CHAPTER – 8
DEVELOPMENT OF SMART PHONE BASED
OPTO-FLUIDIC DEVICE

8.1 Overview

With the advances in microfluidics technology newer forms of reliable and accurate opto-fluidic devices are continuously evolving[1][2]. These devices carries a significant potential streamline healthcare by providing cost effective and personalized healthcare especially for low income countries [3]. Microfluidic based point of care (POC) devices promotes a paradigm shift from conventional clinical laboratory setting to near-patient settings. This helps physicians in providing well timed treatment by timely accessing information about patient’s health. Moreover at the same time these POC devices also assist patient to personally monitor his health at home [4]. The tedious procedure of travelling samples to clinical settings and long waiting queues can be circumvented. However, the approaches for fabricating such medical devices are largely based on expensive techniques [5]. The substitution of silicon based fabrication by polydimethoxysiloxane (PDMS) chips is considered as a significant technological advancement in low cost microfluidics [6], [7]. PDMS, a biocompatible elastomer polymer is widely exploited for fabricating microfluidic devices using soft lithography techniques [8]–[11]. It is widely believed that elimination of expensive lithography materials and equipments would be helpful in building more cost effective microfluidic assemblies, especially for disease diagnostics [12], [13]. As reported earlier, using photolithography the current time for concept to device is 24 hour [14]. Out of this most of the time is spent in preparing mould and maintaining clean room facility. This creates a void for development of a low cost technique for fabricating fluidic devices.

In order to provide a cost effective alternative to lithography process various groups have developed some non-conventional techniques for fabrication of PDMS cartridges [13], [15]–[17]. Amongst them, the most promising alternatives are printing circuit board (PCB) technique and 3D printing. PCB technique utilizes conventional PCB to quickly fabricate master moulds for microfluidic channels [16]. PCB fabrication method requires no clean room facilities, cost effective and can be easily developed. However, a major drawback of rough supportive materials like epoxy and paper phenolics under copper foils causes difficulty in device sealing [12]. Moreover, increase in surface roughness results in poor adhesion to glass surface restricting its applications in diagnostics. The roughness of PCB
does not pose a problem with other thermoplastic polymers, but for laboratories PDMS is more familiar than other thermoplastics [13], [18].

To circumvent this issue, many groups remove partial copper layer with subsequent polishing and washing steps to mould PDMS [19], [20]. Still the protocol requires multiple surface modifications by plasma to seal the device. Some groups have reported the use of PDMS base instead of glass to easily adhere fabricated PDMS channels [13]. PDMS posses a non functional surface an in comparison to glass as it is difficult to functionalize PDMS surface. This restricts the application of such devices to mixing and droplet generation purposes. However for performing immunoassays and other complicated analytical techniques, an easily functional surface like glass is desirable.

In this study, an extremely simple approach has been presented to produce microfluidic wells using a novel punching method. The problem of PDMS adhesion to glass was solved by preparing a two layer device with different elastomer-curing proportions. Almost all the techniques reported in literature use plasma or other surface treatment methods to produce adhesion between glass and PDMS surface. In the reported strategy however no specialized equipments like plasma, PCB copper clads were used to fabricate device. The chapter also highlighted some major drawbacks of PCB and 3D based mould preparation techniques. Towards practical applicability of device, it has been utilized to design fluid cell for urea sensing in drinking water.

8.2 Experimental

8.2.1 Reagents and apparatus

PDMS elastomer and curing agent was procured from Dow corning USA. PCB copper clad and biopsy punch was procured from local market. 3D printing was done by using 3D printing facility at UIET. Sodium dodecyl sulfate polyacrylamide gel electrophoresis apparatus was procured from Himedia laboratories, Bangalore. Reagents like urease, urea and phenol red were procured from SRL chemicals, Mumbai.

8.2.2 3-D printing based microfluidic devices

Various designs for microfluidic channels were created in solidworks. Then, STL file is generated which is further processed in Slicer software to generate the G codes. PolyLactic Acid (PLA) wire was used as a feed wire in 3D printer. Other than this, Acrylonitrile Butadiene Styrene (ABS) was also used. All parts are printed with a printing
nozzle of 400 microns. The printed moulds were used for fabrication of PDMS. In brief, 1:10 ratio of elastomer and curing agent was mixed and degassed in vacuum. Following this protocol, PDMS was cured at 80°C for 2 hours.

8.2.3 Printed circuit board design and Fabrication

PCB was constructed using etching of an electrically conducting copper clad. To selectively etch copper, firstly design was printed on a glossy sheet and transferred to copper clad by heating process. Selective etching was achieved by placing it in a solution of FeCl$_3$. PDMS was then cured on using PCB as substrate in same way as describes in 2.2.

8.2.4 Punching technique for microfluidic device

In this technique Biopsy Punch is used to make cut outs in PDMS layers and produce the microwells. Firstly, two different plain layers of PDMS were casted each of 1mm thickness. The composition of both the layers was different. For first layer the ratio of curing agent to base was kept at 1:5 w/w and second layer was 1:20 w/w (ratio between curing agent and base). First layer was taken and cut down into a rectangular piece of 23mm x 35mm. Then using Biopsy-Punch of 5mm 4 holes in a column are cut out from layer keeping a distance of 3mm between each hole and also the edge of PDMS layer. Then similar parallel column is made on other side. So, total 8 wells were made. Along this, a same dimension rectangular piece is taken out from second layer. Then both layers are aligned together and placed on each other in a order and small holes were created using Biopsy Punch of 2mm in new layer to use it as for inlet and outlet purpose. After this, for bonding of layers on glass surface, the two layers and a glass slide were aligned and pressed together for an interval of an hour at a temperature of 80°C.

8.2.5 Urease assay

To immobilize urease on glass surface of microwells, firstly an electrolyte Polyethylenimine (75 Kda) was coated overnight. Then 10µg/ml of urease solution was filled in well for 2 hours at room temperature. The wells were then washed 3 times with water followed by addition of standard urea solution.

8.3 Results and Discussions

8.3.1 3-D printing for mould fabrication

3-D printing technique has been reported in manufacturing masters for soft lithography and directly printing microfluidic channels [9], [21], [22]. Recently,
Embedded SCAffold RemovinG Open Technology (ESCARGOT) was reported using 3-D printed scaffolds for microfluidic applications [15].

In this report, the work was started by fabricating 3D printed moulds for PDMS microfluidic device. 3D design was developed using solidworks and stored in STL format that was further processed into G codes in slicer software. These G codes were used by 3D printer and following master moulds are developed. Along with these master moulds, some other basic shapes were also printed to fabricate devices with different fluidics. These include spiral shape, circular well cum microchannel and a multi port shape (see Figure 8.1).

Few designs of sample tray device, like micro channel and micro wells has been developed using designing software for the initial study.

Figure 8.1 – (a-b) Pattern of a micro-channel designed using solid works, the conduit is of square cross-section having a dimension of 2x2 sq. mm. The flow will pass through it, and provides a site for reaction (b) micro wells of cylindrical cross section of radii 2 mm and height 4 mm. (c) Design of multi port microchannel on a glass slide (d) General arrangement of spiral channels on glass slide (e) arrangement of circular wells on glass slide.
All the designs were printed using polylactic acid (PLA). These shapes were carefully arranged and bonded on glass slide using permanent resin. After the fabrication of master mould, PDMS was casted followed by careful removal of pattern. The devices thus prepared carry a high degree of surface roughness making them inappropriate for usage in diagnostic applications. This poor surface roughness also results in weak adhesion to glass substrate and results in fluid leakage. Figure 8.2 clearly illustrates large rough ridges along fluidic surface. These rough surface ridges moreover contribute to issues like dead volumes and inconsistent surface modifications. This unpredicted increase in surface roughness also increases uneven adsorption of molecules [23]. Moreover, intricate patterns with prepared using 3D printed moulds result in fluid leakage (Figure 8.2c and 8.2f).

![Figure 8.2 – Various fluidic designs printed using 3D printing. (a) to (c) 3D printed moulds using PLA, (d) to (f) respective fluidic compartments fabricated using moulds. (f) Digital photograph taken by flowing luminescent graphene quantum dots through channel for visualizing leakage.](image)

8.3.2 Printed circuit board technique

Next we investigate the printed circuit technology for fabrication of microfluidic channels. Firstly, the channel drawings were printed using laser printer on a glossy sheet.
The use of glossy sheet instead of normal paper facilitates the transfer of pattern from paper to PCB copper clad. The transfer of pattern from paper to clad can be easily carried out by using laminator or household iron. After transfer of pattern, the clad was placed in FeCl₃ (20 wt/vol%) for etching of free copper. The etching must be carefully monitored as placing for prolonged times may result in etching of copper from channels also. After successful fabrication of mould PDMS was casted on the PCB mould. Figure 8.3 illustrates various fabrication steps in PCB technique. However, the fabricated channels result in poor adhesion to glass surface. This is due to epoxy and phenollic base of board which results in elevated surface roughness and poor affinity towards glass.

![Figure 8.3](image)

**Figure 8.3** – Step wise illustration of various steps in PCB technique. (a) Laser printing on glossy sheet (b) transfer of pattern to copper clad (c) etching in FeCl₃ solution (d) etched board (e) removal of ink by ethanol (f) PDMS microfluidic chip.

8.3.3 Dual Layer Punching (DLP) technique for microfluidic fabrication

In both the above mentioned techniques, increased surface roughness and poor adhesion to glass remains major stumbling blocks in fabrication of device. Many groups have reported use of surface modification techniques for increasing adhesion properties of PDMS after curing. However, this is also widely believed that these modification techniques result in increase in overall cost of the system and moreover also restricts fabrication to expensive laboratory settings only.

In order to avoid this problem bonding two layers of semicured PDMS together has been established and widely used. However, drawbacks like difficulty in functionalizing PDMS surface and a less rigid support to fluidic device surface still
To overcome these drawbacks along with above discussed limitations of 3D printing and PCB technology we invented a new method; Dual Layer Punching (DLP) to produce microfluidic reaction chambers. A block diagram of steps involved in DLP is shown in Figure 8.4.

![Block diagram of DLP technique.](image)

**Figure 8.4** – Block diagram of DLP technique.

DLP employs a commonly used biopsy puncher to make reaction wells in two different PDMS layers. The thickness of PDMS layers is controlled by building layers in a commonly used SDS PAGE gel apparatus with glass spacers. By controlling the thickness of glass spacer’s layer thickness can be easily controlled. Biopsy punches are available in variable sizes ranging from 2mm to 8mm in diameter and thus volume of reaction wells can also be easily controlled. Figure 8.5 shows digital photographs of DLP fabricated chips.

![Digital photographs of steps in DLP technique.](image)

**Figure 8.5** – Digital photographs of steps in DLP technique (a) SDS PAGE apparatus with glass spacer to produce controlled thickness PDMS films (b) microwells punched by biopsy punch (c) assembling layers in glass to produce microwells.
In this work a 1 mm thick glass spacer was employed to control PDMS layer thickness. Both the layers were composed of different ratio of curing agent and elastomer. For base layer (First) it was kept at 1:5 w/w and for second layer was 1:20 w/w. Biopsy punch of 5mm dia punches holes of first layer and of dia 2mm in second layer to use it for inlet and outlet purpose. The excess of curing agent in first layer promotes bonding on glass surface and other layer. Subsequent thermal conditioning at temperature of 80º C provides strong bonding.

8.3.4 Smart phone device drawing and parts

Smart phone holder accessory was developed to provide a stable and constant interface between the sample holder and smart phone. The holder serves the purpose of providing a fixed position to smart phone and sample holder that can be easily inserted. This accessory is also important as it provides an environment that is free from outer disturbances of light and shadows which varies from place to place and can affect the capturing of solution pictures by CMOS sensor of mobile handset. Generally it is used for capturing fluorescence, light intensity or RGB of images. As the sizes of different mobile phones varies and also their position of cameras are not same, so, accessory is initially designed for a particular handset of Samsung namely Samsung Galaxy J2 pro. Dimensions of mobile handset are as follows- Length-142.4 mm, Breadth-71.1 mm and Thickness-8 mm. The drawings are provided in Appendix and assembled device is shown in Figure 8.6. A set of detailed drawings corresponding to smart phone based device are provided in Appendix.

![Figure 8.6](image)

Figure 8.6 (a) 3D view of complete accessory marked with all its parts (b) Actual view of smart phone device
8.3.5 Smart phone based urea diagnosis assay

The developed microwells were utilized for urea sensing using urease and phenol red as sensing element. Urease was immobilized on glass surface in microwells to carry out the further urea sensing operations. A number of protocols have been established for coating protein on glass surface. Polyelectrolyte coating is one of the simplest methods for protein coating as it does not require any tedious bioconjugation steps. In this report we utilized polyethyleneimine a cationic polyelectrolyte for immobilizing urease on glass. In order to check efficiency of the PEI towards urease coating initial kinetics study was carried out in polystyrene microwells on plate reader. Figure 8.7 shows an increase in urease immobilization in the presence of poly-electrolytes.

![Figure 8.7](image)

**Figure 8.7** – Effect of PEI on urease immobilization. In the presence of electrolyte urease coating is tremendously increased as compared to control.

Urease hydrolyzes urea into ammonia and carbon dioxide and the production of ammonia elevates the pH of solution which is subsequently detected by commonly used pH responsive dye phenol red (Figure 8.8). Phenol red colorimetric transition occurs from yellow to pink as pH of solution increases. After fabrication of optofluidic device, different known concentrations of urea ranging from 100 to 1000 µg·mL\(^{-1}\) were added to solution and color change was estimated using smart phone based device on image j platform software at 8bit RGB scale.
Figure 8.8 – Applicability of developed DLP microwells for urease sensing using smart phone. (a) Visual image (b) image j analysis (c) smart phone based detection system.

In this scale each primary colour has 256 states ranging from 0-255, where 0 means dark or black and 255 shows highest intensity. RGB graph in Figure 8.12a, variation in red, green and blue colour intensity is visible. Clearly, intensity in green band illustrates a linear relationship with equation $y = -0.0519x + 120.91$ and $r$ squared value of 0.9296. Further we also analyze signals from RGB bands to get more linearity. But however none of the relation from RGB, RG, RB, GB shows us better linearity than only green band (Figure 8.9 b).

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\begin{align*}
\text{RGB} &= \frac{R+G+B}{3} & \text{Eq. 8.1} \\
\text{RG} &= \frac{R+G}{2} & \text{Eq. 8.2} \\
\text{RB} &= \frac{R+B}{2} & \text{Eq. 8.3} \\
\text{GB} &= \frac{G+B}{2} & \text{Eq. 8.4}
\end{align*}
\]

Figure 8.9 – (a) RGB values calculated by analyzing image of wells (b) further analyzed color coding from wells.
8.3.6 Siloxane immunoassay fluid cell cartridge

Next, we incorporate assay developed in Chapter 6 to the DLP fluid cartridge. BSA was coated on glass surface of microwells overnight at 4°C. Followed by subsequent washing and blocking steps gold nanoparticle labeled antibody probe was added in wells. After subsequent washing, optimized silane solution was added to the wells (refer Chapter 6 for details). However, it must be noted that propanol was used instead of ethanol due to its higher boiling point in comparison to ethanol. Figure 8.10 shows siloxane based fluid cell cartridge for detection of analytes. The cartridge is very similar to pregnancy test strips available in market and can be easily exploited for point of care testing of various antigens without requiring any instrument.

![Siloxane based fluid cell cartridge](image)

**Figure 8.10** – Siloxane based fluid cartridge for detection of antigens (BSA) (i) E represents empty well, C is control (only GNP-IgG probe coated overnight), T is test, B is blocked GNP-IgG probe. (ii) and (iii) visual photographs of cartridge.

8.4. Conclusions

In conclusion we have developed a convenient DLP method for microwell fabrication. The technique dominates over already reported PCB and 3-D printing in terms of ease and simplicity. Moreover, DLP does not require any surface modification
for fabrication of channels, thus significantly reducing cost and saving time required for microwell fabrication. The microwells produced can be easily exploited for immunoassays and other diagnostic applications. In this paper, urea sensing is reported using DLP fabricated microwells and a smart phone based device. Due to ease of fabrication, minimal requirement of equipments and use of PDMS the method provides a low cost alternative to researchers who intend to carry out basic microfluidic diagnostics.
8.5 References


