to the bead holder though a double sided adhesive (DSA) with a cutout flow chamber. Fluid is delivered to a bubble trap prior to entry into the flow chamber. The flow chamber is flushed of air with a bubble trap membrane at the opposite end of the bead array. A schematic of the flow in this chamber is shown in Figure 1A. The DSA drain channel underneath the bead holder is assembled on top of a plastic slide. Figure 1B shows the flow trajectory of delivered fluid as the fluid flows around the bead and down the drain.

3.2. Epoxy mold

While brass molds cut using computer numerical controlled (CNC) micromilling are typically used directly for hot embossing, direct wear of the mold after a few iterations delays rapid fabrication of thermoplastic parts. Similarly, direct hot embossing using the silicon is not ideal as the brittle wafer is prone to breaking. Long micromilling times of up to two weeks for high resolution molds and cumbersome steps for anisotropic etching necessitate an alternative mold material for hot embossing. For the development of prototype molds for development efforts it is critical to have efficient options to develop new prototype parts. While a few groups have used epoxies as molds for hot embossing, these molds were not able to withstand high temperatures and pressures needed for polyethylene.(Bhattacharyya and Klapperich 2005; Garstecki et al. 2006b) As such, the choice of this particular PT4925 aluminum epoxy allowed for resistances to temperatures up to 185°C and compressive strengths up to 21,120 psi. While the wear has not been fully tested for this mold, 40 successive iterations have not damaged the mold. The ability to cast this mold in under six hours is much preferred to the longer fabrication times for micromilling brass or etching silicon after a few embossing iterations. The processes here described are thus ideal for rapid prototyping of plastic parts as may be required to service the initial stages of clinical testing. Clinical validation of lab on a chip structures to date has received little attention yet this next step is required for this field to mature where broad-scale clinical implementation is possible.(Chin et al. 2012) Figure 3A and B show scanning electron microscopy of a single silicon well and epoxy replicate.

While the casting of the epoxy over the silicon wafer allows for quick replications of the mold, silicon is prone to cracking due to silicon's brittle material properties. Further, subsequent etching of silicon can lead to variations in dimensions from batch to batch. As such, methods to preserve the silicon mold for future casting ensured reproducibility. One method implemented here, uses surface treatment to facilitate the removal of the mold from the wafer. To achieve this goal, application of Ease Release 200 as a coating to the surface of the silicon, followed by 10 minutes to dry enabled low bonding between the epoxy and the wafer. Under no surface application, the wafer bonded permanently to the epoxy. With the application, the wafer was easily released from the epoxy. No re-etching of the wafer was required.

Moreover, while the wafer is preserved, proper handling is required due to the brittleness of silicon. A secondary option to preserve the pyramidal structures is to cast the positive features of the epoxy into polydimethylsiloxane (PDMS). While PDMS does not bond well with the epoxy, application of hydrophobic treatment with silane on the surface of the epoxy under a vacuum ensured no bonding with the epoxy.(Mabey et al. 2004) As such, casting of the epoxy can be performed onto the more versatile PDMS mold instead of the silicon mold.

3.3. Thermoset polymer

Efforts by Becker et al. have examined a collection of potential polymers for applications in microfluidics.(Liu et al. 2006) Examination of a collection of common thermoplastics and their initial feasibility for use in the p-BNC bead microcontainer led to the choice of polyethylene. Several factors were taken into account to eliminate thermoplastics that are not suitable. These included optical reflectivity, the ability to be recycled easily,(Lee et al. 2009; Sung et al. 2009) hot embossing temperature, and performance indicators, including signal to noise, signal to background, and mean fluorescent intensity (MFI).

While many thermoplastics exist, those with high autofluorescence properties are not amendable to fluorescence-based sensors where signal to noise and signal to background would be low. A table containing the autofluorescence values at an excitation wavelength of

495nm is provided as supplemental material. Moreover, while several thermoplastics do meet the low background noise requirements, such as cyclic olefin copolymer (COC), polycarbonate (PC), polyoxymethylene (POM), and polymethylmethacrylate (PMMA), factors such as cost and machinability create challenges for low cost fabrication. For example, while COC is often used in microfluidics due to its optical transparency, its cost is a barrier to low cost, single use devices. Further, while PC has low autofluorescence, assays run under PC exhibited high background noise. Further, difficulties in hot embossing through-holes in PMMA within the temperature and pressure limitations of the epoxy limited its use. Moreover, embossing of through-holes parts in POM met challenges. The opaque material became semi-transparent after embossing and the resulting embossed part was too thin. As such, polyethylene was chosen for its low cost, compatibility with hot embossing, and low inherent optical noise.

3.4. Through-hole embossing

Because of the need to emboss through-hole parts, complications arose to protect the tips of the mold. Several methods have been explored previously using either aluminum or acetate buffer layers, and alignment of complementary top and bottom molds.(Gitlin et al. ; Walker and Beebe 2002b; Zhao et al. 2002) Yet, challenges exist in alignment and choice of complimentary thermoplastics as a buffer layer for polyethylene. Without any appropriate steps taken, the mold and the underlying stainless steel plate would have dented and prevented subsequent use in cycles. As such, the choice of elastomer rubber, with a durometer of 75A, served as a buffer layer to reduce complications in damaging the mold.

Hot embossing requires a processing temperature that is above the glass transition temperature of the thermoplastic. Low-density polyethylene of the type used here has a glass transition temperature of about -10°C .(Liu et al. 2006) However, embossing results vary depending on the embossing time, pressure, and temperature. In order to keep embossing process consistent and quick, 10 minutes and 680 kg/platen were chosen. Thicknesses of the part obtained under embossing temperatures ranging from 140°C to 180°C are shown in Figure 3D (RSD: 19.3%). At the low temperature of 140°C , through-holes were not visible on the parts. However, arrays with complete through-holes were observed at 160°C . Comparisons of the mold and embossed polymer at 160°C are shown in Figures 3B and C. The size of the openings of the microwells can be increased by increasing the embossing temperature. Reductions in the thickness of the part will decrease as the temperature increases. This plateau effect is due to the limitation of the mold in completely penetrating the buffer layer.

3.5. Performance

A dose curve was obtained to characterize the range as well as to determine the detection limit of the assay. Points were chosen based on previously reported ranges for the protein CRP.(Kim et al. 2009b) Subtraction of the signal on negative controls beads removed the offset from nonspecific binding and background noise. Figure 4A shows data from a representative assay run with 50 ng/mL of CRP. Here, in addition to the CRP bead sensors, loaded on the chip with 8x-redundancy, are two calibrator beads coupled to a fixed amount of fluor, as well as two negative control beads coupled to an antibody irrelevant to the CRP target. Intensities from negative control beads were subtracted from the intensities from the CRP beads. This array was exposed to increasing concentrations of CRP, including no antigen condition, to derive the dose response shown in Figure 4B (mean RSD: 18.8%). This 12 minute assay, demonstrated a limit of detection at 0.3 ng/mL, as the 3 standard deviations above the 0 antigen run (CI: 99.9%). The dynamic range of the assay, spanning 3 orders of magnitude, is suitable for the concentration of CRP in saliva for the detection of acute myocardial infarction where the cutoff for positive cases is 4.13ng/mL.(Sretavan et al. 1988)

For the detection of CRP in serum, the sample would need to be diluted prior to introduction into the device and/or the use of on-chip dilution sequence.

3.6. Cost Considerations

In the macroscopic world, polymers have found widespread success as a result of low costs and fast fabrication times. (Wang et al. 1989) Microfluidics technology can benefit from the use of polymers, which offer benefits over traditional microfabrication materials such as silicon and glass. (Wang et al. 1989) High reproducibility, low per unit costs, and the ability to quickly produce high quantities in short times allow for commercialization of microfluidic devices. (Semetey et al. 2006; Yuan et al. 1989) While initial infrastructure costs are high, the ability to quickly produce polymer parts, as well as the low cost of raw materials, makes polymers very attractive for both laboratory scale production. Figure 5A shows the costs associated per part for silicon etching and polymer hot embossing. Costs include both equipment and labor. While mass production can certainly reduce costs, the cost of a silicon chip plateaus at \$10. (MNX 2010) In contrast, hot embossed polymer chips cost 1000X less. Furthermore, etching of a silicon wafer can take about a day to complete. In contrast, simultaneous application of four molds, each containing 12 arrays, can produce 96 parts in half an hour. While not industrial scale, the ability to produce such quantities is appealing for research grade testing and serves as an attractive pre-commercial step prior to moving to scalable injection molded plastic approaches.

Figure 5B provides a representative breakdown of the manufacturing and supply costs of individual components for fabrication of a pre-commercial bead-based approach. It is worth noting that the costs of the sensor substrate, agarose, is negligible. For example, 1 g of agarose can result in 265,000 beads (280 μm diameter) with 2% agarose content, has a cost of only \$13.70 (#A9045; Sigma-Aldrich). Polyethylene with a thickness of 250 μm costs ~\$0.80 per m^2 (#86255K61; McMaster-Carr). With a footprint less than 2 cm^2 , each polyethylene microchip costs less than a \$0.01. As such, the cost structure of bead-based approaches are similar to those of paper-based diagnostics where costs are in the few cents. (Li et al 2011; Liu et al 2007; Phillips et al 1988) However, independent of the materials base (paper or plastic), the costs of reagents are an expensive component in immunoassays. For example, the research cost of 12 agarose beads with 280 μm diameters with a capturing antibody load for CRP of 6mg/mL costs ~\$0.50 and the cost of detection antibody is ~\$0.32. When scalable plastics are brought into the equation, the costs of reagents, often overshadowed in the cost of raw materials, significantly contribute to final costs of a diagnostic device. It should be noted that as these devices scale, reagent costs are expected to be reduced 10x or more relative to these initial conservative projections. Furthermore, while often neglected in the overall cost considerations, packaging constitutes a major portion of overall device costs. Packaging costs here consist of bonding, surface modification, reagent loading, printing, and final card assembly. (Kim et al. 2010b; Lee et al. 2010) We provide an estimate of the final cost for packaging to be ~\$1. This cost correlates with previous estimates whereby packaging constitutes for 50-90% of the final device fabrication cost, with 80% being the accepted norm. (Chae et al. 2009; Kim et al. 2010a; Kim et al. 2009c)

4. Conclusion

The ability to quickly replicate designs from silicon to the much more affordable polyethylene thermoplastic has the potential to move LOC devices forward towards applications that are amendable at the point-of-care. Furthermore, in cases where optical transparency is not required, often for the non-imaging regions of a test device, the use of polyethylene is a much affordable alternative to other relatively expensive thermoplastics such as cyclic olefin copolymer. The transition from silicon to polyethylene offers a ~3 log

reduction in material costs. In the development of microfluidic immunoassays, the reductions in costs of materials can offset the costs of reagents.

Further, the methodology described here to fabricate tapered, through-hole polymer devices is important for developing more advanced, multilayer microfluidic devices in polymers. As demonstrated here, when coupled to agarose beads in this unique flow through system, diagnostic tests can be rapidly performed to detect low concentrations of analytes. Moreover, the unique array structure described here can be expanded to support large multiplexed panels of biomarkers simultaneously.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- We discuss the fabrication of microfluidic through-hole polyethylene-based microarrays.
- Polyethylene is an affordable and disposable plastic that is amendable to the point of care.
- Hot embossing thickness varies depending on temperature.
- Method for release of epoxy based mold from etched silicon wafer is discussed.
- Cost of materials for commercialized bead-based devices is discussed.

Figure 1.

A) The microfluidic card device consists of layers of laminate and plastics. B) Flow trajectory showing fluid delivery to an array of bead sensors.

Figure 2.

A) A master containing multiple arrays of etched wells is produced using standard lithography and anisotropic etching of KOH to produce wells to hold agarose beads. B) An aluminum-based epoxy, poured over the silicon wafer, is cured, released, and hard baked. C) The epoxy stamp and thermoplastic is sandwiched between pairs of elastomer rubber, stainless steel sheets, and Kapton film during the embossing process.

Figure 3.

A) SEM image of etched silicon microcontainer shows pyramidal square through-hole structure with a top opening of $500\mu\text{m} \times 500\mu\text{m}$ and bottom opening of $100\mu\text{m} \times 100\mu\text{m}$ used to hold a $290\mu\text{m}$ bead. B) SEM image of casted PDMS shows very consistent replication of features. C) SEM image of polyethylene, embossed at 160°C , shows good replication of pyramidal pit. D) Variation in thickness of final polyethylene-based microchip as embossing temperature changes.

Figure 4.

A) Epifluorescent images showing results from 50ng/mL CRP delivery with 2 calibrator beads (top left), 2 negative control beads (top right), and 8x redundancy CRP specific bead sensors. B) The 7 point dose curve with a limit of detection of 0.3 ng/mL.

Figure 5.

A) The economics of scaling up for silicon and plastics. The cost of a silicon chip levels off at ~\$10 while the cost of a plastics chip can go down to pennies. B) Breakdown of costs for individual components of agarose bead-based approach for the detection of CRP.