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Flow Valve Diagnostics for Label-Free, Quantitative Biomarker Detection: Device Fabrication, Surface Modification, and Testing

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A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

Flow Valve Diagnostics for Label-Free, Quantitative
Biomarker Detection: Device Fabrication,
Surface Modification, and Testing

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Diseases are often diagnosed by detection of disease-specific biomarkers in fluid samples. However, many state-of-the-art detection methods require a lab with complex machinery, trained operators, and/or lengthy analysis time. In contrast, point-of-care (POC) devices are brought to the patient's location, they are easy to use, and results are obtained almost immediately. Many current POC devices are too difficult to be used without a skilled assistant, and although many are able to detect analytes above a threshold value, they give little or no quantitative information. This work presents the development of polymer-based microfluidic devices capable of sensing and quantifying biomarkers in fluid samples in a straightforward manner using a novel biomarker assay termed "flow valve diagnostics". In this assay, an antibody-modified polydimethylsiloxane (PDMS) microchannel constricts due to the binding force between antibodies and antigens, stopping fluid flow. The flow distance is measured and correlated to antigen concentration. This detection method is an improvement over other methods because it is an innovative, non-instrumented, label-free, easy-to-use approach. These devices are small, portable, disposable, inexpensive, and thus ideal for use in POC testing.

I have successfully fabricated flow valve devices with standard micromachining techniques, including photolithography, replica molding with PDMS, and plasma oxidation. Following fabrication, I compared two methods for attaching receptor biomolecules (e.g., antibodies) to the microchannel surfaces: non-specific adsorption and silanization with 3-glycidoxytrimethoxypropylsilane (GOPS). I used laser-induced fluorescence to determine that silanization with GOPS was the better method for biomolecule attachment. Finally, I tested antibody-modified flow valve devices with target antigens to determine if the antibody/antigen binding force was strong enough to cause channel pinching and flow stoppage. By modifying the device design and using higher antigen concentrations, I was able to show that flow valve devices can detect antigens in a concentration-dependent manner. Future work to improve the device design and to modify and test these devices with different receptor/target pairs will bring flow valve diagnostics closer to becoming a valuable asset in biomarker detection and POC testing.

Keywords: biomarker detection, point-of-care testing, label-free, quantitative, PDMS

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1. INTRODUCTION

1.1 Motivation

In the past 20 years, the high interest in microfluidics is a clear indication that it is indeed a small world after all. Microfluidics deals with the precise control of fluid samples at the sub-millimeter scale. Separation scientists developed microfluidics to improve the analytical performance of methods such as chromatography through miniaturization. This led to the idea of a "miniaturized total analysis system" (μ-TAS) or "lab-on-a-chip,"[1] a device capable of integrating sample preparation, handling, analysis, and detection.

Microfluidics has several advantages when compared with traditional analysis methods. First, the micromachining techniques used to make microfluidic devices come from the semiconductor industry. Because of this, microfluidic devices share many of the same desirable qualities as electronic microchips, including small size, high speed, low cost, scalability, and portability. Second, by nature of their size, microfluidic devices require only small sample volumes. This is a distinct benefit when dealing with limited sample volumes or samples containing dangerous chemicals. The small size of microfluidic devices also leads to lower reagent consumption and thus lower cost. Finally, a microfluidic device has the potential to reduce human error and contamination through integration and automation of sample preparation and handling.

1.2 History

The first use of micromachining for miniaturization and integration with electronics was a gas chromatograph with an injector and thermal conductivity detector integrated on a 5-cm diameter silicon chip.[2] Although this device was published in 1979, silicon-based miniaturization of analytical methods did not receive much attention until the late 1980s and early 1990s. In 1990, a miniaturized open-tubular liquid chromatograph with a conductometric detector on a 5 × 5 mm silicon wafer sparked new interest in creating complete analytical systems on a single, small chip.[3] Although this liquid chromatograph was not functional, the theoretical separation efficiency was 8,000 and 25,000 plates in 1 and 5 minutes, respectively. These efficiencies were calculated from the physical dimensions of a channel (such as internal and external column diameter and the thickness between channel walls) and not actual experimental data, however, this work showed that miniaturization of such a device was both feasible and could give acceptable separation efficiency in a minimal amount of time.

Many of the first μ-TAS setups utilized capillary electrophoresis (CE) to separate mixtures.[4-8] This advance revolutionized the field of microfluidics and made it a popular platform for research as it allowed for rapid analyte movement and separation without valves or pumps. One of the first examples of a μ-TAS capable of rapid analysis was a planar chip that used CE to separate amino acids in 15 seconds with a separation efficiency of 75,000 theoretical plates and the same separation in less than 4 seconds with a separation efficiency of 600 theoretical plates.[4]

The materials used to make microfluidic devices have changed as the field has developed. Early microfluidic devices were fabricated with materials familiar to the semiconductor industry, namely silicon and glass. Glass was a more popular substrate for microfluidic devices because of its electrical and optical properties. The chemical makeup of glass allows for the application of high separation voltages, and because glass is transparent it can be used with UV-visible optical detection methods. In recent years polymers have gained prevalence as a material for microfluidic devices, especially because polymers are inexpensive and because polymer-based microfluidic devices are relatively easy to produce.[9] Polymers commonly used to make microfluidic devices include polymethylmethacrylate (PMMA) and polydimethylsiloxane (PDMS).

1.3 Biomarker Detection and Point-of-Care Testing

As the field of microfluidics became more popular it started to gain the interest of not only electrical engineers and analytical chemists, but also biologists and biochemists. Microfluidics showed great promise in biology and biochemistry because of its ability to precisely separate and control reagents, allowing for careful monitoring of complex biological systems. Publications soon began to appear using a microfluidic platform for biological, clinical, and medical applications, such as genetic analysis[10] and clinical diagnostics.[11]

In order to simplify clinical diagnostics and make them more accessible, researchers started to design microfluidic devices capable of sensing biomarkers. A biomarker is a substance produced by an organism that can indicate a disease state or

its physiological condition. Thus, measuring the levels of specific biomarkers can help clinicians diagnose, monitor, and treat illness. Traditional methods for biomarker analysis include enzyme-linked immunosorbent assays (ELISAs),[12] microarrays,[13] electrochemical methods,[14] and mass spectrometry,[15] but not all are particularly suited for miniaturization and simplification. Most of these methods must be carried out in a clinical or research laboratory by skilled technicians. These procedures may also be expensive, call for complex machinery, or require a long wait between the time of the test and receiving results.

In contrast, point-of-care (POC) testing is a type of clinical diagnostics that brings biomarker testing to the patient's location and can provide immediate, straightforward information about an illness. Additional advantages include low cost, disposability, potential for multiplexing, and low sample and reagent consumption. These characteristics make POC testing an attractive option for at-home self-diagnosis, humanitarian efforts in developing countries, or when rapid and/or frequent testing is necessary. Because POC testing shares many of the same qualities as microfluidic testing, microfluidics is an ideal system for POC testing.

1.4 Current Directions in POC Testing

According to the World Health Organization, the ideal microfluidic POC device would be ASSURED: Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users.[16] Much of the current research in POC testing is focused on developing devices with most or all of these attributes. Two areas

of POC testing that follow the ASSURED model and are especially relevant to this thesis are lateral flow tests (a.k.a. immunochromatographic tests) and microfluidic paper-based analytical devices (μPADs).

Lateral flow tests are simple immunoassays (see Section 1.8.1) that are well-suited for POC testing. In a lateral flow test, sample is applied to a solid dipstick and flows by capillary action along a nitrocellulose strip. As the sample flows, it mixes with a colored reagent (e.g., gold nanoparticles[17, 18]) conjugated to antibodies specific to the target analyte. Further along the strip is a test region containing a different antibody specific to the same target. If the antigen of interest is present in the sample, it will bind at the test region, producing a colored line. Presence of a second colored line called the control line indicates that the test worked properly. The best and most well-known example of a lateral flow test is the home pregnancy test.[17] Lateral flow tests have also been used to detect infectious agents, metabolic disorders, drugs, and toxic compounds.[19]

μPADs are POC devices made of patterned paper[20] and were developed to be inexpensive, diagnostic tests for use in developing countries. μPADs are made by defining hydrophilic channels and hydrophobic barriers on chromatography paper with photolithography (see Section 1.5.1). In an assay, sample travels up the channels and into test zones containing assay reagents, leading to a color change (colorimetric assay) or an electrochemical response (electrochemical assay) that is associated with the concentration of the analyte. In colorimetric assays, the μPAD may be photographed or scanned and sent to an off-site laboratory where trained personnel can analyze the

image and recommend further action.[21] Thus far, μPADs based on colorimetric assays have been used for analysis of glucose, proteins, pH, and alkaline phosphatase.[21-24] μPADs based on electrochemical detection have been used for analysis of heavy metal ions, glucose, lactate, and uric acid.[25, 26]

Lateral flow tests and μPADs are good platforms for POC testing due to their simplicity, ease-of-use, portability, speed, low cost, and lack of instrumentation. However, these methods would be more useful if they provided accurate quantitative information about biomarkers. Although qualitative information about biomarkers may be sufficient in some cases, quantitative information gives experts a better idea about the state and severity of a disease, giving patients the best chance to receive the most beneficial and timely treatment.[27] Generally, lateral flow tests give only qualitative information, indicating whether or not a target is present above a threshold level. μPADs can give quantitative information, but accurate quantification depends on consistent image lighting, expert examination, and increased analysis time. So although lateral flow tests and μPADs are very useful, an assay that follows the ASSURED model and gives quantitative information about biomarkers would be a great asset to POC testing.

1.5 Micromachining

Many of the micromachining techniques used to fabricate microfluidic devices have origins in the semiconductor industry. These techniques allow for excellent control over micrometer-sized features and lead to reproducible devices. Because a general

knowledge of these techniques is useful for understanding my work, I will discuss some of the relevant techniques. For more detailed information on micromachining, please refer to the book *Fundamentals of Microfabrication: The Science of Miniaturization*.[28]

1.5.1 Photolithography

Photolithography is one of the most important micromachining techniques due to its many applications and utility across several scientific disciplines, including microfluidics. Photolithography is a process that uses light to transfer a design from a pattern of transmissive and non-transmissive features, called a photomask, to a light-sensitive chemical known as a photoresist. This is often one of the first steps in producing a microfluidic device. Different photolithographic methods and different photoresists may be used depending on the desired feature type, size, and resolution, the choice of substrate for pattern transfer, or to enable integration with subsequent separation and detection methods.

The first step in transferring a pattern from a photomask to a photoresist is heating a substrate to remove water or other solvents from the surface (Figure 1.1a). At this point, an adhesion promoter or primer may be evaporated or spun onto the substrate to ensure better interaction between the wafer and the photoresist (Figure 1.1b). A thin, uniform layer of photoresist is deposited on the substrate by spin-coating at several thousand revolutions per minute (Figure 1.1c). This is followed by heating the substrate to evaporate excess solvent in the photoresist (Figure 1.1d). In the next step, the photoresist is exposed to UV light through a photomask (Figure 1.1e). The

photomask protects some regions of the photoresist from UV exposure while other regions undergo photochemical reactions. After exposure, the substrate is immersed in a developing solution. Depending on the type of photoresist, the developer will dissolve either the areas exposed to UV radiation or the areas protected from UV radiation. Last, the substrate is heated again to further improve photoresist adhesion and to make the photoresist more robust (Figure 1.1f).

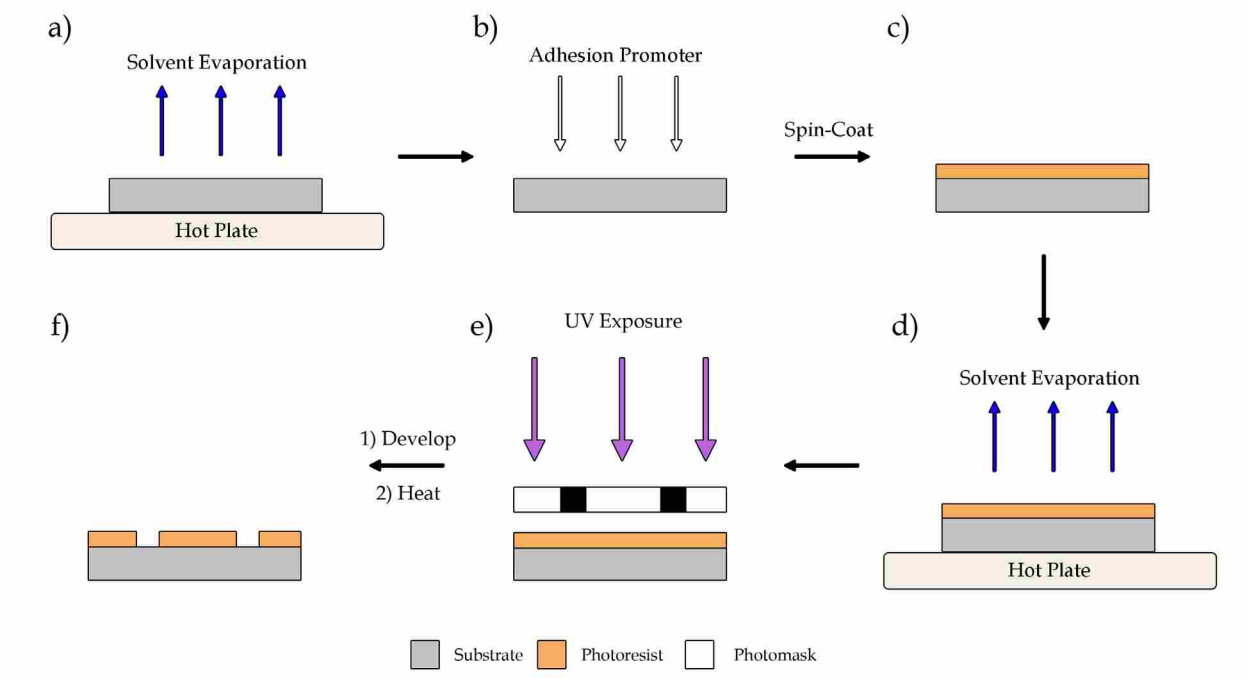

Figure 1.1 – Patterning with photolithography.
Photolithography is a technique that is used to transfer patterns from a photomask to a photoresist. The basic steps of pattern transfer are a) solvent evaporation, b) adhesion promoter deposition, c) photoresist deposition, d) a second solvent evaporation, e) exposure to UV radiation through a photomask, and f) development followed by heating to generate a photolithographic pattern.

As mentioned above, the type of photoresist dictates which parts of the photoresist dissolve in developing solution. The two general categories of photoresist

are called positive and negative, but as only positive photoresists were used in this thesis I will only discuss the former (Figure 1.2). When positive photoresists are exposed to UV light, the main or side polymer chains in the unprotected areas of the resist break apart, causing the exposed areas to become much more soluble in the developer. When positive resists are developed, the photoresist pattern left on the wafer matches the pattern on the photomask.

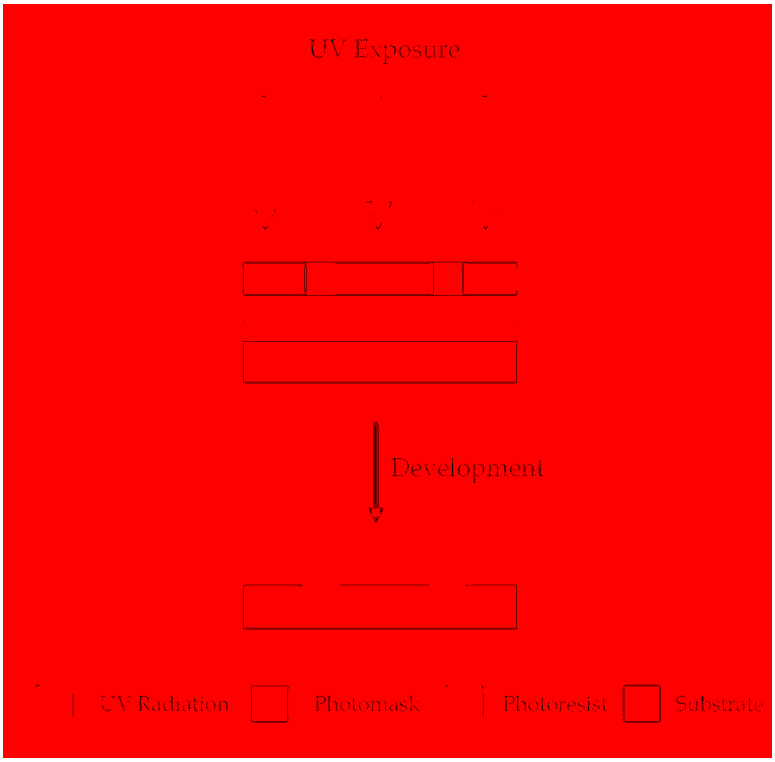

Figure 1.2 – Behavior of positive photoresists.
After UV exposure through a photomask and development, positive photoresists leave a raised image identical to the pattern on the photomask.

1.5.2 Soft Lithography: Replica Molding

Soft lithography refers to a collection of non-photolithographic techniques capable of generating micrometer- and even nanometer-sized features.[29] These techniques are called "soft lithography" because they rely on elastomeric organic

stamps or molds rather than rigid inorganic materials (i.e., silicon) for pattern transfer and structure fabrication. One such technique is replica molding (Figure 1.3). Replica molding is a method for duplicating the structure, features, or morphology of a master mold. Replication of a master mold is straightforward, consistent, and inexpensive, and duplicates the copy the master mold with nanometer (<100 nm) resolution.[29]

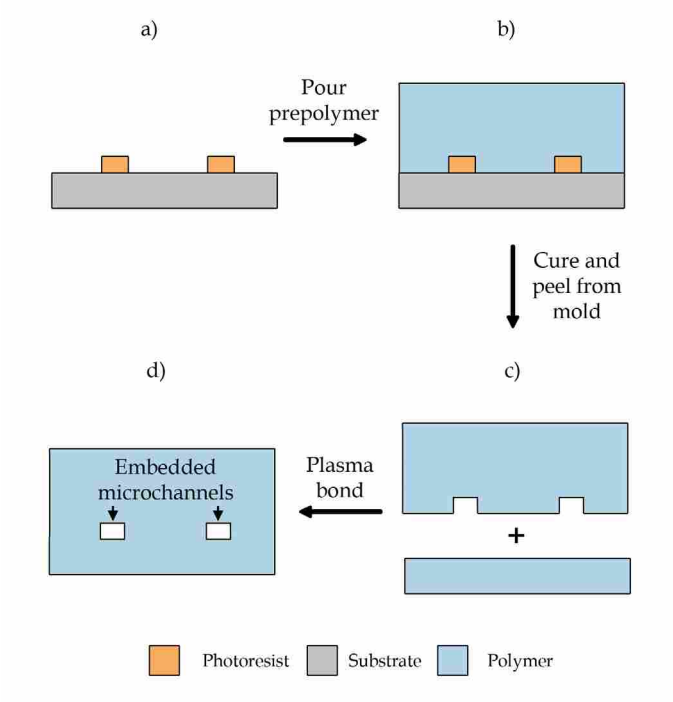

Figure 1.3 – Replica molding.
A soft lithographic method for creating duplicates of a master mold. (a) A master mold is fabricated. (b) Prepolymer is poured over the master mold and cured to harden the polymer. (c) The duplicate is peeled off the master mold. (d) The duplicate is bonded to a polymer slab to form embedded microchannels.

Replica molding begins with the creation of a master mold by standard photolithography (Figure 1.3a). Until it breaks or its features degrade, the master mold may be used repeatedly to produce new duplicates. Next, pre-polymer is poured on the master mold and the pre-polymer is cured (Figure 1.3b). Finally, the polymer is peeled

off the master mold (Figure 1.3c) and the duplicate may be bonded with a polymer slab to create a microfluidic device with embedded microchannels (Figure 1.3d).

Due to several desirable properties, PDMS (Figure 1.4) is a polymer commonly used for replica molding. As an elastomer, PDMS can replicate micrometer and sub-micrometer sized features. For the most part, it is chemically inert,[30] and it is optically transparent down to ~300 nm,[29] making PDMS devices compatible with detection schemes such as laser-induced fluorescence. Last, PDMS is inexpensive and durable.

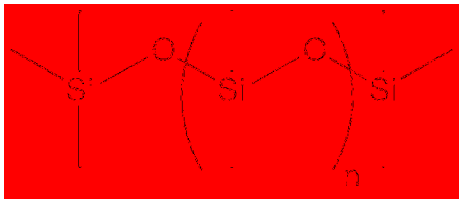

Figure 1.4 – Structure of polydimethylsiloxane (PDMS).
PDMS is a silicon-based polymer popular for fabrication of microfluidic devices.

1.6 PDMS Surface Modification

Although PDMS offers some advantages over silicon and glass for the fabrication of microfluidic devices, PDMS often requires modification to fit the specific needs of the experiment. There are many methods for PDMS surface modification, but I will focus on the two methods I use when making devices: plasma oxidation and silanization.

1.6.1 Plasma Oxidation

Plasma oxidation or activation is an easy method for changing the surface chemistry of PDMS. Without modification, PDMS is chemically inert and hydrophobic. These characteristics make it difficult to use PDMS for CE or with aqueous samples.

Exposure to O_2 plasma oxidizes PDMS and creates silanol groups on its surface,[31, 32] making PDMS hydrophilic and capable of forming the electric double layer necessary for stable electro-osmotic flow.[33] However, the hydrophilicity is temporary, and if oxidized PDMS is left exposed to air the polymer chains will migrate to the surface and the polymer will revert to its original hydrophobic state.[34] This reversion can be slowed if PDMS is immersed in water immediately after oxidation and if it remains stored underwater, PDMS can remain hydrophilic for months.[34, 35]

Plasma activation also provides a simple method for irreversibly bonding PDMS to glass or to itself. Plasma activation forms siloxane radicals on the surface of PDMS, and if it is brought into conformal contact with another piece of activated PDMS or glass, covalent siloxane bonds are formed.[33] This an easy method for forming embedded, hydrophilic microfluidic channels.

1.6.2 Silanization

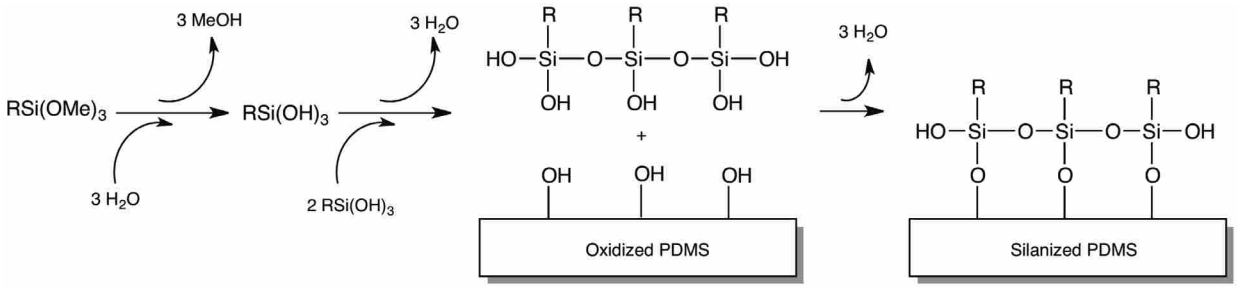

Figure 1.5 – Silanization of PDMS.
A surface modification method for forming self-assembled monolayers on PDMS. The silane R-group may be chosen to give PDMS a specific chemical functionality.

Silanization is a method for forming self-assembled monolayers on silicon-based surfaces such as PDMS (Figure 1.5). To silanize PDMS, the polymer is first plasma

oxidized to generate silanol groups on the polymer surface. After oxidation, the PDMS is exposed to the chosen silane, and the silanol groups react with the silane to create new siloxane bonds and form the self-assembled monolayer. The silane may have a specific terminal –R group to confer the desired chemical functionality to PDMS.

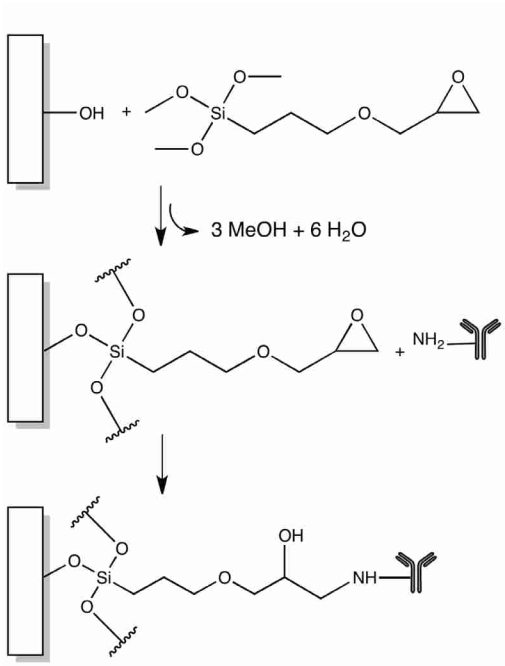

Figure 1.6 – Antibody attachment with 3-glycidoxypropyltrimethoxysilane (GOPS).
GOPS-silanized PDMS may form a covalent attachment with an antibody when the epoxy end group reacts with amine groups on the antibody.

The chemical functionality of the silane end group makes it possible to conjugate biomolecules to PDMS. Of particular interest to this thesis is the ability to functionalize PDMS with antibodies. One readily available silane that can react with antibodies is 3-glycidoxypropyltrimethoxysilane (GOPS) (Figure 1.6). GOPS has an epoxy end group that can react with the amine side chain of the amino acid lysine to form a covalent attachment.

Although GOPS silanization is convenient method for attaching antibodies to PDMS, this method has its drawbacks. Water is required to facilitate bonding between GOPS and oxidized PDMS. However, water may also react with the epoxy end group on GOPS and destroy its ability to bond with amine groups on an antibody, defeating the purpose of the silanization. To alleviate this problem, I used fresh GOPS to silanize PDMS and reacted the silanized PDMS with antibody immediately after silanization (see Section 2.3).

1.7 Detection Schemes

The most important aspect of a diagnostic POC device is its ability to detect and quantify the biomarkers of interest. This can be particularly difficult when working with small sample volumes and trying to detect biomarkers only present in limited amounts. There are currently many methods for detecting biomarkers, but not all of them are easily integrated with sample preparation and analysis as would be necessary for a μ-TAS, nor are all of them suitable for POC testing. Therefore, a new detection scheme that meets the requirements of a POC μ-TAS would be a great addition to the field.

1.7.1 Electrochemical Detection

Electrochemical detectors operate by monitoring the resistance of a solution or current from an electrochemical process in a solution and are respectively known as conductometric or amperometric detectors.[36] Conductometric detectors work because the resistance of a solution of buffer is often different from the resistance of a solution

with both buffer and analyte. In an amperometric detector, an applied potential forces the analyte to undergo a redox reaction, producing a current that is related to the analyte concentration.

One of the earliest examples of electrochemical detection on a POC device was a blood oxygen monitor developed by Clark and Lyons in 1962.[37] This work used electrodes to monitor blood oxygen, carbon dioxide, and pH. Clark and Lyons also suggested the possibility of combining an electrode with an enzyme-containing membrane to additionally monitor blood glucose concentration, which would work as follows: (1) The enzyme glucose oxidase catalyzes the oxidation of glucose to gluconic acid. (2) Electrons from glucose are transferred to an oxidizing agent, which in turn transfers these electrons to an electrode, producing a current proportional to the amount of glucose in the blood. This idea led to the development of modern blood glucose monitoring systems, one of the most successful and widely used POC devices today.

1.7.2 Laser-Induced Fluorescence

Laser-induced fluorescence (LIF) is one of the most sensitive analytical detection techniques. In this method, a laser excites fluorescent molecules from an electronic ground state to a high-energy excited state. After a short time (10^{-5} to 10^{-9} s), the molecules return to the ground state and emit light with a longer wavelength than the laser.[38] The emitted photons are detected by a photomultiplier tube (PMT) or a charge-coupled device (CCD) and converted to an electrical signal.

To achieve maximum sensitivity with LIF, several factors must be considered. First, background emission from sources other than the analyte must be eliminated or minimized. These sources include scattered laser light, fluorescence from the buffer and the device, Rayleigh scattering, and Raman scattering. Minimization of background emission may be achieved with optical filters and irises.

Like any detection method, LIF has both advantages and disadvantages. LIF is highly sensitive and can detect single particles.[39] It is also a useful method for visually monitoring an analyte's location in and progress through a device. A disadvantage of LIF is the need for large, delicate, and expensive hardware. In addition, analytes may need to be labeled before they can be detected, and signal may decrease over time due to photobleaching.

1.8 Biomarker Assays

A biomarker assay is a complete analytical procedure for assessing the presence and/or the amount of biomarker present in a sample. Traditional biomarker assays such as immunoassays are specific and sensitive, but do not translate easily into a POC device. In this section I will introduce a new biomarker assay that I have helped develop called "flow valve diagnostics." This assay is both quantitative and fit for POC applications.

1.8.1 Immunoassays

An immunoassay is a biochemical test that detects the presence of or measures the concentration of specific analytes in a sample using antibody/antigen interactions.

In these tests, binding of an antigen and an antibody leads to a detectable response, often a color change, which indicates the presence and amount of analyte. Depending on the type of immunoassay, either the antigen or the antibody may be the analyte of interest. The sensitivity of an immunoassay depends on specific binding between the antibody and the antigen without interference from other compounds in the sample. Some examples of immunoassays are enzyme-linked immunosorbent assays (ELISAs) and lateral flow tests (see Section 1.4).

The sandwich ELISA method detects antigen in a sample by using two monoclonal antibodies. A primary antibody is adsorbed to the bottom of a well of a microtiter plate. Sample is added to the well. If the sample contains the antigen specific to the primary antibody, the antigen will bind. A second, enzyme-linked antibody is added to the well. This secondary antibody binds to a different epitope of the antigen of interest, and thus "sandwiches" the antigen between the two antibodies. After unbound secondary antibody is removed by washing, the enzyme activator is added to the well, which causes the solution to change color. The rate of color formation is proportional to the amount of antigen in the sample. These tests can detect less than a nanogram of analyte.[40]

The ELISA is one of the most sensitive and specific biomarker assays, but it is not very compatible with POC testing. First, ELISAs are comprised of several steps, making them time-consuming and laborious. Second, they are only cost-effective if performed on many samples at once (e.g., 96 samples in a microtiter plate). Last, trained personnel

are required to perform the assay. Until these disadvantages are overcome, the ELISA will remain a lab-bound technique.

1.8.2 Flow Valve Diagnostics

The Woolley laboratory has developed a novel biomarker assay termed "flow valve diagnostics" that is well suited to POC testing (Figure 1.7). In this new method, a flexible PDMS microchannel coated with receptor molecules (Figure 1.7a) acts as a self-constricting valve due to binding of receptor and target molecules (e.g., antibodies and antigens). When a sample containing target molecules flows through a receptor-coated PDMS microchannel, the binding force between target and receptor causes the microchannel to constrict and prevent fluid from flowing further in the channel (Figure 1.7b). This flow stoppage can be detected by the naked eye because the refractive

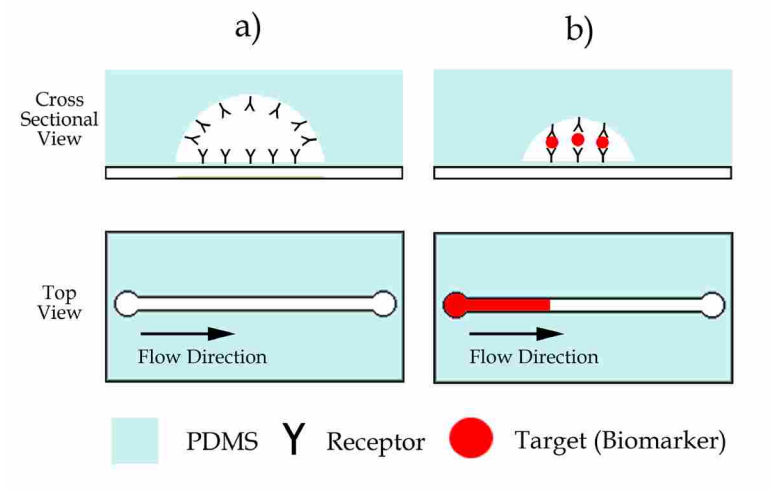

Figure 1.7 – Flow valve diagnostics.
A receptor-functionalized polymer microchip capable of detecting the presence of biomarker in a label-free manner. Features are not to scale and have been exaggerated to show detail. a) Sample containing no target flows freely through the channel. b) Sample containing biomarker results in target/receptor binding, causing the flexible polymer to constrict and stop fluid flow.

indices of air in an empty microchannel and fluid in a filled microchannel are sufficiently different that it is easy to distinguish the empty portion of a microchannel from the filled portion of a microchannel.

One of the great advantages of flow valve diagnostics is that it is a quantitative POC technique. Preliminary work done by Debolina Chatterjee using the model target/receptor system of streptavidin-biotinylated-bovine serum albumin (BSA) indicates that there is a linear relationship between flow distance (i.e., how far the sample flowed in the microchannel before valve constriction prevented further flow) and $\log_{10}$[streptavidin].[41] Therefore, the flow distance can be measured and used to ascertain the target concentration in a sample. As there are few simple, inexpensive, and quantitative POC methods, flow valve diagnostics has the potential to meet a specific need in the realm of POC testing.

To make flow valve devices generally applicable, I would like to use disease-specific antigens and their corresponding antibodies. Consequently, the focus of this thesis is modifying PDMS microchannels with antibodies and performing flow valve assays with antigen solutions.

1.9 Thesis Overview

In this thesis I will discuss developing flow valve devices by modifying the surface chemistry of PDMS to allow for the attachment of biomolecules such as antibodies. Chapter 2 introduces the device fabrication and surface modification processes. Chapter 3 presents testing of antibody-modified flow valve devices. Last,

Chapter 4 discusses conclusions that may be drawn from my work and future directions for this research.

1.10 References

(1) Manz, A.; Graber, N.; Widmer, H. Miniaturized Total Chemical-Analysis Systems - a Novel Concept for Chemical Sensing. *Sens. Actuator B-Chem.* **1990,** *1*, 244-248.

(2) Terry, S.; Jerman, J.; Angell, J. Gas-Chromatographic Air Analyzer Fabricated on a Silicon-Wafer. *IEEE Trans. Electron Devices* **1979,** *26*, 1880-1886.

(3) Manz, A.; Miyahara, Y.; Miura, J.; Watanabe, Y.; Miyagi, H.; Sato, K. Design of an Open-Tubular Column Liquid Chromatograph using Silicon Chip Technology. *Sens. Actuator B-Chem.* **1990,** *1*, 249-255.

(4) Harrison, D.; Fluri, K.; Seiler, K.; Fan, Z.; Effenhauser, C.; Manz, A. Micromachining a Miniaturized Capillary Electrophoresis-Based Chemical-Analysis System on a Chip. *Science* **1993,** *261*, 895-897.

(5) Harrison, D. J.; Fan, Z. H.; Seiler, K.; Manz, A.; Widmer, H. M. Rapid Separation of Fluorescein Derivatives using a Micromachined Capillary Electrophoresis System. *Anal. Chim. Acta* **1993,** *283*, 361-366.

(6) Manz, A.; Harrison, D.; Verpoorte, E.; Fettinger, J.; Paulus, A.; Ludi, H.; Widmer, H. Planar Chips Technology for Miniaturization and Integration of Separation Techniques into Monitoring Systems - Capillary Electrophoresis on a Chip. *J. Chromatogr.* **1992,** *593*, 253-258.

(7) Manz, A.; Fettinger, J. C.; Verpoorte, E.; Ludi, H.; Widmer, H. M.; Harrison, D. J. Micromachining of Monocrystalline Silicon and Glass for Chemical-Analysis

Systems - a Look into Next Century Technology Or just a Fashionable Craze. *Trac-Trends in Analytical Chemistry* **1991,** *10*, 144-149.

(8) Seiler, K.; Harrison, D. J.; Manz, A. Planar Glass Chips for Capillary Electrophoresis - Repetitive Sample Injection, Quantitation, and Separation Efficiency. *Anal. Chem.* **1993,** *65*, 1481-1488.

(9) Becker, H.; Gaertner, C. Polymer microfabrication technologies for microfluidic systems. *Anal. Bioanal. Chem.* **2008,** *390*, 89-111.

(10) Mastrangelo, C.; Burns, M.; Burke, D. Microfabricated devices for genetic diagnostics. *Proc IEEE* **1998,** *86*, 1769-1787.

(11) Schulte, T.; Bardell, R.; Weigl, B. Microfluidic technologies in clinical diagnostics. *Clin. Chim. Acta* **2002,** *321*, 1-10.

(12) Humpel, C. Identifying and validating biomarkers for Alzheimer's disease. *Trends Biotechnol.* **2011,** *29*, 26-32.

(13) Ray, S.; Reddy, P. J.; Jain, R.; Gollapalli, K.; Moiyadi, A.; Srivastava, S. Proteomic technologies for the identification of disease biomarkers in serum: Advances and challenges ahead. *Proteomics* **2011,** *11*, 2139-2161.

(14) Li, T.; Yang, M. Electrochemical sensor utilizing ferrocene loaded porous polyelectrolyte nanoparticles as label for the detection of protein biomarker IL-6. *Sens. Actuator B-Chem.* **2011,** *158*, 361-365.

(15) Cazares, L. H.; Troyer, D. A.; Wang, B.; Drake, R. R.; Semmes, O. J. MALDI tissue imaging: from biomarker discovery to clinical applications. *Anal. Bioanal. Chem.* **2011,** *401*, 17-27.

(16) Peeling, R. W.; Holmes, K. K.; Mabey, D.; Ronald, A. Rapid tests for sexually transmitted infections (STIs): the way forward. *Sex. Transm. Infect.* **2006,** *82*, V1-V6.

(17) Leuvering, J.; Thal, P.; Waart, M.; Schuurs, A. Sol Particle Agglutination Immunoassay for Human Chorionic-Gonadotropin. *Fresenius Zeitschrift Fur Analytische Chemie* **1980,** *301*, 132-132.

(18) Nielsen, K.; Yu, W. L.; Kelly, L.; Bermudez, R.; Renteria, T.; Dajer, A.; Gutierrez, E.; Williams, J.; Algire, J.; de Eschaide, S. T. Development of a lateral flow assay for rapid detection of bovine antibody to Anaplasma marginale. *J. Immunoassay Immunochem.* **2008,** *29*, 10-18.

(19) Posthuma-Trumpie, G. A.; Korf, J.; van Amerongen, A. Lateral flow (immuno) assay: its strengths, weaknesses, opportunities and threats. A literature survey. *Anal. Bioanal. Chem.* **2009,** *393*, 569-582.

(20) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E. Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices. *Anal. Chem.* **2010,** *82*, 3-10.

(21) Martinez, A. W.; Phillips, S. T.; Carrilho, E.; Thomas, S. W., III; Sindi, H.; Whitesides, G. M. Simple telemedicine for developing regions: Camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. *Anal. Chem.* **2008,** *80*, 3699-3707.

(22) Abe, K.; Suzuki, K.; Citterio, D. Inkjet-printed microfluidic multianalyte chemical sensing paper. *Anal. Chem.* **2008,** *80*, 6928-6934.

(23) Li, X.; Tian, J.; Nguyen, T.; Shen, W. Paper-Based Microfluidic Devices by Plasma Treatment. *Anal. Chem.* **2008,** *80*, 9131-9134.

(24) Martinez, A. W.; Phillips, S. T.; Butte, M. J.; Whitesides, G. M. Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angew. Chem. -Int. Edit.* **2007,** *46*, 1318-1320.

(25) Nie, Z.; Nijhuis, C. A.; Gong, J.; Chen, X.; Kumachev, A.; Martinez, A. W.; Narovlyansky, M.; Whitesides, G. M. Electrochemical sensing in paper-based microfluidic devices. *Lab Chip* **2010,** *10*, 477-483.

(26) Dungchai, W.; Chailapakul, O.; Henry, C. S. Electrochemical Detection for Paper-Based Microfluidics. *Anal. Chem.* **2009,** *81*, 5821-5826.

(27) Ludwig, J.; Weinstein, J. Biomarkers in cancer staging, prognosis and treatment selection. *Nat. Rev. Cancer* **2005,** *5*, 845-856.

(28) Madou, M. J. *Fundamentals of Microfabrication: The Science of Miniaturization;* CRC Press LLC: Boca Raton, FL, 2002; 2nd ed.

(29) Xia, Y.; Whitesides, G. Soft lithography. *Annu. Rev. Mater. Sci.* **1998,** *28*, 153-184.

(30) Kunzler, J. Silicone hydrogels for contact lens application. *Trends Polym. Sci.* **1996,** *4*, 52-59.

(31) Chaudhury, M. K.; Whitesides, G. M. Direct Measurement of Interfacial Interactions between Semispherical Lenses and Flat Sheets of Poly(dimethylsiloxane) and their Chemical Derivatives. *Langmuir* **1991,** *7*, 1013-1025.

(32) Chaudhury, M.; Whitesides, G. Correlation between Surface Free-Energy and Surface Constitution. *Science* **1992,** *255*, 1230-1232.

(33) McDonald, J.; Duffy, D.; Anderson, J.; Chiu, D.; Wu, H.; Schueller, O.; Whitesides, G. Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis* **2000,** *21*, 27-40.

(34) Morra, M.; Occhiello, E.; Marola, R.; Garbassi, F.; Humphrey, P.; Johnson, D. On the Aging of Oxygen Plasma-Treated Polydimethylsiloxane Surfaces. *J. Colloid Interface Sci.* **1990,** *137*, 11-24.

(35) Delamarche, E.; Bernard, A.; Schmid, H.; Bietsch, A.; Michel, B.; Biebuyck, H. Microfluidic networks for chemical patterning of substrate: Design and application to bioassays. *J. Am. Chem. Soc.* **1998,** *120*, 500-508.

(36) Grieshaber, D.; MacKenzie, R.; Voeroes, J.; Reimhult, E. Electrochemical biosensors - Sensor principles and architectures. *Sensors* **2008,** *8*, 1400-1458.

(37) Clark, L. C.; Lyons, C. Electrode Systems for Continuous Monitoring in Cardiovascular Surgery. *Ann. N. Y. Acad. Sci.* **1962,** *102*, 29-45.

(38) Skoog, D. A.; Holler, J. F.; Crouch, S. R. *Principles of Instrumental Analysis;* Cengage Learning: Independence, KY, 2006; 5th ed.

(39) Dittrich, P.; Manz, A. Single-molecule fluorescence detection in microfluidic channels - the Holy Grail in mu TAS?. *Anal. Bioanal. Chem.* **2005,** *382*, 1771-1782.

(40) Berg, J. M.; Tymoczko, J. L.; Stryer, L. Proteins Can Be Detected and Quantitated by Using an Enzyme-Linked Immunosorbent Assay. In *Biochemistry;* W. H. Freeman and Company: New York, 2002; 5th ed., pp 101-102.

(41) Chatterjee, D.; Mansfield, D.; Anderson, N.; Subedi, S.; Woolley, A. "Flow valve" microfluidic devices for simple, detectorless and label-free analyte quantitation. *Anal. Chem.* **2012,** submitted.

2. FABRICATION AND SURFACE MODIFICATION OF FLOW VALVE DEVICES

2.1 Introduction

In this chapter I present fabrication methods for flow valve devices. I used well-established micromachining techniques to create many PDMS flow valve devices. Briefly, the fabrication process begins with making a master mold (Section 2.2.1), followed by replica molding of PDMS on the master mold to create the layer of the flow valve device containing the microfluidic channels (Section 2.2.2). The channel layer of the device is bonded to a PDMS slab, after which the embedded microchannels are functionalized with receptor molecules (e.g., antibodies) to prepare the devices for use in flow valve assays (Section 2.2.3).

2.2 Experimental

2.2.1 Mold Fabrication

The mold fabrication process is summarized in Figure 2.1. Glass was chosen as the mold substrate because it breaks less easily than silicon. Also, initial device fabrication attempts indicated that PDMS was less likely to permanently adhere to glass than to silicon. The wafer was rinsed in acetone and isopropyl alcohol. After rinsing, any residual organic material was removed in a Planar Etch II plasma etcher (Technics West, San Jose, CA) with an oxygen plasma for 3 min at 250 W. Following the etching, water was removed from the wafer by drying in an Ultra-Clean 100 oven (Lab-Line Instruments, Melrose Park, IL) for 15 min at 150 ^{o}C. Hexamethyldisilizane (HMDS, SPI

Supplies, West Chester, PA) was evaporated onto the wafer to promote photoresist adhesion (Figure 2.1a). Next, the wafer was spin-coated with a positive photoresist, heated to remove any residual solvent, and allowed to rehydrate in air at room temperature for 45 min.

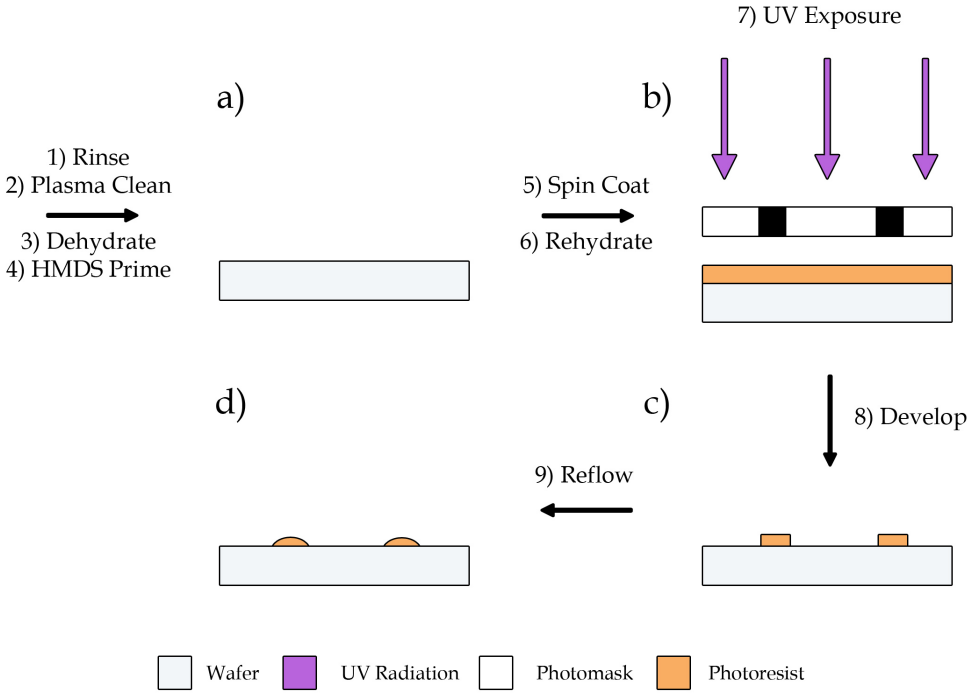

Figure 2.1 – Mold fabrication for flow valve devices.
A master mold for replica molding of flow valve devices is created using standard photolithographic techniques. a) After cleaning, dehydrating and priming the glass wafer, it is ready for photoresist depostion. b) Following spin coating of the photoresist and rehydration, the photoresist is exposed to UV light through a photomask. c) The wafer is developed to dissolve exposed photoresist. d) The wafer is reflowed to achieve a semicircular feature geometry.

I chose to use AZ series positive photoresists for pattern definition on my molds, specifically AZ 3330F, AZ P4620, and AZ 50XT (AZ Electronic Materials, Branchburg, NJ). These photoresists allowed me to achieve various channel heights when optimizing the device design while also offering reflow capabilities (see Table 2.1). Initial

fabrication was done with AZ 3330F, giving channels from 5-7 μm tall. When I desired taller channels, I moved to the thicker photoresist AZ P4620, and I was able to make channels 7-13 μm tall. For channels taller than 13 μm, I used AZ 50XT.

Table 2.1 – Select Photolithographic Recipes for Specific Microfluidic Channel Heights

Channel Height (μm)	Photoresist	Spin Speed (rpm)	Spin Time (s)	Soft Bake Temp/Time (°C/min)	Exposure Time (s)*	Post-Exposure Bake Temp/Time (°C/min)	Developer	Developing Time (s)	Reflow Temp/Time (°C/min)
~6.8	AZ 3330F	600	60	90/1	17	90/1	AZ 300MIF	~45	250/5
~8	AZ P4620	3000	40	90/5	40	n/a	1:4 AZ 400K:Water	~90	150/5
~17	AZ 50XT	3200	60	60/ 10 then 125/3	20	n/a	1:2 AZ 400K:Water	~480	125/3

*At 10 mW/cm^2

After photoresist rehydration, the wafer was exposed to UV light from a 250 W mercury lamp in a MA150 CC Karl Suss Aligner (Karl Suss America, Waterbury Center, VT) through a photomask (Figure 2.1b), followed by a post-exposure bake, if necessary for the photoresist (see Table 2.1). The wafer was then developed (Figure 2.1c) in the appropriate developer (AZ Electronic Materials; see Table 2.1) and reflowed to achieve a semicircular feature geometry (Figure 2.1d).

Reflowing, or heating the photoresist to a temperature above its melting point after exposure and development, changes microfluidic channel geometry from rectangular to semicircular.[1] A semicircular channel geometry is desirable for flow valve channel constriction because it is easier for a curved channel to pinch shut from the sides toward the center than a rectangular channel.[2] Unger *et al.* demonstrated the validity of this principle for valves closed by actuated pressure, with semicircular channels closing easier than rectangular channels.[3]

I used two photomask designs to make device molds (Figure 2.2). The first design contained five flow valve devices with 35 mm-long straight channels (Figure 2.2a), while the second design contained 4 three-lane devices, 2 five-lane devices, and 3 nine-lane devices, all with serpentine channels of varying length (Figure 2.2b). Of the serpentine devices, I mainly used the five-lane devices, which had five 36 mm-long channels connected by rounded corners. The straight channel devices were simple and easy to use, but did not allow for a low limit of detection. On the other hand, the serpentine devices were more difficult to work with, but were able to achieve a lower limit of detection due to the longer channels.

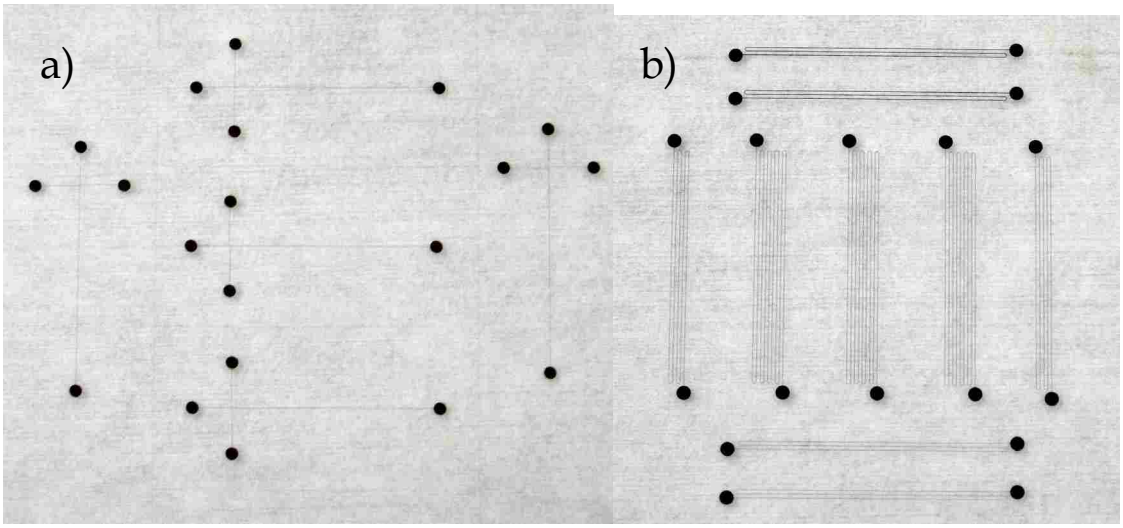

Figure 2.2 – Photomask designs for device fabrication.
a) The straight channel design produces 5 devices with 35-mm long channels. b) The serpentine channel design produces 4 three-lane devices, 2 five-lane devices, and 3 nine-lane devices with straight channels of varying length connected by rounded corners.

The height and width of the channels on the completed master molds was determined with an Alpha-Step 200 profilometer (Tencor Instruments, Milipitas, CA).

2.2.2 Flow Valve Device Fabrication

Flow valve devices were prepared by casting PDMS against a master mold (Figure 2.3). PDMS (Dow Corning, Centennial, CO) was prepared by mixing the base and curing agents in a 10:1 ratio and was degassed for 30-60 min. PDMS was then poured on the master mold to a thickness of 0.5 mm and cured at 80 °C for 45 min (Figure 2.3a). A separate layer of the device was prepared by spinning a thin slab of PDMS onto glass microscope slides and also curing at 80 °C for 45 min (Figure 2.3b).

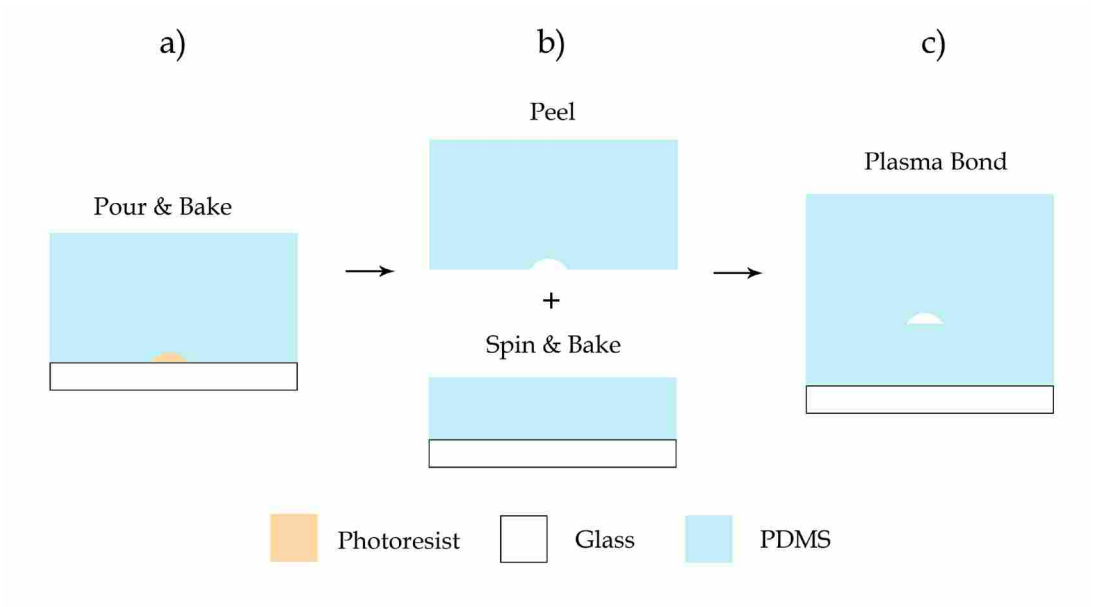

Figure 2.3 – Flow valve device fabrication.
Replica molding enables the fabrication of many flow valve devices with a single master mold. a) Pre-polymer is poured over the master mold and baked. b) Cured PDMS is peeled off the master mold, and the second layer of the device is prepared by spinning and curing PDMS on a glass slide. c) The two PDMS layers are joined by plasma bonding to complete the device.

To complete the devices, the patterned PDMS was peeled off the master mold (Figure 2.3b) and bonded to the PDMS-covered slides (Figure 2.3c). After both layers were exposed to an oxygen plasma they were bonded by being brought into conformal contact with one another,[4] forming a flow valve device with embedded channels. The

devices were either stored in water or used immediately to prevent loss of hydrophilicity.[5, 6]

2.2.3 PDMS Surface Modification for Antibody Attachment

The first method I tried to attach antibodies to PDMS was non-specific adsorption. Biomolecules tend to adsorb non-specifically to PDMS,[7] and this characteristic is usually detrimental to microfluidic systems. However, as non-specific adsorption was an acceptable method for functionalizing PDMS with biotinylated-BSA during proof-of-concept testing (see Section 3.2), I wanted to see if I could take advantage of this trait as an easy method to attach antibodies to the channel walls.

Immediately following plasma oxidation and bonding of the device, 1.5 µL of fluorescein isothiocyanate (FITC)-conjugated rabbit polyclonal anti-streptavidin (Abcam, Cambridge, MA, 10 mg/mL in 0.02 M phosphate, 0.15 M NaCl, 0.01% sodium azide, pH 7.2) was introduced into a designated liquid reservoir and allowed to adsorb for 60 min. To prevent the antibody from drying out, the devices were kept in a humid environment by keeping them in a Petri dish with a moist paper towel. After antibody adsorption, excess solution was removed from the channels by applying vacuum. Unadsorbed antibody was removed by rinsing the channels with 1 µL of distilled water or phosphate buffer.

I also explored silanization as an alternate method for attaching antibodies to PDMS. A silane solution was prepared by mixing 0.4-1 mL GOPS (Sigma-Aldrich, St. Louis, MO) in 20-40 mL toluene (Sigma-Aldrich) or methanol (Sigma-Aldrich).

Immediately following plasma oxidation, the flow valve devices were submerged in the silane solution for 60 min. After silanization, the devices were removed from the silane solution and rinsed with solvent to remove unreacted GOPS and the channels were drained of any residual silane solution. A 1.5 μL sample of FITC-conjugated anti-streptavidin (1 mg/mL in 0.02 M phosphate, 0.15 M NaCl, 0.01% sodium azide, pH 7.2) was pipetted into the channel reservoir, filling the channel, and reacted with the GOPS for 60 min. The devices were once again covered with a damp paper towel to prevent the antibody solution from drying out. Finally, excess solution was removed from the channels and the channels were rinsed with water or phosphate buffer.

2.2.4 Characterization of Modified Devices with Laser-Induced Fluorescence

I used LIF to characterize the antibody-modified devices to determine whether or not the methods were efficiently modifying the channel surfaces. This also allowed me to decide which modfication method, non-specific adsorption or silanization, was the best method for attaching antibodies to PDMS.

The LIF system consisted of a 625 mW LED (MBLED, Thorlabs, Newton, NJ) passed through a filter cube (FITC-LP01-Clinical-OMF, Semrock, Rochester, NY) and an upright microscope (Axio Scope, A1, Zeiss, Thornwood, NY), which was connected to a CCD camera (CoolSNAP HQ2, Photometrics, Tucson, AZ). Exposure time was 500 ms. Devices were imaged after GOPS silanization but before antibody adsorption and also following antibody adsorption and rinsing with distilled water.

During surface modification with FITC-anti-streptavidin, care had to be taken to avoid photobleaching of the FITC. The fluorescently conjugated antibody was stored in an opaque container and was only pipetted in a dark room. Devices reacting with FITC-anti-streptavidin were covered and kept in a dark room until imaging was complete. The laser was shuttered in between images to prevent photobleaching of the antibody by the laser.

Images were processed and analyzed with ImageJ software.[8] A square box was drawn on each image and probed for average fluorescence intensity and standard deviation.

2.3 Results and Discussion

The fabrication procedure described in Section 2.2.2 produced PDMS devices with semicircular channels with width and height matching that of the master mold used to make the replica. Figure 2.4 shows photographs of a completed straight channel device (Figure 2.4a) and a serpentine channel device (Figure 2.4b).

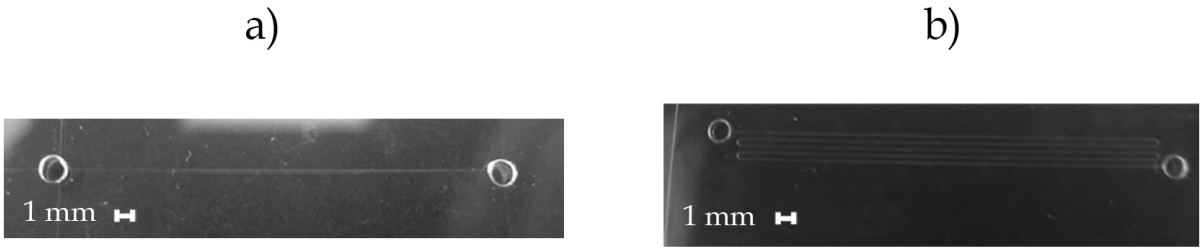

Figure 2.4 – Completed a) straight channel and b) serpentine channel devices.
a) The straight channel devices had channels 35 mm-long and were made using the long arm of an existing offset-T design. b) The serpentine channel devices consisted of five 36 mm-long channels connected by rounded corners.

To ensure that plasma bonding produced enclosed channels and that dust or other debris was not blocking the channels, a 1 μL sample of distilled water was added to one channel reservoir and allowed to flow through the channel. If the water completely filled the channel without difficulty the device was considered testable, otherwise the device was considered defective and was thrown away.

Fluorescence images were obtained for antibody in complete devices with enclosed channels. Figure 2.5 shows images of a plasma oxidized but unsilanized device before, during, and after exposure to FITC-anti-streptavidin.

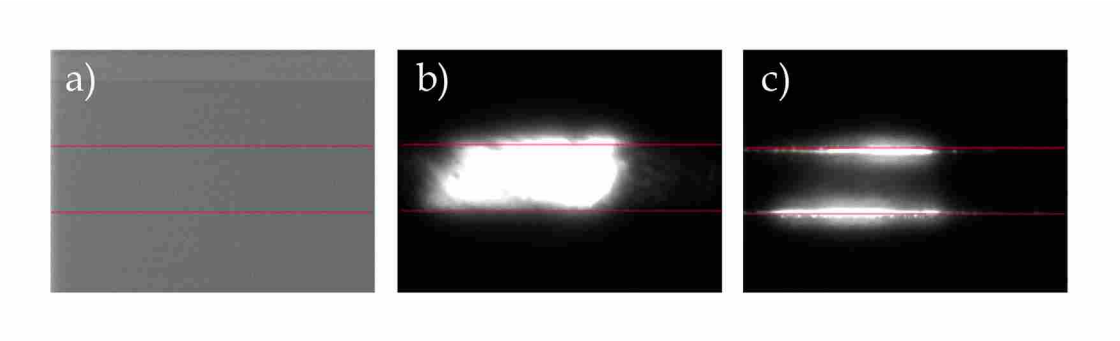

Figure 2.5 – LIF images of a plasma oxidized, unsilanized flow valve device.
The red lines indicate approximate channel boundaries. Channel width is 50-60 μm. a) An air-filled microfluidic channel before introduction of the antibody. b) The microfluidic channel filled with FITC-anti-streptavidin. c) The microfluidic channel after flushing out unadsorbed antibody with distilled water.

Fluorescence images of plasma oxidized but unsilanized devices indicated that antibody could adsorb to PDMS by non-specific adsorption alone, but most often the primary location of antibody adsorption was at the channel edges and not the entire surface of the channel. This increased fluorescence at channel edges was also apparent in GOPS-modified PDMS devices (Figure 2.6).

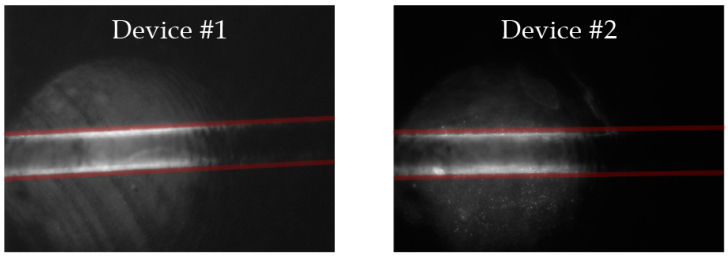

Figure 2.6 – Increased fluorescence at channel edges in two silanized devices.
Fluorescence images of two GOPS-silanized devices after removing unreacted antibody. The red lines indicate approximate channel boundaries. Channel width is 50-60 μm. The fluorescence at the channel edges is more intense than fluorescence in other parts of the channel.

There are two possible explanations for the accumulation of fluorescence at the channel edges. First, due to the channel geometry it was favorable for liquids to pool along the edges of the channel where high surface tension would allow them to escape the vacuum that drained liquid from the channel. Second, the top down perspective of the microscope in addition to the curvature of the channel walls (Figure 2.7) produced an edge effect that could make it appear as if there was more fluorescence at the edges of the channel.

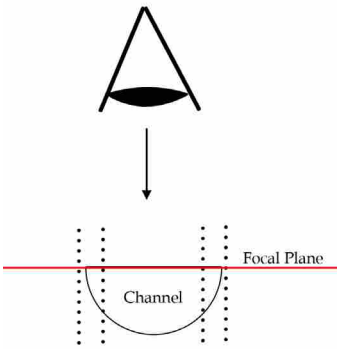

Figure 2.7 – Microscope edge effect.
The top-down microscope perspective combined with narrow depth of field and curved channel edges could lead to deceptively bright fluorescence signal at the channel edges.

Difficulty draining the enclosed channels of unadsorbed antibody led to testing for antibody adsorption on GOPS-modified PDMS slabs. It was much easier to rinse antibody from a flat PDMS slab than from an enclosed channel. Figure 2.8 shows LIF images of a GOPS-modfied PDMS slab before, during, and after exposure to FITC-streptavidin. These images indicate that attaching antibodies to PDMS through silanization was a viable option and that it appeared to attach more antibodies than non-specific adsorption.

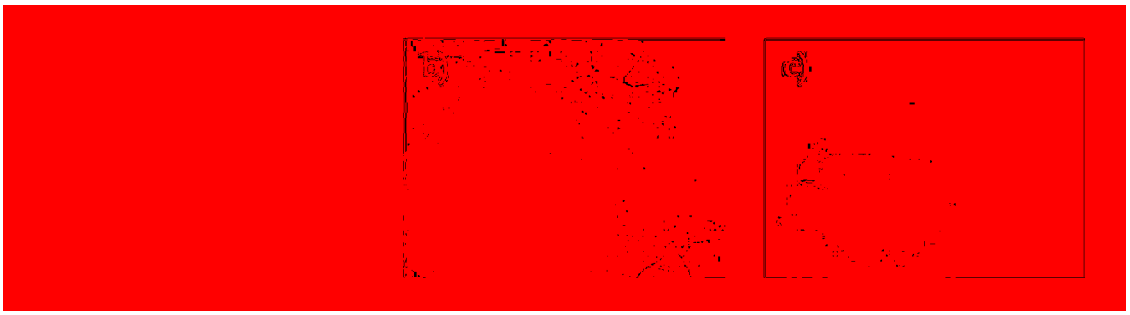

Figure 2.8 – LIF images of a GOPS-modified PDMS slab.
a) A GOPS-silanized PDMS slab before introduction of the antibody. b) The slab with FITC-anti-streptavidin on the surface. c) The slab after rinsing off unadsorbed antibody with distilled water.

Although imaging PDMS slabs allowed for better removal and rinsing of unadsorbed antibody, lack of an adequate focal point such as a channel edge led to many unfocused images and thus inaccurate representations of fluorescence. Thus, I imaged unenclosed PDMS channels exposed to the air. This gave me a channel edge to focus on while still allowing for easy rinsing and removal of unadsorbed antibody. The microscope edge effect is still apparent in images obtained by this method, but it allowed for a better comparison of antibody attachment on unsilanized and silanized PDMS.

Fluorescence images (Figure 2.9) show two unsilanized (but plasma oxidized) PDMS devices (two leftmost columns) and two GOPS-silanized PDMS devices (two rightmost channels) before (top row), during (middle row), and after (bottom row) exposure to FITC-anti-streptavidin. After rinsing, the GOPS-silanized PDMS channels had an average fluorescence intensity of 2300 ± 1200 and 1700 ± 1000 while the unsilanized PDMS channels had an average fluorescence intensity of 1200 ± 700 and 500 ± 200. The large standard deviations indicate that there was significant variability in fluorescent intensity from point to point in the channels. Despite the large standard deviations, these results point to GOPS-silanization offering a higher surface coverage of attached antibodies on PDMS.

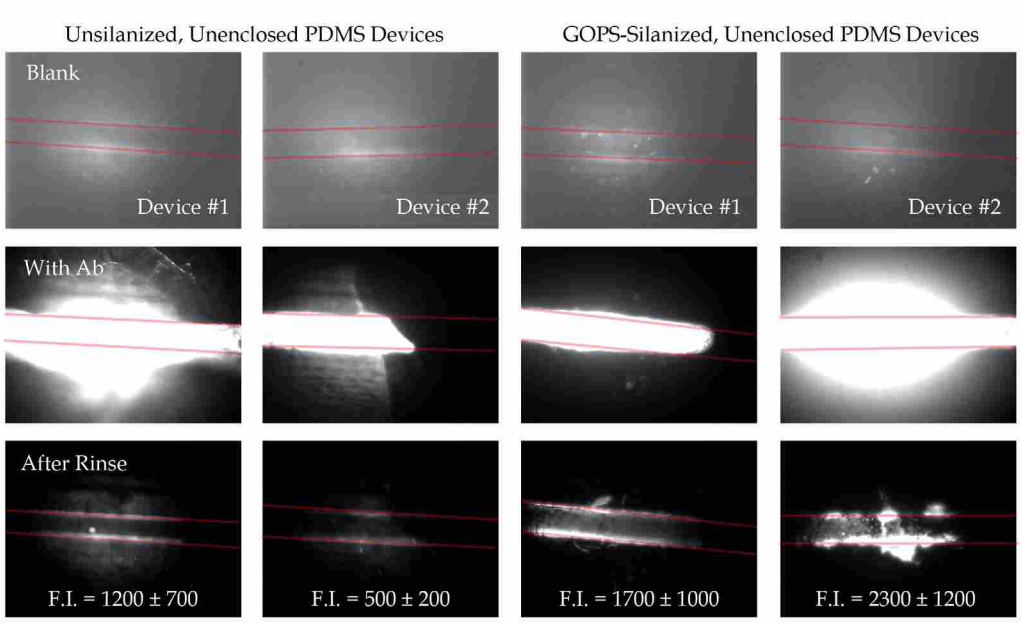

Figure 2.9 – Comparison of antibody adsorption in unsilanized and silanized unenclosed PDMS devices.
LIF images of FITC-anti-streptavidin in two unsilanized (only plasma oxidized) PDMS devices and two GOPS-silanized PDMS devices. The red lines represent approximate channel boundaries. Channel width is 60-70 μm. The top, middle, and bottom rows respectively show the PDMS before, during, and after antibody exposure.

Silanization is also advantageous because it ensures a covalent attachment between the antibodies and the PDMS and because it should make more antibodies attach in the proper orientation for antigen binding. However, disadvantages to the silanization method include the time required, GOPS' reactivity with water, and PDMS solvent compatibility. First, the current silanization attachment method is 2-3 times longer than attachment by non-specific adsorption. Further testing could be done to see if the silanization and/or the antibody attachment steps could be shortened with no loss in the amount of antibody attachment. Second, GOPS' reactive terminal epoxy ring is sensitive to reaction with water, and if GOPS and water react, GOPS can no longer react with antibodies. Therefore, care must be taken to prevent exposing GOPS to water before it can react with the antibodies. This leads to the final issue, PDMS solvent compatibility. Because GOPS is water reactive, the silanization solvent is normally hydrophobic, with toluene being the normal solvent of choice. However, PDMS is incompatible with many organic solvents as it absorbs some solvents and swells.[9] This swelling is particularly detrimental as it can cause PDMS plasma bonds to break, compromising the integrity of a device.

I used several methods to overcome these issues with the silanization method for antibody attachment. First, I purchased a new, septum-capped bottle of GOPS that had not been exposed to water, as even minimal water exposure will cause a bottle of GOPS to degrade in 6-8 months. Second, all GOPS solutions were used immediately and flow valve devices were tested immediately after all silanization steps to prevent the GOPS from reacting with water in the air or solvent. I did, however, have to compromise on

the silanization solvent. Toluene made the PDMS swell, breaking the plasma bond between the two device layers, so I had to find a new solvent. I tested three common solvents: acetone, ethanol, and methanol. According to Lee *et al.*,[9] these three solvents would not significantly swell PDMS, with methanol swelling PDMS the least, so I chose to use methanol as the silanization solvent. Although methanol is hydrophilic, I used the purest methanol available (≥99.9%) to reduce the likelihood of water contamination.

2.4 Conclusions

This chapter presents the fabrication of flow valve devices using standard micromachining techniques, including photolithography, replica molding, and plasma oxidation. The devices may be modified by plasma oxidation and silanization to facilitate biomolecule attachment. LIF analysis of unsilanized and GOPS-silanized devices indicated that silanization is the better method for attaching antibodies to PDMS microchannels.

2.5 References

(1) Barber, J.; Lunt, E.; George, Z.; Yin, D.; Schmidt, H.; Hawkins, A. Integrated hollow waveguides with arch-shaped cores. *IEEE Photonics Technol. Lett.* **2006,** *18*, 28-30.

(2) Chatterjee, D.; Mansfield, D.; Anderson, N.; Subedi, S.; Woolley, A. "Flow valve" microfluidic devices for simple, detectorless and label-free analyte quantitation. *Anal. Chem.* **2012,** submitted.

(3) Unger, M.; Chou, H.; Thorsen, T.; Scherer, A.; Quake, S. Monolithic microfabricated valves and pumps by multilayer soft lithography. *Science* **2000,** *288*, 113-116.

(4) McDonald, J.; Duffy, D.; Anderson, J.; Chiu, D.; Wu, H.; Schueller, O.; Whitesides, G. Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis* **2000,** *21*, 27-40.

(5) Morra, M.; Occhiello, E.; Marola, R.; Garbassi, F.; Humphrey, P.; Johnson, D. On the Aging of Oxygen Plasma-Treated Polydimethylsiloxane Surfaces. *J. Colloid Interface Sci.* **1990,** *137*, 11-24.

(6) Delamarche, E.; Bernard, A.; Schmid, H.; Bietsch, A.; Michel, B.; Biebuyck, H. Microfluidic networks for chemical patterning of substrate: Design and application to bioassays. *J. Am. Chem. Soc.* **1998,** *120*, 500-508.

(7) Mukhopadhyay, R. When PDMS isn't the best. *Anal. Chem.* **2007,** *79*, 3253.

(8) Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012,** *9*, 671-675.

(9) Lee, J.; Park, C.; Whitesides, G. Solvent compatibility of poly(dimethylsiloxane)-based microfluidic devices. *Anal. Chem.* **2003,** *75*, 6544-6554.

3. TESTING OF FLOW VALVE DEVICES

3.1 Introduction

With the flow valve device fabrication and surface modification processes established, the next step in device development was to prove that these devices could be used to detect and quantify biomarkers. To do this, I performed two types of tests. First, I studied proof-of-concept tests with biotin acting as the receptor bound to the microchannel walls and streptavidin acting as the target (biomarker) to be detected. Second, once the effectiveness of the proof-of-concept tests was verified by other group members, I began performing tests with anti-streptavidin (receptor) and streptavidin (target) as a more realistic approximation of the target/receptor interactions that would be useful for analyzing biological fluid samples.

3.2 Proof-of-Concept Testing

Initial testing of flow valve devices was with a simple, effective, and well-studied target/receptor pair: streptavidin and biotin. The streptavidin-biotin bond has a dissociation constant (K_d) of ~10^{-14}, making it one of the strongest known non-covalent interactions.[1] Streptavidin is also a tetramer with four identical subunits and thus can bind up to four biotins at one time. Due to these qualities, the streptavidin-biotin system was ideal for proof-of-concept testing.

The proof-of-concept testing procedure has been described elsewhere[2] but is summarized here for reference and convenience. Immediately after plasma bonding

and oxidation, the microchannels of the flow valve devices were filled with biotinylated-bovine serum album (biotinylated-BSA, Thermo Scientific, Rockford, IL, 2 mg/mL in 0.14 mM citrate buffer, pH 6.8) by capillary action. The biotinylated-BSA was allowed to adsorb to the channel walls for 15 min. After that time period, unadsorbed biotinylated-BSA was flushed from the channel using phosphate buffered saline (PBS, 10 mM, pH 7.2). Last, PBS was removed from the channel and 1 μL of streptavidin solution (New England Biolabs, Ipswich, MA) of known concentration in PBS was pipetted into the reservoir. Flow distance was recorded with a ruler and images were obtained with a digital camera.

Results from the proof-of-concept testing indicated that $\log_{10}$[streptavidin] and flow distance share a linear relationship.[2] Therefore, for a given device design, one is able to create a standard curve and subsequently determine the concentration of unknown samples by measuring flow distance.

3.3 Antibody/Antigen Testing

After the success of proof-of-concept testing I wanted to demonstrate the wide applicability of flow valve devices by testing them with antibody/antigen pairs. Because the best way to detect a biomarker (e.g., antigen) is by using its complementary antibody, study of an antigen/antibody interaction gives more valuable information about detecting biomarkers in a biological system using the flow valve method than the streptavidin-biotin interaction.

For antibody/antigen testing, antibodies were attached to the channel walls by the method given in Section 2.2.3. I chose to use anti-streptavidin as the antibody so that I could continue to use streptavidin as the antigen, as this would provide a good comparison to proof-of-concept tests. Following rinsing and drying of the channel, a 1.5 μL sample of streptavidin of a given concentration was pipetted into the channel reservoir and began to move through the channel by capillary action. During initial testing I was only concerned with whether or not the antibody/antigen interaction was strong enough to cause the fluid to stop in the channel. I expected that the fluid would travel further in these channels than in any of the proof-of-concept tests because the anti-streptavidin/streptavidin interaction is weaker than the biotin/streptavidin interaction, so it would likely take more extensive binding to stop flow.

Initial tests gave varying results as to whether or not the fluid in the channel had stopped moving as a result of the flow valve effect. Some trials seemed to indicate that flow was stopping, while others indicated that flow was not stopping but instead the fluid flow rate significantly slowed. I thought this might be due to the relative weakness of the antibody/antigen interaction. However, as the streptavidin samples did seem to flow more slowly than the blank samples, I thought that the anti-streptavidin/streptavidin interaction might be causing the channel to constrict to the extent that liquid flow would slow but not enough that it would stop completely.

The hypothesis of limited channel constriction led to timed flow testing. Instead of waiting for the liquid to stop flowing and recording only the final flow distance, I placed rulers parallel to the channels and recorded the flow distance at 15 sec intervals

until either the flow had stopped, the fluid had flowed to the end of the channel, or a specified amount of time (~15 min) had elapsed. This allowed me to look for trends in fluid flow to see if there was a time or point where the flow consistently slowed.

Initial timed flow trials were performed in 5-lane serpentine devices with channels 12.6-13.8 µm tall and 78-86 µm wide. To determine if these antibody-modified devices exhibited concentration dependent-flow distance, I tested two streptavidin concentrations: 1.0 mg/mL and 0.33 mg/mL. The average flow distance of several timed trials (eight with 1 mg/mL and four with 0.33 mg/mL) is shown with error bars representing one standard deviation in Figure 3.1.

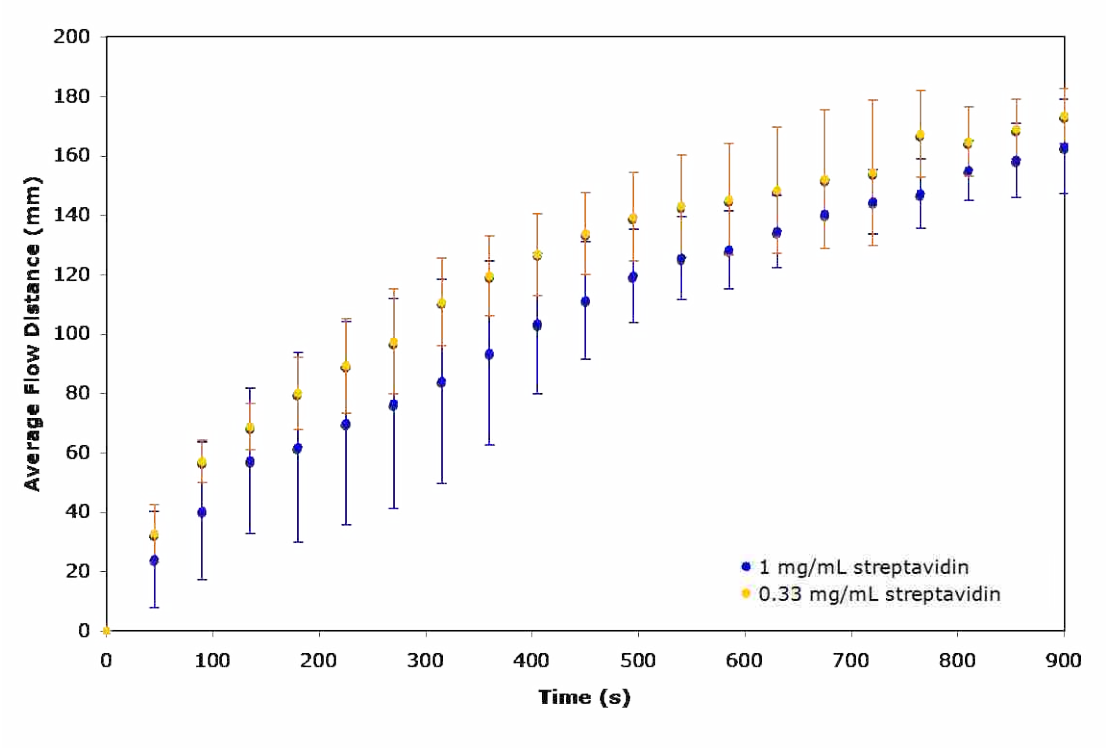

Figure 3.1 – Average flow distances in anti-streptavidin modified flow valve devices for two streptavidin concentrations in 12.6-13.8 µm tall channels.
Timed flow tests were used to look for trends in flow rate slowing that could have been caused by limited channel constriction. Although the trials indicate concentration-dependent flow distance, overlapping of error bars (one standard deviation) likely meant that these streptavidin concentrations were near the limit of detection for these devices.

Analysis of the results from these initial timed flow trials appeared to indicate that there was some concentration dependence; that is, more dilute streptavidin samples (0.33 mg/mL) generally flowed a greater distance than more concentrated streptavidin samples (1.0 mg/mL) at a given time. However, repeated experiments sometimes gave varied flow distance profiles for the same concentration, leading to overlapping standard deviations for different concentrations.

Overlapping of the error bars for the 0.33 mg/mL samples and the 1 mg/mL samples indicated that these concentrations could not be differentiated easily in these antibody-modified flow valve devices. Therefore, I decided to try modifying the device design by reducing the height of the microfluidic channels to alter the concentration dependence of flow. A shorter channel should need less flow distance before the channel constricts enough to slow or stop fluid flow. Thus, the new mold had devices with channels 9.5-10.4 μm tall and 62-70 μm wide. I made anti-streptavidin modified devices with this mold and performed timed flow tests with 0 mg/mL streptavidin (blank) and 1 mg/mL streptavidin. The results are shown in Figure 3.2.

This second set of timed flow trials in shorter channels does verify that shorter channels have a reduced flow distance for the same concentration than taller channels, as the 1 mg/mL streptavidin samples in these devices began slowing at a flow distance of ~90 mm, as opposed to the 1 mg/mL samples in the 12.6-13.8 μm tall channels, which began slowing at a flow distance of ~120 mm. However, the overlapping error bars of

the blank and 1 mg/mL samples in these channels indicated that this concentration was near the limit of detection for the modified device design.

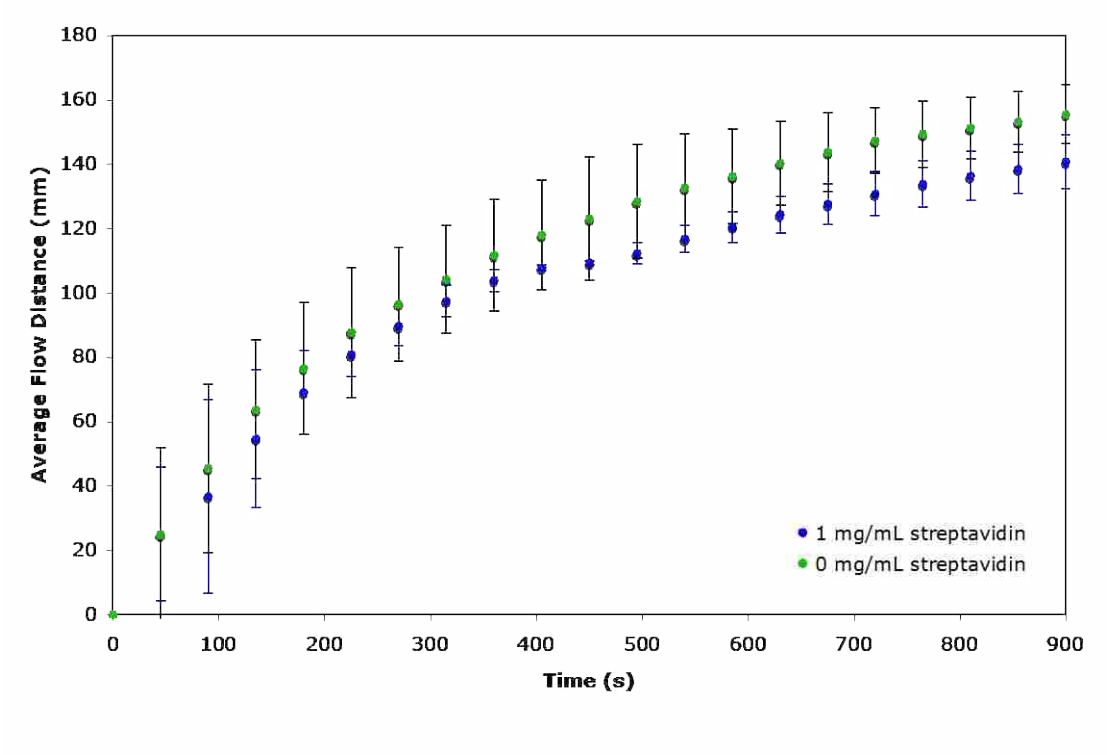

Figure 3.2 – Average flow distances in anti-streptavidin modified flow valve devices for blank and 1 mg/mL streptavidin in 9.5-10.4 μm tall channels.
The overall flow distance for 1 mg/mL samples was less than the flow distance for 1 mg/mL samples in taller channels. Overlapping of the error bars (one standard deviation) for 0 mg/mL and 1 mg/mL samples indicates that 1 mg/mL streptavidin is near the limit of detection for this device design.

To ensure that the streptavidin samples were above the limit of detection for these flow valve devices I tested a more concentrated sample. I obtained 1.0 mg of lyophilized streptavidin and reconstituted it to 10 mg/mL with distilled water (Thermo Scientific, Rockford, IL, 10 mg/mL in 20 mM potassium phosphate, pH 6.5). This concentrated streptavidin was tested in channels 12.6-13.8 μm tall and 78-86 μm wide (similar to the experiments in Figure 3.1).

The results for the 10 mg/mL streptavidin trials are given in Figure 3.3. The data clearly show that the 10 mg/mL streptavidin samples traveled less distance (~80 mm) than the 1.0 mg/mL streptavidin samples before fluid flow slowed and stopped. Also, the error bars for the 1.0 mg/mL streptavidin samples stopped overlapping with the error bars for the 10 mg/mL streptavidin samples at approximately the distance where the 10 mg/mL samples began to slow (~80 mm). In all, these results demonstrate a concentration-dependent flow valve effect and suggest that with further development an antibody/antigen system is a viable option for biomarker detection in flow valve devices.

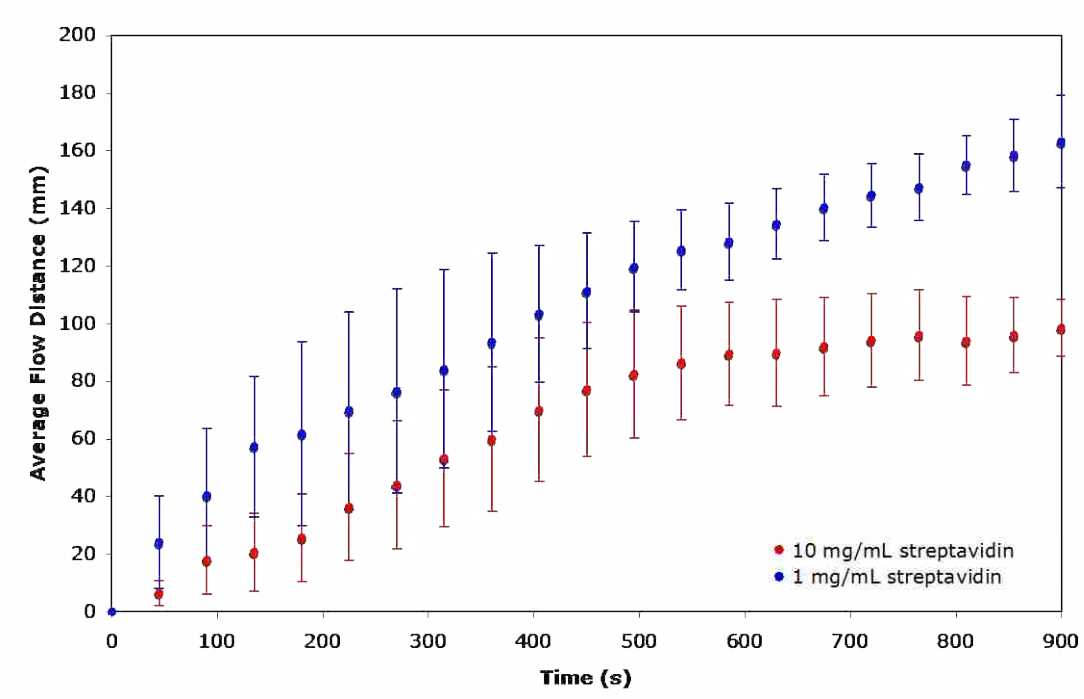

Figure 3.3 – Average flow distances in anti-streptavidin modified flow valve devices for 1 and 10 mg/mL streptavidin samples.
Different flow distances and non-overlapping error bars (one standard deviation) for 1 mg/mL and 10 mg/mL streptavidin samples signify the feasibility of using an antibody/antigen interaction for detection of biomarkers in flow valve devices.

3.4 Conclusions

These experiments suggest that quantitation of antigens by use of flow valve devices is possible through an antibody/antigen interaction which leads to channel constriction and the slowing or stoppage of fluid flow. Although my results were with an antigen concentration above the typical biomarker concentrations in fluid samples, the flow valve devices could be modified to improve limits of detection. More research must be done with new device designs and different antibody/antigen pairs to further demonstrate the viability of this concept.

3.5 References

(1) Green, N. Avidin and Streptavidin. *Meth. Enzymol.* **1990,** *184*, 51-67.

(2) Chatterjee, D.; Mansfield, D.; Anderson, N.; Subedi, S.; Woolley, A. "Flow valve" microfluidic devices for simple, detectorless and label-free analyte quantitation. *Anal. Chem.* **2012,** submitted.

4. CONCLUSIONS AND FUTURE WORK

4.1 Conclusions

I have described the fabrication process for a new kind of POC test called flow valve diagnostics (see Chapter 2). These devices are easy to make using established micromachining techniques, allowing for parallel processing of several devices at one time. The microchips are small and made of inexpensive materials. The PDMS microchannel may be modified using silane-based surface chemistry to attach biomolecules, such as antibodies, to the channel surface (see Chapter 2). I evaluated attachment of antibodies through non-specific adsorption on oxidized PDMS and to PDMS coated with a reactive silane (GOPS). LIF experiments indicated that GOPS-silanization was a more effective method for attaching antibodies to PDMS.

Flow valve devices can detect and quantify biomarkers due to the interaction between the receptor biomolecules attached to the channel surface and target biomarkers in the sample solution (see Chapter 3). The receptor/target interaction causes the flexible PDMS microchannel to constrict like a valve, slowing and/or stopping fluid flow in the channel. The distance the sample flowed may then be correlated to biomarker concentration in the sample. I have demonstrated the use of an antibody/antigen system for concentration-dependent detection of antigen samples. Devices modified with anti-streptavidin were able to detect 10 mg/mL streptavidin and were able to distinguish these samples from 1 mg/mL streptavidin.

4.2 Future Work

4.2.1 Improve Limits of Detection

Although experiments were able to demonstrate proof-of-concept with flow valve systems, my experiments using anti-streptavidin and streptavidin were not very successful below a target concentration of 1 mg/mL. In fact, the best results were achieved with a 10 mg/mL sample solution, a concentration far higher than the biological levels of most diagnostic biomarkers. For flow valve devices to be useful for testing biological samples, the limit of detection must be significantly improved.

There are several device parameters that can be modified to improve the limit of detection. First, the channel height dictates how quickly channel constriction occurs. Shorter channels pinch more quickly than taller channels; however, shorter channels may also lead to clogging and thus incorrect results. Therefore, an optimal channel height must be determined that allows for efficient channel constriction and a low limit of detection while avoiding clogging.

Second, the cross-sectional shape of the channel affects channel closure. While the Woolley group has already determined that a rectangular channel will not close due to the flow valve effect, a trapezoidal channel shape may work even better than a semicircular channel shape. It may also be easier to control the angle of trapezoidal channel walls than it is to control the angle of semicircular channel walls, which is limited by the reflow step.

Third, the thickness of the top PDMS layer containing the microfluidic channel also affects channel constriction. The thinner the top PDMS layer, the easier it is for the PDMS to flex and constrict. Recent work[1] shows that a PDMS layer 0.45-0.5 mm thick is ideal for testing with biotin/streptavidin. While it is possible to fabricate a PDMS layer <0.45 mm thick, PDMS becomes difficult to work with and susceptible to tearing at these thicknesses. I have done some experiments with a commercial 0.250 mm-thin silicone film (Bisco Silicones HT-6240, Rogers Corp., Rogers, CT) and found this film to be easier to work with than lab-made thin PDMS. However, I was not able to achieve consistent bonding for proof-of-concept testing in those initial tests. Therefore, it should be useful to improve device fabrication with thin silicone films, perhaps by treating the film prior to plasma bonding to improve adhesion or through alternative bonding methods, such as thermal bonding. Successful integration of a thin silicone film could help improve the limit of detection.

Finally, the viscosity of the sample solution plays a role in how fast the channel constricts. Viscous samples flow more slowly under capillary action, providing more time for receptors and targets to cross-link and narrow the channel.[1] Sample viscosity can be increased by adding glycerol before testing, but this adds an additional handling step and dilutes the sample.

One unexplored parameter that may be used to improve the limit of detection is the amount of receptor attached to the channel walls. If the modification method is not attaching a sufficient number of biomolecules to the channel, it could take a long time for channel shrinkage to occur (or it may never occur) despite a sample with high

biomarker concentration. GOPS may not be the best silane to use for antibody attachment due to its susceptibility to hydrolysis. Another silane that could be used to attach biomolecules to PDMS is 3-aminopropyldiethoxysilane (APDIES), which confers amine functionality (Figure 4.1). The amine functional group can further be activated with glutaraldehyde and can then readily react with amine groups on biomolecules to form a covalent attachment. APDIES silanization does not require a solvent; however, the silanization procedure takes more time and requires use of the toxic chemical glutaraldehyde. It would be valuable to determine which silanization method attaches more antibodies to the channel walls. This could be studied by silanizing PDMS and attaching fluorescent antibodies with each method, then obtaining LIF images and comparing the fluorescence intensity of GOPS-silanized PDMS to that of APDIES-silanized PDMS or other attachment methods.

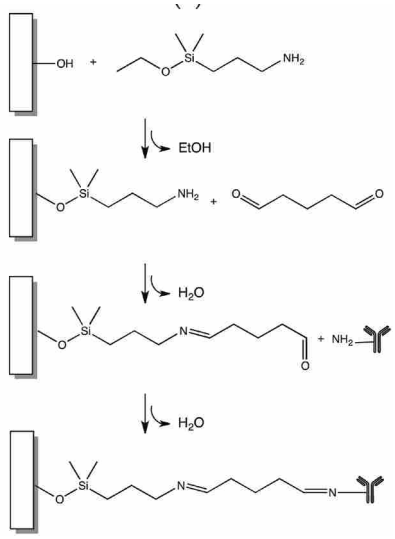

Figure 4.1 – APDIES silanization.
APDIES is an alternate silane to GOPS that can be used to attach biomolecules to PDMS.

4.2.2 PDMS Modification with 2 Monoclonal Antibodies

Receptor molecules attached to the PDMS channels must be able to bind to multiple locations on the target biomarker in order for channel constriction to occur. Thus far, I have worked with polyclonal antibodies, which are capable of binding multiple epitopes of the same antigen. Another method that could achieve the same outcome is to use two monoclonal antibodies. Monoclonal antibodies can bind only a single epitope on an antigen, but using two monoclonal antibodies that bind to different epitopes would enable channel narrowing as antigen flowed through the channel. Modification with two monoclonal antibodies could be done by mixing equal amounts of each antibody before adding them to the silanized channel.

Monoclonal antibodies are more specific than polyclonal antibodies,[2] which could be an advantage if a sample contained non-target antigens with epitopes similar to those on the target antigen. Monoclonal antibodies are also more homogeneous than polyclonal antibodies,[2] so monoclonal antibodies could potentially give more reproducible results. However, monoclonal antibodies are much harder to produce and thus more expensive than polyclonal antibodies, making flow valve devices modified with two monoclonal antibodies more costly.

4.2.3 PDMS Modification and Testing with DNA/RNA Oligomers

Biomarkers are not limited to antigens. Certain DNA or RNA oligomers can also be used as diagnotic biomarkers. Therefore, it would be useful to modify flow valve devices with DNA/RNA receptors and test these devices with the complementary

oligomer sequence. The sequences of the receptors and targets must be carefully designed so that it is most favorable for the target to bind multiple receptors and less favorable for the target to completely base pair with a single receptor, as the former scenario would allow for channel pinching and the latter would not (Figure 4.2).

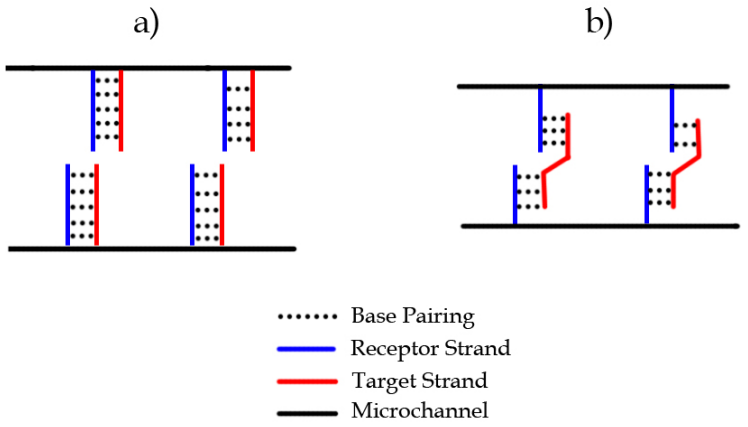

Figure 4.2 – Importance of nucleic acid oligomer design for flow valve diagnostics.
a) If the oligomer sequences are not carefully designed, it will be more favorable for target strands to base pair with a single receptor strand and the channel will not constrict. b) Well-designed oligomer sequences will allow the target to base-pair with multiple receptor strands, leading to channel constriction and flow stoppage.

4.3 Conclusions

Future studies with flow valve devices should include improving the limits of detection by modifying the device design, functionalization with two monoclonal antibodies instead of a polyclonal antibody, and functionalization and testing with DNA/RNA oligomers. All of these options should lead to improved biomarker quantitation and broader applicability for flow valve devices. These prospects make flow valve diagnostics an exciting new biomarker assay method with great potential to enhance POC testing.

4.4 References

(1) Chatterjee, D.; Mansfield, D.; Anderson, N.; Subedi, S.; Woolley, A. "Flow valve" microfluidic devices for simple, detectorless and label-free analyte quantitation. *Anal. Chem.* **2012,** submitted.

(2) Abcam. Polyclonal and monoclonal: A comparison. http://www.abcam.com/index.html?pageconfig=resource&rid=11269&pid=11287 (accessed Jul 17, 2012).