Development of a capillary-driven, microfluidic, nucleic acid biosensor

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ABSTRACT

An ideal point-of-care device would incorporate the simplicity and reliability of a lateral flow assay with a microfluidic device. Our system consists of self-priming microfluidics with sealed conjugate pads of reagent delivery and an absorbent pad for additional fluid draw. Using poly(methyl methacrylate) as a microfluidic substrate, we have developed a single-step surface modification method which allows strong capillary flow within a sealed microchannel. For our biosensor design, we have incorporated aspects of lateral flow assays within our device. Conjugate pads within the device held trapped magnetic nanoparticles which were released when the sample entered the chamber. In addition, nucleic-acid-probe-conjugated horseradish peroxidase was released on a second conjugate pad. The complex consisting of the magnetic nanoparticle, target nucleic acid and HRP was immobilized over a magnet while a co-reactant stream containing H_2O_2 was merged with the channel. A downstream electrode attached to a potentiostat was able to quantify the signal. This new format of biosensor will allow for a smaller and more portable point-of-care biosensor. The design also allows to commercial-scale manufacturing and low materials cost. The potentiostat can be miniaturized to allow for a truly handheld system which and conduct assays on a disposable chip.

Keywords: microfluidics, biosensors, surface modification

1 INTRODUCTION

Rapid detection technologies with high selectivity and sensitivity for pathogenic bacteria are critical in food safety, environmental monitoring, and clinical diagnosis. Conventional identification methods include culture and colony counting which involve a pre-enrichment step or a selective enrichment step followed by a biochemical test [1] and [2], polymerase chain reaction (PCR) or reverse transcriptase PCR (RT-PCR), immunological techniques and fluorescence in situ hybridization. Although these approaches are powerful and confirmative, most of them are technically complex, time consuming, cost-intensive, and they do not afford the necessary detect ability and specificity towards the target. Biosensors offer several advantages over existing techniques (e.g., limited hands-on time, high through-put screening, improved detectability, real-time analysis and label-free detection methods and devices) [3].

Recent advances in micro- and nano-fabrication technologies have provided unique advantages for developing pathogen biosensors. The sensor probe created with similar or smaller dimensions of a bacterial cell could provide high sensitivity and a low detection limit [4]. The use of nanoparticles as functional probes for detecting pathogens have raised great expectations with respect to generating enhanced signal-to-noise ratios, reducing response times and using them in multiplexed systems [3].

2 MATERIALS AND METHODS

2.1 Device fabrication

2.1.1 Channel design

Our system consists of self-priming microfluidics with sealed conjugate pads of reagent delivery and an absorbent pad for additional fluid draw. The channel has a total length of 0.25 m and a width of 400 μm. The height of the channel was targeted at approximately 80 μm resulting to a volume of approximately 8 μl. Two inlet poles respectively allow for sample and chemiluminesence buffer additions. (Fig.1 b)

2.1.2 Copper Master Stamp Fabrication

We fabricated a copper-based stamp with various microchannel arrays and demonstrated successful replication of the stamp microstructure on poly methyl methacrylate (PMMA) substrates. Microfluidic channels were fabricated by means of photolithography and electroplating (Fig.1 a). A copper plate with a polished mirror finish was cut into 5cm×5cm squares, and cleaned with 1% Microsoap-90 (Cole Parmer, IL, USA) at 60℃ in ultrasonic cleaner (Branson, CT, USA), rinsed with DI water 3 times, then dried with N_2 gas. Next, the copper plate was treated in UV Ozone cleaner (Jelight, CA, USA) for 1h, rinsed with DI water and again, N_2 dried. A negative photoresist (SU-8 2050, MicroChem, MA) was deposited onto the center of the copper square and spun for 5s at 500rpm, 100r/s and then 30s at 1000rpm, 300r/s to obtain a thickness of approximately 80 μm. The plate was then

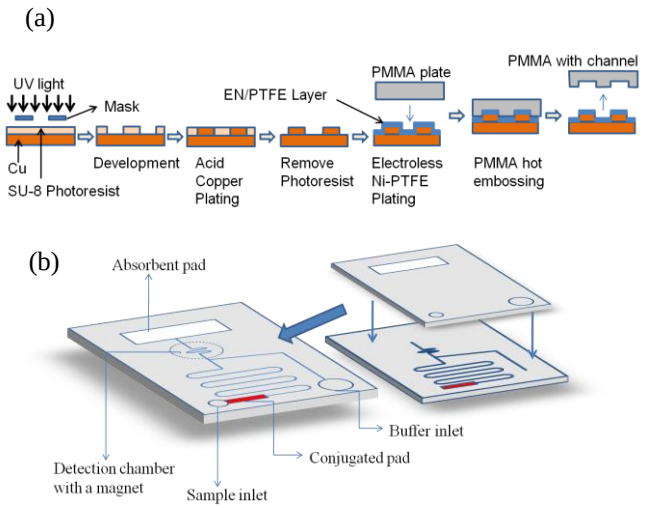

Figure 1: Device fabrication. Copper Master Stamp was fabricated through Photolithography, electroplating, electroless Ni-PTFE plating (b) demenstration of PMMA chip fabrication through hot embossing, laser ablasion, and UV assistant thermal bonding.

baked at 95℃ on a level surface for 5min followed by gradual cooling to 37℃. The resist was patterned by exposure to UV light (SUSS MA6 Mask Aligner) through a 365nm long pass filter to obtain vertical side walls. A post-exposure bake for 6min at 95℃ was followed by developing for 3min in SU-8 developer with mild agitation, then rinsed with Isoproponal alcohol, and dried with N_2 gas.

Electroplating was performed at room temperature using Acid Copper Plating solution (Caswell, NY, USA), which at a current density of 0.582mA/mm^2. Current was applied to the electroplating bath to produce approximately 80 µm tall copper structures where SU-8 did not cover the copper surface, resulting in the raised microchannel design. After plating, the SU-8 layer was stripped off the copper plate by immersed in 95℃ deionized water for 20min, SU-8 layer peeled off due to the differences of thermal expansion factor.

2.1.3 Electroless Ni-PTFE plating of copper master stamp

A release layer was deposited on the stamp surface by electroless Ni-PTFE plating to increase the performance of copper stamp during de-embossing. Here, we developed a novel method of electroless Ni-PTFE plating for the enhancement of copper mold performance.

Niklad ICE (MacDermid, CT, USA) is a new technology for producing a composite of electroless nickel phosphorus and occlude sub-micro particles of Teflon® (polytetrafluroethylene, better known as PTFE). A new Niklad ICE bath is made up with 20% by volume ELNIC 101-C5(MacDermid, CT, USA) and Niklad ICE as illustrated by the following table:

Elnic 101 C5(ml/L)	Desired volume% PTFE in Deposit	Niklad ICE(ml/L)
200 ml/L	<11%	1.0-2.0
200 ml/L	11-17%	2.0-3.0
200 ml/L	20-23%	4.0-5.0
200 ml/L	>24%	6.0-7.0
200 ml/L	48%	15.0

Tab. 1 Niklad ICE bath

2.1.4 PMMA chip fabrication

Hot embossing was used to fabricate microfeatured channel patterns on PMMA substrates using a hot press (Carver, NJ, USA). The microfeature in the copper-based (copper plate coated with SU-8) stamp includes microchannel arrays of approximately 80 µm in depth and 400 µm in width. PMMA discs of 1mm thickness were utilized as the molding substrate. Prior to hot embossing, PMMA sheets were cut into 50 mm×50 mm squares, ultrasonicated in 15% 2-propanol for 15min at 25℃, and rinsed 3 times with DI water, then placed in 80℃ vacuum oven (1k Pa)(Lab-Line, IL) for 12h to remove any volatiles that could result in outgassing during embossing process. The PMMA substrate was then sandwiched between the copper master and a glass plate for the duration of the embossing cycle. We found 16KN applied force and 120℃ embossing temperature were optimum for transferring the microstructure to the PMMA substrate. Then the copper master, PMMA and glass plate were removed and cooled to room temperature. During the cooling process, the PMMA would easily peel off from the copper master with the aid of EN-PTFE release layer.

The embossed PMMA discs were then visually inspected under microscope. Inlets, outlets and chambers for conjugated pad and absorbent pad were made by laser ablasion.

UV-assistant thermal bonding was used to bond plain polystyrene plates on the fluidic platforms. Prior to bonding, the embossed PMMA disc (the side with channel patterns was up) and a plain polystyrene disc were UV treated for 10 min in UVO Cleaner. Then the conjugated pad was put into the PMMA disc, and the UV treated

Fig. 2 Photo of final chip

polystyrene surface was combined with the PMMA channel surface. Then the combined chip was put into the hot press. A force of 2000 N was applied with an embossing temperature of 75℃ for 2min. The final chip dimensions were 4cm×2.7cm×0.3cm (l×w×h) (Fig.2).

2.2 Yeast RNA detection

We use yeast rRNA as target through Chemiluminescence assay detection. The reporter enzyme (horseradish peroxidase HRP) is not conjugated directly to the probe, but is linked to it through Streptavidin-biotin bridge. The signal was achieved via a biotin-streptavidin system (BSAS). The HRP–luminol–H_2O_2 chemiluminescent system with high sensitivity was chosen as the detection system.

2.2.1 Pretreatment of conjugate pad

Modified horseradish peroxidase (HRP)-streptavidin and oligonucleotide-biotin were used for the conjugation of HRP-oligonucleotide via the interaction between biotin and streptavidin. The HRP and oligonucleotide-biotin solutions with were mixed and incubated at room temperature with agitation for 45 minutes.

Commercial Dynabeads-streptavidin (Invitrogen, Oslo, Norway) and oligonucleotide-biotin are used for the conjugation of Dynabeads-oligonucleotide via the interaction between biotin and streptavidin. The Dynabeads and oligonucleotide solutions were mixed and incubated at room temperature with agitation for 45 minutes.

The conjugates from previous two steps were mixed together at room temperature, applied to the conjugate release pad and dried under vacuum.

2.2.2 Chemiluminescence assay detection

The target nucleic acid sequence (Eurofins mwg operon, AL, USA) was applied to the chip and pulled into the chamber via the capillary forces of the conjugate pad. In the conjugate chamber, the hybridization of the probes formed a sandwich complex and continued to flow toward the capture zone under the capillary forces of the hydrophilic channel. With the aid of a magnet, the beads (with or without bound target) were localized onto the surface of microchannels. luminol–H_2O_2 solution was added and the transformation of luminol intermediate generated detectable chemiluminesent signal, which was proportional to the number of captured targets.

3 RESULTS AND DISCUSSION

3.1 Characterization of EN/PTFE plating

3.1.1 Atomic force microscopy (AFM) examination

The copper mold surface with different concentration of EN-PTFE was examined.

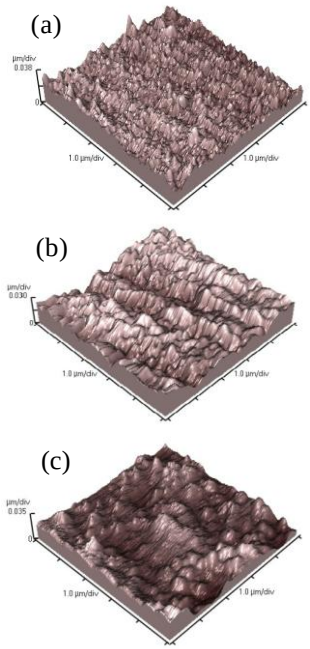

Fig.3 AFM micrographs of the copper master stamp with different concentration of EN/PTFE plating. (a) Pure copper (b) EN-24%PTFE (c) EN-48%PTFE

AFM images showed surface roughness, the surface roughness of plain copper plate is the highest, with EN-PTFE plating, the surface become smoother.

3.1.2 Scanning electron microscope (SEM) Examination

The copper mold surface with different concentration of EN-PTFE was examined. SEM micrographs of copper plates surfaces are shown in Fig 4.

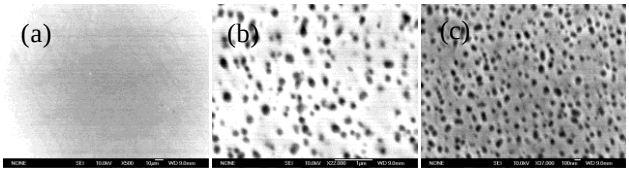

Fig 4. SEM Examination (a) pure nikel plating (b) EN-24%PTFE (c) EN-48%PTFE

SEM images showed surface characteristic of copper mould, black spots represent concentration of PTFE particles on the copper mould.

3.1.3 Contact Angle Measurement

The most useful methods for characterizing wettability on solid polymer surfaces are contact angle measurements[5][6]. Any drop of liquid in contact with a solid surface will exhibit a contact angle θ, which is a quantitative measure of the wetting of a solid by a liquid [7]. It is defined geometrically as the angle formed by a liquid at the three phase boundary where a liquid, gas and solid intersect. Contact angle is measured using a contact angle goniometer.

Water contact angle measurements evaluate hydrophilicity and hydrophobicity. Low values of θ indicate that the liquid spreads, or wets well, while high values indicate poor wetting. Large contact angle means high hydrophobicity, which indicates high PTFE concentration on the sample surface.

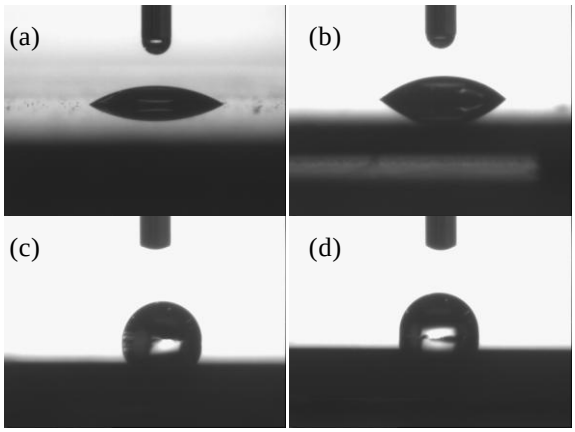

Fig.5 Contact Angles of different molds (a) pure Ni plating, θ =33.69 (b) plain copper plate (99%), θ =40.18 (c) EN-24% PTFE plating, θ =97.51 (d) EN-50% PTFE plating, θ =101.78

The contact angles for the water increased from approximately 40 degrees (hydrophilicity) to approximately 101 degrees (hydrophobicity) in proportion to the plated PTFE concentration.(Fig. 5)

4 CONCLUSION

Microfluidic channels were fabricated using copper masters with co-electroplated with nickel (for corrosion resistance) and PTFE (for rapid release). Within the poly (methyl methacrylate) (PMMA) microchannels, fluid transport was achieved through capillarity flow generated by the UV surface modification and the application of an absorbent pad. Nucleic acid-tagged horseradish peroxidase (HRP) and magnetic beads were embedded in conjugate pad for release once the sample was applied.

The target nucleic acid was applied to the chip and pulled into a chamber containing the conjugate pad. There the hybridization of the probes formed a sandwich complex and continued to flow toward the capture zone. With the aid of a magnet, the beads (with or without bound target) were localized onto the surface of microchannel. Enzyme substrate was added and the transformation of luminol intermediate generated detectable chemiluminesent signal, which was proportional to the number of captured targets. Assays were conducted at or near real-time with results obtained within 15 min of sample application. The results showed that the miniaturized integrated microfluidic biosensor was rapid, low-cost and has appreciable sensitivity range. The highly sensitivity, simplicity and portability of this point-of-care biosensor enabled its widely application for resource-limited setteings.

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