UNDERGRADUATE REPORT

Fabrication of Microfluidic Devices for Rapid Screening of Biological Agents

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UG 2001-3

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Abstract

In this paper, design and fabrication techniques are presented for a new microfluidic device that can be used for a wide variety of biological applications. The device consists of a channel as well as inlet and outlet reservoirs. Along the channel bottom is a pattern of alternating electrodes that can be used to control flow of charged species such as proteins. Driving electrodes are made from thin film metals as well as polypyrrole (PPy), a conducting polymer. The channel walls and covering are both constructed out of transparent or translucent polymers such as polydimethylsiloxane (PDMS) and SU8 to allow visual observation of biological activity. Deposition of chitosan, a biopolymer, is also discussed as a binding agent for adhesion of other biological materials to the channel bottom.

Introduction

Microelectromechanical systems (MEMS) that utilize microfluidic channels are useful for a wide variety of biological applications. Their size makes it easy to compress many devices into a small space, making many biological experiments possible on one 4 inch silicon wafer. These experiments could be used to screen for the function of a particular gene or protein. Different reagents could be secured to the bottom of the

channel in each device on a wafer. An aqueous solution of proteins could then be passed through the channel and the reagents with which the protein reacts can be observed.

The goal of this project is to create a microfluidic device that will aid the biologist in such trials. The advantage of MEMS technology is that very small volumes of biological material will be required to many tests. Large quantities of blood need not be drawn from patients, for example. Also, amplification techniques such as polymerase chain reaction (PCR) need not be used for genetic screening trials.

The device must meet a number of specifications in order to be of use. Often, the biologist designs trials so that successful reactions will emit visible light. Consequently, the device should allow this light to be observed under a microscope. The device should also be inexpensive and quick to fabricate. A biologist could then tailor a particular channel design to the needs of each experiment. Many new designs could be tested without monopolizing an entire research budget.

There should also be a system in place to control flow in the channel. This would allow macroscopic injection of sample fluids to a large reservoir. Once in the reservoir, the control system can dictate which devices and which areas on the device will receive a sample.

Materials

The choice of materials and soft lithographic techniques for fabrication coincides with the desire for inexpensive, efficient prototyping. First, PPy is chosen as the conducting material [1]. It performs comparable to metal and is much cheaper and easier to deposit [2]. Second, building channels out of SU8 is much faster than bulk or surface

micromachining of silicon when high aspect ratios and sharp edges are required [3,4]. Third, PDMS is a cheap, transparent polymer that can be cast as a liquid and cured to a solid at moderate temperatures within 2 hours.

Use of SU8 for channel formation is also desirable due to its translucence after polymerization. SU8 is an epoxy resin that functions as a negative photoresist. When exposed to UV light, a Lewis acid catalyzes the hydroxyl coupling of the SU8 epoxy rings, forming a solid structure. In order to achieve thick structures, the SU8 should have a high viscosity. Consequently, XP-SU8-50 (Microlithography Chemical Corporation, Newton, MA) was used [5]. SU8 is also advantageous because it can produce very sharp edges and well-defined, high aspect ratio structures [5]. Furthermore, complex multi-layer surfaces can be created [6].

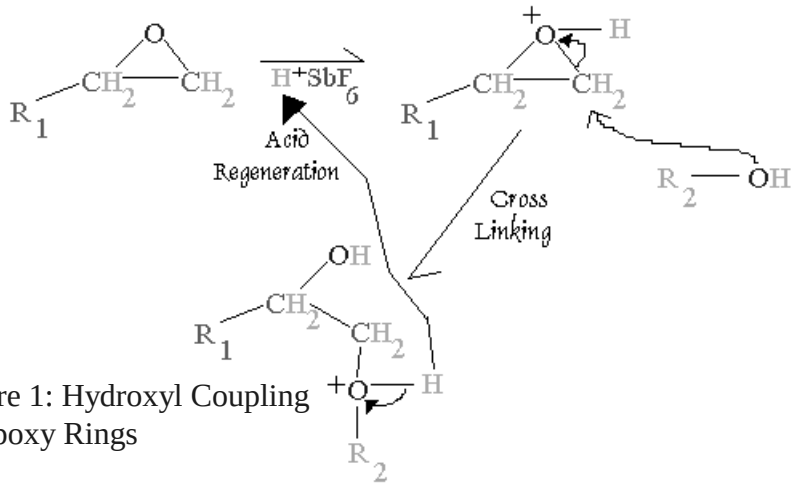


Figure 1: Hydroxyl Coupling of Epoxy Rings

Systems using pumps and valves are the obvious choice for macroscopic flow control. While attempts are being made to scale these systems down to the microscopic level, complete flow systems have yet to be perfected [7,8]. A pattern of electrodes running down the length of the channel will allow us to transport charged species electrokinetically. Many biological materials such as proteins carry charges depending on the pH of the surrounding solution. By applying positive or negative charges to the electrodes, the charged species can either be repelled or attracted to the electrodes. In

this manner, flow through the channel can be controlled. One drawback to this system is that uncharged species will not be controlled and may diffuse freely throughout the channel and reservoirs. For biological applications, however, the species of interest are usually charged, while the solvent is usually uncharged.

Often, it is desirable to fix some biological agent in the channel while flowing other agents through the device. In order to bind biological material to silicon, some adhesive must be used. For this device, chitosan is used. Chitosan is a biopolymer comprised mainly of repeating glucosamine units. It is soluble in aqueous solutions below its pK_a of 6.3 [9]. However, the presence of electrolyte salts prevents chitosan from dissolving, even at lower pH values.

In order to deposit chitosan, Microposit 1813 photo resist (Shipley Company, Inc., Marlborough, MA) can be deposited and patterned, exposing only the channel bottom. Next, the wafer should be submerged in an aqueous solution of chitosan for 30 minutes. A mixture of 40 parts acetone and one part phosphate buffer washes away the photoresist while preventing chitosan from being removed. After photoresist removal, the wafer should be washed in de-ionized (DI) water to complete the silicon / chitosan bonding process. Afterwards, electrodes can be used to repel the amine group of chitosan in DI water, allowing for deposition only on the silicon surfaces of the channel bottom.

Chitosan can be detected through the use of the enzyme tyrosinase in a solution of phenol. Tyrosine catalyzes the reaction between phenol and molecular oxygen to form a quinone. Chitosan then binds at the para position on the qunione causing the entire complex to change colors. The exact color depends on the additional functional groups

on the phenol. Once the deposition process is tested, insect cells can be flowed through the channel to bind to chitosan.

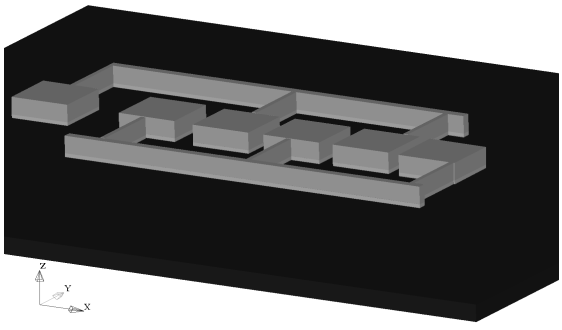
Fabrication

In order to fabricate the device, we first deposited chromium metal 90 Angstroms thick. This seed layer adheres well with silicon as well as gold. Next, gold was deposited 2000 Angstroms thick. Gold was used for its good conductivity as well as its chemical inertness.

Electrodes were then patterned using photolithography. A primer of hexamethyldisilazane (HMDS) was first deposited on the gold. Photoresist (1813) was spun on top of the primer and soft baked at $100^{O}C$ for one minute. 1813 was then exposed to UV light. The total dose of light was ~150 mJ/cm^2. After development for 45 seconds, 1813 was then hard-baked at $120^{O}C$ for 10 minutes. Gold Etchant: Type TFA (Transene Company, Inc., Danvers, MA) and Chromium Mask Etchant (Transene Company, Inc., Danvers, MA) removed the exposed areas of metal to reveal the pattern of electrodes.

To complete the electrode formation, PPy was electrochemically deposited on the gold with a thickness of 1-3 μm. PPy acts as the conducting material for the electrodes and may be used in future devices as a cheap alternative to metal.

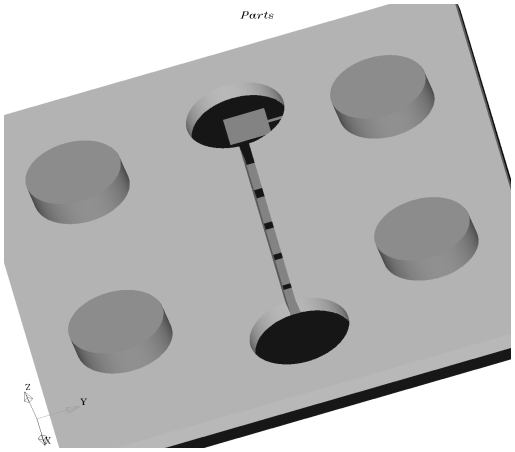
Figure 2: Electrode Formation



Microfluidic channels were then constructed on top of the electrodes using

soft lithographic techniques. First, SU-8 was spun to a thickness of 100 μm and pre-baked at 95^{O}C for 30 minutes. Pre-baking is essential for removal of the SU-8 solvent. Next, the mask for the channels was aligned with the electrodes. The center of the last electrode was aligned with the center of the outlet reservoir to permit flow completely through the channel. After alignment, an exposure dose of ~1040 mJ/cm^2 was given. Next, a second layer of SU8 was spun to a thickness of 150 μm and pre-baked at 95^{O}C for 30 minutes. This pre-baking also served as a post-baking step for the first layer in which additional cross-linking of the exposed SU8 takes place. The mask for the pegs was then aligned on top of the SU8 channel walls and given an exposure dose of ~1340 mJ/cm^2. After a 10 minute post bake at 95^{O}C, the wafer was placed in developer and agitated for 2 hours to remove all non-polymerized SU8. Finally, the wafer was cleaned in oxygen plasma for 5 minutes. The channels are 3.980 mm in length and 50 μm in width. The reservoirs are 800 μm in diameter as are the pegs.

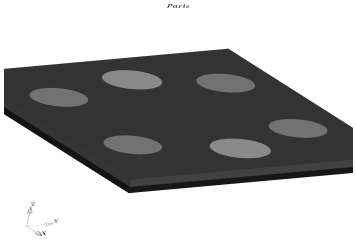
Figure 3: SU-8 Structures



A second silicon wafer was then used to make the channel encapsulation out of PDMS. SU8 structures 200 μm thick were first patterned on the wafer to act as the molding for PDMS. These structures resemble the pegs on the first wafer with the exception that they are slightly larger to allow for easier alignment. Additionally, two more pegs that aligned with the reservoirs were also created. This would create a channel covering while leaving inlet and outlet ports accessible for injection or removal of liquids. Next, the wafer was coated in a vacuum with the vapors of tridecafluoro-1,1,2,2-

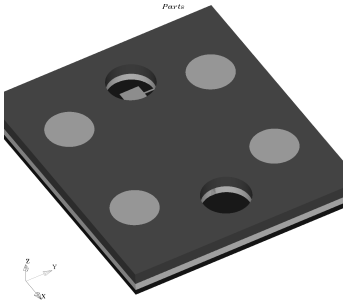
tetrahydrooctyltrichlorosilane (United Chemical Technologies; Bristol, PA). The chamber was evacuated until the silane was observed to boil. The pump was then switched off and the wafer was allowed to remain in the vapor for 10 minutes. The chamber was then opened and the wafer removed. Next, PDMS (Sylgard 184, Dow Corning) was mixed in a 10:1 ratio with its curing agent for 10 minutes. It was then poured onto the wafer and spun to a thickness of 90 μm. After baking in a box furnace at 65 ^{O}C for 2 hours, the PDMS was then peeled from the wafer, giving a covering for the channel.

Figure 4: PDMS Molding



The PDMS covering was then aligned using surface tension provided by drops of methanol. Methanol was removed and the PDMS rested on the first layer of SU8, aligned by the pegs of SU8 layer two, creating the covering.

Figure 5: Covered Device



Results

Devices were observed after creating the electrodes and 2-layer SU-8 structures. The channel was clear, but some PPy was removed as a result of the oxygen plasma cleaning recipe. As a result, gold is exposed in some parts of the channel. PPy can be redeposited or the power of the

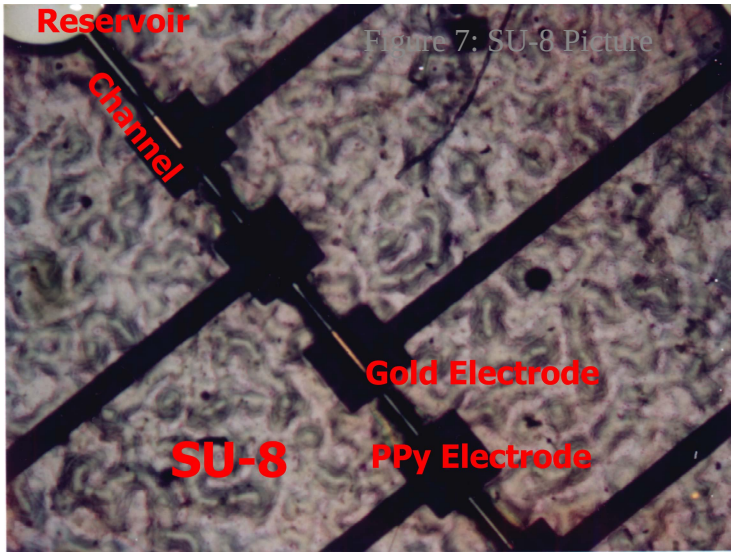


Figure 7: SU-8 Picture

cleaning recipe can be reduced. Reservoirs, 800 µm in diameter were also clear with sharp, well-defined edges.

The channel covering of PDMS was 50 µm thick and sat on top of the pegs as predicted. The alignment between the SU8 pegs and reservoirs was off, however. As a result, only 1/3 of the area of the reservoirs was exposed to the air. For manual injection of fluid, this poses no problem. Better alignment would be necessary if the DNA printing machine was to be used for injection. PDMS did cover the entire channel, preventing evaporation. When covered, complete evaporation of water was prevented for 12 hours.

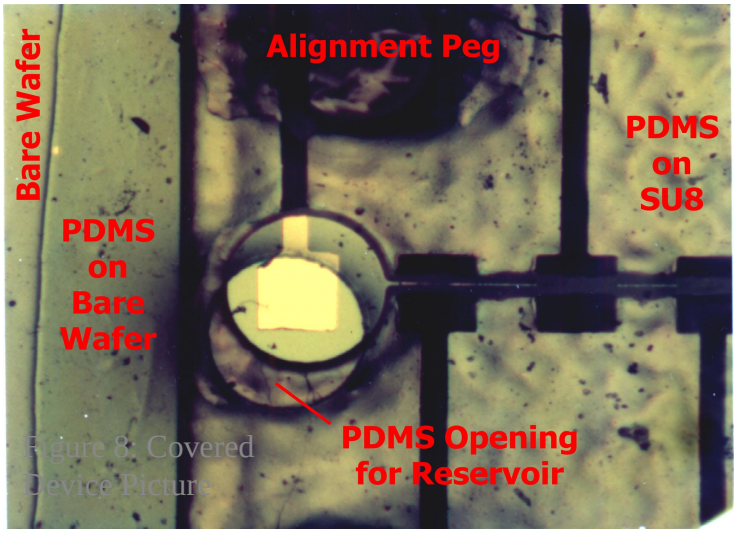


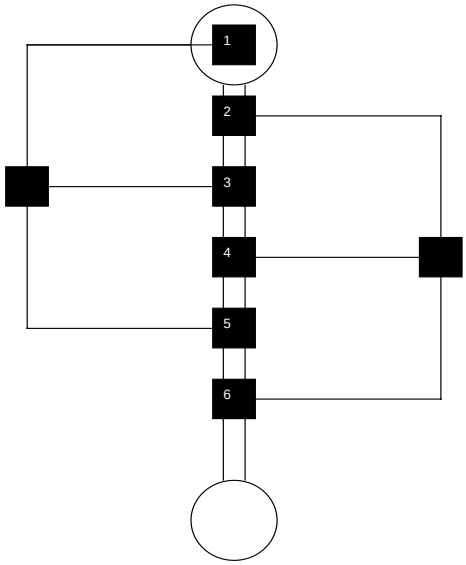
Figure 8: Covered Device Picture

A total of 48 devices with 6 electrodes each were fabricated on one silicon wafer 4 inches in diameter. Of these 288 electrodes, only one did not function properly, due to dust falling on the wafer during patterning of 1813 for electrodes. This rendered the device useless. One SU8 device became released from the wafer during the development and cleaning process. Additionally, the pegs on 6 devices did not become fully cross-linked to the channel layer, causing them to float away during development. This leaves 40 fully functional devices out of 48 possible, an 83% yield.

Proposed Flow Control System

In order to control flow through the device, 2 sets of 3 electrode squares (0.4 x.0.4 mm) are placed down the length of the device in an interlocking pattern. The first electrode is placed 680 μm from the inlet of the channel while the last electrode is centered in the outlet reservoir. This system depends on diffusion to move material close to the first electrode, but permits flow control completely through the channel. Diffusion should be aided by the injection of fluid into the inlet reservoir in which turbulent flow is temporarily created. This aids mixing and, consequently, the diffusion process.

Figure 9: Electrode Design



During the diffusion process of a positively charged species, all electrodes should be kept at a positive potential. This allows capillary action to move an uncharged solvent throughout the channel, but prevents the positively charged sample from doing the same. Next, electrodes 1,3,5 can be changed to negative potential while electrodes 2,4,6 are kept at positive potential. This pulls the species down the channel to the first electrode, but prevents it from continuing further. Finally, electrodes 2,4,6 can be changed to negative potential of greater magnitude than that of 1,3,5.

This scheme allows the concentration gradient of the species to diffuse it from lower potential to higher potential. Once close to the electrode of higher potential (1,3,5), electrokinetic forces pull the species to that electrode. The potential difference between 2,4,6 and 1,3,5 then causes the species to move towards electrodes 2,4,6. It should be

noted, however, that the concentration gradient of the species makes it unfavorable to flow backwards (i.e. from electrode 3 to electrode 2). In this manner, flow can proceed from electrode 1 to electrode 6, in the outlet reservoir. Eventually, the species will diffuse throughout the channel and there will be no more concentration gradient. After this happens, the species can only be kept in the channel, not directed into the outlet reservoir. Only removal of species from the outlet reservoir can restore the concentration gradient, allowing for flow control. Flow can be stopped at any time by changing the potential of all electrodes to positive. Some particles will be suspended between electrodes, while the rest will be pushed into the closed reservoirs. With these techniques, a species can be moved through the channel and eventually suspended within the channel to observe a particular interaction.

Discussion

While PDMS is often employed in microfluidic devices, this method of channel encapsulation is unique. A pattern of alignment 'pegs' allows for manual covering and removal using surface tension of methanol. This quick and easy process seals the channel against low pressures, and is useful to prevent evaporation of solvent or diffusion of foreign matter into the channel.

Rapid prototyping of microfluidic systems can be aided with this system. The designer can remove the covering and remove the contents of the channel. New agents can be deposited into the channel and the cover realigned. The channel structure can be reused for another trial by simply changing the contents inside.

One limitation of this device is the need for charged species to obtain flow control. Because flow is controlled electrokinetically, uncharged molecules will diffuse with regard to their concentration gradient. No control is offered over such molecules with this device. It is, therefore, the job of the biologist to design trials to incorporate charged species as the flowed species. The uncharged species should be fixed to the channel and charged material flowed past it. Often, this limitation is not significant in biological applications because species of interest usually carry charges. The charge of proteins, for example, can be controlled through pH regulation.

Flow control in this device is not very powerful. Diffusion of species down its concentration gradient can only be prevented or accelerated, not reversed. This should be taken into account when designing applications for the device.

One last issue is that of packaging. There must be a practical method for injecting liquid into hundreds of completed devices for trials. The arrangement of devices is such that a DNA microarray printing machine can be used to inject a controlled volume of liquid into the reservoirs. The center of each inlet reservoir is 9 mm from the next. This is a standard spacing for some microarray machines, but the design can easily be modified to accommodate different dimensions. Furthermore, a reservoir diameter of 800 μm is much larger than the injection needle diameter of 125 μm. Although injection was done manually, the printing machine can be used for further trials.

Conclusion

The design of a new microfluidic device has been presented. It consists of circular inlet and outlet reservoirs as well as a narrow channel defined in SU8 photoresist.

The channel covering prevents leakage under moderate pressures as well as evaporation and is versatile and durable enough to be removed and replaced many times.

Flow within the channel can be controlled through a system of alternating electrodes running along the channel bottom. A method for flow control using these electrodes has been proposed, but not tested. With this system, however, one cannot flow a species against its concentration gradient.

Nonetheless, the structural material of the device has been deposited in high yield. Once perfected, the process used to fabricate these devices could easily be modified to meet the specifications of a particular biological experiment, producing completed prototypes in 2-3 days from completion of channel design.

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