Design and Testing of a Hydrogel-Based Droplet Interface Lipid Bilayer Array System

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Abstract

The research presented in this thesis includes the development of designs, materials, and fabrication processes and the results of characterization experiments for a meso-scale hydrogel-based lipid bilayer array system. Two design concepts are investigated as methods for forming Droplet Interface Bilayer (DIB) arrays. Both concepts use a base of patterned silver with Ag/AgCl electrodes patterned onto a flat polymer substrate. In one technique, photopolymerizable hydrogel is cured through a mask to form an array of individual hydrogels on top of the patterned electrodes. The other technique introduces a second type of polymer substrate that physically supports an array of hydrogels using a set of microchannels. This second substrate is fitted onto the first to contact the hydrogels to the electrodes. The hydrogels are used to support and shape droplets of water containing phospholipids, which self-assemble at the surface of the droplet when submerged in oil. Two opposing substrates can then be pushed together, and a bilayer will form at the point where each pair of monolayers come into contact.

The photopatterning technique is used to produce small arrays of hydrogels on top of a simple electrode pattern. Systems utilizing the microchannel substrate are used to create mesoscale hydrogel arrays of up to 3x3 that maintained a low resistance (~50-150 kΩ) connection to the substrate. Up to three bilayers are formed simultaneously and verified through visual observation and by recording the current response behavior. Arrays of varying sizes and

dimensions and with different electrode patterns can be produced quickly and inexpensively using basic laboratory techniques. The designs and fabrication processes for both types of arrays are created with an eye toward future development of similar systems at the microscale.

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1 Introduction

This thesis presents the design, fabrication, and testing of a mesoscale hydrogel-based droplet interface bilayer (DIB) array system. Lipid bilayers can be formed artificially in a laboratory to replicate the structure of naturally occurring cell membranes. DIBs are lipid bilayers formed at the interface between two aqueous droplets coated with lipid monolayers. Hydrogels have also been used to support monolayers in these systems which improves durability and allows increased control over monolayer shape. Some recent work has focused on fabricating large networks of bilayers in transportable packages. This work introduces hydrogels as a solid aqueous phase in a DIB array system, and presents fabrication processes for mesoscale systems with a focus on future microfabricated networks.

1.1 Lipid Bilayers

Lipid bilayers appear in nature as a primary component of the cell membrane in living organisms [1, 2]. The membrane protects the cell and regulates which chemicals can enter and leave [2]. Phospholipids are molecules which contain a hydrophilic head group and a hydrophobic tail group [1]. A bilayer consists of two layers of lipids, called monolayers, with the tails facing each other and the heads facing outward, toward the aqueous outside environment. Artificial lipid bilayers have been formed that mimic the structure and properties of cell membranes [1-4].

Droplet interface bilayers (DIBs) have emerged in recent years as a common, effective method of bilayer formation [5-7]. When a droplet of aqueous lipid solution is submerged in oil, the lipids will self-assemble at the edge of the droplet with the heads facing inward. To form a DIB, two such droplets are formed, such as those represented in Figure 1-1(a). When the

monolayer supporting droplets are pushed together, as shown in Figure 1-1(b), the lipid tails force the oil out of the gap. The two monolayers eventually pull together to form a bilayer.

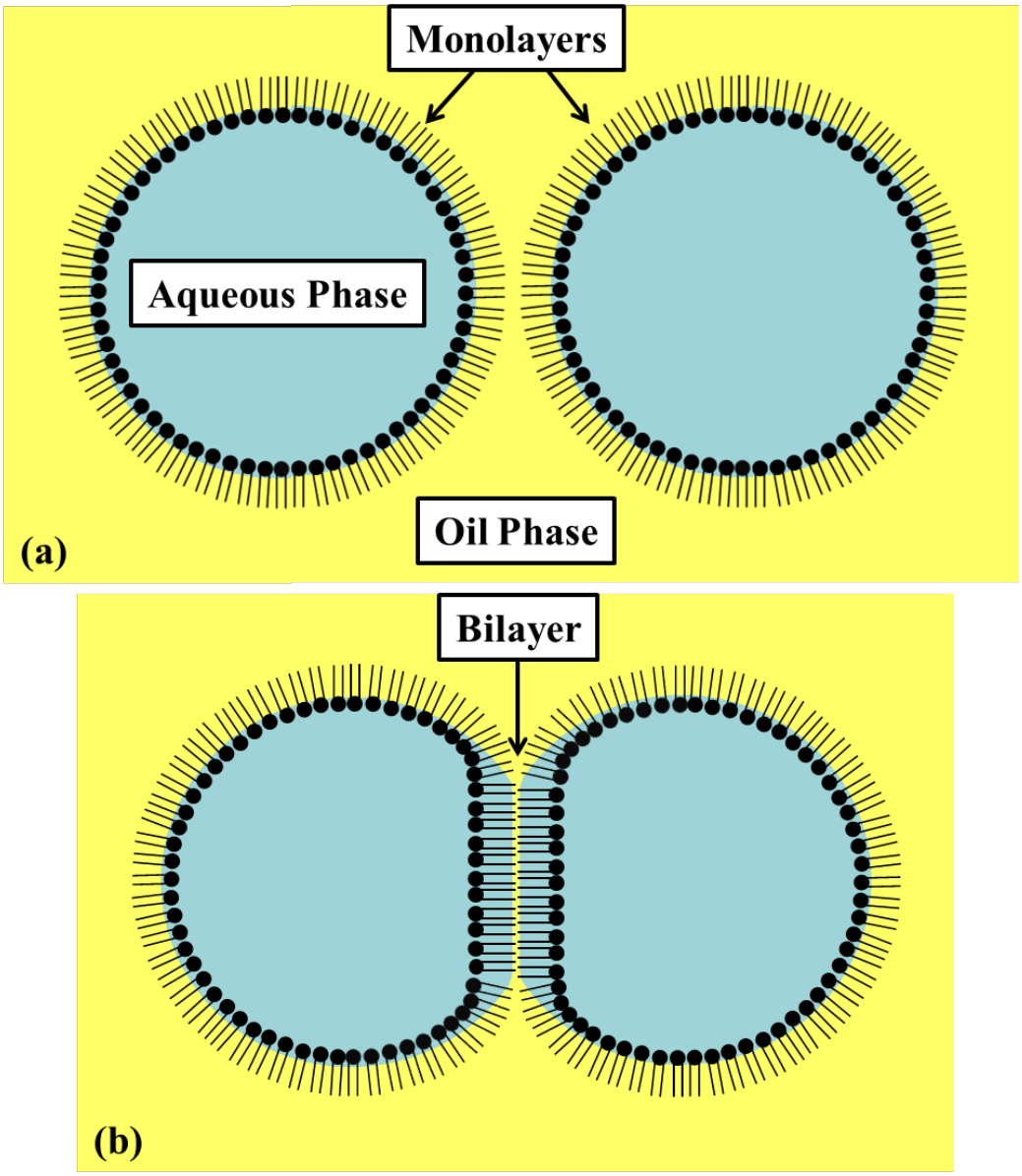

Figure 1-1. Two steps of bilayer formation process. (a) Lipids self-assemble at the oil-water interface around two aqueous droplets. (b) When the droplets are pushed together, the oil is forced out, and the lipid tails come together to form a bilayer.

This is a relatively simple method for forming durable, long-lasting bilayers. A process for forming DIBs called the regulated attachment method (RAM) was recently introduced by Sarles, et. al [8]. In this process, a flexible polymer substrate holds a droplet of aqueous lipid solution submerged in hexadecane oil. An electrode is inserted into each end of the substrate to be submerged in the droplet. When the substrate is compressed, the shape of the polymer splits the droplet into two parts. The droplets are allowed to rest, so that the lipids form a monolayer at the oil-water interface. When the pressure on the substrate is released, the droplets come together to form a bilayer. This formation technique provides a relatively simple way to produce durable bilayers in a package that can be transported and handled [9-11].

1.2 Applications and Previous Work

In order to alter the properties of the bilayer, proteins and peptides can be incorporated into the aqueous lipid solution, which adjusts the permeability of the bilayer to certain chemicals [11-13]. As a result, DIBs have found many recent applications including sensing [14-18] and drug delivery [19-21]. In one example, bilayers infused with alamethicin peptides will switch between discrete conductance levels in response to external stimuli including physical deformation [2, 22]. Recent work by Najem, et. al has used hydrogel-supported DIBs to support mechano-sensitive channels (MscL) that alter the conductance of the bilayer under mechanical loads [23, 24].

Recent work has investigated the use of hydrogels as a solid aqueous phase for supporting DIBs [8, 25]. Hydrogels can be formed into a variety of shapes, and can be used to support monolayers whose shape matches that of the hydrogel. This enables the creation of a new type of DIB system with complex geometries and large, robust bilayer networks [25]. In addition, the properties of many hydrogels can be altered by adjusting their concentration,

contents, or curing process, and hydrogels will also react to changes in their environment [26-30]. One recent application for hydrogel based DIBs is to create artificial hair cells that mimic the functionality of the inner ear. In these systems, a stiff fiber is embedded into one of a pair of solid, lipid-supporting hydrogels, and vibrations due to changing fluid flow over the fiber are detected by observing changes in the current through the bilayer [31-34]. Previous work has also investigated the used of hydrogels in microfabricated DIB systems [35-37]. Some hydrogels, such as Poly(ethylene glycol) dimethacrylate (PEG-DMA), can be cured by exposure to ultraviolet light, meaning that they can be easily patterned into complex or microscale shapes for supporting lipid bilayers [36].

To date, most of the work involving lipid bilayers has focused on systems with one membrane. For many applications, however, it is advantageous to create a system with an array of bilayers that can improve on the durability and functionality of single-bilayer systems [9, 38]. Several groups have worked recently to develop packaged bilayer array systems to provide a robust platform for bilayer experimentation [39-42]. These systems focus on using repeatable processes for forming long-lasting bilayer arrays for supporting channels for many applications including sensing. In some sensing systems, it is advantageous to form a network of interconnected DIBs with different types of gating properties [2, 38]. Another type of DIB array uses a microfluidic device to deposit lipid-containing droplets in compartments within an enclosed polymer substrate [43, 44]. While some methods for bilayer network formation have been explored, there has not previously been extensive work done to incorporate hydrogels as a solid aqueous phase in an array system.

1.3 Contributions

- A process is developed for photopatterning PEG-DMA hydrogels onto a flat polymer substrate. The process is inexpensive and can be performed using simple laboratory equipment including a laser printer, UV curing gun, hot plate, and simple machined substrate mold. In the future, this technique could be scaled down to produce microscale hydrogel-based bilayer arrays.
- The adhesion of patterned hydrogels to a polyurethane substrate, silver paint, and silver epoxy is investigated. Different methods of adhesion promotion are also tested.
- Conductive silver with silver chloride electrodes is patterned onto polymer substrates using adhesive masks and surface patterned indentations. Surface indentations prove effective in preventing the dried silver paint from peeling off the substrate.
- A system is developed using microchannel supported hydrogels to form arrays of DIBs. The system can be created using common laboratory techniques, and its functionality is demonstrated by forming arrays of up to three bilayers. The design and fabrication process allows for new systems with different array sizes, dimensions, and electrode patterns to be produced in a matter of hours using only a 3D printer and basic laboratory equipment.
- Reusable molds are created using additive manufacturing to form compact polymer substrates with detailed features including 230 μm diameter microchannels. These substrates support electrodes, hydrogels, and wire connections in an integrated, flexible structure.

- Experiments showed that PEG-DMA hydrogels can be supported and cured in microchannels, and reliable electrical connections can be formed by contact on a flat electrode.
- A procedure is developed for maintaining the hydration, shape, and integrity of PEG-DMA hydrogels for up to two hours while individually curing large hydrogel arrays.
- An experimental setup is developed to form and evaluate bilayer arrays formed using this method. This allows for visual observation of the bilayer formation in addition to electrical interrogation.

1.4 Thesis Summary

Chapter 2 will provide a brief overview of the theory describing the behavior of bilayers. This will form the basis for many of the measurements taken during later experimentation. This chapter will also describe the test equipment and setups used to perform experiments and collect data. The next section in this chapter discusses work done to produce a bilayer array supported by photopatterned hydrogels. The fabrication and intended bilayer formation processes are outlined along with the results from this work.

Next, a new array system is designed using microchannels in a polyurethane substrate to support hydrogels. First, a flow chart and overview of the fabrication and bilayer formation process is presented. The first system created to demonstrate this method is described in detail, and the results from single bilayer experiments are presented. Using the knowledge gained from these tests, a second, smaller scale system is developed to form larger arrays. This chapter details the fabrication process for this system and shows refinements made to each step. The

functionality of this system is demonstrated through experiments involving single and multiple DIBs.

The final chapter summarizes the results from each array system and the improvements made to their design and fabrication. Finally, the conclusions drawn from this work are presented along with some potential directions for future work.

2 Experimental Setup and Photopatterned Hydrogel Arrays

In this work, hydrogels are used to support lipid-containing aqueous droplets used to form droplet interface bilayers. This chapter discusses the methods for forming and testing bilayers and introduces the theory behind the electrical models used to describe their behavior. The experimental setups and procedures used to form and observe bilayer arrays are also presented. Finally, the fabrication process and results for a DIB array system are shown. This system is made using a pair of polymer substrates with arrays of photopatterned hydrogels designed to support aqueous lipid droplets.

2.1 Experimental Setup

2.1.1 Bilayer Electrical Modeling

Lipid bilayers are commonly represented using an electrical circuit of a resistor in parallel with a capacitor [16, 45]. Figure 2-1(a) shows a visual representation of a bilayer formed between two droplets of electrolyte solution. The electrical circuit used to model the behavior of a bilayer appears in Figure 2-1(b) and includes resistors to account for the resistance in the electrodes and aqueous phase of the system.

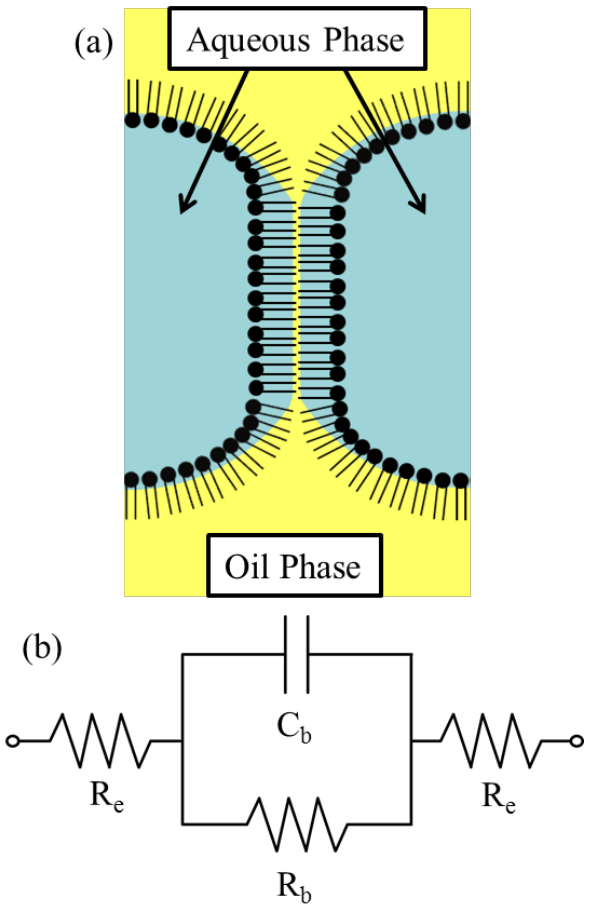

Figure 2-1. a) Droplet Interface Bilayer (DIB) consisting of two aqueous droplets surrounded by an organic solvent. LIpids assemble into a monolayer at each interface and form a bilayer between the two droplets. b) Electrical circuit representation of a bilayer. C_b and R_b represent the capacitance and resistance of the bilayer, and R_e represents the resistance of the aqueous solution and electrode.

The charge q in a capacitor is given by

$$q = CV \tag{2-1}$$

where C is the capacitance and V is the voltage applied to the capacitor. Since current is defined as the change in charge over time, the current across a capacitor is

$$i = \frac{d(CV)}{dt} = C\frac{dV}{dt} + V\frac{dC}{dt} \tag{2-2}$$

Expanding this equation to describe the bilayer by including the parallel resistor, R_b gives

$$i_b = C_b \frac{dV}{dt} + V \frac{dC_b}{dt} + \frac{V}{R_b} \tag{2-3}$$

However, the resistance of a bilayer is relatively high, so, if no DC voltage is applied to the system, the second and third terms of Equation 2-3 can be approximated as zero. Therefore, the current across a bilayer can be given as

$$i_b \approx C_b \frac{dV}{dt} \tag{2-4}$$

Because of this, bilayer formation can be verified by applying a triangle wave voltage signal between the electrodes and measuring the current response. The current response of a bilayer membrane is a square wave with an amplitude proportional to the capacitance of the system. The capacitance of a bilayer increases as the area of the bilayer increases. When two monolayers come into contact, bilayer formation begins in one point and spreads until reaching equilibrium. A square wave response whose amplitude gradually increases to a steady state indicates that the capacitance, and therefore the area, of the bilayer is growing. This pattern is characteristic of bilayer formation and is used in this work to identify and verify DIBs.

2.1.2 Test Equipment

When evaluating the array systems presented in this thesis, bilayer formation is monitored using a data acquisition (DAQ) system consisting of a Digidata 1440A digitizer (Molecular Devices) and an Axopatch 200B patch clamp (Molecular Devices). The system is operated using AxoScope software from Molecular Devices. The Axopatch system is able to take low-noise measurements from low current systems. The Axopatch amplifier has a noise floor of less than .13 pA, and the digitizer noise floor is 1 mV, or 1 pA with an amplifier gain of

1. With this gain, the system can measure differences in current as low as .3 pA. To further reduce interference from outside noise sources, all experiments are performed within a Faraday cage. Voltage signals are applied using an Agilent 33220A Wave Form Generator. For experiments presented here, a 10 Hz, 10 mV peak-to-peak triangle wave signal is used for bilayer verification. The patch clamp used for this study is only capable of recording one input at a time, so for tests involving multiple bilayers, a circuit is designed to switch between input channels. The circuit uses a high performance analog multiplexer (ADG406, Analog Devices) that can switch between up to 16 input channels. The multiplexer is controlled using digital output signals from the digitizer, which is controlled externally through the AxoScope software interface. This means that measurements can be taken from each bilayer in a system quickly and without manual interference to the system during an experiment.

2.1.3 Hydrogel Resistance Measurements

Once an array of hydrogels is fabricated, the resistance of each hydrogel and electrode is measured to detect connectivity problems and evaluate hydrogel quality. Both of the fabrication methods presented here involve a cured hydrogel on top of a silver electrode treated with chloride. The resistance in these tests is measured between the wire attached to the patterned electrode and a thin silver wire, which has been immersed in Clorox for 15 minutes, held against the top of the hydrogel where the bilayer will form.

2.1.4 Enclosed Array Test Setup

The hydrogel-based bilayer array system is designed to form droplet interface bilayers when two sets of hydrogels are moved into close proximity. In the fully enclosed system, mechanical force is used to compress the substrates and move the hydrogels together. Both the top and bottom substrates are made from flexible polyurethane and are surrounded by short walls

that contact each other when the system is assembled. When the system is compressed, these flexible walls allow the hydrogel substrates to be pushed together. To pull the bilayers apart, the pressure is simply released, and the substrates return to their original shape. The design of these substrates is discussed in greater detail in Chapter 4.

For experiments involving an enclosed array, the compressive force is applied using a 3-axis micromanipulator operated with a joystick controller. An arm is fabricated from acrylic to attach to the manipulator and apply a force evenly on the top surface of the array. A diagram of this test setup, along with a representation of the DAQ system is shown in Figure 2-2.

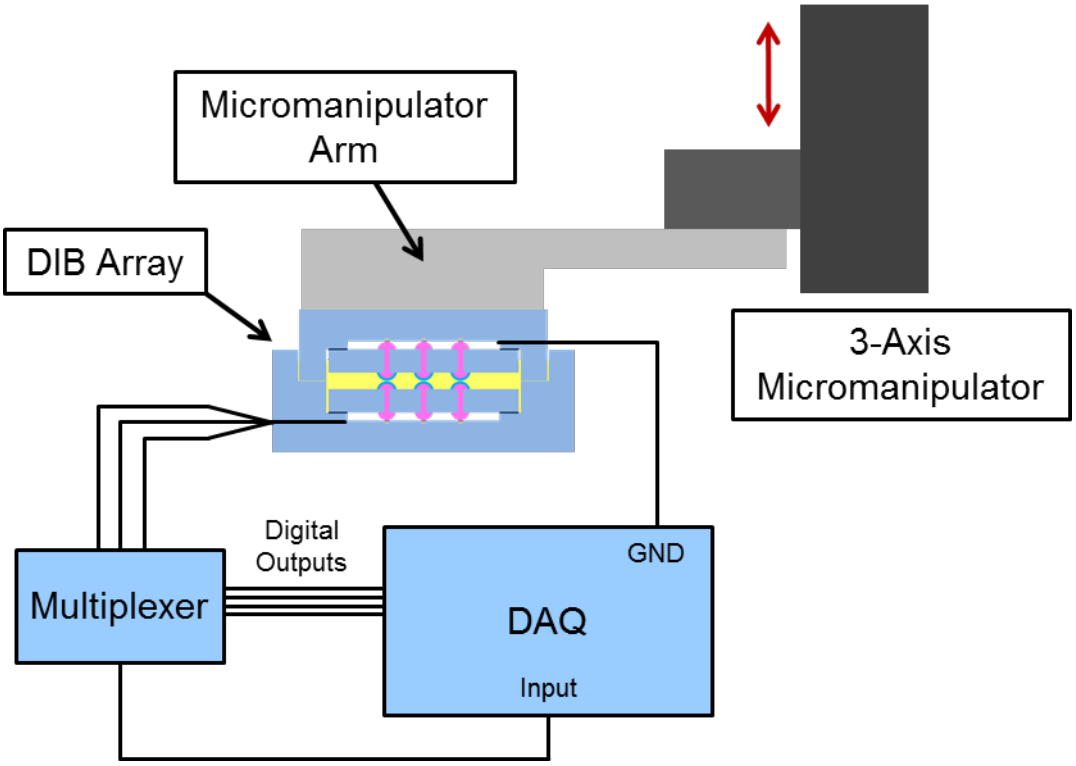

Figure 2-2. Diagram of test setup for experiments with bilayer arrays. A multiplexer controlled remotely through the DAQ system switches between input channels to take measurements from each bilayer individually. A variable compressive force can applied to the array using a 3-Axis Micromanipulator.

In Figure 2-3, the experimental setup for a three bilayer array is shown. Wires connected to each electrode on the bottom substrate are attached to separate ports on the breadboard which are wired to the inputs of the multiplexer IC chip. The three wires coming from the top substrate are all attached to ground. The manipulator arm is vertically actuated by the 3-axis micromanipulator, and the horizontal degrees of freedom allow the arm to be aligned once the array substrate is put in place.

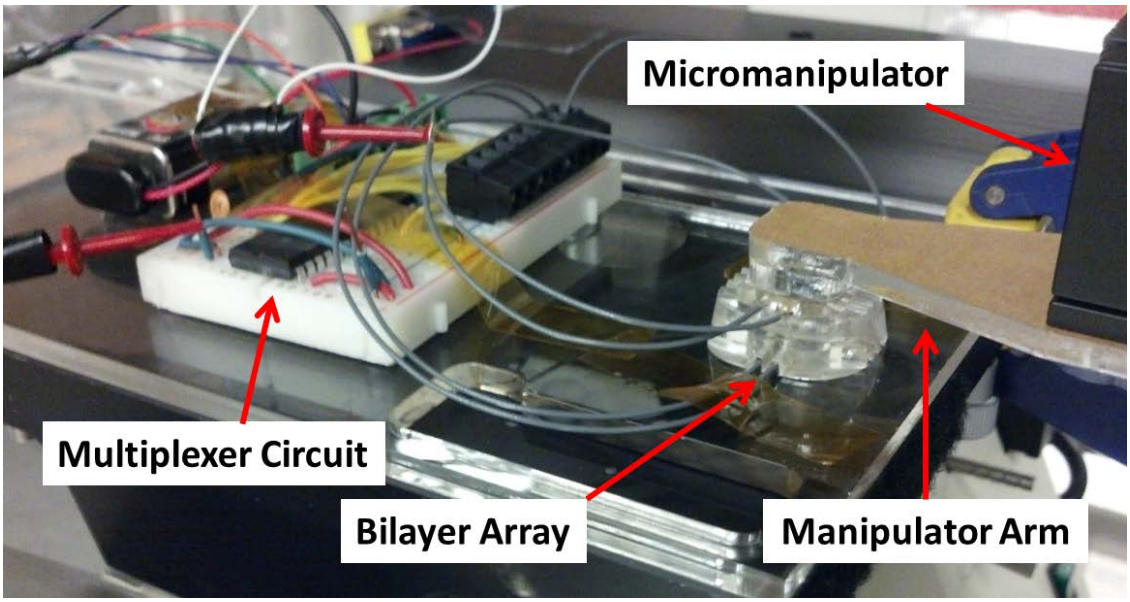

Figure 2-3. The test setup for the enclosed array system. The micromanipulator is used to push down on the array substrates forcing the hydrogels together. The multiplexer circuit used to switch between bilayers in the array is shown with connections to the DAQ system, out of frame.

2.1.5 Oil Container Test Setup

While an enclosed array is compact and shows promise for future applications, it does not allow for any direct sightlines to observe bilayer formation. To allow this, an alternative test setup is conceived that includes a separate oil container with a clear front. The bottom substrate is attached to the bottom of the container, and the top substrate is attached to the 3-axis

manipulator. This eliminates the need for walls to be built in to the substrates, and the hydrogel interactions can be viewed and recorded during a test.

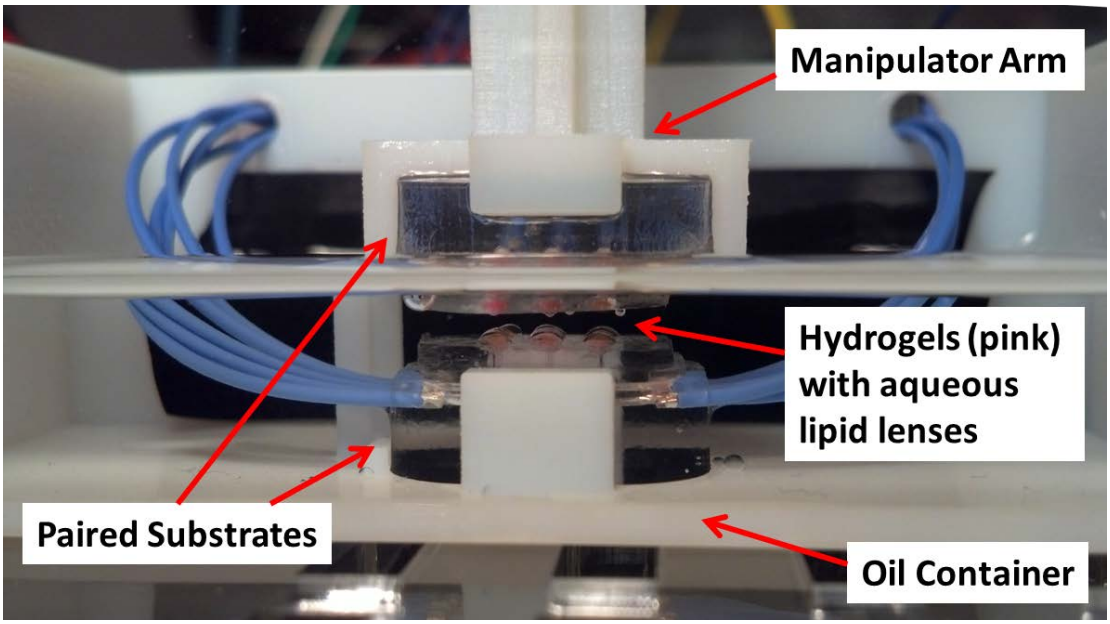

Figure 2-4. Test setup with oil container. The 3D printed container has a glass front and a slot on the bottom for attaching one of the substrates. An arm is designed to hold the top substrate and attach to the 3-axis micromanipulator. Wires are held by holes in the container above the level of the oil.

The oil container, shown in Figure 2-4, is made using a 3D printer (Objet Eden260V). It is rectangular with one open side and a slot on the bottom for holding the lower substrate of the array. A glass slide is epoxied in place on the front to complete the container. A second 3D printed piece is made to attach to the micromanipulator used in the earlier test setup. This holds the top array substrate so that its height can be adjusted when forming bilayers. The container and arm are designed to enforce horizontal alignment of the substrates as they approach. This test setup uses the same DAQ system and multiplexer circuit described in the previous section.

Eliminating the polyurethane walls around the hydrogel array means that a camera can be used to record the interactions of the hydrogels and bilayers. When using the joystick operated actuator to adjust the distance between substrates, the camera provides instantaneous visual feedback about the state of the array and the required action. This camera view is also invaluable for diagnosing problems and analyzing results.

2.2 Photopatterned Hydrogel Arrays

A system is designed to support DIB arrays using photopatterned hydrogels. The fabrication process for this system is based on common microfabrication techniques but is adapted for use with inexpensive laboratory processes. The system consists of a printed electrode pattern on a flat polyurethane substrate. Hydrogels are patterned over the electrodes by shining UV light through a photomask. The hydrogels are designed to support lipid containing droplets that will form monolayers when submerged in an oil phase. By creating two of these monolayer arrays and bringing them together, an array of bilayers can be formed and interrogated. This section will discuss details of the fabrication process along with work done toward improving and refining this system's design.

2.2.1 Materials

Poly(ethylene glycol) dimethacrylate (PEG-DMA; MW = 1000 g/mol) polymer from Poly-Sciences, Inc. is used as the hydrogel for this study, and Irgacure 2959, from Ciba is used as a photoinitiator. The hydrogel solution contains 80 % (w/v) PEG-DMA and 0.5 % (w/v) Irgacure 2959 in a solution of 500 mM KCl and 10 mM MOPS. Rhodamine B (Sigma-Aldrich; Dye content: 90%) was used with a 10 mM concentration to color the hydrogel solution.

2.2.2 Substrate Fabrication

The substrate is made using polyurethane (PU, ClearFlex 50, Smooth-On, Inc.). PU is clear and flexible, and is ideal for DIB systems because it will not absorb uncured hydrogel, the oil phase (Hexadecane), or the aqueous lipid solution. It is also hydrophobic, which means that the lipid solution will form a clearly defined lens, and it can be easily molded into complex geometries [6]. The circular substrate, shown in Figure 2-5 and represented in Figure 2-9(a), is 25 mm in diameter with a 3 mm high, 2.5 mm thick wall around the outside.

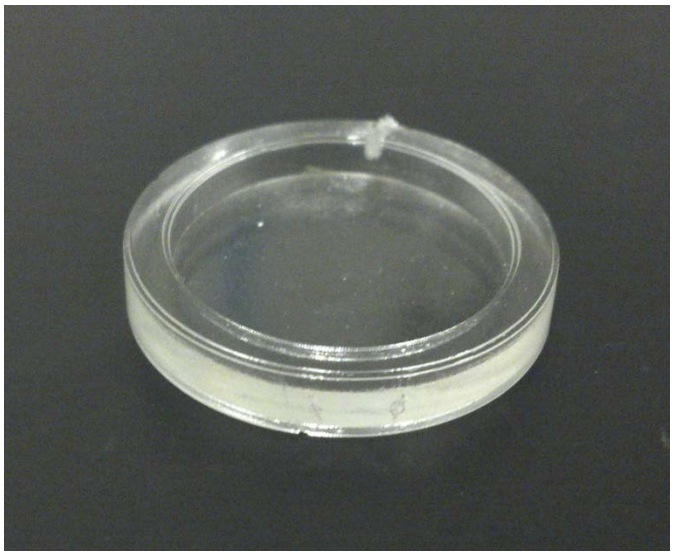

Figure 2-5. Circular polyurethane substrate designed to contain oil phase used in bilayer experiments.

The substrate is designed to contain the oil used in the DIB system. The mold is created by milling the substrate pattern into a block of acrylic. The PU is formed by mixing parts A and B in a 1:2 ratio and degassing under a vacuum. To further eliminate air bubbles, the PU is heated to 50˚C while mixing, and the mixture is heated again after degassing to temporarily reduce its viscosity for filling the molds. Mold release (Smooth-On, Inc.) is sprayed into the molds before pouring the PU so the cured parts can be easily removed. After pouring in the PU,

the molds are cured in an oven at 70˚C for one hour. Then, the substrates are removed from the molds, cleaned using de-ionized (DI) water, and heated to 80˚C for 15 minutes to evaporate any remaining oils.

Electrodes are added to the surface of the substrates to establish an electrical connection with the hydrogels. The electrodes are made by applying silver paint (Leitsilber 200, Ted Pella, Inc.) over a mask of clear tape. Silver epoxy (Chemtronics CW2400) was also tested as an electrode material, but silver paint was less viscous and proved easier to shape into detailed patterns. The tape can be used to form a variety of simple patterns for arrays of different dimensions. Figure 2-6(a) shows the substrate with the tape applied as a mask, and in Figure 2-6(b) the silver paint is applied over the mask. After allowing the paint to partially dry for about one minute, the mask is removed to leave behind the desired electrode pattern. Next, Clorox® is applied to the electrodes at points corresponding to the eventual hydrogel locations to form silver chloride. A separate mask, as shown in Figure 2-9(c), can be used for this step to more precisely apply the Chloride. The strips of silver paint for a 2x2 array before and after chloriding are shown in Figure 2-6(c) and Figure 2-6(d) respectively. In some experiments, to more securely attach the hydrogels to the PU, an adhesion promotor (trimethylsilyl methacrylate 98%) is added to the substrate and allowed to dry at 80˚C for 15 minutes.

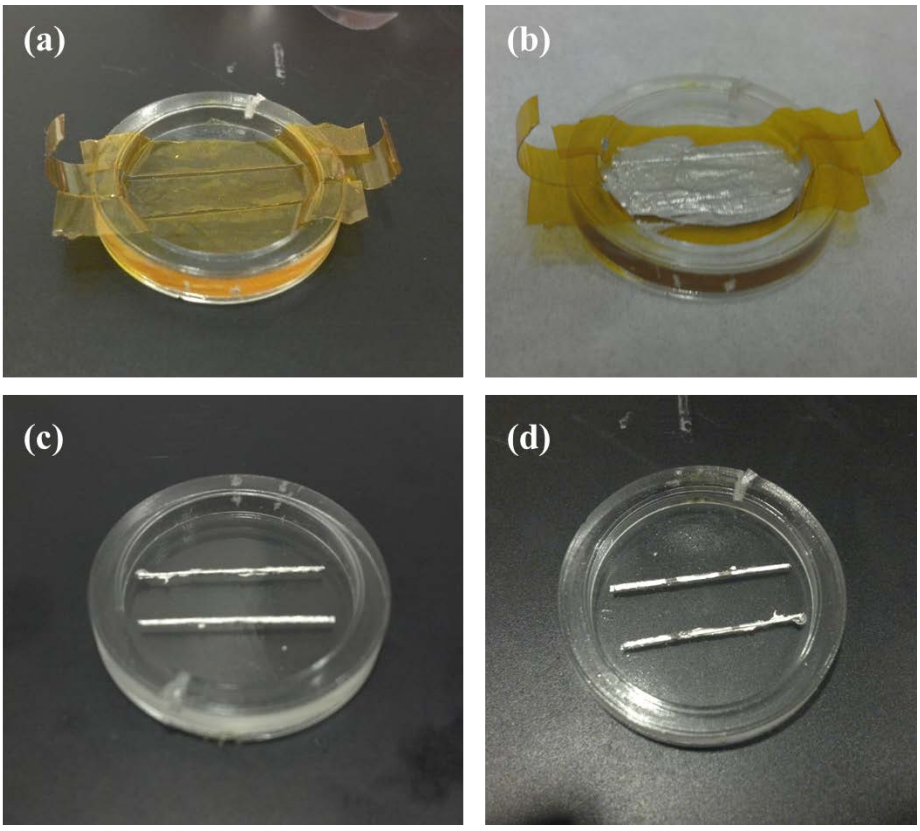

Figure 2-6. (a) Strips of tape applied as a mask to surface of substrate. (b) Silver paint applied to substrate over mask. (c) Thin, silver electrodes after removal of tape. (d) Electrodes with 2x2 pattern of chlorided patches.

2.2.3 Hydrogel Patterning

Hydrogel solution is added to the substrate on top of the dried silver paint electrodes so that each of the Silver Chloride connection points is covered. A glass slide covered in a thin film of polyethylene (PE) is then placed over the hydrogel so that the top of the hydrogel droplet is flat against the slide. The PE allows the glass slide to be easily separated from the hydrogel after curing. A photomask with the desired hydrogel array pattern is printed using a laser printer and placed on top of the glass slide, as demonstrated in Figure 2-9(d). Each hydrogel is cured through free-radical polymerization by exposure to 365 nm ultraviolet light for three minutes at an intensity of 1 W using a spot UV source (LED-100, Electro-Lite Corp.) Figure 2-7 shows the

spot source being used to cure hydrogels in a 2x2 array. While using a spot UV source, it is necessary to cure each hydrogel individually through the holes in the mask to ensure a consistently intense beam of light perpendicular to the substrate.

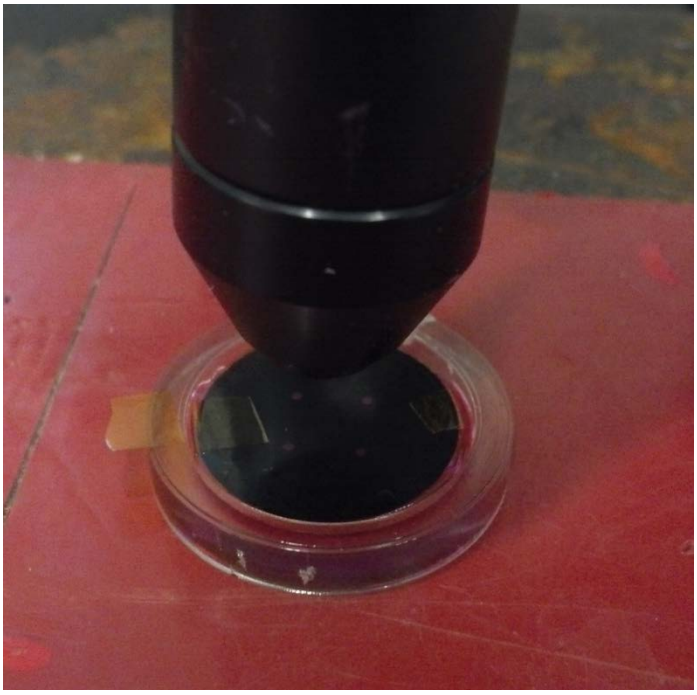

Figure 2-7. Hydrogels in the process of curing for a 2x2 array. A printed mask over a glass slide covers the liquid hydrogel on the substrate.

Once curing is completed, the mask is removed, and the remaining hydrogel is removed using DI water. Hydrogels of any shape can be patterned using this method, limited only by the resolution of the laser printer. In this experiment, masks with circular holes were used to form arrays of cylindrical hydrogels as shown in Figure 2-8. The hydrogels shown in the figure are 1 mm in diameter and 2 mm in height.

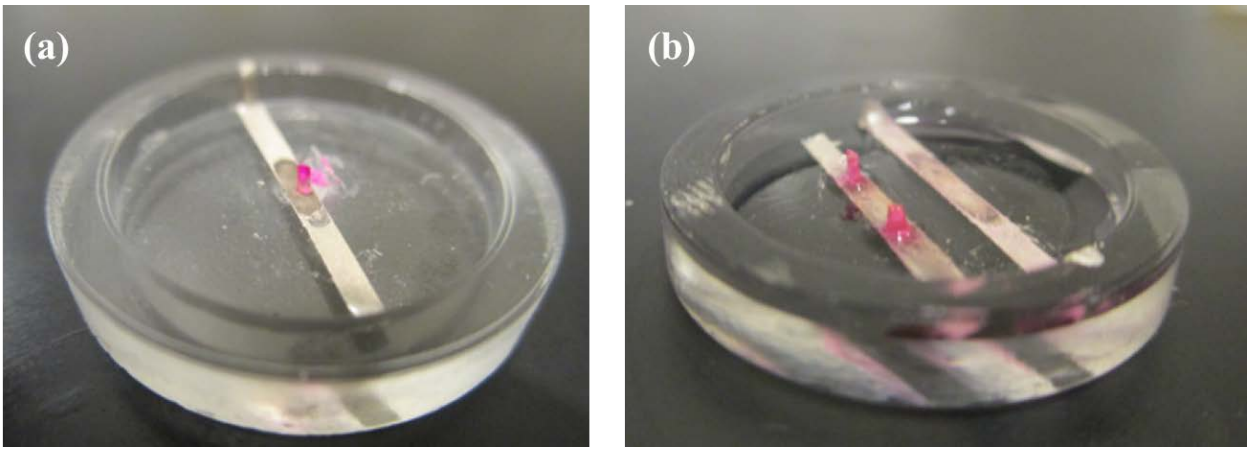

Figure 2-8. (a) Single cured hydrogel on a polyurethane substrate with silver electrode strip. (b) 2x1 hydrogel array on a substrate with two electrodes.

2.2.4 Bilayer Formation

Figure 2-9 shows the fabrication process for an array of hydrogels patterned over a set of silver electrodes. In order to form bilayers, a droplet of lipid solution (prepared with DPhPC, Avanti Polar Lipids, Inc.) would be added to each hydrogel on two substrates. The bottom substrate would be filled with oil (Hexadecane), and the two substrates would be brought together until the two sets of droplets come into contact and form bilayers. The electrodes would allow the current across each bilayer to be monitored.

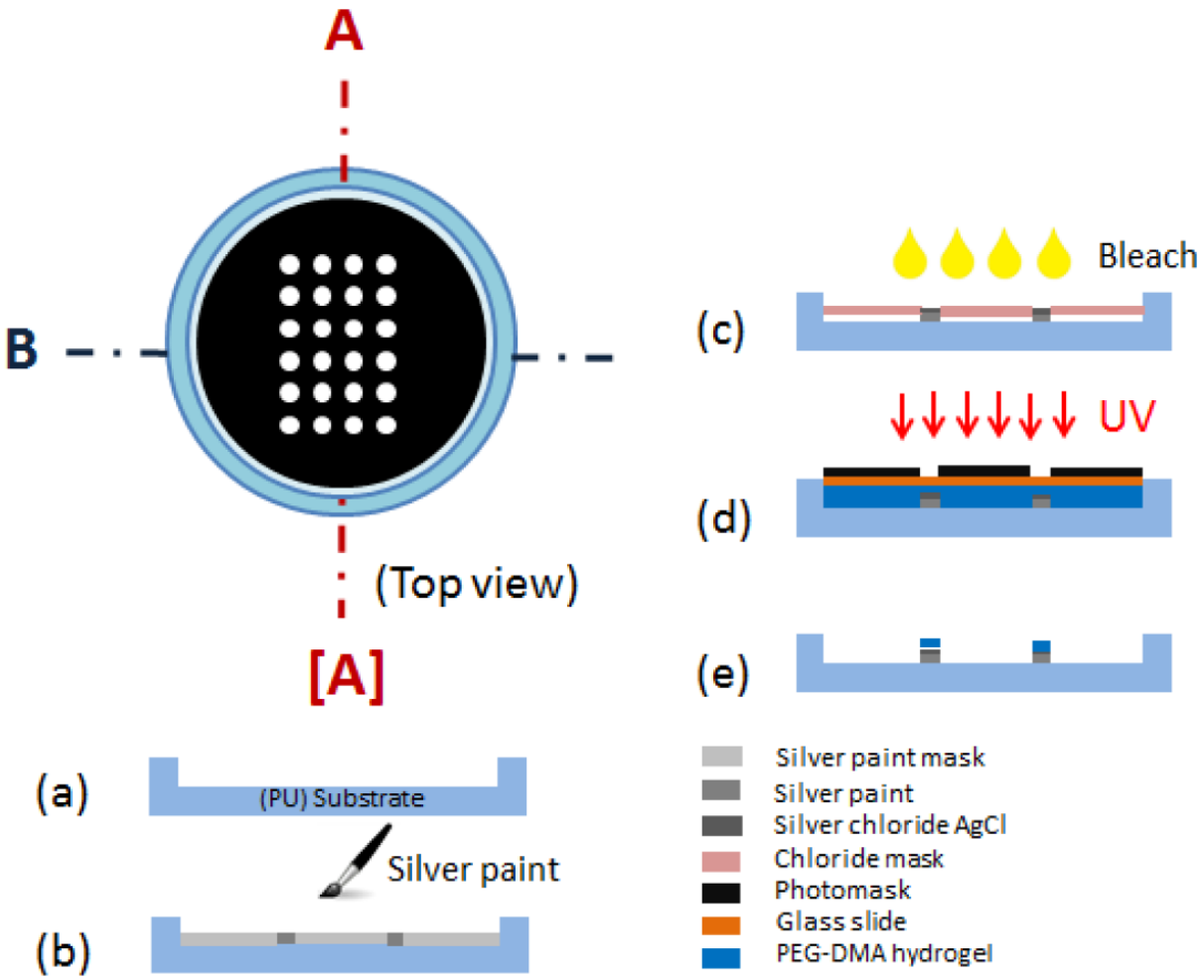

Figure 2-9. Cross-sectional flow chart of hydrogel fabrication process. (a) Polyurethane substrate prepared using mold. (b) Silver paint electrodes patterned using mask. (c) Sections of electrodes treated with chloride to form Silver Chloride connection points. (d) PEG-DMA hydrogel deposited onto substrate and cured by shining UV light through a photomask. (e) Excess hydrogel removed to reveal finished array. This figure was originally presented by Najem, et al. [36]

2.2.5 Results

The fabrication method presented in this section is demonstrated by producing the small hydrogel arrays shown in Figure 2-8. These arrays can be produced using simple, low cost laboratory processes. Once the mold is fabricated, it can be used to create many substrates with

minimal preparation time. Electrode material is deposited, patterned, and chlorided by hand. Photomasks are made using a laser printer on a transparency slide. As a result, this process saves significant time and cost when compared with traditional photolithography techniques. While experimenting with this array system, several fabrication steps and design factors were altered in order to improve and refine the process.

Before curing the hydrogel with UV light, the hydrogel is deposited on the substrate manually. However, due to the hydrophobic nature of the polyurethane surface, the aqueous hydrogel solution forms into a rounded droplet. This means that spin coating is impractical for this system as the droplet maintains its shape and resists spreading. The solution for this problem is to place a glass slide over the hydrogel at a uniform distance from the substrate. This allows the UV light to reach the hydrogel with minimal diffraction while ensuring that the cured hydrogels have the desired height. One factor that must be considered with this solution is the adhesion between the cured hydrogels and the glass. In some cases, when the glass slide is removed after curing, the hydrogels attach more firmly to the glass slide and are pulled from the substrate. This problem can be eliminated by treating the substrate to improve adhesion or by altering the glass surface to ensure separation. Care must also be taken to prevent contamination of the hydrogel from contact with the glass slide.

While the hydrophobic nature of polyurethane makes it ideal for droplet formation in DIB systems, it also tends to hinder strong adhesion with hydrogels. The silver paint also does not adhere strongly to the substrate, so curing the hydrogels completely on top of the electrodes, as shown in Figure 2-8, does not alleviate this problem. The use of an adhesion promotor (trimethylsilyl methacrylate 98%) is not found to significantly improve the bond between the hydrogels or electrodes and the substrate. While the hydrogels will temporarily stick to the

substrate, they will swell when water is added, causing stress at the interface with the polyurethane. This could be avoided by using PEG-DMA instead of polyurethane as a substrate material, so the substrate swells and contracts at the same rate as the bilayer supporting hydrogel. However, this proves impractical as a PEG-DMA substrate does not reliably maintain its shape, and using one sacrifices the benefits of a hydrophobic polyurethane surface.

During experimentation, many of the cured hydrogels were observed to have slightly tapered sides, with a larger diameter at the base, rather than vertical as would be expected when using a collimated UV source. The spot source used (LED-100, Electro-Lite Corp.) is not completely collimated, but, for small hydrogels such as the ones in this study, near vertical walls would be expected. The observed tapering appears to result from a slight diffraction as the light passes through the mask, glass slide, and hydrogel. Further study would be needed to more precisely predict the final shape of the hydrogels.

3 Microchannel Supported Hydrogel-Based Bilayer Arrays

As an alternative to bonding hydrogels directly to a flat substrate, a new array system was designed to physically fix each lipid-supporting hydrogel in place. This system is similar to the photopatterned system in that is consists of two opposing arrays of hydrogel supported lipid lenses which are brought together to form bilayers. Each opposing hydrogel array consists of two separate polyurethane substrates. One substrate supports a planar electrode pattern with outside electrical connections and the other holds photopolymerized hydrogels in an array of microchannels. On one end of the microchannels, the hydrogels are pressed against the silver chloride electrodes, and the other end is exposed to the oil phase to allow for the formation of a lipid monolayer lens.

Like the previously presented photopatterned array system, the substrates are molded from polyurethane, electrodes are patterned using silver paint, and PEG-DMA hydrogels are deposited and cured with UV light. Because the hydrogels are physically restricted from moving, this design eliminates the problems with adhesion to the substrate and results in a more robust, stable system.

3.1 Array Formation Process Overview

The first step in the fabrication process is to use 3D printed molds to form the polyurethane substrates. The base substrate, shown in Figure 3-1(a), consists of a flat surface with slight imprints marking the desired electrode pattern. These patterns are surrounded by walls which form a container. Silver paint is patterned into the indentations to form the electrodes, and a patch at the end of each electrode is treated with chloride to form silver chloride. These patches are used to electrically connect with the hydrogels. A cross sectional view of the substrate at this point is shown in Figure 3-1(b). Next, a second polyurethane

substrate is formed, consisting of a flat, rectangular base with an array of microchannels. This microchannel substrate, Figure 3-1(c), is used to support the hydrogel structures. Aqueous hydrogel is added with a syringe to the top end of each microchannel. Capillary forces pull the solution through the channels while leaving a relatively large droplet on top of the substrate. The hydrogel is then cured by exposing it to UV light, as shown in Figure 3-1(d). After each hydrogel is cured, a drop of non-curing PEG-DMA solution is deposited on the top end to avoid dehydration. In Figure 3-1(e), the substrate is flipped over, and the curing process is repeated on the opposite end of each channel. Each hydrogel is now fixed in place with two droplets connected through a channel. After all the hydrogels are cured, the excess PEG-DMA solution is removed, and the microchannel substrate is attached to the base substrate with cyanoacrylate adhesive. This process, shown in Figure 3-1(f), holds the bottom ends of the hydrogels onto the electrodes and forms a seal around the outside edge of the electrodes.

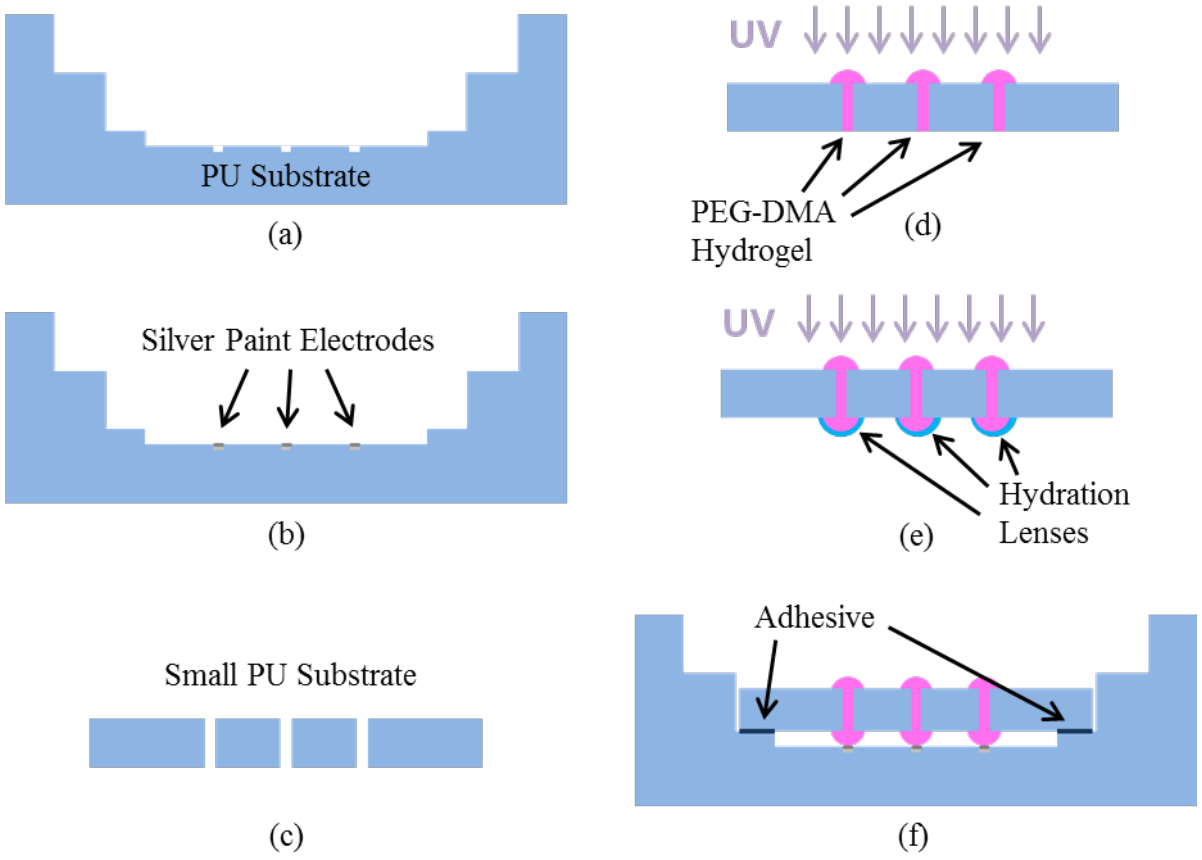

Figure 3-1. Cross-sectional view of steps in substrate fabrication process. (a) A polyurethane substrate is made with an indented electrode pattern and walls to contain the hexadecane oil. (b) Electrodes are formed using silver paint, and patches of silver chloride are added to connect to hydrogels. (c) A smaller PU substrate is made with an array of microchannels. (d) PEG-DMA hydrogel is added to the channels and cured with UV light. (e) Liquid PEG-DMA solution is added to cured hydrogels to maintain hydration. The substrate is then turned over, and more hydrogel is added and cured. (f) Excess PEG-DMA is removed, and the microchannel substrate is attached to the larger substrate with electrodes.

In conjunction with this substrate fabrication process, a second substrate is made using the same method. The only difference is that the second substrate does not include the outer container walls and is designed to fit snugly into the first. Once the substrates are fabricated, the base substrate is filled with hexadecane oil until the hydrogels are submerged. As shown in Figure 3-2(a), the adhesive seal prevents the oil from interfering with the connection between the

hydrogels and electrodes. Next, as shown in Figure 3-2(b) and (c), a syringe is used to deposit a droplet of aqueous lipid solution onto the top of each hydrogel in the base substrate, and the second substrate is flipped and inserted into the first. Then, in Figure 3-2(d), the entire system is compressed to push the hydrogels closer together until the lipid solution droplets spread between the pair. In Figure 3-2(e), the compression is released, allowing the hydrogels to separate. This separation pulls the coalesced droplet apart so that both the top and bottom hydrogels hold a droplet of lipid solution. The system is allowed to rest so that the lipids in the solution can assemble at the oil-water interface and form a monolayer. After five minutes, the system is gradually compressed again until the opposing droplets come into contact, as shown in Figure 3-2(f). At this point, the bilayers will gradually form and grow in area, and the process can be observed by using an Axopatch system to view the current response.

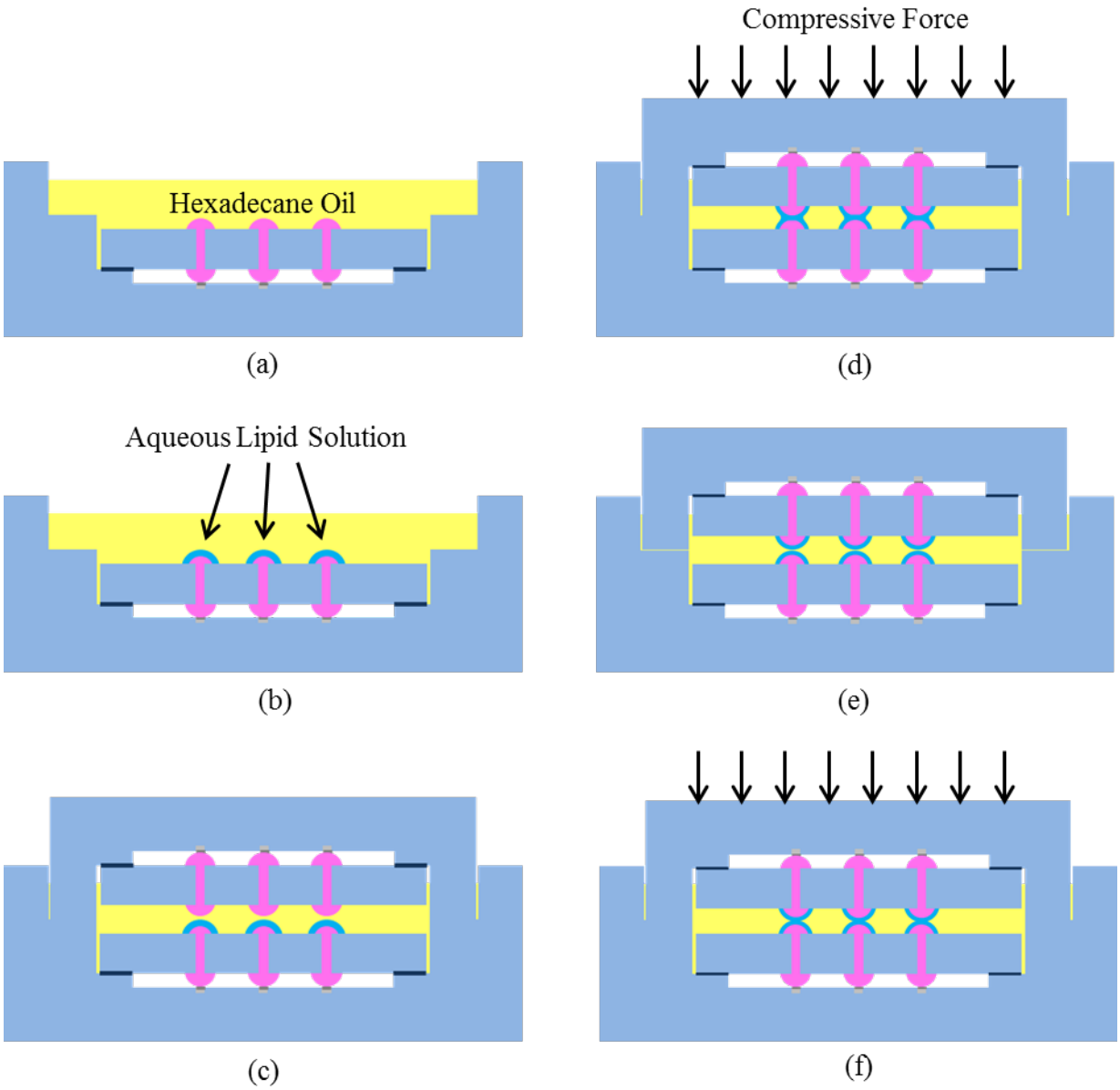

Figure 3-2. Bilayer Array Formation Process. (a) The base substrate is filled with hexadecane to submerge the tops of the hydrogels. (b) Droplets of aqueous lipid solution are added to each hydrogel. (c) The second substrate is placed on top of the bottom substrate so that the two sets of hydrogels face each other. (d) The substrates are compressed together so the lipid solution spreads between the two sets of hydrogels. (e) When the compressive force is released, the hydrogels move apart, separating the coalesced droplets. Lipid monolayers form around each hydrogel-supported droplet. (f) The substrates are pushed together again until the opposing monolayers come into contact and form bilayers.

3.2 Substrate Design

Two different types of polyurethane substrates are used to support this hydrogel array system. One holds a reconfigurable silver electrode pattern with built in wire connection points. In fully enclosed systems, this substrate will also form a container to hold the oil used in bilayer formation. When a larger external container is used, the piece is designed to fit snugly at the bottom of the container or to an actuated arm while allowing a direct line of sight to the hydrogels. The other, smaller substrate holds the hydrogels using an array of microchannels. Once the hydrogels are cured, this substrate is attached to the larger substrate so that the portions of the hydrogels protruding from the bottom of the channels contact the patterned electrodes. The ends of the hydrogels facing away from the electrodes are exposed to the oil phase and can be used to support lipid monolayer lenses.

To create more detailed and smaller hydrogel arrays, additive manufacturing is used to create molds for the polyurethane substrates. This method provides for the fabrication of precise, detailed features and parts with fewer design restrictions than milling. Parts can be made quickly, and, since only a CAD model is needed, substrate molds can be reconfigured with minimal turnaround time. A 3D printer (Objet Eden260V) is used in these experiments to form molds from VeroWhite, a hard, opaque plastic, similar to acrylonitrile butadiene styrene (ABS). Two surface finishes can be produced by this printer, and both are used in molds for this system. A glossy finish results in a smooth surface with slightly rounded edges that ease the removal of cured substrates. A matte finish is achieved by printing a layer of support material around the surface of the object and creates sharper edges that more precisely replicate the original 3D model.

3.3 Large Scale Enclosed System

3.3.1 Fabrication and Assembly

The first system created with this design was an enclosed 3x3 array 42 mm on a side and 14 mm tall. While the eventual goal was to form arrays at smaller scales, a system of this size leaves a greater margin for error and is easier to work with in a laboratory setting. To form an enclosed array, one electrode supporting substrate is designed with a large wall around the edge. The opposing substrate is designed to fit snugly into the first when turned upside down.

These polyurethane substrates are fabricated using the 3D printed molds in Figure 3-3. In order to create features on the top and bottom of the substrate, a two part mold is used. The base mold forms the shape for the outside of the array system and indentations for epoxy connections to the electrodes. Where possible, features are tapered to facilitate easy removal of the cured substrate. A top mold is designed to sit on top of the bottom mold and creates the inner bowl of the substrate, including the electrode pattern. When the two parts are assembled, the ends of the electrodes contact the epoxy indentations forming a through-hole in the finished substrate.

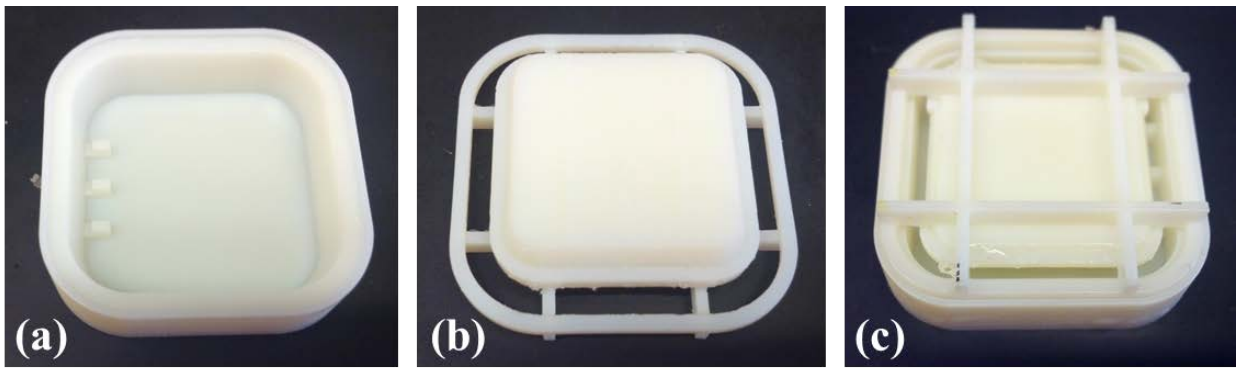

Figure 3-3. (a) Base mold with pattern for three epoxy connection indentations. (b) Top mold piece to form oil container shape. The surface of this piece is also used to imprint the electrode pattern into the substrate. (c) The two mold pieces assembled and ready to receive polyurethane.

The polyurethane used to make these substrates is made using the process presented in Chapter 3. As seen in Figure 3-3(c), a gap between the two mold parts is used to add liquid polyurethane. After filling the mold, a weight is added to prevent the top piece from rising, and the assembly is heated in an oven for one hour.

The polyurethane substrate produced by this mold is shown in Figure 3-4. The substrate in this figure is shown after the electrodes have been patterned and the wires have been attached with epoxy.

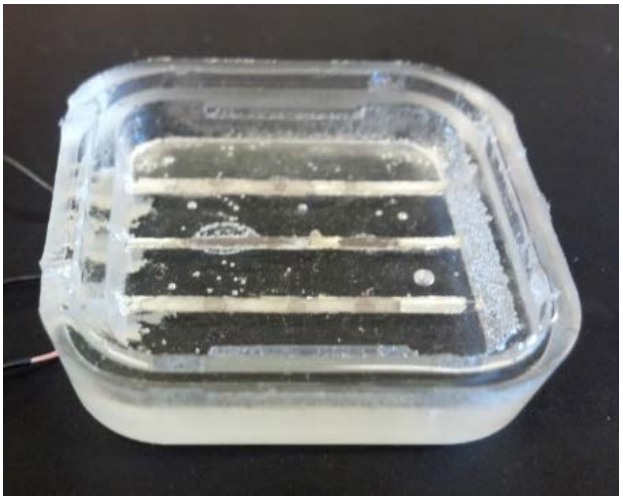

Figure 3-4. Large scale base substrate with wires attached with epoxy and three electrodes painted on the bottom.

The shape of the inner basin of this base substrate is designed to snugly accommodate the microchannel substrate holding the hydrogels. The base substrate also includes a pair of tabs to hold the hydrogel substrate in place and press the bottoms of the hydrogels into the electrodes.

After the substrate is cured and cleaned, a small amount of silver epoxy (Chemtronics CW2400) is used to fill the holes at the ends of the electrodes. Once this epoxy partially dries,

additional epoxy is used to attach wires to the edge of the substrate. Next, the electrodes are patterned onto the substrate surface using a tape mask. The epoxy plugs electrically connect the electrodes to the epoxied wires and ensure that none of the oil used to fill the substrate at a later stage can escape.

A second substrate, shown in Figure 3-5, is fabricated using a similar method as the base. The dimensions of the inner basin and electrodes are identical to the first substrate, but there is no second wall for containing oil.

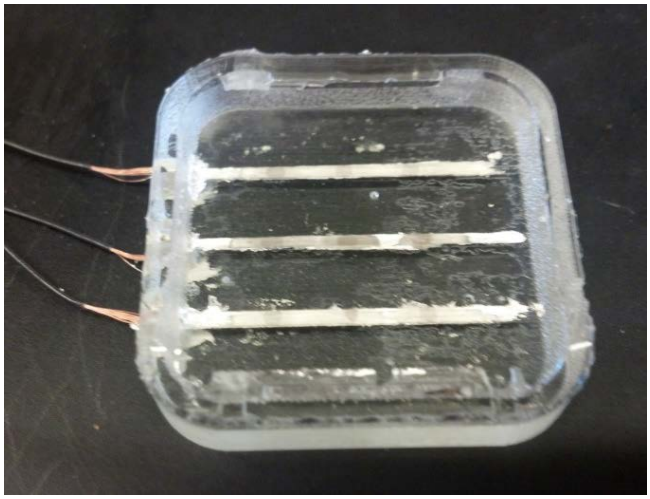

Figure 3-5. Large-scale top substrate, complete with patterned electrodes with epoxied wire connections.

Figure 3-6 shows the mold used for forming the smaller substrates with an array of microchannels for supporting hydrogels. The assembled mold consists of two 3D printed pieces bolted together with a cork gasket between them. An array of small holes on each part of the mold is used to support segments of .009 in. (230 μm) music wire (Malin Co.) which form the channels. While the molds for the base substrates used a glossy finish to ease part removal, this mold is made with a matte finish so that the ends of the channels are more precisely shaped.

When a glossy mold was used initially, some of the resulting microchannels had small outcroppings or imperfections at the edges. This interfered with the filling of the channels with hydrogel, and would occasionally produce bubbles, preventing an electrical connection through the channel. The mold is also designed to stand upright so that any bubbles left in the polyurethane after degassing can escape through the open top of the mold instead of collecting on the surface of the substrate.

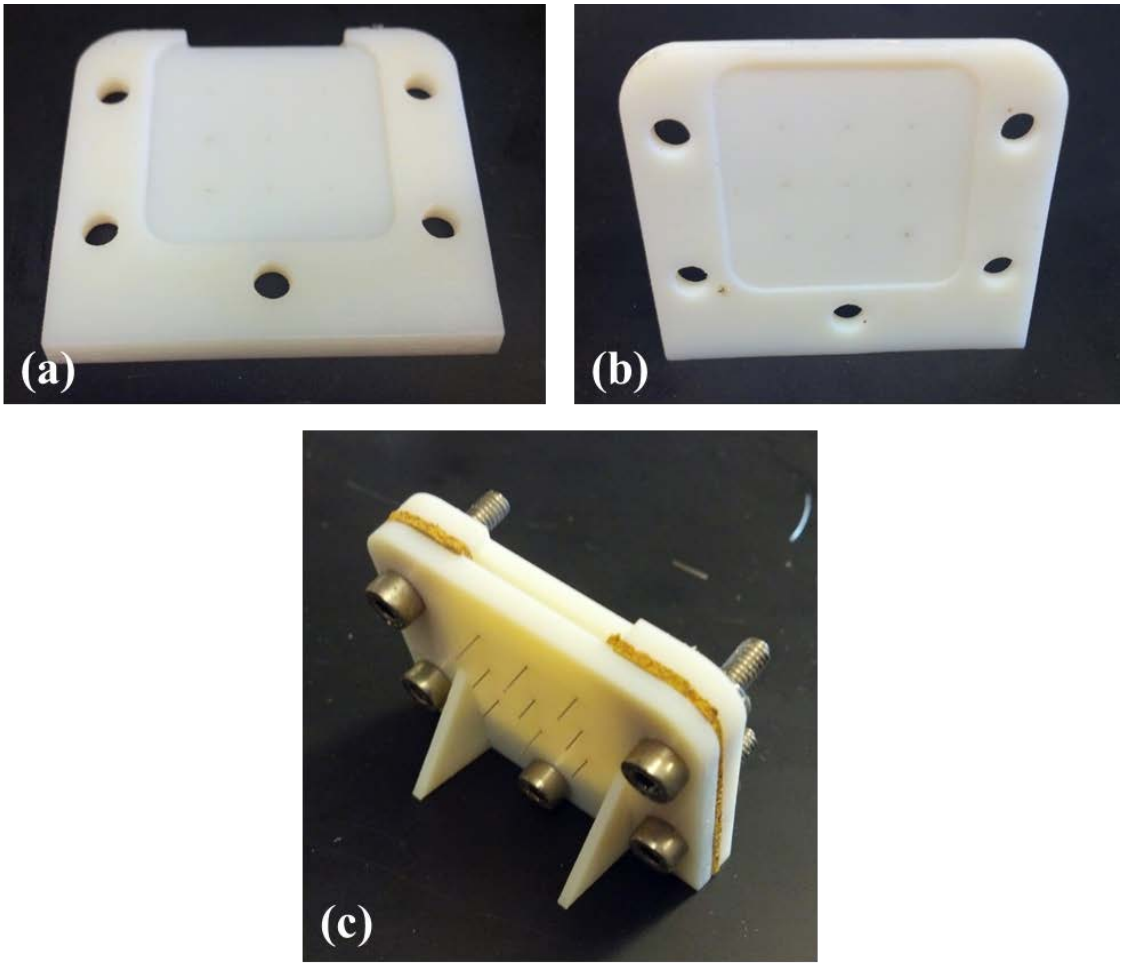

Figure 3-6. Mold for forming microchannel substrate. (a) and (b) 3D printed mold pieces defining shape of substrate. Each of these parts also includes a 3x3 array of small holes. (c) The two parts are assembled using bolts with a piece of cork as a gasket. Wires are inserted into each set of small holes to form microchannels in the polyurethane substrate.

After the polyurethane filled mold is allowed to cure in an oven, the wires are removed, and the mold is disassembled to release the finished microchannel substrate. Once the substrate is cleaned, it can be used to support a hydrogel array.

The PEG-DMA used in this system is prepared using the method presented in the previous chapter. However, it is made with a concentration of 40% w/v instead of 80% to reduce curing time. A droplet of aqueous hydrogel is deposited on top of each channel in the array, and then cured in turn with UV light. The substrate is then flipped, and the process repeated to form solid hydrogels held in place by the microchannels. Next, a droplet of lipid solution is added to the top side of each hydrogel. The substrate is then placed on the base substrate so that the tabs hold it in place and press the hydrogels into the electrodes. Figure 3-7 shows the substrates after this step. Although the two substrates in this figure are designed to support a 3x3 array, only the three channels along the top row are filled with hydrogels.

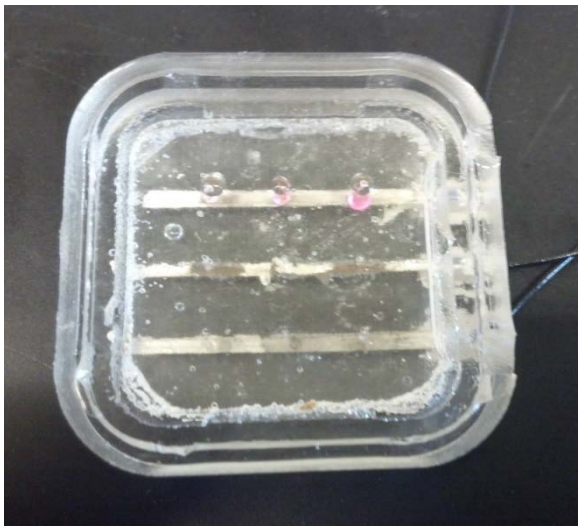

Figure 3-7. Base and microchannel substrates assembled. This system includes three hydrogels, all along the top row of the 3x3 array.

The final step in the array assembly process is to make a second set of substrates with a set of hydrogels to match those on the first substrate. The second set is turned to face downward and placed into the first set. Figure 3-8 shows the fully assembled array system with a second substrate set inserted into the substrates from Figure 3-7.

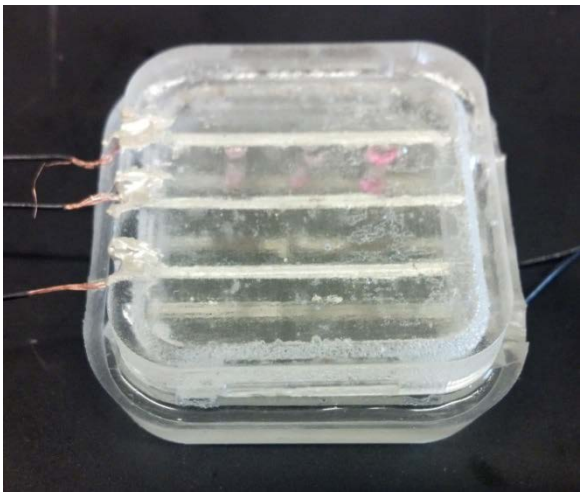

Figure 3-8. Assembled pair of opposing hydrogel arrays.

3.3.2 Micropipette supported hydrogel test

Before running experiments with a fully assembled array system, a test is set up to form a bilayer using one part of the hydrogel array with a second hydrogel supported monolayer formed using a micropipette. The method for forming the micropipette based monolayer is similar to the one developed by Najem, et al. [46] Using a proven method for supporting one of a pair of monolayers, the behavior of one hydrogel supported by one set of substrates can be observed. Replacing the top substrate also allows for visual inspection of the bilayer formation interactions.

3.3.2.1 Test Setup

First, the bottom portion of the array system is fabricated using the method shown above. For this test, the polyurethane substrates for a 3x3 array are created, but only one of the microchannels is filled with hydrogel.

A borosilicate flat tip micropipette with an outer diameter of 1 mm and an inner diameter of 0.5 mm is used in this experiment. The micropipette is filled with aqueous PEG-DMA hydrogel, and a Silver Chloride wire is inserted as an electrode. The hydrogel is cured with UV light to fix the electrode in place and form a solid support for a lipid solution droplet. The micropipette is then attached to a manually operated micromanipulator, and the silver wire is connected to ground.

Once the hydrogels are prepared, the base substrate is filled with hexadecane oil so that the top of the hydrogel is submerged. The wire connected to the hydrogel is attached to the Patch Clamp, and the substrate is placed under a microscope inside a Faraday cage. As shown in Figure 3-9, the micropipette is oriented to point downwards at an angle toward the hydrogel in the base substrate. The micromanipulator can be used to finely alter the distance between the end of the pipette and the base hydrogel. This microscope setup is not ideal for capturing images of the bilayer formation because an opaque silver electrode blocks light from underneath the substrate and the bilayer plane is at a diagonal to the vertical line of sight. However, the visual feedback is useful during the experiment for adjusting the micropipette position to form a bilayer.

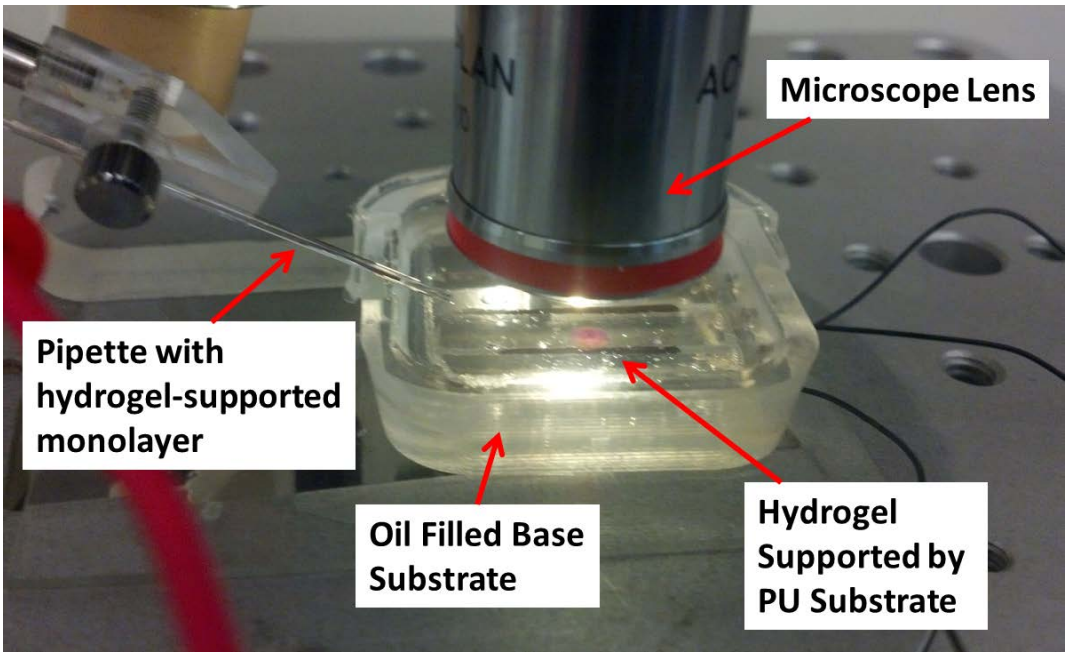

Figure 3-9. Image of the test setup using one half of an enclosed hydrogel based array system. A pipette is used in place of the opposing half of the array to support a hydrogel based lipid lens.

Once the setup from Figure 3-9 is assembled, a droplet of lipid solution (DPhPC) is added to the hydrogel on the base substrate. The micropipette is then moved towards the hydrogel until the end touches the droplet forming a single, coalesced droplet between the two hydrogels. The hydrogels are then separated so that each one has a droplet of lipid solution and the micropipette droplet is still submerged in the oil. Five minutes are allowed for the lipids to self-assemble into a monolayer lens around each droplet. Then, using the magnified image as a guide, the pipette is maneuvered towards the base hydrogel until the droplets begin to touch. As the bilayer forms, the current is measured and recorded by the Axopatch.

3.3.2.2 Results

This test setup demonstrates the array system's ability to form and evaluate lipid bilayers. Only one bilayer is formed at a time, and, out of ten attempts, only one successfully produced a

bilayer. However, the test shows that a stable bilayer can be formed and verified. The low success rate is mainly caused by the viewing angle of the pipette interacting with the hydrogel based monolayer. This means that, in these cases, the bilayer may begin forming before the operator is aware and before a clear current response appears. In each of the failed attempts, the droplets coalesced before and clear signal appeared to represent a forming bilayer. As outlined in Chapter 2, bilayers are detected by applying a triangle wave voltage signal between the electrodes and seeing a square wave voltage response. Since the bilayer behaves as a capacitor, the amplitude of the response signal depends on the area of the bilayer. As the bilayer forms, it gradually increases in size, so a square wave current of steadily increasing amplitude indicates that a bilayer is forming. Figure 3-10 shows the response observed during this test.

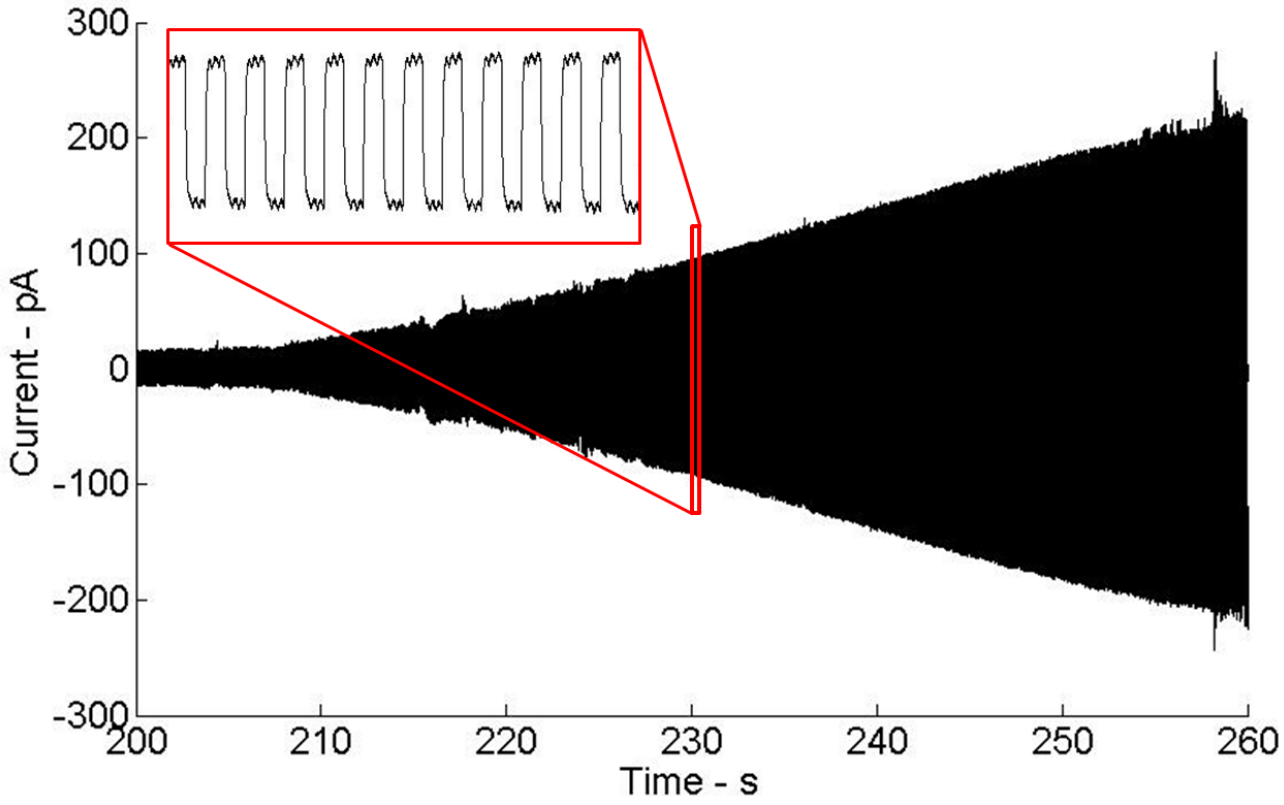

Figure 3-10. Plot of current response of substrate-pipette bilayer system to a 10 Hz triangle wave voltage input. Red outlined insert shows current over about one second.

The smaller image outlined in red shows the waveform of the signal and includes just over one second of data. The frequency of the signal is 10 Hz, the same as the input, and, apart from a small amount of 60 Hz noise, the signal closely matches the expected square waveform. The larger plot shows the increasing amplitude of this wave as the bilayer slowly forms over a 60 second period.

While DIBs had previously been created using hydrogels, this test demonstrated the feasibility of this substrate design and fabrication process. The result shows that a strong electrical connection can be formed by pressing the hydrogel into a painted electrode and that the

substrate and wiring design result in a consistent connection and a sealed container for oil. Although the bilayer was only formed briefly, its electrical response closely matches the expected current response. This test indicates that the system design is promising, and the next step is to use the complete enclosed array system.

3.3.3 Single Bilayer Enclosed System Test

The next test is to use the second half of the enclosed array system to further demonstrate the viability of this design. In this experiment, the base substrate, shown in Figure 3-11(a), is the same as the one used in the previous test. The second substrate, in Figure 3-11(b), uses the same electrode design as the base, but its outer dimensions match the dimensions of the inside of the larger bottom substrate. Both microchannel substrates are identical, and as in the last test, one hydrogel is fabricated in a 3x3 array. Two of the corners of the top substrate are trimmed to allow excess air to escape when the system is assembled, preventing spillage of the hexadecane.

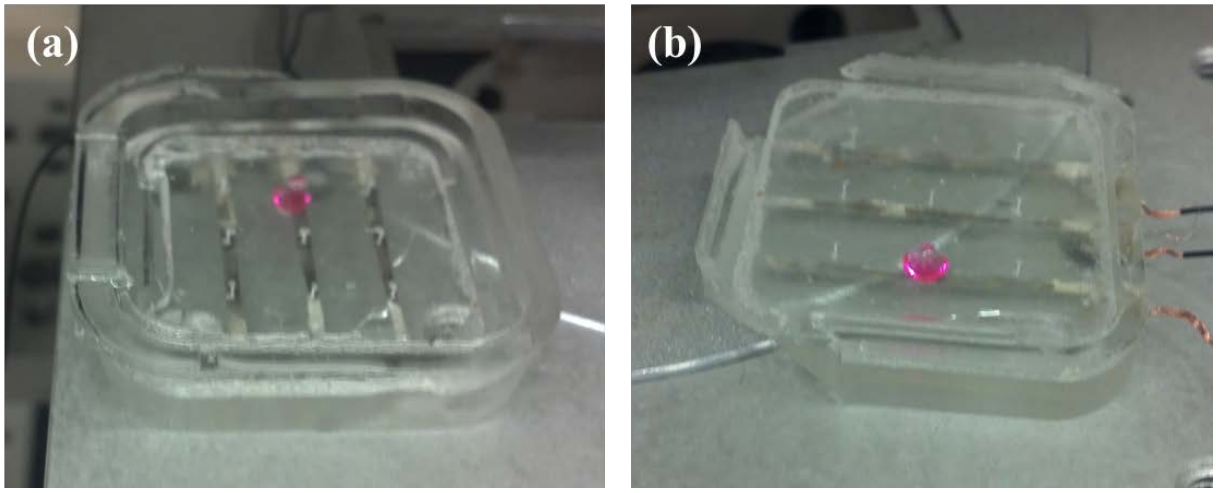

Figure 3-11. (a) Base substrate with one out of nine channels filled with a cured hydrogel. (b) Top substrate with one cured hydrogel placed directly opposed to the hydrogel in the base substrate.

3.3.3.1 Test Setup and Procedure

Once the substrates and hydrogels are fabricated, the base substrate is filled with oil, and a droplet of lipid solution is added to the hydrogel. The top substrate is turned over and placed into the base so that the two hydrogels are vertically aligned but not in contact. Once the electrical connections are made, one electrode to the patch clamp and one to ground, the system is aligned underneath the manual micromanipulator used in the previous experiment to hold the micropipette.

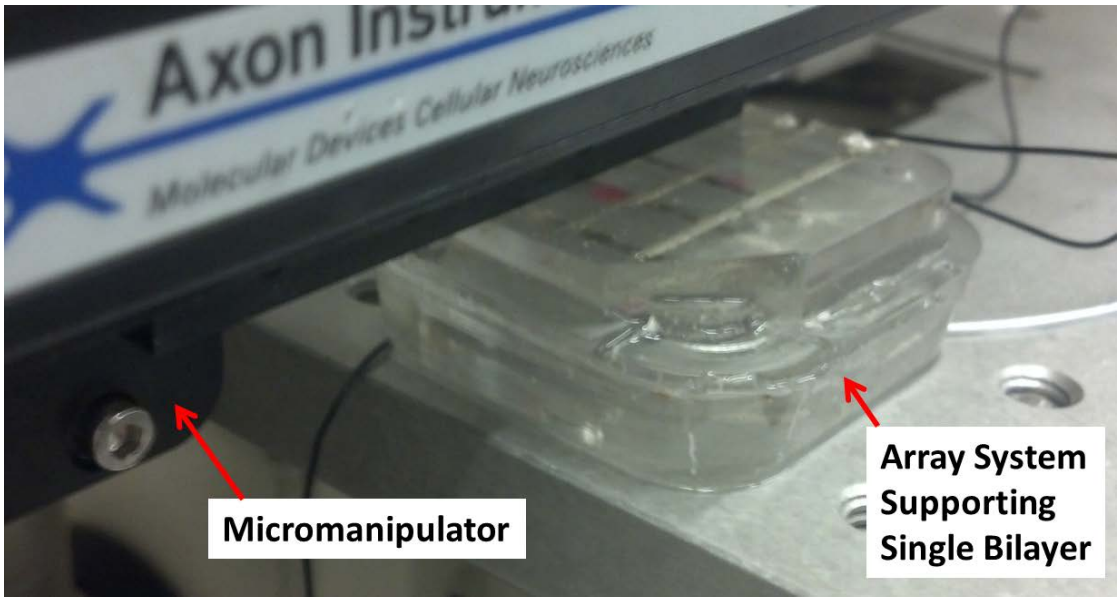

Figure 3-12. Experimental setup for enclosed array system with one bilayer. A manual micromanipulator is used to compress the system to form a bilayer.

Although the polyurethane is clear in appearance, the matte textured surface and the patterned electrodes make it difficult to obtain clear images of the interactions inside the substrate. In the array system shown in Figure 3-12, a hydrogel can be seen in pink near the top left edge of the substrate. However, the optimal viewing angle for a bilayer in this system would

be from the side, which is effectively blocked by the edges of the substrates. Therefore, only the current response of the bilayer can be used to aid in bilayer formation.

To begin the experiment, the micromanipulator is moved down onto the substrates and used to push the hydrogels together until the lipid droplet spreads to the upper hydrogel. This is determined by observing the current response of the system to a triangle wave. When the hydrogels are both surrounded by one droplet, a very high current, indicating a closed circuit, is observed. The manipulator is then raised, allowing the substrates to decompress and the hydrogels to separate, resulting in an open circuit. Five minutes is allowed for a monolayer lens to form around each hydrogel. A bilayer is formed by pushing the two hydrogels together slowly until the amplitude of the current response starts to increase. At that point, the system is held in place as the bilayer forms and the amplitude rises to a steady value.

3.3.3.2 Results

Forming a bilayer manually without visual feedback proved unreliable, and multiple attempts were needed in order to settle on the required positioning of the top hydrogel. However, bilayers were created using this system, showing promise for future experimentation. The data gathered from one of the bilayers is shown below in Figure 3-13.

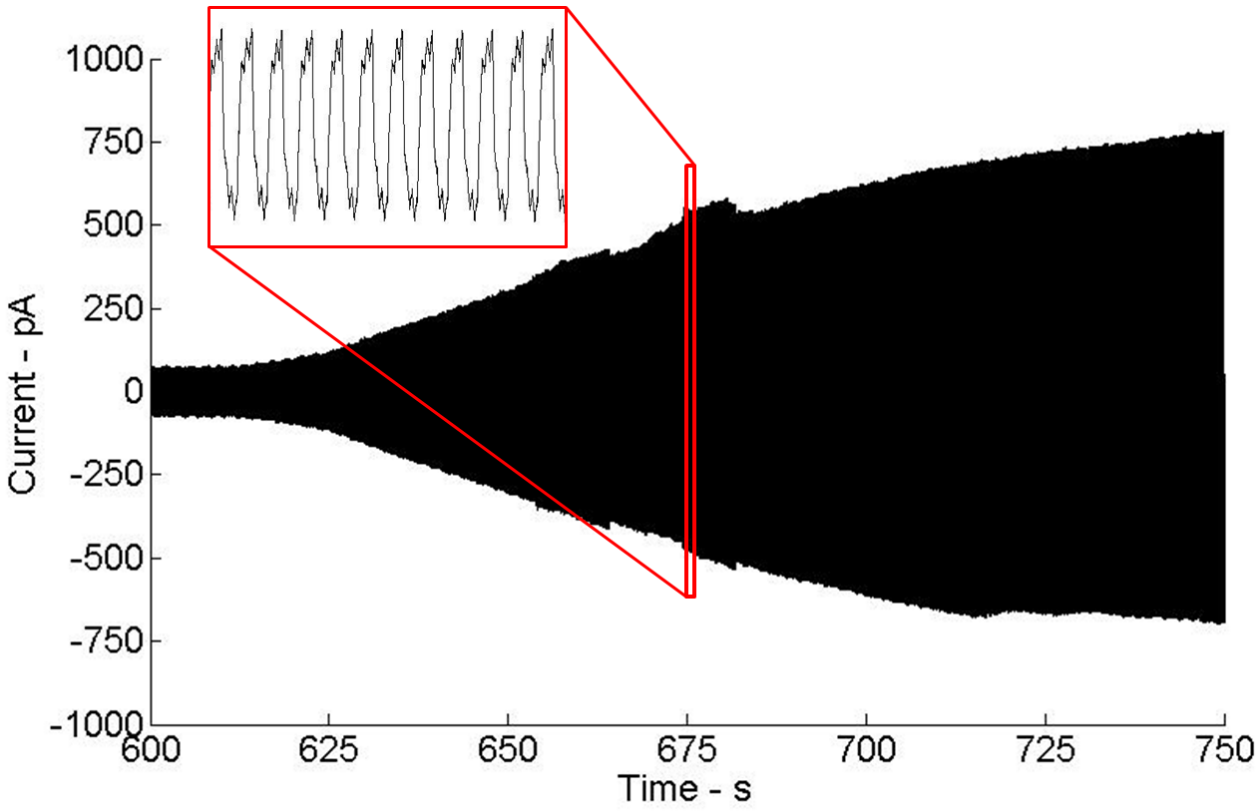

Figure 3-13. Current response to 10 Hz triangle wave of bilayer formed using enclosed array system. Red outlined insert shows response over about 1.2 seconds.

The amplitude of the current response in this test rises gradually, as expected. This test resulted in a higher current than the micropipette test, which is likely due to the substrate-based hydrogel being larger than the end of the pipette. While the general response is similar to the previous test, the waveform, as seen in the insert of Figure 3-13, does not appear as a square wave. Disregarding the 60 Hz noise signal, the current tends to rise, or lower, gradually toward each peak or valley. This effect becomes more evident over time as the bilayer forms, as illustrated in Figure 3-14. In a system with a resistor and capacitor in parallel, this behavior indicates that the resistance is much lower than would normally be expected in a DIB system.

This would suggest that some ions are allowed to leak through the bilayer, which may be a side effect of the large bilayer area.

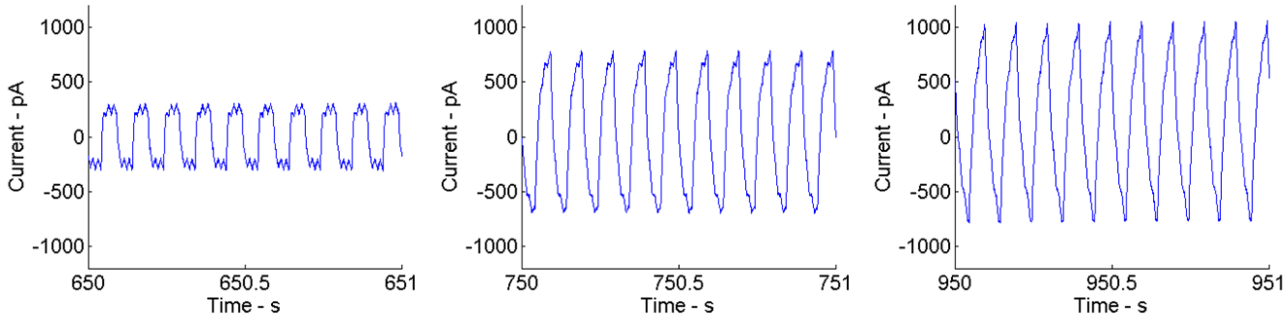

Figure 3-14. One second samples of current response data from the enclosed array system test. As the experiment progresses, the bilayer becomes progressively leakier, and eventually, the response nearly matches the closed circuit response.

Despite the problems with bilayer stability, a DIB was formed using this array system and test setup. The bilayer was able to be monitored and verified with an Axopatch system, which shows that the electrodes behaved as expected and maintained a connection for the whole experiment. The hydrogels maintain contact with the electrodes, even when the microchannel substrate is suspended from above. This experiment shows that this hydrogel-based system is a viable method for forming arrays of DIBs.

3.3.4 Discussion

The system presented here is the first enclosed DIB array system to use opposing pairs of substrates to physically support hydrogel-based bilayers. The substrates are made from polyurethane and formed using 3D printed molds. The substrates use detailed features that would be virtually impossible to fabricate using machined molds and which are necessary for the system to function. Electrodes are patterned with silver paint and connected to wires using

conductive epoxy. Hydrogels are formed using simple laboratory techniques and cured quickly with UV light. Experiments showed that the system is able to support hydrogel-based DIBs while providing a reliable electrical connection for analysis.

This enclosed array design is useful as a proof of concept and is capable of supporting a single bilayer. The substrates have been used to support multiple hydrogels with reasonably low (~2-4 MΩ) resistance electrical connections. However, forming multiple bilayers simultaneously requires more precision and consistency in fabrication and testing procedures. In order to form simultaneous bilayers, both the bottom end of the hydrogel, contacting the electrode, and the top, supporting the lipid lens, must be identical for each part of the array. Even if droplet sizes are precisely controlled, surface imperfections in the polyurethane can cause variations in shape and liquid hydrogel can sometimes flow through the channel between droplets before curing. Also, when each hydrogel is cured independently in turn, some in the array might receive additional UV light while adjacent hydrogels are being cured. Over time, water can evaporate from the hydrogels, causing shrinkage and, in extreme cases, forming cracks. The molds used to create the microchannel substrates do not always form smooth, well-defined edges. Since these substrates are physically held in place at their edges by the larger substrates, they do not always rest perfectly horizontally, especially if there is some variation in height of the bottom ends of the hydrogels. Another difficulty in reliably forming bilayers with this system is that the substrate design does not allow a line of sight to view bilayer formation. Generally, when DIBs are formed, two droplets are moved together until they appear to touch, at which point the current response signal amplitude will begin to gradually rise [46]. Removing the visual feedback from this process introduces some manual estimation and makes it more difficult to consistently form bilayers. To improve upon this array design, and provide a platform for

forming multiple bilayers simultaneously, a new set of substrates and fabrication and testing processes are developed using the knowledge gained from these experiments.

3.4 Reduced Scale Array System

Using the results from the large scale system experiments, a new, smaller scale DIB array system is developed. Like the previously described array, this system is designed around a 3x3 bilayer array, although smaller variants are also created. The dimensions of the system are about 18 mm on a side and 10 mm tall, and this is intended to be as small as possible using the available laboratory fabrication techniques. In addition, several alterations are made to the fabrication processes to improve the precision of the system and allow for the formation of simultaneous bilayers. Further experiments with the new design lead to more changes to improve the system's reliability. In order to see the bilayers form, an alternative test setup is developed that uses a separate oil container to eliminate the need for polyurethane walls on the substrates. This setup, along with improvements to the substrate design, fabrication processes, and testing procedures, is used to form an array of hydrogel-supported DIBs simultaneously.

3.4.1 Fabrication Process

A new set of 3d printed molds is designed to make the new polyurethane substrates. The first set of substrates used an electrode design similar to the one in the larger scale system. The pattern is imprinted into the base of the substrate, and epoxy is used to attach wires. The molds used for the base substrate are shown in Figure 3-15. This mold consists of two pieces, but, unlike the previous molds, the bowl shape and electrode patterns are formed by the bottom piece. This means that any bubbles in the polyurethane will escape instead of being trapped on mold surface. The top piece of the mold, shown in Figure 3-15(b), forms a space for the epoxied wire

connections while allowing the substrate to rest on a flat surface. The X shape is purely for structural support, and the gaps allow space for pouring the uncured polyurethane.

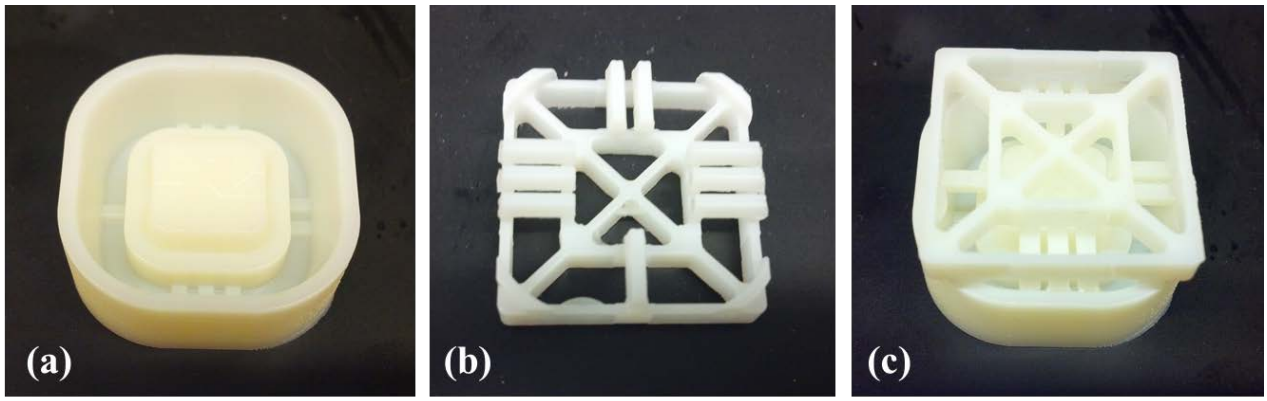

Figure 3-15. Set of 3D printed molds used to create the base substrate. The primary mold (a) forms the basic bowl shape of the substrate and the electrode pattern. The second piece of the mold (b) forms space for wire to be epoxied and attached to the electrodes and fits into the first piece (c).

The substrate created from this mold is shown on the left in Figure 3-16. It is designed to support a 3x3 hydrogel array and the electrode pattern addresses each hydrogel individually. The substrate on the right is the top substrate designed to rest inside the outer wall of the base. It is fabricated using a similar two piece mold. The notches around the outer edge of the base substrate allow wires from the top substrate to extend horizontally away from the array. This ensures that an evenly distributed compressive force can be applied to the top of the system without pinching any wires.

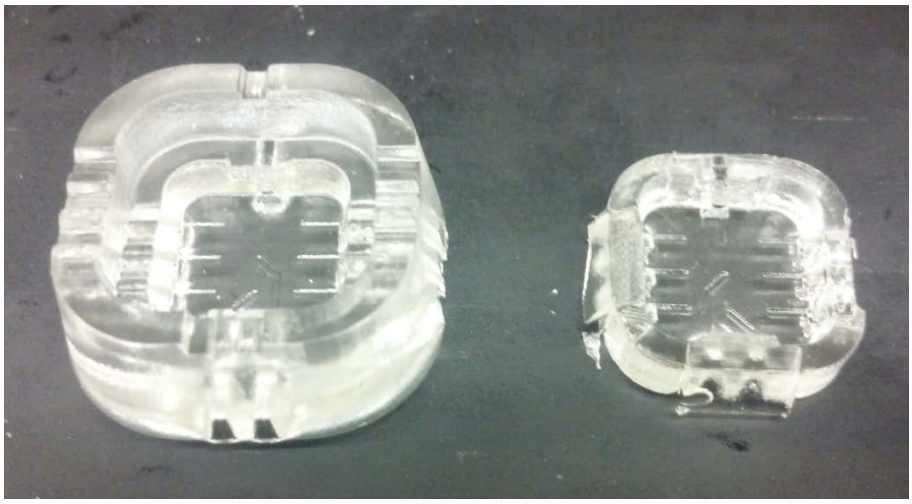

Figure 3-16. Polyurethane substrates for enclosed bilayer array system. The base substrate is on the left, and the top substrate, which fits into the base, is on the right.

Once the substrates are cured, removed from the mold, and cleaned, a small amount of epoxy is pushed into the opening at each electrical connection point and allowed to dry. Next, a larger amount of epoxy is used to attach a wire at each point. Dividing this into two separate steps ensures that the epoxy will completely seal the opening to prevent leakage and that there will be a consistent electrical connection. Finally, silver paint electrodes are patterned using the indentations in the substrate as a guide, and chloride is added at each hydrogel connection point. The finished base and top substrates are shown in Figure 3-17.

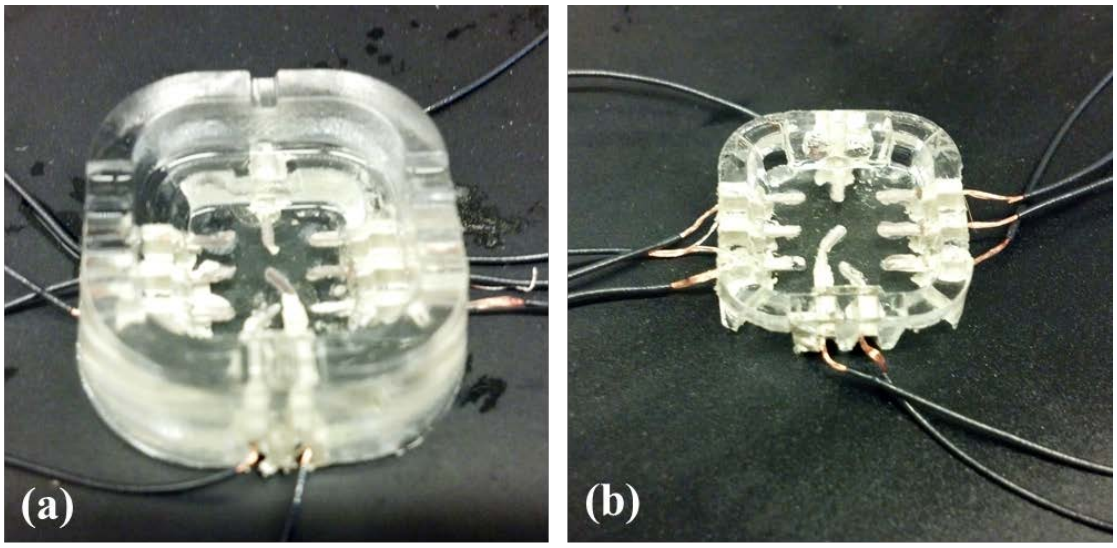

Figure 3-17. Base (a) and top (b) substrates with silver chloride electrodes and wires attached with conductive epoxy.

Next, the two microchannel substrates are fabricated using a similar method as previous designs. Two 3D printed pieces with an array of small holes are attached face to face with a gasket in between to form a seal. In this design, a laser cut rubber gasket, seen in Figure 3-18(a), is used to produce a clean, precise edge to the finished substrate. Once the second piece is attached, thin wires are inserted through each of the holes. The fully assembled mold, shown in Figure 3-18(b), is then propped up on its side, polyurethane is added, and it is placed in an oven to cure.

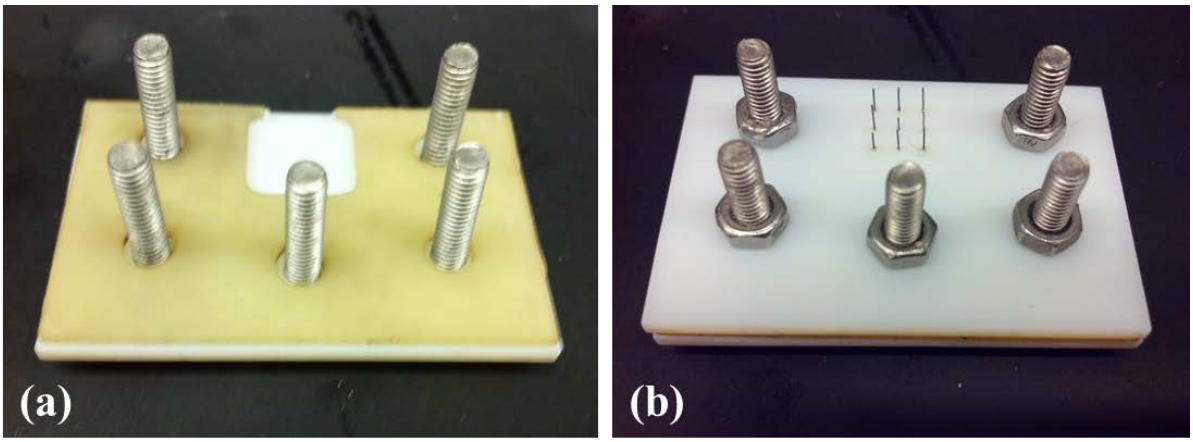

Figure 3-18. Mold for forming microchannel substrate. (a) One 3D printed side of the mold with rubber gasket in place. (b) Assembled mold with second 3D printed piece and a 3x3 array of wires to form microchannels.

After curing, the microchannel substrates are removed from the molds and cleaned. At this point, the process for forming hydrogels is relatively unchanged from the large scale system. A precisely measured droplet of aqueous hydrogel is added to the top end of one of the microchannels and exposed to UV light for one minute. The process is repeated for each channel in turn, the substrate is flipped over, and a droplet is added and cured to the opposite end of each channel. The microchannel substrates are then inserted into the base and top substrates where a pair of tabs holds them in place. The assembled base and top substrates with 3x3 hydrogel arrays are shown in Figure 3-19.

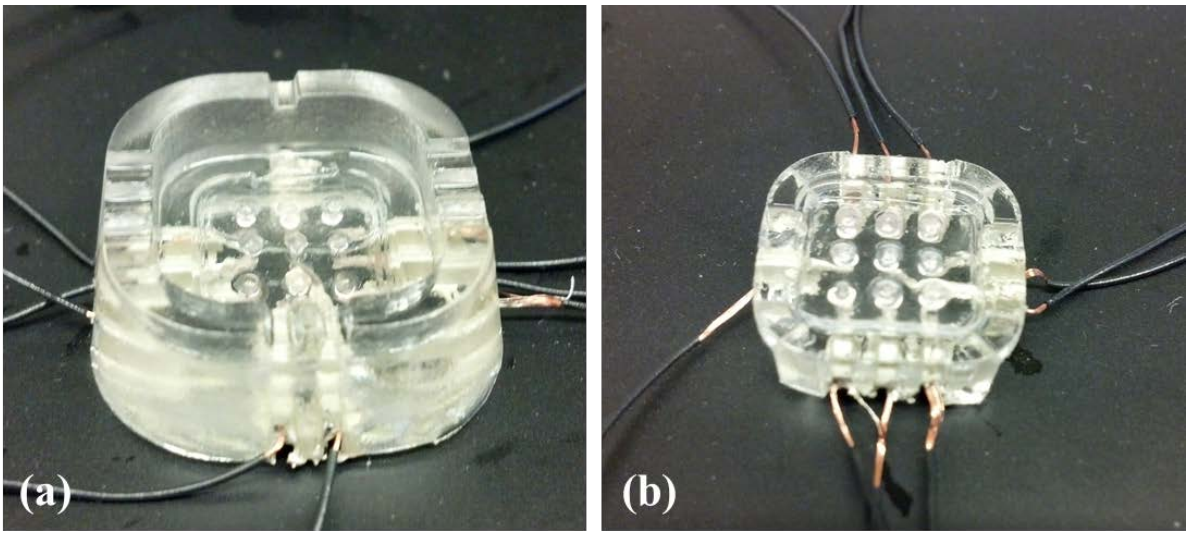

Figure 3-19. Microchannel substrates with 3x3 hydrogel arrays assembled with (a) base substrate and (b) top substrate.

To form bilayers, the base substrate in Figure 3-19(a) is filled with hexadecane, and a droplet of lipid solution is added to each hydrogel. The top substrate in Figure 3-19(b) is flipped and inserted into the base. The top surface of the combined substrates is flat and can be compressed using a micromanipulator.

3.4.2 Fabrication Process Results and Adjustments

The array system shown in the previous section shows improved reliability and fabrication precision compared to the large scale system. However, producing consistent 3x3 arrays of hydrogels with reliable electrical connections has proven difficult, and more problems arise when creating bilayers for experiments. As a result, many aspects of the design are refined and improved to create a more effective system.

3.4.2.1 Substrate and Electrode Design

In the original substrate design, the microchannel substrate is held in place with two tabs build in to the base substrate. These are designed to prevent the smaller substrate from falling

out when the array is moved or turned upside down and to press the hydrogels into the electrodes underneath, maintaining an electrical connection. However, this does not form a seal around the edge and allows the hexadecane to fill the area underneath the microchannel substrate, as seen in Figure 3-20(a). While preparing for an experiment, the connectivity of each hydrogel is measured by touching a silver chloride wire to the top end of the hydrogel and measuring the resistance from that point to the wire attached to the electrode. Immediately after placing the hydrogels on the electrodes, most showed a connection with a low resistance of about 50 to 150 kΩ. While attempting to form bilayers, however, many of the connections fail. Additional testing shows that many of these failures occur shortly after oil is added to the system, indicating that some of the oil may be coming between the hydrogels and the electrodes. The solution to this issue is to create a seal around the edge of the microchannel substrate that prevents the oil from reaching the bottom side of the hydrogels or electrodes. This is achieved by attaching the microchannel substrate to the base with cyanoacrylate adhesive. The adhesive is applied around the edge of the base substrate and, when the second substrate is attached, it forms a dry area for the hydrogels and electrodes to interact. A cross-sectional view of this is shown in Figure 3-20.

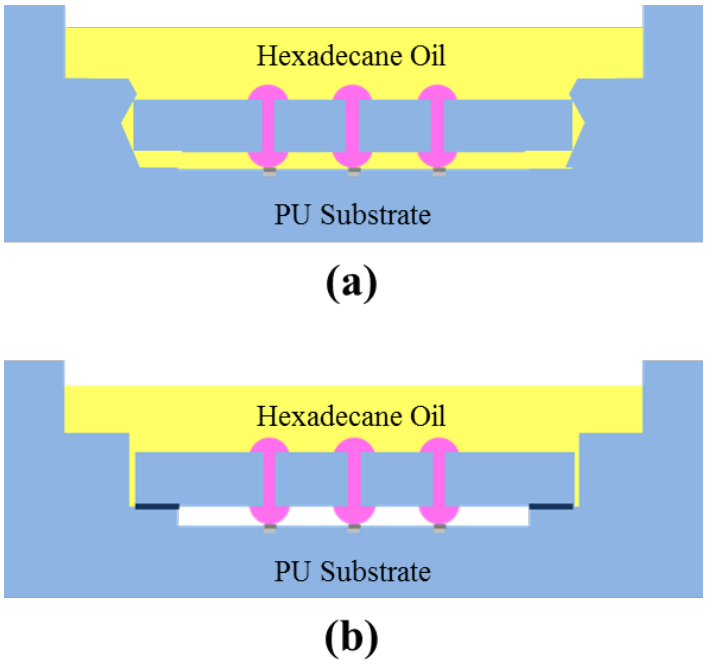

Figure 3-20. (a) Base substrate with hydrogel-supporting substrate held in place with tabs. (b) Base substrate with hydrogel-supporting substrate glued in place, preventing oil from reaching electrodes.

This method for attaching the hydrogels also holds the microchannel substrate in place more firmly, so it cannot shift and break contact with the electrodes. Unfortunately, since the adhesive is permanent, substrates used with this design cannot be reused.

Preventing the oil from interacting with the hydrogel-electrode interface improves the reliability of the system and the electrical connections. In order to further improve the robustness of the electrode system, the base and top substrates are redesigned. When the silver paint used to form the electrodes dries, it tends to become brittle, and a thin layer can easily form a crack if it is disturbed. Imprinting the electrode pattern onto the substrate helps prevent delamination due to shear forces, but any flexing of the substrate or agitating the wires can still

cause connectivity problems. Also, in some tests, the epoxy used to attach the wires does not adhere strongly to the polyurethane and can slightly detach from the electrode.

The first problem area to be addressed is the epoxy connections. A new base substrate is designed to eliminate the need for epoxy by gluing wires directly to the substrate. Eliminating the epoxy connections also eliminates the need for a second piece of the mold. The simpler, newly designed mold is shown in Figure 3-21.

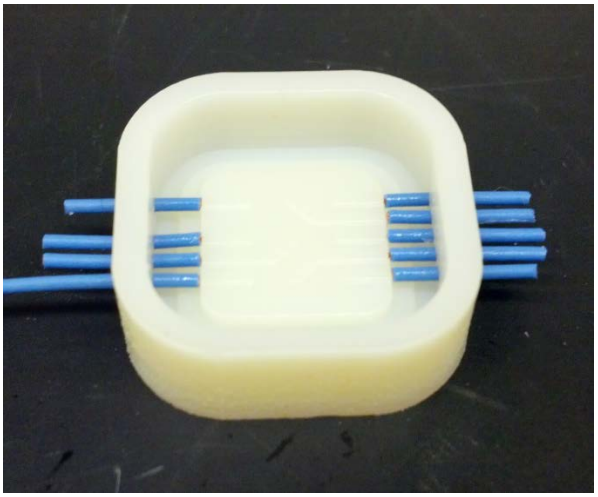

Figure 3-21. Base substrate mold for 3x3 array. The wire segments form openings in the side of the substrate for attaching wires to electrodes.

Removing the top part of the mold means that the underside of the substrate is smooth and that any bubbles can escape while curing. For each electrode in the pattern, a segment of wire the same diameter that will eventually be attached to the substrate is inserted through the side of the mold. After curing, the wires are removed, and the substrate is removed from the mold. The resulting substrate is shown in Figure 3-22.

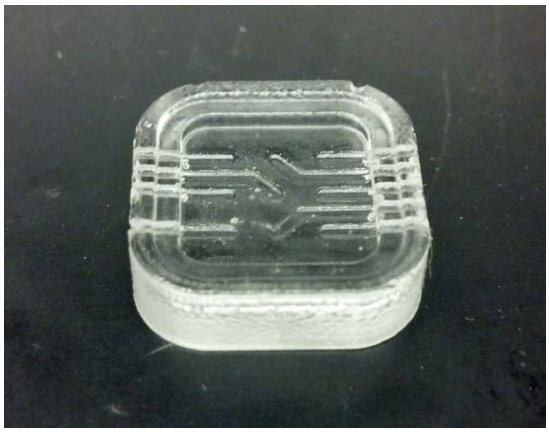

Figure 3-22. Base substrate for 3x3 array after removal from the mold.

The substrate shown in the figure is designed for the alternative test setup presented in Chapter 2, so it does not include the walls for containing oil. The substrate is designed for a 3x3 array of bilayers addressed individually, so it has nine holes along the outside edge. A wire is glued in each hole with cyanoacrylate, and the electrodes are patterned and treated with chloride. With the wires glued in place instead of epoxied, the connections can be designed closer together, potentially for further downscaling. Figure 3-23 shows the completed base substrate

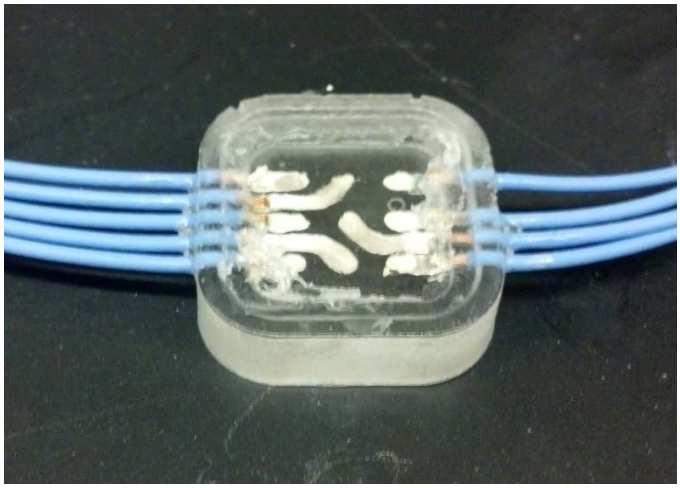

Figure 3-23. Base substrate for 3x3 array with electrodes patterned and wires glued into place.

Wires attached using this method are firmly attached to the substrate and will not lose their connection to the electrodes when the system is handled or agitated. The glue also forms a strong seal, so oil will not seep into the closed area of the substrate.

Another adjustment to the substrate fabrication procedure is to alter the ratio used to make the two part polyurethane for the base and top substrates. Using a 1:1.5 instead of a 1:2 ratio of parts A and B results in a significantly stiffer polyurethane substrate. Because the dried silver paint of the electrodes is thin and brittle, and flexing of the substrate stretches the electrode surface and causes cracking. A single crack in the paint will irreversibly damage its electrical connectivity. Stiffening the substrate reduces the chance of large amounts of flexing and, therefore, protects the electrodes.

3.4.2.2 Hydrogel Fabrication

In order to form multiple bilayers in an array, each monolayer lens should be of equal size, and each opposing pair of monolayer should be equidistant. Since the shape of the lens is closely defined by the shape of the hydrogel that supports it, it is important to ensure that each hydrogel in an array is equal in size. Even though a precision syringe is used to deposit droplets when forming hydrogels, some variations in the final shape and size are observed. Some changes in behavior between adjacent hydrogels are also observed as a result of the photocuring procedure. Several adjustments are made to the array fabrication process to account for these problems and produce more consistent hydrogel performance.

First, the design of the microchannel substrate is changed to include a small indentation at the ends of each channel. Figure 3-24 shows the new substrate design for a 3x3 array. On this substrate, each indentation is 1.5 mm in diameter and 0.5 mm deep.

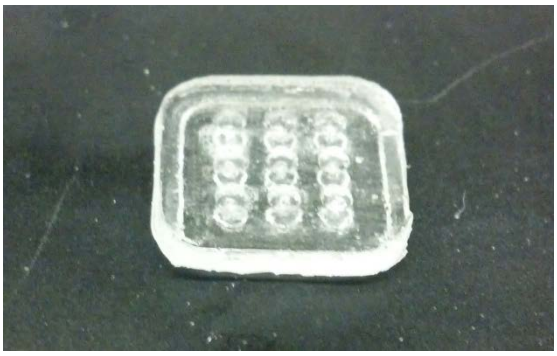

Figure 3-24. Microchannel substrate with an indentation at the ends of each channel to hold the hydrogel droplets in place.

When an aqueous hydrogel droplet is deposited near one of the channels, it fills in the indentation, which automatically centers it over the channel. The diameter of the droplet is controlled by the diameter of the indentation which, in turn, controls the height of the droplet. This design requires a greater volume of hydrogel, but creates the precision needed to form bilayer arrays.

When tests are done with arrays of multiple hydrogels, some of the hydrogels fail to maintain their structure. In some cases, the hydrogel end facing away from the electrodes will break away from the rest of the hydrogel and come out of the substrate completely. In other cases, the hydrogel will appear normal, but show no electrical connection during or after the experiment, despite showing a low resistance immediately after curing. Further investigation reveals that this problem occurs more frequently on the hydrogels cured earliest, and it never occurs on the most recently cured hydrogel. Also, in tests performed with a single pair of hydrogels in this array system, the hydrogels reliably stayed in one piece and maintained an electrical connection to the electrode. This indicates that the problem is a result of the additional resting time needed while the other hydrogels are being cured. Also, while the light from the UV

curer is focused on a small spot that encompasses on hydrogel at a time, some of the light can be observed to reach adjacent hydrogels. This could give those hydrogels additional effective curing time, and the heat produced by the curer could also affect the hydrogels.

One likely explanation for the hydrogel breaking problem is that the additional heat and resting time after curing is causing some of the water held by the hydrogels to evaporate. PEG-DMA, like other hydrogels, contracts when it dries out [30]. The hydrogels in this system consist of two droplets on either side of a comparatively rigid substrate connected by a thin stem. When the hydrogel contracts, the step can crack, breaking the electrical connection and allowing one end to release from the substrate. One possible solution to this is to cure all of the hydrogels simultaneously. This would be the preferred solution for a similar, smaller scale system created using microfabrication methods. However, since using this curing method would greatly increase the cost of this process, an alternative solution was found by adding a step to the curing process.

In the new process, after one side of the hydrogel is cured, a droplet of water is deposited on top of it. This means that even if some of the water in the hydrogel evaporates, there will still be enough to maintain full hydration. The substrate is then flipped over, and another droplet of hydrogel is deposited and cured. This side of the cured hydrogel is also hydrated with a water droplet. The hydration process for a single hydrogel is shown in cross-sectional view in Figure 3-25.

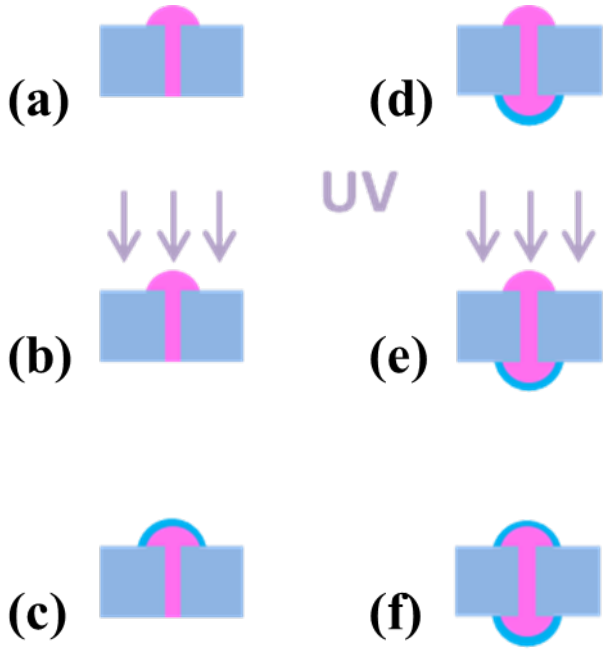

Figure 3-25. Process for maintaining hydration of PEG-DMA hydrogels. (a) Aqueous hydrogel is deposited on the polyurethane substrate at the top end of a microchannel. (b) The hydrogel is cured with UV light. (c) A droplet of water is deposited on the hydrogel. (d) The substrate is flipped and another droplet of hydrogel is added to the opposite end of the channel. (e) The hydrogel is cured. (f) A water droplet is added to the newly cured end of the hydrogel.

This process is repeated for each hydrogel in the array. While the total process takes longer, the additional water prevents the hydrogels from cracking. At this point, the excess water is removed from the sides of the hydrogels that will contact the electrodes using a syringe. The microchannel substrate is then glued into place, sealing the bottom of the hydrogels off from the outside and preventing future evaporation. The water droplets on the tops of the hydrogels maintain hydration while the second set of hydrogels is prepared. The excess water on the top of each hydrogel is removed just before the start of an experiment when lipids are added.

Using droplets of water to maintain hydration resulted in consistently shaped hydrogels that did not break easily and produced a low resistance for electrical signals. However, a new

problem arises during the next step in preparing for an experiment when droplets of lipid solution are added to the hydrogels. Normally, the water in the hydrogel behaves as a droplet whose shape is defined by the shape of the hydrogel. When another water droplet is placed in contact, the two droplets coalesce into a larger droplet surrounding the hydrogel. The hydrogels that had been hydrated after curing, however, do not absorb the droplet of lipid solution. When the system is submerged in oil, the droplets float away after failing to attach to the hydrogel. Droplets added to hydrogels in air rest on the hydrogels without absorbing into them, although, in some cases, the droplets will absorb slowly over a period of hours. Another observation is that this effect does not occur on the most recently cured hydrogel, even though a drop of water is added after curing, and removed before testing.

To examine this phenomenon, a simple test is devised to observe the effect of each variable. A 2.0 μL droplet of PEG-DMA hydrogel (40% w/v) without dye is deposited on a clean, flat polyurethane substrate. For a control test, the hydrogel is cured for one minute and allowed to sit without the addition of more water. After five minutes, a droplet of water is deposited on top of the hydrogel. As expected, the droplet absorbs into the hydrogel almost immediately. This test is repeated with waits of 15 and 30 minutes, and, in both cases, the water absorbs into the hydrogel. At 30 minutes, the water absorbs noticeably slower, although this is generally as long as a hydrogel would be sitting during a normal fabrication process. Next, the test is repeated with a small (2.0 μL) of water added to the hydrogel after curing. First, the excess water is left on the hydrogel for one minute and then removed. After allowing the hydrogel to rest for 15 minutes, a drop of water is deposited and absorbs readily, with almost no observable difference from the control test. When the hydrogel is hydrated for three minutes and allowed to rest for fifteen, the water absorbs significantly slower, and with five minutes of

hydration, there is no apparent absorption. However, after five minutes of hydration and five minutes of rest, the droplet absorbs slowly, and with no rest, the droplet absorbs instantly. These results indicate that increasing the time of hydration or the rest time after hydration causes the hydrogel to resist absorbing additional water droplets. Replacing the water with the KCl solution used in the hydrogel has no apparent effect on this phenomenon. Next, the test is performed using a large droplet (~50 μL) of water to hydrate the hydrogel. This greatly reduces the hydration and resting time needed to hinder droplet absorption. For example, after five minutes of hydration and five minutes of rest, the water does not noticeably absorb into the hydrogel. After just one minute of rest, the hydrogel absorbed the droplet significantly slower than one hydrated with a small droplet. The last variable examined is the curing time. Hydrogels are cured for one, three, six, and nine minutes, hydrated with a large drop of KCl solution for five minutes, and allowed to rest for five minutes. At six and nine minutes of curing time, there is a slight improvement in the absorption rate, although the effect of this variable is less than the effect of hydration or resting time.

These results show a pronounced effect of hydration time and resting time on the absorptivity of the hydrogels. Curing time causes a slight change, but curing each hydrogel in an array for more than nine minutes is impractical for an array and increases the required hydration time. It is also observed that increasing the volume of water or solution used for hydration accelerates the effects of the hydration and resting time. This leads to the hypothesis that when a droplet of water surrounds the hydrogel, uncured PEG-DMA diffuses through the droplet. When the excess water is removed, much of the PEG is also removed, leaving behind a lower concentration. The hydrogel with this lower concentration dries more quickly at the outer edges, and the dehydrated hydrogel does not readily absorb water. This would explain the effect of

hydration time and droplet size, as time is needed for diffusion and diffusing over a larger volume leads to a lower concentration. Because the failure to absorb only occurs after a resting period, does not inherently prevent absorption, but causes an accelerated change in the hydrogel that creates this effect. This also explains why longer curing time has a slight effect, since curing for a longer period leaves less uncured PEG. While this hypothesis cannot be verified without further analysis of the molecular changes in the hydrogel, it appears to explain the observed behavior of the system.

Based on this hypothesis, a solution to the problem is attempted to allow the fabrication of usable hydrogels. Instead of using water or KCl solution to hydrate the hydrogels, a 40% w/v solution of PEG-DMA is made without the curing agent (Irgacure 2959). This solution is deposited on each of the hydrogels after curing and maintains hydration while preventing a drop in PEG-DMA concentration in the hydrogel. The previous tests are performed using a PEG solution for hydration, and no loss of absorptivity is observed, even after over 30 minutes of hydration time. This means that arrays of hydrogels can be fabricated and used for testing, and this result lends support to the proposed hypothesis.

These changes to the fabrication process enable the production of consistent, structurally sound hydrogels that can support lipid monolayers. However, some hydrogels tend to lose connection to the electrode when submerged in oil. The resistance of each hydrogel is measured before the substrate is submerged in oil, and almost every fabricated hydrogel produces a strong connection. Some of the hydrogels are found to slide slightly along the channel when submerged in oil with a lipid solution droplet. Therefore, the curing time for each hydrogel is increased to improve adhesion to the substrate. After some experimentation, the final curing time for this

system is chosen to be three minutes. This improves the adhesion of the hydrogels enough to prevent sliding while keeping down the overall fabrication time.

These changes to the fabrication process produce substrates that can be used to form and evaluate arrays of DIBs. Alterations to simplify and improve the precision of polyurethane substrates result in more reliable electrical connections that can withstand test conditions. Changes in the hydrogel fabrication process alleviate problems with hydrogel structure and connectivity and provide a consistent support for lipid monolayer lenses.

3.4.3 Results

The tests using this substrate design are performed using the alternative test setup that allows a direct line of sight to the hydrogels and bilayers. The first experiments are done with a single bilayer supported by the array system, and further tests are performed with larger arrays including 2x1 and 3x1. These array dimensions allow a clear view of each bilayer as it forms. Arrays of larger dimensions are also tested, but they are more difficult to view clearly and yielded mixed results.

3.4.3.1 Single Bilayer Array

The first test to be performed is a single DIB supported by this array system. This simplifies the test setup and serves as a tool for diagnosing any problems with the general design. For convenience, the substrates molds for a 3x3 array are used for the single bilayer system, although a custom system design could be created by printing a new set of molds. The base substrates are only made with one wire connection and electrode, and the microchannel substrates only contain one open channel. The bottom substrate and hydrogel used in this experiment is shown in Figure 3-26.

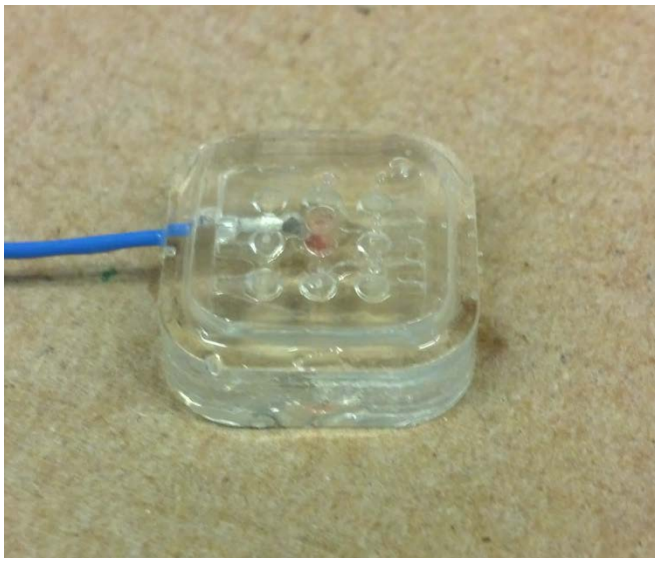

Figure 3-26. Bottom half of an array system supporting a single hydrogel.

The substrates are fabricated and electrodes patterned using the methods presented above. The hydrogels in this test are cured for nine minutes on each side and hydrated with a PEG-DMA solution. The test setup shown in Chapter 2 is used to allow visualization of the bilayer formation. With only one bilayer, the multiplexer circuit is not needed, and the electrode is attached directly to the Patch Clamp. Once the fabrication process is complete, the resistance of each hydrogel is measured to ensure a strong connection. In this test, the resistance of the bottom hydrogel and electrode is 85 kΩ, and the resistance of the top is 133 kΩ. Next, the bottom substrate is inserted into the bottom of the oil container, and hexadecane is added to completely submerge the hydrogel. The droplet of PEG solution on the hydrogel is removed with a syringe, and a 1.0 μL droplet of lipid solution is added. Next, the second substrate is attached to an arm connected to a joystick controlled micromanipulator. The horizontal axes of the 3-axis manipulator are used to align the pair of substrates before lowering the top substrate into the oil.

The top substrate is then carefully lowered until the droplet of lipid solution spreads between the two hydrogels. This can be viewed using the camera and observed through the current response on the Axopatch. When the droplet is coalesced, the response shows a closed circuit, which appears as a large amplitude triangle wave. The hydrogels are then pulled apart to separate the lipids into two droplets, one around each hydrogel. The system is allowed to rest at this point to allow the lipids to assemble into a monolayer at each interface. The top substrate is then slowly lowered until the droplets slightly touch. The camera view is useful for accomplishing this because the current response does not begin to change instantaneously when the droplets touch. The camera view of the droplets coming into contact is shown in Figure 3-27.

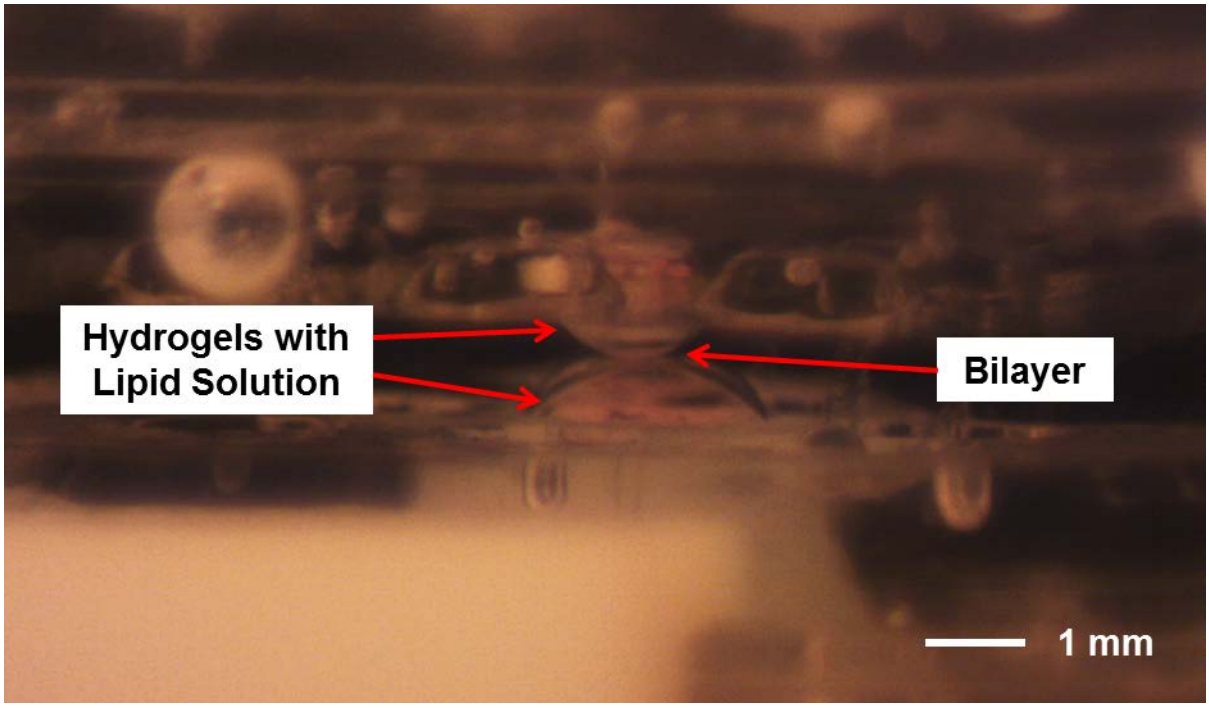

Figure 3-27. A bilayer begins to form as two hydrogel-supported monolayers come into contact. The hydrogels are lightly dyed pink, and the monolayers appear as a droplet surrounding them. Each hydrogel is 1.5 mm across.

At this point, the system is allowed to rest as the bilayer gradually expands. The current response is recorded, and bilayer formation is confirmed by the characteristic, gradually increasing square wave response. The current response to a 10 Hz, 10 mV peak-to-peak voltage input of this system can be seen in Figure 3-28.

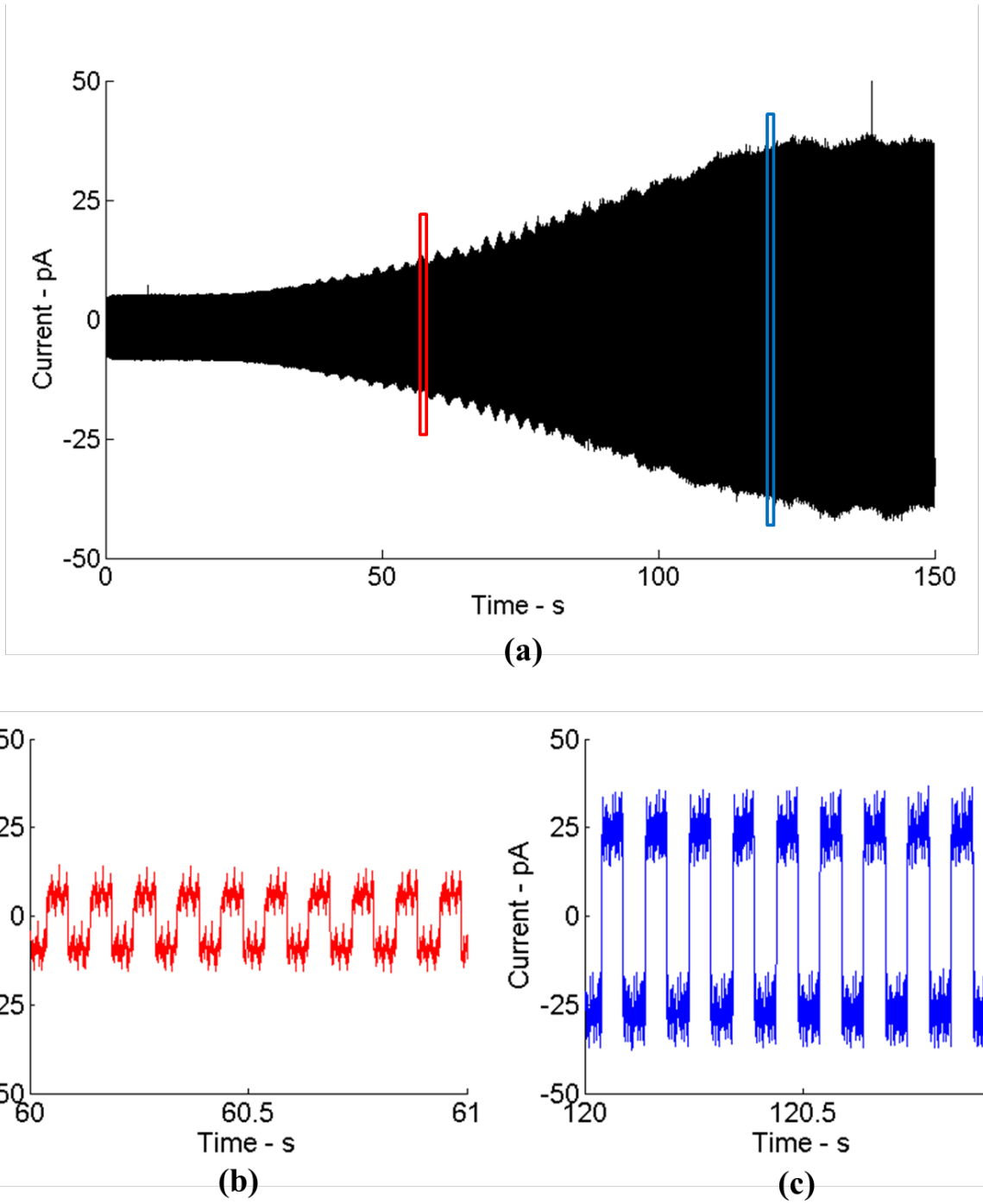

Figure 3-28. (a) Current response to triangle wave voltage applied across DIB in array system. (b) and (c) show the square waveform of the response at two different times as the bilayer expands and the amplitude grows.

Figure 3-28(b) and (c) show samples of the waveform of the response at two different points 60 seconds apart. Despite a noticeable, but small amount of noise, both plots clearly show the expected square wave response. These waveforms are representative of the response through the whole experiment, and indicate a stable, high resistance bilayer. In order to verify the robustness of the hydrogels cured using the revised method, the resistance of each is measured again after the experiment. The resistances of the bottom and top hydrogels are 97 kΩ and 129 kΩ respectively, similar values to those measured prior to the test.

The video of this bilayer forming is analyzed to estimate the area of the bilayer. In this test, a single bilayer is formed and allowed reach steady state. Then, the substrates are pushed together, increasing the area and capacitance of the bilayer. At the first step, the diameter of the bilayer is estimated to be 0.55 mm for an area of 0.24 mm^2. This corresponds to a square wave current response of ±27 pA. In the voltage input signal, $\frac{dv}{dt} = 0.2\frac{V}{s}$, and using Equation (2-4), the capacitance of this bilayer can be estimated to be 135 pF. When the droplets are compressed, the diameter of the bilayer increases to 0.68 mm for an area of 0.36 mm^2. When the area of the bilayer increases by a factor of 1.5, the capacitance should increase by the same factor. The larger bilayer showed a current response signal of ±39 pA indicating a capacitance of about 195 pF. This shows an increase by a factor of 1.44, similar to the change in area. This shows that the bilayer is demonstrating predictable capacitive behavior and that the area of bilayers produced using this method can be altered by simply changing the distance between the substrates.

The results from this test show that this array system is a viable method for producing stable droplet interface bilayers. The electrodes, wires, and hydrogels maintain a consistent electrical connection for the duration of the test. The lipids are able to form lenses around the

hydrogels and formed a stable DIB when pushed together. The alternative test setup produced clear images of the interface that are useful for forming a bilayer and analyzing the results. The next step is to replicate this result with multiple sets of hydrogels supporting multiple bilayers.

3.4.3.2 3x1 Bilayer Array

After the success of the single bilayer test, an array is created to support three bilayers formed simultaneously. This experiment uses the same setup as before, but the multiplexer circuit is used to switch between the three input channels. For this test, each hydrogel is cured for three minutes on each side instead of nine, but the fabrication process is otherwise the same as in the single bilayer test. The hydrogels and substrates used in the test are shown, submerged in oil with lipid lenses, in Figure 3-29.

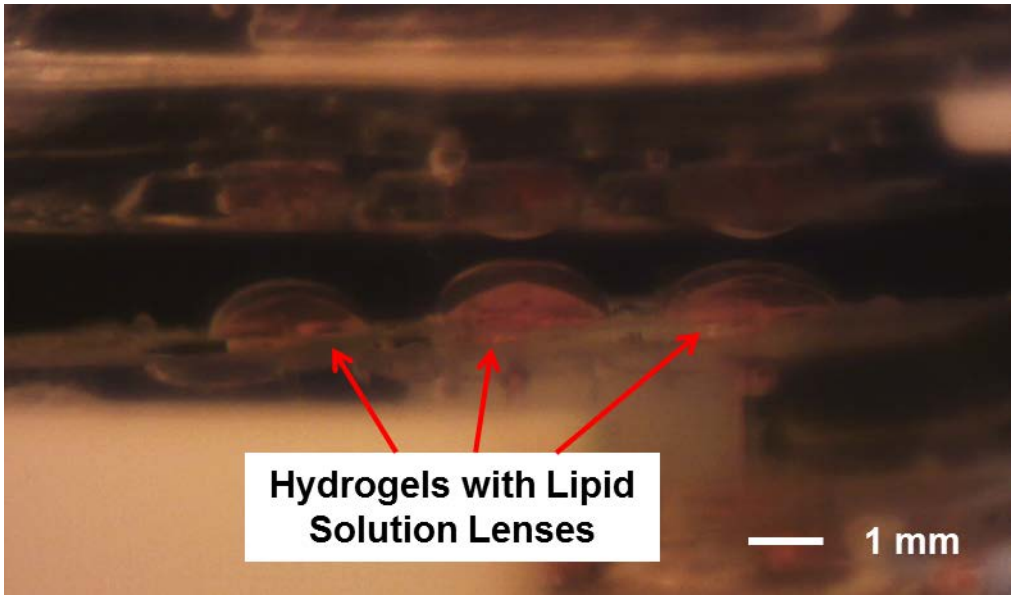

Figure 3-29. 3x1 array before bilayer formation. A lens of lipid solution can be seen surrounding each hydrogel. The top set of hydrogels is slightly obscured by the substrate, but the tips, where the bilayers will form, are still visible.

The resistance of each hydrogel is measured before being submerged in oil. The resistances of the hydrogels on the bottom substrate are, from left to right in the image, 73, 78, and 102 kΩ. The resistances of the top hydrogels are 51, 56, and 69 kΩ. All of these values are within the expected range and indicate a strong electrical connection. Due to a slight imperfection in the substrate, the top left hydrogel is shorter than the others but maintains a connection and supports a lipid lens. During the experiment, the center and right hydrogel pairs form simultaneous bilayers, but, because of the height difference, coalesce before the leftmost hydrogel pair comes into contact. Figure 3-30(a) shows the rightmost pair of hydrogels with bilayers formed simultaneously, and in Figure 3-30(b), the substrates are pushed closer together to form a bilayer using the leftmost hydrogels.

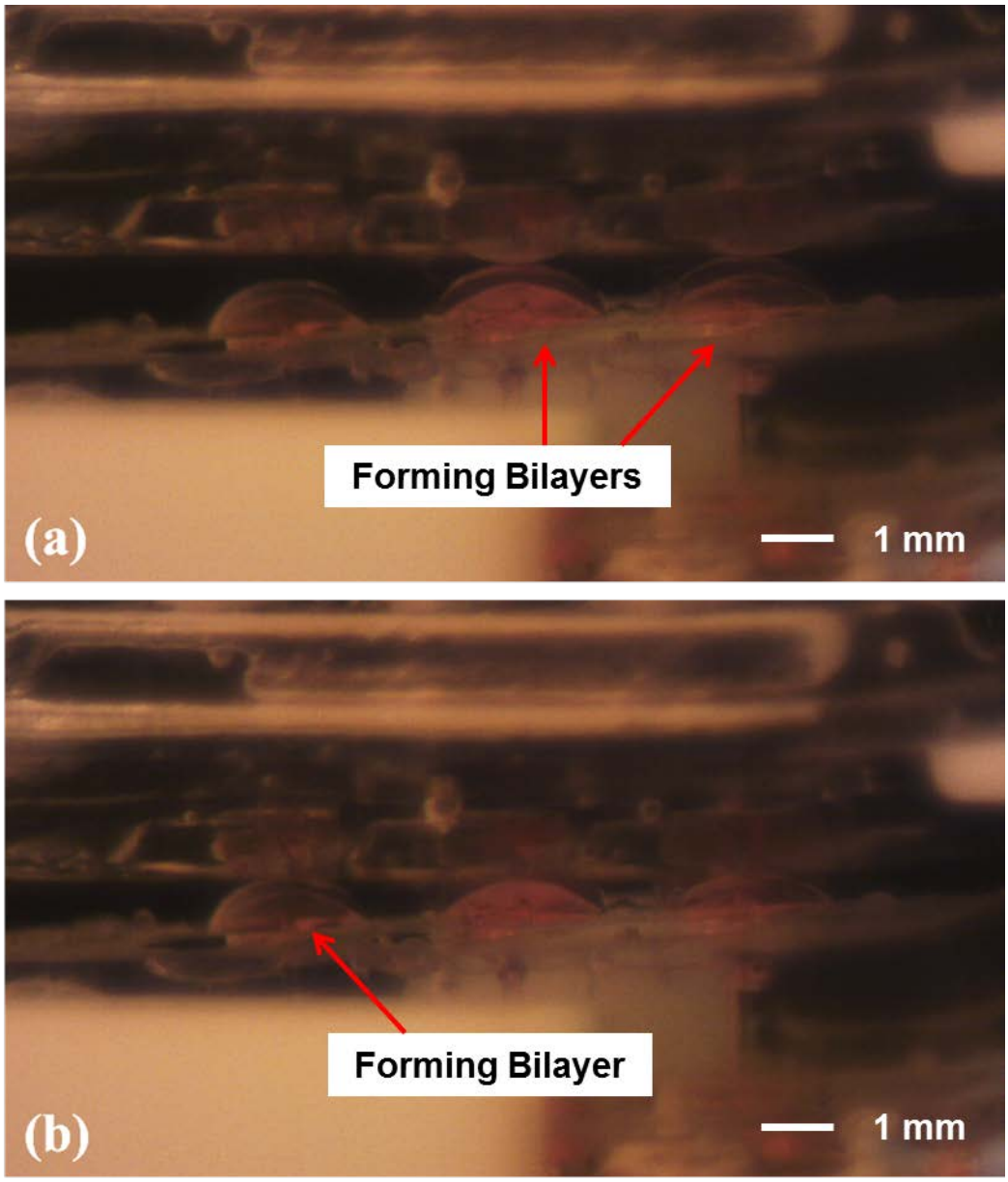

Figure 3-30. Droplet interface bilayers formed in a 3x1 array. (a) The center and rightmost bilayers are formed, and the leftmost droplets are not in contact. (b) The leftmost bilayer is formed, and the other two droplet pairs are coalesced.

While in this case, one of the hydrogels has a different height and prevents the formation of three bilayers, there is some flexibility to the droplets that allows multiple bilayers to be formed despite slight variations in height. At the start of the formation process, the response of

the center bilayer is recorded to verify bilayer formation. At the same time, the rightmost bilayer appears to be forming on the video, but cannot be recorded simultaneously.

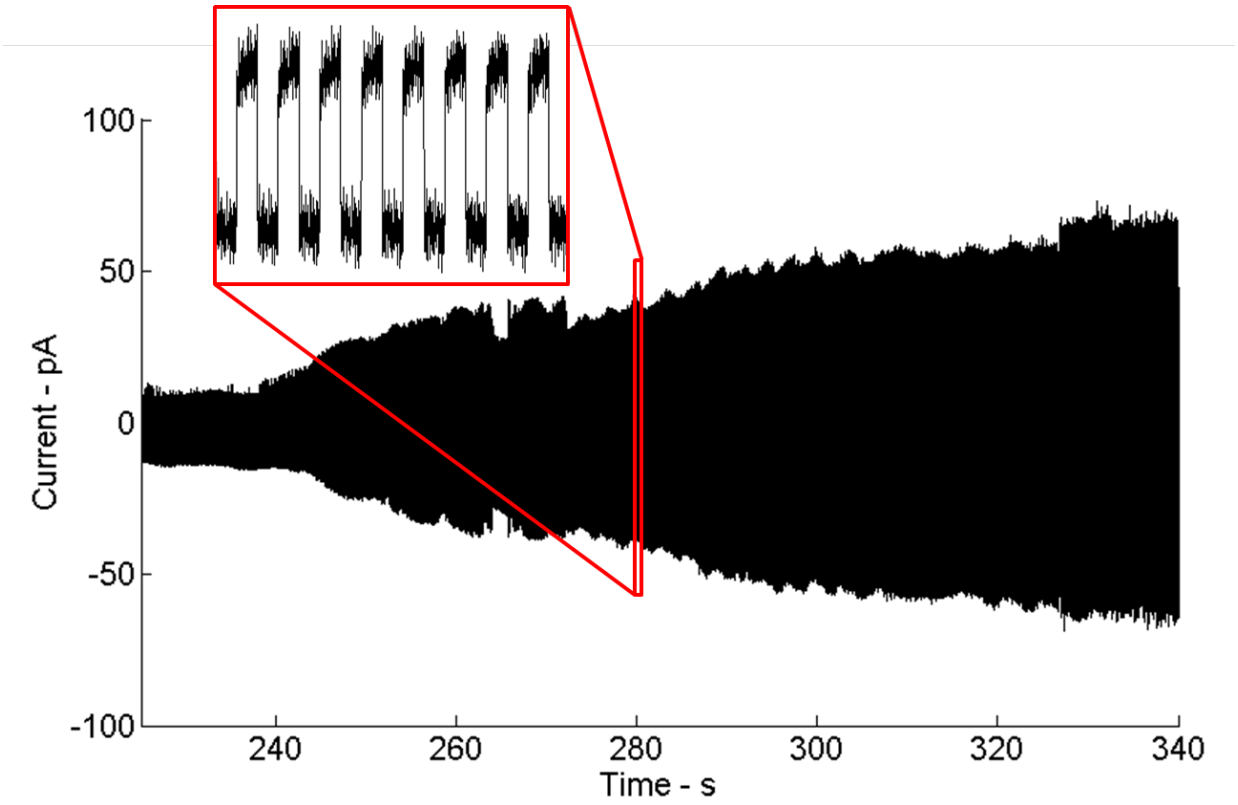

Figure 3-31. Plot of current response of center bilayer in 3x1 array to a 10 Hz, 10 mV peak-to-peak voltage input. The waveform in the red insert shows a sample of about 0.8 seconds of the signal. The time references the start of the recording and not necessarily the beginning of bilayer formation.

Figure 3-31 shows the current response of the center bilayer as it forms. The plot shows the characteristic response with a gradually increasing amplitude, and, a closer look reveals that the waveform follows the expected square wave pattern. At around the 260 to 280 second mark on the plot, the amplitude appears to abruptly drop, rise, and drop again. This is a result of switching on and off the light used to illuminate the test setup for filming. When the light is plugged in, the noise level increases, so when the camera is not needed, the light is switched off

so a cleaner signal can be collected. Once the amplitude of the response reaches steady state, the input is switched to the bilayer on the right. Since the bilayers form at the same time, it is already at steady state. A one second sample of steady state data from each of these two bilayers is shown in Figure 3-32.

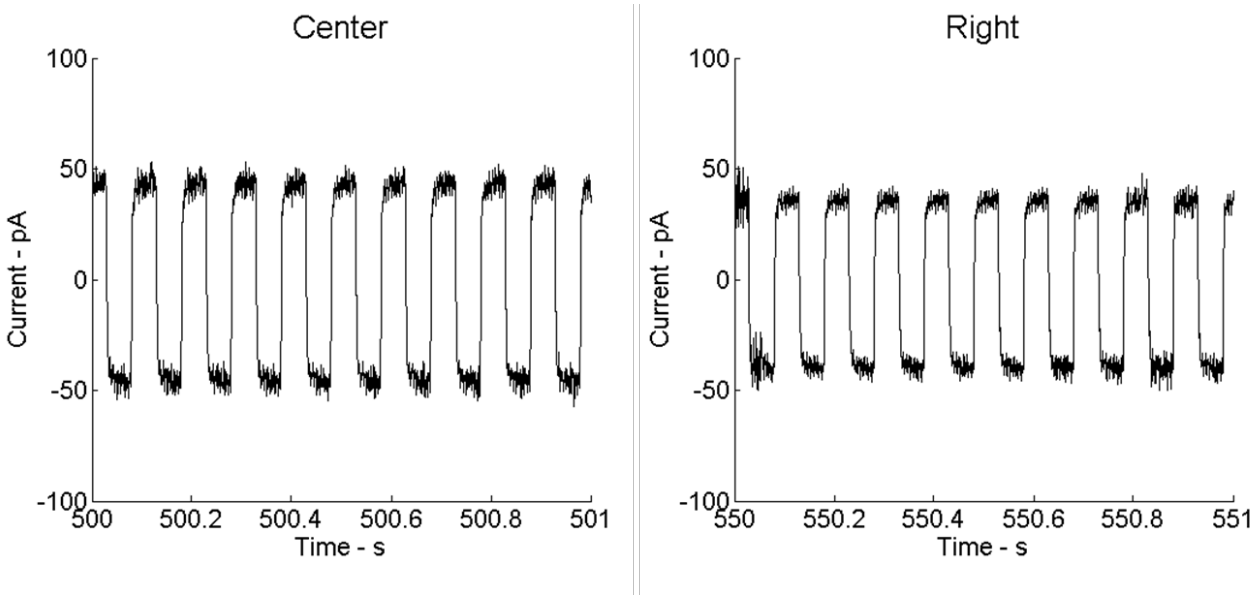

Figure 3-32. Waveforms of the center and rightmost bilayers. This data is recorded with both bilayers at a steady state, and no changes are made to the system between these samples.

Each of the two bilayers gives the expected response with very little leakage. Their amplitudes are similar, indicating that they are of similar size. This is supported by the image in Figure 3-30(a), in which the hydrogels and droplets of the two rightmost bilayers appear nearly identical.

While this array is not capable of supporting all three bilayers at once, the next step in the experiment is to move the substrates further together to form a bilayer with the leftmost hydrogel pair. This position is shown in Figure 3-30(b) and results in the center and right droplet pairs

coalescing and creating a closed circuit. The current response of the leftmost bilayer as it forms is shown in Figure 3-33.

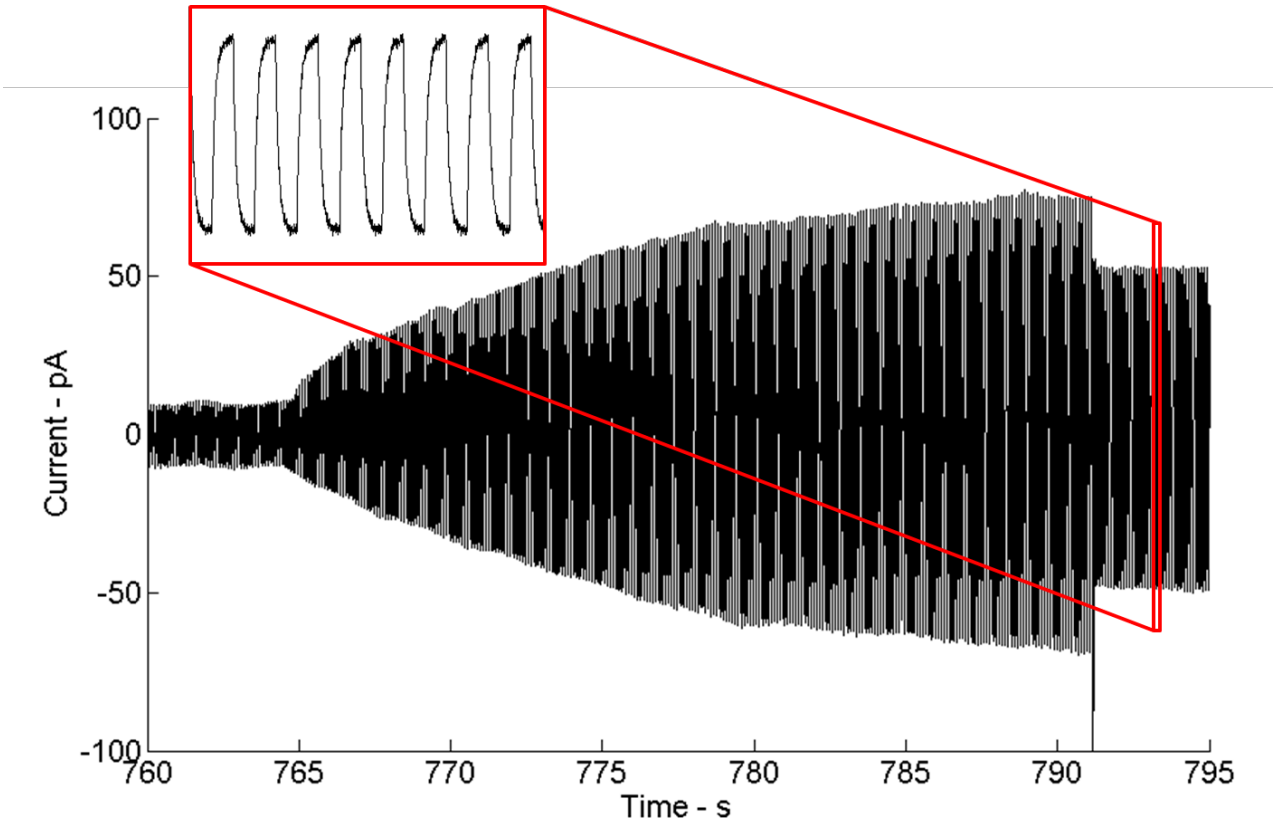

Figure 3-33. Plot of current response of leftmost bilayer in 3x1 array to a 10 Hz, 10 mV peak-to-peak voltage input. The waveform in the red insert shows a sample of about 0.8 seconds of data.

This plot shows a similar pattern of increasing amplitude as the center bilayer. However, while the previous bilayer took over 100 seconds to form and reach a steady state, this bilayer reaches steady state in just over 30 seconds. Also, the waveform for this bilayer does not follow an ideal square wave, which indicates that there may be some leakage. The drop off in amplitude at around 791 seconds on the plot is due to the light being turned off to reduce the noise level. The waveform sample is taken from this low noise data at steady state. Despite the

non-ideal waveform, this current response shows a capacitive bilayer with a similar area as the other two in the array.

The results of this test demonstrate the capability of this system for producing arrays of bilayers. It shows that this design and fabrication process can produce stable bilayers using simple, common laboratory techniques. However, while three bilayers are formed, the limited available precision causes one of the bilayers to form at a different height than the others. This experiment expands upon the results of the single bilayer test and shows that each part of the system works with an array of bilayers. It also shows that three minutes per side is an adequate curing time, and that hydrating the hydrogels with PEG-DMA solution prevents cracking and allows rehydration. Although the limited precision available with inexpensive techniques hinders the creation of large arrays, the fabrication methods have been shown to produce 3x3 arrays of viable hydrogels and electrode connections.

3.5 Discussion

A fabrication and testing process is developed for a meso-scale, hydrogel based DIB array system. The new test setup allows a close up view of the bilayer formation process to aid in forming DIBs and provides additional feedback for improving the system. The oil container provides a reusable and reasonably controlled test environment, and the multiplexer circuit enables fast switching between bilayers on separate channels. The molds used to form the polyurethane substrates are easy to use and allow a quick turnaround time when creating arrays with different dimensions and electrode patterns. The substrates themselves are shown to be durable, and they allow constant connectivity between the wires, electrodes, and hydrogels. The hydrogel curing process is revised to improve their precision and consistency enough to form arrays that stay fixed in place, contact the electrodes, and support lipid monolayer lenses.

Experiments demonstrate the effectiveness of this array system by forming a single bilayer and a 3x1 array. In each test, the bilayers are stable and show the characteristic capacitive behavior. The most important factor limiting the size of the arrays that can be produces is the precision available for fabrication processes. This process is designed to be done inexpensively in a laboratory, so many of the fabrication steps are performed by hand or with handheld tools. Improving on this precision would require either more expensive technologies such as flood UV curing systems or the development of automated fabrication methods. Producing arrays at a smaller scale would require a transition to microfabrication techniques. Despite, the limitations in precision, these experiments show that this type of system can be used to support arrays of DIBs supported by hydrogels.

4 Summary and Conclusions

4.1 Summary

This thesis presents the development of fabrication and testing processes for a hydrogel-based DIB array system. Two methods of array formation are presented, one with photopatterned hydrogels attached directly to a substrate with electrodes and another with polyurethane microchannels to fix lipid supporting hydrogels in place. Arrays of varying dimensions are fabricated using each process, and the microchannel system is used to form arrays of stable DIBs. The fabrication processes for both systems can be performed using only basic laboratory techniques and can be adapted to new designs with minimal turnaround time. Many of the fabrication steps are similar to microfabrication processes that could be used to produce microscale arrays.

4.1.1 Photopatterned Hydrogel Array

A fabrication process is presented for forming arrays of lipid bilayers supported by photopatterned PEG-DMA hydrogels. Simple polyurethane substrates are prepared using a reusable machined mold and provide a flexible, relatively hydrophobic substrate for supporting hydrogels. Silver paint electrodes are deposited onto the substrate surface over an adhesive mask. This method allows for the creation of a variety of simple electrode designs. The electrodes are then treated with Chlorine to form Silver Chloride connection points for the hydrogels. Hydrogel is deposited onto the substrate as an aqueous solution and cured with UV light through a laser printed photomask. The hydrogels are designed to support droplets of aqueous lipid solution that, when submerged in oil, form a monolayer at the interface. The last step in the process would be to combine the substrate with a second similar substrate, such that each hydrogel-supported monolayer is paired with an opposing monolayer to form a DIB.

One problem that arises when patterning the hydrogels onto the polyurethane surface is that, due to the surface's hydrophobic nature, the hydrogels do not adhere strongly to the substrate. When a glass slide is used to support the photomask, the cured hydrogels tend to stick to the slide after it is removed. Even if nothing is placed over the hydrogel when curing, it does not stick to the substrate, and any slight agitation will break it loose. Using an adhesion promotor (trimethylsilyl methacrylate) to chemically bond the hydrogel to the substrate proves ineffective. Using a PEG-DMA substrate, which would form strong adhesion, is not practical as it is not as durable or hydrophobic as polyurethane.

While adhesion problems present the largest barrier preventing the use of this system for bilayer arrays, other issues also arose during testing. The silver paint electrodes showed a tendency to break away from the substrate surface, especially when submerged. The dried paint is brittle, and does not adhere well to polyurethane. In later designs, this is avoided by imprinting the electrode pattern directly into the substrate to provide additional structural support and minimize the influence of shear forces. This feature, however, is more difficult to implement with a machined mold, and became more practical and less time consuming with the introduction of 3D printing techniques. Later substrates are also made with stiffer polyurethane to reduce the stress on surface-based electrodes. Another observation is that the cured hydrogels appear to have tapered edges, instead of vertical, as would be expected when using a vertical UV source. This may be a result of the specific light source used, but the effect could potentially impose a lower limit on hydrogel packing density and make it difficult to accurately predict cured hydrogel size.

4.1.2 Microchannel Based DIB Arrays

In an effort to solve the adhesion issue from the previous system, a new hydrogel-based bilayer array system is designed to mechanically fix the hydrogels in place. This system includes a base substrate similar to the one used in the photopatterned system. The substrate forms a container for oil and supports silver paint electrodes attached to external wires. A second polyurethane substrate contains an array of microchannels (230 μm in diameter) that hold hydrogels in place. This substrate, with hydrogels in place, is attached to the base so that each hydrogel contacts a corresponding electrode. The opposite end of each hydrogel supports a lipid monolayer lens. A second array of hydrogel-supported monolayers is brought into contact with the first, forming DIBs.

The first system created using this design is a relatively large scale system (42x42 mm for a 3x3 array). This system is used to form single bilayers that are verified by applying a triangle wave voltage input. The first test uses a pipette supported hydrogel instead of a second substrate and displays a stable, high resistance bilayer. A second test using a pair of substrates is used to produce a somewhat stable bilayer with a lower resistance. Because of the enclosed nature of this system, the bilayer is formed by manually adjusting the distance between the substrates without visual input. This means that the bilayer often cannot be formed with one attempt. Also, the fabrication process limits the precision that can be achieved when forming the substrates and hydrogels, and depositing the lipid solution. These problems effectively prevent the formation of multiple bilayers using the large scale system.

To form larger arrays of bilayers, a new system is created at a smaller scale (A 3x3 array is 18x18 mm) and with greater precision in fabrication. In this system, the microchannel substrate is fixed in place with the base to ensure a consistent connection between the hydrogels

and electrodes. Also, hydrogels are cured for a longer period and kept hydrated during the curing process. This helps improve their structural integrity and prevents them from moving within their channels. These changes to the process reduce the resistance between the connecting wires and the top of the hydrogels from 2-4 MΩ to 50-150 kΩ. Altering the substrate to more precisely hold hydrogel droplets and using a precision syringe to deposit hydrogels means that the height and shape of each hydrogel in the array is more precisely controlled. Stiffening the base substrate and gluing wires in place help to prevent breaks in the electrical connection to each bilayer. Also, a new test setup is developed that provides more meaningful results and assists in bilayer formation by allowing visual access to the interactions between opposing substrates. Using this test setup, arrays of one, two, and three bilayers are formed. The bilayers can be observed forming visually and electrically, by applying a triangle wave input and observing the individual response of each bilayer.

4.2 Contributions

- A process is developed for photopatterning PEG-DMA hydrogels onto a flat polymer substrate. The process is inexpensive and can be performed using simple laboratory equipment including a laser printer, UV curing gun, hot plate, and simple machined substrate mold. In the future, this technique could be scaled down to produce microscale hydrogel-based bilayer arrays.
- The adhesion of patterned hydrogels to a polyurethane substrate, silver paint, and silver epoxy is investigated. Different methods of adhesion promotion are also tested.
- Conductive silver with silver chloride electrodes is patterned onto polymer substrates using adhesive masks and surface patterned indentations. Surface

indentations prove effective in preventing the dried silver paint from peeling off the substrate.

- A system is developed using microchannel supported hydrogels to form arrays of DIBs. The system can be created using common laboratory techniques, and its functionality is demonstrated by forming arrays of up to three bilayers. The design and fabrication process allows for new systems with different array sizes, dimensions, and electrode patterns to be produced in a matter of hours using only a 3D printer and basic laboratory equipment.
- Reusable molds are created using additive manufacturing to form compact polymer substrates with detailed features including 230 μm diameter microchannels. These substrates support electrodes, hydrogels, and wire connections in an integrated, flexible structure.
- Experiments showed that PEG-DMA hydrogels can be supported and cured in microchannels, and reliable electrical connections can be formed by contact on a flat electrode.
- A procedure is developed for maintaining the hydration, shape, and integrity of PEG-DMA hydrogels for up to two hours while individually curing large hydrogel arrays.
- An experimental setup is developed to form and evaluate bilayer arrays formed using this method. This allows for visual observation of the bilayer formation in addition to electrical interrogation.

4.3 Conclusions

This work presents a novel system for forming lipid bilayer arrays using hydrogels in polymer substrates. Arrays of up to nine hydrogels and three bilayers are created using this fabrication process. The process uses inexpensive materials and fabrication techniques and can be adapted to produce arrays of various dimensions and electrode layouts and at different scales. The hydrogels maintain a consistent connection to the electrodes and produce low resistances suitable for many bilayer applications. Bilayer arrays produced stable, simultaneous DIBs with low leakage. Bilayer array sizes are only limited by available fabrication precision, which could be improved by using more precise, specialized equipment or automating some of the processes. Despite limitations in precision in this instance, the fabrication processes are designed for adaptation to microfabrication techniques. Polyurethane can be molded into finely detailed features in microfabricated devices, silver is commonly patterned to form electrodes in MEMS processes, aqueous hydrogel solution will fill small channels, and photocuring is easily implemented at small scales. The bilayer network system presented here provides a useful experimental tool and forms the basis for a new class of biomolecular material.

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