BUBBLE FREE ELECTRODES FOR ELECTROOSMOTIC PUMPING

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BY

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ABSTRACT

Electroosmotic (EO) pumping is a widely used microfluidic pumping technique due to its advantages such as simple design, applicability to wide range of conductive fluids and favorable scaling of electrical forces. Electrodes are necessary to establish the electrical forces and hence fluid flow inside the microchannel. One of the major problems associated with inert electrodes such as gold or platinum used in EO pumping is gas evolution, whereas redox electrodes have a shorter life time. In this thesis, EO pumping is demonstrated using two different bubble free electrodes with longer life time.

The first of the two types demonstrated is thick silver/ silver chloride electrodes embedded in poly di-methylsiloxane (PDMS) micro channels. However, embedding thick electrodes inside PDMS channels using traditional micro fabrication will lead to a gap near the electrodes which can result in leakage of the working fluid. A new surface micromachining technique in PDMS has been developed to address the leakage problem.

Surface micromachining with PDMS as a structural material and photoresist as a sacrificial material allows the fabrication of PDMS microchannels on substrates with significant topography. Adhesion of the structural layer with the substrate is characterized for different prepolymer ratios using standard tensile test. 1:3 (curing agent: base) combination was found to have the highest bonding strength with an adhesion strength of 7.2 MPa. The effectiveness of this technique is demonstrated by the fabrication of microchannels with embedded 6μ m thick silver/ silver chloride electrodes. The microchannels are leak proof and conformal contact between the PDMS and electrode is confirmed through SEM. The release time for microchannels was reduced to 1 min irrespective of the length of the microchannel. The flexibility and versatility of this technique for fabrication of multi layered microchannel structure is demonstrated through a microfluidic valve. The valve closure occurred at 6.37 kPa.

The Voltage-current density of the Ag/AgCl electrodes was characterized. The limiting current density of the electrode is 2.7 mA/cm². The capacity of the electrodes is 1680 μ A-sec. EO pumping has been demonstrated with an applied voltage of 1-2 V and a maximum velocity of 44 μ m/sec is obtained.

The second bubble free electrode demonstrated is an inert liquid electrode which has an extended life unlike the metal electrodes. The device consists of two upper PDMS microchannels and a lower microchannel with a 6μ m thick nanoporous polycarbonate sheet placed in between them. An electro osmotic pump has been demonstrated using this liquid electrode configuration with a maximum velocity of 118 μ m/sec at 60 V.

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When I began my studies at McMaster University, the idea of completing a thesis in a relatively unknown field seemed overwhelming. As the years have passed, it has come together slowly, step by step, and in the end, finally come into focus. All of this would not have been possible without the help and support of countless people.

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CHAPTER 1

MOTIVATION AND ORGANIZATION

1.1 MOTIVATION

Pumps are used in microfluidics to precisely control the flow of liquid from one part of the system to another part of the system. Microfluidic pumping [Laser 2004] can be done using various techniques such as piezoelectric, thermopneumatic, electrostatic and electroosmotic (EO) pumping. However, electroosmotic pumping is the most favored pumping technique due to its advantages such as simple design, applicability to a wide range of conductive fluids and a favorable scaling of electrical forces. Electrodes are required to manipulate the electrical field forces in order to control the fluid flow inside the microchannel. One of the major problems [Guzman 2006] associated with inert electrodes such as gold and platinum used in EO pumping is electrolytic gas evolution, whereas redox electrodes have a shorter life time. In this thesis, we address the problem of gas evolution while extending typical lifetimes of these electrodes.

1.2 OBJECTIVE

The objective of this research thesis is to develop gas free electrodes with longer lifetimes in order to demonstrate bubble free EO pumping. This is accomplished by developing two types of electrodes. One is a 6 μ m thick Ag/AgCl redox electrode with higher capacities and the second is a novel liquid

electrode with an enhanced lifetime in which the liquid diffusing through a 6 μ m thick nanoporous membrane acts as an electrode in driving the EO pump.

1.3 ORGANIZATION

The thesis is organized as follows:

Chapter-2 begins with the introduction of microfluidics and is followed by a discussion on the scaling of forces in microfluidic regimes for different actuation mechanisms. The advantages, disadvantages of different types of microfluidic pumping are reviewed as well. The advantages of electroosmotic (EO) pumps over other pumping methods are discussed briefly and the mechanism of the electroosmotic pump is discussed in detail.

In Chapter-3, major problems associated with inert metal electrodes such as gas evolution and poor localized flow control are discussed while previous work done by researchers to overcome the limitations are reviewed.

Chapter-4 discusses the Ag/AgCl redox electrodes along with their associated advantages and disadvantages. After a brief introduction of the problems of embedding thick electrodes inside microchannels, soft lithography and the properties of PDMS, this chapter highlights a newly developed surface micromachining technique for overcoming the problems of embedding thick electrodes inside a microchannel. The characterization of the bond strength with various compositions of PDMS prepolymer is also done to achieve maximum bonding strength of the final device.

In Chapter-5, characterization of the developed surface micromachining technique is performed through fabrication of channels embedded with thick

electrodes and construction of multilayered microchannels. Leakage, SEM characterization of the channels and pressure characterization of the valve membrane are demonstrated.

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Fabrication and characterization of the Ag/AgCl electrode is discussed in chapter-6. Voltage-current density characterization of the silver/silver chloride electrode is carried out in order to understand the behavior of the electrodes. Lifetime characterizations of the electrodes are done at various current levels while capacity utilization of the electrodes is calculated. EO-flow is demonstrated using these electrodes.

A new liquid electrode for gas free electroosmotic pumping is reported in chapter-7. The V-I characterization of the electrodes was performed in order to find out the behavior of the system. Diffusion characterization using fluorescent dye is carried out for visualization. Electroosmotic flow is demonstrated using these electrodes. Velocity - voltage characteristics of the flow are also studied in this chapter.

Chapter-8 highlights the three major contributions in this thesis: the embedding of thick electrodes using surface micromachining technique in PDMS, methods of extending Ag/AgCl electrode capacity and liquid electrodes. The future improvements required to overcome the current problems are also discussed.

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CHAPTER 2

MICROFLUIDIC PUMPING

2.1 INTRODUCTION TO MICROFLUIDICS

Microfluidics deals with the manipulation, engineering and behavior of liquids inside microchannels at microliter, nanoliter or picoliter volumes. The past 15 years has seen a dramatic interest in the development of microfluidic Lab-on-Chip devices for automation of laboratory procedures for genomic and proteomic applications. Bulk manufacturing of the devices gives the advantages of reduced manufacturing time and lower cost. Miniaturization of the devices allows for automation which has the benefits of reduced analytical time, reduced analytical errors and increased reproducibility while parallel processing of samples offers high throughput. The smaller dimension of the microchannel gives the advantage of reduced reagent handling cost [Beebe 2002].

Microfluidic devices are being used as portable diagnostic devices for the detection of a wide range of diseases such as diabetes [Pugia 2005], HIV [Sia 2004], cancer [Pilarski 2004] and cardiovascular disease [Wolf 2004]. In biomedical applications, they are being used for drug design [Pang 2004] and drug delivery [Bourouinay 1997]. Among the numerous biological and biochemical processes demonstrated are polymerase chain reaction [Liu 2002], immunoassays [Bange 2005], electrophoretic separations [Lagally 2000] and DNA sequencing [Paegel 2003]. Other applications of microfluidic devices include microelectronic cooling and ink jet printing [Delamarche 2005, Reyes 2002].

2.2 SCALING OF FORCES IN MICROFLUIDICS

Scaling laws helps us to understand the scaling of different forces and the forces which dominate in the microdomain. When the length dimension decreases, the influence of different forces such as gravity, surface tension and pressure inside the microfluidic domain varies. According to the scaling laws [Trimmer 1996, Wautelet 2001], surface tension and Van der Waals forces scale linearly with length, however, these forces are difficult to manipulate. Electrical forces and pressure forces scale to the square of the length dimension. Gravitational and magnetic forces dominate at the macro level but are not so significant at the micro level. This makes electrical and pressure forces to be more useful and dominant over gravitational and magnetic forces in the microfluidics regime.

2.3 MICROFLUIDIC PUMPING

Pumps [Gravesen 1993] are an integral part of microfluidic systems. Pumps are required for the local controlled movement of fluid inside microchannels. Pressure force and electromagnetic field force are mainly used for pumping the liquids as these forces scale favorably in the microfluidic regime. Different types of pumping have been demonstrated using these forces. The key pumping methods have been summarized [Laser 2004] in Table 2.1.

Туре	Working Parameters	Force	Working fluid	Pressure (kPa)	Flow	
					(ml	Control
					min ⁻¹)	
Syringe Pump [Laser 2004]	Piston movement - 9.5 nm/min	Pressure force	Conductive and Dielectric	1.4	8333	No
Electrostatic pump [Laser 2004]	Voltage- 75V to 200V Frequency- 100 to 400 Hz	Pressure force	Conductive and Dielectric	29	0.16- 0.26	Reasonable (High frequency)
Piezo electric pump [Laser 2004]	Voltage – 100V to 200V Frequency- 200 to 3000 Hz	Pressure force	Conductive and Dielectric	0.78- 304	0.0006- 16	Reasonable (High frequency)
Thermo Pneumatic [Laser 2004]	Voltage-5V to 10V Frequency- 6 to 20 Hz	Pressure force	Conductive and Dielectric	5.1 - 16	0.53- 0.0063	No (Low frequency)
Magneto hydrodynamic pump [Laser 2004]	Current- 1 to 100μA Magnetic flux - density- 0.44 T	Lorentz force	Conductive	0.001	0.018- 0.063	Good
Electro hydrodynamic pump [Laser 2004]	Voltage- 100V to 600V	Electrical force	Dielectric	0.25- 0.43	2 x10 ⁻⁵ -14	Good
Electro osmotic pumping [Laser 2004]	Voltage- 40V to 6.7 kV	Electrical force	Conductive	1	5.4 x 10 ⁻⁶ - 20,000	Good

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 Table 2.1: Comparison of the various types of pumping in microfluidic devices

Syringe, piezoelectric, thermopnuematic and electrostatic pumps are mechanical pumps. In these pumps, mechanical actions such as piston movement or diaphragm movement are converted to pressure. A syringe pump (Appendix2: Cole Parmer data sheet) uses piston movement for applying external applied pressure to pump the fluids. Even though the syringe pump is versatile for pumping a wide range of fluids, the pressure generated is applied to all regions of the microfluidic network and therefore there will be no localized control of fluid while pumping.



Figure 2.1: Schematic representation of piezoelectric pump.

A piezoelectric film [Koch 1997, Laser 2004] is one that develops a strain under application of an electrical field. This phenomenon is used in the piezoelectric pump for actuating the fluid inside the microchannel. These pumps generally consist of an electrode material and a thin piezoeelctric diaphragm (thickness range 0.3 mm to 0.018 mm) necessary for the actuation (Fig. 2.1). The applied voltage (50 V-1200 V) using electrodes across the piezoelectric disk will cause the thin membrane to deform and introduce a pressure gradient across the bottom microchannel. This pressure force is used for pumping the fluid. Fast mechanical response with frequencies of up to 3400 Hz and high actuation forces can be achieved with these pumps. However, complex designs and reduced reliability due to moving parts comprise the major limitations of these pumps.



Figure 2.2: Schematic representation of an electrostatic pump [Woias 2005].

An electrostatic pump [Bourouina 1997, Laser 2004] consists of a diaphragm, electrodes, valves and the pumping chamber. Figure 2.2 schematically represents an electrostatic pump. When a high voltage is applied to the capacitor electrodes (counter electrode), the pump diaphragm electrostatically gets attracted towards the electrode and at the upper threshold gets fully attached to the counter electrode. When the capacitor electrodes are discharged, the pump diaphragm relaxes to its rest position. Depending upon the charging and discharging rate of the capacitor electrodes, this pump can be operated with different frequencies (300–400 Hz). The main limitation is the long term performance of the device. Due to the build-up of surface charges at the insulator inside the capacitor, the internal electrical field strength is reduced and the long-term performance of the device is compromised. Moreover, design and fabrication of the diaphragm, electrodes and valves can be complex. The presence of moving parts limits the reliability of the pump.



Figure 2.3: Components of thermopneumatic pump.

The thermopneumatic pump [Grosjean 1999, Laser 2004] employs a membrane and a heating element (Fig. 2.3) wherein the heat generated by the heating element expands a secondary fluid actuating the membrane to pump the working fluid inside the microchannel. The rate of heat transfer into and out of the secondary fluid determines the response rate of the thermopnuematic pumps. Hence the pumping occurs in relatively lower frequencies (typically 0.5 Hz to 10 Hz) and hence lesser flow rates (maximum 530 µl/min). Due to the large surface area to volume ratio in microfluidic devices, the rate of heat loss is more and it is difficult to achieve a desired temperature and hence very little control over the flow rate of fluid. It is also desirable to have a thermally efficient heater to minimize the amount of power necessary to heat the liquid. The presence of moving parts reduces the reliability of the pump. Electroosmotic, electrohydrodynamic and magnetohydrodynamic pumps are non- mechanical pumps which use electromagnetic forces for pumping liquids. The electromagnetic pumps are simple to design and fabricate because of the absence of moving parts.

Magnetohydrodymanic pumps [Jang 2000, Laser 2004] use a Lorentz field force for pumping the fluid. Charged particles experience a resultant force (Lorentz force) when placed in a perpendicular electrical and magnetic field. This resultant force acting on the ions causes the displacement of the ions in a mutually perpendicular direction to that of both fields. These moving ions will drag the liquid with them and hence the pumping occurs. Magnetohydrodynamic pumps can pump only highly conductive liquids. The limited magnetic flux density achieved with the miniaturized permanent magnets (max 1 T) and electromagnets (0.1 T) restricts the maximum achievable flow rate to 100 μ l/min.

Electrohydrodynamic (EHD) [Bart 1990, Laser 2004] pumps work on the principle of charge injection and charge collection. The EHD pumps have a simple design with one emitter electrode and one collector electrode. The pump usually operates with a high applied voltage of 100-600 V. When a large electrical field is applied across the electrodes, the emitter electrode will inject charge into the working dielectric fluid. Due to the columbic force of attraction, the emitted charge travels through the dielectric fluid and gets collected at the collector electrode. The moving charge drags the liquid due to viscous drag and hence pumping occurs. A maximum flow rate of 14 ml/min can be achieved using this pump. Pressures of up to 0.43 kPa can be achieved using this pump. The main limitation of this type of pumping is that only dielectric fluids can be actuated and hence excludes most biological fluids.

2.3.1 ELECTROOSMOTIC PUMP

Electroosmotic (EO) pumps are widely used in the field of microfluidics. Advantages of these pumps include minimum dispersion, simple fabrication methods, high operating pressures, no moving parts and accommodation of a wide range of conductive fluids [Chen 2002] including organic solvents such as acetonitrile ($<10^{-6}$ S/m), deionized water (10^{-3} S/m) and highly conductive aqueous solutions (>1 S/m). Electroosmotic [Rice 1965, Harrison 1993, Chen 2002] pumping occurs when an electrical field is applied in a channel with a charged wall.

2.3.2 EOF MECHANISM



Figure 2.4: Formation of a fixed Stern layer and a partially mobile diffuse layer.

When an aqueous solution comes into contact with a silica-based material such as PDMS or glass, the walls acquire negative charge as a result of the deprotonation of surface silanol groups to form SiO⁻ ions. The negatively charged surface attracts the positive ions present in the solution and repels the co-ions from the solution. The net excess positive ions in the region of the surface form an electrical double layer (EDL) as shown in Fig. 2.4. The electrical double layer has two parts, one is the inner layer called the Stern layer and the outer layer is called

a diffuse layer. The thickness of the double layer is given by the Debye length (λ_D) .



Figure 2.5: Schematic of potential difference across the electrical double layer. Zeta potential is the potential across the shear plane. The Stern plane is typically the width of an atom and will be equal to 0.1 nm.

In the Stern layer, the ions are strongly attracted to the wall surface by strong electrostatic forces ($E\sim10^7$ to 10^8 V/m) and are immobile. The thickness of the Stern layer is approximately the radius of a hydrated ion (Angstroms). In the diffuse layer, both ordered electrical forces and disordered thermal forces act on the ions. Hence they are not completely mobile. When an electrical field is applied, the charges at the diffuse layer move and causing the plane between the stern layer and the diffuse layer to move at the interface termed the shear plane. Zeta potential (ζ) is the potential at the shear plane. The zeta potential varies with

the substrate and typically lies between 1 to 200 mV [Kirby 2004]. The variation in potential difference occurring across the double layer is shown in Fig. 2.5.



Figure 2.6: Schematic diagram of electroosmotic flow inside microchannels.

The surface is negatively charged leading to the introduction and accumulation of an excess of positively charged ions. When an external electrical field is applied, the positive charges move towards the negative electrode dragging the liquid with them due to viscous drag and hence electroosmotic pumping occurs. The center line represents the axis of symmetry with respect to the y-axis and the flow profile in the lower half of the channel (below the axis of symmetry) represents the same profile in the upper half of the channel (above the axis of symmetry).

When an electrical field is applied through the external electrodes, the ions in the diffuse layer will move towards the oppositely charged electrode due to the columbic force. When the ions move towards the opposite electrode (electro migration), they drag the liquid with them due to the viscous interactions and hence the electroosmotic pumping of the fluid occurs. Figure 2.6 shows the schematic of electroosmotic pumping inside the microchannel. Since the Stern layer is immobile due to the high electrostatic attraction, the charges will not move and hence is taken as the equivalent of a microchannel wall. Thus, the Stern layer can be neglected and zeta potential (ζ) is assumed equal to the wall potential. Thus electroosmotic flow can be understood in terms of the ζ potential because the phenomenon is only directly related to the mobile part of the electrical double layer.

Assuming fluid to be a continuum, the governing equations are the continuity and Navier -Stokes equations.

The continuity equation [Panton 1995] for a 2-D incompressible fluid is given by:

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} = 0 \tag{2.1}$$

where u and v are the velocity components in the x and y directions.

When the depth of the microchannel is much smaller than the length and width and when the flow is creeping flow, the Navier-Stokes equation can be approximated to 1-D fully developed laminar flow [Panton 1995, Probstein 1994].

$$\frac{dP}{dx} = \mu \frac{\partial^2 u}{\partial y^2} + F \tag{2.2}$$

Where dP/dx is the assumed uniform pressure gradient along the flow direction, μ is the viscosity of the fluid and F is the body force acting on the fluid. Due to the electrical charges and the associated electrical field, the body force is replaced by electrical body force in the equation 2.2 by: [Chen 2002]

$$\frac{dP}{dx} = \mu \frac{\partial^2 u}{\partial y^2} + \rho_e E_x \tag{2.3}$$

 ρ_e is the charge density of the polar medium.

The electrical field is E_x related to the potential gradient $\nabla \phi$ [Chen 2002] by:

$$E_x = -\nabla\phi \tag{2.4}$$

(a))

The potential gradient is related to the charge density by the Poisson equation

[Chen 2002]:

$$\nabla^2 \phi = -\frac{\rho_e}{\varepsilon} \tag{2.5}$$

where ε is the permittivity of the electrolyte.

The potential gradient can be written in terms of concentration of the solution as [Chen 2002]:

$$\frac{d^2\phi}{dx^2} = -\frac{1}{\varepsilon} \sum c_i z_i e \tag{2.6}$$

where c_i is the concentration of the i^{th} species, z_i is the valency of the i^{th} species, e is the electronic charge, .

Concentration of the species inside the double layer can be written in terms of the potential distribution using the Boltzmann equation [Chen 2002]:

$$c_i = c_i^0 \exp(\frac{-z_i e}{kT}\phi)$$
(2.7)

where k is the Boltzmann constant, T is the temperature of the solution, c_i^0 is the concentration of the solution in bulk of the electrolyte.

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By the Debye-Huckel approximation, $\frac{z_i\phi}{kT} \ll 1$ and hence $\exp(\frac{-z_ie}{kT}\phi) \approx 1$ -

 $\frac{z_i e}{kT}\phi$ and substituting this term in c_i :

$$c_i = c_i^0 \left[1 - \left(\frac{z_i e}{kT} \phi\right) \right]$$
(2.8)

Rewriting 2.8 in terms of equation 2.7:

$$\frac{d^2\phi}{dx^2} = -\frac{1}{\varepsilon} \sum c_i^0 (1 - \frac{z_i e\phi}{kT}) z_i e$$
$$= -\frac{1}{\varepsilon} \sum z_i e c_i^0 - \sum \frac{c_i^0 z_i^2 e^2 \phi}{kT}$$
(2.9)

The term $\sum z_i ec_i^0$ in the above equation is zero as the liquid in the bulk is electrically neutral. Hence equation 2.9 becomes:

$$\frac{d^2\phi}{dx^2} = \kappa^2\phi$$

where $\frac{1}{\kappa}$ is the characteristic length scale called the Debye layer, λ_D .

$$\lambda_D = \frac{1}{\kappa} = \sqrt{\frac{\varepsilon KT}{e^2 \sum z_i^2 c_i^o}}$$

Thus $\frac{d^2 \phi}{dx^2}$ is dependent only on ϕ

The modified Navier-Stokes equation [Chen 2002] for the electrical field gradient is:

$$\frac{dP}{dx} = \mu \frac{\partial^2 u}{\partial y^2} - \varepsilon \nabla^2 \phi E_x$$
(2.10)

The above equation can be rewritten in terms of the individual potential components across a homogenous microchannel surface. When a surface is homogenous, the potential can be divided into two parts as:

$$\phi = \phi_1 + \Psi \tag{2.11}$$

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where ϕ_1 is the potential due to externally applied electrical field and is constant, Ψ_1 is the potential across charged surface[Chen 2002]:

$$\nabla \phi_1 = 0 \tag{2.12}$$

Substitute equation 2.11 and 2.12 in equation 2.10,

$$\frac{dP}{dx} = \mu \frac{\partial^2 u}{\partial y^2} - \varepsilon \nabla^2 \Psi E_x$$
(2.13)

When there is no pressure gradient inside the microchannel, the equation simplifies to:

$$\frac{dP}{dx} = 0$$

Then, equation 2.13 reduces [Chen 2002] to:

$$\mu \frac{\partial^2 u}{\partial y^2} = \varepsilon \nabla^2 \Psi E_x \tag{2.14}$$

The boundary conditions applied for solving the equation are:

(1) At the bulk of the microchannel $(y \rightarrow \infty)$,

$$\frac{d\Psi}{dy} = 0 \qquad \frac{du}{dy} = 0$$

(2) $\Psi = \zeta$, u=0 at x = 0.

Integrating equation 2.14 twice and applying the boundary conditions, the velocity at the bulk of the channel is found to be [Chen 2002],

$$U_{EOF} = \varepsilon \zeta E/\mu.$$
(2.15)

The equation is called Helmholtz-Smoluchwski equation.

Thus the electroosmotic flow depends upon the applied electrical field, zeta potential of the microchannels, permittivity constant and viscosity of the fluid. The properties of the microchannel and the working fluid are constant for a given system to be used and hence the electrical field is the only variable parameter for a given system to achieve higher flow rates. Thus by applying a higher electrical field, higher flow rates can be generated. Electrical field is applied using electrodes and the applied electric field depends on the external applied voltage and the distance between the electrodes. By controlling the distance between the two electrodes, the electrical field can be manipulated inside the microchannel. By fabricating a number of electrodes inside the microchannel, electrical field can be switched on and off at the various points inside the microchannel and hence the fluid can be engineered at the desired point. Thus a very good local control can be achieved in pumping the fluid. Fabricating electrodes inside the microchannel will ensure that only a part of the fluid is exposed to the applied electrical field. But due to the inherent limitations of the chemical reactions occurring at the electrodes, fabrication of electrodes inside the channel is nearly impossible. In the next chapter, limitations of the electrodes for pumping and previous work done by researchers to overcome the limitations are discussed.

CHAPTER 3

BUBBLE- FREE ELECTRODES FOR ELECTRO OSMOTIC PUMPING

3.1 ELECTRODES FOR ELECTROOSMOTIC FLOW

Electroosmotic (EO) pumping is the most versatile method of pumping used in microfluidics. As discussed in the previous chapter, excellent flow control requires localized manipulation of an electrical field in the flow conduit. The strength of the electrical field is a primary determinant in EO pumping as it establishes the velocity field of the electroosmotic flow. Localized manipulation of electrical field can be achieved by placing the electrodes in proximity of one another, anywhere between $100 - 500 \mu m$, within the microchannel. However, conventional metal electrodes introduce problems such as gas evolution that preclude their placement within the confines of the microchannel itself.

3.2 LIMITATIONS OF THE ELECTRODES FOR EO-PUMPING

The main disadvantage of inert metal electrodes, such as gold, used for EO pumps is bubble evolution [Selvaganapathy 2002, Guzman 2006] and fluctuation of electrolyte pH. An inert metal electrode doesn't participate in chemical reaction with the aqueous electrolyte and hence when the inert electrodes are operated in aqueous electrolytes with more than 1 V, water electrolysis will occur leading to the evolution of hydrogen at the cathode and oxygen at the anode. At the anode:

$$2H_2O \longrightarrow 4H^+ + 4e^- + O_2 \qquad E^O = -1.23 V$$
 (3.1)

At the cathode:

 $2H_2O + 2e^- \longrightarrow H_2 + 2OH^- E^O = -0.83 V$ (3.2)

 E^{O} represents the standard reduction potential with respect to a standard hydrogen electrode at 300 K, 1 atm and 1 Molar electrolyte concentration. The negative sign indicates that spontaneous evolution of hydrogen and oxygen is not thermodynamically favorable.

Due to the surface tension between the bubble and the channel wall, bubbles tend to clog the channel and result in an increased resistance to fluid flow. Another major limitation is that of pH change occurring near the electrodes. According to reactions 3.1 and 3.2, the pH of the solution near the anode increases due to the accumulation of hydrogen ions and decreases near the cathode due to the accumulation of hydroxyl ions. Thus, associated changes in pH cause inconsistency in the performance of the device. However, the use of a buffered solution as the electrolyte will prevent fluctuations in pH can but only until the inevitable depletion of the buffer occurs. One solution to the pH depletion and stabilization problem is to give the electrodes more access to the buffer solution. By placing electrodes in the reservoirs at the end of the microchannel allows the electrodes to have increased access to the buffer solution with an increased stabilization time. This placement of electrodes along the reservoir and open to the atmosphere also allows for the escape of the evolving gas bubbles to the atmosphere.

3.2.1 LIMITATIONS OF PLACING ELECTRODES AT THE RESERVOIR

Placing the electrodes at the reservoir causes associated problems such as higher voltages, Joule heating and limited control options. Firstly, the electrical field between the electrodes is reduced due to the increased distance between the electrodes. The same electrical field strength could be obtained with a smaller interelectrode distance and with a reduced operating voltage. Secondly, EO pumps with higher operating voltages consume more power and introduce Joule heating. Thirdly, due to the placement of the electrodes at the reservoirs, the biomolecules inside the bulk fluid get exposed to high electrical fields preventing the localized control of flow.

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Bubble free electrodes are a good solution to the above problem as it would allow low operating voltages and local pumping control. Bubble free electrode operation allows for the placement of the electrodes inside the microchannel, thereby decreasing the interelectrode distance and the associated operating voltage. Also, as mentioned in the Chapter 2, when electrodes are operated in a bubble-free mode, an array of electrodes can be placed inside the microchannel in order to provide localized control of the fluid. This will help in the evolution of low voltage microfluidics systems.

3.3 BUBBLE FREE ELECTRODES FOR EO PUMP

A number of researchers have worked on bubble free electrode operation techniques that include redox buffered electrolytes, hydrogen storing palladium electrodes, ion exchange membrane electrodes, and periodical asymmetric current input. Moini *et al* [Moini 1999] used hydroquinone (10mM in 0.1% acetic acid) as a redox buffer additive for the suppression of the bubble evolution reaction in his demonstration of capillary electrophoresis. Redox buffers have a decomposition potential below that of water (reaction 3.1 and 3.2) which makes them undergo alternate reactions before the water decomposition reaction thereby suppressing the gas evolution reaction. Hence the redox buffer resists a change in pH of the solution by holding the potential at the electrodes below that necessary to decompose the water. Master's Thesis – Balasubramanian- McMaster- Mechanical Engineering

Hydroquinone _____ p-benzoquinone +2 H^+ +2 $\text{e}^ \text{E}^0$ = 0.699 V (3.3)

Equation 3.3 shows the hydroquinone redox buffer undergoing a redox reaction at 0.699 V to form p-benzoquinone that suppresses the oxygen evolution reaction leading to the reduction of gas evolution. Moini *et al* tested several electrodes including stainless steel, gold-coated stainless steel and platinum with the redox buffer. No reduction of bubbles was observed at the stainless steel and gold-coated electrodes while bubble formation was reduced, though not completely eliminated, in the case of the platinum electrodes. The authors attributed the reduction of bubbles at the platinum electrode to the reduced potential of platinum in the presence of hydroquinone. It should be noted that the introduction of redox buffers may not be compatible with biological samples as well as can interfere in the analyte results of the microfluidic analytical system.

Zeng *et al* [Zeng 2001] observed no bubbles inside the microchannel when a fluid was pumped at pressures above 7 atm. Their group used platinum electrodes and fabricated a porous polymer inside the microchannel. The high surface area offered by the porous polymer increases the number of ions and resulting in enhanced flow rates. The pores of the porous polymer increase the resistance to the backflow allowing for high pumping pressures. At high pumping pressures, there may be recirculation of electrolytically generated gases that could undergo further reaction at the electrodes to form water or else the flow rate is so high that the saturation of gas to form bubbles in the liquid might not be reached. The limitation of these pumps is the requirement of high pressures to operate them bubble free which in many cases could be deemed impractical.

Selvaganapathy *et al* [Selvaganapathy 2002] followed the current reversal technique at the time of bubble nucleation to avoid bubble formation. By using

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the nonlinear current voltage characteristics of the electrochemical system, his group applied asymmetric, zero averaged alternating current to produce a non-zero net voltage. They demonstrated a bubble free EOF pump based on this principle. The actuation frequency was about 1 Hz with an applied current pulse of about 1 nA, -0.5 nA for period of 1/3 and 2/3 seconds respectively. This waveform produced an equivalent net DC voltage of 1.43 V without any bubble formation. They were also able to decrease the operating voltage by fabricating gold electrodes 50 µm apart. However, strict control over the current is required to prevent bubble formation.

Brask *et al* [Brask 2005] used an ion exchange membrane as an electrode to demonstrate EO pumping. An anion exchange membranes will allow only anions to pass through them. The anion exchange membrane was used for separating the electrodes and the flow channels while the platinum electrodes were placed at the top reservoirs. A maximum flow rate of 3 μ l/min was obtained with an applied voltage of 29 V. Anion exchange pumps can be used for solutions containing low anionic salts. If the salt concentration is high, then the ion exchange membranes get saturated with salt and the performance of the membrane will degrade because of the reduced tendency to exchange ions. Ion exchange membranes are highly permeable to water and they swell in water due to their hydration property which will complicate the microfabrication process.

Brask *et al* [Brask 2006] demonstrated bubble free AC electroosmotic pumping by producing palladium cathodes and palladium anodes. Palladium has the ability to absorb hydrogen and store the hydrogen gas in its atomic lattice which will prevent hydrogen gas evolution when used as a cathode (reaction 3.4). When this hydrogen-stored palladium is used as an anode, oxidation of the hydrogen (reaction 3.5) will be favored rather than the oxygen formation reaction (reaction 3.6) due to the reduced reduction potential of hydrogen ion formation.

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At the cathode

$$2H_2O + 2e^- \longrightarrow H_2 \text{ (stored)} + 2OH^- E^0 = -0.83 \text{ V}$$
 (3.4)

At the anode

H₂ (stored)
$$\longrightarrow$$
 2H⁺ + 2e⁻ E⁰ = 0 V (3.5)

$$2H_2O \longrightarrow 4H^+ + O_2 + 4e^- E^0 = -1.23 V$$
 (3.6)

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Thus no bubbles will evolve. However, there is a capacity limit for the palladium electrodes to get saturated with hydrogen where their performance will cease. As such, Brask operated the electrodes in AC mode where the current reverses after a certain period of time. When the palladium electrodes are operated in AC mode, the hydrogen stored in the cathodic half cycle will get oxidized in the anodic half cycle due to the lower reduction potential of hydrogen $(E^0 = 0 \text{ V})$ than that of oxygen $(E^0 = -0.83 \text{ V})$. But the symmetric reversal of the current in the AC mode produces no net EO flow. So they designed a rectifying valve to generate the flow. They demonstrated their pumping in 5mM phosphate buffer solution with an applied AC current of peak 2 mA AC. The pump cannot be operated in the higher current region due to the diffusion limitation of hydrogen gas into the palladium lattice. The diffusion constant of the hydrogen into the palladium lattice is $1 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$. Within a given time frame, especially at higher currents, the amount of hydrogen produced at the electrodes will be much faster than the saturation of hydrogen into the palladium metal lattice. They achieved a maximum flow rate of 12 µl/min.

The same redox buffer principle described by Moini *et al* can be applied to electrodes to form redox anodes and redox cathodes. Redox anodes undergo reaction or dissolution at much lower voltages before the oxygen evolution

reaction takes place. Metals such as Cu, Fe, Zn, and Ag were used as redox anodes [Blades 1991, Berkel 2001]. During electrolysis, redox anodes may either get converted to a metallic salt or will undergo dissolution inside the solution. In redox cathodes, electrodes in the form of metallic salts get reduced to the metal form. This conversion reaction is potentially more favorable than the water decomposition reaction and hence hydrogen evolution is suppressed.

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3.4 SUMMARY

The main limitation of inert metal electrodes used for electroosmotic pumping is the gas evolution; hence they cannot be placed inside the microchannel. The associated problems are higher voltage, Joule heating and loss of localized control. Bubble free electrode operation is a good solution and will overcome aforementioned problems as it would allow for localized pumping control. A significant amount of research has been done on bubble free electrode operation techniques that include redox buffered electrolytes, hydrogen storing palladium electrodes, ion exchange membrane electrodes and periodical asymmetric current input. Redox electrodes which undergo potentially more favorable chemical reactions than the water decomposition reaction can also be used for bubble free electrode operation.

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CHAPTER 4

SURFACE MICROMACHINING TECHNIQUE FOR EMBEDDING THICK ELECTRODES

4.1 Ag/AgCl REDOX ELECTRODES

When the anode - cathode undergoes oxidation and reduction reactions with the aqueous electrolyte at much lower voltages than that of water decomposition reaction, the electrodes can be operated without the generation of bubbles. Silver/silver chloride ($E^0 = 0.22$ V) [Gives 1961] electrodes are a common example of redox electrodes. Other comparable similar redox electrodes [Gives 1961] are Cu/CuSO₄ with $E^0 = 0.34$ V [Gives 1961], mercury/mercurous chloride electrode ($E^0 = 0.268$ V) [Gives 1961] and Ti immersed in quinhydrone electrolyte (Hydroquinone: Benzoquinone in ratio of 1:1) with $E^0 = 0.699$ V [Gives 1961]. Copper as a redox anode will not remain as a stable electrode as it undergoes dissolution in acid based electrolytes to form CuSO₄. Quinhydrone is a liquid and hence microfabrication of devices with this liquid electrode is a major concern. Mercury/mercurous chloride electrodes are toxic, semi-solid and hence cannot be microfabricated.

When silver/silver chloride is used as a redox electrode, silver metal forms the anode and silver-chloride salt forms the cathode part of the electrochemical system. Chloride based electrolytes such as potassium chloride and sodium chloride are used for the reaction 4.1 to proceed in the forward direction to form stable silver chloride. When connected as electrodes, silver is oxidized to form silver chloride at the anode and silver chloride is reduced to form silver at the cathode. Silver chloride undergoes much lesser dissolution and hence remains more stable as an electrode. The potential window of this reaction is much lower than 1 V. Hence the decomposition of water will not occur during the presence of this Ag/AgCl electrode couple.

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 $Ag + Cl^ AgCl + e^- E^0 = \pm 0.22 V$ (4.1)

4.2 PROPERTIES OF Ag/AgCl REDOX SYSTEM

Two important properties of the redox system to be discussed are the nonpolarizable nature of the silver/ silver chloride electrodes and concentration polarization near the electrodes.

The Butler- Volmer equation describes the relationship between the current density and the overpotential [Rieger 1987, Franks 2005]. Overpotential is the extra voltage needed to force the electrode reaction to proceed at a required rate. Butler- Volmer equation is given by:

$$i = i_0 \left(e^{-(\alpha n \eta F / RT)} - e^{-((1 - \alpha) n \eta F / RT)} \right)$$
(4.2)

where,

i is the applied current density (current/ area).

 i_0 is the exchange current density (i.e.: at the cathode or anode at equilibrium).

F is the Faraday constant (96,500 coulombs).

 α is the cathodic transfer coefficient.

n is the number of electron transfers occurring in the reaction 4.1

 η is the overpotential (mV).

R is the gas constant (J/mol-K).

T is the temperature at which the reaction is occurring (Kelvin).

For systems with a higher exchange current density (higher i_{0}), the increase in applied current density will not have much impact on the overpotential (η). When the charge (electron) transfer reactions occurring at the electrodes are much faster, the generated charges at the electrode surfaces are consumed faster. Thus, there will be no accumulation of charges near the electrode surface and the charge transfer reaction will not contribute to an increase in voltage even when a large current density is applied through the electrodes. Thus, a system having infinite i_0 is said to be ideally non-polarizable and zero i_0 is said to be polarizable. In actual cases, i_0 will exist between these two extremities. Silver/silver chloride electrodes have a high ion exchange current density [Kingma 1983] and are non-polarizable.

The rate-limiting step is the concentration polarization, which means that only the concentration of the electrolyte near the electrodes affects the limiting (maximum) current density that the electrodes can be operated with it.



Figure 4.1: Concentration profile of the redox ions near the electrode surface.

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Chlorine ions have to diffuse from the bulk of the microchannel to the electrode surface for the conversion reaction to proceed forward (reaction 4.1). Due to the silver/silver chloride conversion reaction, the concentration profile of the chlorine ions near the electrode surface reduces with respect to time and a concentration gradient is set up. In order to overcome the concentration gradient, the chlorine ions diffuse from the bulk of the electrolyte surface to the electrode surface as shown in Fig. 4.1. C_B is the concentration of the chlorine ions (equivalent to bulk electrolyte concentration) near the electrode surface at the initial time t_0 , C_{δ} is the concentration near the electrode surface at t_n . But when the electrodes are operated at a higher current density, the consumption rate of the chlorine ions will be higher near the electrodes and hence the diffusion flux of chlorine ions from the bulk electrolyte will not be able to supply a sufficient rate of chlorine ions for the reaction 4.1. Therefore, the concentration of Cl⁻ at the surface becomes zero and alternate reactions (3.1, 3.2) will occur leading to water hydrolysis and consequently gas evolution. Thus the current density of the redox system is determined only by the concentration gradient near the electrodes and not by the charge-transfer reaction.

Lower reaction voltage and a non-polarizable nature make silver/silver chloride electrodes ideal for use in bubble free microfluidic devices. Dunphy *et al* [Guzman 2006] used the concept of Ag/AgCl electrodes and demonstrated spatially controlled microfluidics for low voltage (<1 V) applications. They microfabricated 0.5 μ m thick silver silver-chloride electrodes with a reduced interelectrode spacing of 40 μ m and used a 10mM buffer solution containing 100mM NaCl as their fluid. Their group demonstrated electrophoretic movement of beads at 0.4 V. The operating time of their device is limited to less than 1 second due to the reduced electrode thickness. However, by switching polarity between the electrodes, the device was operated bubble free for some tens of minutes [Guzman, 2006].

Though Ag/AgCl electrodes are the preferred modality over other bubble free EO pumping methods (as discussed in chapter 3) due to their ease of fabrication and flexible biocompatible electrolyte system (i.e.: lower concentrations of KCl or NaCl), the lifetime of the Ag/AgCl electrodes remains a major concern. The lifetime of the electrodes is directly proportional to the quantity of the electrode material available for reaction. Thus by increasing the amount of the Ag/AgCl electrodes, the reaction time can be increased further.

4.2.1 FABRICATION OF Ag/AgCl ELECTRODES





Figure 4.2: Fabrication and cross-sectional view of thick electrode devices inside glass and silicon. 4.2A shows the schematic of a diagram of a microfluidic device embedded with electrodes.

Early fabrication of silver/ silver chloride electrodes was performed on silicon and glass substrates using bulk micromachining techniques. Electron beam evaporation or sputtering is used for creating thin layers of electrodes [Suzuki 2000].



Figure 4.2 B: shows the manufacturing process flow of the device (Cross section AA').

Processes such as screen printing [Wang 2001] and electroplating [Bao 2003] are used for producing thick electrodes. The final step is the bonding of the substrate containing the microchannel and the substrate containing the electrodes as shown in Fig. 4.2b. The major drawback associated with embedding electrodes inside glass and silicon is assurance of a good bond between the substrate with its associated substrate to form the final functional device.



Figure 4.3: Gap in bonding obtained when fabricating electrode structures inside silicon or glass substrates (Cross section BB'). 4.3A shows there is no bonding obtained due to the topography at the electrode. B shows gap in bonding when the electrode mold is not completely filled.

Leakage of the fluid is a major concern when sealing of the device is not hermetic. The leak-free bonding of the electrode substrate with the microchannel substrate depends on the topography of the electrodes. When there is uneven topography at the surface, such as electrodes protruding from the surface, hermetic bonding cannot be achieved (Fig. 4.3a) as there will be a gap formed due to the rigidity of the glass and silicon materials. In another case, there will be a gap in bonding (Fig. 4.3b) near the electrode surface when the bulk micromachined electrode mold is not completely filled with the electrode material.

Planarization can be done to even out irregular topography making the wafer flat or planar. The disadvantages with the planarization process [Zantye 2004] are long process times and high cost. Poor metal adhesion with the substrate could lead to delamination of the electrode. Pitting or blisters can be

formed in the electrode surface due to the abrasives used in the process of planarization.

Polymers have the advantages of moldability and flexibility to form a conformal bond. Polymers are easily moldable to form channels with straight sidewalls. Parylene [Mutlu 2004] is one such polymer which has been used for producing microchannels embedded with thick electrodes. The coatings obtained with parylene are completely conformal and of uniform thickness and pinhole free. Parylene and conventional surface micromachining techniques were combined to create microchannels embedded with thick electrodes. Surface micromachining of parylene was carried with photoresist as the sacrificial layer and parylene as the structural layer. Metal electrodes were patterned on glass or silicon wafers. Sacrificial photoresist was patterned on this substrate in the form of channels. Parylene (typically 3-5 µm) was deposited on this substrate using chemical vapor deposition (CVD). Openings for the electrical connections and the reservoir holes for injecting liquid into the microfluidic channel were patterned using oxygen plasma etching. The release of the channel is performed by the sacrificial removal of the photoresist with a solvent such as acetone. The time taken to release the entire channel depends on the length of the channel and is a diffusion-limited process. Approximately 30 minutes per 1 mm dissolution of the photoresist in acetone is required regardless of the microchannel width and height [Walsh 2001]. As such, the fabrication process is time-consuming as the sacrificial removal of photoresist is a slow diffusion limited process and not a convective process. Forcing convection of acetone can make faster release of the microchannels but the thickness of the parylene limits the forced removal of sacrificial resist. The fluid cannot be pressurized as the thin parylene structure may get distorted. The thickness of the parylene layer also limits the fluidic interconnections to be made at the reservoirs. By casting thick layers of polydimethyl siloxane polymer, convective release can be done resulting in faster

release of the channels. The thick polymer layer also allows the integration of fluidic interconnects into the fabricated device.

4.2.2 POLYDIMETHYL SILOXANE (PDMS)

PDMS has been widely used in the fabrication of microfluidic devices [Whitesides 2002]. Its primary advantages in the context of microfluidic technologies are its low cost and biocompatibility. PDMS is a silicone-based material available commercially in the form of base and curing agent. The base contains short chemical chains containing vinyl groups (-CH = CH₂) while the curing agent contains long chains containing hydrosiloxane (Si-H) groups. The base and curing agent are mixed together at the manufacturer's suggested ratio of 10:1 with subsequent curing at 65°C to form the solid PDMS. Figure 4.4 shows the structure of solid cured PDMS.



Figure 4.4: Structure of cured PDMS.

PDMS is a preferred material [Whitesides 2002] for microfluidic devices for a number of reasons. The PDMS prepolymer flows and conforms to features of as small as 10 nm. This allows the shape of the master to be replicated with minimum resolution up to 10 nm. Cured PDMS is an elastomer and is flexible in nature with a typical Young's Modulus of 750 kPa. Hence it conforms and

adheres reversibly to micron level smooth (typically 1 μ m rough) and ultra clean surfaces. The silicone elastomer is inert and does not react with most chemicals. The PDMS surface can be made reactive by plasma oxidation. Cured PDMS is also non-toxic and hence biocompatible while being permeable to gases and nonpolar organic solvents. These combined properties make PDMS an excellent platform for microfluidic devices that incorporate living cells. Moreover, the elastomer is electrically and thermally non-conductive. This allows for the manufacturing of PDMS devices with electrical connections in which crosstalk between electrodes will be minimal. PDMS surfaces exhibit low fluorescence and hence will not interfere with fluorescence detection systems. PDMS also [Chaudhary 1991] has low interfacial surface energy (~20 erg/cm²) and hence can be easily delaminated from the master mold without causing any damage to the master mold. Its hydrophobic surface can be made hydrophilic by plasma oxidation and other similar surface modification techniques. One disadvantage however is that protein molecules, which are typically hydrophobic, may get adsorbed to nonspecific sites. However, the surface can be modified by coating chemicals thus preventing the tendency for surface adsorption. The prepolymer can be mixed with different conductive materials to form conductive membranes and hence can be used in different electrical applications. PDMS is also much cheaper than silicon and glass. In summation, these various numerous flexible material properties along with the rapid prototyping technique make PDMS as a versatile material in manufacturing microfluidic components such as valves, switches, pumps and mixers [Whitesides 2002].

4.2.3 BULK MICROMACHINING OF PDMS

The primary method for fabrication of microchannels in PDMS is similar to the bulk micromachining technique commonly used in silicon microfabrication. A number of groups have demonstrated this technique by the casting of PDMS

over microfabricated molds to reliably replicate structures such as microchannels, micropumps and microvalves [Duffy 1998, Quake 2000, Satyanarayana 2005, and Wu 2005].

The molded PDMS layers contain open microchannels consisting of three walls. They are subsequently peeled from the mold and bonded with PDMS, glass or silicon substrates which enclose the microchannel structure to form the fourth wall. The process flow for this technique, which we refer to as bulk micromachining, is shown in Fig. 4.5.



Figure 4.5: Bulk micromachining process of PDMS.

Briefly, the bulk micromachining process starts with the creation of an SU-8 or silicon mold using standard lithographic techniques to create a negative of the shape that is desired. Liquid PDMS pre-polymer usually in the ratio of 1:10 (Curing agent: Base) is mixed, degassed and then poured over the mold. Liquid pre-polymer is subsequently cured for 1-hr at 65° C and then delaminated [Whitesides 2002]. This layer is subsequently attached to another PDMS or glass

layer using bonding technique to form the microchannel. The bonding technique is the most crucial step in the bulk micromachining technique. The bonding step determines the quality of the adhesion of PDMS to the substrate. A good bond will result in a tightly sealed device. A number of bonding techniques such as surface activation by oxygen plasma [Whitesides 1996] and PDMS prepolymer as an adhesion layer stamped by microcontact printing [Satyanarayana 2005, Wu 2005] have been demonstrated.

Whitesides [Duffy 1998] introduced plasma oxidation of PDMS for irreversible bonding of PDMS. Due to its process simplicity and irreversible bonding outcome, plasma oxidization is widely used for bonding cured PDMS. PDMS is plasma oxidized for a minute at 50 Watts in a plasma asher. When plasma oxidized, the methyl groups $(Si-CH_2)$ at the surface of the PDMS get oxidized to form silanol (Si-OH) groups. When a conformal contact is made, Si-O-Si covalent bonds will be formed resulting in irreversible bonding between the two substrates. The limitation of this technique is that plasma oxidized PDMS should be aligned and bonded with the substrate in less than 10 minutes. Otherwise, silanol groups formed at the surface will be lost and hence, no bonding will occur. Also in order to get a good bond, the PDMS surface should be smooth and dirt free. This technique can be used to bond PDMS to substrates including Si, glass, polyethylene, glassy carbon, quartz, silicon nitride, polystyrene and limited to substrates including PMMA, polycarbonate, polyimide. Bhattacharya [Bhattacharya 2005] characterized the bond strength at various conditions of plasma oxidation time, power and achieved maximum bond strength of 0.50 MPa.

Quake *et al* [Unger 2000] created monolithic PDMS valves by bonding two PDMS substrates wherein one of the substrates has excess curing agent and another having more base. His group cast PDMS substrates of 1:30 (excess base) and 1:3 (excess curing agent). They aligned the two substrates and heated them

together for 1 hr at 65°C. Thus the excess curing agent and excess base formed monolithic PDMS, similar to the properties of bulk PDMS. They operated their device at 100 kPa. The flexibility of this method is that it can be used for creating "n" number of bulk PDMS layers by bonding alternating layers of excess curing agent and excess base. They called this method of fabrication as "multilayer soft lithography". But this method cannot be used for bonding substrates other than PDMS.

Satyanarayana *et al* [Satyanarayana 2005] and Zare *et al* [Wu 2005] used 1:10 ratio PDMS prepolymer as an adhesion layer for bonding PDMS with other substrates. PDMS dissolved in toluene was spun at high rpm of 1500 for 60 seconds to form 400 nm thin PDMS layer and using contact printing, this thin layer of PDMS was transferred to their substrate and was used as intermediate glue for bonding PDMS with the other substrate. They characterized their bonding strength using burst pressure test and their devices can withstand pressure in the range of 200-700 kPa.

Zare *et al* [Wu 2005] experimented with both epoxy glue and PDMS as glue for bonding PDMS with the other substrates. They used the same technique of contact printing but they used 8000 rpm for 8 - 9 minutes to form $1 - 1.5 \mu m$ thick PDMS layer. Zare *et al* used air test to characterize their bonding strength and found that the bonded PDMS-glass cannot be separated without causing damage to the glass.

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These mentioned bonding techniques rely on conformal bonding of top and bottom substrates for a leak free microchannel. When tall structures such as thick electrodes (>5 μ m) and porous polymers are present on one of the substrates, their bonding method fails as the young's modulus (approximately 750 kPa) of PDMS limits the bonding to only micron level smooth substrates [Whitesides 2002]. Figure 4.6 represents the adhesion issue during the bonding step of the bulk micromachined PDMS especially when there is a sudden local variation in topography of the bonding substrate such as thick electrodes. Previous researchers [Lee 2005, Chang 2002, Liu 2001, Kovarik 2005] demonstrated embedding electrodes of approximately 500 nm thickness inside bulk micromachined channels.



Figure 4.6: Adhesion problem of PDMS with the non-planar substrates especially when there is a sudden variation in the topography.

Martin *et al* [Martin 2000] fabricated 6 μ m thick carbon microelectrodes on a glass plate and reversible sealed PDMS microchannel to form channels with thick embedded electrodes. They reported a small section of gap (8 μ m) on one side of the channel near the fabricated electrodes. But there was no leakage from their PDMS microchannel. This can be due to the hydrophobic nature of PDMS channel along with the surface tension of the working fluid adding resistance to flow down the small gap. Also there is a possibility that the electrodes crosssection may not be rectangular due to their processing technique for fabrication of the microelectrodes. A curved cross-section will reduce the sudden variation in topography and hence is conducive for conformal contact of PDMS. Also, they embedded only one thick electrode inside the microchannel and the other electrodes were placed across the reservoirs of the channel. But placing the working electrodes near by (typically 500 microns or lesser than that) will reduce the resistance losses occurring in the working fluid and the device can be operated with minimum power. In general, all the microfluidic applications including EHD, MHD, EOF, electro-wetting pumps requires minimum electrode-toelectrode distance. But a tight sealing cannot be achieved with their technique when the electrodes are placed close to each other.

Dunphy and her colleagues [Guzman 2006] used PDMS adhesive layer transfer technique for embedding silver/silver-chloride electrodes inside bulk micromachined PDMS microchannels. A thin layer of PDMS was used as a thin adhesive layer to bond the PDMS layer and the glass substrate having 500 nm thick silver electrodes having inter-electrode spacing of 300 μ m. They could not embed thick electrodes inside microchannel due to the reason that leakage of working fluid may happen near the electrodes. Thus the lifetime of their electrodes is limited to less than 1minute.

4.2.4 SURFACE MICROMACHINING OF PDMS

Surface micromachining, using photoresist as the sacrificial and PDMS as structural material allows creation of microfluidic channels on top of this nonplanar topology already present on the substrate provided adequate bonding strength exist between PDMS layer and the substrate. Figure 4.7 represents the gap-free bonding obtained using surface micromachining structures.



Figure 4.7: Advantage of surface micromachining technique in creating gap free bonding with substrates having tall structures.





Figure 4.8 conceptually demonstrates the developed surface micromachining technique. Positive photoresist is patterned on the substrate to

form microchannels. Plasma oxidation of substrate was carried out followed by the casting of PDMS. Interconnect ports are opened at the reservoirs and then sacrificial etching of the photoresist was carried out using acetone to release the microchannels.

However, traditionally used composition of PDMS pre-polymer (1:10 curing agent: base) did not yield PDMS layers with appropriate adhesion to the substrate. Like the conventional casting technique, there is no adhesion of 1:10 PDMS to substrate and delamination of PDMS occurred. Various pre-polymer ratios were made and their bonding strength characterized using conventional tensile test to choose the appropriate polymer ratio. The versatility of this technique in creation of not only embedded electrode structures but also multilayered channels as required in construction of microvalves is also demonstrated.

4.3 CHARACTERIZATION OF BONDING STRENGTH

4.3.1 PREPARATION OF THE SAMPLE

The bond strength of PDMS to the silicon substrate was characterized using standard tensile test. Figure 4.9 schematically represents the tensile test arrangement. PDMS prepolymer with curing agent: base was mixed in the ratio of 1:3, 1:5 and 1:10. AZ P4620 photoresist was patterned on silicon wafer in the form of a well with length 6 mm, width 6 mm and depth of 10 μ m. Plasma oxidization was carried out for 1 min at 50 W. The PDMS liquid pre-polymers were spun at 1000 rpm on this plasma oxidized Si. Another piece of plasma oxidized Si is placed on this layer. Samples of Si/ PDMS/ Si were then cured at 65°C for 1 hr. Photoresist was then removed using acetone. Epoxy glue was used to bond the above-prepared sample with aluminum gripper. Bond strength

between silicon-aluminum gripper using epoxy glue was less than that of PDMS-Si bond strength. But by increasing bonding area between epoxy glue-silicon approximately 10 times more than the bonding area of Si-PDMS, we were able to perform the pull test. Instron 4400R was used for testing the bond strength at a pulling rate of 1mm/min.

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Figure 4.9: Arrangement for tensile testing of PDMS bond strength. Plasma oxidized Si surfaces are bonded with PDMS. Si/PDMS/Si is then bonded with Al gripper using epoxy glue.

4.3.2 CALIBRATION OF INSTRON TENSILE TESTER

The output of the load cell (voltage) was calibrated against the added weights. Force loads are applied in five equal steps starting from no load condition to load of 50 pounds. The corresponding voltage was measured using multimeter and a graph (Fig. 4.10) was plotted to measure the calibration data. The slope of the graph gives the change in the voltage per unit weight and is equal to -0.045 mV/pound. This data is used for conversion of the output voltage in to pounds and then the corresponding pressure. The accuracy of the measurement is limited to the error in the multimeter ($\pm 0.001 \text{ mV}$) and the corresponding error in the calibration curve will be ± 0.22 pounds (0.027 MPa for 36mm² sample area).



Figure 4.10: Calibration curve for pull test.

4.3.3 RESULTS OF PULL TEST

Figure 4.11 shows the bond strength achieved for different PDMS ratios. Six samples of the three different PDMS ratios (1:10, 1:5, and 1:3) were tested for the adhesion strength. The maximum bond strength achieved is 7.2 MPa for 1:3 ratio PDMS and was chosen for use. Failure of the 1:3 PDMS occurred in the PDMS bulk whereas the other two ratios failed in the PDMS-Si interface. Maximum bond strength achieved for the 1:3 PDMS is comparable to the ultimate tensile strength of the same [Mata 2005].

This compares favorably with other techniques for improved adhesion including plasma oxidation [Bhattacharya 2005]. Also, the ultimate tensile strength of the ratios below than that of 1:3 PDMS are lesser than that of 1:3 PDMS [Mata 2005] and hence cannot be used.



PDMS prepolymer ratio

Figure 4.11: Bond strength achieved by tensile tests of Si/PDMS/Si sandwich with different ratios of PDMS pre-polymer (Crosslinker : base).

4.4 SUMMARY

Silver/silver chloride electrode has the advantages of reduced gas evolution and ease of fabrication. The lifetime of Ag/AgCl depends on the volume of electrode material. Hence fabricating thick Ag/AgCl electrodes will increase the lifetime of the EO pump. The conventional technique of making PDMS microfluidic devices

cannot be used for embedding thick electrode structures due to adhesion problems related to uneven topography. Surface micromachining, using photoresist as the sacrificial and PDMS as structural materials allows creation of microfluidic channels on top of this non-planar topology already present on the substrate provided appropriate bonding strength exist between PDMS layers. Adhesion of the structural layer with the substrate was characterized for different prepolymer ratios using standard tensile test and 1:3 (Curing agent: base) combination was found to be the best with maximum adhesion strength of 7.2 MPa.

CHAPTER 5

CHARACTERIZATION OF SURFACE MICRO-MACHINING TECHNIQUE

5.1 FABRICATION OF MICROCHANNELS EMBEDDED WITH THICK FILM ELECTRODES

A PDMS surface micromachining technique is developed to overcome the adhesion problem affecting traditional bulk micromachining technique when embedding thick electrodes inside microchannels. The surface micromachining technique has been demonstrated by fabricating thick electrodes inside microchannels.

Masks were made from high-resolution (30µm) laser printing of the mask design on transparency sheets. These were subsequently cut to size and mounted on glass plates 4"x 4" size. PDMS prepolymer (Sylgard-184) was obtained from Paisley Canada. AZ P4620 photoresist was obtained from Mays Chemical Company, IN. Non-Cyanide silver plating solution was obtained from Shor international, NY. Pressure sensor, PX185-030D5V used for characterizing the valve was obtained from Omega.

5.1.1 FABRICATION METHODOLOGY

The process flow for fabrication of the embedded thick-film electrodes in the microchannel is depicted in Fig. 5.1. Thermally grown SiO_2 (300 nm) coated silicon wafers were purchased from University wafers. SiO_2 acted as an insulating

layer on the silicon substrate. Titanium (20 nm) was deposited as the adhesive layer followed by the deposition of gold (200 nm) as the seed layer using electron beam evaporation (Process A). Positive photoresist AZ P4620 was spin cast at 2000 rpm to form a 10 μ m thick layer and patterned using lithography to form rectangular electroplating mold of 2.5 mm length, 500 μ m width and 10 μ m height as shown in Fig. 5.2 (Process B).



Figure 5.1: Process flow of fabricating single microchannel with embedded thick electrodes.

Electroplating of silver was carried out using commercially available noncyanide silver bath. Researchers [Bao 2003] have tried silver plating of electrodes from cyanide-based electrolyte, but with polishing as a tool to make smooth surfaces. Silver-plating from non-cyanide silver baths has not been well characterized in the field of micro electro mechanics. Non-cyanide silver baths don't exhibit good throwing power (ability to plate uniformly) compared to cyanide based baths. But due to lab safety, health regulations and disposal issues, cyanide-based bath is not used for our plating purpose. Soluble silver electrode was used as the anode. Shor international electroplating solution based on silver nitrate solution was used for plating silver. Stirring was done using magnetic stirrer to achieve uniform concentration of silver ions near the plating structures. Uniformity of plating in micron level structures is difficult to obtain as the current density increases from the center of the plating mold towards the edges [Luo 2005]. This variation in the current density tends to develop rough plated surfaces. The electrical field variation inside the micron level structures tends to develop different profiles ranging from rabbit ear profile to the cap-like profile [Luo 2005].



Figure 5.2: Shows the electroplated structure with the current thief windows.

One solution is using large current thief windows to reduce the concentration of electrical fields and divert them from the plating area

[Romankiw 1974]. Four current thieves were opened in the plating mask near the electrodes for our case. Various current densities ranging from 1 mA/cm² to 0.5 mA/cm² were tested for creating electrode structures. Plating at a current density of 0.8 mA/cm² for 5 hrs with Shor International plating bath gave much better results than the other current density parameters. 6 μ m thick (average) electrodes were created using these parameters with an average surface roughness of 1 micron.

After the plating, electroplating mold was dissolved in acetone (Process C). Positive photoresist AZ4620 was spun at 2000 rpm to from 10 μ m photoresist layer and lithographically patterned as a mask in the form of the electrode structure with the electrode pads. Gold etchant and Titanium etchant (buffered HF) was used to etch the unmasked seed layer (Process C). Subsequently AZ P4620 resist was spun cast and patterned (Process D) in the shape of the intended microchannel (400 μ m width and 25 μ m height). Figure 5.3 shows the patterned photoresist in the form of channels on the fabricated thick electrodes.



Figure 5.3: Patterned photoresist in the form of channels embedded with electrodes.

Interconnects were created at the gold electrode pads by soldering the copper wires to the same. Plasma oxidization for 1min @ 50 W was performed to improve adhesion of this substrate to subsequent layers. PDMS prepolymer (1:3 curing agent: base) was cast over mold and cured over temperature at 65°C for 1 hr (Process E). Liquid PDMS can flow and conforms upto 10 nm. This allows the features to get replicated upto 10 nm level resulting in a conformal bonding between the non-planar substrate and the cured PDMS. It should be remembered that bulk micromachined PDMS cannot be bonded with the substrate having thick electrodes as there will be adhesion problem (as discussed in chapter-4).

Reservoir holes were punched manually through the polymerized PDMS using 20 gauge blunt needles (0.8 mm) and acetone was forced through one of the reservoirs to remove the sacrificial resist (Process F). The release time was approximately 1 min. The flexibility of PDMS material allows penetration of acetone in between the sacrificial resist and PDMS. The adhesive forces prevent delamination of PDMS from the substrate upon application of these forces. The hollow channels formed after the photoresist removal was washed in IPA, distilled water to remove the chunks of photoresist. The traditional method for sacrificial material removal using diffusion of the solvent takes several hours depending on the length of the microchannels [Walsh 2001]. Acetone tends to swell the PDMS [Favre 1996, Lee 2003] upon exposure over hours but does not affect its bonding to the substrate [Lee 2003]. Moreover since our release process occurs very quickly (in minutes) we do not see swelling in the PDMS microchannels constructed.

5.1.2 CHARACTERIZATION OF THE MICROCHANNEL WITH EMBEDDED ELECTRODES

Single electrode microchannel was filled with yellow dye to determine the water tight sealing of the device. Inlet and outlet of both the upper and lower channels were open to the atmosphere and so filing of the dyes were done with minimum applied pressure. When filled, there is no leakage near the electrodes of the microchannel, as seen in Fig. 5.4 which confirms the irreversible sealing of the device. It should be noted that the bulk micromachined PDMS channels with thick electrodes would be having leakage near edges of the electrode, as there is a sudden variation in topography.



Figure 5.4: Fabricated microchannels with embedded electrodes.

SEM was used to characterize the shape of microchannels. SEM samples were prepared by dicing the device and sputtering ~50 nm of gold on the samples. Figure 5.5 shows the SEM cross-sectional pictures of the straight and curved PDMS microchannel. The curved microchannels were fabricated by post baking the photoresist at 120°C for 10 minutes before casting the PDMS pre-polymer.

The resist reflows at this temperature to form the curved microchannel mold. The PDMS pre-polymer subsequently flows and conforms to replicate the shape of the mold.



Figure 5.5 shows the SEM cross-section of the fabricated PDMS microchannels. A shows the microchannel with straight wall. B shows microchannel with curved wall surface.

Figure 5.6 shows the cross sectional picture of PDMS with electrode surface (BB' in Fig. 5.4) which confirms that there is no gap between the non-planar substrate and the PDMS material. PDMS Liquid pre-polymer can flow up to 10 nm [Whitesides 2002]. This property of PDMS ensures a gap free bonding between the PDMS and the different layers.



Figure 5.6: SEM illustrating no gap between the PDMS layer and a topographically non-planar substrate.

5.2 FABRICATION OF MULTILAYERED PDMS MICROCHANNELS

5.2.1 MONOLITHIC PDMS VALVES

The versatility of this surface micromachining fabrication method to create multilayer microchannel structure has been demonstrated by the fabrication of a microvalve through the construction of multilevel microfluidic network.

Valves, pumps, mixers and detectors forms an active part of a microfluidic network. Valves are important in a microfluidic system to achieve flow control. Various types of microfluidic valves have been designed and used. Among the different types of valves, electrostatic and electromagnetic actuations are popular because of their low power consumption and the controllability [Oh 2006]. A flexible, transparent polymer value is one of the innovations accomplished by Quake et al [Unger 2000]. Quake and his group used the flexible property of polymer PDMS for constructing multilayer microfluidic networks and demonstrated a monolithic valve, where a thin membrane of typically 30 micron was deflected downwards by the application of pneumatic pressure and closed the channel. In addition to the combined advantages of soft lithography like simple design, ease of fabrication, this type of polymer valve has other advantages over silicon or glass based valves such as low dead volume and the possibility of dense integration. This developed surface micromachining technique is used for demonstrating multilayered structures on PDMS by construction of microfluidic valves.

5.2.2 FABRICATION OF MICROFLUIDIC POLYMER VALVE

The surface micromachining technique described above can be used to fabricate PDMS structures on silicon, glass and PDMS. Figure 5.7 represents the

process flow for a surface micromachined PDMS microvalve. A sacrificial resist AZ P4620 was patterned for the microchannel (400 μ m width, 10 μ m height) and post baked at 120°C to form curved microchannels (Fig. 5.5 B). Plasma oxidation for 1min @ 50 W was done followed by spinning of 1:3 PDMS at 900 rpm to form a 30 μ m-thin valve membrane. Characterization of the thickness obtained at different spinning speed is performed for 1:3 PDMS ratio and found to be different from that for the 1:10 PDMS ratio [Chou 2006].



Figure 5.7: Process flow for fabrication of Multilayer microfluidics network such as a microvalve.

In the characterization process, 1:3 ratio of PDMS pre-polymer was spun for a period of 50 seconds at different RPM. Subsequently the wafer was mounted on a leveling table and heated to 65° C for 1 hr to form thin PDMS membranes. Then SEM was used to measure the thickness of these membranes. Figure 5.8 shows the thickness of spin coated PDMS versus spinning rate.



Figure 5.8: Spin curve of 1:3 of PDMS (Crosslinker : base).



Figure 5.9: Micro valve formed at the intersection of the two channels in different levels. The control channel (top) is filled with blue liquid while the bottom channel is filled with green liquid. The valve region is green indicating that the valve is open.

Another layer of microchannels (600 μ m width, 10 μ m height) were created again by patterning photoresist using the same procedure as described above. The PDMS was plasma oxidized to increase adhesion of the sacrificial resist layer. Reservoirs holes were punched and sacrificial photoresist was removed to form the control channel and main channel. The top view of the fabricated microvalve is shown in Fig. 5.9.

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The bottom channel (horizontal) is the flow channel through which fluid flow has to be controlled. The top channel (angled) is the control channel where pressure can be applied to close the valve. The intersection of the two channels is separated by the thin PDMS membrane and pressure differential across the membrane which is controlled by the pressure in the control channel can deflect it and close the valve. After fabrication and release it was noticed that the inside surface of the top control channel was not smooth and showed striations. A possible explanation for this was the process conditions and sequence. When the second sacrificial resist layer was spun on the first PDMS structural layer (valve membrane in this case), permeation of the solvents from the photoresist occurred through the thin PDMS layer into the resist in the flow channel causing it to swell. When the photoresist was baked at 120° C, the thin PDMS membrane wrinkled due to vaporization of the solvent adsorbed by the flow channel resist.

This was reduced significantly when the temperature was ramped slowly from 35° C to 120° C, resulting in the balance between the rate of evaporation of the solvent and the rate of permeation through the membrane. Furthermore, cracks were observed in the top photoresist layer during post-baking at 120°C for 1 hr. Reduction in the post-baking time reduced the cracks significantly. This indicates that the cracks in the photoresist are due to drying and subsequent cracking due to stress introduced in the layer. These are then replicated on to the top microchannel when PDMS is cast on this structure as seen in Fig. 5.9 and 5.11.

5.2.3 PRESSURE CHARACTERIZATION OF THE VALVE

In order to characterize the pressure, at which the valve closes, a syringe pump is connected to the one end of the upper control channel. The other end of the control channel is connected to the one end of the pressure sensor. The output of the pressure sensor is connected to a computer to store the output pressure. Figure 5.10 schematically represents the experimental set-up for characterizing the closing pressure of the microvalve. It should be noted that control channel runs perpendicular to the plane in Fig. 5.10.



Figure 5.10 Experimental set-up for the pressure characterization of microfluidic valve.

However since it is at an angle the inlet and outlet ports don't line up. Syringe pump was used with the flow rate of 1μ l/min to gradually increase the pressure in the control channel. At an applied pressure of 6.37 kPa, valve membrane deflected downwards and closed the flow channel. Quake *et al* [Unger 2000] operated the 100 µm x 100 µm valve membrane at a pressure of 50 kPa. The lower pressure was obtained in our case was primarily due to larger diaphragm of the valve (400 µm x 600 µm).


Figure 5.11: Control channel is pressurized deflecting the valve membrane and closing the bottom channel. The valve region is blue in color, which shows the functioning of the valve.

To show the functioning of the valve, the upper control channel was filled with the blue liquid and lower flow channel with the green liquid. The junction region of the both channel is green in color, as shown in Fig. 5.9 which indicates that there is fluid flow in the flow channel and the valve is open. When 6.37 kPa is applied in the top channel, the thin valve membrane deflects and thus closing the lower channel. The color in the junction region changes to blue, which indicates that the liquid in the flow channel has been displaced due to the deflection of the PDMS membrane downwards thus closing the microchannel. Figure 5.11 demonstrates the functioning of the valve.

5.2.4 SIMULATION OF VALVE

In order to verify the experimental results, simulation of the valve functioning was performed in Solid Works. The valve region was defined (750 μ m x 500 μ m x 10 μ m) as shown in Fig. 5.12.



Figure 5.12: Constructed valve region in Solid Works.

Grid spacing was defined with 9659 elements and 17020 nodes. The properties of the 1:3 PDMS (Young's modulus =900 kPa, Poisson ratio =0.49) and silicon were fed into the materials section of the software. Linear elastic isotropic model was chosen. Fixed boundary conditions were given at the edges, faces, surface of silicon. The simulation was performed with an applied pressure of 6.37 kPa normal to the elastomeric PDMS surface.



Figure 5.13: Simulation of the valve at the pressure of 6.37 kPa.

75% of the valve closed perfectly and is in conformal contact with the surface as shown in Fig. 5.13. Experimentally 90% of the valve closed completely as seen from Fig. 5.11. The difference between the results of experiment and simulation can be attributed to the variation in the curvature of the fabricated channels, thickness of the fabricated PDMS thin valve membrane.

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5.3 SUMMARY

A new surface micromachining technique for PDMS microchannels has been developed to address the leakage problem affecting traditional PDMS microchannel process with tall structures or sudden topographical transitions. PDMS pre-polymer composition of 1:3 ratio of curing agent; base was identified as the one producing the best adhesion. The versatility of this technique has been demonstrated by fabrication of microchannels with thick film electrodes as well as multilayer PDMS microvalve. A limitation of this technique is the requirement of photolithographic facility in fabricating the devices. However surface micromachining allows alignment of multiple layers with greater precision and allows complex structures to be fabricated. This technique also overcomes one of the major problems associated with surface micromachining, namely long release times by pressurizing the solvent through the microchannels and achieving release time of ~ 1 min. This work demonstrates the flexibility of the surface micromachining technique in incorporating other structures inside PDMS microchannels and will lead to increase functionality and complexity of PDMS based microfluidic devices.

CHAPTER 6

FABRICATION AND CHARACTERIZATION OF Ag/AgCl ELECTRODES

6.1 CHLORINATION OF SILVER ELECTRODES

The 6 μ m thick silver electrodes embedded in the microchannels were fabricated through the surface micromachining technique of PDMS as mentioned in the previous chapter. One of the silver electrodes was connected to the positive terminal and the other electrode was connected to the negative terminal of a voltage supplier for forming Ag/AgCl electrodes. Chlorination was carried out with 1M KCl and applied voltage of 1 V for 45 minutes to form silver chloride electrode. The silver electrode which was connected to the positive terminal forms the silver chloride by the following chlorination reaction.

 $Ag + Cl^{-} \longrightarrow AgCl + e^{-} E^{0} = \pm 0.22 V$ (6.1)

Negative electrode formed the inert electrode and didn't participate in any reaction including water decomposition reaction since the applied voltage was lesser than decomposition potential of water (1.23 V). The formed silver chloride is grey in color. Color change from white to grey was observed under microscope and was used as the indicator for the termination of the chlorination reaction.

6.2 CHARACTERIZATION OF SILVER-SILVER CHLORIDE ELECTRODES

6.2.1 VOLTAGE-CURRENT CHARACTERIZATION

Voltage - current characterization of the electrodes provides information about the limiting current density that the electrode can be operated without the gas evolution. As discussed in chapter-4 (Section 4.2), Ag/AgCl redox system reaches a limiting current density due to the concentration polarization at the silver electrode. Keithley 2400 LV controlled with the lab view software was used for characterizing the electrodes. The applied voltage to the electrodes was increased linearly from 0 V to 3 V with scan rate of 2 V/s. 100mM KCl in 10mM phosphate buffer was used as electrolyte solution. Fig. 6.1 shows the voltage current characterization of the microfabricated silver/ silver chloride electrodes.



Figure 6.1: V-I characterization of the Ag/AgCl electrodes.

There are 3 types of regions in the Fig 6.1. Region A represents the charge transfer polarization at the electrode surface (Section 4.2) while, region B is the ohmic losses resulting from resistance to the flow of ions in the electrolyte and electrons through the electrodes and interconnects. Region C is due to the concentration polarization at the silver electrodes. As discussed in chapter 4, concentration polarization will occur because of the mass transfer limitation of the chloride ions. Moreover, the diffusion of the chloride ions to the anode surface determines the gas evolution and limiting current density. The limiting current density of this system was found to be $2.2 \text{ mA}/\text{Cm}^2$.

6.2.2 LIFE TIME CHARACTERIZATION OF THE ELECTRODES

Lifetime characterization was performed by passing current of $1-3\mu A$ in order to know the total capacity of the device without gas evolution. Visual observation under microscope was used for confirming the time at which the gas evolution occurred. The experimental and theoretical life time were used for calculating the capacity utilization of the electrodes. The theoretical life time was computed using Faraday's first law of electrolysis. According to Faraday's first law, mass of the substance depleted or deposited at the electrode is directly proportional to the amount of the charge passed through it.

$$M = \frac{mIt}{nF}$$
6.2

Where, M is the mass of the electrode reacted (grams), m is the molecular weight of electrodes (108 gram for Ag, 143.5 gram for AgCl), I is the current passed through the electrode (Amperes), t is the time through which it is passed (sec), n is the number of electron transfer involved in the system (n=1 for Ag/AgCl system), F is the Faraday constant (F = 96500 Ampere- sec).

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Figure 6.2: Experimental and computed theoretical life time of the electrodes.

Equation 6.2 can be rearranged to find the theoretical time.

$$t_{theo} = \frac{MnF}{mI}$$

$$6.3$$

Using equation 6.3, the theoretical time period of the electrode consumption for both silver and silver chloride electrodes was computed. The computed average experimental capacity of the electrode (It) is 1680 µA-sec. The capacity of the previously demonstrated 0.5 µm Ag/AgCl electrode is 3.2 µA-sec [Guzman 2006]. Ratio of the experimental capacity to theoretical capacity gives the capacity utilization of the electrodes. Capacity utilization of the electrodes $=\frac{It}{It_{theo}} \times 100\%$. Table 6.1 shows the capacity utilization calculation.

Current	Theoretical	Theoretical	Experimental	Experimental	Capacity
(μΑ)	life	Capacity	lifetime (sec)	Capacity	utilization
	time(sec)	(µA-sec)		(µA-sec)	(%)
1	1794	1794	1752	1752	97.65
2	897	1794	834	1668	92.97
3	598	1794	540	1620	90.28

Table 6.1 Capacity utilization of the electrodes at various current.

The reduction in capacities of the electrodes at higher current densities may be because of the increased activation and resistance overvoltages. The average capacity utilization of the electrodes is 93.6%.

6.3 DEMONSTRATION OF EOF

EOF has been demonstrated by applying voltages upto 2 V. To aid the visualization of the EOF, 1 μ m bead solution was added to the 100mM KCl in 10 mM phosphate buffer solution.

Electroosmotic flow velocity was measured using the movement of beads. Keithley 237 voltage source meter was used to apply voltages up to 2 V. In order to minimize the influence of electrophoretic force acting on the beads, the flow measurement was taken at 1 cm far away from the electrode edges. Modeling of the electrical field in a microchannel (400 μ m) with two electrodes separated by 500 μ m was done using FEMLAB software to find out the distribution of the electrical field. All the boundaries except at the electrodes were insulated. Potential of 2 V was set at one electrode and a potential of 0 V was set at another electrode. Thus, a potential difference of 2 V was set as boundary condition between the two electrodes. Fluid medium was chosen as 100mM KCl with conductivity of 1.12 S/m. As shown in the Fig. 6.3, it is confirmed that electric

field intensity is more in between the edges of the electrodes facing each other. The electric field intensity is weak outside the electrodes. Hence, electrophoretic force will not influence the movement of the beads outside the electrodes.

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Figure 6.3: Electrical field (E_x) distribution between the two electrodes.

Tracking of beads was done by using frame-by frame analysis methodology as shown in Fig. 6.4. The movement of beads was captured as movie file (15 fps) using camera connected with microscope. The microchannel was magnified 425 times and flow through the channel was captured as a movie file. Then the movie file was imported in to the Adobe Premier Software and each one second movie was divided into 15 frames per second. Then the individual frames were printed in A4 sheet for the duration of analysis and the positions of the beads with respect to the time were tracked.



Figure 6.4: Methodology of measuring velocity

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Figure 6.5 shows the movement of the bead at an applied voltage of 2 V. The bead velocity is a function of the position of the bead inside the microchannel as the flow profile will be parabolic (pressure driven) outside the electrical field. Thus the bead velocity doesn't represent the average flow velocity. The liner used to calculate the position of the beads has an accuracy of 1 mm. Since 1mm corresponds to a magnification factor of 425, the error associated with the measurement is $1/425 \text{ mm} (2.35 \mu \text{m})$.



Figure 6.5: Movement of beads at an applied voltage of 1V. 6.5A shows the initial position of the bead at 12 sec. B shows the initial position of the bead at 13 sec.



Figure 6.6: Electroosmotic flow velocity with respect to the applied voltage.

Maximum achieved velocity is 44 μ m/sec with an applied voltage of 2 V. Higher voltages (more than 2V) could not be applied as there was gas evolution due to the diffusion limited reaction associated with the chloride ion availability.

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6.4 SUMMARY

The fabricated 6 μ m thick electrode using surface micromachining technique has increased capacity (1680 μ A-sec) due to the increased volume of the electrodes; hence the lifetime of the electrodes is much longer (10 minutes @ 3 μ A). The computed capacity utilization of the electrodes is 93.6%. EO pumping has been demonstrated and a maximum velocity of 44 μ m/sec at an electrical field of 40 V/cm was achieved. By decreasing the electrode spacing to 50 μ m, higher electrical field up to 400 V/cm can be generated. The disadvantage of the device is chloride based dependent electrolyte system and bubble evolution at higher currents or voltages as bubbles due to the diffusion limitation of chloride ions

CHAPTER 7

LIQUID ELECTRODE

7.1 INTRODUCTION

The bubble free metal electrodes including silver/ silver chloride have a limited life time because of the limited capacity of the electrodes and the consumption of the electrodes due to the chemical reactions (reaction 4.1). If the working electrode is inert such as gold and doesn't undergo any chemical reaction, then the life time of the electrodes will be increased. However, the inherent problem with the inert metal electrodes is dissociation of water to form gas bubbles inside the microchannel.

Jonsson [Jonsson 2006] *et al* constructed a bubble free electrocapture device using a nafion membrane as a separator. Nafion membrane is a cation exchange membrane with high conductivity. Their device consisted of two upper reservoir channels and a bottom flow channel with a nafion membrane placed in between the top and bottom channels (Fig. 7.1). Polyimide and glass were used to construct the top and bottom channels. Voltage was applied through stainless steel connectors placed in the reservoirs. The nafion membranes at the intersection of the top and bottom channel functions as the electrodes. Due to the possibility of short circuiting, the distance between the two electrodes is approximately 10 mm and hence much higher voltage (300 V) is needed for functioning of the device.



Figure 7.1: Schematic representation of the nation electrode device [Jonsson 2006].

Takamura *et al* [Takamura 2003] fabricated photopolymerized acrylamide gel as separators for EO-pumping. These gel electrodes undergo no reaction and hence no gas evolution occurred near the electrodes. Platinum metal was placed in the reservoir and was used for applying the electrical field. They demonstrated EO pumping and achieved a pressure of 200 Pa in a single microfluidic channel with applied voltage of 80 V. Their group demonstrated another new low voltage electroosmotic pump by fabricating a series of long narrow channels followed by one wide channel. A forward electric field is created in the narrow channels which produces a high pressure EOF flow and reverse electric field is created in the wide channel which produces a low pressure EOF. The difference in pressure causes the flow towards the outlet. Thus the resultant pressure head depends on the "n" number of series channels. With an applied voltage of 10 V and 10 series channels, they achieved a pumping pressure of 25 kPa. The main limitation of the pump is complex fabrication.

Combining Jonsson's concept of separating the top and bottom channel with a membrane and Takamura's concept of gel electrodes, a new bubble free electroosmotic pump with electrodes which doesn't undergo any chemical reaction and hence having a much better life time than the other electrodes such as silver-silver chloride, palladium electrode has been developed. The developed EOF device consists of two fluid filled upper channels separated vertically from a lower channel using a 6 μ m thick nano porous (10 nm pore size) polycarbonate sheet as shown in Fig. 7.2. Metal connectors are placed along the reservoirs of the top channels for applying the electrical field. The pores of the membrane at the intersection of the top and bottom channel together with the filled fluid acts as electrodes. The lower channel is the flow channel where the pumping of the fluid occurs.



Figure 7.2: Schematic representation of the final device.

The cylindrical nanochannels of these PC (polycarbonate) membranes have been used as microfluidic interconnects [Kuo 2003, Chatterjee 2005] between the various microfluidic channels operating in the different planes. These nanoporous membranes are used to control the flow in our EO pump. Several groups [Ismagilov 2001, Kuo 2003, Chatterjee 2005] have incorporated these membranes to form three dimensional microfluidic devices with functionalities of molecular separation, chemical analysis, and biological analysis.



Figure 7.3: SEM picture showing the PC membrane with vertical pores

The distinct advantage of using PC membrane over gel electrodes is the possibility of constructing devices with reduced interelectrode spacing and hence better fluidic manipulaion. Firstly, vertical pores (Fig. 7.3) of the PC membrane allow the possibility of constructing devices with reduced interelectrode distance (500 μ m) by avoiding short circuit through the pores between the two electrodes as shown in Fig. 7.4a. In the membranes having random pore orientation, conductive fluid from both the upper channels can spread through the network of pores causing a current leakage as shown in Fig. 7.4b. Consequently, the devices have to be fabricated with maximum interelectrode spacing. Otherwise, when fabricated with reduced distance between the two electrodes, EOF in the lower channel is reduced significantly as there is a secondary current path through the interconnected pores. Secondly, the reduced interelectrode spacing allows better fluidic manipulation (Chapter-2, Section: 2.3.2) at reduced voltages.



Figure 7.4: Schematic diagram comparing PC membrane having vertical pores with polymerized gel having connected pores. 7.4 A shows the flow of current in single direction. 7.4 B shows there are two paths for the current flow including the interconnected pores of the membrane.

PC membrane also functions as a separator physically separating the different working fluids in different planes. When the upper channels and lower channels are filled with different working fluids, nanoporous membrane will act as separator enhancing minimal physical contact of the liquids between them, thus reducing convective transport and mixing. In the absence of any external forces such as electrical field or pressure gradient, the nanoporous membrane will allow only diffusive transport through the nanopores.

Hence, this device configuration allows filling of top channels with conductive solution to reduce the overall resistance losses occurring in the device. The upper channels were filled with the conductive 1M KCl (conductivity 11.2 S.m⁻¹) solution and the lower channels were filled with the working fluid (10mM phosphate buffer). The copper metal electrodes were placed in the reservoir of the upper channels and the voltage was applied through the copper electrodes.

7.2 MEMBRANE CHARACTERIZATION

Fig. 7.5 shows the fabrication process of the device for the characterization of membrane. SU-8 master of upper electrode channel mold (20mm x 1mm x 150 μ m) was created using standard photolithography (Process A). PDMS pre-polymer was mixed in the ratio of 1:10 (curing agent: base), degassed and cast on the mold and cured at a temperature of 65°C for 1 hr (Process B). The cured PDMS was delaminated (Process C). The above said method was used for the fabrication of the flow channels (30mm x 200 μ m x 150 μ m) in the PDMS (Process A1 to C1). Inlet and outlet holes created (Process D) formed in the PDMS electrode channel reservoirs using a blunt 20 gauge needle (0.8mm). Several researchers [Ismagilov 2001, Kuo 2003, Chatterjee 2005] have used the plasma oxidation technique to bond the PDMS layers with the PC sheet.

But a major concern in using plasma oxidation technique is the leakage occurring near the edges of the PC sheet. This leakage problem was addressed by using microcontact printing to bond the PDMS layers with PC sheet. Transfer technique [Satyanarayana 2005, Wu 2005] was used for the creation of the final device. In this method, a thin adhesive layer $(1\mu m)$ was formed on the bare wafer by spinning 1:10 PDMS prepolymer at 4500 rpm for 4 minutes.

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Figure 7.5: Fabrication methodology of the device for membrane characterization.

The molded PDMS layers with electrode microchannel and flow microchannels were then placed on the adhesive layer with channel side downwards facing the wafer. Thus the adhesive layer of liquid PDMS is transferred to the positive relief of the cured PDMS molds. The elastomeric stamped PDMS is then lifted from the wafers (Process E). The top PDMS electrode channels are then aligned perpendicularly with the bottom PDMS flow channel having a 6 μ m thick porous polycarbonate sheet (10 nm pore size) placed in between them (Process F). Thus the membrane is stamped an area of 0.4 mm².

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After the stamping process, PC membrane is lifted from the PDMS elastomers containing upper, lower channels (Process G) and bonded between two layers of PDMS elastomer with access holes (Process H) and baked at 65°C for one hr. Inorder to calculate voltage drop across the membrane, V-I characteristics of the membrane were obtained with 1M KCl in one compartment and 10mM phosphate buffer in another compartment separated by the membrane. Copper wires were used as electrodes and connected across the reservoir to the voltage source. Electrode areas were at least 10 times more than that of the stamped area in order to reduce the electrode polarization. Electrodes were placed much closer (typically 1 cm) to the membrane in order to reduce the IR drop occurring across the solution. Agilent 4156C was used as the voltage source. Before the characterization, the membrane was pretreated by applying voltage of 20 V for 20 minutes. This pretreatment helps in attaining the equilibrium concentration of the two fluids inside the pores of the membrane. Voltages were scanned from 0 V to 10 V at a rate of 1 V/sec.

The current voltage behavior is found to be linear as shown in Fig. 7.6 and the resistance is found to be 26.5 k Ω . Thus the voltage drop across the membrane is not too high; hence majority of the resistance will fall across the liquid part and

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not across the membrane. Therefore, the membrane can be used as fluidic interface for application of the potential to manipulate the electroosmotic flow.



Figure 7.6: Characterization of the membrane showing voltage-current behavior.

7.3 MECHANISM OF THE PUMP

When a voltage is applied using two metal electrodes placed along the reservoir of the top channel, ionic current flows between electrodes through the 10 nm vertical pores of porous polycarbonate membrane. When the circuit is closed, there are five resistances occurring in series for the flow of the current across the microfluidic channels and the nanofluidic channel as represented in Fig. 7.7.

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Fig 7.7: Electrical resistance in series across the microchannel.

The Resistance R_{U1} and R_{U2} represent the electrical resistance in the upper electrode channels and the resistance R_{P1} and R_{P2} represents the resistance across the porous membrane. R_B is the resistance across the bottom flow channel. The resistance R_{U1} , R_{U2} , R_{P1} and R_{P2} are reduced by filling the upper microfluidic channel with a conductive solution. The potential difference between V_1 and V_2 at the lower bottom side of the channel provides the electrical field which pumps the liquid. The combined action of electrical resistance and the fluidic resistance determines the EO flow path.



Figure 7.8: Schematically represents the fluidic resistance across the top and bottom channel. If the fluidic resistances across the porous membrane (RFP1, RFP2) are lesser then the resistance in microfluidic channels (R_{FB1} , R_{FB3}) then the flow will occur only in the electrical field path i.e: R_{FP1} - R_{FB2} - R_{FP2} .

Figure 7.8 represents the resistance to the fluid flow. R_{FU1} , R_{FU2} represent the flow resistance across the upper channels and the resistance R_{FP1} , R_{FP2} represents the flow resistance across the porous membrane. R_{FB1} is the flow resistance across the bottom channel.

The EO flow rate through the porous membrane is represented by the following equation [Yao 2003].

$$Q_{Porousmembarane} = \frac{\psi}{\tau} \left(\frac{(-\Delta P A a^2)}{(8\mu L)} \right) + \frac{\psi}{\tau} \left(\frac{-\xi \varepsilon A f(a^*) V_{eff}}{(\mu L)} \right)$$
(7.1)

Where Ψ is the porosity (0.2%), ΔP is the pressure difference, A is the cross sectional area of the membrane, a is the pore radii (5 nm), V_{eff} is the effective voltage applied (20 V), μ is the viscosity of the electrolyte (10⁻³ kg. m⁻¹. s⁻¹), L is

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the thickness of the membrane(6 μ m), ζ is the zeta potential (22.8 mV for PC membrane) [Kirby 2004], ε is the permittivity of the electrolyte (80x 8.854x 10⁻¹² C. V⁻¹ m⁻¹), f(a*) is the non-linearized function obtained from Debye –Huckel approximation and can be written as [Yao 2003]

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$$f(a^*) \approx 1 - \left(\frac{2I_1(a^*)}{(a^*I_0(a^*))}\right)$$

where I_1 is a first order modified Bessel function. a^* where a^* is dimensionless distance parameter and is written as $a^* = a/\lambda$ in which λ is the Debye length and for symmetric electrolyte.

$$\lambda = \left(\frac{\varepsilon KT}{2e^2 Z^2 n_{\infty}}\right)^{0.5}$$

where K is the Boltzman constant $(1.38 \times 10^{-23} \text{ J.K}^{-1})$, n_{∞} is the bulk electrolyte concentration (1M KCl concentration), e is the electronic charge $(1.602 \times 10^{-19} \text{C})$, z =1 for symmetric electrolyte, T is the temperature (298 K).

When the pressure term in the first half of the equation tends to become zero, equation 7.1 reduces to 7.2.

$$Q_{Porousmembarane} = \frac{\psi}{\tau} \begin{pmatrix} -\xi \varepsilon A f(a^*) V_{eff} \\ /(\mu L) \end{pmatrix}$$
(7.2)

Evaluating $f(a^*)$ as 0.90 using the Bessel function appendix (Appendix 3), the flow rate is computed as $1.7 \times 10^{-22} \text{m}^3 \text{.s}^{-1}$.

Helmholtz-Smoluchwski equation (Chapter-2, Section 2.3.2) is used for computing the approximate EO flow rate through the PDMS microfluidic flow channel.

$$Q_{\text{Miicrofluidicchannel}} = \frac{\zeta_{PDMS} \varepsilon V_{eff} A}{\mu L}$$
(7.3)

Where, Zeta potential of the native PDMS (ζ_{PDMS}) is 16mV. The flow rate through the channel is computed as 1.26 x10⁻¹⁴ m³ s⁻¹.

The net flow rate ($Q_{Channel} - Q_{Porous\ membrane}$) acts as dynamic pressure for the liquid to flow through the other electrode side of the porous membrane (through R_{FP2} in Fig. 7.6). The computed net flow rate through the bottom flow channel is 1.26 $\times 10^{-14}$ m³ s⁻¹.

The ratio of the flow resistance between the porous membrane and the microfluidic channel determines the flow path. Eliminating the second part (electrokinetic flow part) of eqn 7.1, the flow resistance through the porous membrane is

$$R_{Porous\ membrane} = \frac{\Delta P}{Q} = \begin{pmatrix} 8\,\mu L \\ \psi Aa^2 \end{pmatrix}$$

The computed flow resistance for the flow through the porous membrane is 8.15 x 10^{24} g m⁻⁴ s⁻¹.

The computed flow resistance across the microfluidic channel will be

$$R_{Microfluidicchannel} = \Delta P / Q = \begin{pmatrix} 8 \mu L / A a^2 \end{pmatrix}$$

The computed flow resistance for the flow through the porous membrane is $3.45 \times 10^{13} \text{ g m}^{-4} \text{ s}^{-1}$. Thus the ratio of the flow resistance through the porous membrane is 10^{10} magnitude higher than the flow resistance through the microfluidic channel. Hence the majority of flow will occur through the microfluidic channel and only very little occurs through the membrane.

7.4 FABRICATION METHODOLOGY OF EO-PUMP

The fabrication methodology of EO pump is mentioned (as in the process of A, A1 to F) in the Fig. 7.4. After the adhesive bonding of PDMS layers containing microfluidic channels and the PC membrane, this transferred adhesive layer was then cured by placing the entire device on a 65°C hotplate for 1 hr. The reservoir holes for the flow channel were punched through the PDMS substrate. Thus a microfluidic device consisting of two layers of channels that cross at 90 degrees and that are separated by a porous membrane is fabricated. Figure 7.9 shows the fabricated bubble free liquid electrode pump.



Figure 7.9: Fabricated bubble EO pump

The fabricated device was characterized for leakage by filling the upper and lower channels with dyes. Inlet and outlet of both the upper and lower channels were open to the atmosphere and so filing of the dyes were done with minimum applied pressure. The upper channels were filled with blue dye and the lower flow channel was filled with yellow dye. No leakage was found to occur from the channel for an observed period of 30 minutes. Picture of the bubble free electrode device filled with dyes is shown in Fig. 7.10.



Figure 7.10: No leakage of dye is occurring from top channels

7.5 SHORT CIRCUITING TEST

The dye test used for finding the leakage of the device is not highly sensitive. The dye will not be visible in low concentrations or when there is some dye present in small nanometer gaps formed between the upper electrode channels. Any diffusion occurring between the two electrode channels through these gaps will lead to the short circuiting and hence malfunctioning of the device. The insulating property of PDMS elastomer was used to confirm if any leakage or short circuiting occurs between the two electrode channels. PDMS elastomer is an insulator but electrical breakdown occurs when applying electrical field strength of 20 V/micron. McDonald *et al* [McDonald 2001] utilized this property of PDMS and demonstrated single use disposable device with 20 μ m thick PDMS as an electrical breakdown of PDMS elastomer occurred leading to an irreversible small opening in PDMS which acted as a flow connection between the two separated channels and hence causing the pumping of the liquid from one channel to another channel. We used this insulating property PDMS to find out if there is any

short circuiting between the two upper channels. If there is no short circuiting between the two upper channels, there will be no flow of current until the breakdown voltage of PDMS elastomer.

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PC sheet was stamped completely with PDMS (app 500 nm thick) and was used for forming the bubble free device as shown in the Fig. 7.4 (process A, A1 to F). Upper channels were filled with 1M KCl and lower channel was filled with 10mM phosphate buffer. This device was tested for short circuiting by applying step voltage (step size 1V) between the two electrode channels. Fluctuating current lesser than 0.1 nA was observed until an applied voltage of 24 V. However, approximately 5 nA of current is observed at a voltage of 25 V. This shows that at 25 V, conduction occurs between the two electrode channels as a result of PDMS electrical breakdown. Thus it is confirmed that that there is no short circuiting between the two channels.

7.6 VOLTAGE-CURRENT CHARACTERIZATION OF ELECTRODES

Voltage current characterization was done to understand the behavior of the system including any non-linearity. Voltage was applied through the copper electrodes in the reservoir and the electrodes were pretreated with 20 V for 20 minutes before studying the voltage current behavior. Agilent 4156C was used as high voltage source meter. A scan rate of 20 V per second was used for applying the voltage from -100 V to 100 V. The behavior of the system is much linear (Fig. 7.11) and the resistance is found to be 0.26M Ω . Similar V-I characteristics have been obtained for experiments conducted by the previous researchers [Chatterjee 2005].



Figure 7.11: Voltage current Characterization of the electrode showing the resistor characteristics.

7.7 DYE TRANSPORT CHARACTERIZATION

In the absence of the membrane, when the upper and lower channels of the constructed device are filled with solutions having different concentrations, the diffusion of the ionic species will take place from one channel to another channel due to the concentration difference existing in the solutions. But the presence of the PCTE membrane in between the two channels will allow negligible diffusion of the species (in the absence of external forces) as the species has to pass through the 6 μ m thick pores with pore diameter of 10 nm. The transport of the species between the top and bottom microchannels may also vary depending upon the magnitude of the electrical field and the time through which the electrical field is applied. When a voltage is applied, electrokinetic flow of the fluid through the

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nanochannels of the PCTE membrane similar to the microfluidic channel occurs and hence the convection of the ionic species takes place between the two channels.

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Figure 7.12: Transport of the fluorescein dye from top channel to bottom channel at various time and voltage. 7.12 A shows the schematic of the flow channel in between the electrode regions we are observing. B shows no diffusion at zero voltage (30 minutes). C shows some transport of dye (20 V, 2 minutes).D shows increased amount of dye at 20 V, 4 minutes. E shows there is no dye between the electrodes at 50 V, 2 minutes (as the velocity in the bottom channel is higher than the velocity of the dye transport through the pores). F shows that the dye is at 1.5 mm away from the electrode surface.

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Fluorescent microscope images were used to characterize the transport of the fluorescent dye across membrane with and without voltage for different periods. Fluorescein is a water soluble fluorescent dye used in the fluorescent microscopy. Excitation of the fluorescein occurs at 494 nm and emission occurs at maximum of 521 nm. The lower channel was first filled with 10mM phosphate buffer solution. 0.1% (wt) fluorescein dissolved in 1M KCl solution was then filled in the upper channel. Figure 7.12 shows the diffusion profile of the fluorescent dye at different voltages and through different time regime. Fig. 7.12a shows that there is no diffusion of the dye when no voltage was applied for 30 minutes.

The transport of the dye increased with respect to time and with respect to the applied voltage (Fig. 7.12b, 7.12c). However, at an applied voltage of 50 V, no dye is visible between the electrodes (Fig. 7.11d) since the flow rate of the fluid flow at the bottom channel is more than the flow rate of dye passing through the membrane and hence is observed at a distance of 1.5 mm from the electrode edges (Fig. 7.12e).

7.8 DEMONSTRATION OF EOF

EOF was demonstrated by applying voltages upto 70 V. 1M KCl was filled in the upper channel and 10mM phosphate buffer was filled in the lower channels. The EO pump was pretreated at 20V for 20 minutes. To aid the visualization of the EOF, 1% of the 1µm bead solution was added to the 10mM phosphate buffer solution.

Electroosmotic flow velocity was measured using the movement of beads. Voltages from 10 V to 70 V were applied using Keithley 237 voltage source meter to observe the movement of the beads. In order to minimize the influence of electrophoretic force acting on the beads, the flow measurement was taken at one cm far away from the electrode edges. Movements of the beads are shown in Fig. 7.13. The bead velocity is a function of the position of the bead inside the microchannel as the flow profile will be parabolic (pressure driven) outside the electrical field. Thus the bead velocity doesn't represent the average flow velocity.



Figure 7.13 Movement of the bead at 50 V. 7.13 A shows the initial position of the bead. B shows the final position of the bead.

With a magnification of 800 times, tracking of beads was done by using frame-by frame analysis methodology (Chapter-6 Fig. 6.4). The error in the process of measurement is $1.25 \,\mu$ m.

Figure 7.14 shows the graph of the EOF velocity with respect to the applied voltage. The velocity linearly increases and gets saturated to value of 60 V with corresponding value of 118 μ m/sec. This can be due to the attraction of the beads towards the membrane causing sites of pore channels to get clogged or due to deflection of the membrane inside the microchannel.



Figure 7.14: Electroosmotic flow velocity with respect to the applied voltage.

7.9 ELECTROPHORETIC BEAD TRAPPING

Electrophoresis is the movement of an electrically charged substance under the influence of an electrical field. This movement is due to the Coulomb force acting on the electrically charged particle. When a solid body gets in contact with the polar liquid charge separation occurs (Chapter-2) and the charges induced at the solid body gets attracted due to the columbic force of attraction and moves along the electrical field. Electrophoresis is the movement of the solid substrate where as in electro osmotic flow solid substrate (wall of microchannel) remains fixed and the liquid will be moving due to the columbic-viscous force interactions. This electrophoretic phenomena was observed in our microchannels when the demonstrating electroosmotic pumping.



Figure 7.15: Electrophoretic accumulation of the beads. A shows the picture of the device at 100V (t=0). B shows the accumulation of the beads at an applied voltage of 100V (t=10 seconds). C shows the accumulation of the beads under 100V (30 seconds). D shows the dissipation of the beads under -100V (10 seconds). E shows the condition at the electrodes with an applied voltage of -100V (30 seconds).

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When the fluid is subjected to electrical field in the microchannel, fluid experiences electroosmotic flow. The beads suspended inside the fluid within the electrical field experience two types of force, electrophoretic force and electroosmotic force. The electrophoretic force is absent outside the electrical field. The electroosmotic flow tends to move the beads using the drag force and the electrophoretic flow attracts or repels the beads near the electrode surface. Thus depending on the polarity of the electrode and the magnitude of the applied voltage, beads tend to concentrate near the electrode surface. Accumulations of beads were observed only near the positive electrode at 100V (Fig. 7.15 b, c). When the electrode was switched as negative electrode, the beads dissipated (Fig. 7.15 d, e).

7.10 SUMMARY

A new bubble free electroosmotic pump with liquid electrodes is reported. The device fabricated consists of two upper channels and one lower channel separated by a nanoporous membrane. The nanoporous membrane acts as an electrical interconnect connecting the upper and lower channels. Resistance of the porous membrane was determined using voltage-current characterization and found to be 26.5 k Ω . EOF pumping is demonstrated with maximum velocity of 118 µm/sec. This pump can be operated until the evaporation of the fluid from the top channel. Thus the advantages of this device are long life time and independent working fluid system (unlike the Ag/AgCl system on chloride salt based electrolyte as mentioned in chapter-4).

CHAPTER 8

CONTRIBUTIONS AND FUTURE WORK

8.1 CONTRIBUTIONS

This thesis proposes three major contributions to the field of microfluidics. One is the novel surface micromachining technique of PDMS developed for embedding thick electrodes inside the microchannel. Second one is the thick Ag/AgCl electrodes having longer life time with reduced gas evolution. The other one is the bubble free liquid electrode, in which a polymer membrane having a network of nanofluidic connections with water diffusing through it acts as electrode.

8.1.1 SURFACE MICROMACHINING TECHNIQUE IN PDMS FOR EMBEDDING THICK Ag/AgCl ELECTRODES

Silver/Silver chloride (Ag/AgCl) electrode has the advantages of reduced gas evolution and have been traditionally been used for bio, chemical and pH sensing applications. Lately they are being investigated for microfluidic applications because of their reduced bubble evolution capability. Using Ag/AgCl electrodes for electro osmotic pumping gives advantages such as reduced bubble evolution, low operating voltage and good flow control. The lifetime of Ag/AgCl depends on volume of electrode material. Hence thick Ag/AgCl electrodes will increase the lifetime of the electrodes and allow long bubble free pumping. The conventional technique of making PDMS microfluidic devices cannot be used for embedding thick electrode structures due to adhesion problems due to topography.

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The surface micromachining technique developed with PDMS as structural material and photoresist as sacrificial material addresses the leakage problem affecting traditional PDMS microchannel process with tall structures or sudden topographical transitions. Various liquid PDMS pre-polymer ratios were tested for adhesion with the silicon substrate using pull test. 1:3 PDMS prepolymer was found to have a bonding strength of 7.2 MPa and was used for the pull test. Thick Ag/AgCl electrodes (6 μ m) are fabricated by using this technique. Leakage tests show that there is no leakage of the dye near the thick electrodes. SEM characterization of the device was done and showed that gap free bonding between PDMS and the non-planar substrate is obtained. This technique also overcomes one of the major problems associated with surface micromachining, namely long release times by pressurizing the solvent through the microchannels and achieving release time of ~ 1 min.

The flexibility of this technique is demonstrated by construction of multilayered microfluidic structures such as microvalve. The valve functioned at an operating pressure of about 6.37 kPa. This work demonstrates the flexibility of the surface micromachining technique in incorporating other structures inside PDMS microchannels and will lead to increase functionality and complexity of PDMS based microfluidic devices.

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8.1.2 LONG LIFE Ag/AgCI ELECTRODES FOR EO PUMPING

The thick Ag/AgCl electrodes fabricated have higher capacity (1680 μ A-sec) and longer life time (10 minutes @ 3 μ A). The bubble free electro osmotic pumping has demonstrated using these electrodes. The maximum EO velocity achieved is 44 μ m/sec. But the limitation of these redox electrodes is that the chloride salts are necessary in the working fluid for bubble free operation of the device. Diffusion limitation process of the chloride ion transport near the electrodes
render using these electrodes to limited voltages and hence only limited velocity can be achieved.

8.1.3 LIQUID ELECTRODES FOR EO PUMPING

An inert electrode doesn't undergo chemical reaction and hence the operating time of the device is more. But inert electrode causes electrolysis of water and hence gas evolution occurs near the surface. An inert electrode with no gas evolution will have much longer life time and is the need of the hour. Other bubble free electrode created is a novel liquid electrode which undergoes no reaction at the surface and hence acts as an inert electrode. The device fabricated in PDMS consists of 6 μ m thick porous membrane (10 nm pore size) in between the upper channels and the lower channel. The upper channels are filled with a conductive solution. When a voltage is applied through copper metal connectors in the upper channel, the liquid which diffuses through the pores of the membrane acts as electrode and the pumping occurs in the lower channel. The bubble free EO flow pump reported is having atleast 30 minutes life time. Maximum velocity of 118 μ m/sec is achieved with an applied voltage of 60 V.

7.2 FUTURE IMPROVEMENTS

Pre-baking and curing parameters for the sacrificial and structural layers of surface micromachining technique has to be optimized as baking for a little longer time will leave the sacrificial photoresist with lesser solvents. When the solvents are evaporated from the photoresist, it will be tough to release the microchannel by pressurizing acetone.

Wrinkles were observed in the PDMS valve membrane due to the penetration of the solvents from the resist through the PDMS causing it to swell and shrink. Optimization of the baking steps has to be done to reduce the shrinkage occurred in the membrane. Cracks due to stress and drying were also noticed in the top of the PDMS microchannel due to the slow baking. Baking parameters has to be optimized to reduce the amount of the cracks.

The commercial non-cyanide silver electroplating bath used for electroplating produced a rough electrode surface. When the sacrificial resist layer is patterned in the form of microchannels above the rough electrodes, the photoresist get trapped in between the rough surfaces and cannot be removed entirely during the releasing of channels. Better silver plating solution has to be formulated and plating parameters has to be optimized for forming smooth plated structures.

Saturation of the velocity with respect to the voltage in liquid electrodes EOF has to be further investigated in the future. The width of the upper electrode channel has to be varied to find out the effect of the electrode area on the maximum velocity.

The fabricated Ag/AgCl and Liquid electrode EO-pumps have not contributed to any observable pressure. These pumps can be integrated into Lab-On-Chip applications only if they can produce high pressures. High pressures can be produced by fabricating multistage pumps with multiple electrodes and also by fabricating porous polymers inside the microchannel.

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APPENDIX 1

MICROFABRICATION PROCESS AND PARAMETERS

MASTER MOLD FABRICATION USING SU-8 PROCESS:

- 1. Cleaning of Silicon wafer with HF for 2 minutes.
- 2. Rinsing of wafer with water for 5 minutes.
- 3. Cleaning of Silicon wafer with acetone for 2 minutes.
- 4. Rinsing of wafer with Isopropyl alcohol.
- 5. Cleaning of wafer with water for 5 minutes.
- 6. Plasma oxidation of wafer at 60watts for 45 seconds.
- Spinning of SU-8 at 500 rpm for 5 seconds then continuous ramping to 1800 rpm in another 5 seconds.
- 8. Spinning SU-8 coated wafer at 3000 rpm for 30 seconds.
- 9. Pre-baking the wafer at 65°C for 15 minutes
- 10. Pre-baking the wafer at 95°C for 1 hr.
- 11. Exposing the wafer at 95 seconds at 7.1 Watts/ seconds.
- 12. Post exposure baking at 65°C for 10 minutes.
- 13. Ramping the hot plate and post exposure baking at 95°C for 25 minutes.
- 14. Developing the wafer at SU-8 developer with stirring for 30 minutes.
- 15. After the development, spray Isopropyl alcohol on the developed wafer.
- 16. If small white dots are visible after spraying Isopropyl alcohol, then development has to continue.
- 17. Dry with blowing nitrogen gas
- 18. Do a flood exposure (without any mask) at 50 watts for 30 seconds.

THICK Ag/AgCI ELECTRODE FABRICATION METHODOLOGY:

- 1. Cleaning of Silicon dioxide coated silicon wafers with acetone for 2 minutes.
- 2. Rinsing of wafer with Isopropyl alcohol.
- 3. Cleaning of wafer with water for 5 minutes.
- 4. Depositing Cr metal at 1A/sec for 200 seconds using electron beam evaporation.
- 5. Depositing Au metal at 1A/sec for 2000 seconds using electron beam evaporation.
- 6. Spin the positive photoresist AZ 4620 at 1800 rpm for 40 seconds
- 7. Pre-baking the wafer at 12°C for 1 minute
- 8. Exposing the wafer at 65 seconds at 7.1 Watts/ seconds to form sacrificial electroplating mold.
- 9. Developing the wafer with AZ-400K developer until the patterns are clearly visible.
- 10. Rinse with distilled water for atleast 5 minutes.
- 11. Dry with blowing nitrogen gas to remove moisture.
- 12. Electroplate silver metal inside electroplating mold with a current density of for 3 hrs.
- 13. Dissolve the electroplating mold in acetone.
- 14. Spin AZ 4620 at 2000 rpm for 40 seconds.
- 15. Pre-baking the wafer at 120°C for 1 minute
- 16. Expose the photoresist at 65 seconds at 7.1 Watts/ seconds using electrode metal pad mask.
- 17. Developing the wafer with AZ-400K developer until the undeveloped photoresist is dissolved.
- 18. Rinse with distilled water for atleast 5 minutes.

- 19. Dry with blowing nitrogen gas to remove moisture.
- 20. Etch the gold layer using gold etchant for 30 seconds.
- 21. Rinse with water for at least 5 minutes.
- 22. Etch the exposed chromium layer using chromium etchant.
- 23. Rinse with water for at least 5 minutes.
- 24. Dissolve the photoresist mask using acetone.
- 25. Spin AZ 4620 at 2000 rpm for 40 seconds.
- 26. Pre-baking the wafer at 120°C for 1 minute
- 27. Expose the photoresist at 65 seconds at 7.1 Watts/ seconds using microchannel mask.
- 28. Developing the wafer with AZ-400K developer until the undeveloped photoresist is dissolved.
- 29. Rinse with distilled water for atleast 5 minutes.
- 30. Dry with blowing nitrogen gas to remove moisture.
- 31. Solder copper wire for electrical connections at the metal pad.
- 32. Plasma oxidize the wafer for 45 seconds at 60 watts.
- 33. Cast 1:3 PDMS on the wafer.
- 34. Cure the wafer for 1 hr at 65°C for 1 minute.
- 35. Using glass pipettes, Punch holes through the PDMS at the reservoir of the channel.
- 36. Pressurize acetone through the punched holes to remove the photoresist.
- 37. After the releasing of the channels, rinse the channel with propanol.
- 38. Rinse the channel with water.
- 39. Pass 1M KCl solution through the channels.
- 40. Connect one of the Silver electrodes to the positive terminal and the other electrode to the negative terminal of the Keithley 237 voltage source unit.
- 41. Chlorinate the positive silver electrode to form silver chloride at 1V.

LIQUID ELECTRODE FABRICATION METHODOLOGY:

- 1. Form the Electrode master mold using SU-8 process.
- 2. Form the Channel master mold using SU-8 process.
- 3. Mix 1 part of PDMS Curing agent to 10 parts of PDMS base.
- 4. Pour the liquid PDMS over the electrode master mold.
- 5. Pour the liquid PDMS over the electrode master mold.
- 6. Cure both the molds with PDMS at 65°C for 1 hr.
- 7. Peel the cured PDMS from both the molds.
- 8. Using glass pipettes, punch holes through the PDMS replica (with microchannel) at the reservoir of the channel.
- 9. Spin 1:10 liquid PDMS at 4500 RPM on a silicon wafer for around 4 minutes.
- 10. Stamp the PDMS substrate having microchannel with liquid PDMS by placing on the silicon wafer.
- 11. Place PC sheet on the stamped PDMS channel replica.
- 12. Stamp the PDMS substrate having electrode channels with liquid PDMS by placing on the silicon wafer.
- 13. Align both the replicas and place them conformally on a hot plate at 50°C for 1 hr to allow the curing of the PDMS glue.
- 14. Using glass pipettes, punch holes through the PDMS at the reservoir of the electrode channel.

MULTILAYERED MICROFLUIDIC DEVICE:

- 1. Clean Silicon wafer with HF for 2 minutes.
- 2. Rinse wafer with water for 5 minutes.
- 3. Clean the wafer with acetone for 2 minutes.
- 4. Rinse wafer with Isopropyl alcohol.
- 5. Clean wafer with water for 5 minutes.
- 6. Spin AZ 4620 at 2000 rpm for 40 seconds.
- 7. Pre-bake the wafer at 120°C for 1 minute
- 8. Expose the photoresist at 65 seconds at 7.1 Watts/ seconds using flow channel mask.
- 9. Developing the wafer with AZ-400K developer until the undeveloped photoresist is dissolved.
- 10. Rinse with distilled water for atleast 5 minutes.
- 11. Dry with blowing nitrogen gas to remove moisture.
- 12. Plasma oxidize the wafer at 60 watts for 45 seconds.
- 13. Spin 1:3 liquid PDMS on the wafer at 900 rpm for 50 seconds.
- 14. Cure this PDMS by placing it on the hotplate for 60°C for 1 hr.
- 15. Plasma oxidize the PDMS at 60 watts for 45 seconds.
- 16. Spin AZ 4620 at 2000 rpm for 40 seconds.
- 17. Pre-bake the wafer on a hotplate at 40°C for 5 minutes.
- 18. Ramp the temperature to 120°C and heat at 120°C for 1 minute.
- 19. Expose the photoresist at 65 seconds at 7.1 Watts/ seconds using control channel mask.
- 20. Develop the wafer with AZ-400K developer until the undeveloped photoresist is dissolved.
- 21. Rinse with distilled water for atleast 5 minutes.
- 22. Dry with blowing nitrogen gas to remove moisture.
- 23. Plasma oxidize the wafer at 60 watts for 45 seconds.

- 24. Cast 1:3 PDMS on the wafer.
- 25. Cure the wafer for 1 hr at 65°C for 1 minute.
- 26. Using glass pipette, punch holes through the flow channel reservoirs and control channel reservoirs.
- 27. Pass acetone through the punched holes to release the channels.
- 28. After the releasing of the channels, rinse the channel with propanol.
- 29. Rinse the channel with water.

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APPENDIX 2

COLEPARMER DATA SHEET

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Technical Appendix

BInstruction Manual (851 KB)

Technical Library (roll over link for article summary) <u>Metering Pumps Selection Guide</u> The In's and Out's of Metering Pumps <u>Guide to Liquid Pumps</u> <u>More Related Technic Articles</u>

APPENDIX 3

BESSEL FUNCTION CHART



Bessel function chart [Yao 2003]

APPENDIX 4

Ag/AgCl EO VELOCITY DATA

Voltage (V)	Initial time (sec)	Final time (sec)	Position 1 (cm)	Position 2 (cm)	Movement of beads (µm)	Velocity (µm /sec)
0	0	1	7.1	6.8	0.71	
1	12	13	27.5	26.6	21.15	20.44
2	16	17	21.5	19.6	44.65	43.94

APPENDIX 5

LIQUID PUMP VELOCITY DATA

Voltage (V)	Initial time (sec)	Final time (sec)	Position1 (cm)	Position2 (cm)	Movement of beads (µm)	Velocity (µm /sec)
0	11	12	12	11	12.5	
10	20	21	28.5	26.2	28.75	16.25
20	26	27	11.7	7.5	52.5	40
30	31	32	25.3	19.7	70	57.5
40	36	37	19.1	11.5	95	82.5
50	44	45	17.5	8.7	110	97.5
60	48	49	13	2.5	131.25	118.75
70	57	58	28.4	17.9	131.25	118.75

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