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A novel approach on fluid dispensing for a DNA/RNA extraction chip package

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ABSTRACT

Micro fluidic package with integrated reservoirs has been developed for DNA /RNA extraction application. A membrane based pump which consists of a reservoir to store reagents and a pin valve to control the fluid is developed to dispense the reagents into the chip. A programmable external actuator is fabricated to dispense the fluid from the membrane pump into the DNA chip. An elastic and high elongation thin rubber membrane is used to seal the membrane pump and at the same time prevent actuator from mixing with different reagents in the micro fluidic package. Break displacement during actuation of membrane pump sealing material is studied with different ratios of PDMS and other types of rubber materials. The fluid flow from the reservoir to the chip is controlled by a pin valve which is activated during the external actuation. A CFD simulation is performed to study the pumping action during the external actuation and is validated with experimental results.

Keywords: microfluidics, DNA, RNA, sample preparation, fluidic simulation, CFD, actuation, reservoir

1. INTRODUCTION

In the medical field, there is increasing need for new health technologies for diagnosing and treating communicable and non-communicable diseases. Identification of DNA/RNA has proven useful to many applications such as forensic, diagnostics and drug discovery. Conventional methods are available to separate DNA/RNA from the human sample. Conventional method uses non-portable equipments which are expensive and test one sample at a time. However, many applications request DNA /RNA analysis at the point of care or even in field. In view of this a fully integrated, outdoor safe, low cost, easy operating and portable DNA/RNA identification is required to meet the ever increasing future demand of DNA/RNA use. Miniaturization can reduce the sample size and adopting the analogy of using microchips in electronics can make the DNA/RNA extraction faster and cheaper [1]. Silicon chips micro machined to make a filter, mixer and binder to do the same functions do in a lab, lab on a chip. In the lab fluids/reagents are stored in the bottles and test tubes for the analysis. When we shrink the lab in to a chip, a package encapsulating the chip must self contain reagents and providing control mechanism to dispense reagents and ensure their dispensing volume, flow rate and timing to meet analyses protocol requirement. [2].

Development of a bio-microfluidic package for DNA lab on a chip has been described. DNA extraction consists of a filter, binder and a PCR (Polymer chain reaction) part. (Fig.1) The DNA extraction chip is fabricated in silicon and filter, binder and PCR chambers are formed by surface micromachining method. The DNA extraction chip wafer is bonded to a glass wafer by anodic bonding method and is done at the wafer level using a wafer bonder. The glass wafer is used so that optical method can be used for detection. The filter is used to separate the DNA from the blood and a binder is used to bind the DNA onto the chip. The bound DNA particles are removed from the chip by a process called elution. The collected elution sample containing DNA particles is mixed with the primer and injected into the PCR chamber of the chip for amplification.

Packaging of the DNA extraction chip is done using a polymer material, PDMS (Polydimethylsioxane) [2]. A schematic view of the package layout is shown (Fig.2).

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PDMS is a biocompatible, transparent material and has good resistance to chemicals. Rectangular channels with dimension 500um are made on the bottom PDMS substrate and are connected to the micro channels of an extraction chip. In the DNA extraction chip, DNA extraction process requires blood and other reagents, such as high salt, ethanol, water, air etc. Except blood other fluids can be stored in the package and can be injected into the package during the DNA extraction process. Reservoirs are fabricated on the PDMS package and the fluids are stored in the reservoir [3]. The novelty in this package is the integration of reservoir in the package for storing reagents for DNA extraction chip and eliminating the contamination issue from tubes and bottles used in the normal clinical laboratory.

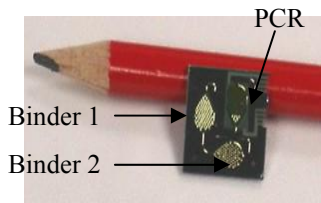


Fig.1 DNA Extraction chip

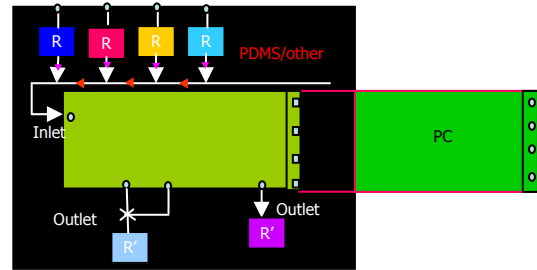


Fig.2 Schematic view of microfluidic package with reservoirs and valves (R: reservoir)

In this paper a membrane based pump integrated with a pin valve is developed to dispense the fluid in a controlled manner. The developed membrane pump is integrated into the DNA/RNA extraction package. The membrane pump has got a storage space, called as reservoir and is filled with the fluid. A thin membrane of highly elastic, biocompatible material is bonded to seal reservoir space. An external actuator pushes the membrane down and the resulting displacement of the membrane pushes the fluid into the channel through a pin valve. The fluid displaced inside the reservoir is proportional to the external actuation. The displacement of the membrane is controlled by an external actuation and is proportional to the fluid flow.

2. METHODOLOGY

2.1 Membrane pump

The membrane pump has got a reservoir which is filled with reagents. The reservoir has a conical shape cavity covered with a thin layer of flexible film as shown in Fig.3(a). The actuator diameter is same as the bottom diameter of reservoir. The volume of dispensed liquid is equal to the volume of film deformation as shown in the dash line area in Fig.3(b).

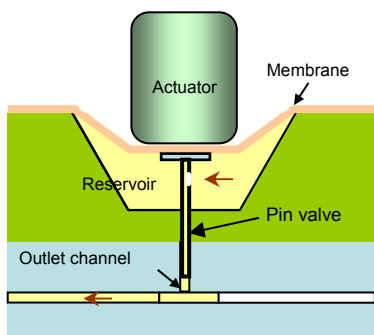


Fig.3 (a) Cross section view of membrane pump

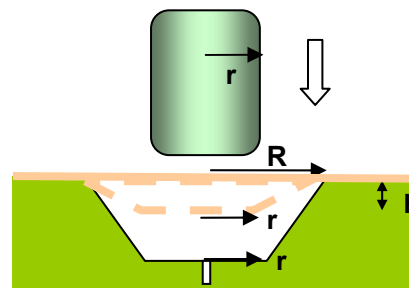


Fig.3 (b).External actuator acting on reservoir

Consider the volume of the liquid inside the reservoir is V , top radius of the reservoir is R , height of the reservoir is 'h' and radius of the actuator is 'r'. Then the relation between flow rate and actuator speed is as follows.

$$\text{Volume (V)} = (R^2 + rR + r^2) \times h \times \pi / 3$$

$$dV/dt = [(R^2 + rR + r^2) \times (\pi / 3)] \times dh/dt$$

Since $dV/dt = Q$, flow rate; And $dh/dt = S$, actuator speed;

Flow rate (Q) = Constant x actuator speed (s)

Therefore the liquid flow rate is proportional to the actuator speed. The advantage of conical shape reservoir is to have a minimum dead volume and easy membrane deformation during the actuation.

2.2 Membrane characterization setup

The membrane with good elongation is desirable for smooth liquid dispensing. Good elongation membrane also helps to minimize push force required from actuator, so that reduced actuator size. Six bio-compatible materials are selected in this study. They are made to be 0.5mm thick membrane. The samples are cut as dumbbell shape as shown in Fig. 4. Samples are fixed on the Instron tensile test machine as clamping to ends on fixtures. The bottom load cell is non-movable. A computer controls top fixture to move upward and pull long the specimen. During the pulling action, the displacement of top fixture and load are transiently recorded. The test setup is as shown in Fig.5.

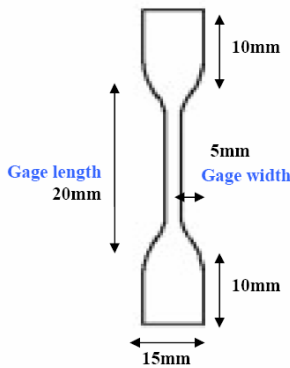


Fig.4 Dumbbell shaped membrane specimen for elongation test

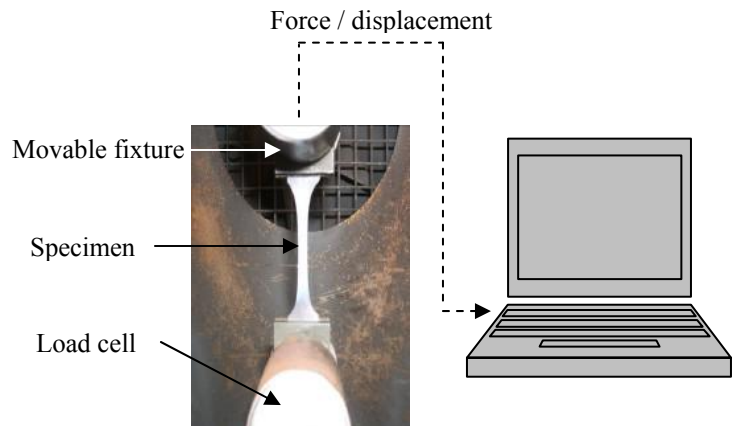


Fig.5 elongation test set up

To calibrate the membrane pump dispensing performance, a cartridge using selected membrane was conducted dispensing test. A computer controlled actuator push the membrane and dispense the liquid from reservoir. The flowrate of liquid flow was transient recorded.

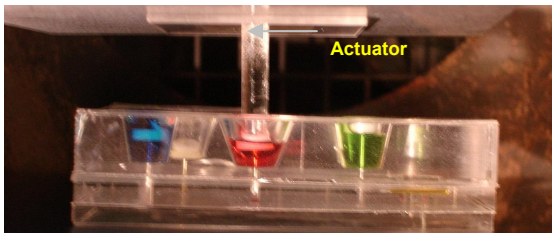


Fig.6 Calibration set up

2.3 CFD simulation:

The pumping mechanism of the membrane pump is studied using CFD (Computational Fluid Dynamics) simulation. A transient CFD simulation is resulted due to the interaction between the membrane and the fluid. In the simulation of the membrane pump the maximum displacement and the principal strain is in the center of the reservoir and is shown in Fig.7(a) & (b). The velocity distribution of the fluid flow in the membrane pump at the junction of the reservoir and outlet is seen denser at the outlet location of reservoir and in the case of the channel the distribution become lighter

when moving away from the outlet location of the reservoir (Fig.8(a) & (b)). In this analysis it is understood that the velocity distribution is not uniform along the fluid flow from the membrane pump to the micro channel.

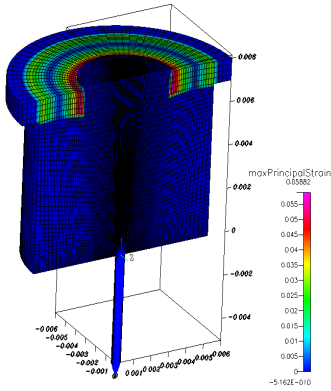


Fig.7(a) Membrane deformation

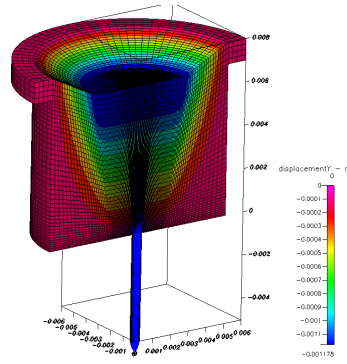


Fig.7(b) The stress distribution

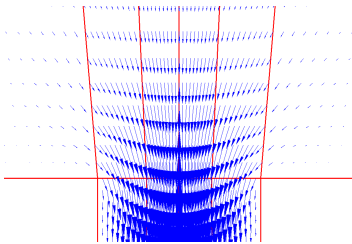


Fig.8(a) Velocity distribution at the bottom of reservoir

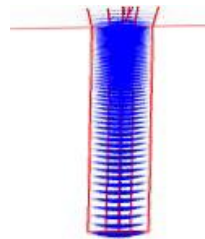


Fig.8(b) Velocity distribution in the outlet channel

Ideal volume flow rate,

$$H=U \cdot t$$

$$V = \frac{1}{3} \pi (R_1^2 + R_2^2 + R_1 R_2) U t$$

(1)

$$q = \frac{\partial V}{\partial t} = \frac{1}{3} \pi (R_1^2 + R_2^2 + R_1 R_2) U = a \text{ constant}$$

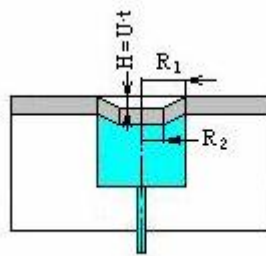


Fig.9 Displacement

A theoretical formula for volume flow rate is given in expression (1). There is a large deviation between theoretical average flow rates and measured. The relative error is as large as 24.2 %. There are two factors contributing to the

errors. One of them is that the membrane volume change is not considered. Another is that the shape of deformed membrane is different from that shown in Fig.9.

Membrane volume change & deformed membrane shape are affected by membrane thickness, R1/R2, Back pressure P_b .

Based on the above deviation, the following empirical formula is proposed here.

$$q = \lambda \frac{1}{3} \pi (R_1^2 + R_2^2 + R_1 R_2) U$$

Additional factors such as membrane thickness, back pressure P_b are considered in the new theoretical formula

$q = \lambda \frac{1}{3} \pi (R_1^2 + R_2^2 + R_1 R_2) U$ where λ is the modifying factor considering the membrane volume change and deformation. Generally, the modifying factor is less than unit $\lambda < 1$.

$$\lambda = f(R_1/R_2, \delta, \Delta P_b) \tag{2}$$

Where R_1/R_2 is the radii ratio, δ is the membrane thickness and the pressure difference between the back pressure and environmental atmospheric pressure $\Delta P_b = P_b - P_{atm}$.

3. RESULTS

3.1 Membrane selection

Different materials are tested for membrane to seal the reservoir. Selection of the membrane material is based on the longest elongation of the membrane with smaller force Table 1&2. Different mixing ratios are tried for PDMS based membrane and different rubber materials are tried with different grades in the final selection process.

Table 1 Experimental data on break displacement of biocompatible elastomer membrane

Material	10:1 PDMS	15:1 PDMS	20:1 PDMS	Rubber 60A H6003	Rubber 90A IE829	Rubber 60A IE830
Break displacement (mm)	29.7	31.9	37.6	79.9	22.9	126.8
	26.4	35.2	40.1	82.6	25.4	126.8
	23.5	36.5	38.8	75.8	24.2	126.8
	31.6	38.0	35.3	83.9	26.7	126.8
	25.7	36.1	37.9	74.9	31.4	126.8
	28.1	37.5	39.4	75.9	29.8	126.8
	29.4	32.0	38.9	80.5	22.1	126.8
	30.4	35.1	36.9	77.2	27.6	126.8
	25.9	33.7	38.5	76.1	26.1	126.8
	27.4	38.9	39.7	79.3	30.5	126.8
	28.2	31.4	40.6	78.6	29.8	126.8
	31.4	37.9	35.6	80.4	27.3	126.8
	30.6	33.5	38.6	83.4	30.1	126.8
	26.9	32.2	42.9	73.9	27.2	126.8
	28.2	37.5	37.6	79.6	28.4	126.8
	Average (mm)	28.23	35.16	38.56	78.8	27.3

Table 2. Experimental data on break force of bio-compatible material

Material	10:1 PDMS	15:1 PDMS	20:1 PDMS	Rubber 60A H6003	Rubber 90A IE829	Rubber 60A IE830
Break force (N)	1.95	1.87	2.09	63.8	45.3	63.7
	1.62	2.13	2.47	65.9	46.6	58.9
	1.53	2.26	2.28	60.3	45.8	60.1
	2.11	2.62	1.95	66.4	49.3	61.7
	1.57	2.21	2.14	59.7	56.3	57.7
	1.82	2.35	2.34	60.5	53.0	64.9
	1.94	1.83	2.29	65.8	43.7	65.6
	1.98	2.09	2.06	61.9	51.5	59.4
	1.58	2.09	2.17	61.5	47.7	62.6
	1.73	2.71	2.35	63.0	55.6	57.2
	1.84	1.79	2.48	62.9	53.2	61.4
	2.01	2.56	1.99	64.5	50.4	66.2
	2.01	1.97	2.18	66.2	55.1	59.8
	1.69	1.83	2.53	59.1	50.1	63.5
	1.84	2.54	2.14	63.6	52.4	62.8
	Average (N)	1.81	2.19	2.23	63.0	50.4

From the above analysis it is concluded that the higher elongation material yield higher break force and is not desirable for the current fluidic package application. So the rubber materials are found to be not suitable due to higher breaking force even though they have large break displacement. Final selection is made on PDMS membrane with 15:1 ratio which has better break displacement and comparable break force with standard PDMS ratio, 10:1.

3.2 Experimental validation of Membrane pump simulation

The membrane pump is fabricated and tested to validate the simulation effect (Fig.10). Fluidic is pushed by an actuator piston connected to a pneumatic pump. The volume of liquid collected from the membrane pump during the regular intervals of actuation during testing, by simulation and theoretical methods are shown in Table 3. Average volume of liquid with 3 calculation is tabulated and found that the relative error with simulation to experimental is only with in

2% while with theory is about 24% (Table 4). Volume flow rate with time is plotted with respect to time and is found to be correlating with the simulation results. The initial waving part seen in the simulation is due to bouncing effect of the membrane pump due to the elastic nature of the membrane used in the membrane pump (Fig.11).

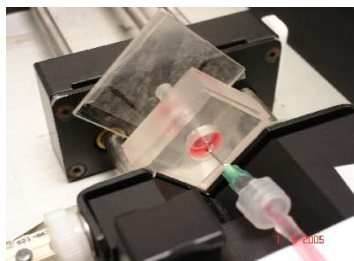


Fig.10 Membrane pump testing

Table 3. Time history of volume flow rate q ($\mu\text{l}/\text{min}$)

t (min)	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
Testing		9.3	9.7	9.7	8.9	10.1	9.7	10.1	9.3	9.7	10.1	8.9
CFD	11.32	9.56	9.20	9.23	8.89	8.65	*					
Theory	12											

Table 4. Average volume flow rate ($\mu\text{l}/\text{min}$)

	Theory (ideal)	Testing (in 6 minutes)	CFD (in 3 minutes)
q	12	9.66	9.46
Relative error	24.2%		2%

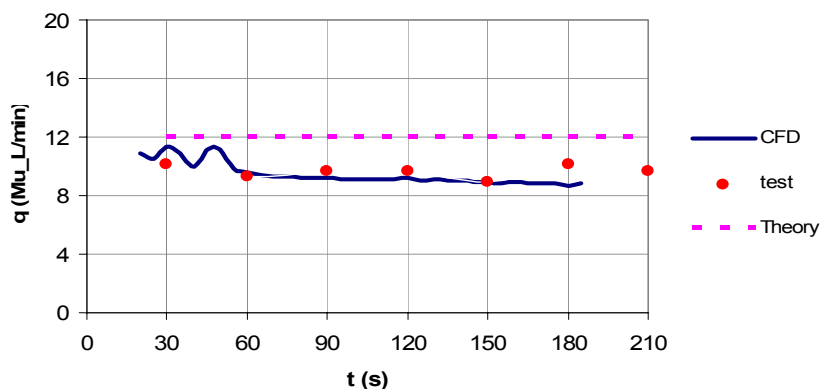


Fig.11 Volume flow rate plot for all three methods ($\mu\text{l}/\text{min}$)

4. CONCLUSION

A microfluidic package is developed for DNA/RNA extraction. A membrane pump which is used for dispensing the fluid to the DNA/RNA chip is developed. Simulation study of the membrane pump has showed the maximum displacement and principal strain at the centre of the micropump. In the case of the velocity distribution the maximum density is found at the reservoir /outlet junction and in the case of the micro channel the velocity density found to be decreasing trend towards the chip direction. Experimental correlation study has done with simulation and theoretical calculation and found that simulation result correlate well with experimental result with a relative error of 2%.

Membrane selection for pump is done based on the breaking force and break distance and found that the PDMS with ratio of 15:1 has met the requirements for this application. Higher breaking distance material shown higher force and is not suitable for membrane pump application.

Calibration of the micro pump after integrating into the microfluidic cartridge is performed. A linear relation of fluid flow with respect to external actuation is achieved for fluid flow from the membrane pump to the chip.

5. ACOWLEDGEMENTS

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