ABSTRACT

SHAY, TIMOTHY WILLIAM. Hydrogel and Microfluidic Enabling Technologies for Wearable Biomonitoring Devices: Sweat and ECG Sensing (under the direction of Dr. Orlin D. Velev and Dr. Michael D. Dickey)

Wearable devices in healthcare can greatly benefit from the development of new microfluidic sampling methods for sensing biomarkers in sweat. Sweat contains many useful bioindicators that could enable traditional blood based testing techniques in a non-invasive wearable device. Current commercial wearable devices are limited to a combination of motion and heart rate sensors, which provide little insight to the health of the user. Several research groups have shown that sweat sensing patches can be used to analyze the physiological status of the user, but they have the drawbacks that they only work during periods of high sweat rate and cannot manage the flow of fluid to direct it away from their sensors after testing, resulting in higher error over time.

This thesis will present new microfluidic technologies that may enable the next generation of wearable biosensors. The goal of our research is to harness the osmotic properties of hydrogels with a paper microfluidic network to enable continual sweat sensing. These technologies will solve issues related to sensing during low sweat rate and fluid management. We first describe hydrogels that are doped with glycerol or sodium chloride to create a high osmotic pressure in order to pump sweat from the body, mimicking the sweat gland itself, and into our device. Flowrate control and sensing were demonstrated for this pumping mechanism in a lab setting. We then investigated evaporation as a means to continually drive fluid flow through a paper microfluidic network. Evaporation off of the back end of a microfluidic device enables long-term operation of a sweat collection device. Flow control and sensing modalities were demonstrated for this platform. We show that even with
the accumulation of salt due to evaporation, we can still achieve pumping for durations of up to 10 days.

These two technologies, osmotic hydrogel pumping and paper based evaporative pumping, were integrated into a single device to be tested on human subjects. During periods of high sweat rate, the paper microfluidic strip acts as a wicking material to draw sweat from the skin and pass it through a microfluidic channel. During periods of low sweat rate, the osmotic properties of the hydrogel draws fluid from the body, which can then be wicked through the paper microfluidic channel. This microfluidic pumping method ensures the continual sampling of fresh sweat with minimal mixing before sensing has occurred. We present initial results that show the feasibility of this concept on a proof of principle scale. We also introduce possible extensions or modifications of these concepts that may further develop the field of wearable sweat sensing devices.

We will also show how this same hydrogel interface can also be used as a soft electrode to perform ECG measurements on the user. The hydrogel can be made conductive through the addition of ions. We can then implement the use of a liquid metal to create our electrodes, resulting in a device comprised entirely of soft materials. The electrical properties of this system were first tested through impedance spectroscopy. Working prototypes were then created and tested on human subjects, showing better performance than commercial electrodes.

The technologies introduced in this thesis will have a large impact on the future of wearable non-invasive biosensing devices. We have presented and tested prototype devices on a proof of principle level that perform passive sweat collection and management. Non-invasive continual biochemical sensing is a field of untapped potential and will enable patients
to more closely monitor serious medical conditions. These devices will ultimately help patients better monitor their own bodies, which will result in better health for the individual.
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Hydrogel and Microfluidic Enabling Technologies for Wearable Biomonitoring Devices: Sweat and ECG Sensing

by
Timothy William Shay

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APPROVED BY:

Dr. Orlin D. Velev Committee Co-Chair

Dr. Michael D. Dickey Committee Co-Chair

Dr. Jan Genzer

Dr. Balaji Rao

Dr. Michael Daniele
DEDICATION

I would like to dedicate this thesis to my family who have always been supportive of me,

including my decision to move 1000 miles away to attend graduate school.
BIOGRAPHY

Tim Shay was born in Green Bay, Wisconsin to Bill and Judy Shay. Tim has an older brother Dan, who is 5 years older and lives in Milwaukee, WI with his wife Katrina. Tim attended the University of Wisconsin-Madison where he double majored in Chemical Engineering and Applied Mathematics. During his undergraduate pursuit, he had internships/co-ops with the City of Green Bay’s Department of Public Works, Andersen Windows and Kimberly Clark. Following the completion of his doctorate, he will be moving back to the Midwest to pursue an industrial career, as well as to bring him closer to his favorite sports teams (Green Bay Packers, Wisconsin Badgers, Chicago Cubs and Milwaukee Bucks).
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I also want to thank the members of the ASSIST center. ASSIST provided an interactive research atmosphere that most students do not get to participate in. It allowed me to learn about many more facets of engineering that I likely would not have without the center. But more importantly, the people of the center were great to work with and created a friendly social environment.

I want to thank the very friendly staff in the chemical engineering department who have always been extremely helpful with pretty much any questions I have ever had. They helped us graduate students get oriented here at NC State when we first joined the program, and are still working hard to make sure everything is in order for us to graduate.

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Chapter 1

General Introduction of Wearable Health Sensing Devices, Microfluidics, Hydrogels and Liquid Metals

1.1 Introduction

Growing and aging populations are stressing the healthcare ecosystem like never before, resulting in various social and economic problems. More importantly, these healthcare problems are inhibiting the ability of people to have their health needs addressed, resulting in adverse health and wellbeing effects. Most medical visits can consume large amounts of time for both the patient and the physicians/staff performing the needed tests. One method to mitigate these issues is to create sensors that can perform tests that are traditionally performed in a hospital setting during the normal day-to-day routine of a patient. This requires the development of wearable biosensors that can non-invasively perform medical tests while the user goes about their normal routine.¹

Industry has been heavily involved in the emerging wearable sensing market, but with mixed to negative results in most situations. Companies such as Fitbit, Nike, Microsoft, and Garmin (to name a few) have all been involved in the development of wearable health monitoring devices. Interest in these wearables began with high expectations, but has decreased over time due to a multitude of reasons. The largest reason is the lack of sensing modalities available on these devices. Most of these health tracking devices utilize accelerometers and gyroscopes to measure physical activity of the user, while a few also offer heart rate sensing. While these technologies are good breakthroughs in this space, these
sensing modalities offer a very small sliver if information of the overall health status of the user. Another drawback to these devices is that they often utilize many sensors that can rapidly drain batteries. This requires frequent charging of batteries, which leads to decreased use for the user. These issues indicate that at this point users like the concept of wearable health monitors better than the actual devices themselves.\(^2\)

In order for wearable health sensors to be widely accepted and beneficial for the user, new and efficient noninvasive sensing methods need to be developed and incorporated. Therefore, we are exploring methods for harvesting sweat from the body for biochemical sensing purposes. We aim to achieve this through the research and development of passive non-invasive methods utilizing hydrogels and paper microfluidics. Sweat was chosen as a target body fluid for the multitude of health indicators present in it. The hydrogel/body interface we create will also be electrically conductive, which will also enable electrocardiogram (ECG) sensing for monitoring the heart.

The first chapter of this thesis provides a background of current state of the art wearable biosensors, including a theoretical background on the scientific fundamentals and technologies we use to overcome the present device shortfalls. The following chapters discuss how we (1) used osmotically tuned hydrogels to create a passive microfluidic pump for sweat collection, (2) utilized paper microfluidics used for a long-term continual passive pump, (3) merged hydrogels and paper microfluidics to create working sensing prototypes and (4) characterized the electrical properties of hydrogels and a liquid metal to create flexible ECG electrodes.
1.2 Sweat Sensing

1.2.1 Biology of Sweating

Sweating is a natural mechanism performed by the body primarily for temperature control. While sweat is viewed as an unpleasant fluid that is “unclean” and causes odor, it actually provides a plethora of bioanalytes that can provide indicators to the health of the body. The eccrine gland is the predominant type of sweat gland that can be found across the human skin. This gland consists of a secretory coil at its base with a dermal duct that connects the coil to the surface of the skin (Fig 1.1). Upon signaling from the brain to produce sweat, a chain reaction of ion pumping takes place, which creates a high concentration of salt in the secretory coil. This concentration gradient between the secretory coil and the rest of the body creates an osmotic pressure difference which drives the flow of water into secretory coil.\(^3\) Osmotic pressure is a common biological phenomenon, which results from the imbalance in concentrations of two solutions separated by a semipermeable membrane, as described in Equation 1.1.

\[ \Delta \Pi = i(\Delta C)RT \]  

(1.1)
Figure 1.1 Diagram showing the microfluidic network of the eccrine gland. During sweating, active ion pump drives chloride ions into the secretary coil, creating a concentration difference with the interstitial fluid. This creates an osmotic pressure gradient, driving fluid into the secretory coil. As fluid is continually pumped into this coil, it passes through the dermal duct and exits on the surface of the skin as sweat.3

The flux of fluid into the secretory coil draws along many of dissolved bioanalytes present in the interstitial fluid and blood of the body. These bioanalytes can be used as biomarkers for monitoring many different health conditions. A few of these bioanalytes and what health indicators they represent are listed in Table 1.1.
Table 1.1: Listing of various biochemical that can be found in both sweat and blood (as well as their concentration ranges). These bioanalytes can be sensed as an indicator for various health conditions.

<table>
<thead>
<tr>
<th>Bioanalyte</th>
<th>Indicator For</th>
<th>Sweat (mM)</th>
<th>Blood (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Diabetes</td>
<td>0.02 – 0.4</td>
<td>4 – 12</td>
</tr>
<tr>
<td>Sodium (Na(^{+}))(^{5,6})</td>
<td>Hydration</td>
<td>10 – 100</td>
<td>135 – 100</td>
</tr>
<tr>
<td>Chloride (Cl(^{-}))(^{5,7})</td>
<td>Hydration</td>
<td>10 – 100</td>
<td>96 – 106</td>
</tr>
<tr>
<td>Potassium (K(^{+}))(^{8})</td>
<td>Hydration</td>
<td>4 – 24</td>
<td>5 – 6</td>
</tr>
<tr>
<td>Ammonium (NH(_{4}^{+}))(^{9})</td>
<td>Liver Failure</td>
<td>0.5 – 8</td>
<td>20 – 50</td>
</tr>
<tr>
<td>Ethanol(^{10})</td>
<td>Alcohol Use</td>
<td>2.25 – 22.5</td>
<td>2.25 – 22.5</td>
</tr>
<tr>
<td>Cortisol(^{11-13})</td>
<td>Stress</td>
<td>2.21 – 38.6 x 10(^{-5})</td>
<td>12.4 – 40 x 10(^{-5})</td>
</tr>
<tr>
<td>Lactate(^{14-17})</td>
<td>Exertion</td>
<td>5 – 60</td>
<td>1 – 7</td>
</tr>
</tbody>
</table>

It is necessary to understand the relationship between sweat and blood levels of bioanalytes if their sweat concentration is to be used as a reference for the rest of the body. Many studies have been performed to analyze these correlations for specific biomarkers. One such study looked at the correlation between glucose levels in sweat and blood. They measured both sweat and blood glucose levels of multiple diabetic patients and used this data to create a predictive model to determine blood glucose from measured sweat glucose levels. These predicted glucose concentrations show good agreement with reference blood glucose concentrations, thus demonstrating the proof of principle that sweat glucose levels can be used to accurately monitor blood glucose levels\(^{4}\). Studies have also been performed in a similar manner for cortisol, a hormone that is an indicator for stress. These results also show a distinct correlation between serum and salivary cortisol\(^{11}\). Lactate\(^{18}\) and ethanol\(^{10}\) concentration correlations between sweat and blood have also been found (Fig. 1.2). These examples demonstrate that correlations between blood and sweat analyte levels can be used for sensing purposes.
Figure 1.2 Plots showing correlations of bioanalytes between blood and sweat. (a) Plot showing estimated blood glucose levels (determined from sweat glucose) versus a reference blood glucose sample. The upper and lower lines are ± 20% from a direct correlation. (b) Plot showing measured salivary cortisol versus measured cortisol from serum. (c) Plot displaying measured ethanol concentration in blood versus sweat. (d) Plot displaying measured lactate concentration in capillary blood versus sweat.

Each of these studies is important as they provide correlations between concentrations of analytes in sweat and in the body. These correlations are required if medical action is to be taken based off of sweat concentrations. It has been shown that sweat rate does effect analyte
concentration in sweat.\textsuperscript{3} As sweat travels through the dermal duct of the sweat gland, bioanalytes are able to permeate back into the body through the walls of the sweat gland. Higher sweat rates therefore result in higher analyte concentration, as there is a lower retention time for the chemicals to diffuse back into the body. This indicates that sweat rate monitors may also be required to create precise correlation curves between sweat and blood concentration levels. Measurements taken from sweat can also produce higher than expected analyte concentrations due to evaporation of the water from the sample. While there are multiple factors affecting analyte levels in sweat, there are enough resources to accurately create correlation curves enabling accurate biochemical sweat sensing.

1.2.2 Past Sweat and Non-invasive Biosensing Devices in Industry

Sweat testing was first performed in the 1940’s as a method to diagnose cystic fibrosis by detecting elevated chloride levels.\textsuperscript{8} Drug testing was also performed to determine if a person has been consuming illegal narcotics.\textsuperscript{19,20} For many of these tests, patients would need to physically exert themselves to release large enough quantities of sweat for sensing. This sweat was collected with a wipe or absorbent patch and then tested in a lab setting. This method of sweat collection testing is inconvenient and time consuming for both the patient and the medical staff performing the test.

Apart from physical exertion, sweat glands can be stimulated chemically to produce elevated sweat rates. The chemical substance pilocarpine can be introduced to the sweat glands to promote sweating. This development lead to the creation of the Macroduct\textsuperscript{®}, a sweat
collection device for cystic fibrosis testing (Fig 1.3). Pilocarpine is transferred into the skin via iontophoresis, a process where two electrodes interfacing the skin are used to drive the chemical into the skin via electrophoretic flows. The Macroduct® device is then strapped onto the forearm where the pilocarpine was introduced. The Macroduct® consists of a coiled tube that has an opening that interfaces the skin. Sweat released from the body is collected in this coiled tube and then analyzed in a lab afterwards. This device has the advantage that the patient is not required to perform physical activity and the device collects the fluid in a coiled tube, from which it is easy to test. The Macroduct® has also been shown to provide accurate chloride readings for human subjects. However, this does not avoid the drawback that lab testing is still required for the collection of sweat, which is costly and time consuming. Reverse iontophoresis has also been known to irritate the skin, an inconvenience for the subject.

Figure 1.3 (a) Image of the electrodes placed on an infant’s arm used to drive the pilocarpine into the skin via iontophoresis. (b) Picture of a Macroduct® device on an infant’s hand collecting sweat. A blue dye is placed in the tubing to track the collection of sweat. (c) Image of a Macroduct® device.

The GlucoWatch G2 Biographer is a commercial device used for measuring blood glucose through noninvasive means. This device is worn as a wristband and interfaces the
body with two electrodes. A low DC current is drawn between these electrodes, driving the movement of ions through the body. Human skin has a net negative charge, resulting in the interstitial fluid being rich with sodium ions (Na\(^+\)). During operation, there is a large flux of sodium ions towards the cathode, resulting in a convective flow that is able to carry along uncharged molecules, such as glucose. Glucose sensing is performed on a biosensor integrated into the cathode.\(^{23}\) This technology enables non-invasive glucose sensing as frequently as every 20 minutes. A schematic and image of the device can be seen Fig 1.4.

\[\text{Figure 1.4} \quad \text{(a) Profile diagram of the GlucoWatch and the process of reverse iontophoresis. Electric fields are used to drive interstitial fluid to the cathode for biochemical sensing.} \quad \text{(b) Photograph of the skin interface of the actual device showing the two electrodes required for reverse iontophoresis. The cathode has multiple adjunct electrodes to act as the working and reference electrodes for glucose sensing.}\(^{23}\)\]

Multiple clinical studies were performed on the GlucoWatch to determine its accuracy. Initial studies performed showed that the GlucoWatch had good agreement of measured analytes to blood tested samples, albeit with variance in the data.\(^{24}\) A further study focused on glucose measurements has been carried out under hypoglycemic conditions, when glucose...
levels are lower. The results showed that the GlucoWatch had a median difference of 26 mg/dL from reference samples. This resulted in a false-alarm rate of 51% when the alarm was set to 60 mg/dL.\footnote{25} This latter study highlighted the perils of using such devices as a primary means to measure glucose levels near critical concentrations.

The GlucoWatch had other design flaws as well. This device required a three hour warmup time. Finger stick blood tests were still required for calibrating the device, defeating the purpose of using this device to avoid painful finger pricks. The electric current from the reverse iontophoresis was also found to be discomforting and even painful for some users.\footnote{26} Due to these multiple issues with the device, users were dissatisfied and the company stopped production. This case study highlights the need for non-invasive biosensors that are accurate, comfortable and work for long terms in order to be effective and accepted by the user and general public. While noninvasive and sweat sensing methods have been prevalent in industry for decades, there are currently no commercial wearable sweat sensing devices.

1.2.3 \textit{Academic Investigations of Sweat Sensing Devices}

Sweat collection and sensing research has grown rapidly in recent years in academia. The research groups of Jason Heikenfeld and Ali Javey have both taken similar to each other approaches to creating sweat sensing devices. Their research groups have created various patch type devices utilizing biochemical sensors that interface the skin to measure bioanalyte levels during sweating. The sweat sensing devices from Heikenfeld’s lab have been capable of measuring sodium levels in sweat. Their devices are fabricated in two sizes, one the size of
a band aid and another slightly larger that could be placed on a body area such as the knee. The electronics and circuits are fabricated on thin flexible materials. Pd and Ag metals are then deposited on the device to create the working and reference electrodes. Chloridization is performed on the reference electrode to create an Ag/AgCl electrode. A sodium ion-selective membrane is then adhered to the working electrode to create an ion-selective electrode. Adhesives purchased from 3M are used to adhere the device to the skin. Testing performed in a lab setting has successfully demonstrated the ability of this device to measure sodium levels (Fig 1.5). Further research has been performed to investigate whether the presence of an oil membrane between the sensor and skin can help reduce sample contamination. The oil film prevents spreading of the sweat on the surface of the skin. This reduces contamination of sweat prior to sensing and may result in higher testing accuracy.
Figure 1.5 (a) Photos of the flexible sodium-sensing device. (b) Schematic of the electrode portion of the device. An ion-selective membrane is placed over the working electrode to allow for sodium ion detection (sodium is demonstrated in this figure). (c) Calibration curve for sodium levels and (d) resulting tests from step changes in the sampled solution between 20 and 70 mM sodium.

Javey’s lab has created a similar device which utilizes electrodes directly interfacing the skin to perform biosensing (Fig. 1.6). Sensors are fabricated on a flexible polyethylene terephthalate (PET) substrate. These electrodes can be customized to be able to sense for temperature, lactate, glucose, sodium and potassium concentration. Sodium and potassium sensors utilize ion-selective membranes, similar to the work of the Heikenfeld group. The lactate and glucose sensors utilize lactate and glucose oxidase to perform amperometric sensing. Temperature is measured through resistance based sensing techniques. These sensors
were packaged as a wearable device that can be used on either the wrist or the forehead. In both situations, a strap is used to attach the device to the body. Subjects have worn these devices and performed various exercises to stimulate sweat while the sensors monitored the body.²⁹

**Figure 1.6** (a,b) Images of the sweat sensing device. (c) A schematic of the electrode and printed circuit board arrangement. (d,e) Measurements of multiple bio signals (physical and biochemical) during a trial with a human subject.²⁹
John Rogers’ group (Illinois/Northwestern) has specialized in creating flexible and wearable electronics. Their early work focused around creating thin film electronic devices that were very flexible. The flexibility was created by depositing a thin film of gold on a flexible polymer substrate. This work transitioned to the creation of epidermal electronics, also known as electronic temporary tattoos. Sensing was first performed with this system by measuring an electrocardiogram (ECG) and hydration levels. Their flexible electronic system was then patterned onto a hydrophilic porous material for interfacing skin for the purpose of sweat collection and testing. With this system, they have been able to test for pH, copper and iron concentrations in sweat using colorimetric methods.

Each of these groups took the approach of creating sensors that interface directly to the skin to electrochemically sense sweat that has been released from the body. One problem with this method is that these devices are unable to manage the fluid after sensing. The earlier collected sweat that has been tested will inherently mix with new sweat and provide error in the reading. Contamination will also occur as sweat contacts the skin. Therefore, the Rogers’ group adapted a new strategy for their next generation sweat sensing device and created a flexible microfluidic silicon device that is adhered to the skin. Microfluidics allow for management of the sweat by transporting it through a continual network. Their device is able to measure sweat rate, lactate, chloride, glucose and pH through visual colorimetric means. Chemical sensing is performed through depositing commercially available assays onto paper discs that are located at the entrance to the microfluidic channels. Colorimetric assays
were used for bioanalyte sensing and sweat rate was determined through visualizing the progression of sweat flow through the outer microfluidic channel.

A smartphone can be used as a convenient means to take a photo of the device and analyze the colors for concentration. These devices were tested on human subjects while exercising. The sensing output was compared to in-lab tests performed separately as a reference. Good agreement was found between the prototype and the in-lab testing. This work was then expanded to create a device where the collected fluid is stored in separate chambers in the microfluidic device. Each chamber corresponds to sweat collected at various times throughout the test. This fluid can be analyzed in vitro and produce chronological data for various sweat analytes such as potassium, sodium and lactate.
A recent development has seen the introduction of paper microfluidics into wearable sweat sensing devices as a means to wick fluid from the body for biosensing. This device utilized a paper microfluidic strip sandwiched between two thin plastic films. One end of the paper strip interfaces a reservoir which contacted the skin to collect sweat. The back end of the paper strip is exposed to the environment to allow for evaporation of the collected sweat. Electrodes were interfaced to the paper to detect pH, sodium and lactate concentrations.
concentration. This device incorporates the use of a microfluidic network to mitigate the errors associated with directly interfacing electrodes to the surface of the skin.

Figure 1.8 (a) Schematic of the sweat-sensing devices comprised of a paper microfluidic strip sandwiched between two thin PMMA layers. Electrodes interface the paper strip near the inlet of the device. (b) Photos of the device by itself and during use on a human subject. (c) Test results of the measured sodium, pH and lactate of the sweat collected over time.
These academic studies have made strides in creating wearable real time sweat sensing devices, but there are inherent drawbacks to these designs that limit their capabilities. Many of these devices can exhibit inaccuracy in testing due to the lack of management of the earlier or excessive sweat that has been released from the body. While recent efforts have incorporated microfluidic networks to collect sweat, these devices still require high sweat rates to function well. These sweat rates were achieved by having patients exert themselves physically. This method is fine for athletic performance, but normal sweat rates are much lower and will prove difficult to monitor and collect continually.

Long-term management of sweat has not been addressed in the literature and remains a challenge. As sweat evaporates, salt and other solutes will accumulate, which will inevitably inhibit the evaporation rates on the device. Therefore, further research is required to determine how microfluidic networks can be used to collect, pump, and evaporate off sweat to enable long-term sweat sensing. This will be the primary focus of this dissertation.

1.3 The Electrocardiogram

1.3.1 Biology of the Electrocardiogram

Monitoring cardiovascular health is key to preventing heart related medical issues. One of the most effective methods of non-invasively monitoring the heart is through the use of electrocardiogram (ECG). The ECG shows the electrical activity produced by the heart. These electrical signals arise from the depolarization of the heart’s muscles. The flux of ions across
the cell membranes in the muscle creates a voltage that is constantly fluctuating. Measuring these signals makes possible to record the ECG.\textsuperscript{37}

These electrical signals are normally monitored by interfacing the body with between 3 and 14 electrodes (Fig 1.9a). An example ECG can be seen below. The signal can be broken up into five specific impulses that occur during the heartbeat (Fig 1.9b). The various waves and complexes created from these impulses are correlated to a specific function in the heart. Medical experts are able to examine these waves to look for abnormalities that may indicate issues with the heart. The time delay between ECG signals may also be used to determine heart rate of the user if implemented in a wearable device.\textsuperscript{37,38}

\textbf{Figure 1.9} (a) An example of a commercial ECG electrode and (b) a representation of a normal ECG showing the waves and intervals.
1.3.2 Past ECG Electrode Designs and Materials

The magnitudes of the electrical signals produced by the heart are on the order of millivolts. This requires the use of low impedance electrodes to measure this signal with minimal noise. To achieve this, a high conductivity interface is required to be placed between the electrode and the skin. The ECG electrode types are generally broken into two classes, dry and wet electrodes. Dry electrodes generally utilize a solid printed metal or conductive material to interface the skin directly. Wet electrodes utilize an electrolytic gel or fluid to interface a metal electrode to skin.

Screen printing has been used extensively for dry electrodes (ECG and others) to create thin metal films of specific shape. Through screen printing, pastes and nanowire/nanotube solutions can be deposited on flexible substrates. One research group has screen printed silver paste into textiles to create flexible ECG electrodes.\(^{39}\) Another setup cast a silver nanowire (AgNW) solution in a specific pattern and encased it in PDMS. This creates a stretchable electrode that has been used for ECG and hydration sensing.\(^{40,41}\) Deposition of metal directly onto PDMS has also been used to create a dry ECG electrode.\(^{42}\) However, high interfacial impedances between electrodes and skin have prevented dry electrodes from being utilized on a large scale. Dry electrodes often provide a poor electrical connection to skin, resulting in a higher noise ECG signal.\(^{43,44}\)

Wet electrodes, which utilized liquids and gels are often used as an interface material for ECG electrodes as they are soft and can conform to the skin. They can also help rehydrate the stratum Corneum, a layer of dead skin that provides a high electrical impedance. The Cui
research group utilized a polyacrylate hydrogel to create ECG electrodes. The hydrogel was
swollen in a solution of sodium chloride to increase ionic strength and decrease the resistivity.
A silver/silver chloride wire was then inserted into one end of a pre-cut hydrogel disc to
complete the electrode. These electrodes have displayed comparable impedances to current
commercial ECG electrodes. Most commercial electrodes utilize a wet interface.

1.4 Microfluidics

1.4.1 Surface Tension and Capillary Pressure

Devices that collect and manage sweat will operate with small amounts of fluid, as
sweat rates are usually small. Fluids behave much differently on smaller scales than large ones
due to the increased importance of surface tension over gravitational forces. Surface tension
is created by the cohesive forces between molecules in liquids. Interfaces of liquids, whether
with solids, gases or other liquids, have an inherent pressure due to their intermolecular
interactions. These interfaces often create curved surfaces because of the balance of adhesive
and cohesive forces of the materials. A pressure difference arises from this curvature and is
determined from the Young-Laplace equation:

\[ \Delta P = \gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right) \]  

(1.2)

This equation relates the Laplace pressure to the surface tension (\( \lambda \)) and the principle
radii (\( R_i \)) of curvature of the liquid interface. The radius of curvature is largely controlled by
the interactions between the liquid and other surfaces, namely the fluid’s wettability on a solid
surface. In a three phase system comprising a solid (S), a liquid (L) and a gas (G), there are three distinct surface tensions that balance each other and define the wetting contact angle, as described by the Young equation. \[ \gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cos \theta \] (1.3)

When dealing with fluids in small geometries, it is important to understand these principles as it affects how the fluid will flow through microchannels. Small volumes of fluid, microliters for example, are often used in microfluidics. In these systems, fluid is transported through well-defined channels with heights and widths of less than one millimeter. The Laplace pressure \( p_c \) in these systems can be defined as follows

\[ p_c = \frac{2 \gamma \cos \theta}{r} \] (1.4)

Most microfluidic systems use water, which has a fixed surface tension. Therefore, hydraulic radius \( r \) and wettability \( \theta \) are the parameters that can be adjusted to modify the flow in the system. Microfluidic materials with a high surface energy, and therefore small contact angle, create a positive pressure resulting in a capillary flow of fluid into the channel. Low surface energy materials strongly resist flow into a microfluidic channel. By assuming a Poiseuille flow in a laminar regime, the pressure drops from hydrodynamic resistances can be estimated and correlated to the capillary pressure. This results in the following equation, known as the Washburn equation, which describes the transient water rise in a capillary channel over time : \[ p_c = \frac{2 \gamma \cos \theta}{r} \] (1.4)
\( h^2 = \frac{r \gamma \cos \theta}{2 \eta} t \)  \hfill (1.5)

This equation neglects gravity, as capillary forces are generally much larger than gravitational forces exerted on small volumes of fluid. The Washburn equation works well under initial periods of capillary action, but deviates as capillary rise increases causing the gravitational force to increase. To determine the equilibrium value of capillary rise, the capillary force can be balanced against gravity \((g)\) to determine the equilibrium height \((h)\).

\[ h = \frac{2 \gamma \cos \theta}{\rho g r} \]  \hfill (1.6)

These principles also apply for liquids infused in porous materials. While not uniform, porous materials often have interconnected void regions that behave similarly to capillary channels. This explains why materials such as paper towels wick fluid through their matrix. The same equations as above can be utilized by replacing the channel radius with an average-equivalent pore radius.

These principles are important to understand because they govern how water will behave on microscales. Surface and geometrical modifications to a microchannel can alter the capillary pressure and dictate whether or not capillary action will pull water through a channel or porous material.
1.4.2 PDMS Microfluidics Fabrication

The need for management and control of small volumes of fluids led to the development of the microfluidics field. Microfluidics systems normally utilize small channels that have heights and widths ranging from 10 – 1000 micrometers. The field of microfluidics expanded after the soft lithography was introduced to create poly(dimethysiloxane) (PDMS) microfluidic networks.49,50 The first step in soft lithography is to design the microfluidic system using computer software. This design is then printed onto a transparent sheet as a negative (the channel regions are transparent while the rest is solid black). A photoresist is applied to a silicon wafer through spin coating to achieve a uniformly thin film. The photomask is then placed on the wafer and UV light is applied to polymerize the selected regions of the photoresist (as designated by the photomask). The unreacted photoresist is removed with a developing solution. The remaining cured polymer creates a microfluidic mold, which PDMS is then polymerized on. This imprints the shape of the microfluidic system into a sheet of PDMS. An oxygen plasma treatment can be performed to create hydroxyl groups on the surface of the PDMS. Two PDMS pieces that have been oxygen plasma treated will form a chemical bond when interfaced to each other. This method is used seal the PDMS microfluidic system. This method is described in Fig 1.10.
Figure 1.10 Schematic showing the steps of soft lithography for creating PDMS microfluidics. (a) Photoresist is spun-coat onto a silicon wafer. Photolithography is used with a photomask to create a master mold to which PDMS can be (b) polymerized on and (c) removed. (d) The PDMS with the imprinted microchannel can be bonded to another sheet of PDMS or glass through an oxygen plasma treatment to close the microchannel.\textsuperscript{51}

While this fabrication method is simple and widely used, many other techniques for creating microfluidic channels have been introduced. For example, recent developments have seen microfluidic devices being created through various 3D printing processes (Fig 1.11). One such 3D printing technique utilizes a liquid metal (EGaIn) to print a design on the surface of a substrate. The liquid metal has an oxide skin that holds its shape in response to low stresses. The liquid metal is then printed on the surface of a silicon wafer and a polymer resin is then
used to encapsulate this design. The liquid metal can then be removed after polymerization, resulting in a hollow microchannel. This method is convenient as pre-made photomasks are not required and the liquid metal can be reused multiple times. Channel widths as small as 70 \( \mu \text{m} \) can be created. This procedure can be repeated to create 3D microfluidic networks.\textsuperscript{52}

Another technique uses a standard 3D printer that ejects acrylonitrile butadiene styrene (ABS). The microfluidic network is first printed using ABS. The printed ABS is then inserted into liquid PDMS, followed by PDMS polymerization. This encapsulates the ABS design in the cured PDMS. Acetone is then used to dissolve away the ABS, resulting in void regions that create the resulting microfluidic network.\textsuperscript{53} While the resolution of this technique is not as high as the soft lithography methods, this technique allows for complex 3D networks to be created in very few steps.
Figure 1.11  (a) Steps showing how a liquid metal can be used to 3D print microfluidic channels. The liquid metal is first printed onto a 2D substrate. A polymer resin is used to encapsulate the liquid metal. After polymerization, the liquid metal can be removed and then refilled with a target solution.  (b) Steps showing how ABS can be 3D printed, cured in PDMS and removed by immersion in acetone to create a 3D microfluidic network in just two steps.
While we only focused on a few of the multiple available techniques, there are many more methods to create PDMS microfluidic networks. This versatile material has opened the door for researchers to easily fabricate microscale fluidic platforms for various sensing, reacting and separating operations.

1.4.3 PDMS Microfluidic Biosensing Methods

Microfluidics have grown to prominence in biomedical testing due to their ability to perform sensing on very small volumes of fluid. This introduction will focus on two such modes of sensing: optical and electrochemical.\textsuperscript{54}

Electrochemical biosensing is often performed through the use of an enzymatic reaction in a three electrode system. Enzymatic biosensing utilizes chronoamperometry across a series of embedded electrodes in the microfluidic channel. The required enzyme (glucose oxidase for glucose sensing, for example) will be deposited on one of the electrodes (working electrode). The working electrode will be held at a constant voltage relative to a reference electrode. This constant voltage can be determined through cyclic voltammetry. The voltage with peak current is selected, as it provides the largest reaction rate resulting in better sensitivity. A third electrode (counter electrode) is used to apply current to ensure a constant potential between electrodes.\textsuperscript{55}

An example of how cyclic voltammetry can be used to obtain a calibration curve for a glucose electrode is shown in Fig 1.12. When the solution of interest comes into contact with the electrodes, the enzyme selectively reduces its target molecule on the working
electrode. This reduction results in the loss of electrons, which can be measured by the electrode. Measuring the current of the electrode provides a direct correlation to the rate of the reaction being performed, which can then be correlated to the analyte concentration in solution.\textsuperscript{56}

Figure 1.12 (a) Cyclic voltammograms of an enzymatic glucose sensor with varying concentrations of glucose in solution. (b) A calibration curve obtained from the peak current measured versus the glucose concentration.\textsuperscript{57}

Optical sensing in microdevices can be performed both on and off the microfluidic platform.\textsuperscript{58} Off-chip sensing requires the microfluidic device to be analyzed using larger measurement equipment that are not integrated into the microfluidic channel. These testing methods may require the use of a microscope or spectrophotometer. On-chip sensing utilizes MEMS devices integrated into the microfluidic device. These devices are much more complex than the sensing devices and need to be minimized in size to fit onto the microfluidic platform. These optical detection methods operate by principles of absorbance,\textsuperscript{59–64} fluorescence,\textsuperscript{65–76} chemiluminescence\textsuperscript{77–80} or refractive index.\textsuperscript{81–85} These methods of sensing have been used for
many different analytes in various systems. Sensing methods have been previously used for
detection of many of the target bioanalytes present in sweat including sodium, potassium, 
chloride,\textsuperscript{86,87} ammonium,\textsuperscript{88} cortisol,\textsuperscript{89,90} and glucose.\textsuperscript{91} These many sensing modalities 
demonstrate the versatility and feasibility of using PDMS microfluidics for biosensing 
operations.

1.4.4 Active Microfluidic Pumping Mechanisms

The PDMS microfluidic channels are naturally hydrophobic and resist the flow of water. This creates a need for an external pumping force to drive fluid flow through the microchannels, leading to the development of various pumping mechanisms implemented in microfluidic networks. Many microfluidic pumps are active, meaning that electrical energy is required for actuation. Pumping mechanisms can largely be broken up into two different groups, displacement and dynamic pumps.\textsuperscript{92} Both pumping mechanisms incorporate concepts from larger scale fluid pumps, but have been designed to work on the microscale.

Micro-displacement pumps commonly utilize piezoelectric materials to actuate a diaphragm. These pumps are designed to draw fluid into a chamber and discharge it through an outlet during actuation.\textsuperscript{93} The pumps are normally micromachined through various etching and bonding steps. Displacement pumps have been created to utilize multiple chambers for increased performance.\textsuperscript{94,95}

Dynamic pumps that utilize no moving parts to pump fluid have also been created. These pumps can be broken into three different classes; electrohydrodynamic, electroosmotic and magnetohydrodynamic. Electrohydrodynamic pumps use electrostatic interactions with
ions in a dielectric fluid to drive flow.\textsuperscript{92,96,97} Electroosmotic pumps utilize the electric double layer that is created when a fluid contacts a surface. An electric field is applied across a channel to drive the flow of the counter ion to induce fluid flow.\textsuperscript{98} Magnetohydrodynamic pumps utilize magnetic fields to drive the motion of ions and induce concurrent fluid flow.\textsuperscript{99}

1.4.5 Passive Microfluidic Pumping Mechanisms

Many active microfluidic pumps suffer from poor efficiency characteristics. While higher power consumption may not be problematic in a lab setting, implementation of microfluidic pumps into wearable devices will require low to no power to prevent draining the energy required to run the rest of the system. This creates the need for passive microfluidic pumping mechanisms that require no electrical energy input.

Passive microfluidic pumps have been created that rely on either evaporation, capillary action or osmotic pressure to engender fluid flow. These pumps rely on pressure and potential differences that rely on no electrical energy input. Capillary pumps operate due to the pressure difference at the water surface tension, while other osmotic and evaporative pumps utilize differences in chemical composition.

Passive pumps have been that utilize evaporation operate on the basis of the humidity difference between the atmosphere and liquid water. This mechanism, along with capillary action, is how plants and trees pump water throughout their trunk, branches and leaves.\textsuperscript{100,101} Evaporation has been used on the back end of microfluidic systems to continually pump water through a microchannel. One such device has utilized a large opening at the end of the microfluidic network that is exposed to the atmosphere (Fig 1.13a). As water evaporates from
a curved meniscus at this outlet, capillary action pulls water from the reservoir through the microchannel to the replenish the lost water.\textsuperscript{102} Another evaporation pump has utilized a filter paper plug at the exit of the microchannel to facilitate evaporation.\textsuperscript{103} A separate pump mimics the xylem in plants to achieve microfluidic pumping, as evaporation from leaves is how trees pump fluid to drive fluid flow.\textsuperscript{104}

Osmotic forces have been used to create passive micropumps.\textsuperscript{105,106} In these systems a saturated brine solution is separated from ambient solution by a semipermeable membrane (Fig 1.13b). This concentration difference creates an osmotic pressure gradient that drives fluid flow into the brine solution. The osmotically drawn fluid is pumped out through an attached tube to be tested.

Capillary action has been used to create passive pumps. One capillary pump utilized arrays of tightly packed hydrophilic pillars in a microfluidic device to create a pumping force (Fig 1.13c). Water would wet these microstructures due to surface tension effects. As the water menisci expand around these structures, they come into contact with and wet the next hydrophilic structure. This process continues as more and more of the hydrophilic structures are wetted, which creates the pumping action seen in the device.\textsuperscript{107}
Figure 1.13 Examples of past pumps in the literature based off of (a) evaporation,\textsuperscript{102} (b) osmotic pressure\textsuperscript{106} and (c) capillary action.\textsuperscript{107}

These passive pumping mechanisms are important as they provide a method to mechanically pump fluid without the use of electrical energy. Future wearable devices that utilize microfluidic networks will need to use adaptations of these technologies to reduce the already high strain on power requirements in order to achieve a longer battery life.

1.4.6 Paper-based Microfluidics

While PDMS microfluidic devices provide many opportunities for lab-based sensing, they require complex fabrication methods, which prevent their large scale use in non-lab settings. They also require methods to pump the fluid through the microfluidic network, which can be expensive and introduces unnecessary complexity. This has led to the development of new materials for microfluidic devices.
One of the more promising materials investigated for microfluidic operations was filter paper, which has many qualities that lend itself naturally to microfluidic liquid handling. Filter paper is both porous and very hydrophilic. This creates a high capillary pressure that is able to wick water into its matrix. Therefore paper microfluidics are able to draw in water passively without the use of an external power. Since the first publication of paper microfluidics in 2007,\textsuperscript{108} this field has grown greatly in respect to fabrication methods, biochemical sensing modalities and flow control.

Paper microfluidics can be implemented in many ways that can be scaled up for manufacturing processes. The paper substrates used in microfluidic systems are inherently homogenous, meaning that fluid introduced to the system would spread uniformly in all directions in the paper. To guide the fluid flow, the paper must be altered to direct flow. The simplest method to control flow is by cutting the paper to the specific design of the microfluidic network. This can be achieved with knife\textsuperscript{109} or laser cutting\textsuperscript{110,111} operations. These patterning techniques often result in a mechanically weak substrate as much of the paper has been removed\textsuperscript{112}.

Patterning filter paper to control fluid flow can also be performed through various printing and lithographic techniques. These methods modify the paper to create hydrophobic regions, preventing capillary action from driving fluid into these areas. The first paper microfluidic devices were fabricated through photolithographic techniques.\textsuperscript{108} A paper substrate was saturated with a photoresist and then exposed to UV light through a predesigned photo mask. Areas with polymerized photoresist became hydrophobic while un-polymerized
material was washed away, leaving the paper in its porous hydrophilic state. Wax printing methods have been utilized in multiple manners to pattern hydrophobic regions into paper.\textsuperscript{113–115}

The measure of sorptivity defines how a porous material is able to absorb fluid via capillary action. Fluid wicking through a porous material is analogous to capillary rise in hydrophilic channels. Equilibrium capillary rise can be described utilizing the same capillary rise equation for channels by applying the average pore radius ($r_p$). However, due to the higher surface area to volume ratio of porous materials, hydrodynamic resistances are much higher. Darcy’s law describes these pressure gradients relative to the flow of fluid ($Q$) through the paper device, viscosity ($\mu$), permeability ($\kappa$) and cross-sectional area ($A$). This equation can be used to predict and adjust the flowrate of paper microfluidic devices.\textsuperscript{116}

\[
\nabla P = -\frac{\mu}{\kappa A} Q
\]

Flow control has been implemented in some devices to limit the total amount of fluid collected or control the velocity of the fluid front. The modified Washburn equation describes how pressure drives fluid flow through a porous material. Utilizing this principle has allowed researchers to create paper microfluidic prototypes where the front of fluid flow advances at a constant velocity (Fig 1.14a).\textsuperscript{117} This was achieved by utilizing paper channels with decreasing widths. The total flowrate of fluid will continually decrease as the fluid path length, and pressure drop, increase. This problem was mitigated by tapering the channel such that the velocity remains constant, even as total flow decreases.
Paper microfluidic devices have been created that can have step decreases over time in the total flowrate. These flow patterns are achieved through careful design of the paper microfluidic geometry. The flowrate is determined by the capillary pressure of the hydrophilic pores and limited by the area of paper capable of wetting. Creating microfluidic devices with step changes in area will decrease the total flowrate possible in the device. This was demonstrated for periods of up to one hour.\textsuperscript{118}

![Figure 1.14](image)

**Figure 1.14** (a) Paper microfluidic devices fabricated to achieve a desired velocity profile over time.\textsuperscript{117} (b) Paper microfluidics capable of step changes in fluid flow due to their geometrical design of the wicking region.\textsuperscript{118}

One method for limiting the total amount of fluid collected in a paper device is to utilize a dissolvable bridge.\textsuperscript{119} Two separate paper microfluidic strips are connected through a solid bridge composed of a porous water-soluble material. This enables fluid to flow from one strip through the bridge to the second strip. As the fluid is transported through the bridge, it dissolves it. When the bridge becomes completely dissolved, the fluid is prevented from
passing to the second paper strip. Paper microfluidic devices can also be fabricated with 3D networks, which allow for even more complex system and flow designs.  

Sensing on paper microfluidics is traditionally performed using indicators that have been deposited on the paper prior to testing. The tested fluid enters a region where assays have been deposited as it wicks through the paper. These analytes then react with the assays to create a visual color change. The first paper microfluidic device utilized this concept by incorporating a colorimetric sensor to measure glucose and protein levels in human urine. Many glucose based paper sensors have been described since this first invention.  

Electrochemical sensing can be performed on a paper substrate with methods similar to the ones in PDMS microfluidics. One group utilized paper microfluidics to separate plasma from blood and then perform biochemical glucose sensing. Their paper device wicks away the plasma, together with the dissolved glucose, from the whole blood sample towards a detection zone. This detection zone uses a three electrode system to amperometrically measure glucose levels. Enzymatic testing on paper strips has also been performed in a similar manner for lactate and uric acid.  

This section has introduced the field of microfluidics. Both PDMS and paper based microfluidics have been utilized in this thesis for the purpose of creating a sweat collection platform. Much of the work to be presented focuses on furthering our knowledge of how passive pumping methods can be created and integrated into these systems.
1.5 Hydrogels

1.5.1 Background and Fabrication

Hydrogels are hydrophilic crosslinked polymer networks that may be comprised of over 99% water. Hydrogels can be crosslinked through either chemical or physical bonds. Physically crosslinked hydrogels rely on polar interactions to link the chains of the hydrogel networks together, whereas chemically crosslinked hydrogels will have a series of chemical bonds that hold the polymer chains together. The very hydrophilic nature of the polymers creates a high osmotic pressure which causes them to swell in water. Degree of swelling in solution can be described using the Flory-Rehner theory. This theory relates the total osmotic pressure of the gel to the polymer-solvent interactions ($\Pi_{mix}$), the stress on the crosslinked chains while elongated ($\Pi_{el}$), and the non-uniform distribution of mobile ions between the gel and the solution ($\Pi_{ion}$). The osmotic pressure difference between two solutions separated by a semi-permeable membrane can generally be described by using the Morse equation, which is based off of concentration differences.

\[
\Pi_{gel} = \Pi_{mix} + \Pi_{el} + \Pi_{ion}
\]

\[
\Delta \Pi = \Pi_{gel} - \Pi_{Solution} \approx i(\Delta C)RT
\]

Free radical initiation is the most common method used to chemically crosslink hydrogels. In this process, the hydrogel monomer, crosslinker and initiator are dissolved in water. Acrylamides and acrylates are commonly used monomers for chemically crosslinked hydrogels. Heat or UV light is then applied to initiate the polymerization of the hydrogel.
Interest in physically crosslinked hydrogels has grown in recent years. Physical hydrogels rely on physical bonds, as opposed to chemical bonds, between molecules to act as crosslinks. A reason for the growth in researcher’s interest is that physically crosslinked hydrogels do not utilize chemical initiators. For many hydrogel applications, especially in biological settings, the initiator must be removed prior to use, resulting in an extra cleansing step that can be time consuming. Physically crosslinked hydrogels are fabricated through a different process. We will describe a fabrication method used to create alginate hydrogels, as it is one of the most commonly used physical hydrogels. A solution of sodium alginate is first prepared. Calcium ions are then introduced into the system to initiate the gelation process. Calcium ions are divalent and have a stronger affinity for the alginate and therefore create stronger bonds. These stronger physical bonds between the calcium ions and alginate result in the formation of a hydrogel matrix. The addition of a chelating agent to an alginate hydrogel will remove the calcium ions from the matrix and therefore result in the disassembly of the hydrogel.

1.5.2 Hydrogel Actuators

Swelling and de-swelling of hydrogels under various stimuli can be harnessed and controlled to create soft actuators (Fig 1.15). This is performed by creating hydrogels with specific chemical moieties whose intermolecular interactions are affected by an external stimuli. The simplest examples of this are pH-responsive hydrogels, which involve hydrogels that contain acidic and/or basic groups as part of the polymer network. For example,
hydrogels with acidic moieties will deprotonate in basic (high pH conditions). This turns a polar component into an ionic one, therefore increasing the osmotic pressure of the hydrogel, leading to increased swelling and a volumetric change. It is this volumetric change that can be harnessed through careful design for physical actuation.

Temperature responsive hydrogels can be created utilizing poly(n-isopropylacrylamide) (PNIPAM). When PNIPAM hydrogels are heated above 32 °C, which is their lower critical solution temperature (LCST), a reversible change occurs where the hydrogel releases water from its matrix. Below the LCST the hydrophilic regions dominate and hold water. Above the LCST the increased thermal energy causes entropy to grow larger and the hydrophobic interactions become dominant, resulting in the release of water.¹⁴¹

Hydrogels have been used as actuators in various microfluidic processes. One early example utilized hydrogel plugs as valves in microfluidic systems. Researchers polymerized pH responsive hydrogel plugs in two outlets of a “T” shaped microfluidic channel. One hydrogel swelled under acidic conditions while the other swelled under basic conditions. Upon swelling, the hydrogel constricts flow through the microfluidic channel. Depending on pH of the solution pumped through, either one or both of the hydrogel plugs would expand and constrict flow through the resulting microchannel.¹⁴²
Figure 1.15 Demonstrations of responsive hydrogels as actuators. (a) Patterned bilayers systems of ionic and temperature responsive hydrogels. These designs will bend to take different shapes depending on the stimuli applied.\textsuperscript{141} (b) Use of pH responsive hydrogels as valves in microfluidic channels. Acidic and basic hydrogels were used to swell under varying pH solution to prevent flow through their respective microchannel.\textsuperscript{142}

Responsive hydrogels have been used as actuators in many more arrangements than the two examples discussed above.\textsuperscript{143–147} The commonality between all of these actuators is that it is the osmotic pressure difference between the hydrogel and solution that results in actuation. These osmotic pressure changes in the hydrogel were initiated by external stimuli. These devices demonstrate the ability of hydrogels to be used under actuation by osmotic pressure.

1.5.3 Hydrogels for Biomedical Applications

The high water content, biocompatibility, flexibility and versatility of hydrogels have made them a popular material for use with biomedical applications. Hydrogels can be tuned to be very tough, allowing them to be used with bone and cartilage to increase joint
functionality. Wound dressings made from hydrogel have been around for many years. Hydrogels can be used as a scaffold material to facilitate cell growth for tissue engineering. The versatility and biocompatibility of hydrogels also makes them an ideal material for use in biological sensing operations, which is largely the topic of the research described in this thesis.

1.6 Liquid Metal

1.6.1 Properties of EGaIn

Recent years have seen tremendous interest in wearable devices. These devices need to be soft and flexible, to accommodate user comfort. Electronic devices are traditionally created using lithography techniques. Many of the materials used in such devices, such as metal interconnects, are very rigid and inflexible. To increase flexibility, metals are commonly made thinner and deposited on flexible substrates. These metal films are inherently more flexible and show good durability for light strains. Unfortunately, the performance of these devices decreases greatly for strains above 10%, greatly limiting the applications of these materials (Fig 1.16a).

Inherently soft materials are needed to make extremely flexible electronics. This has led to the use of a eutectic gallium indium alloy liquid metal (EGaIn) for stretchable electronics. EGaIn is a eutectic metal alloy comprised of 75% gallium and 25% indium that has a melting point at 15.5°C, allowing it to remain a liquid at room temperature. EGaIn spontaneously forms a gallium oxide skin when exposed to oxygen or air, which is strong.
enough to overcome the high surface tension of the liquid and hold its shape on small length scales. This led to devices that utilize EGaIn in microfluidic networks to create soft and stretchable electronics.

One early use for EGaIn was in stretchable wires (Fig 1.16b). Poly[styrene-b-(ethylene-co-butylene)-b-styrene] (SEBS) was used to extrude small hollow fibers. SEBS is an ultra-thermoplastic elastomer, making it ideal for this use. EGaIn was injected into these hollow fibers with a syringe. It was shown that the addition of the liquid metal into the core of the fiber had no noticeable effect on the mechanical properties of the fiber, allowing it to retain its high stretchability. These wires were able to endure strains of up to 800%, while maintaining a strong enough electrical connection to be utilized as a wire for earphones.
Figure 1.16 (a) Images showing how metal films on flexible substrates inherently crack when exposed to repeated strain.\(^{154}\) (b) Images showing ultrastretchable EGaIn wires.\(^{157}\) These wires can be strained orders of magnitude greater than thin metal films.

The benefit of using liquid metal in electronics is that the fluidic nature of the material allows for soft and stretchable circuit components. EGaIn has been used to create many flexible electronic devices in recent years.\(^{157–168}\)
1.6.2 Past EGaIn and Hydrogel Devices

Hydrogels and EGaIn are both flexible materials that can be used to create soft devices. Therefore, it was a natural progression to utilize these materials to create flexible electronics. One effort involved constructing and testing a soft biomimetic memory device (Fig 1.17). Most common electronics and memory storage devices are created with rigid silicon based materials. Yet one of the most advanced memory devices is composed of entirely soft gel based materials, the brain. The brain is a water based material that utilizes ionic conduction, similar to a hydrogel, for electrical operations.\textsuperscript{169}

Studies were performed to examine the electrical properties of EGaIn and hydrogel. Under DC conditions, the oxide skin on the EGaIn can either be increased through further oxidation or decreased through reductive potentials. This oxide serves as an electric insulator that can decrease current flow. By utilizing acidic hydrogels in contact with the EGaIn, the formation of the oxide skin can be limited to increase conductivity for their backwards current, whereas forward conductivity is governed solely by the electrolyte concentration of the hydrogel.\textsuperscript{170} This idea was expanded to create a four bit memory device composed of hydrogel and liquid metal. By controlling the oxide skin and creating conductive and resistive states, they essentially created the same on/off mechanism that is used in memory devices.\textsuperscript{169} This was the first soft electronic device created using both liquid metals and hydrogels.
Figure 1.17 (a) Schematic of how various acidic and basic hydrogels were used to construct each interconnect of the (b) 4-bit memrsistor based entirely off of soft materials.\textsuperscript{169}

1.7 Layout of the Dissertation

My graduate research was focused on exploring novel principles and technologies that can enable wearable health monitoring devices. This chapter has served to introduce the field of wearable sweat sensing devices and the materials that I have used to enable their capabilities. Chapters 2, 3 and 4 will look at methods and materials that may assist with sweat collection and management for real-time sweat sensing. Chapter 2 presents a passive microfluidic pump that we created using osmotically tuned hydrogels. We doped hydrogels with varying levels of NaCl or glycerol to create a high solute concentration, and therefore a high osmotic pressure. When these hydrogels come into contact with a membrane, the hydrogels passively pump water through by means of osmosis. This pumped fluid is then guided to an adjacent microfluidic channel where sensing can be performed. Chapter 3 presents a novel method for removing pumped fluid through the process of evaporation. We created paper microfluidic strips with large surface area that promote evaporation of fluid after the paper strip has been
saturated. As fluid is evaporated, capillary action causes more fluid to wet the material, which creates a fluid flow throughout the entire paper device. We explore the effects and limitations that the geometry of the paper microfluidic device has on its long-term operation. Chapter 4 describes the merger of these two past ideas. We combine these technologies into a single platform that we test in both a lab setting and on real human subjects.

Chapter 5 investigates how we can use these same hydrogels for ECG sensing. We also utilize a liquid metal, instead of a traditional solid metal electrode, to create a soft flexible device. We characterize how modifying the hydrogels pH and ionic strength affect the conductivity of the system, and demonstrate this concept with a working prototype.
1.8 References


Chapter 2

Hydrogel-Enabled Osmotic Pumping for Microfluidics

2.1 Introduction

Wearable health monitors and activity trackers are rapidly developing classes of devices. Most of these devices utilize MEMS sensors such as accelerometers and gyroscopes to obtain data on motion of the user and correlate these data to physical activity. These platforms only allow for limited capabilities for health monitoring with variable accuracy and do not provide the same measure of health vitals as a biochemical test. Biochemical tests are still routinely performed in a doctor’s office on blood drawn using a needle and then later tested in a laboratory for various analytes. Thus, blood sampling and analysis — although informative — is an invasive, uncomfortable, and slow process. As a result, individuals typically get biochemical tests done infrequently and often only in response to a health concern.

Health monitoring strategies that are non-invasive and easy to implement could enable more proactive health monitoring. Sweat monitoring provides one such opportunity. Sweat is a bodily fluid that is constantly released through the skin and contains numerous biomarkers such as glucose, lactate, cortisol and various ionic species which can also be tested to determine user health.2–9

Biochemical sensors have been used to measure sweat that comes to the surface of the skin.10–12 Hydrophilic capillary channels can draw sweat off the body to measure ion concentration.13 This approach relies on the presence of excessive sweat on the skin, which can occur during periods of exertion. It is also possible to pump interstitial fluid through the
skin using reverse iontophoresis.\textsuperscript{14,15} This approach, however, may lead to skin irritation and necessitates the need for additional power on wearable devices that are already power-starved.

We discuss the results of a technique that noninvasively collects and pumps aqueous fluids into microfluidic devices without any external power. This work was motivated as a first step toward sweat harvesting through the use of passive pumping. Passive microfluidic pumps may utilize capillary forces, osmotic pressure and evaporation.\textsuperscript{16–20} These devices all have the drawback that they require a pool of free fluid at the inlet, which only occurs on the body during periods of high sweat rate. To create an effective pump that withdraws fluid from sweat glands, we sought a pumping mechanism that would act as an extension of the sweat gland. Sweating is a process driven by osmotic pressure differences created in the sweat gland.\textsuperscript{2} When the body produces sweat, it pumps sodium and chloride ions into the lumen of the secretory coil located in the skin. This results in an ionic concentration difference between the secretory coil and the surrounding cells, further creating an osmotic pressure difference driving fluid flow from the cells to the coil. The only place for this fluid to flow is through the eccrine gland to the surface of the skin.
Figure 2.1  (a,b) Schematic of the novel sweat collection concept device. The hydrogel discs are doped with solutes (NaCl or glycerol) at a higher concentration than the body, which creates an osmotic driving force of fluid into the device. This fluid is then collected and transported through a microfluidic channel. Biosensors for continual sensing applications can be implemented within this channel. (c) The hydrogel discs have been designed to facilitate flow and direct it to the adjacent microfluidic channel.

The device described in this paper utilizes hydrogels with variable osmotic strength to mimic the pumping mechanism found in the skin. Hydrogels are hydrophilic crosslinked polymers that can contain up to 99% water by mass. They have been used in many applications such as soft actuators and drug delivery.\textsuperscript{21–26} The very high water content can make hydrogels biocompatible and useful for biomedical operations.\textsuperscript{27} The hydrogel interface for skin was chosen for its biomimetic properties. Microfluidic channels capture this pumped fluid and continuously transport it into the device. Here, a dialysis membrane serves as a mimic for the skin as a first step. Dialysis tubing was chosen as a skin model, as it allows for osmosis through the membrane while separating the fluid reservoir from the environment. Dialysis tubing has been used as a viable skin simulant earlier due to its similarities to biological membranes.\textsuperscript{28} Hydrogels doped with solutes create an osmotic driving force to pull fluid through the membrane and into an engineered microfluidic network. This device could act as a platform that could serve as a natural interface of the sweat gland and onto which various biosensors
could be implemented. A schematic of this device is shown in Fig. 2.1. This paper reports data on the flow rate and osmotic characteristics of this fluidic device in contact with a dialysis membrane as a preliminary proof-of-principle model for future sweat studies.

2.2 Materials and Experimental

2.2.1 Materials

The hydrogels were formed using acrylamide as the monomer, n,n’-methylenebisacrylamide as the crosslinker and 2-hydroxy-4’-(2-hydroxyethoxy)-2-methylpropiophenone as the photoinitiator (Sigma). Microfluidic devices were fabricated on the basis of polydimethylsiloxane (PDMS, Sylgard 184 Dow Corning). Microfluidic molds were generated using SU8-100 photoresist (Microchem) on silicon wafers. Sodium chloride (Sigma) and glycerol (Acros) were used to dope the hydrogels.

A phosphate buffer solutions (PBS) solution, prepared from tablets (Sigma), was used as an aqueous system that mimics the ionic strength and osmotic pressure of sweat. PBS has an ionic strength of ≈150 mM. Sodium chloride, the most predominant salt in sweat, can vary in concentration within the 10-100 mM range.

2.2.2 Device and Hydrogel Fabrication

Polymerization of hydrogel sheets was initiated under a 100 mW/cm² mercury lamp for 1 min. The monomer solution is 4 M acrylamide, 1% crosslinker by mole and 0.002 g/mL photoinitiator. Hydrogels were allowed to equilibrate in their respective solutions of glycerol
or saline for at least 24 hours after polymerization is complete. Hydrogel disc designs were created in CorelDraw and cut from equilibrated hydrogel sheets using a CO₂ laser cutter. Semicircle notches of 0.375 mm radius were also cut into the hydrogel discs to create a region of low hydrodynamic resistance to guide fluid flow during operation. This predesigned path for the fluid allows for a guided and controlled flow with consistent flow rates. These hydrogels were stored in solution to maintain osmotic pressure until testing.

The PDMS microfluidic devices were fabricated using soft lithographic techniques. First, molds were generated using SU8-100 photoresist on silicon wafers by means of photolithography. A 10:1 mixture of PDMS was prepared and degassed using a Thinky mixer AR-1000. PDMS was then cured on both a mold and on a flat surface in a 70 °C oven for 1 hour. Metal leather punch dyes ranging from 2 to 12 mm in diameter were used to cut the holes in PDMS. Oxygen plasma treatment was then used to bind the pieces together to create the microfluidic devices. The microfluidic channels have a width of 485 µm, height of 200 µm and vary in length from 1 – 2 cm.
2.2.3 Experimental Procedure

Figure 2.2 Photographs (a) of one of the hydrogel discs and (b) the PDMS microfluidic device. (c) The hydrogel discs are placed in the PDMS devices and clamped onto a small diffusion cell with a piece of dialysis membrane separating the gel from the fluid. (d) The diffusion cell may be placed beneath a microscope for observation.

Testing was performed on a model system to demonstrate that doped hydrogels are able to osmotically withdraw fluid through a membrane and pass it to a microfluidic channel. Methods and schematics for the experimental setup are shown in Fig 2.2. The hydrogel discs were blotted with tissue paper to remove excess water from the surface. The hydrogel disc was then placed in the opening of the PDMS device (Fig. 2.2b) and a razor blade was used to cut off any hydrogel extending past the surface of the PDMS to create a smooth interface. Dialysis tubing (Fisherbrand, 12-14kD MWCO) was cut and placed on the bottom side of the hydrogel disc to act as a skin-like membrane (Fig. 2.2c). The PDMS device was then clamped
in place on a diffusion cell and the bottom chamber was filled with PBS, which models an isotonic solution of sweat in the body. A compact diffusion cell (Fig. 2.2d) was constructed to fit onto the stage of a microscope to enable visualization of flow through the channels in real time. Fluorescent magnetic microparticle (Bangs Laboratories, COMPEL Magnetic, COOH Modified, 5.8 µm) tracking was used to measure flowrate over long durations. These particles would be deposited on the surface of the PDMS well that houses the hydrogel prior to testing. Glucose sensing at later stages was performed using a commercial TRUEresult® meter with TRUEtest® glucose strips.

2.3 Results and Discussion

2.3.1 Osmotic Driving Force and Notched Hydrogel Design

Hydrogels were doped with either sodium chloride or glycerol by immersing the gels in their corresponding solutions and allowing the hydrogel to equilibrate. The presence of these solutes in the hydrogel matrix creates the osmotic pressure differential that drives fluid through the membrane. Equation 1 predicts the osmotic pressure (\(\Pi\)), which relates directly to the Van’t Hoff factor (i), universal gas constant (R), temperature (T) and the concentration difference between the hydrogel and the sweat.

\[
\Pi = iRT\Delta C = iRT(C_{Gel} - C_{Sweat})
\]  

Selection of chemical species used to create a high osmotic pressure difference between the hydrogel and the PBS beneath a membrane is critical to device operation. Fixed species on the hydrogel backbone, acrylate moieties for example, create a high osmotic pressure
difference with the PBS but only drive fluid flow into the hydrogel, resulting in a swelling of the hydrogel with no pumping of fluid in the microchannel.

The creation of a “push and pull” mechanism with the fluid requires hydrogels with mobile dissolved solutes. This process is described in Fig. 2.3. Mobile species create an osmotic pressure difference with the solution, which pulls the fluid through the membrane. The fluid drawn across the membrane forms a thin fluidic layer between the hydrogel and the membrane. The solute dissolved in the hydrogel diffuses into this fluidic layer based on the concentration gradients between the hydrogel and liquid layer, resulting in a higher concentration in the fluidic region. This increased concentration keeps the osmotic pressure difference through the membrane with the PBS high, continually driving fluid flow through the membrane into this fluidic region. This region is analogous to the secretory coil in the sweat gland. As more fluid is drawn into the fluidic region, a notch in the hydrogel (an important feature of the device) collects and guides the fluid into the microchannel. This pumping action can be seen in video S1. Streamlines in this fluidic region were observed with the help of a long exposure digital imaging (2 seconds) using fluorescent microparticles inserted at the interface between the hydrogel and membrane prior to testing. The motion of these particles is captured as a streak in the long exposure image and follows the streamlines in this fluidic region. The images show that there is flow in this region that leads towards the notch in the hydrogel disc.
Figure 2.3 (a) Schematic of the mechanism of fluid flow from the isotonic solution in the lower chamber through the microchannel. Isotonic solution and dissolved solute from the hydrogel mix in the fluidic layer. Fluid is passively pumped continually into this fluidic layer, while the excess fluid is pushed into the adjacent microchannel, guided by the notch in the hydrogel. (b) Microscope images over time showing how fluid drawn initially through the membrane fills up the notch channel in the hydrogel. The fluid then enters the microchannel and continues flowing through it. (c) A long duration (2 sec) fluorescent micrograph traces the flow in this fluidic layer to the notch in the hydrogel disc. The immobile particles have adhered to the gel and are moved with the flow.

2.3.2 Hydrogel Effects on Flow Rate: Molarity and Geometry

Visually monitoring the flow through the microchannel allows for flowrate to be calculated. Flowrate was calculated during initial periods of flow by tracking the front of the fluid through the microchannel. Multiple micrographs are taken to track the fluid. Velocity of the fluid front is calculated by dividing the change in distance by the time difference in-between images. This provides the average velocity, which can be multiplied by the cross-
sectional area of the channel to obtain flowrate. The equation used and an example of images can be seen in Fig 2.4.

$$Q = LW \frac{\Delta x}{\Delta t} = LW \frac{x_{i+1} - x_i}{t_{i+1} - t_i}$$

(2.2)

**Figure 2.4** Images over time showing the progression of the front of fluid flow. Measuring the velocity of the fluid front allows for total flowrate to be calculated.

We measured the flowrate through the microfluidic device while varying osmotic pressures within the hydrogels by varying concentrations of NaCl (1-6 M) and glycerol (2-12 M). These flowrates vary as a function of concentration, as shown in Fig. 2.5. Increased concentrations of solute in the hydrogel create higher osmotic pressure differences through the membrane, which results in an increased flowrate through the microchannel. Flowrates for hydrogels doped with sodium chloride and glycerol both follow a similar correlation with concentration of solute. A linear trend and an intercept comparable in magnitude to the
background (PBS) salt concentration are seen of lower concentrations, as expected based on Equation 2.1.

Further tests performed using hydrogels equilibrated in 6 M NaCl with varying diameters and thicknesses revealed how hydrogel geometry affects the flowrate. Osmotic pressure is a driving force per unit area. Therefore, it is expected that flowrate would be directly proportional to the surface area of the gel. Hydrogel thickness does not affect surface area or osmotic pressure and therefore should not affect the flowrate. Both of these model expectations were confirmed by the results plotted in Fig. 2.5.
Figure 2.5 (a) Hydrogels doped with NaCl and glycerol show a similar and direct effect of solute concentration based on measured flowrate. This agrees with the osmotic pressure equation. (b) Flowrate shows a linear correlation to the surface area of the hydrogel disc. (c) Hydrogel thickness has no effect on the initial flowrate achieved by the hydrogel as it does not affect osmotic pressure or surface area. Larger thickness should allow for longer pumping times as more total dissolved solute is stored in the hydrogel.
We compared osmotic pumping of PBS versus an artificial sweat simulant in order to validate the use of PBS for our lab testing. Artificial eccrine solution was purchased from Pickering Laboratories. Pumping tests were performed using a hydrogel disc equilibrated in 12 M glycerol. Three trials were performed and the average flowrate is shown in Fig 2.6.

![Graph showing flowrates for PBS and artificial eccrine sweat solution](image)

**Figure 2.6** Comparison of pump rates for hydrogels swelled in 12M glycerol for PBS and an artificial eccrine sweat solution. These rates fall within each other’s standard deviations and there is no statistical significance between the two.

These flowrates fell within the range of one standard deviation of each other. Therefore we can state that there is no statistical difference between the pumping rates for the two solutions. This validates the use of PBS in our lab setting for testing. The artificial eccrine solution had a pH of 4.5 and the PBS used has a pH of 7.4. These tests also show that pH has no sizeable effect on the pumping mechanism. These results were expected as variations in
pH and presence of proteins in solution have no meaningful effect on the solution’s osmotic strength, which is the basis for the pumping mechanism.

2.3.3 Long Duration Pumping

Particle tracking within our microfluidic devices provided a method to measure long term flow rates for the PDMS devices. Photographs taken of fluorescent particles using a long exposure show streaks with lengths proportional to velocity. Magnetic fields applied briefly at the onset of the experiment perturb these particles, which then get pulled by the convective fluid flow and travel through the microchannel where they can be tracked.

The process described for measuring flow rate relies on measuring the maximum velocity of a laminar flow through a square microfluidic duct. In laminar flow, there are well defined models for the velocity profile through a cross section. In a square channel with width ranging from \(-\frac{1}{2}W\) to \(\frac{1}{2}W\) and height from 0 to \(H\), the velocity can be modeled using the equation below:\(^{30}\)

$$u_x(y,z) = \frac{4h^2\Delta P}{\pi^3 \mu L} \sum_{n=1,3,5,\ldots}^{\infty} \frac{1}{n^3} \left[ 1 - \frac{\cosh\left(\frac{\pi ny}{h}\right)}{\cosh\left(\frac{\pi nw}{2h}\right)} \right] \sin\left(\frac{n\pi z}{h}\right)$$

(2.3)

The flowrate can be calculated as the product of the average velocity and the cross sectional area. Linear relations can be made between the average velocity in the channel and the maximum flowrate by a correlation value \((\alpha)\), which can be measured experimentally. The maximum velocity is obtained from the velocity profile at the center of the channel \((w = 0, h = \frac{1}{2} H)\). Average velocity can be determined by dividing the flowrate by the cross-sectional area.
Flowrate can likewise be determined by integrating the velocity across the cross-sectional area.

$$u_{avg} = \alpha u_{max}$$

$$u_{max} = u \left(0, \frac{1}{2} h\right) = \frac{4h^2 \Delta P}{\pi^3 \mu L} \sum_{1,3,5,...} \frac{1}{n^3} \left[1 - \frac{1}{\cosh\left(\frac{n\pi w}{2h}\right)}\right] \sin\left(\frac{n\pi}{2}\right)$$

$$Q = \int \int_{\frac{w}{2}}^{h} u_x(y,z) dydz = \frac{h^3 w \Delta P}{12 \mu L} \left[1 - \sum_{1,3,5,...} \frac{192 h}{(n\pi)^5} \tanh\left(\frac{n\pi w}{2h}\right)\right]$$

$$\alpha = \frac{Q}{WHu_{max}}$$

With the channel dimensions used (W=485 µm, H=200 µm), α is found to be 0.51 by combine Equations 2.5, 2.6 and 2.7. This value can be used to correlate maximum velocity to average velocity, and therefore the total flow rate. Fluorescent microscopy was used with five micron diameter fluorescent particles. As can be seen in Fig. 2.7, these particles are small enough that they can easily fit in the region of highest velocity. It can be seen that there is a rather large region in the center of the channel where the velocity is between 90-100% that of the maximum.

$$Q = \alpha WHu_{max} = \alpha WH \frac{\Delta l}{\Delta t}$$
Figure 2.7 (a,b) Setup of how we use fluorescent microparticles and a microscope to detect particle motion. (c) A velocity field of laminar flow in a rectangular microchannel. Particles oriented in the center are focused on and are approximated as being at the maximum velocity.

Images were captured using a 1 second exposure in fluorescence mode (Fig 2.8). This long exposure causes moving particles to appear as a streak. The length of this streak (l) corresponds to the distance the particle has traveled during the exposure time (t). Tracking only particles that are located in the center of the microchannel allows the maximum particle velocity to be calculated, which can be used Equation 2.8 to determine flowrate.
Figure 2.8 Example images of flowing particles tracked with long exposures. The length correlates to how fast the particle is moving. The camera is focused in the middle of the microchannel. The circles in the micrographs are particles that are stuck to the walls of the microchannels (which is why they are out of focus).

The data collected from these long duration tests show how the flowrate decreases over the two hour duration of observation (Fig. 2.9). The fluid pulled into the fluidic region slowly depletes the solute in the hydrogel. This in turn decreases the concentration in the hydrogel which lowers the osmotic pressure difference. The lower pressure differential results in lower flowrates.
Figure 2.9 Graph of flowrate and fluid accumulation over time measured using tracer particles demonstrating that flow can be achieved for over two hours. Flow decreases over time due to the dilution of the dissolved solute in the hydrogel.

2.3.4 Fluorescent Fluid Tracking

The doped hydrogels have shown the capability of drawing in fluid and pumping it to a microfluidic channel for long durations. This device needs to be able to pump both the fluid and the analytes in solution to serve effectively for sensing purposes. The chamber beneath the membrane was filled with PBS solution with a dissolved fluorescent dye to demonstrate the pumping. The emergence of this dye in the channel was then followed over time by the fluorescent microscope. Initially there is no fluorescent material in the channel and it appears dark. As the hydrogel pumps the fluorescent fluid from the chamber, it makes its way through the membrane and into the channel where the fluorescence increase can be measured. Fig. 2.10 shows the resulting images and data plot. A calibration curve created using ImageJ correlates the mean gray value of the fluorescent channel to the concentration of the fluid in the chamber. A 0% concentration represented no fluorescent material while a 100%
concentration corresponds to the fluorescence intensity of the pure fluid loaded in the chamber. The first measurable data was collected after 2.7 minutes, at which time the fluorescence intensity was around 3% of the concentration in the chamber. After 11 minutes, the concentration leveled out to 32% of that in the chamber. This test conclusively demonstrates that this technique is able to pump both solution and analytes into the microfluidic channel, where sensing can take place. Leveling off at 32% may be due to the dialysis membrane blocking flow of fluorescent material through or leaching of the fluorescent dye into the hydrogel.

**Figure 2.10** (a) Fluorescence microscope images of the microfluidic channel over time as the fluorescent emission of the fluid in the microchannel increases. (b) A calibration curve was created to correlate the fluorescent intensity to a relative concentration with 100% being equal to what is in the chamber below the membrane. (c) Concentration of the fluorescent dye is shown over time. These results demonstrate the ability of the device to passively pump analytes through the microfluidic channel for sensing purposes.
2.3.5 Glucose Monitoring

Testing was also performed to determine whether the device will be able to pump glucose as a step toward sensing health-relevant fluid components. As the device pumps fluid it accumulates at the outlet of the microfluidic channel forming a droplet. Analysis of this droplet can be correlated to the chemical makeup of the fluid extracted through the membrane. Glucose was added to the isotonic solution in the diffusion cell below the membrane to achieve a 50 mg/dL reading (a biologically relevant concentration) measured using the commercial glucose meter. As the accumulated fluid increases, one can measure the glucose concentration in the droplet by the commercial instrument. Wiping the fluid away after measuring ensures that freshly pumped fluid can re-accumulate. The results can be seen in Fig. 2.11. There is an initial 15 minutes induction time where fluid needs to initially flow through the channel and form a large enough droplet such that the glucose monitor can test it. For three trials, glucose concentration was successfully measured within 10% error after 25 to 30 minutes.
Figure 2.11 Glucose measured at the outlet of the device over time for three different trials. These data demonstrate the ability of the device to passively pump biological relevant solutes and suggest that the osmotic technique can used for continual non-invasive monitoring of glucose.

In all three trials performed, the measured glucose levels decreased a bit after accurate initial readings. This may be due to the decreasing flowrate in the device and some loss of glucose via diffusion in the hydrogel. As flowrate decreases, the pumped fluid spends more time in the fluidic region at the bottom of the hydrogel disc. It is likely that some of the glucose is then lost by side diffusion into the hydrogel. Longer retention times in this region would lead to more leaching of glucose and therefore a lower reading on the fluid that has reached the outlet of the microfluidic device. This problem can be addressed in future devices by design changes and sensor calibration. This test was performed as a proof of concept for sensing an easily detectible bioanalyte. This pumping device is merely a platform to which an array of biosensors may be used.
2.4 Conclusion

A technique for osmotic liquid sequestering and passive microfluidic pumping has been demonstrated. The test device uses solute-doped hydrogel discs that interface with a microfluidic network. The hydrogel composition and geometry control the flowrate for flow up to two hours. Pumping with both glycerol and NaCl demonstrate that there is flexibility in choosing dissolved solutes for future applications. Glucose solution withdrawal and monitoring was successfully demonstrated and could prove to be a step towards non-invasive continual glucose monitoring.

The technique of osmotic sweat sequestering seems to pose a number of potential advantages, which include the use of biomimetic materials and non-invasive fluid withdrawal from the model skin. The passive, yet long-term, pumping could deliver the collected samples to an array of microsensors for long-term monitoring. The pumping process is “powered” on the chemical potential of the solute infused in the hydrogel, and our data suggest that this can allow for the devices to operate from tens of minutes to many hours. The pumping duration can be increased further by implementing microfluidic valves to reduce the flowrate which would enable longer sampling periods. For example, reducing flowrate to 25 nL/min could theoretically enable flow for up to 12 hours. Creating a larger microfluidic backpressure may also allow for better control and slower flowrates, which can increase the sampling time.

The energy needed for this type of pumping is derived directly from the chemical potential of the dissolved species infused in the hydrogel patch. Even longer-lasting flowrates may be attainable by using thicker hydrogel discs, controlling the release of the dissolved
solute or using solute with limited solubility. As we demonstrate, the osmotic gradient can be achieved by using glycerol solution, which could be safe and non-irritating in contact with the skin. In another practical perspective, the replacement of a hydrogel patch as a driving source can be much simpler and less expensive than the replacement of electrochemical power sources (batteries).

This is an early stage successful demonstration of a method that will require additional work before it can be introduced in future real world devices that could be tested in more realistic sensing from human skin. While our preliminary data indicate that such hydrogel devices can withdraw sweat samples from human skin, real subject testing and clinical development are massive tasks for future development. Future work will also be aimed at obtaining longer flowrates, integrating sensors into the microfluidic channel and testing on human patients when such a device interfaces actual skin rather than a model system. Sensors placed in the microfluidic channels would enable the collection of rapid, more reliable, and continuous data collection.

2.5 Acknowledgements

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2.6 References


Chapter 3

Evaporative Paper Microfluidic Pumping

3.1 Introduction

Paper-based microfluidic devices have found numerous applications in recent years due to their low cost, manufacturing simplicity and easy use for point of care diagnostics. Paper is hydrophilic and porous, enabling it to wick small amounts of water for biochemical sensing applications in which fluid volume is limited. As research on paper microfluidics has grown, the sensing techniques and modalities have expanded greatly to biologically relevant analytes including glucose, lactate, nitrates and cholesterol. Fabrication of these devices can be simple by utilizing various wax patterning, printing, lithographic and cutting methods.

Most existing paper-based devices are for only one-time use, as capillary flow into the paper ceases upon saturation. The lack of controlled long-term flow in paper microfluidics prevents applications requiring continual monitoring. Long term paper based microfluidic sensing could prove useful for applications ranging from fluid management for sweat sensing, environmental monitoring and food quality control. Researchers have begun exploring how they can control the flow of fluid into paper based devices to prolong sensing duration or incorporate areas of mixing and separation.

Quasi-continual sensing can be performed in the short term by controlling the rate at which the paper strip saturates. Flow control has been achieved utilizing this principle through various geometrical designs in both 2D and 3D. The drawback of these methods is that...
flow inevitably ends after the paper has been fully saturated, thus reducing the capillary pressure.

We sought evaluate the efficiency of continual long term capillary pumping through paper microfluidics by utilizing evaporation. Traditional microfluidic devices (i.e. those that do not use paper) have utilized evaporation at large openings at the end of a microchannel to drive fluid flow.\textsuperscript{30} One device utilized a paper plug at the outlet of a microfluidic channel to facilitate evaporation for pumping\textsuperscript{31}. Another microfluidic pump was inspired by the evaporation occurring in trees\textsuperscript{32}. These devices have all demonstrated the ability of evaporation to be used for continuous microfluidic pumping, but they all have complex designs that utilize difficult fabrication techniques. They have also not been tested extensively for long durations (greater than 15 minutes).

A microfluidic platform capable of managing fluid flows for long durations could prove to be an integral component in sweat sensing devices. There have been great advances in the field of wearable sweat sensors in the past few years\textsuperscript{21,33–36}. A key limitation to these technologies is the ability to passively manage the sweat that has been analyzed for long durations. The evaporative pumping mechanism shown here could move fluids, such as sweat, across a sensor and allow for long-term continual monitoring.

We have constructed and characterized a new class of passive paper pumps that utilize evaporation to draw fluids for more than 10 days, a thousand times greater than current paper-based microfluidic systems. During operation, fluid is initially drawn through the device via capillary wicking. This process lasts on the timescale of minutes, depending on the size of the
device. Evaporation takes over as the dominant pumping force after saturation. As shown in
**Fig 3.1**, evaporating water from the pad allows capillary action to continually replenish this
evaporated fluid in the pad creating a pull that continually draws fluid from the sampling region
through the channel to the evaporating pad. This enables the paper microfluidic to pump fluid
for long durations after the paper saturates with fluid. Continual evaporation of solution results
in accumulation of solute at the edge of the evaporation pad, slowly decreasing the flow over
time. We envision this device as a possible sweat management platform for future non-
invasive wearable biosensors.
Figure 3.1 Paper microfluidic strips are fabricated with 3 sections; an interface section that draws fluid, a closed channel with an adherent electrode and a pad with a large surface area that utilizes evaporation to continually drive fluid through the device. Wicking initially saturates the paper (5-30 minutes). Evaporation then removes water from the pad, allowing for capillary action to pump more fluid through the channel to this pad. Even with salt accumulation, this pumping can last for up to 10 days.
We show fabricated paper strips can passively pump fluid for sensing purposes for long durations. We will first demonstrate flow rate control and modeling based on geometric control of the strips. We will then show the limitations of pumping based on hydraulic resistance and salt accumulation for long-term use. Finally, we performed sensing in colorimetric and electric modes to demonstrate the efficacy of the platform for sensing and monitoring applications.

3.2 Materials and Experimental

3.2.1 Materials

Whatman quantitative filter papers, hardened, asheless, Grade 542 were used. Our designs consist of three main features: a sampling interface where the paper is in contact with the desired fluid, a linear encased segment acting as the microfluidic channel where sensors will be interfaced and an open large surface area segment providing the pumping energy via evaporation. Strip designs were created in CorelDraw and a CO₂ laser cutter was used to cut the paper strips.

3.2.2 Visual Dye Monitoring

Paper strips were placed on an acrylic sheet prior to testing with 1 cm of the linear segment hanging below. A 1 cm wide PDMS strip is placed over the top of the paper channel and pressed against the acrylic to hold the paper to the acrylic and limit evaporation in this region (see supplemental information). A black piece of cardstock is then attached to the back side of the acrylic to act as a dark backdrop during testing. The acrylic is held vertically 2cm
above the lab table with the strips hanging below. A Canon EOS Mark5 DSLR camera was placed on the table ranging from 15-30 cm away and focused on the strip. Manual mode was used with an ISO of 400 and exposure of 1/13th seconds. Six black (UV) LED lights, from an ACLOROL 5050 LED strip, were placed approximately 2 inches in front of the paper strips to illuminate the fluorescent dye. A cardboard enclosure surrounded the setup to block external light from affecting the images. The camera took photos every 30 seconds to obtain time-lapse images of the fluorescent dye moving through the paper. All tests were performed in ambient lab conditions (~70 °F and 40 % RH). Fluctuations in these conditions may occur and can be a cause of error in measurements.

The strips are initially suspended with the sampling region interfacing DI water to allow for wicking to fully wet the strip. Once fully wetted, a solution of a fluorescent fluid is placed at the bottom of the strips. It is the motion of this dye that is tracked. Depending on the test performed, the sampled solution may be switched mid test between DI water and samples with varying dye concentration.

A Matlab loop was created that analyzed color intensity for every image in the series. The first image from the test was opened in Matlab and separated into its red, green and blue (RGB) components. The green component was focused on, as it is the color of the dye used. Color intensity was then measured on horizontal lines that cross the “channel” of the paper strip. Intensity values were averaged for pixels that covered up the strip to obtain an intensity for that strip at the height of the line. This was performed for each of the horizontal lines and
the values were stored in a vector. The loop then opened the next image in the series and repeated this process until all images had been analyzed. This process is described in Fig 3.2.

Figure 3.2 Explanation of how color intensity is quantified. (a) Images are opened in Matlab and then divided into their red, green and blue components. (b) Only the green image is analyzed, as a green dye is tracked. (c) Color intensity can be measured across a horizontal line covering the entire image. (d) Intensity values are averaged over a range for each paper strip and inserted into a vector. This process is repeated for all images in a run.

This test setup and analysis was used to quantify the images taken during testing. Depending on the lighting of the strips, there were variations in the measured intensity of fluorescence in the paper. Intensity also appeared to spike briefly on the first step before leveling off to a constant value. Therefore the intensity values were normalized on a 0 – 1 scale with 0 representing the lowest intensity measured and 1 being the maximum value calculated after intensity leveled off. This allowed for strips to be analyzed on a similar scale.
The Matlab output image files is the intensity of dye over time for specific locations on the paper strip. The time delay between intensities changes on the same strip are used to calculate dye velocity.

3.2.3 Electrical Sensing

Gold interdigitated electrodes were interfaced with the channel portion of the paper microfluidic device in our setup. The fingers on the electrode have widths of 50 µm and are spaced 25 µm apart. There are 29 total fingers, each with a length of 3 mm. The two leads on the electrodes were connected to a Gamry potentiostat on which single frequency potentiostatic electrochemical impedance measurements were performed at 1 MHz with 10 mV. Impedance was measured every 15 seconds.

3.3 Results and Discussion

3.3.1 Proof of Evaporative Pumping

We performed tests under various conditions to prove that evaporation is the driving force for this pumping mechanism. Paper strips were set up in our test method and allowed to fully wet through capillary action. We then placed these strips under different environmental conditions to observe how limiting evaporation affected the flow of dye through the paper microfluidic. One strip was exposed to the normal lab conditions (~70F and 40 % RH), one strip was placed in a humidly chamber (90% RH) and a third strip was entirely covered by PDMS. The results of these tests can be seen in Fig 3.3.
Figure 3.3 Images showing evaporative pumping under three conditions. The top row shows normal pumping through evaporation in ambient conditions (70F 40%RH), the middle row shows pumping under humid conditions (90% RH) and the bottom row shows pumping when the entire device (evaporation pad included) is covered with a strip of PDMS to limit evaporation. All paper strips were allowed to fully saturate with water prior to testing.

Under normal conditions, the dye travels through the channel into the evaporation pad. In the humidity chamber, the dye travels through the channel and into the pad, but to a much lesser extent than is seen under less humid conditions. This indicates that pumping is slower, which is expected in a more humid environment. The last strip had a PDMS sheet covering the entire device (evaporation pad included). The dye was not able to fully travel through the length of the channel over a two hour span in this setup, indicating that evaporation has been mostly prevented resulting in no fluid pumping.

Evaporation of water requires a large amount of energy, which is drawn from the surrounding materials in the environment. We used an infrared (IR) camera to visualize the
temperature drop present on the evaporation pad for our paper strips. **Fig 3.4** shows a 5 °F temperature drop on the evaporation pad, indicating that evaporation is present in this region of the paper microfluidic strip.

![Figure 3.4 IR image showing the temperature drop on the evaporation pads of paper microfluidics strips due to evaporative cooling effects.](image)

**3.3.2 Alternating Fluid Flow**

We first demonstrated the continual pumping of a fluorescently dyed solution to visualize and trace the fluid movement. The sampling region of the paper strips interfaced a reservoir of DI water. During this initial priming, capillary action wicked water to fully wet the strip. After saturating the paper with water, we switched the solution in the reservoir between a fluorescent dye and DI water every twenty minutes. We measured the flow rate by
monitoring the intensity of the alternating bands of fluorescent dye that passed through the paper.

The pumping of fluid can be visualized in Fig 3.5a based on the movement of the bands of fluorescent dye. The green intensity of the fluorescent dye was measured at the locations denoted by the red and blue dotted lines and plotted versus time in Matlab. The resulting measurements produce two oscillating signals resulting from the presence or absence of dye in the reservoir (Fig 3.5b).

![Figure 3.5](image)

**Figure 3.5** (a) Images of a paper strip during testing where the sampling fluid was oscillated between DI water and a fluorescent dye every 20 minutes. (b) Image analysis shows the intensity of the dye oscillates in the encased channel. One measurement was performed at the bottom of the channel (red) and the other was measured near the top of the channel (blue). (c) Velocity of the dye was calculated at each step and remains constant over a 2 hour span.
The time delay between the two intensity signals represents the time it takes for the dye to travel the distance between the two points of measurement. Velocity of the dye traveling through the channel can be calculated by dividing the gap length between the locations of the measurements by the time difference between the two signals.

These paper devices have a steady state flowrate over small timescales due to the constant path length of fluid flow during operation. The wetted path length in conventional paper microfluidic devices increases during operation, resulting in a higher hydrodynamic resistance and therefore a declining flowrate. Evaporation based pumping has a constant wetted path length, resulting in constant flowrates that do not diminish within a few hours, as shown in Fig 3.5c.

3.3.3 Velocity Model and Limits

We developed a generalized fluid transport model to predict fluid velocity based on evaporation rates and the geometrical design of the paper channel. We also show the limitations to total fluid flowrate that arise from the force balance between the capillary pressure and the hydraulic resistances encountered throughout the paper.

The dye velocity through the channel was measured and modeled for paper devices of varying evaporation pad areas and channel widths. This information is important to determine the time delay from the moment a target analyte contacts the paper to the time it reaches a sensor. This model accounts for three important factors: 1) evaporation rate based on pad size,
2) velocity of dye through the paper channel based on channel geometry (cross-sectional area) and 3) chromatographic effects between the dye and paper substrate.

Evaporation rate was determined by measuring the mass of water evaporated over time in the paper strip. We assume there is a constant evaporation flux \((H)\) over the entire area \((A)\) of the evaporation pad, as capillary action will keep the paper wetted. This assumption allows for the calculation of the mass flow rate of water going through the device \((\dot{m})\):

\[
\dot{m} = HA \quad (3.1)
\]

The flow rate of water through the paper channel in the microfluidic device can be obtained from a mass balance using the fluid velocity \((v)\), channel width \((w)\) and moisture content of the paper \((M\, \text{mg/cm}^2)\):

\[
\dot{m} = vwM \quad (3.2)
\]

Lastly, chromatographic effects of the dye were taken into account. They dye lags behind the fluid flow due to a balance of intermolecular forces between the dye with both the fluid and paper substrate. This lag can be defined using the retention factor \((R_f)\). The retention factor is calculated by placing a drop of dye on a piece of chromatography paper, dipping the bottom of the paper in the solvent and tracking the distance traveled by the solvent \((D_{sol})\) and the dye \((D_{dye})\). This ratio of distances traveled is proportional to the ratio of velocities:

\[
R_f = \frac{D_{dye}}{D_{sol}} = \frac{v_{dye}}{v_{sol}} \quad (3.3)
\]
The mass evaporated equals the mass flow rate through the channel via mass conservation. Incorporating the retention factor and rearranging the equation allows for formulation of an equation for dye velocity. This model, plotted in Fig. 3.6a, fits the data well.

\[
v_{dye} = \frac{R_H A}{w M}
\]  

(3.4)

While increasing the area of the pad is the only way to increase the flow rate, the channel geometry can limit the maximum flow rate. Flow rate depends on the evaporation rate of water from the pad and how quickly capillary wicking of fluid traveling through the paper device can replenish the evaporated fluid. Fluid transport through the device encounters hydrodynamic resistance, which induces an associated pressure drop. Balancing this hydrodynamic pressure drop with the capillary pressure defines the flow rate through the channel. The pressure drop (\(\Delta P\)) over the channel length (\(L\)) can be correlated to the flow of water (\(Q\)) through the paper device, viscosity (\(\mu\)), permeability (\(\kappa\)) and cross-sectional area (\(A\)) using Darcy’s Law\(^{37}\):

\[
\frac{\Delta P}{L} = -\frac{\mu}{\kappa A} Q
\]  

(3.5)

We assume that the pressure drop of the fluid in the evaporating pad is negligible due to the much larger cross-sectional area of the fluid flow. The maximum flow rate is limited by the evaporation rate,

\[
Q_{\text{max}} = \frac{\Delta P w h \kappa}{\mu L} = \frac{H A}{\rho}
\]  

(3.6)

Thus, the maximum wetted area (\(A_{\text{max}}\)) that can be achieved for a given channel width while the device is operating at its steady state is given by Equation 3.7:
We measured the maximum area of water coverage with varying channel widths and large evaporating pads to verify $A_{max}$. The results are plotted in Fig 3.6b. The maximum wetted area follows a linear relationship with channel width as predicted by the model. The deviation from the origin may be due to edge effects of the paper strip or the neglected pressure drop in the evaporating pad. This graph defines the regions in which we can operate our device. Operation with evaporation pads with areas above this line result in a pad that is not fully saturated, as capillary wicking is not able to replenish water rapidly enough for the expected evaporation rate based on pad area. Smaller evaporation areas can be used as evaporation becomes the limiting factor and capillary action is able to fully replenish evaporated water. These results show the flow control and limitations made possible with evaporation-based pumping through a porous media.
Figure 3.6 Geometric control and limits to flow rate within a paper microfluidic device. (a) Plot of the measured dye velocity in the paper channel relative to the size of the evaporation pad for various widths of channels. Velocity increases with increased area and decreased channel width, as expected. The theoretical model was plotted for each channel width and shows agreement with empirical data. (b) Plot showing the maximum wetting area achieved on the evaporation pad vs. the channel width. Smaller channel widths have a higher pressure drop which hinders the flow of fluid through the channel reducing the maximum wetted area of the evaporation pad.

3.3.4 Long Term Viability

The limits of uninterrupted pumping were examined to determine the maximum operating lifetime. Real-world applications of microfluidics, such as biosensing of sweat
within a wearable device, utilize solutions that contain salt and other dissolved species. These analytes will accumulate on the evaporation pad over time and eventually precipitate as the concentration exceeds the solubility limit. This deposited salt will hinder the evaporation rate of fluid from the pad based on the increased osmotic pressure from the higher salt concentration as well as a hindrance to evaporation caused from the precipitated salt crystals on the paper. Paper strips were infused with phosphate buffer solution (PBS) for long durations to observe the effects of salt accumulation on flow rate. PBS was used to simulate technologically relevant fluids, such as sweat. Precipitated salt was first observed at the outer edge of the evaporation pad on the second day of testing. A solid salt crust grew each day as more salt precipitated, whereas pumping DI water has no accumulation of analytes resulting in a homogenous wet paper strip (Fig 3.7a).

There is a continual flow of water through the paper to the edge of the evaporation area during operation. The evaporation of water concentrates the salt dissolved in solution. Salt begins to precipitate and forms a solid crystal deposit on the exterior of the paper once the salt reaches its solubility limit. The pumping mechanism continues to precipitate salt from the periphery of the pad inwards (Fig 3.7b).

Dye velocity was measured once a day over a 10 day span for multiple days (Fig 3.7c). The area of the evaporation pad not covered in salt crystals was also measured daily (Fig 3.7d). Both the dye velocity and uncovered area decrease over time as salt accumulates. Integration of the velocity over time shows a total fluid uptake of roughly 13 mL of solution. By
comparison, a saturated paper strip of this size would contain 0.025 mL, approximately 1/500th of the amount of fluid pumped through evaporation.

Dye velocity is proportional to the evaporating area, as shown in equation (4). The area available to evaporate water for pumping decreases as a direct result of salt accumulation. The rate of salt accumulation relates directly to flow rate. These correlations relate fluid velocity and area.

\[ v = \alpha_1 A \]  
\[ \frac{dA}{dt} = -\alpha_2 v \]  

Using these correlations, a system of first order differential equations were formulated with the solutions below:

\[ v = v_0 e^{-\frac{t}{\tau}} \]  
\[ A = A_0 e^{-\frac{t}{\tau}} \]  

Both of these models match the data with good agreement. A plot of velocity vs. area for each day verifies the assumption of velocity being proportional to area (Fig 3.7e). A linear trend with an intercept at the origin agrees with the assumptions of our model. This behavior of decreased evaporation rate due to an increase in dissolved and precipitated salt is analogous to that of the evaporation of saline water from soil.  

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**Figure 3.7** Long-Term Pumping and Effects of Salt Accumulation. (a) Images and micrographs showing the evaporating pad of the paper strip under operation of DI water and PBS. With PBS, salt accumulates at the end of the pad over time, where DI water results in a homogenous paper substrate. (b) Schematic showing the mechanism of salt accumulation. Salt concentration first builds up at the edge of the strip as water evaporates. Excess salt begins to precipitate along the edge, resulting in a front of salt crystals moving inward from the edge of the pad. (c) Velocity of a fluorescent dye was measured over a 10-day span to determine the effect of salt accumulation on fluid flow. (d) The area of the paper pad not covered by salt crystals was measured over this span. Both velocity and area show a negative exponential decay over time. (e) Velocity was plotted vs. area remaining with a linear trend.

### 3.3.5 Sensing Demonstration

These continual pumping paper microfluidic devices were tested to determine their performance with several sensing mechanisms. Solutions with varying dye concentration (0 – 0.2%) were pumped through the paper channel while measuring the resulting color over time. These tests were performed using multiple step changes as can be seen in Fig 3.8a.

The response of the measured intensity to the change in sampled dye depends on the pumping rate and channel width. The steady state intensity was used to create a correlation curve with the dye concentration (Fig 3.8b). There is a linear relationship between color intensity and dye concentration. This correlation was expected, as concentration of the dye is the only parameter altered and has a direct effect on color intensity. This demonstrates the ability of this pumping device to be used for colorimetric sensing applications.

Many microfluidic sensing applications often involve electrochemical measurements through interfaced electrodes. Interdigitated gold electrodes were adhered to the paper channel with the leads connected to a potentiostat (Gamry Reference 600) for high frequency...
impedance testing. Solutions of varying NaCl concentration were pumped over time while recording the impedance response.

The impedance signal changed rapidly in response to changes in the salt concentration at the reservoir (Fig 3.8c). A correlation curve for impedance vs. salt concentration shows an inverse relationship between impedance and salt concentration ranging from low salinities, 0.002 M, up to 0.25 M. Impedance leveled off at salt concentrations above this salinity. This is likely due to large surface impedances between the paper and electrode that dominate the measured impedance. This problem can be solved by engineering a better electrode and interface.
Figure 3.8 Colorimetric and electric sensing in a paper microfluidic device. (a) Plot showing measured intensity in a paper channel while the concentration of dye in the solution being sampled was adjusted via step changes. (b) Intensity was plotted vs. dye concentration, which can be used as a correlation curve. (c) Electrical sensing during continual evaporative pumping. Impedance at 1 MHz was measured using a gold interdigitated electrode interfaced onto the paper strip. The impedance was monitored over time as the sensing solution was varied between saline solutions ranging from 0.002 to 2 M with DI water intermittently (note that impedance was plotted inversely on the y axis). (d) Impedance vs. salt concentration. An inverse relationship can be seen up to 0.2 M, at which point interface and electrode resistances were dominant and led to a leveling of impedance. These plots show the ability of this pumping mechanism to work for both electrical and colorimetric sensing applications.
3.4 Conclusion

Continual evaporation based passive microfluidic pumping has been demonstrated on paper substrates. Paper microfluidic devices, which can be easily fabricated through laser cutting, have been tested to observe their ability to continually pump fluid for long durations via evaporation. We have shown and modeled the flow rate of fluid through these devices based on geometric properties of the paper device. Pumping of a PBS solution was performed for 10 days continuously which resulted in a total accumulated flow of over 13 mL. Both colorimetric and electrical sensing applications were demonstrated using this paper microfluidic pumping platform.

This pumping mechanism requires no power and thus can be used with most paper microfluidic sensing devices to achieve continual long-term monitoring where power consumption may be limited. For example, this technology may allow paper to be used effectively in the rapidly developing field of wearable biosensors. Evaporation-based pumping may enable paper to be used as a substrate for wearable sweat sensing technologies. Paper is currently used for many single use biochemical sensing operations performed on fluids such as blood or drops of sweat. Many current research endeavors in sweat sensing utilize a material to wick sweat from the body, but do not have a way of handling that fluid for long term after testing has been performed\textsuperscript{21,33}. Management of sweat has been one of the roadblocks for these technologies, as guiding sweat flow can be difficult without using an additional power input. The simple evaporation-based pumping platform shown here may be able to fulfill these needs and fill an integral role for next generation of wearable technologies.
3.5 Acknowledgements

We acknowledge the funding of this study by the NSF-ASSIST Center for Advanced Self Powered Systems of Integrated Sensors and Technologies Center (EEC-1160483). We also acknowledge fellow colleagues Dr. Michael Daniele for his assistance with this work.
3.6 References


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Chapter 4

Human Subject Testing of Prototype Hydrogel-Paper Microfluidic Devices

4.1 Introduction

The previous two chapters of this dissertation discussed passive methods for pumping fluids through microfluidic networks for the purpose of creating a sweat collection device. Our next goal was to combine the osmotic hydrogel pumping platform with the evaporative paper microfluidic system into a working prototype to be tested on human subjects. The combination of these technologies would enable (1) the paper to wick away sweat from the body during periods of high sweat rate while evaporating it away on the back end and (2) the hydrogel to promote sweat collection during periods of low sweat rate. A schematic of these devices can be seen in Fig 4.1.

While a paper microfluidic-based sweat sensor has been demonstrated previously,\(^1\) we believe our design has multiple benefits that may enable more versatile and longer-term operation. Our device will utilize the passive osmotic hydrogel pump described in chapter 2 to promote sweat rate during periods of low sweat. All prior sweat collection devices require high sweat rates to operate. The effects of salt accumulation due to evaporation had also not been studied previously. Salt accumulation will inherently reduce evaporation and limit the lifetime of the device. We have studied how this process works and affects the system, which will allow us to design the microfluidic devices accordingly.
Figure 4.1 (a) 3D renderings of how the microfluidic device works. The design is conceptually identical to the original hydrogel pump with the exception of the paper strip that is placed in the microfluidic channel and wraps around the bottom of the hydrogel disc. (b) A block diagram demonstrating how this device operates. (1) The hydrogel provides an osmotic pressure gradient to draw sweat from the skin. (2) Capillary wicking draws the fluid pumped by the hydrogel through the paper microfluidic network. (3) Evaporation on the back end of the paper strip enables continue long-term operation of this device.

To prove the efficacy of these new devices, we first demonstrate the pumping ability of this device in a lab setting on a diffusion cell. We then demonstrate the ability of this platform to draw sweat from human subjects in an internal review board (IRB) approved testing. Lastly, we perform a brief chemical analysis of the collected sweat to show that chloride has been collected from the body using our device.
4.2 Materials and Experimental

4.2.1 Materials

The hydrogels were formed using acrylamide as the monomer (4 M), n,n’-methylenebisacrylamide as the crosslinker (0.04 M) and 2-hydroxy-4’-(2-hydroxyethoxy)-2-methylpropiophenone as the photoinitiator (0.002 g/mL, all from Sigma). Whatman quantitative filter papers, hardened, ashless, Grade 542 were used for the paper-portion of the device. The paper designs consist of three main features; a sampling interface where the paper contacts the desired fluid, a linear encased segment acting as the microfluidic channel where sensors will be interfaced, and an open large surface area segment for evaporation. Strip designs were created in CorelDraw and a CO₂ laser cutter was used to cut the paper strips. Microfluidic devices were fabricated from polydimethylsiloxane (PDMS, Sylgard 184 Dow Corning). Microfluidic molds were generated using SU8-100 photoresist (Microchem) on silicon wafers.

Glycerol (Acros) was used to dope the hydrogels. A phosphate buffer solutions (PBS) solution, prepared from tablets (Sigma), was used as an aqueous system that mimics the ionic strength and osmotic pressure of sweat. Blue food coloring was used as the dye in the IRB tests. A colorimetric chloride assay (Sigma) was used to measure chloride concentration in the paper strips.
4.2.2 Paper Washing

The Whatman paper contained significant amounts of chloride, which overpowered the chloride signal that should be present in human sweat. This resulted in erroneous readings showing higher chloride concentrations than expected. Therefore, a washing method was developed to remove any excess chloride from the paper prior to testing. The laser cut paper strips were placed in excess DI water for one hour to rinse the chloride (prior to fabrication of the PDMS device). The water was then removed, and the solution refilled with fresh DI water and allowed to rest for another hour. This process was repeated four times to ensure that nearly all the chloride had been removed.

Warping or bending of the paper strips occurred when the paper strips were allowed to dry in a warm oven, preventing their use in the microfluidic system. We placed the paper strips between two flat pieces of acrylic held together by binder clips and then placed it in a vacuum oven set to 75°C for one hour to remove the water. Sandwiching the paper between acrylic sheets prevented bending or warping during the drying process. This method resulted in chloride-free paper strips while maintaining structural integrity. These paper strips were then implemented to create the PDMS microfluidic devices.

4.2.3 Device and Hydrogel Fabrication

Polymerization of hydrogel sheets was initiated under a 100 mW/cm² mercury lamp for 5 minutes. The monomer solution is 4 M acrylamide, 1% crosslinker by mole and 0.002 g/mL photoinitiator. Hydrogels were allowed to equilibrate in their respective solutions of
glycerol for at least 24 hours after polymerization was completed. The solution was then removed and replaced with a fresh glycerol solution. This washing process is used to ensure that unreacted monomer and photo initiator is removed from the hydrogel. Hydrogel disc designs were created in CorelDraw and cut from equilibrated hydrogel sheets using a CO₂ laser cutter. Semicircular notches of 0.375 mm radius were also cut into the hydrogel discs to create a region of low hydrodynamic resistance to guide fluid flow during operation. This predesigned path for the fluid allows for a guided and controlled flow with consistent flow rates. These hydrogels were stored in solution to maintain osmotic pressure until testing.

The PDMS microfluidic devices were fabricated using soft lithography techniques. First, molds were generated using SU8-100 photoresist on silicon wafers by means of photolithography. A 10:1 mixture of PDMS was prepared and degassed using a Thinky mixer AR-1000. PDMS was then cured on both a mold and on a flat surface in a 70 °C oven for 1 hour. A metal leather punch dye (6.8mm in diameter) was used to cut the holes in PDMS. Oxygen plasma treatment was then used to bind the pieces together to create the microfluidic devices. The precut and rinsed paper strips were aligned with the microfluidic channel just prior to binding the PDMS sheets. This encapsulated the paper microfluidic strips in the PDMS channel. The microfluidic channels have a width of 1000 µm, height of 500 µm and vary in length from 1 – 2 cm.
4.3 Results and Discussion

4.3.1 Diffusion Cell Testing

The paper microfluidic sweat collection devices were first demonstrated in a lab setting on a diffusion cell to demonstrate that the hydrogel pumping mechanism can be used in conjunction with paper microfluidics. We performed a test where a PBS solution with a green fluorescent dye was pumped through the paper microfluidic device (Fig 4.2a). The green dye can be seen traveling through the paper microfluidic channel and into the evaporation pad. A second test was performed (Fig 4.2b) where a red fluorescent dye was introduced into the diffusion cell chamber 30 minutes into the test. The red dye can be seen passing through the paper microfluidic channel as well, indicating that there is continual flow in the microfluidic device. These tests demonstrate that our paper microfluidic network is compatible with the osmotic hydrogel pumping mechanism.
Figure 4.2 (a) Time-lapse images showing the pumping of a green fluorescent fluid into the paper microfluidic device. The circular paper disc interfaces the hydrogel disc on its top and the dialysis membrane on the bottom. The chamber beneath the dialysis membrane is a PBS solution with fluorescent dye. (b) Image from a second test where a red dye was introduced into the diffusion cell chamber after 30 minutes of pumping.

4.3.2 Initial IRB Testing

We acquired permission from the NC State Institutional Review Board (IRB) to perform testing with our materials on human subjects. These tests were used to verify that the
osmotic pumping platform would work on human skin, which is a much more complex membrane than the dialysis membrane used in prior testing.

A drop of blue dye was first placed on the inner forearm near the wrist of the participants. The dye was left on the skin for five minutes, which allowed for the dye to penetrate into the skin and sweat glands. All excess dye was then removed with tissue paper. The purpose of the dye was to be a trackable visual marker as it transports into the paper microfluidic system.

Hydrogel discs that have been equilibrated in either DI water or 12 M glycerol solution were used as the osmotic pump for these tests. A hydrogel disc was removed from solution and blotted with tissue paper to remove all excess water on the surface. The hydrogel disc was then inserted into the opening on the bottom of the PDMS microfluidic device and excess hydrogel extending past the bottom PDMS surface was removed with a razor blade. The bottom circular region of the paper microfluidic that extends from the microfluidic channel was then folded over the hydrogel to act as an intermediate layer between the hydrogel and skin during testing.

The microfluidic device was then adhered to the wrist, with the paper interface on the dyed region of the forearm, using a Velcro strap. During testing, the Velcro strap would temporarily be removed so photographs could be taken to visually monitor the microfluidic device. Results of one of the IRB tests can be seen in Fig 4.3.
Figure 4.3 Photographs of the microfluidic device on a subject’s arm during an IRB approved test taken every 15 minutes. The blue stained skin can be seen beneath the interface of the paper strip with the body. Tests were performed for a hydrogel disc swelled in DI water and one swelled in 12 M glycerol. Over the course of 90 minutes, the blue dye can be seen penetrating into the paper microfluidic channel for the glycerol hydrogel. The DI hydrogel showed no pumping ability at all. This shows the ability of the osmotic hydrogel to drive the flow of analyte from skin into our device.

These images for the glycerol doped hydrogel show the blue dye penetrating into the paper microfluidic channel over the course of 90 minutes. These results confirm that our device was pumping dye (and suggests it pumps sweat from the body), as all excess dye was removed prior to the start of the test. The hydrogel swelled in DI water, which should not have provided any osmotic pumping ability, drew no fluid or dye into the paper microfluidic channel. This confirms our hypothesis that it is the osmotic pressure difference created by the hydrogel that is driving sweat flow from the body to the device. A remarkable feature is that the participant in this study was sitting in a chair during the whole experiment (no physical exertion). Prior research endeavors in sweat collection devices have had their participants
perform physical tasks to promote sweat rate, such as running or biking, while ours were in a calm relaxed state.

4.3.3 Colorimetric Chloride Assays

A colorimetric chloride assay was used to detect the presence of salt in the fluid collected in the paper strips. Neither the hydrogels nor prepared paper strips used contained a measurable amount of chloride. Therefore, the presence of chloride in the paper strip definitively shows that this device has pumped sweat from the body and through the paper microfluidic channel.

A colorimetric chloride assay kit was purchased from Sigma-Aldrich. We modified the protocol to meet the needs of our system. We first filled a microcuvette with 900 µL of DI water. The paper substrate to be tested was then placed into the water and allowed to rest for 10 minutes. This helps ensure that any salt from sweat collected by the paper is extracted by the DI water. The paper sample is then removed and 100 µL of the chloride reagent is added. The reagent becomes blue in the presence of salt. Absorbance of the solution is measured after 15 minutes has passed at a wavelength of 600 nm.

Tests were first performed in a lab setting on a diffusion cell to verify this method in a controlled environment. Solutions of varying chloride concentration were pumped through the paper microfluidic devices. The paper strips were designed to have a 6.8 mm diameter disc at the end of the channel. This disc is cut from the rest of the device and analyzed after saturation of the device has occurred. This method is shown in Fig 4.4a. A calibration curve was first
created using known levels of sodium chloride added to a paper disc. We injected 3 µL of NaCl solutions (0, 25, 50, 100, 150 and 200 mM) onto 6.8 mm diameter paper discs, which were then inserted into 900 µL of DI water. The calibration curve for the measured absorbance vs moles of chloride present can be seen in Fig 4.4b.

![Schematic diagram](image)

**Figure 4.4** (a) Schematic showing how we remove the paper disc at the end of the channel and perform a colorimetric assay on it to determine chloride concentration. (b) Calibration curve showing 600 nm absorbance over a range of chloride levels in our 1 mL system. (c) Comparison of results when tested on a diffusion cell with DI water and PBS as well as from human subject tests.

Tests were first performed on a diffusion cell with DI water and PBS as control experiments (Fig 4.4c). The DI water tests showed no chloride present in the pumped fluid, as was expected. We measured chloride at 110 mM in the PBS pumped solution. PBS has a
chloride concentration of approximately 140 mM. The lower measured chloride concentration is likely due to the dilution of our sample from the water and glycerol present in the hydrogel, or the impediment of chloride ions to pass through the dialysis membrane.

Human subjects were then tested to determine if we could collect chloride in sweat. The microfluidic devices were strapped onto the inner forearm of patients until enough fluid was collected such that the paper was fully saturated, at which point the chloride assay was performed on the paper disc. We measured chloride levels at 120 mM from our human subjects. The normal range of chloride in sweat is from 10 – 100 mM. The elevated measurement obtained is likely due to evaporation of fluid from the paper during testing. Roughly one hour was required to collect sufficient sweat for testing. As water evaporates from the paper pad, the analyte concentrations increase. We did not notice this effect in the diffusion cell tests with PBS. Diffusion cell tests have higher pumping rates, requiring less time for saturation and therefore less total evaporation. While our measurements were higher than expected, this test demonstrated the purpose of proving that our osmotic hydrogel device can work to pump sweat and bioanalytes through a wearable paper microfluidic platform.

4.4 Conclusion

In this chapter we combined the concepts and microfluidic platforms discussed in the first two chapters to create a working prototype which was tested both in a lab setting and on human subjects. We first demonstrated that paper microfluidics are compatible with the hydrogel pumping mechanism by testing the system in a lab setting using a diffusion cell. We next showed that our devices could passively draw sweat from the body of a human subject.
This was demonstrated by visually observing the pumping of a blue dye that had been soaked into the skin as well as measuring the presence of chloride in the pumped solution.

This is the first time a sweat collection device has been able to collect fluid under periods of low exertion. All prior sweat sensing devices require the user to exert themselves physically, often by running or biking.\textsuperscript{1,4–8} The ability to sample sweat during periods of low sweat rate enables continual monitoring for biomarkers that are of interest during normal daily operations.

4.5 Acknowledgements

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4.6 References


Chapter 5

Soft and Flexible ECG Electrodes Created with Hydrogel and Liquid Metal

5.1 Introduction

The electrocardiogram (ECG) is currently the best noninvasive method used to monitor the heart’s health.\(^1\) The ECG measures the electrical activity produced during the pumping motion of the heart. The electrical signal comes specifically from the flux of ions through cell membranes during polarization. Two electrodes can be used at a minimum, but up to fifteen electrodes, placed across the body, can be used to monitor very precise functions of the heart.

These electrical fluctuations are in the range of 10 mV. Obtaining a clean low-noise ECG signal requires low impedance electrodes. The largest impedance in the system arises at the electrode-skin interface, specifically the stratum corneum. This is the layer of dead skin that accumulates on the surface of the epidermis. An abrasive cream is commonly applied prior to testing to remove this resistive layer during normal ECG measurements.\(^2,3\) This extra step may also irritate the skin.

ECG electrodes fall into two primary categories; dry and wet. Dry electrodes are comprised of printed metal or polymers that interface the skin directly. While these electrodes are successfully implemented\(^4\), they often are stiffer that the skin (i.e. mechanically mismatched), have large areas, are affected by sweat and can have poor connection with the skin which can reduce the signal quality.\(^5,6\) Advances have been made to improve flexibility in these dry electrodes by utilizing flexible materials as the electrode material.\(^7-9\) Wet electrodes contain a conductive fluid (usually an electrolyte or an organic fluid) that creates a
continuous electrical connection between the electrode, normally silver/silver chloride (Ag/AgCl), and the skin, improving performance.\textsuperscript{10} Hydrogels can be used as the wet material in bio electrodes due to their conductive and biocompatible nature.\textsuperscript{11–14} Hydrogels are hydrophilic crosslinked polymers that can contain up to 99\% water by mass. They have also been used in various other applications ranging from soft actuators and sensors to drug delivery.\textsuperscript{15–21} The very high water content can make hydrogels biocompatible and useful for biomedical operations.\textsuperscript{22} Past studies have shown that the electrical properties of hydrogels can be easily modified based on the polymer structure and additives, which enables their use in soft and stretchable electronics.\textsuperscript{23,24} This makes them an ideal material for electrically interfacing the skin. Hydrogels can also be modified to have varying levels of stiffness and flexibility, which can improve skin contact and performance.

We sought to create an ECG electrode using entirely soft and stretchable materials by combining hydrogels with liquid metal electrodes. We investigated a eutectic mixture of gallium and indium (EGaIn), a liquid metal at room temperature, as a material to replace the traditionally solid metal electrodes used in commercial devices. EGaIn is a eutectic metal alloy comprised of 75\% gallium and 25\% indium that has a melting point at 15.5°C, allowing it to remain a liquid at room temperature.\textsuperscript{25} EGaIn, which is considered to have low toxicity, can be injected into microfluidic channels to create stretchable wires and antennas.\textsuperscript{26} EGaIn spontaneously forms a gallium oxide skin that is strong enough to hold the shape of the liquid on small length scales.\textsuperscript{27} The benefit of using liquid metal in electrical devices is that it is soft due to the fluidic nature of the material. It can also self-heal when damaged.\textsuperscript{28} Stretchable
electronics will likely be important for enabling on-body sensors for health monitoring. EGaIn has been used to create many flexible electronic devices in recent years.29–40 Hydrogels have been used with EGaIn in the past to create biomimetic electronic devices. Doped hydrogels were used to control the presence of the oxide skin on EGaIn in order to dictate the electrical properties of the interface under DC conditions. This concept was used to create diodes41 and a soft four bit memory device.42

The goal of this paper is to study and characterize hydrogels with a liquid metal electrode to create a truly soft electrode. We will first examine the electrical characteristics of both the hydrogel and its interface with the liquid metal. The hydrogel can be modified to have varying ionic strength and pH which affect the electrical properties of both the hydrogel and its interface with the EGaIn. We show that by optimizing the materials, we can create and test a working ECG electrode with a signal-to-noise ratio that is slightly better than commercial electrodes.

5.2 Materials and Experimental

5.2.1 Materials

The hydrogels are created using combinations of acrylamide (AAm) and acrylic (AA) acid as the monomer, n,n’-methylenebisacrylamide as the crosslinker and 2-hydroxy-4’-(2-hydroxyethoxy)-2-methylpropiophenone as the photoinitiator (all from Sigma-Aldrich). Hydrogels were either polymerized with pure acrylamide (pAAm) or a mixture of acrylic acid and acrylamide (pAA-AAm). Sodium chloride (Sigma-Aldrich) was added to the monomer
mixtures prior to polymerization to increase mobile ion concentration. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used to modify the pH of the hydrogel. These salts and acids/bases were added to the monomer mixture prior to polymerization. EGaIn was purchased from IndiumCorp. The PDMS used was Sylgard 184 (Dow Corning).

5.2.2 Impedance Spectroscopy

We created a test method to measure the electrical properties of the EGaIn-hydrogel interface with a controlled geometry. The impedance of this interface must be minimized to produce an effective ECG electrode. We performed tests on hydrogel plugs polymerized in Tygon tubing to examine the impedance (Fig 5.1). After injecting the solution of hydrogel monomer with a syringe into 0.05 inch ID Tygon tubing, we polymerized it under a 100 mW UV light for five minutes. Polymerization of the hydrogel in tubing controls both the length and cross sectional area of the hydrogel cylinder. EGaIn was then injected into the Tygon tubing with a syringe such that the metal was flush with the hydrogel.

Electrochemical tests were performed using a Gamry Reference 600 potentiostat. Copper wires were inserted into the EGaIn and then connected to the potentiostat for two electrode sensing. Potentiostatic electrochemical impedance spectroscopy (PEIS) was performed using a 10 mV RMS voltage between 1 Hz and 100 kHz (unless otherwise stated). This method can also evaluate the electrodes impedance with the skin. Similar methods have been used to evaluate electrode impedance.4,12
Figure 5.1 (a) Diagram showing how hydrogels and EGaIn could be implemented to create an ECG electrode. The electrical impedance between the hydrogel and EGaIn must be minimized to improve signal to noise ratio during ECG measurements. (b) The test method that allows measurement of the electrical characteristics of both the hydrogel and hydrogel-EGaIn interface using potentiostatic electrochemical impedance spectroscopy. The working and workings sense leads are connected on one end and the counter and reference leads are connected to the opposite end.

5.2.3 Tensile Testing

Tensile testing was performed on hydrogel pieces to determine their Young’s modulus. The hydrogels were first polymerized in a petri dish to create a sheet 3 mm thick. A laser cutter was then used to cut the hydrogel into a dog bone (ASTM D412) shape for testing. The cut hydrogels had a gauge length of 15 mm and width of 5 mm. Testing was performed on an Instron operating at a speed of 3 mm/min.
5.3 Results and Discussion

5.3.1 Control of Hydrogel Resistance

Hydrogel resistivity was first calculated from the measured impedance for the hydrogel plugs. PEIS was performed on pAAm hydrogels that were polymerized with 2M NaCl. The hydrogels tested had varying hydrogel lengths and the resulting impedance spectra can be seen in Fig 5.2a. The impedance is highest at lower frequencies due to a capacitive impedance that arises from the EGaIn hydrogel interface, which is consistent with the impedance being the same regardless of the length of the gel. Capacitive effects are inversely proportional to frequency and therefore the contribution of capacitance to impedance decrease at high frequencies. Thus, at high frequencies the impedance is a sum of all of the resistances in series in the system. The bulk materials in series are the hydrogel plug, EGaIn, copper wire and alligator clips connecting the setup to the potentiostat. Because the hydrogel resistance is orders of magnitude more resistive than the metallic components, we assume the hydrogel resistance dominates the impedance measurement. The impedance values at 100 kHz were plotted vs. hydrogel length (Fig 5.2b) and a linear trend with a zero intercept can be seen. This is expected as resistance (R) is equal to the electrical resistivity ($\rho$) times the ratio of the hydrogel length (l) to the cross sectional area (A) according to Ohm’s law.

We also measured the impedance of hydrogels with varying amounts of NaCl to determine the effect of ionic strength on the hydrogel resistivity. As expected, increased salt concentration lowered the hydrogel resistance (Fig 2c,d). Resistivity shows an inverse relationship with salt concentration. The larger deviation in resistivity at low salt concentration
arises from error in the testing caused by reaching the maximum limits of the measureable impedance.

Figure 5.2 (a) Impedance tests results for pAAm hydrogels with 2M NaCl of varying lengths. (b) The 100 kHz impedance was plotted as a function of hydrogel plug length. At these high frequencies, impedance from capacity effects are minimized, and the impedance measured is the sum of the resistances in series. Both the EGaIn and copper have negligible resistances. Therefore the measured impedance can be approximated as the hydrogel resistance, which agrees with the linear trend. (c) Impedance spectra of pAAm gels of similar lengths with varying amounts of NaCl (0.001 M – 1 M). (d) Hydrogel resistivity was obtained from the 100 kHz impedance measurements and calculated based on the geometry of the hydrogel plug. Resistivity is plotted vs. NaCl concentration and follows an inverse relationship with salt concentration.
5.3.2 Control of Low Frequency Impedance

While modifying the ionic strength of the hydrogel lowers high frequency impedances, it has a minimal effect on the low frequency impedances (1-50 Hz), which is the range of importance for ECG sensing. We hypothesized that the high impedance at low frequencies is due to the EGaIn-hydrogel interface effects since a resistive oxide skin forms between the EGaIn and hydrogel. This oxide skin can be removed by loading the hydrogel with hydrochloric acid (HCl) prior to polymerization to reduce the pH. PEIS was performed on hydrogels with pH ranging from 7 to 1.5 with 2M NaCl (Fig 5.3a).

The addition of HCl lowered the hydrogel pH and decreased the low frequency impedance. Hydrogels were also created with basic properties by adding NaOH to the hydrogel solution, as basic conditions (pH>10) will also remove the amphoteric oxide skin. A spectra of impedance values taken at 1 Hz was plotted vs pH for both HCl and NaOH modified gels (Fig 5.3b). Impedance drastically declines at both low and high pH, which correlates to the removal of the oxide skin. This correlates well to the Pourbaix diagram for gallium species.43

Hydrogels with acrylic acid moieties were created and tested to determine if the pH effects observed with HCl could be obtained with hydrogels that have acidic groups bound to the polymer backbone. Hydrogels with 20% acrylic acid (80% acrylamide) were created (pAA/AAm) and tested. These gels had a pH of approximately 2.5 and were polymerized with varying amounts of NaCl to observe effects of ionic strength. Hydrogels with low pH and high NaCl concentration behaved very similarly to those with HCl, with a decreased low frequency
impedance (Fig 5.3c). But hydrogels with low ionic strength did not exhibit the decreased low frequency impedance, although the pH was below 3. This indicates that hydrogels must have an extreme pH to remove the oxide and a high ionic strength to achieve decreased low frequency impedances (Fig 5.3d).

Figure 5.3 (a) Impedance spectra of PAAm gels with 2M NaCl and varying amounts of HCl to decrease the pH. (b) The 1 Hz impedance of these hydrogels and others modified with NaOH to make basic hydrogels were plotted as a function of pH. The impedance decreases as pH is lowered below 4 and raised above 10, which coincides with the removal of the oxide skin. (c) Impedance spectra of pAA-AAm (pH ~ 2.5) gels with varying NaCl concentrations. (d) The 1 Hz impedance of these hydrogels plotted as a function of ionic strength.
An acidic hydrogel interface may be problematic as an electrode interface depending on the use case. For example, utilizing these gels as a skin interface for an ECG electrode may cause skin irritation. Neutral pH hydrogels unfortunately result in poor impedance properties due to the presence of the oxide skin at the hydrogel-EGaIn interface.

To combat these issues, a bilayer hydrogel system can be implemented that can satisfy both of these concerns simultaneously. This bilayer can be created such that a layer of a pAA-AAm gel interfaces the EGaIn while a neutral layer of pAAm hydrogel interfaces the skin. A schematic of this arrangement can be seen in Fig 5.4. Gels combined this way create a pH gradient that will satisfy both surfaces’ requirements while ionic strength can remain uniform in the system, preventing swelling or de-swelling of the hydrogels. We tested the impedance characteristics of this system (Fig 5.4c). In principle, the PEIS spectra of the multilayer gel should appear the same as a single plug of pAA-AAm gel of the same size. Methyl red, a pH indicator, was placed in both hydrogels to illustrate the pH differences between the two hydrogels.

Three sets of hydrogels were created and tested (Fig 5.4d). One pAAm gel, one pAA-AAm gel and the third set was a trilayer gel with pAA-AAm gel on the exterior and a pAAm gel on the interior. All of these gels had an ionic strength of 2M. The trilayer gel and pAA-AAm gel have nearly identical impedance spectra, whereas the pAAm gel had a relatively large low frequency impedance. The resistivity was the same for all three configurations, as interface effects do not alter this bulk property. These tests show that a bilayer hydrogel system can be implemented that has the same electrical properties of a purely acid hydrogel. This
allows for a low impedance hydrogel-EGaIn impedance to be created while maintaining a neutral hydrogel-skin interface to prevent harm to a user.

Figure 5.4 (a) Schematic demonstrating how a bilayer hydrogel ECG electrode could be created to utilize an acidic interface to lower EGaIn-hydrogel impedance and a neutral hydrogel to interface the skin. Both gels would have the same ionic strength resulting in uniform resistivity. (b) Hydrogel plugs with the pH indicator methyl red were created to show two gels of different composition could maintain pH while interfacing each other. (c) Testing was performed by creating a trilayer plug with the acidic hydrogel interfacing EGaIn. (d) Impedance spectra of a pAAm, pAA-AAA and trilayer hydrogel system are plotted. The trilayer gel shows the same electrical characteristics of the pAA-AAA gel, while all three gels have the same resistivity (inset).

5.3.3 Crosslinker Concentration Effect on Hydrogels

The hydrogels interfacing the skin should be soft for user comfort and to ensure good conformity to the skin. Softness can be achieved in hydrogels through changing the crosslink
density. We tested multiple NaCl infused pAAm hydrogels to determine the effect of crosslink density on Young’s modulus and electrical properties (Fig 5.5).

As expected, increased crosslinker concentration results in a stiffer hydrogel, while having a minimal effect on hydrogel resistivity. The slight increase in resistivity may be due to the lower water content of these higher crosslinked hydrogels or the more tortuous path for movement of charge through the gel. Low frequency impedance for acidic hydrogels decreased as crosslinker concentration increases. A possible explanation for this trend is that EGaIn may make better contact with a stiffer surface, which would help decrease the electrical impedance. These impedances are all lower than impedances encountered with neutral hydrogels.
Figure 5.5 Hydrogel characterization is shown for hydrogels of varying crosslinker. (a) Young’s modulus, (b) hydrogel resistivity and (c) 1 Hz impedance were plotted against hydrogel crosslinker concentration. Young’s modulus and resistivity were measured for pAAm gels with 2M NaCl. The 1 Hz impedance data was obtained for a 2M NaCl hydrogel with added HCl.
5.3.4 Modeling

Impedance results were modeled using an equivalent circuit model to describe this hydrogel-metal system. Equivalent circuit models are effective at describing electrochemical systems using ideal electric circuit components (resistors, capacitors, etc.). The Randles circuit is commonly used to model a system containing a solution in contact with an electrode. The resistor in series ($R_{gel}$) represents the resistance due the hydrogel in our system. The resistor ($R_{CT}$) and capacitor ($C_{DL}$) correspond to the charge transfer resistance and double later capacitance created at the interface of an aqueous phase and solid interface. The Warburg element ($Zw$) can be used to represent impedance as a result of diffusion in solution, while the general constant phase element (CPE) can be used to mathematically model further deviations from ideality.\textsuperscript{44}

Three different equivalent circuit models were tested on our system (Fig 5.6). An impedance spectra ranging from 0.1 to 100 kHz was obtained using a pAAm gel with 1M NaCl with a pH of 1.5 (modified with HCl). One model tested was the Randle’s circuit while the other two models were Randles circuits modified with constant phase elements. The first modified Randles circuit utilizes the Warburg coefficient, which accounts for the diffusion of ions in solution with a fixed phase angle of $45^\circ$. The last model utilizes a constant phase element with an unspecified phase angle, which is commonly used to represent non-idealities from an ideal double layer capacitance.
Figure 5.6 Three equivalent circuits were modeled for an acidic hydrogel (pH=1.5) with 1M NaCl to see which models fit the system. (a) A Randles circuit shows general agreement but fails at lower frequency where the model levels off and test results rise. (b) A modified Randles circuit with a Warburg Element (used to model diffusion) shows good agreement with impedance magnitude across the spectrum but deviates from the phase angle data at low frequency. (c) A constant phase element shows great agreement between the data and model for both the impedance and phase angle. While CPEs have no direct physical interpretation, they are often used to represent a deviation from an ideal capacitor.

All of the models tested show a general agreement to the system. Randles circuit accurately describes high frequency impedances but deviates from the data at low frequencies. This is the result of the model predicting a leveling off of frequency to match the charge transfer resistance. The measured impedance increases at low frequency and deviates from this model. The Warburg element accurately models the impedance spectra, but the phase angle deviates at lower frequency. The unspecified constant phase angle accurately predicts both the impedance and phase angle across the entire frequency spectra. These tests were performed to demonstrate that these models could be used for optimizing electrode design for future tests.
5.3.5 Prototype ECG Electrodes

Prototype ECG electrodes were created and tested using PEIS as well as in a real ECG setup. Designs and images of the electrodes can be seen in Fig 5.7. The electrodes are created utilizing multiple soft lithography steps (detailed in supplemental document). EGaIn was then injected into the microfluidic channel where it will interface the hydrogel in a 3 mm wide region. A 10 mm diameter hydrogel disc can then be placed into the PDMS opening device prior to testing. A copper wire was inserted into the back end of the microfluidics device to interface the EGaIn. This wire was then connected to the monitoring equipment. This device setup allows us to utilize multiple layers of hydrogel discs into our device. We can insert one pAAm hydrogel disc equilibrated in a phosphate buffer solution (PBS) or utilize the bilayer system mentioned earlier to allow for an acidic interface with the EGaIn and a bio-friendly interface with the skin. The pAAm hydrogels used were equilibrated in PBS prior to testing to both achieve an ionic strength isotonic to the body and remove any unreacted monomer and initiator.
Figure 5.7 (a) 3D schematic of our ECG electrodes created using soft lithography. EGaIn is
injected after the PDMS layers are oxygen plasma treated and bonded. Hydrogel discs are
inserted prior to testing. (b) Photographs of the electrodes that show their extreme flexibility.
(c) Diagrams of two separate ways hydrogel discs can be used for testing. One hydrogel disc
equilibrated in PBS or a bilayer system may be used.
We first performed PEIS on electrodes placed 2 cm apart on the inner wrist of a participant. Hydrogel discs are placed in the opening of the PDMS and held to the wrist using a Velcro strap, in order to maintain good contact with the skin (supplemental). PEIS tests were performed in the same manner as earlier, a 10 mV signal from 100 kHz to 1 Hz.

PEIS testing was performed on two sets of prototype electrodes, a single PBS layer and acidic/PBS bilayer, as well as one set of commercial electrodes to obtain a comparison to current commercial ECG electrodes from Covidien Medi-Trace (Fig 5.8a). The electrodes all exhibit similar impedance at high frequencies, as this impedance is comprised predominately of the capacitance of the skin interface with the electrode. The impedances differentiate at low frequencies. The PBS gel electrode has a comparable 1 Hz impedance as the commercial electrode, while the bilayer gel electrode has an impedance almost one order of magnitude lower.

An ECG test was performed lastly to verify that these hydrogel-liquid metal electrodes would work in a live setting. A Bitalino ECG recording device was used to obtain the signal. A two electrode system was used with one electrode placed on the interior of each wrist. The resulting ECG waveforms were measured (Fig 5.8b).
Figure 5.8 (a) PEIS was performed for various electrodes on a human subject’s wrist. Two prototype electrodes were tested. PBS equilibrated hydrogels were used for one set of prototype electrodes. The other set utilized a bilayer setup where a PBS hydrogel interfaced the skin while an acrylic acid hydrogel interfaced the EGaIn. Commercial ECG electrodes were tested for comparison. (b) ECG’s were performed using these three sets of electrodes; a commercial electrode, a prototype electrode with a PBS gel and a bilayer gel system utilizing a PBS and acrylic acid gel. (c) A Fourier transform was performed on each ECG signal to obtain a power spectral density plot.

A fast Fourier transform (FFT) was performed on the resulting ECG signals to obtain a power spectral density map (Fig 5.8c). The signal to noise ratio (SNR) was calculated using equation 1. This equation represents the ratio of the power of the signal to the power of the noise in the system. A cutoff frequency of 50 Hz was selected, meaning lower frequency powers are designated as part of the signal and higher frequencies have been designated as
noise. These values were specifically calculated by taking the root mean square ($A_x$) of the signals from the FFT.

$$SNR = \frac{P_{signal}}{P_{noise}} = \left(\frac{A_{signal}}{A_{noise}}\right)^2$$ \hspace{1cm} (5.1)

The SNR values obtained for the commercial, PBS and bilayer electrodes were 29.6, 94.7 and 102.6 respectively. This indicates that our electrodes were able to provide a cleaner signal than the commercial electrodes. This can be qualitatively observed in the ECG readings as the prototype electrodes appear to have less noise.

5.4 Conclusion

We investigated the electrical properties of hydrogels and a liquid metal, EGaIn, for their use in ECG electrodes. We performed PEIS to obtain electrical characteristics of this system over a range of frequencies. The native oxide on EGaIn creates a capacitance that dominates the impedance at low frequencies, which is the range of interest for ECG measurements. However, acidic and basic species in the hydrogels removed the oxide and decreased the low frequency impedance. These hydrogels can be encased with a biologically neutral hydrogel that can safely interface the skin. Prototype electrodes were able to perform as well as, if not better than, current commercial electrodes. The ability to create truly soft and deformable electronics with impedances that can be tuned may allow for integration of these materials into future flexible and bendable wearable health monitoring devices.
5.5 Acknowledgements

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5.6 References


Chapter 6

Summary and Outlook for the Future of Wearable Sweat Sensing Devices

6.1 Summary

Interest in sweat sensing devices has grown dramatically over the past few years. Sweat is a desirable target body fluid as it is a readily available bio-fluid that contains a plethora of bioanalytes that can be correlated to health through non-invasive testing methods. Many research groups have created devices that utilize electrodes which are interfaced directly to the skin to perform continual biosensing. These technologies unfortunately do not provide a method of pumping and managing the sweat that has been released by the body, which can result in inaccuracies over time due to mixing of old and new sweat.

Our goal was to develop technologies that will help further the field of wearable sweat sensing devices. We created novel microfluidic platforms that can passively pump and manage fluids to enable accurate long-term sweat sensing. Battery life is a key component of any wearable electronic device. Therefore, the passive microfluidic pumping methods developed in this thesis work will allow biochemical sensing technologies to be implemented without cutting into the already strained available electricity sources for powering the device.

In Chapter 2 we described a novel osmotic microfluidic pump that we created through the use of doped hydrogels. We used a biomimetic osmotic pumping concept inspired by the natural processes leading to sweating in the eccrine sweat glands of humans. We used hydrogels doped with high levels of glycerol or sodium chloride to achieve pumping. Tests were performed on a diffusion cell using a dialysis membrane to mimic skin. In accordance
of the expectations of our model concept, the flow rate displayed correlations to solute concentration and hydrogel area, but not hydrogel thickness. We performed testing on both fluorometric and glucose sensing abilities of this system as a proof of principle for our osmotic pumping platform.

Chapter 3 introduced the concept of using paper microfluidics for long-term continual pumping. Paper microfluidics have been researched intensely in recent years. The low-cost fabrication methods and simplicity of use has made these paper microfluidic devices very appealing for point of care diagnostics. Up until now, all paper microfluidic devices have been designed to operate on a single use basis. The devices wick fluid in until the paper is saturated, after which testing is performed and the paper strip is discarded. We utilized evaporation off the back end of a paper strip as a means to enable long-term continual collection of fluid. We measured flowrate through visually tracking a dyed solution through the paper channel. We were able to control the flowrate achieved through careful design of the paper “channel” width and area of the evaporation pad on the back end of the channel. Salt accumulation from pumping isotonic solution slowly inhibited evaporation, but continual pumping could still be achieved for durations of up to 10 days. Both colorimetric and electrical sensing tests were performed on this platform.

In the research described in Chapter 4 we combined the previous passive microfluidic pumping mechanisms to create a sweat collection device that was tested on human subjects. The paper microfluidic strip was integrated into the microchannel of the PDMS hydrogel-based osmotic pump. The paper provides an interface that is able to freely wick away any sweat that
the body has naturally produced or sweat that has been drawn from the body by the osmotic hydrogel pump. The back end of this paper strip is left open to the environment to allow for evaporation to continually drive fluid flow. We first showed that these technologies were compatible by using the diffusion cell in a lab setting. Two sets of tests on human subjects were then performed to demonstrate that the proof of principle can be applied on real skin. In one test the skin on the inside of the forearm was dyed. The dye was allowed to penetrate into the sweat glands, and then all excess fluid was removed. Two microfluidic devices were placed on the subject’s body; one had a gel infused with glycerol while the other hydrogel was equilibrated in DI water. As expected, the glycerol doped hydrogel was able to draw sweat into the paper channel, as was observed by the blue dye. The DI hydrogel drew no sweat into the hydrogel, as there was no osmotic driving force to draw sweat from the body. In the second set of tests performed on human subjects, segments of the paper strip were cut out after use and the chloride concentration was measured using a commercial assay. These tests showed the presence of chloride in the pumped fluid, indicating that our device is capable of withdrawing and pumping sweat and the bioanalytes present in it.

This proof of principle device is likely to represent a major breakthrough in the field of wearable sweat sensing devices. Nearly every article published on sweat sensing devices required the subject to perform a strenuous physical activity, such as riding an exercise bike or running. Our devices have shown ability to collect sweat under periods of low sweat rate. Subjects tested in these studies were simply sitting at a desk during the tests, and yet our
devices were able to passively collect enough fluid to be visually observed or tested for chloride levels.

In Chapter 5 we show how our hydrogel devices can be used as a multimodal interface; sweat collection and ECG sensing. The ECG is one of the best ways to non-invasively monitor the heart’s health. The ECG is recorded by measuring the fluctuating electrical signals across the body resulting from heart beats. We aimed to make a flexible ECG electrode comprised of conductive hydrogels with a liquid metal (EGaIn). EGaIn is a non-toxic metal that is liquid at room temperature. Its unique properties make it ideal for applications in flexible and stretchable electronic devices.

We created a test setup where we were able to characterize both the electrical properties of the bulk hydrogel and EGaIn-hydrogel interface through PEIS. Hydrogel resistivity can be easily adjusted by modifying its electrolyte concentration. At low frequencies, the capacitive effects of the native oxide skin of the EGaIn resulted in larger impedances. These low frequencies impedances were decreased by introducing acidic and basic moieties in the hydrogel that removed the oxide skin. We then created prototype electrodes which were encased in PDMS. Electrodes were either designed to use one hydrogel disc that was equilibrated in PBS or a bilayer hydrogel system where a PBS hydrogel interfaced the skin while an acidic hydrogel interfaced the EGaIn. This bilayer system is able to reduce low frequency impedances caused by the EGaIn oxide skin, while also providing a biologically safe interface for the human body. Impedance tests were performed on human subjects for these prototype electrodes and compared to commercial electrodes. Both prototype electrodes
performed as well, if not better, than the commercial electrodes. Finally, we recorded an ECG from human subjects with these three sets of electrodes. All electrodes provided a clean ECG signal. Analysis was performed to determine the signal to noise ratio of each set of electrodes, and it was found that both of the prototype hydrogel electrodes had better signal to noise ratio than the commercial electrodes.

6.2 Outlook

This thesis research has introduced new and exciting technologies that may enable non-invasive continual sweat monitoring. However, these ideas have only been demonstrated on a proof of principle level so far. Further work will be required to quantitatively understand how these devices work on real human subjects. This will entail a larger and more thorough design of experiment for tests performed on human subjects. Flowrate will need to be calculated for all trials and correlated to the hydration levels of subjects, which can be obtained with various commercial hydration monitors. Hydration level may affect the rate at which we can draw sweat from the body. The fluid collected can be tested for the presence of various bioanalytes (glucose, lactate, chloride) in order to obtain more knowledge on how our microfluidic platform operates on human subjects.

We will begin to integrate embedded electrodes into the microfluidic channels which will enable continuous biochemical sensing. One method to achieve this goal will be to screen print electrodes onto a flexible substrate and then interface that substrate to the microfluidic channel. Preliminary designs have been created and are displayed in Fig 6.1. These electrodes can be screen-printed easily with a graphene paste. Silver/silver chloride paste can be applied
to the reference electrode and enzymes can be immobilized on the working electrode for enzymatic sensing modalities (glucose and lactate).

Figure 6.1 (a) Schematic of how we plan to screen print a 3-electrode system to interface to our paper microfluidic strip. Each electrode can be modified to serve the purpose of acting as the working, counter or reference electrode. (b) Photograph of a prototype of this design as fabricated.

Future work could also entail combining the principles of the osmotic hydrogel pump with other materials for skin interfacing and sensing, such as microneedle arrays. Microneedles are arrays of micron sized needles that have been used recently in the field of drug delivery.1 Microneedles are an appealing technology as they are able to penetrate through the stratum corneum, the top-most layer of dead skin cells, and into the interstitial fluid below
Due to their small size and low penetrating depth, they do not cause pain to the user.

Most current uses of microneedles are predominantly in the areas of drug and vaccine delivery, which can be performed in multiple ways. Microneedles can be briefly applied to the skin to puncture the stratum corneum and then removed. A cream or solution containing a specific drug can then be applied to the skin for transdermal injection. Other methods combine the desired drug with the microneedles for a single step application. Microneedles can be coated, which allows for direct injection upon application. Microneedles can be created out of a dissolvable material, which may have the drug or vaccine embedded. This drug or vaccine is then delivered to the body over time as the microneedles dissolve. Hollow microneedles have been fabricated, from which a fluid can be injected into the body.2,3

These applications all involve injecting a material into the body, but recent work has been focused on using microneedle technology for the purpose of biosensing and fluid extraction. In many research efforts the microneedles can be used directly as biosensing electrodes. The microneedles can be modified to serve as the working, counter and/or reference electrode in a 3-electrode setup. This allows for biochemical sensing to be performed directly on the interstitial fluid.

Recent work has seen microneedles used to draw interstitial fluid into a device through capillary action, at which time glucose sensing is performed.4 Hydrophilic microneedles create a large capillary pressure, which can easily draw interstitial fluid from the body. One long-
term issue for these devices, similar to the challenges addressed earlier with paper microfluidics, is that pumping stops after the microneedles have been completely filled.

Figure 6.2 (a) Schematic showing how microneedles are able to penetrate through the stratum Corneum and into the viable epidermis. This region is where the microneedles are able to interface and collect interstitial fluid. (b) We propose interfacing a microneedle array with our hydrogel-paper sweat collection platform to enable better collection of interstitial fluid.

A future extension of the work presented in this thesis could combine microneedle technologies with our passive pumping platforms. The osmotic pressure created from our doped hydrogels is much greater than the capillary pressure of the fluid in the microneedles.
We aim to utilize this pumping mechanism to draw the collected interstitial fluid from the microneedles and pass it along a microfluidic network where biosensing can be performed. The evaporative paper pump can also be implemented on the back end of this system to enable long-term fluid collection. We describe this proposed system in Fig 6.2b.

We believe the technologies introduced here will have a large impact on the future of wearable non-invasive biosensing devices. We have presented devices on a proof of principle level that perform passive sweat collection and management. This system will be studied further and integrated with embedded biosensors to create a continual sweat sensing platform. We will also integrate passive pumping mechanisms with microneedle technology to create an alternate approach that may enable non-invasive biosensing. Non-invasive continual biochemical sensing is a field of untapped potential. Accurate biosensing will enable patients to more closely monitor serious medical conditions. One potential future application could be the combination of wearable biosensors with insulin pumps that can operate under a closed loop control system. This would allow for blood glucose to be both monitored and regulated using less invasive means that currently available. This closed loop system may also be used to obtain better adherence in patients who may have issues administering medication to themselves. These devices will ultimately help patients monitor their own bodies, which will result in better health for the individual.

6.3 Acknowledgements

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6.4 References


