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# RESEARCH ARTICLE

## INTEGRATED MICRO-FLUIDIC PUMP FOR MEDICAL AND ENVIRONMENTAL APPLICATIONS

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**ABSTRACT**

The fabrication and testing of an integrated micro-fluidic sample delivery system for polydimethylsiloxane (PDMS) micro-fluidic channel is reported. Adopting a multi-disciplinary approach to the establishment of core platform technologies, we brought together physical chemistry, biochemistry, surface science and micro-fabrication technology. The fluidic channel is designed in Mentor Graphics® IC layout, and transferred to a flexible transparency which will act as a photo-mask during contact photolithography. A negative photosensitive epoxy resin (SU-8 50) is dispensed and processed upon a silicon substrate. Exposure to a UV source permits the patterning of the epoxy resin, and the fabrication of a re-usable master mould. Casting the PDMS against the mould yields a polymeric replica containing the designed fluidic channel. Finally, the oxidation of the PDMS by a handheld tesla coil permits the irreversible bonding of the polydimethylsiloxane to a selected substrate. This micro fluidic channel was tested in thin film micro-sensor for clinical applications. Potential applications for this integrated micro-fluidic systems are also discussed.



## INTRODUCTION

Micro-fluidics can be defined as the management, manipulation and analysis of fluids in structures on a micrometer scale. The introduction of micro-fluidics offers several advantages, reduction in the reagent consumed, waste produced, cost per analysis, energy consumption and faster analysis time. The term "Lab-on-a-chip" was coined to describe an integrated micro-fluidic and electrochemical micro-sensor device which can execute multiple laboratory type functions (Yoonhee *et al.,* 2006; Suzuki *et al.*, 2003). Lab-on-a-chip (LOC) configurations can be simple, such as one composed of a single channel and sensor or complex where it can contain multiple channels and sensors. Other components such as valves, pump, switches may also be integrated. LOC is a multidisciplinary research field with chemical, biological, physical, biomedical and engineering applications. In the past conventional three electrodes electrochemical micro-sensor were fabricated on a glass substrate by thin film technologies. The PDMS channel was fabricated by the replica moulding technique. In brief, SU-8 2025 was patterned by photolithography and the PDMS polymer was poured onto the SU-8 mould and cured in a convection oven.

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Inlet and outlet holes where punched into the PDMS before it was irreversible sealed to the lower substrate. Hydrodynamic flow can be employed to deliver a sample to a LOC device. In brief, a pressure drop is created across inlet and outlet of the PDMS channel which forces the sample through. The most common approach of generating the pressure drop is with the use of syringe pumps (Terray *et al.,* 2005; Upadhyaya *et al.,* 2006; Amatore *et al.,* 2004; Pepper *et al.,* 2007 ), alternatively an external vacuum can be applied. The delivery and removal of a sample can be accomplished using polyethylene tubing or needles. Holes slightly smaller than the tubing are punched/bored into the PDMS (Woais, 2005; Fujii, 2002; Plecis *et al.,* 2008). For example, a minimum PDMS thickness of 6 mm was required when a needle was employed to deliver the sample as thinner PDMS structures failed to provide sufficient mechanical support. The main drawback of hydrodynamic flow is that it requires external equipment (either a syringe pump or a vacuum pump) to deliver the sample, therefore, it is not ideally suitable for complete LOC miniaturisation.

### Device layout and fabrication

#### *Device layout*

The sample was delivered by a single stroke polydimethylsiloxane nano-pipette pump (SSnPP). Patterned PDMS was reversibly sealed to a planar silicon substrate

containing an IEMS creating a pump capable of delivering nano-liter sized volumes (Figure 1). The sample delivery system consisted of a micro-fluidic channel (Figure 1 (a)) with one end running into an enclosed cavity (Figure 1 (b)) and the other end open to the atmosphere. The application of a pressure to the roof of the cavity (Figure1 (c)) displaced the air within the fluidic channel. The PDMS would return to its original position upon removal of the applied pressure thus creating a negative pressure that will suck the sample into the channel. The sample, positioned at the channel inlet (Figure 1. (f)) was drawn into the micro-fluidic channel flowing over the IEMS in the process. The incorporation of the simple displacement pump in this manner circumvents the need of external pumps traditionally associated with hydrodynamic flow.

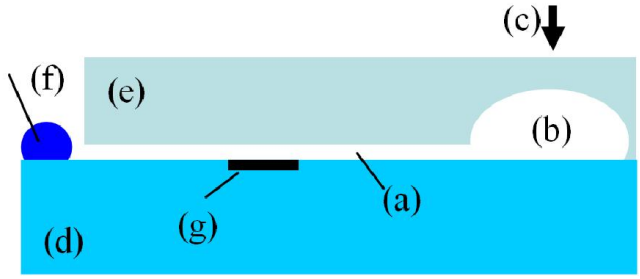



**Figure 1. The SSnPP delivery system was composed of: (a) Micro-fluidic channel; (b) Enclosed cavity; (c) Cavity roof; (d) Silicon substrate; (e) PDMS; (f) Sample; (g) Integrated electrochemical micro-sensor**

### *Integrated Micro-Pump Fabrication*

The silicon wafer underwent a two phase pre-treatment stage where all surface contaminants were removed and a 150 nm thick insulating $SiO_2$ layer was thermally deposited. The dehydrated substrate was prepared for photolithography with the distribution of hexamethydisilane primer and a thick uniform SU-8 layer by spin coating. The resist was soft baked and exposed to UV light through the polyester photomask**.** SU-8 does not crosslink upon exposure to a UV source like traditional photoresists and an additional post-exposure bake was required. Propylene glycol monomethyl ether acetate was employed to remove the unexposed resist and produce a patterned film. To create the cavity a few drops of microposit S1818 were dropped onto the disk shaped SU-8 film. The applied S1818 photoresist formed a sphere due to the solution surface tension. The S1818 was slowly baked at 30 $^{o}$C over several hours. The completed SU-8 mould is displayed in Figure 2. Prior to the application of PDMS the mould was silanised by placing the mould in proximity to Trichloro (3,3,3-trifluoropropyl) silane within a vacuum chamber for several hours. The PDMS pre-polymer and curing agent were mixed thoroughly until it exhibited a white, foam like appearance. The mixture was poured over the SU-8 mould located within a petri dish and placement within a vacuum for 20 minutes removed the bubbles introduced during the pouring and mixing procedure. The PDMS was cured at room temperature for 72 hours. The PDMS was gently peeled from the SU-8 mould (the mould can be reused to cast further PDMS slabs). A negative imprint of the SU-8 was cast into the PDMS. The PDMS slab was dissected into individual SSnPP and heated to 70$^{o}$C for 3 hours. The PDMS was dipped in methanol prior to positioning the fluidic channels above the active area of the electrochemical micro-sensor.

### *Mould Micro-fabrication*

### *SSnPP Geometric Configuration*

The single photo-mask responsible for delineating the SU-8 mould was designed and drawn using Mentor Graphics® IC layout station. The generated GDSII file was converted to gerber file through a software package called Link Cad and was subsequently emailed to JD photo Tools, England. A photo plotter printer, capable of 32,000 dpi, transferred the design onto a flexible polyester substrate using an emulsion based ink. Transparent slits 20 µm to 1.5 mm wide and 1.5 cm long were incorporated into the photo mask so that SSnPP of various sized could be fabricated. On one end of the slit a disk, with a surface area of 5.02 mm$^2$ was connected to complete the SSnPP design.

### *Channel Fabrication*

The PDMS was prepared by thoroughly mixing the PDMS base and curing agents in a 10:1 weight ratio. The agents were mixed until a white frothy like substance was obtained. The mixture was degassed at room temperature in a vacuum desiccators for approximately 15 minutes. Alternatively, the bubbles were removed by simply allowing the PDMS to sit for several hours at room temperature. The mould was placed within a petri dish (SU-8 side up) and the PDMS was poured over it while trying minimising the introduction of bubbles. Two small weights were placed at the wafer's perimeter to prevent floating. If large quantities of bubbles were introduced during the PDMS application the petri dish was placed in the vacuum desiccator for an additional 15 minutes. The PDMS was polymerised at room temperature over a period of 72 hours. It was observed that the S1818 used to generate the SSnPP cavity still contained a large solvent content and if the PDMS was baked in the traditional manner (75 $^{o}$C for several hours) out-gassing of the S1818 destroyed the SSnPP PDMS template. Sufficient polymerisation had occurred after several days to allow the removal of the PDMS from the mould. The freshly separated PDMS was placed on a silanised silicon wafer and heated to 75 $^{o}$C for 3 hours to ensure full polymerisation. The PDMS was then dissected into individual PDMS SSnPP templates. The cross section area of a 115 µm high 50 µm wide micro-fluidic channel is presented in Figure 2.

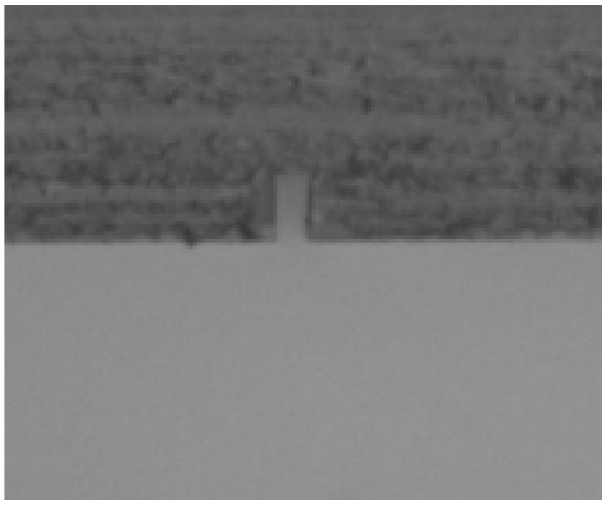


**Figure 2. Cross sectional area of a PDMS microfluidic channel**

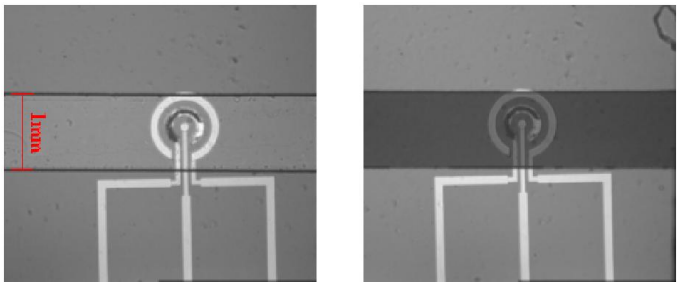
SSnPP's with channels ranging in width from 20 µm to 1.5 mm were fabricated by reversibly bonding the patterned PDMS with several microscope glass slides. Air bubbles were generally created when contact was made between the PDMS and glass substrate however, by placing the SSnPP in a vacuum for 30 minutes this issue was resolved. An aqueous based dye was drawn into each fluidic channel by applying finger tip pressure to the SSnPP cavity. The PDMS channel length was reduced to 1.5 mm therefore, assuming a height of 115 µm, fluidic channels with volumes of 35, 86, 173, 431, 863, 1730 and 2590 $pm^3$ were fabricated. One of the advantages of the SSnPP is that because the channel is filled by a vacuum a reversible seal provides adequate adhesion for a water tight seal. No evidence of water leakage into the PDMS/glass interface was observed under microscopic inspection. In addition, no bubbles were observed within the filled fluidic channels. It was occasionally observed that when the fluid was drawn into the larger volume fluidic channels (0.86 1.7 and 2.59 $nm^3$) it would continue to flow down the channel until the entire sample resided within the cavity, however, this phenomenon was not observed with the smaller volume fluidic channels (35, 86, 173, 431 $pm^3$). At present, because of the large volume of the cavity, excess air is displaced which resulted in additional fluid being drawn into SSnPP fluidic channel and cavity. An additional advantage of the fabricated SSnPP is its reusability, after testing the PDMS can be easily removed, cleaned and repositioned over a different substrate.

### *Integrated Electro-Chemical Sensor Fabrication*

The fabrication procedure for the IEMS was altered slightly prior to integrating the PDMS SSnPP. PDMS adheres to substrates containing the OH group e.g. silicon dioxide Therefore, the polymer passivation layer was replaced with a RF sputtered $SiO_2$ thin film. The micro-fabrication procedure began with the deposition and patterning of the Cr/Pt. A $SiO_2$ thin film was then deposited over the patterned Pt electrodes. The wafer was held stationary during deposition which took 30 minutes. $Si_3N_4$ is generally employed as a passivation layer for electrochemical micro-sensor as it is less permeable than silicon dioxide, however, $SiO_2$ can be selectively removed through photolithography and wet etching. Once the active area (i.e. the three electrodes and contact pads) of the IEMS were delineated with a hard-baked photo-resist template the selective removal of the $SiO_2$ was achieved in a buffer oxide etch over a period of 4 minutes. A third photolithography step was executed to delineate the micro-sensors reference electrode and then a silver thin film was thermally evaporated.

### *SSnPP and IEMS Integration and Testing*

A 1000 µm wide by 115 µm high SSnPP was positioned over an IEMS with a 100 µm working electrode (Figure 3). Precise alignment of the fluidic channel over the IEMS was problematic as the reversible bond occurred upon contact, however, dipping the patterned PDMS slab into methanol prevented spontaneous bonding and acted as a lubricant enabling alignment with a optical microscope. The IEMS with the integrated SSnPP was then heated to 40 $^oC$ to increase the rate of methanol evaporation (alternatively allowing the device to sit for several minutes at room temperature led to the evaporation of the methanol). An additional advantage of using the methanol was that air bubbles between the $PDMS/SiO_2$ interface were not formed during bonding. The channel was filled with a 6 mM $K_3FeCN_6$ + 0.1 M KCl + 0.1 M PBS buffer by applying finger tip pressure pressing to the SSnPP cavity (Figure 3 (b)). No evidence of water leakage into the PDMS/glass interface or bubble formation within the channel where observed under microscopic inspection.




(a) (b)

**Figure 3. Integrated IEMS and SSnPP, (a) empty channel, (b) filled channel**

A well defined $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ couple was obtained at scan rate of 50 mV/s (Figure 4). The reduction peak potential occurred at 0.087 V while the oxidation peak potential occurred at 0.193 V. The formal potential of the redox couple was 140 mV with a peak separation gap of 106 mV. The cathodic and anodic current recorded were 95.96 and 94.76 nA respectively with an $I_{pa}/I_{pc}$ current ratio of 1.01 confirming the reversibility of the process.

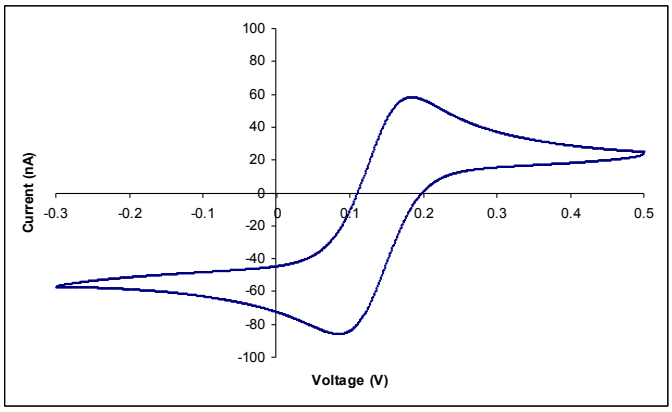



**Figure 4. A $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ couple recorded with a 100 IEMS**

## RESULTS AND DISCUSSIONS

A single stroke polydimethylsiloxane nano-pipette pump was successful fabricated by replica moulding. SU-8 patterned by photolithography was employed as a mould over which an elastomeric polymer (polydimethylsiloxane) was cast. The reversible bonding of the PDMS to a planar substrate produced a single stroke integrated nano-pump. Air within the fluidic channel was displaced with the application of pressure to the pump's cavity which generated a negative pressure that enabled the delivery of a sample to the micro-sensor. Fluidic channels with volumes of 35, 86, 173, 431, 863, 1730 and 2590 $pm^3$ were successfully filled by the nano-pump without the use of any external equipment generally associated hydrodynamic flow and without any damage to the micro-channels. A single stroke polydimethylsiloxane nano-pipette pump was successfully integrated onto a substrate containing a 100 µm working electrode electrochemical micro-sensors

fabricated using thin film technologies, to produce a simple LOC device. A small 1.7 nl volumetric $K_3FeCN_6$ based sample (the channel had a volume of approximately 1.7 $nm^3$) was delivered to the IEMS. A well defined $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ voltammogram was obtained with a formal potential of 140 mV and an $I_{pa}/I_{pc}$ current ratio of 1.01. The electrochemical data obtain compared favourably to that obtained by similar sized IEMS tested without the integration of the polydimethylsiloxane pump. The quantity of the sample delivered to the IEMS was proportional to the volume displaced which resulted in additional fluid being drawn into fluidic channel and cavity. Finally fabricating a cavity with a smaller volume (through multiple SU-8 photolithography steps) will enable a precise volume of liquid to be delivered.

***Potential applications***

An integrated micro- sensor and micro-pump was realized, and it lend itself for biomedical or clinical, and environmental applications. The micro-pump was fabricated using conventional micro-fabrication techniques, therefore it can be integrated easily with micro-fluidic systems. The micro-pump is built onto the sensor therefore there is no need for external equipment or interference with the device. The micro-pump was tested using finger tip pressure, and it can be activated using an external actuator such as electromechanical systems to control the position and rate of application of pressure. This enables the micro-pump to be utilized for other applications such as sample cleaning and/or sensor drying. With the proper layout several micro-pumps can be integrated with channels for multi-agent or multi-sample testing, where the cavity can be used to house some of the reagents needed for the testing which can be released by applying pressure to the cavity. In addition, multi-electrode structures can be housed under different cavities while coated with proper enzyme (Ben Ramadan *et al.,* 2000 and 200; Han *et al.,* 2011; T. Hemelatha *et al.,* 2013) or nanoparticles (Dehghani *et al.,* 2016) to produce multi-analyte electrochemical biosensors for medical or environmental applications. The micro-pump is reusable on the same sensor or on a different sensor. This micro-pump requires very small samples, which means less consumption and less waste and better fluidic management.

**Conclusions**

An integrated micro- fluidic pump was designed fabricated, and tested. It was integrated with a micro-sensor and the combination proved to give excellent results. Ease of fabrication enables the integration of many micro-pumps, micro-sensors and micro channels to produce an integrated multi-function micro-sensor system for biomedical, clinical, and environmental applications using thin film coating to properly test a specific analyte.

## REFERENCES

Plecis, A. and Y. Chen, 2008. *Microel. Engineering,* 85 1334

Terray, A., J. Arnold and S. J. Hart, 2005. *Optics Express* 13 10406

Amatore, C., M. Belotti, Y. Chen, E. Roy, C. Sella and L. Thouin, 2004. *Journal of Eletrochemical Chemistry* 573 333

Dehghani, H., et al 2016. *Int.J.Electrochem. Sci*. 11 2029

Ben Ramadan, Z., K. F. Tipton, and S. Boyce (2000 &2001) Nomenclature Committee of the *International Union of Biochemistry and Molecular Biology (NC-IUBMB).* Nomenclature of recently-recognised enzymes and proposed modifications to existing entries

Fujii, T. 2002. *Microelectronic Engineering,* 61-62 907

Han, Y., S. Song, J. Lee, D. Lee, H. Yoon (2011), *Analytical and Bioanalytical Chemistry,* 400 797

Hemelatha, T., T. UmaMaheswari, G. Krithiga, P. Sankaranarayanan, and R. Puvanakrishnan, 2013. *Indian Journal of Experimental Biology,* 51 777

Pepper, M., N. S. Palsandram, P. Zhang, M. Lee and H. J. 2007. Cho *Sensors and Actuators A: Physical* 134 278

Suzuki, H. and R. Yoneyama, 2003. *Sensors and Actuators B: Chemical* 96 38

Upadhyay, S., N. Ohgami, H. Kusakabe and H. Suzuki, 2006. *Biosensors and Bioelectronics* 21 1230-1236

Woais, M. 2005. *Sensors and actuators B* 105 28

Yoonhee, Y., S. Y. Oh and J. K. Park (2006), Enzyme and Microbial Technology, 39 1112

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