Optimization of a Microfluidic Cartridge for Lab-on-a-Chip (LOC) Application and Bio-Testing for DNA/RNA Extraction

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Abstract

A bio-microfluidic cartridge has been developed with integrated reservoir and valves for Lab on a chip (LOC) applications, e.g. extracting DNA (Deoxyribonucleic Acid) from human blood. A combination of different materials such as thermoplastic and elastomer are used to fabricate the microfluidic cartridge.

A conical shape reservoir with thin membrane sealing is designed to have minimum dead volume in the reservoir after dispensing completely into the chip. An external actuation is used to apply pressure on the thin rubber membrane to push the fluid from the reservoir to the chip and to output collector. The external actuation pushes the fluid through the pin valve and the fluid flow is proportional to the actuation speed.

Material selections and package optimization have done to maximize the performance of the cartridge functionality. Different mixing ratio of PDMS (Polydimethylsiloxane) material based on elongation has been tried to select the membrane to seal the reservoir. To contain the reagents inside the reservoir different thermoplastic material has been tried over the elastomer material. Bonding of similar and dissimilar material is studied for fluidic channel layer selection.

Extraction of DNA/RNA (Ribonucleic Acid) from blood sample is tried and sufficient quantity of DNA has been extracted from this cartridge for PCR (Polymer Chain Reaction) amplification and detection.

1. Introduction

Influence of microelectronics into life science has introduced many developments in bio- applications. One of the main impacts on bio- systems is the miniaturization that has seen clearly on microelectronics industry. Micro and nano level of interaction in bio-systems has emerged new field of research and new application products. Microfluidics is one of the fast emerging fields where fluid flows in micro channels and very little dead volumes are present which eliminate contamination and mixing among the fluids. Convergence of micro fluids and microelectronics results to a new kind of devices, micro fluidic chips. They require channels, reservoirs, filters to process the fluid [1]. Micro fluidic structures are formed on silicon/polymer substrate by micromachining or micro stamping method. Micro fluidic chip offers the ability to work with shorter reaction time, smaller fluid sample/reagent volumes and promises of parallel processes.

Major applications of micro fluidics are on the clinical diagnostics, drug discovery, and bio- terrorism monitoring and therapeutics devices. Fluidic components required for clinical diagnostics is different from the fluidic components required for drug discovery. In most of the cases a common platform can be used to combine the basic modules in order to implement a micro fluidic device for diagnostic applications or drug discovery for pharmaceutical applications.

The lab on a chip (LOC) concept is to realize the functions of bio-laboratory in a silicon chip (Fig.1). The miniaturized bio-laboratories are fabricated by photolithographic process developed in the microelectronics industry to form circuits, chambers, valves and channels in quartz or silicon substrate [1-4]. Fluidic samples can be manipulated by placing valves, pumps in the chip and fluid can be diluted, mixed with other reagents or separated by other process on the same chip. In this paper, a micro fluidic chip for DNA extraction and amplification is described. Silicon substrate is used to form micro fluidic components in the chip.

Packaging of micro fluidic chip is an important factor that supports the chip function by dispensing and controlling fluidic flow in order to realize particular bio-protocol (Fig.2). Fluidic control includes controlling of the fluids’ flow sequence, flow duration, flow direction and flow rate. The package needs to have a mechanism to control each fluid/reagent to follow the protocol. And at the same time prevent reagents from cross mixing and contamination. The package also completes the connection between the micro fluidic chip and other systems such as fluidic source, electrical source and optical sensor.
In the micro fluidic packaging, polymers are generally used for encapsulation. Packaging material is based on the biocompatibility and to the reagents used for the extraction the key parameters of the package development.

2. Design optimization of cartridge

The reservoir has a conical shape cavity covered with a thin layer of flexible film as shown in Fig.3. The actuator diameter is same as the bottom diameter of reservoir. The volume of dispensed liquid is equal to the volume of membrane deformation.

Fig. 3 (a) Original design of the reservoir

Membrane

Needle plug

Al foil

Fluidic port

Base layer

Fig.3 (b) Modified and optimized design of the reservoir

Fig.3 (c) Design optimization of reservoir with Al foil with external actuator

The reservoir design has been modified to reduce the valve opening time. The shape of the reservoir has kept as same while the pin valve supporting part has changed. An injection molded rubber needle plug is used to secure the pin valve in the reservoir. Changing to needle plug helps the pin valve secure straight and the valve opening time is same across the other reservoirs. A blind via hole is made in the needle plug to secure the pin valve. The final optimization of the valve opening is done by inserting an Aluminum foil between the needle plug and the channel layer. The force required to punch through the Aluminum foil is small compared to a thin PDMS membrane used in the first version of reservoirs. The modifications finally reduced the dispensing time by 50% and this eventually reduce the cycle time of the protocol used for DNA extraction used in the cartridge.

3. Material selection

Good elongation membrane helps to minimize push force required from actuator, so that reduced actuator size. Four bio-compatible materials are selected in this study. They are made to be 0.5mm thick membrane. They are used to seal reservoirs of sample cartridges. Each sample cartridges has four reservoirs filled with four reagents respectively. Sample cartridges are fixed on the Instron push test machine as clamping on bottom fixtures. The bottom load cell is non-movable. A computer controls the top actuator to move downward and press the membrane of each reservoir and dispense the fluid from reservoir. During the pushing action, the displacement of top actuator and load are transiently recorded.

Table1 Actuation force on membrane 10:1 PDMS

<table>
<thead>
<tr>
<th>PDMS : Curing agent = 10:1</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reservoir 1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Speed (mm/min)</td>
<td>0.513</td>
<td>0.628</td>
<td>0.853</td>
<td>0.0251</td>
</tr>
<tr>
<td>Extension (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Force (N)</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0.31</td>
<td>1.31</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.21</td>
<td>1.6</td>
<td>3.85</td>
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</tr>
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<td>2</td>
<td>2.91</td>
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<td>7.35</td>
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<tr>
<td>3</td>
<td>4.81</td>
<td>5.6</td>
<td>11.15</td>
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<tr>
<td>5</td>
<td>8.81</td>
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<td>20.59</td>
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<td>35.95</td>
<td>22.6</td>
<td>22.06</td>
<td>40.1</td>
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</table>

Table2 Actuation force on membrane 15:1 PDMS

<table>
<thead>
<tr>
<th>PDMS : Curing agent = 15:1</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reservoir 1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Speed (mm/min)</td>
<td>0.513</td>
<td>0.628</td>
<td>0.853</td>
<td>0.0251</td>
</tr>
<tr>
<td>Extension (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Force (N)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
<td>0.31</td>
<td>0.74</td>
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<td>1</td>
<td>0.86</td>
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<td>2</td>
<td>2.3</td>
<td>2.56</td>
<td>1.91</td>
<td>4.19</td>
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<td>4.15</td>
<td>4.26</td>
<td>3.16</td>
<td>5.99</td>
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<td>5.91</td>
<td>5.91</td>
<td>4.41</td>
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<td>7.46</td>
<td>5.56</td>
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<td>10.79</td>
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<td>6.66</td>
<td>10.59</td>
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<td>13.29</td>
<td>10.11</td>
<td>7.71</td>
<td>12.49</td>
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<td>8</td>
<td>16.84</td>
<td>11.76</td>
<td>8.68</td>
<td>17.4</td>
</tr>
<tr>
<td>9</td>
<td>20.08</td>
<td>20.08</td>
<td>12.49</td>
<td>20.08</td>
</tr>
</tbody>
</table>
Selection of the membrane material is based on the membrane with smaller force. As shown in Table 1, 2, 3&4, different mixing ratios are tried for PDMS based membrane and selected silicon rubber materials are tried in the selection process.

Table 3: Actuation force on membrane 20:1 PDMS

<table>
<thead>
<tr>
<th>Reservoir</th>
<th>Speed (mm/min)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.513</td>
<td>0.628</td>
<td>0.853</td>
<td>0.0251</td>
</tr>
</tbody>
</table>

Table 4: Actuation force on silicone rubber

<table>
<thead>
<tr>
<th>Reservoir</th>
<th>Speed (mm/min)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.513</td>
<td>0.628</td>
<td>0.853</td>
<td>0.0251</td>
</tr>
</tbody>
</table>

From the above analysis it is concluded that the less actuation force is desirable for the current fluidic package application. The PDMS 20:1 and silicon rubber has the smallest actuation force. But the stickiness of the PDMS 20:1 membrane causes difficulty in handling due to less curing agent. So the selected silicon rubber is found to be suitable.

4. Permeability of material

Storing of reagents inside the cartridge for a long time is difficult because it demands a minimum shelf life, six to twelve months. Since some of the reagents are highly volatile, the reservoir material used for storing the reagents should have low permeability and is non-porous. PDMS is a porous material. [5]

Permeability study has done to understand how well the material for reservoir and for the membrane material (Table 5).

Table 5: Permeability of membrane and reservoir material

<table>
<thead>
<tr>
<th>Material</th>
<th>Permeability (g/m²/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubber membrane</td>
<td>0.63</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Thickness of both materials used for the permeability measurements are 0.5mm. Poly propylene has better permeability compared to rubber material for both membrane and reservoir applications. Since the material % of elongation of the poly propylene is shorter than that of the rubber material, rubber material is chosen for the membrane while for the reservoir poly propylene is used. Combining both materials as a membrane and reservoir, the shelf life of the reagents in the container has improved more than 80% compared to PDMS as the material for reservoir. Fig.4 shows a developed cartridge with rubber membrane and PP reservoir.

5. Thermal compression bonding

Bonding of biocompatible polymeric substrates is performed by thermal compression bonding where the bonding parameters are the chuck temperature, time and the force. The bonding strength is measured to study the dependence on these three bonding parameters. The objective is to obtain the optimal bonding characteristics between polymer substrates. Using proper bonding condition the micro fluidic structures on two substrates can be sealed together without any physical distortion due to melting of the material, which results in clogging of micro fluidic channels.

Manufacturability of PDMS is not good compared with other thermoplastic material. Thermoplastic materials which are injection moldable are selected for reservoir and channel layer materials. Different thermoplastic materials such as PP (Polypropylene), Poly carbonate, PMMA (Polymethylmethacrylate, acrylic) are selected. Bonding of these materials, such as similar and dissimilar materials are studied.

5.1 Bonding of PMMA to PMMA for Channel layer

PMMA is a thermoplastic and optically transparent material. It has glass transition temperature Tg at 105°C. The dimensions of the PMMA sample used for this study are 70mm by 55mm, with a thickness of 1mm which meets the requirement of the current micro fluidic cartridge. The upper
substrate consists of all the fluid ports and through holes, while the lower substrate has micro channels. A pair of bonded sample is shown in Fig.5(a)&(b).

![Fig.5 Sample of PMMA to PMMA bonding, (a) side view and (b) top view](image)

Lower temperature range (80°C to 100°C) is used during the initial bonding study because of the Tg of PMMA is 105°C. Even though some signs of bonding interaction are observed at bonding conditions of 100°C, 60kg and 1500s, the interface between the two substrates has air gaps and is not a desirable bonding for fluidic testing. Therefore the temperature has increased to its Tg, while varying the force and time. Based on the modified parameters, the optimized conditions for PMMA with the above mentioned dimensions are 105°C, 50kg and 1200s.(Table6)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Temp (°C)</th>
<th>Force (Kg)</th>
<th>Time (Sec)</th>
<th>Bonding Y/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>0.5</td>
<td>30</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>0.5</td>
<td>100</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>5</td>
<td>100</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>10</td>
<td>500</td>
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<td>1000</td>
<td>N</td>
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</tr>
<tr>
<td>9</td>
<td>105</td>
<td>50</td>
<td>1500</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>105</td>
<td>60</td>
<td>1000</td>
<td>Y</td>
</tr>
<tr>
<td>11</td>
<td>105</td>
<td>50</td>
<td>1200</td>
<td>Y</td>
</tr>
</tbody>
</table>

In the experiment, the inlet pressure increases steadily with time up to a maximum of 176.44 KPa before fluid starts to leak. The pressure encountered in the current biological fluidic testing for the cartridge is usually 20-40KPa and is much lower than this tested pressure value. Therefore, it can be concluded that thermal compression bonding for PMMA is effective and useful and it eliminates the need for adhesive layer for bonding.

5.2 Bonding of PP to PMMA for Reservoir and channel layer

PMMA is not suitable to be reservoir material and store reagents for long time, because PMMA is not chemical resist to some of the key reagents. So polypropylene is selected as the reservoir material based on the permeability, inert to reagents used for extraction. Since reservoir is PP and channel layer is PMMA, a bonding study of PP to PMMA is performed to find the suitability of micro fluidic application. PP and PMMA have different Tg and therefore thermal bonding would not be possible. Bio-compatible adhesive tape was used to bond these two layers in this study (Fig.6).

5.2.1 Sample

The samples are prepared based on the design in Fig.3 (b) with aluminum foil and needle plug to represent the bonding of actual cartridges. The reservoir sample is about 80x60mm and 10mm thick PP layer with 12 reservoirs. The PMMA layer was 3-mm thick, with fluidic ports for fluidic test.

5.2.2 Process development: adhesive tape and thermal compress

To bond PMMA and PP, the Mini-Test Press machine is used. Under the heating condition of 80°C with pressure, the adhesive on the both side tape is soften and wet the interface better, thus maximize the bonding quality.
Optical inspection has done to assess the bonding quality. With process 7 and 8, 90% of bond coverage is able to achieve. Process 7 was selected as final bonding process. The samples are bonded at 80°C, with pressure of 3Mpa for 5mins and cooled down to room temperature with same pressure.

5.2.3 Fluidic testing
Since the bonding interface at the corner and the sides are susceptible to leakage than those at the centre, fluidic testing was conducted on all the 12 reservoirs. It is found that the reservoir-channel interface could withstand the average pressure of 513kPa without leakage, which is much higher than the bio-package requirement (Table8).

Table8 Fluidic testing on max pressure of the sample with 12 reservoirs’ bonding interface (photo attached)

<table>
<thead>
<tr>
<th>Sample with 12 reservoirs</th>
<th>Reservoir</th>
<th>Max Pressure (KPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>517</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>520</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>515</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>520</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>511</td>
<td></td>
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<td>7</td>
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<tr>
<td>8</td>
<td>519</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>498</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>520</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>522</td>
<td></td>
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<td>12</td>
<td>515</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>513</td>
<td></td>
</tr>
</tbody>
</table>

5.2.4 Pull Test
Pull test is performed to study the maximum load the bonding interface can withstand. The sample is assembled with a 20x20mm and 10mm thick PP, a 20x20mm with 3mm thick PMMA and 8mm diameter Al foil. The dimension is same as the single reservoir. Four samples were tested on the MicroTester. Samples are fixed on the pull test machine, on a fixed bottom load cell. A computer controls top fixture to move upward and pull up the specimen. During the pulling action, the displacement of top fixture and load are recorded. The test setup is as shown in Fig.7. The recorded load is shown in Fig.8. The load is build up as top fixture moving up. The load reaches maximum and suddenly drop after the bonding interface is open. The maximum load is recorded as pulling force. The average of pulling force of four samples is 112N. It is observed that after the interface opens the tape and the aluminum foil still remains on the surface of PMMA but not on PP material. (Fig.7)

Fig.7 Tensile test (a) set up and (b) bonding interface is open

Fig.8 Graph of load vs. extension of four samples

A developed bio-cartridge using above mentioned materials and processes is shown in Fig. 9.

Fig.9 Photo of developed cartridge

6. Bio-testing

6.1 Principle
Extraction of DNA from cell nucleus is required to study the DNA pattern. In order to release DNA from cell nucleus, the bio-sample should be lysed and during lysis the cell membrane breaks and the DNA come out from the nucleus. When the lysed blood flows through a silicon micro fluidic chip, DNA binds onto Si channel surface. The remaining particles and the sample flow out of chip as a waste. When
low salt reagent flows through the Si chip, the DNA detaches from Si surface and flow out from chip along with the elution reagent.

6.2 Sample and set up
In this study, DNA from human blood is extracted using a developed bio-cartridge. The cartridge has three main components, Si microfluidic chip, reservoirs and microchannel as link from reservoirs to chip. The chip has a chamber filled with Silicon micro machined pillars. The pillar surface is the area for contacting blood sample. The cartridge has reservoirs to store high salt, low salt solution and other reagents. During the bio-testing, the reagents in reservoirs are dispensed from reservoir to the Si chip by means of pushing the reservoir membrane by an external actuator. The actuating force and distance are controlled by a computer interface. During actuation, the actuator does not getting contact with reagents and avoids cross-mixing and contamination.

6.3 Pre treatment of the cartridge
Binding efficiency of DNA to Silicon chip is important for further amplification (PCR) process. In order to enhance the binding of DNA to silicon chip, pre-treatment on Si pillar surface is necessary. In this study, the package is treated with a mixture of ammonium hydroxide, hydrogen peroxide and water. This treatment solution is pumped into the chip using the peristaltic pump and finally the package is washed with water before bio-testing.

6.4 DNA Extraction
Bio-sample and lysis buffer are mixed in a certain ratio. During the mixing process, cell membrane breaks and DNA is released. The mixture is then injected into the sample port of the micro fluidic package. When the lysed bio-sample flows through microfluidic chip, the DNA binds on the pillars of the silicon chip. The remaining liquid flows out of chip as a waste. A computer controlled actuator pushes all the reservoirs in a sequential form with a predetermined speed based on the protocol. The last reagent is low salt solution, water, which detaches the DNA from Si surface and flushes it out and this process is called as elution. The product of elution is collected into five PCR tubes, which are marked as elution 0 to 4.

6.5 PCR (Polymer Chain Reaction) amplification
All the elutions from this extraction go through PCR thermal cycle with the following condition as shown in Table9. Table10 shows that a certain amount of DNA is eluted and sufficient quantity to be amplified by PCR. From all the elutions a total amount of DNA eluted is about 351.8 ng and is more than sufficient for the normal PCR requirement of 40ng.

Table9 PCR thermal cycle condition

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp</th>
<th>Duration</th>
<th>Cycle no.</th>
</tr>
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<tbody>
<tr>
<td>Initial Denaturation</td>
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<tr>
<td>Denaturation</td>
<td>98.0 °C</td>
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<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>61.5 °C</td>
<td>20 s</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72.0 °C</td>
<td>7 s</td>
<td>28 cycles</td>
</tr>
<tr>
<td>Final Extension</td>
<td>72.0 °C</td>
<td>8 min</td>
<td>1</td>
</tr>
</tbody>
</table>

The gel electrophoresis graph shows elution 0 to 4 have positive amplification and all are containing the targeted DNA. (Fig.10)

Fig.10 All the elutions (0 to 4) contain the target DNA.

Table10 Amount of DNA eluted during extraction

<table>
<thead>
<tr>
<th>DNA extraction Sample ID</th>
<th>Volume of elution (ul)</th>
<th>Average amount of DNA (ng)</th>
<th>Total amount of DNA eluted (ng)</th>
<th>Amount of DNA eluted per ul of blood (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution 0</td>
<td>30</td>
<td>52.22</td>
<td>351.88</td>
<td>3.3513</td>
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<tr>
<td>Elution 1</td>
<td>27</td>
<td>186.01</td>
<td></td>
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<td>Elution 2</td>
<td>24</td>
<td>165.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elution 3</td>
<td>24</td>
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<td>Elution 4</td>
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<td></td>
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<td>10 ng std</td>
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<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100ng std</td>
<td>-</td>
<td>122</td>
<td>-</td>
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</table>

Conclusions:
The micro fluidic cartridge has been further optimized to improve the performance of the extraction. Injection molded needle plugs and selection of suitable membrane material have improved the actuation performance during the extraction process. The reservoir material has been changed from PDMS to polypropylene based on the permeability study so to contain the reagents longer inside the cartridge. Some important results are summarized as following.

1. A membrane material has been selected based on the actuation force and it is found that silicon rubber has the lowest actuation force of 13 N compared to PDMS material with different mixing ratios.
2. Water permeability of Poly propylene plastic (0.02 g/m2/day) is better than rubber material (0.63 g/m2/day) for the selection of reservoir material.
3. Poly propylene (PP) bonding with PMMA could withstand the average pressure of 513kPa without leakage, which is much higher than the bio-package requirement (100 KPa).
4. Total volume of the DNA extracted from the cartridge is about 351 ng from 5 elutions and is better than the minimum sufficient amount of DNA required for PCR amplifications (40ng).

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