Department of Chemical & Biomolecular Engineering

Senior Design Reports (CBE)

University of Pennsylvania Year 2013

THE MICROFLUIDIC MULTI-SURFACE COAGULATION ASSAY: MICROFLUIDIC ANALYSIS OF CITRATED WHOLE BLOOD

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Dear Professors Fabiano and Diamond,

Our group was presented with the task of creating a microfluidic device which would test coagulation on collagen, collagen + tissue factor, and collagen + kaolin surfaces using an input of citrated whole blood. We succeeded in designing a device that not only uses a novel kaolin +lipid + collagen surface but also achieved on chip recalcification of citrated blood. We have named our product the "Microfluidic Multi-Surface Coagulation Assay." Compared with other methods of testing coagulation in the market, our product is faster, more representative of coagulation *in vivo*, and also tests both intrinsic and extrinsic coagulation pathways. It is simple to use, as all the physician or doctor must do is pipette 1 mL of blood onto the chip, place it in the machine, and turn it on to get results within 5 minutes.

With the total market estimated at 10 million per year, and a projected 10% initial market penetration, the Microfluidic Multi-Surface Coagulation Assay is expected to process 1 million samples per year. Within one year, the internal rate of return is estimated at 247% at the price of $100 per chip. The project has a net present value of $176,195,600 assuming 10 years of production. By the third year of production, the return on investment is expected to be 442%, suggesting a profitable project.

Yours sincerely,

Elizabeth Cummings Vamsi Vuppala Melissa Wang

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Chapter 1: Abstract

As technology improves, there is a push to minimize the cost of diagnostics as well as the cost of screening for novel drugs in the pharmaceutical industry. There is no exception in clinical point-of-care settings, and quick, fast, and inexpensive tests are needed to determine if patients require treatment or a change in dosage. The most successful endeavor in the realm of microfluidics is the InkJet printer, invented in 1980[1]. Other examples of successful and developing microfluidic technology include polymerase chain reaction, high throughput DNA sequencing, and point-of-care pathology. It is therefore reasonable that micro-scale, more thorough and diagnostically relevant coagulation studies might be desired.

Several hurdles exist to miniaturizing large scale processes, which stem from flow in the laminar regime. For example, on chip mixing is considered difficult since the Reynolds number reaches a limit so low that the fluid will not mix with particles on different streamlines. The Microfluidic Multi-Surface Coagulation Assay (µMCA) Chip introduces a novel way of recalcifying citrated whole blood on-chip, which simplifies the job of the operator and allows faster determination of results. Simple diffusion and convection of small particles combined with intuitive design make the design of this product not only functional but also inexpensive.

Previous coagulation studies merely test one pathway to coagulation, and many do so in a test tube rather than under flow. The µMCA will test both intrinsic and extrinsic coagulation pathways under arterial shear rates, making results from the device more relevant to diagnostics or patient-specific dosage of coagulants or anticoagulants. In summary, the µMCA provides a streamlined and thorough way to measure patient specific clotting abilities in physically relevant flow regimes.

Chapter 2: Introduction

2.1 Overview

Knowing the clotting ability of individual patients is important for many reasons, including suggested dose of anticoagulants or the determination of possible bleeding disorders in trauma patients. Current tests such as the activated partial thromboplastin time, or aPTT exploit either the intrinsic and extrinsic pathway, and none test blood clotting under flow. Testing under flow simulates a realistic *in vivo* scenario and thus results obtained in this way are more meaningful. Chapter 3 describes methods that exist on the lab-scale to test blood clotting under flow via the intrinsic pathway by linking tissue factor bearing liposomes to collagen surfaces[2].

As described in Chapter 4, during a coagulation event, many complex reactions occur in the blood, and the combination of these reactions defines the coagulation cascade. There are two pathways to coagulation: the intrinsic pathway and extrinsic pathway. The extrinsic pathway what occurs at the surface of an injury, where factor VII binds with tissue factor and initiates the coagulation process. The intrinsic pathway occurs when blood is exposed to a charged surface such as collagen, glass, or kaolin and coagulation ensues. Chapter 5 describes how the Microfluidic Multi-Surface Coagulation Assay Chip will provide a way to measure the functionality of both pathways to coagulation, again in a diagnostically relevant flow regime. There are several important markers to coagulation in the human body. The µMCA Chip measures platelet and fibrin accumulation. Activated platelets initiate the downstream cascade of reactions, and fibrin is the final stabilized product that creates a blood clot. Thus these proteins serve as excellent markers for the extent of coagulation.

Microfluidic technology allows the determination of a large amount of data with a small amount of input reagents and space. The µMCA Chip, as described in detail in Chapter 5, not only provides 6 distinct data sets (platelet and fibrin levels over time on collagen, collagen + kaolin + lipids, collagen + tissue factor surfaces) but provides them faster than the current competition and in a more clinically relevant form. Blood flow in the device is run at arterial shear rates, mimicking *in vivo* conditions.

Chapters 6 and 7 outline the manufacture of the microfluidic chip and cartridge. The fabrication of microfluidic chips is relatively inexpensive due to the small volume of materials required per chip; however, there are several key steps in their manufacture. Polydimethylsiloxane, or PDMS, is used as a base material, since it is optically clear and inert. The chips will be made using a series of techniques-- photolithography and soft lithography. A robotic liquid handling system will deposit the surfaces on a glass microscope slide, which will then be adhered to the fabricated PDMS chip. The chip will be place on a plastic cartridge, which is filled with calcium buffer and a fluorescent dye by another robotic liquid handling system.

Since the µMCA is a medical device, it is regulated by the FDA and must be approved before being sold in the marketplace. This process is outlined in Chapter 8, along with product development, prototyping, and clinical trials. The Microfluidic Multi-Surface Coagulation Assay is a Class II clinical device, and must be approved before use with patients.

Chapter 3 discusses the market for the µMCA chip, including several key areas such as hospitals and pharmaceutical companies. The chip will be useful in hospitals for the time-sensitive treatment of trauma patients who might have abnormal coagulation abilities as well as

heart attack patients and stroke patients. Additionally, the device will be useful for clinical trials and the development and FDA approval of novel anticoagulants or coagulants. Together these applications create an enormous marketplace with no competition that achieves the same functionality as the Microfluidic Multi-Surface Coagulation Assay Chip.

Chapter 9 involves a rigorous financial analysis- from costing the chip, operating assumptions, income and cash flow, and a payback period. The findings detail that the under the assumption of a sales price of $100 per cartridge, the Net Present Value of the product is roughly $176 M. This is an internal rate of return of approximately 247%. Taking into consideration discounts to hospitals and insurance companies, it is estimated the sales price would average around $30, which has a NPV of $45M and an IRR of 137.6%.

2.2 Project Charter and Scope

Project Name	On-Site Clinical Testing of Citrated Blood Samples
Project Champion	Scott Diamond, PhD
Project Leaders	Elizabeth M. Cummings, Vamsi K. Vuppala, Melissa M. Wang
Specific Goals	Design and build a microfluidic device that measures the clotting ability of blood with respect to Ca^{2+}, Mg^{2+}, and Zn^{2+} on Collagen, Collagen and Tissue Factor, and Collagen and Kaolin surfaces.
Project Scope	<u>In-scope:</u> -Basic design of the microfluidic device -Define mixing processes -Define splitting processes -Manufacturing procedure -Economic analysis -Experiment with designs of microfluidic device
Deliverables	Business opportunity Market expansion Technical feasibility Manufacturing capability assessment Competitive product analysis Laboratory data analysis
Timeline	The product design and testing will take place over 3 months

2.3 Innovation Map

The innovation map is a tool that is used to identify relations between aspects of the materials, product, product technology, and the voice of the customer. As discussed by Seider et al., an innovation map for a product contains six levels that are connected[12]. Innovation maps start with the materials and end with customer value proposition. These steps are: Materials Technology, Process/Manufacturing Technology, Product Technology, Technical Differentiation, Products, and Customer-Value Proposition. The innovation map for the Microfluidic Multi-Surface Coagulation Assay is shown in Figure 2.1.

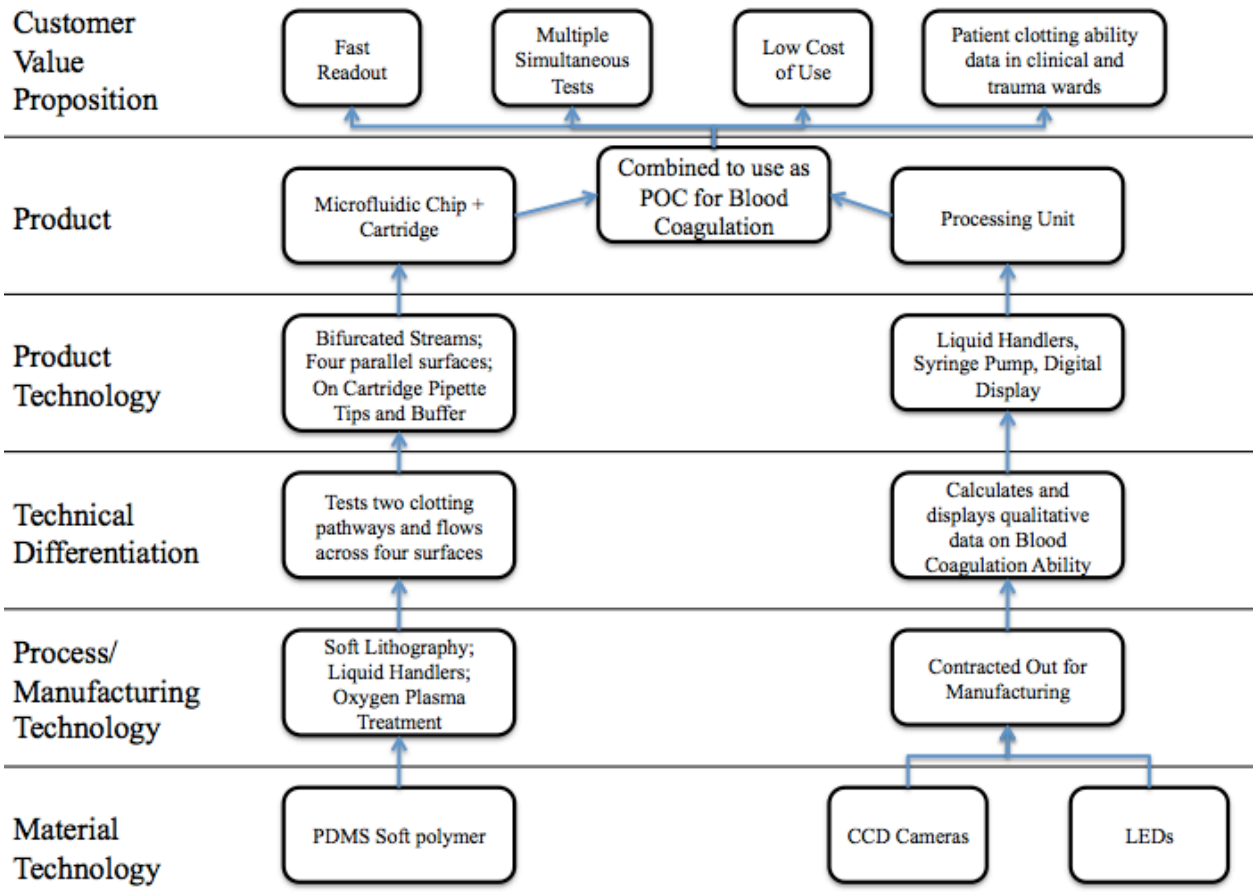


Figure 2.1: Innovation Map. The innovation map shows the new technologies used to provide value

The innovation map in Figure 2.1 shows the Customer-Value Proposition that we are trying to solve using this point of care device. The goal is to create a low cost device with the

ability to produce more data in a shorter amount of time than the previously available options for our customers. These qualities affect the ability of coagulation tests to be used in trauma patients where fast and accurate data concerning the clotting ability of a patient is critical. The final point of care device consists of both the processing unit and the chip-cartridge combination.

This device differentiates itself from other devices on the market by its ability to produce data involving both blood clotting pathways in a shorter amount of time. The chip-cartridge is composed of PDMS soft polymer. Using soft lithography, robotic liquid handling, and oxygen plasma treatment throughout the manufacturing process, the finished device has bifurcated streams that pass over parallel surfaces triggering the different coagulation pathways. The processing unit uses CCD Cameras, LEDs, robotic liquid handlers, and a syringe pump to offer faster study times, which are critical for patient safety in trauma wards and are more efficient for pharmaceutical companies performing clinical studies for potentially life-saving drugs.

Chapter 3: Market Analysis

3.1 Market for Microfluidic Multi-Surface Coagulation Assay

The Microfluidic Multi-Surface Coagulation Assay has the potential to fulfill unmet needs in a variety of markets with the advantage of being able to test blood clotting under flow conditions that mimic both the intrinsic and extrinsic clotting pathways. The initial target markets for the device include clinical laboratories for pharmaceutical companies, biotechnology research groups, hospitals, and point-of-care treatment facilities. A detailed market breakdown can be found in Appendix 13.2. The total market is assumed to be 10 million devices per year, and at 10% market penetration, the Microfluidic Multi-Surface Coagulation Assay is estimated to process 1 million samples in the first year of product launch.

3.1.1 Clinical Applications

Clinical studies are ideal targets for early product adaptation because the same test is repeated multiple times for each participant in the trial. This market generates a predictable demand for the product. The market for anticoagulants is growing, and many major pharmaceutical corporations are pursuing different variations of anticoagulants within their pipelines. The anticoagulant Eliquis, developed by Bristol Myers-Squibb Co and Pfizer Inc., was approved by the FDA in December 2012 as a competitor against the newly released Xarelto from Johnson & Johnson and Bayer and Pradaxa from Boehringer Ingelheim. These three drugs from major pharmaceutical companies are indicators for further clinical trials down the road[4]. Currently, 1,895 clinical trials in the United States are related to conditions involving blood clotting including coagulation management and atrial fibrillation. Enrollment in clinical studies varies widely from hundreds to thousands of patients[3]. In a previous clinical trial for the

anticoagulant Warfarin, 30,000 samples were tested [11].The high volume of trials and the large number of devices that would be utilized make clinical trials a prime market for the Microfluidic Multi-Surface Coagulation Assay.

3.1.2 Point-of-Care Applications

Hospitals are often early adapters of new medical devices and technology due to their established patient base and, in the case of the Microfluidic Multi-Surface Coagulation Assay, a wide potential for application. Point-of-Care (POC) testing is driven by the advantage of bringing diagnostic tests immediately to patients and providing accurate and prompt results with increased diagnostic efficiency. In the United States there are nearly 800,000 strokes and over 900,000 heart attacks annually[5, 6]. For these patients, the Microfluidic Multi-Surface Coagulation Assay can provide accurate and vital information that would allow physicians to discern the proper treatment method. Additionally, patients who have suffered from heart attacks or strokes often require follow-up tests to monitor their condition or to assist in dosage determination or adjustment.

In cases of trauma or emergency care, testing speed and accuracy is even more critical. In 2010, 45.4 million emergency room visits were the result of injuries, with 4.047 million visits resulting from motor vehicle accidents[7]. Although not all of the patients who enter the emergency room after an injury or accident will need results from this device, even if only 10-20 percent of patients needed to be tested for blood clotting, it would still amount to a demand for millions of tests in a single year.

3.1.3 Additional Applications

Further markets for the Microfluidic Multi-Surface Coagulation Assay have been identified and can be considered in the future in order to significantly increase revenue. The Microfluidic Multi-Surface Coagulation Assay has great potential for research applications with the advantage of being able to easily test blood coagulation under flow. Additional use in diagnostics is possible for patients who do not produce enough clot-forming proteins, as in the case of hemophilia. Eventually, the Microfluidic Multi-Surface Coagulation Assay target market could include physician's offices, operating rooms, and even nursing homes and other long term care facilities.

3.2 Product Requirements

The three potential market segments targeted include trauma, cardiovascular, and clinical markets. Working within these markets, the Microfluidic Multi-Surface Coagulation Assay (μMCA) targets different customers with the same goal: testing the patient-specific coagulation ability of blood samples. The customers for this product are hospitals, clinics, and pharmaceutical companies. The stakeholders are Doctors, Nurses, Physician's Assistants, Patients, and Researchers.

3.2.1 Customer Requirements

Considering the needs and requirements of the customers and stakeholders in the device is critical to the design process. Table 3.1 displays the requirements for the customers and stakeholders. Customer requirements are discovered by analyzing the voice of the customer (VOC) and the voice of the market (VOM). The requirements are displayed with their relative importance to the customer. These requirements served as a basis for design of the final product.

Customer Requirements	Product Requirements	Type	Weight (%)
Ease	Safety Simplicity of Use	FTS	15%
High Throughput	Fast Turnaround	NUD	40%

	Multiple Simultaneous Tests		
Accuracy	Reproducibility	FTS	35%
Cost	Include all materials on Chip	NUD	15%

Table 3.1 Customer requirements for the Microfluidic Multi-Surface Coagulation Assay along with the corresponding product requirements are shown. The type and weight indicate the importance of the requirement in the scope of the product.

3.2.2 Critical-to-Quality Variables

The customer and stakeholder requirements need to be translated into characteristics of the end product. Critical-to-Quality Variables are the technical requirements that need to be incorporated in the end device from the Customer Requirements.

Customer/Product Requirements	**Technical Requirement (CTQ)**	**Deliverable**
Safety	No dangerous chemicals or process steps Microfluidic Device Robotic Liquid Handler	Minimum process steps
Ease of use	Microfluidic Device Robotic Liquid Handler Diffusive Mixing	In-Unit liquid handling
Fast Reading Times	Microfluidic Device Robotic Liquid Handler	~ 5 Minutes Process Time + 5 Minutes User Time
Relevant Data	Microfluidic Device Diffusive Mixing Multiple Clotting Surfaces	Surfaces that trigger common clotting pathways
Cost Effective	Low material costs No dangerous chemicals or process steps Microfluidic Device Robotic Liquid Handler Diffusive Mixing Multiple Clotting Surfaces	
Multiple Simultaneous Tests	Microfluidic Device Multiple clotting surfaces	4 surfaces on Chip

Table 3.2 Customer and product requirements for the Microfluidic Multi-Surface Coagulation Assay are shown along with the technical requirements and deliverables that will help the product meet these

3.3 Competition

3.3.1 Standard Tests- aPTT and PT

For many years two tests have been the benchmark of coagulation testing. These tests measure activated partial thromboplastin time (aPTT) or the prothrombin time (PT). The standard test for aPTT does not measure coagulation under flow but measures the time to coagulation of citrated blood in a test tube when calcium and a coagulation activator are added.

The time measured is the time it takes until a clot is formed and is a test of the ability to clot through the intrinsic pathway. PT measures the ability of blood to clot through the extrinsic pathway in a test tube. The test for PT requires that the blood plasma be incubated at 37^{o}C before calcium is added in order to overcome the anticoagulant mixed with the blood. The test is performed with the addition of tissue factor to help observe the time to clot formation for the blood.

The combination of data from these tests offers an understanding of the clotting ability along both the intrinsic and extrinsic pathways. It is important to note that both of these tests do not occur under flow, meaning the data is extrapolated to understand how it occurs in the body where blood is constantly under flow. It is also important to note that the time the test takes is inconsistent due individual clotting ability of each blood sample.

3.3.2 Devices (Siemens PFA-100, Haemonetics TEG 5000, VerifyNow Aspirin)

The current market contains other devices that test for similar factors as the Microfluidic Multi-Surface Coagulation Assay. Table 3.3 provides a summary of the competition including prices and characteristics concerning their functionality. In designing the Microfluidic Multi-Surface Coagulation Assay it was important to assess similarities and differences between our device and the competition to provide significant improvement in design and data while observing the prices that the market is able to handle.

Siemens PFA-100

The current competition in testing blood coagulation under flow is the Siemens PFA-100 System. The PFA-100 system measures platelet dysfunction under high shear stress flow (shear rates of 4,000-5,000/s). It also is able to test for platelet adhesion and aggregation. The system

can either test blood across a collagen and ADP membrane or a collagen and epinephrine membrane. These tests require 800 µL of citrated blood and report the closure time for the clot formation in 5-8 minutes. The product function and market is the assessment of inherited, acquired, or drug-induced platelet dysfunction. The estimated price for the PFA-100 unit is about $30,000 with the cartridge price range between $30-50. Both the unit and cartridges are priced on a per contract basis when sold to a hospital or a lab[8].

AccuMetrics VerifyNow Aspirin

The AccuMetrics VerifyNow Aspirin is a point of care or laboratory device that delivers a qualitative test for the detection of platelet dysfunction. Though the device works for patients on an aspirin regimen it is not meant to diagnose the platelet dysfunction of patients with underlying platelet abnormalities or patients on a non-aspirin regimen. The system delivers data in 5 minutes about how well the blood clots when the patient is on an aspirin regimen by using an agonist to trigger the clotting pathway. The system uses an optical detection mechanism to measure the blood's reactivity to aspirin in the system. The unit itself costs $10,500 while the cartridges are offered sold in packs of 25 for $800. Like the Siemens PFA-100, there is a negotiated contract price established between the customer and the company when sold[9].

Haemonetics TEG 5000

The Haemonetics TEG 5000 Thrombelastograph Hemostasis Analyzer System is a diagnostic instrument that assesses bleeding and thrombotic risks and monitors antithrombotic therapies. Haemonetics also offers a PlateletMapping Assay that relates the potential response to antiplatelet therapy relative to the patient’s overall hemostasis. The TEG 5000 offers many different tests via their reagent kits. The processing unit is able to test two samples

independently of each other. Similar to the other two devices, the costs for the devices and materials are negotiable via contract. The estimated cost for a device is between $15,000 and $25,000 while the different materials are variable depending on the test being performed[10].

Conclusions

After comparing the Microfluidic Multi-Surface Coagulation Assay to the three main competitors on the market and the standard tests performed, the analysis shows the Microfluidic Multi-Surface Coagulation Assay (μMCA) is a better product. The functionality and costs of device versus its competition are shown in Table 3.3 below. All of the products deliver information about the ability of a patient's blood to coagulate via activation. However the μMCA is the only device that can test both the intrinsic and extrinsic pathways under flow simultaneously thanks to the four-surface design. The μMCA offers the combined data of the competition at approximately the same cost. Like the competitive device, it has a fast turnaround time from start to end of the machine; however, as it does not require incubation time, the overall time is shorter. The preparation time for the μMCA is short since the addition of blood to the cartridge and placement of the cartridge into the processing unit are the only preparation steps. The benefits of the Microfluidic Multi-Surface Coagulation Assay are discussed in the following section. The μMCA is able to offer higher functionality at industry standard costs, making the tradeoff between functionality and cost nonexistent.

Competitor	Prices	Functionality
Siemens PFA 100	~$30,000/unit ~$30-50/Cartridge	- Measures dysfunctional platelets under high shear stress. - Flows across collagen/epinephrine or collagen/ADP. But only one at a time.
AccuMetrics VerifyNow Aspirin	$10,500/Unit $32/Cartridge (Sold as: $800/25)	- Measures platelet reaction with aspirin in a cell - Qualitative test for Platelet Dysfunction
Haemonetics TEG 5000 Thrombelastograph hemostasis Analyzer System	$15,000-$25,000/Unit Various costs for cups, pipettes, and reagents	- Assesses bleeding and thrombotic risks, and monitors antithrombotic therapies - Requires Preparatory activity
Microfluidic Multi-Surface Coagulation Assay	$30,000/Unit $100/Cartridge (Full Price) $30/Cartridge (Discount/Rebate Price)	- Measures the clotting of blood on both the Intrinsic and Extrinsic pathway by flowing blood across four surfaces - 5 minutes read time - Minimal setup - Qualitative data

Table 3.3 A summary table of the main competitors of the Microfluidic Multi-Surface Coagulation Assay along with a description of their prices and functions

3.4 Advantages of the Microfluidic Multi-Surface Coagulation Assay

Distinguishing features of the μMCA for determining patient specific blood characteristics make it more efficient to analyze patient coagulation abilities and provide more diverse results. The test is consistently faster than all devices currently on the market. For example, the most similar predecessor is the Siemens PFA-100 System, which gives results in 5-8 minutes, indicating a relatively large range of times until data is produced. The Microfluidic Multi-Surface Coagulation Assay is able to produce data quicker and in a more consistent 5

minutes due to the increase in rate of fibrin accumulation due to the kaolin-lipid-collagen surface (Chapter 5). Additionally, the Siemens PFA-100 System is only capable of measuring for one feature at a time-- Collagen/Epinephrine or Collagen/ADP[16]. The Microfluidic Multi-Surface Coagulation Assay allows simultaneous measurement of both the intrinsic and extrinsic coagulation pathways by flowing blood over 4 surfaces at once.

Another significant advantage of the Microfluidic Multi-Surface Coagulation Assay is its ability to test blood coagulation under flow at arterial shear rates ($200s^{-1}$), mimicking *in vivo* flow conditions and making the results of the test more physically relevant. Other common coagulation tests are the aPTT or the PTT, both of which test coagulation in a tube rather than under flow. In summary, the Microfluidic Multi-Surface Coagulation Assay tests coagulation more quickly, in physically relevant flow regimes, and through both pathways of coagulation.

3.5 House of Quality

The House of Quality (HOQ) relates customer and overall product requirements to one another. Its purpose is to focus on different aspects of the product development process and can be created at different stages in the process. For our product, the HOQ is specifically for the concept-stage of product design. The HOQ consists of six sections. The first section is associated with customer requirements and the second is associated with the quantitative and measurable technical requirements that correspond to at least one of the customer requirements. The third section is a correlation matrix that reveals the relationship between the customer and technical requirements that can be represented either qualitatively or quantitatively and used it indicate whether or not a technical requirement directly corresponds to a customer requirement. The ‘roof’ of the HOQ is a technical correlation matrix that shows the synergies and the

conflicts between the technical requirements. Synergies represent technical requirements that change in the same direction and are denoted by plus signs, conflicts between requirements that change in the opposite direction are represented by a minus sign and for unrelated variables, no sign is shown. The fifth section shows the weighing factors for the customer requirements, which have been previously determined in the customer requirements table. The final section gives the capabilities of the competitors in fulfilling the customer requirements, but is not usually included[12]. The HOQ for the Microfluidic Multi-Surface Coagulation Assay is shown in Figure 3.1.

						1	*Multiple Clotting Surfaces*
					1		*Low Material Costs*
				1			*Diffusive Mixing*
			1				*Robotic Liquid Handler*
		1					*Microfluidic Device*
	1						*No dangerous chemical or process steps*
Customer Requirement	*No dangerous chemical or process steps*	*Microfluidic Device*	*Robotic Liquid Handler*	*Diffusive Mixing*	*Low Material Costs*	*Multiple Clotting Surfaces*	*Weight (%)*
Ease (FTS)							15
Safety	x	x	x				
Simplicity of Use		x	x	x			
Relevant Data		x	x	x		x	
High Throughput (NUD)							40
Fast Turnaround		x	x	x		x	
Multiple Simultaneous Tests		x				x	
Accuracy (FTS)							35
Reproducibility		x	x				
Cost Effective (NUD)	x	x	x	x	x	x	15

Figure 3.1 House of Quality. The HOQ relates the customer requirements to the overall product requirements. The customer requirement are highlighted in green while the technical requirements are highlighted in red.

3.6 Market Projection

The revenue of the Microfluidic Multi-Surface Coagulation Assay will be derived from the initial markets of clinical trials and point-of-care applications. Though there are many possible markets in the future, only these markets will be considered for the purpose of our analysis. After initial product development, it is assumed that the µMCA will be manufactured for 13 years. The global coagulation-testing market is projected to increase at an 8.4 percent annual compound growth rate with the United States projected to make up 43 percent of the market in 2015, so it is assumed that our target markets will grow with the overall market[10].

After time spent developing the product and following the protocol for FDA approval, the µMCA is projected to reach 10% market penetration by the end of its first year of manufacturing. Since the market is so large, market share projection of the first year of manufacturing is 500,000 units or 5 percent market share. This is expected to increase to 750,000 devices sold in the second year of production and then increase to 10% market share at 1 million devices per year in the third year of production. Our estimate is that the Microfluidic Multi-Surface Coagulation Assay will maintain this market share for the remainder of its production years based on the vast size of the market and the relatively simple manufacturing process. According to our current manufacturing assumptions of 330 days of production per year and an 8 hour work day, the number of chips produced per day starts at 1,515 in the first year and increases to 2,275 in the second and 3,030 in the third year. The estimate also assumes that the processing unit has a life of 10 years and every institution will purchase a single device and some additional revenue will be generated from sales and repair of the processing unit.

We believe that the Microfluidic Multi-Surface Coagulation Assay provides a significant improvement to any existing technology to achieve the same result and is both faster and more versatile than its competitors in the market. Microfluidic lab-on-a-chip devices are becoming more commonplace in the healthcare industry and the Microfluidic Multi-Surface Coagulation Assay has the potential to secure its place among what is quickly becoming the new standard of processing devices.

3.7 Patents

The intellectual property of the Microfluidic Multi-Surface Coagulation Assay will be limited primarily by two patents. The first is US20130065260 A1 which discusses a method for a simultaneous assay of thrombin and plasmin generation filed on November 5, 2010[13]. The second is held by Siemens Healthcare Diagnostics, US20070254324 A1 filed on April 12, 2007, which details methods for determination of platelet function under flow conditions[14]. However, neither should pose major obstacles to the Microfluidic Multi-Surface Coagulation Assay because we will file new patents with novel designs of the microfluidic chip and cartridge with four separate surfaces that is not at all covered in the two patents mentioned.

3.8 Superior Product Concept

The Microfluidic Multi-Surface Coagulation Assay has many features that give it an advantage over other competing products. The primary advantage is the fact that the µMCA can test the clotting of a single sample on four distinct clotting surfaces at once. After the double bifurcation, the blood sample flows down four separate channels and channel one is sheathed with calcium buffer and then flows over a different clotting surface. This advantage is important since it saves the physician or hospital both time and money because the patient's response to

both intrinsic and extrinsic clotting pathways (and addition clotting surfaces in the future) can be determined. If a doctor or physician only needs to wait for one test, it is advantageous for the hospital because patients can be diagnosed and treated in a faster timeframe. The µMCA will allow hospitals to see a larger number of patients which in turn will allow the hospital to increase revenue. Furthermore, insurance companies and patients will also save money by having to pay for only a single test. Compared to its leading competitors, the µMCA reduces the need for guess-and-check procedures instead of deciding between multiple coagulation tests.

Additionally, the time it takes for the µMCA to analyze a single sample is shorter than that of our competitors. Physicians can obtain more information from this one test in a shorter time than from any of our competitors. Additionally, many competitors do not test clotting under flow, which provides a more relevant simulation of what occurs in the human body and thus will give the doctor more accurate and applicable data. Our product also has the capability for customization and further development. The lengths of the channels are 4 cm, but clotting surfaces can be deposited anywhere along this channel. Further development of the µMCA could be done to include different surfaces or reagents or different diffusion lengths.

3.9 Other Important Considerations

Other concerns with regards to product design include safety and environmental concerns, especially when working with biological products that will be used for human diagnostics. The manufacturing facility must also be safe for the employees that operate the machines. Material Safety Data Sheets (MSDS) for all reagents used in our manufacturing products are referenced in Appendix 13.1 and are available upon request. None of the reagents used cause any significant environmental concerns.

3.10 Summary

Our market analysis has shown that the µMCA is an innovative new product that satisfies unmet need in the market for blood testing and diagnostics for a reliable technology to test for clotting on multiple surfaces under sheath flow. When it comes to blood coagulation measurement, speed and accuracy are essential and there will always be a need for a faster and more reliable testing method. Through the use of microfluidic technology, the µMCA can fulfill this need and has the ability to surpass all of its competitors on the market. Since microfluidic devices have been introduced to the market already, the Microfluidic Multi-Surface Coagulation Assay can highlight its advantages over existing products. We project that we will achieve sales of 1 million cartridges per year at around 10 percent market penetration, and we will receive some additional revenue from the sale of the processing unit and service contracts and fees.

According to our business model, initially we will target clinical trials and point-of-care markets for the µMCA. We will target pharmaceutical companies, biotechnology companies who both develop drugs and run clinical trials as well as hospitals, and clinical laboratories who administer point-of-care treatments. We also plan to expand to additional research and diagnostic applications in the future.

Chapter 4: Blood Chemistry

4.1 Introduction

Blood serves many functions in the body including oxygen and carbon dioxide transport as well as the transportation of waste products away from cells. One more specialized function which is relevant here is the formation of a blood clot. This process involves a complex series of reactions which is still under study today.

4.2 Coagulation Cascade

The coagulation cascade is a series of reactions that occur in the blood during a clotting event. There are two pathways to coagulation, the intrinsic pathway or the extrinsic, or tissue factor, pathway. The Intrinsic Pathway is initiated by exposure of blood to a charged surface or collagen. The Extrinsic Pathway is initiated when blood comes in contact with tissue factor. Figure 4.1 illustrates the basic reactions that proceed from an initiation event, from activated platelets to stabilized fibrin.

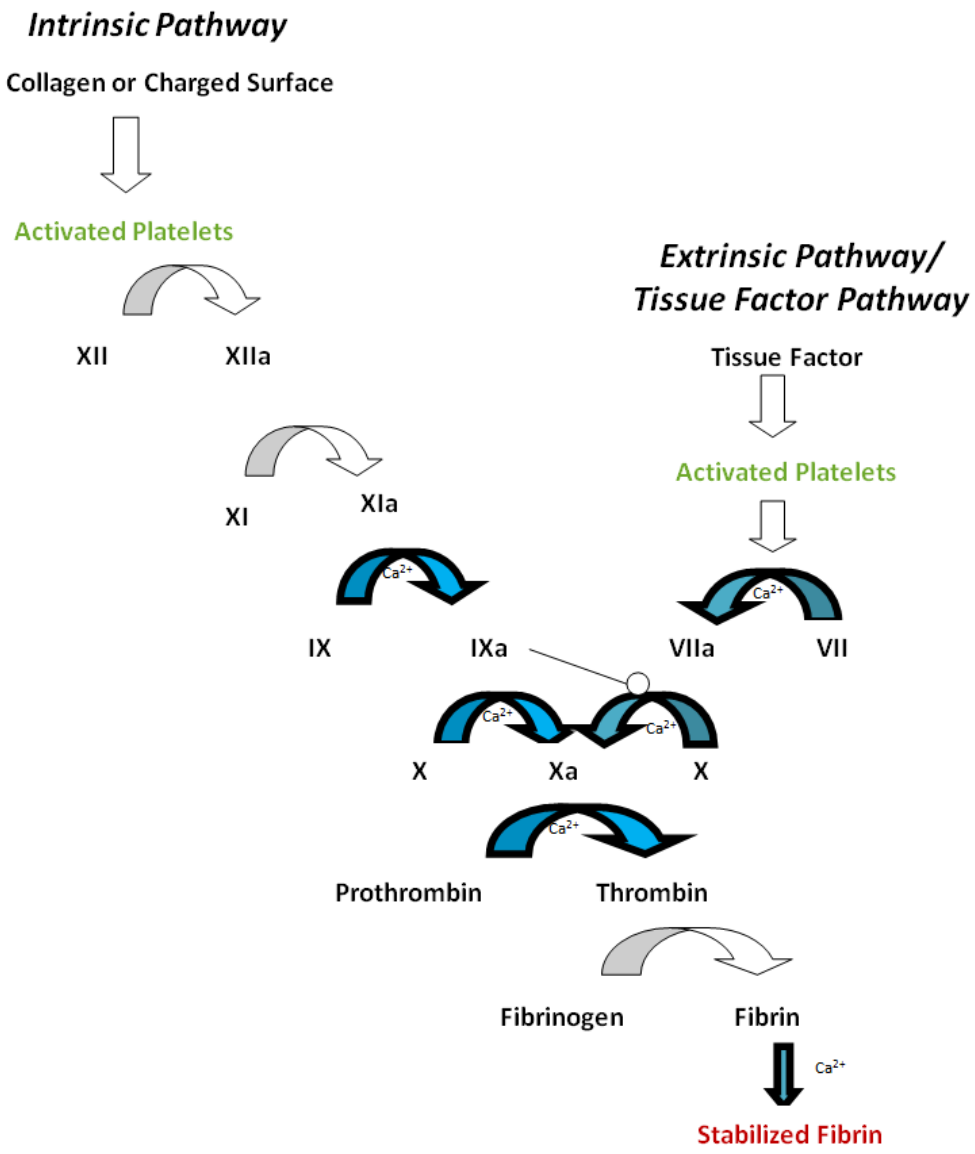


Figure 4.1 Diagram of the coagulation cascade. Both the intrinsic and extrinsic pathways are shown. Each Roman Numeral denotes a protein in the cascade, and proteins above an arrow indicate promoters of a reaction. Blue arrows highlight calcium dependent steps. Platelets are in green and stabilized fibrin is in red, indicating the colors of light under which they will fluoresce in the device.

Every protein in Figure 4.1 promotes reactions downstream of its creation. There is additionally a vast degree of positive feedback within the cascade. For example, thrombin promotes the conversion of Factor XI to XIa, VIII to VIIIa, V to Va, XIII to XIIIa, in addition to the downstream conversion of fibrinogen to fibrin. Once later stages of the cascade have progressed, clot accumulation rapidly increases. The Microfluidic Multi-Surface Coagulation Assay is designed to monitor patient specific coagulation abilities and directly measures both

platelets and fibrin accumulation on a clot-promoting surface. Typical clotting times range from 6-8 minutes, but the kaolin surface on the chip allows a faster clotting time by taking full advantage of the Intrinsic Pathway.

4.3 Coagulation and the Microfluidic Multi-Surface Coagulation Assay

An important feature of the coagulation cascade is the calcium dependence of several steps. Without calcium, these reactions cannot occur and a clot will not form. This can be taken advantage of by using a chelator to inhibit clotting when blood is drawn from a patient. Chelation of blood binds all free calcium, zinc, and magnesium ions in the blood. The blood used for the Microfluidic Multi-Surface Coagulation Assay will be chelated using trisodium citrate, as is standard practice in clinical and hospital settings and thus does not require the additional purchase of specialty coagulation inhibitors. To recalcify the blood and allow clotting to progress, blood will be sheathed with a calcium buffer. The fluids on the chip are in the laminar flow regime and as a consequence distinct layers of flow will remain and blood will remain independent of the recalcification buffer. However, small mobile calcium ions will be transported by diffusion and convection as discussed in Chapter 5 and the blood will be recalcified.

To measure the extent of clotting, fluorescent markers for platelets and fibrin will be mixed into the blood. As these proteins accumulate on the device surfaces, fluorescence levels will increase. These levels will be analyzed and compared with typical values for blood, and thus a patient's clotting abilities can be determined.

Chapter 5: The Microfluidic Multi-Surface Coagulation Assay Chip

5.1 Overview – Lab-on-a-Chip Microfluidic Devices

Lab-on-a-chip technology employs microfluidic technology in order to generate a device that integrates one or more laboratory functions into a chip whose size is on the order of square centimeters. The study of microfluidics is the study of the behavior, flow and precise control of fluids that flow through channels or other areas that are on the micro-scale or smaller. The growing field of lab-on-a-chip microfluidic technology aims to improve on and extend the potential of bioassays as it enables small-scale fluid control and analysis[2]. When compared to more traditional methods of collecting blood samples and sending them to the hospital laboratory for analysis that could take hours or days, lab-on-a-chip presents an attractive alternative that is faster, cheaper, and much less labor-intense. Other advantages of the lab-on-a-chip approach to medical devices go beyond the ease of testing and the convenience of receiving immediate results in the field. The sensitivity of the chip means that the sample size required is much smaller. The microfluidic platforms are also capable of containing a well-defined set of fluidic unit operations such as fluid transport, separation and mixing as well as providing a low-cost product for manufacturer fabrication[18].

The technology of creating fast and efficient micro-scale devices has many appealing applications. Among them include the analysis of enzyme activity and kinetics, testing of drug-protein interactions, protein expression, DNA synthesis, and membrane protein studies[2]. Outside of the medical field, microfluidic platforms have been studied for fields such as environmental monitoring of pollutants and even in basic scientific research to increase the speed of conventionally tedious and time-consuming tests[18].

5.1.1 Chip Diagram

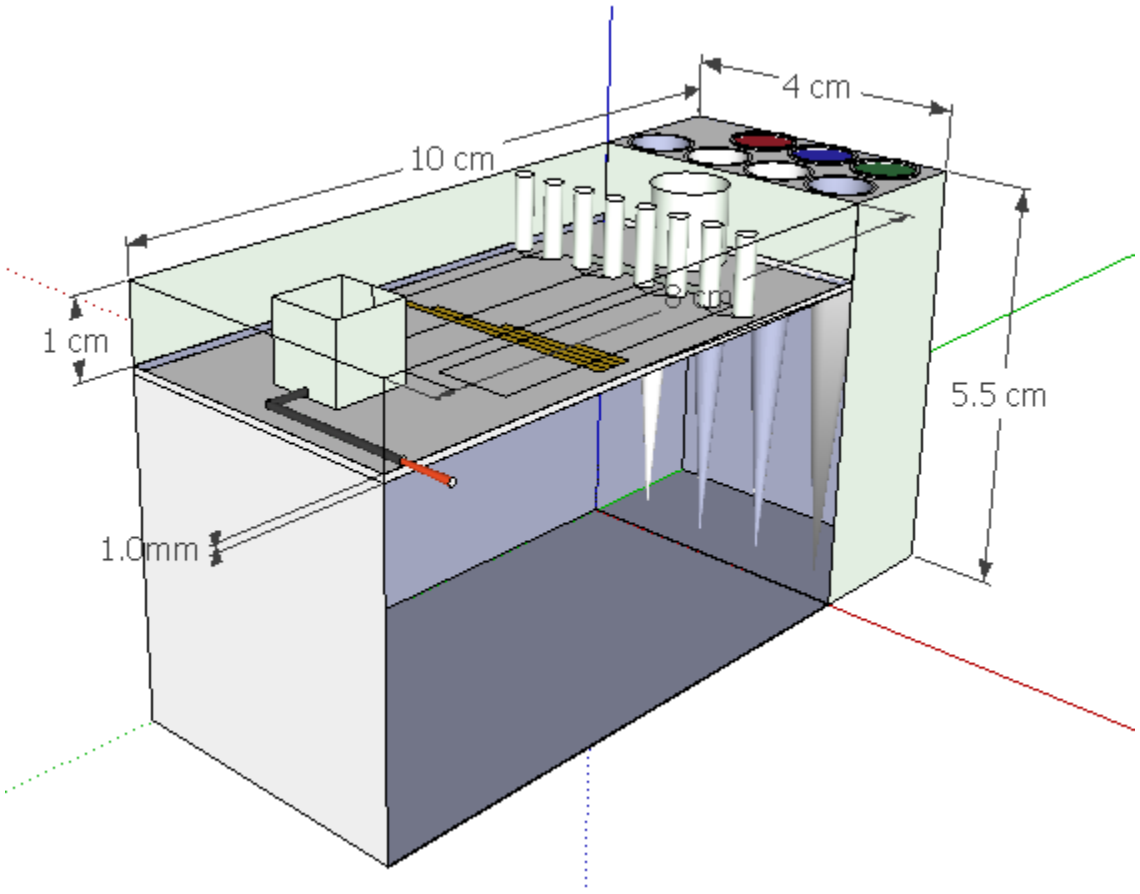


Figure 5.1 3-D model of the Microfluidic Multi-Surface Coagulation Assay device created using Google SketchUp. The cartridge and chip are both pictured with the walls of the chip and cartridge set to transparent so the microfluidic device and pipettes are visible. The red represents citrated blood, blue the dye, green the calcium buffer, and yellow the clotting surfaces.

The 3-D model of our device is shown in Figure 5.1, above. The Microfluidic Multi-Surface Coagulation Assay contains two components, the cartridge and the chip. In the coming sections of this chapter, the design of each component is explored. The principal of the design is that the doctor only needs to add 1 mL of the citrated blood sample to the cartridge before insertion into the processing unit. The device is one-use and disposable and will be sold already loaded with the reagents, clotting surfaces, and pipette tips necessary to conduct the test. After

depositing the sample into the device, the doctor then places the device into an analytical processing unit that contains the software and mechanical capabilities to automatically conduct the test. An overhead view of the device can be seen in Figure 5.2 with colors to indicate the various reagents on the device.

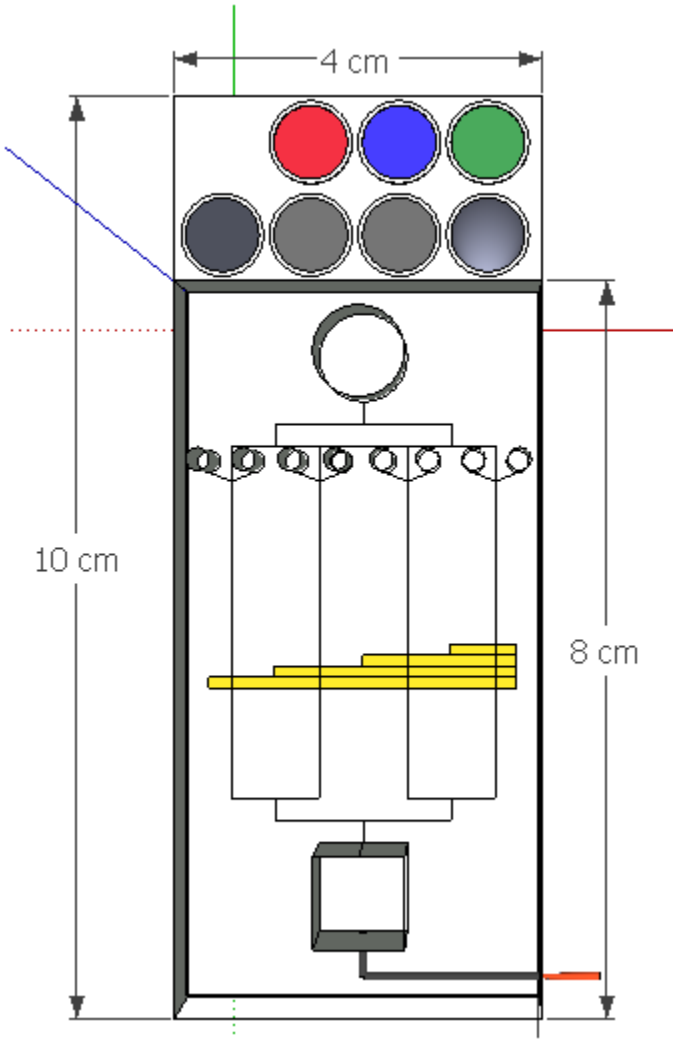


Figure 5.2 An overhead view of the 3-D model of the Microfluidic Multi-Surface Coagulation Assay device created using Google SketchUp. The red represents citrated blood, blue the dye, green the calcium buffer, and yellow the clotting surfaces. The dimensions of the chip are also shown.

5.1.2 Process Flow Sheet

The Microfluidic Multi-Surface Coagulation Assay Chip will take less than ten minutes from blood draw to production of results. These ten minutes are outlined in detail in Figure 5.2. The blue steps are actions required by the physician: blood draw, transfer of 1 mL of citrated blood onto the chip, insertion into the machine, and (roughly 6.5 minutes later), disposal of the

chip in the biohazard bin. Red steps denote liquid handler steps, including pipetting and translational motion of pipettes within the machine. The green bars denote the ‘read’ steps, at which time the machine simultaneously draws blood through the device and reads fluorescence over time.

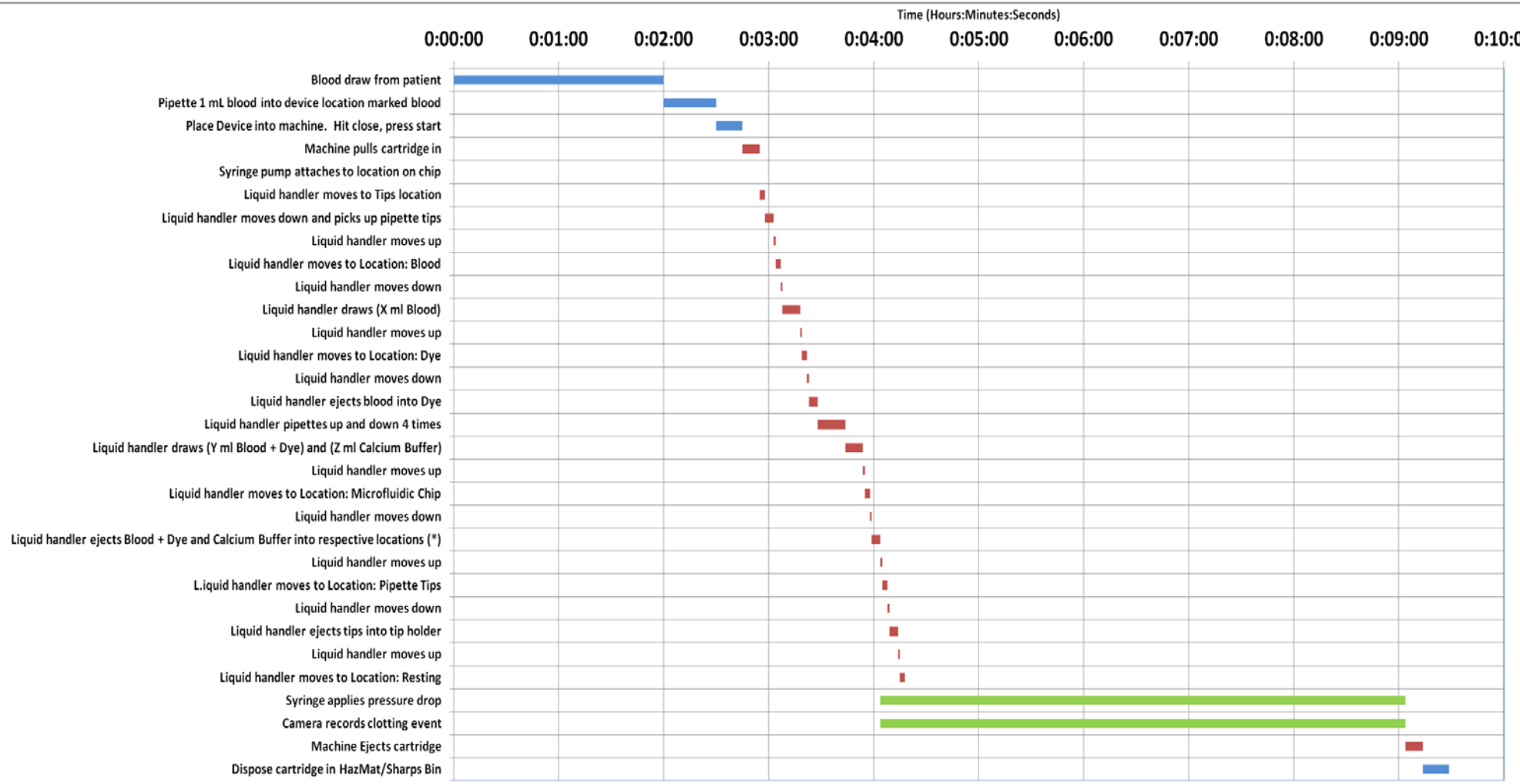


Figure 5.3 Internal Gantt Chart for the Diamond Blood Coagulator Chip steps shown in blue are tasks for the operator (doctor, physician, researcher, etc). Red steps denote jobs for the liquid handler. Green bars denote data collection by the machine, while it simultaneously applies a pressure drop to draw blood across the surfafces and reads fluorescence over time. The whole process is over in less than ten minutes, and at this time data is ready to apply.

5.1.3 Diagrams of Process on Cartridge within Processing Unit

To gain a full understanding of the Microfluidic Multi-Surface Coagulation Assay, the mechanics of how it analyzes blood clotting must be understood. Table 5.1 and Figure 5.1 show a step-by-step process of what occurs on the device from the deposition of the blood sample to when it is inserted into the processing unit until the results are completed.

Color	**Material**
Blue	Dye
Green	Ca^{2+} Buffer
Dark Grey	Empty Wells
Grey	Pipette Tip
Red	Blood
Purple	Blood + Dye Mix
Orange	Ca^{2+} Buffer + Blood + Dye
Yellow	Clotting

Table 5.1: Key for the following Diagrams of the Process on the Chip

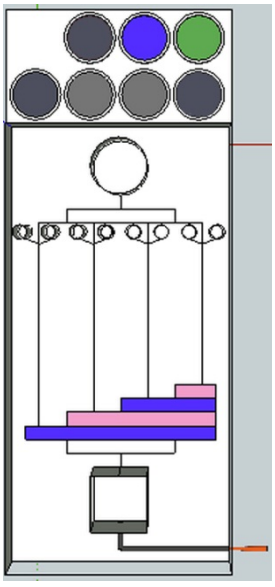


Figure 5.4a: Image of the cartridge right out of the Package

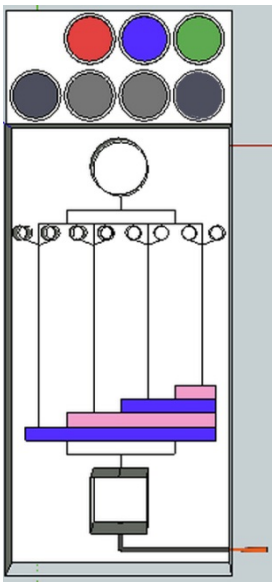


Figure 5.4b: Step 1: Add Blood to the Cartridge

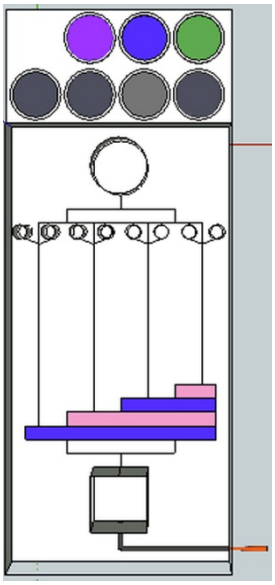


Figure 5.4c: Once in the Processing Unit the Liquid Handler picks up a pipette tip and mixes dye with the blood

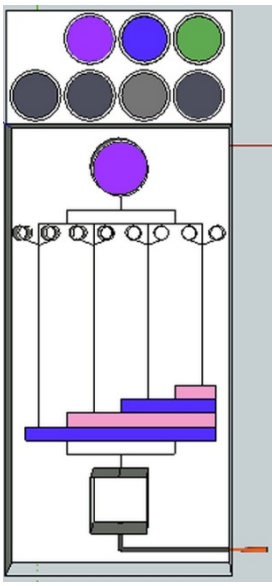


Figure 5.4d: The Liquid Handler then draws some Dye+Blood Mix and adds it to the blood well on the chip

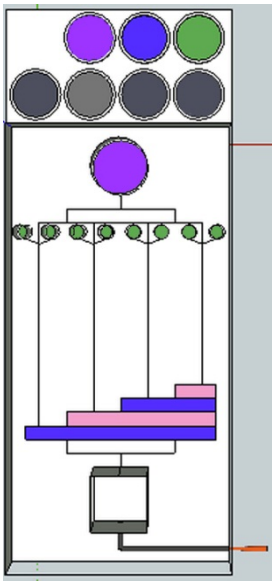


Figure 5.4e: The Liquid Handler then ejects the first tip, picks up the second. It then picks up buffer from the reservoir and adds it to the 8 smaller wells.

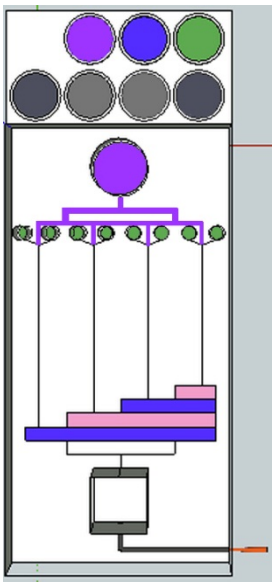


Figure 5.4f: As the Liquid Handler ejects the pipette tip in its original location; the syringe pump begins pulling, starting flow

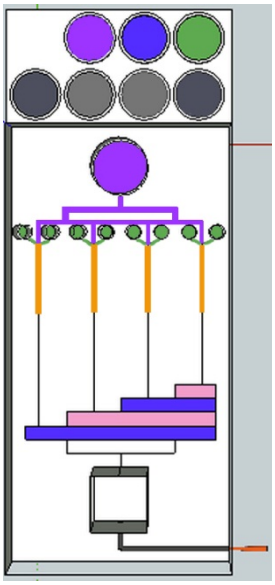


Figure 5.4g: As the liquid starts to flow, the Ca^{2+} diffuses into the Blood+Dye Mix

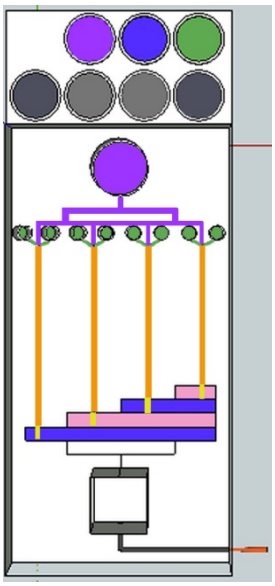


Figure 5.4h: As the calcified blood flows over the surfaces clots begin form

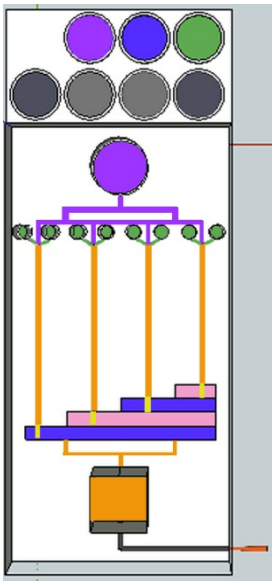


Figure 5.4i: Image of chip after process is finished. Cartridge is ejected and disposed of in a Sharps bin.

5.1.4 Flow through the Microfluidic Chip

After the citrated blood sample is injected into the cartridge and the device has been inserted into the processing unit, the blood sample is mixed with the dye and deposited onto the microfluidic chip portion of the device. Since we are testing blood clotting on multiple surfaces, the flow channel must be divided. Though our goal is only to test three surfaces, dividing the channel into three is much more difficult than dividing the channel into four by bifurcation, so it was decided that we will have four channels and test one of our existing surfaces twice and the fourth surface will be available for future testing on the device. Figure 5.5 shows the double bifurcation that occurs before the calcium buffer is added.

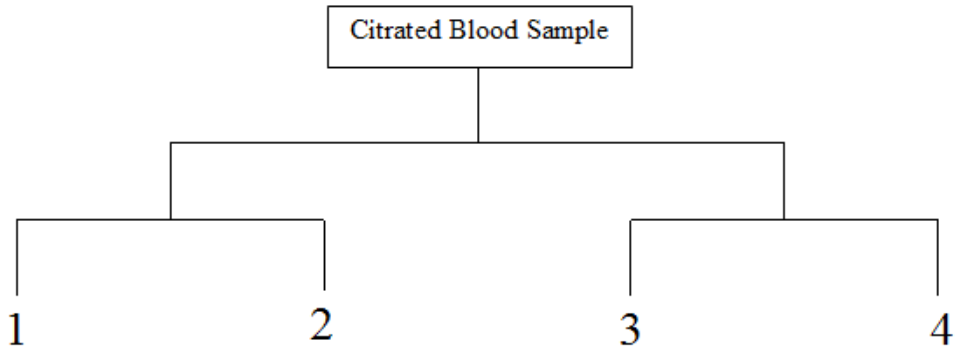


Figure 5.5 Bifurcation of the citrated blood sample. Inside the device, the blood sample is divided into four microfluidic channel, each with a different clotting surface.

The calcium buffer is added to two wells on either side of the four channels, making a total of eight calcium wells. Once the bifurcation is complete, the blood and calcium flow down the channel with calcium buffer forming a sheath around the blood and the Ca^{2+} diffusing into the blood causing recalcification, which will be modeled and examined later in this chapter. Initially, different microfluidic mixing was considered such as the herring-bone mixer and the serpentine mixer, but it was decided that diffusion would be the most simple and reliable

method of on-chip recalcification. The blood and buffer flow through the channel and over the clotting surface where cameras capture images of the blood as platelets and fibrin form. Finally, the blood and buffer mixture flows into the waste reservoir and the entire device is discarded after the test is complete.

5.1.5 SolidWorks Model for Testing

The optimal design of the microfluidic platform was explored by creating several different geometries and testing them in the lab in order to determine which satisfied the necessary recalcification requirements. The 2-D CAD software SolidWorks was used to create four different designs for the channel, as shown in Figure 5.6.

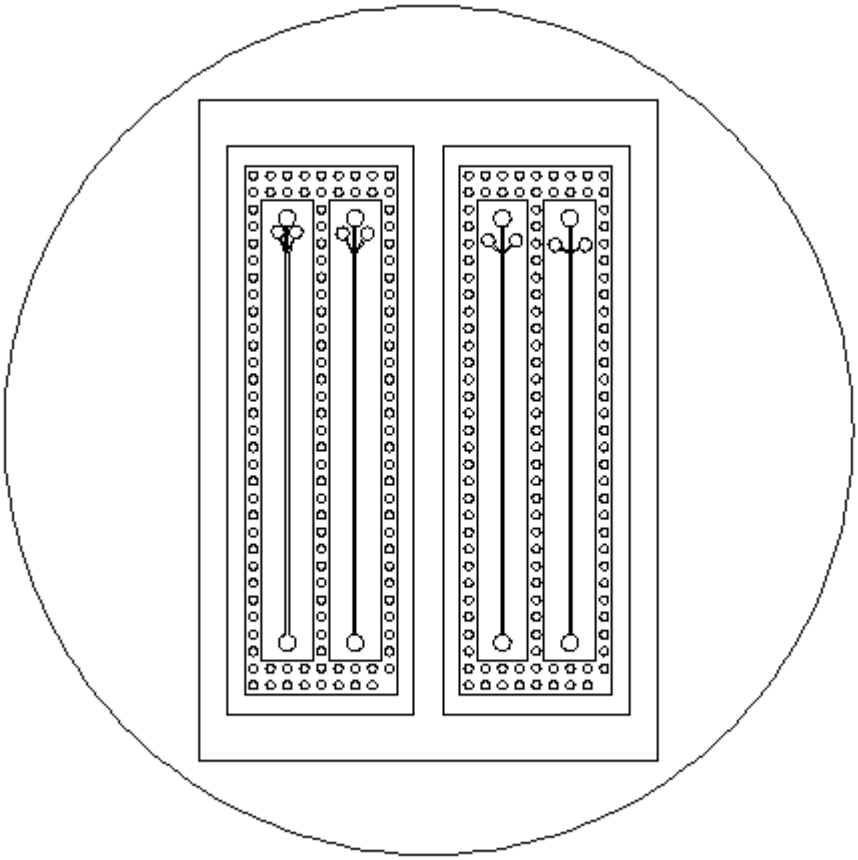


Figure 5.6. A SolidWorks design of the channels used in laboratory testing of the Microfluidic Multi-Surface Coagulation Assay. Angles vary from left to right from 20^0 to 30^0 to 45^0 to finally 60^0.

Each channel is 4 cm in length in order to allow the clotting surface to be deposited along several locations down the channel to determine what length is most optimal. Four different angles were tested: 20^0, 30^0, 45^0, and 60^0. A closer view of the four angles can be seen in Figure 5.7. The experimental process of determining the optimal design is detailed in the subsequent sections.

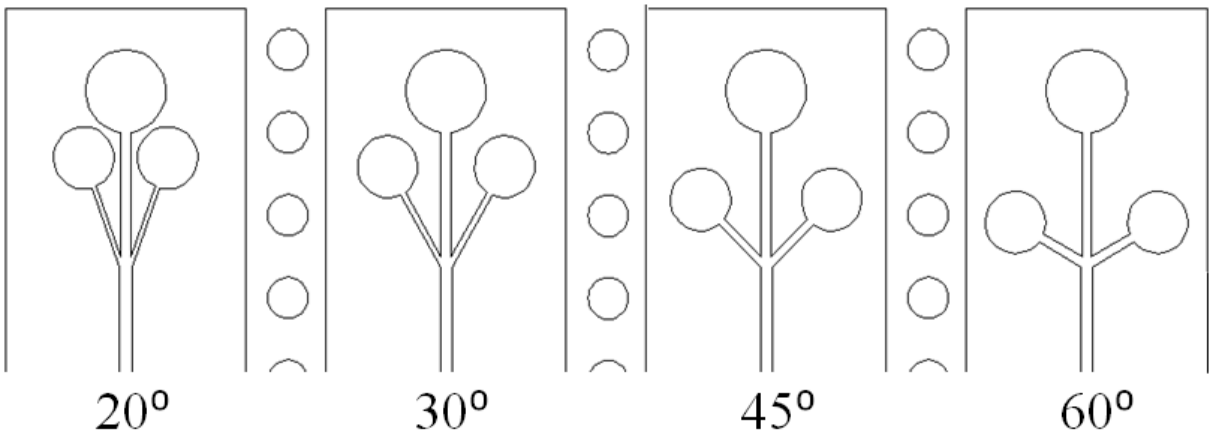


Figure 5.7 A close up of the four angles for the microfluidic diffusive mixing that were manufactured and tested in the laboratory.

5.1.6 Process Conditions and Reagent Volume

The µMCA Chip should be stored at -80°C until use to preserve the biological components of the chip such as antibodies and tissue factor. While the HEPES Buffered Saline based calcium buffer does not need to remain frozen to preserve functionality, for convenience the entire chip can be frozen at -80°C. 1 mL of citrated whole blood should be added to the chip right before placement in the machine. The process itself will occur at room temperature, and thus the chip should be allowed to reach room temperature just prior to use (20-30 minutes).

5.2 On-Chip Recalcification

To overcome the effects of chelation, blood will come into contact with a calcium buffer sheath and calcium will diffuse into the blood and allow clotting to progress. To determine how long the blood needs to be in contact with calcium to achieve a centerline concentration of 10mM in calcium ions, three methods were used. An analytical solution to the transient mass transfer equation was the first and most basic method used to give an approximate length for diffusion. Following this solution, the flow through the chip was modeled using COMSOL. Afterwards, the chip was tested in the lab to confirm our predictions.

5.2.1 Transient Diffusion in a Semi-Infinite Plane Sheet

The flow regime was modeled as a semi-infinite plane sheet, and thus diffusion in the x direction as a function of time was determined. By the time flow is well developed within the channels, concentration of calcium along the y axis, or at a point along the flow channel, can be determined using average velocity and the value of concentration at a given time. Equation 5.1 along with boundary and initial conditions in equations 5.2-4 specify the transient one dimensional mass transfer problem. Figure 5.8 provides a simplified diagram of the problem.

$$\frac{\partial^2 C}{\partial x^2} = \frac{1}{D}\frac{\partial C}{\partial t} \quad (5.1)$$

$$c(t = 0, x) = 0 \quad (5.2)$$

$$c(t, x = 1) = c_1 \quad (5.3)$$

$$\left.\frac{\partial c}{\partial x}\right|_{x=0} = 0 \quad (5.4)$$

where D= diffusivity of calcium in blood= $1.25*10^{-9}$ m^2/sec
C= concentration of calcium in the blood, mol/L
x= distance from the center of the channel, m
y= distance from mixing point in the channel, m

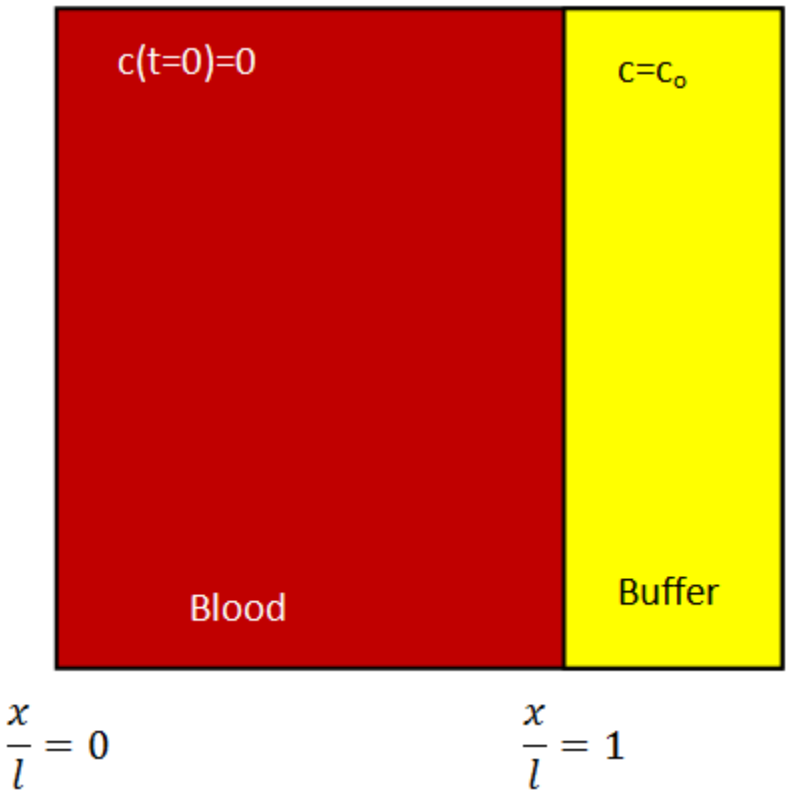


Figure 5.8 Diagram of boundary conditions applied to solve Equation 1. Blood flow region is depicted in red and the buffer flow region is depicted in yellow. Half of the flow regions are shown, as conditions are symmetric within the channel. The concentration of calcium in the blood at t=0 is defined as 0, and the concentration in the buffer is defined as a constant, c_o. The x axis increases along the bottom of the figure. Figure not drawn to scale.

To solve, it was assumed that the rate of diffusion from the right was equal to the rate of diffusion from the left. Additionally, diffusion was neglected in the y and z direction. The solution takes the form of the error function:

$$\frac{c(t)-c_o}{c_1-c_o} = \sum_{n=0}^{\infty}(-1)^n erfc\left[\frac{(2n+1)\ell-x}{2\sqrt{Dt}}\right] + \sum_{n=0}^{\infty}(-1)^n erfc\left[\frac{(2n+1)\ell+x}{2\sqrt{Dt}}\right] \quad 5.5$$

c(t)= concentration at point x in the plane sheet
c_1= concentration at x=l
c_o= concentration in the plane sheet/ buffer
l= half width of blood flow
x= location along the x axis
t= time (sec)

The result stems from the significant assumption that concentration in the buffer and thus at the interface remains constant. To mimic these results in the laboratory, a higher concentration of

calcium in the buffer can be used. The results are collected and graphed in Crank's *The Mathematics of Diffusion*[18], and reproduced here:

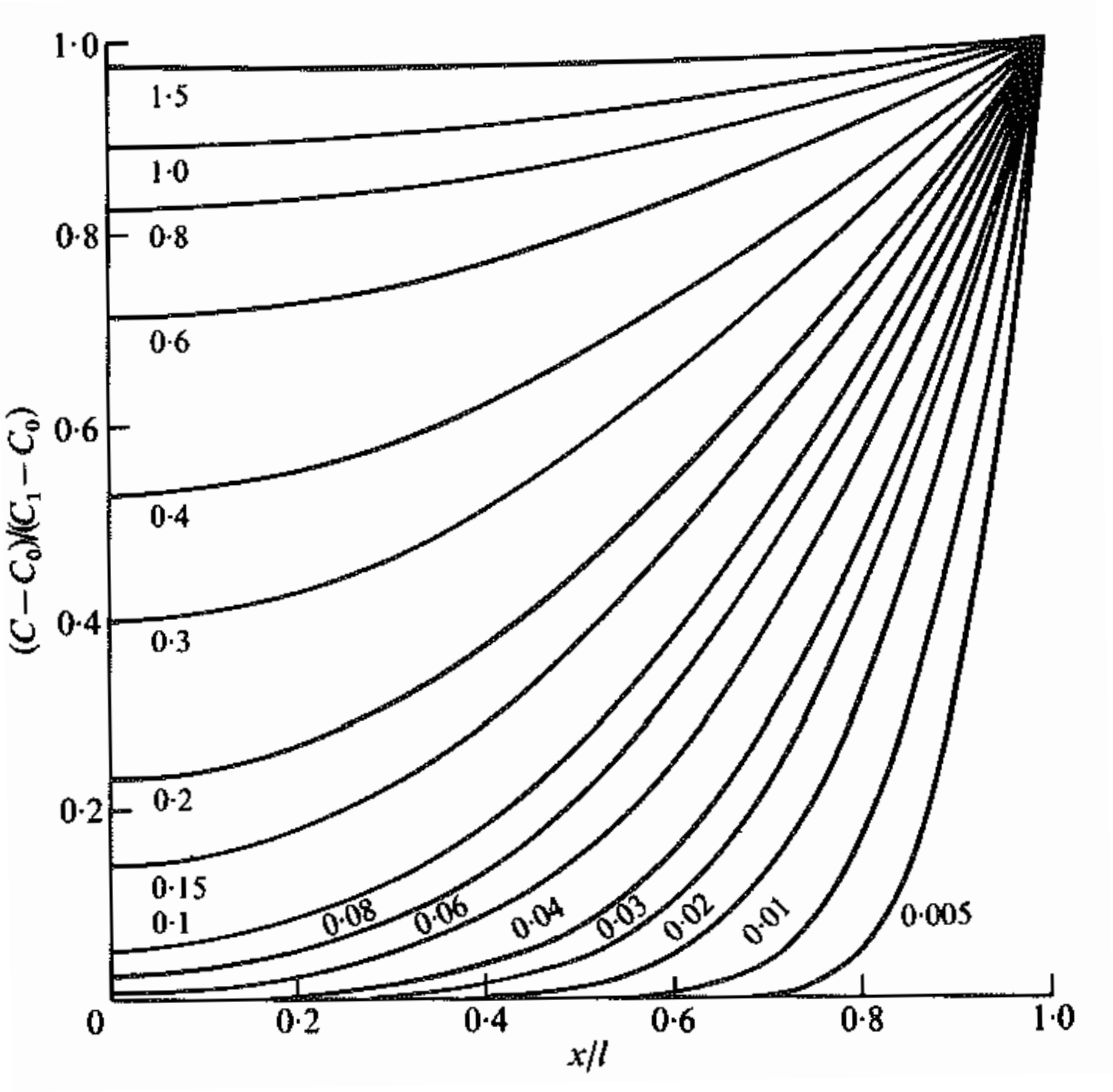


Figure 5.9 Concentration over time in a sheet with a constant source at x=l. Lines are plotted on values of Dt/l^2, and variables are defined in Equation 5.2. (Crank)

Using Figure 5.9, and assuming a buffer concentration of 20mM in calcium, it was determined that it would take 4.75 seconds to achieve a concentration of 10mM in calcium at x=0. Using a superficial velocity of 0.4 cm/sec, this equates to 1.94 centimeters of flow.

5.2.2 COMSOL

Introduction to COMSOL

COMSOL Multiphysics® is a finite element, solver, and simulation software that can be used to model and calculate different situations within a given model. The software offers many different modules that can be used individually or in a coupled system. To model the Microfluidic Multi-Surface Coagulation Assay a model of the mixing channel was imported from SolidWorks®. The microfluidic channel was modeled in 2D on SolidWorks® for different lengths of the channel in which sheath flow occurs. Design of the SolidWorks® model is discussed Chapter 6. Some of the different physics modules offered include a laminar flow model and a dilute species diffusion model. A combination of these models and the flow path from SolidWorks® were used to estimate characteristics of the model[23].

Design Strategy using COMSOL

The objective of using the COMSOL simulation was to simulate the mixing of the streams using parameters found in the analytic solution before the prototype was built. We were testing to find the optimal angle of mixing and concentration of buffer to use to compare to the prototype tests.

The analytical solutions were used as a basis for some of the parameters used in the model. A list of the parameters used for the study is located in Table 5.2. The COMSOL simulation studies the mixing of the flows without the analytical assumption of infinite concentration in the sheath flow. We however still ignore the z-axis under the two-dimensional fluid flow assumption. Using the shear rate found in the analytical solution a flow rate was determined which was set as the goal exit velocity for the cross sectional area of the device. The

estimated pressure drop we expected to use with the syringe pump was 200 Pa (1.5 mmHg). The Ca^{2+} buffer concentration was found by running various concentrations on COMSOL at the 30° mixing angle. The chosen buffer concentration 40 mM. It is a slight overestimation because we are looking to minimize costs by using the lowest concentration necessary while still bringing the centerline concentration to the required amount. Due to limitations in COMSOL in characterizing multiple fluids in the system, the viscosity and density of the both fluids were estimated as that of blood. The last parameter was the length of the flow channel. The original simulations were performed across the entire length of a 4.4 cm channel designed for lab testing different surface locations along the flow channel. As the velocity was set at the exit, we performed a second study for a shorter flow channel (2cm). This study was conducted using the COMSOL optimal mixing angle of 30^{0} and concentration of 40mM.

Parameter	Value	Units
Density	1.06	g/ml
Viscosity	3	cP
Ca^{2+} Concentration	40	mM
Mixing Angles	20, 30, 45, 60	Degrees
Target Flow Rate	8.09×10^{-5}	mL/s
Target Velocity	4.087	mm/s
Target Shear Rate	200	s^{-1}
Estimated Pressure Drop (Pa)	200	Pa
Estimated Pressure Drop (mmHg)	1.5	mmHg
Flow Channel Length	2, 4.4	cm

Table 5.2 The parameters for building a model to test recalcification on COMSOL are shown.

The centerline concentration for each of the models was found by creating an array of points along the centerline and a concentration variable (Figure 5.10b). Once the study was performed a plot graph was created showing the concentration at each of the points at its location along the flow channel. Initial data gathering was a problem as the data showed errors

due to the quality of the mesh COMSOL was using as the mathematical basis for calculations. The data presented was all studied under the "Finer" COMSOL mesh setting.

Results from COMSOL

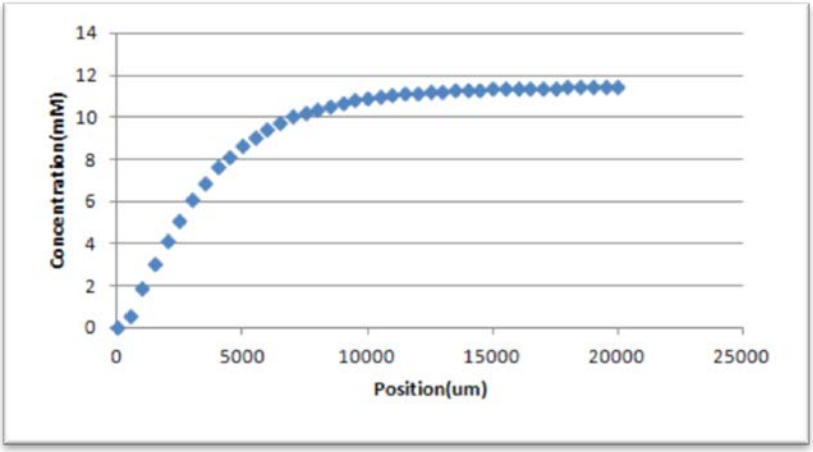
The studies observing the different angles were all tested at a buffer concentration of 40 mM. It was discovered that both the 45° and 60° mixing angles the buffer diffused extremely fast and as such caused a quick step up in concentration and continued along the flow channel at that equilibrium concentration. This is most likely caused by the angle of incidence for the larger two angles forcing the buffer stream into the blood, creating wider buffer paths (Figures 5.12 and 5.13). This is not ideal for use because it narrows the blood clotting area on the surface. The ideal mixing creates a sheath and allows the calcium to gently diffuse into the blood (Figures 5.11). The angle of incidence did not allow for as much mixing along the channel for the 20° mixing angle. Thus 30° was found to be the optimal angle according to these COMSOL simulations.

Angle (degrees)	Center Line Concentration	Distance to 10mM Center Line Concentration
20	8.5 mM	Does Not Reach
30	11 mM	16 mm
45	26 mM	Immediately Higher
60	31 mM	Immediately Higher

Table 5.3 Comparison of Center Line Concentrations at the Different Mixing Angles. Parameters: 40 mM Initial Concentration

The simulation testing the 2 cm flow channel showed that the correcting the length of the flow channel reduced the distance to the 10mM centerline concentration calculated in the analytical section. With the 30° mixing angle and 40mM Ca^{2+} buffer the centerline concentration reaches and stays at the required centerline concentration for a short amount of time. This was found to be optimal design studied on COMSOL.

a)



b)

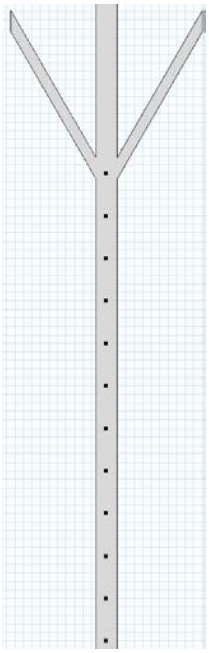


Figure 5.10: a) Graphs of calcium concentration vs. position in micrometers for 30° mixing angle, 2cm Flow channel , and 40 mM inlet buffer concentration. b) COMSOL model of 30° mixing angle and 2 cm flow displaying collection data points along the y-axis.

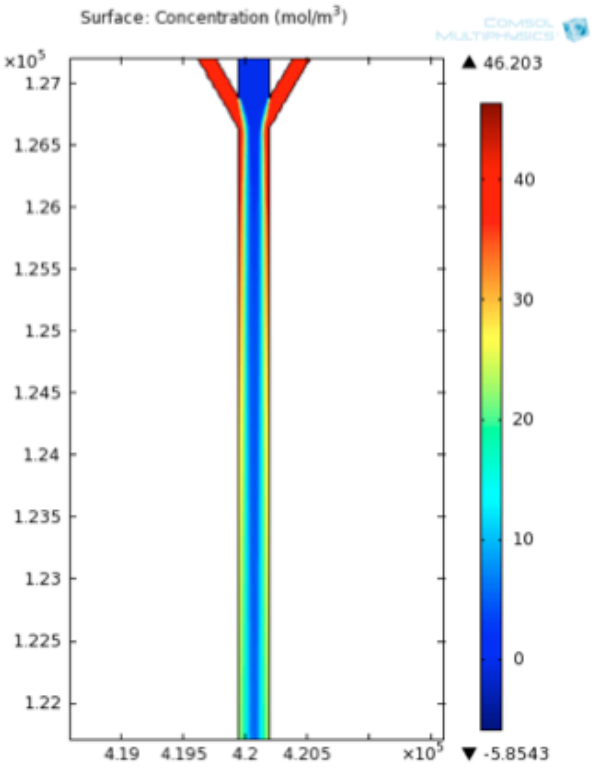


Figure 5.11: COMSOL Simulation of mixing at 30° and 40mM Inlet Concentration

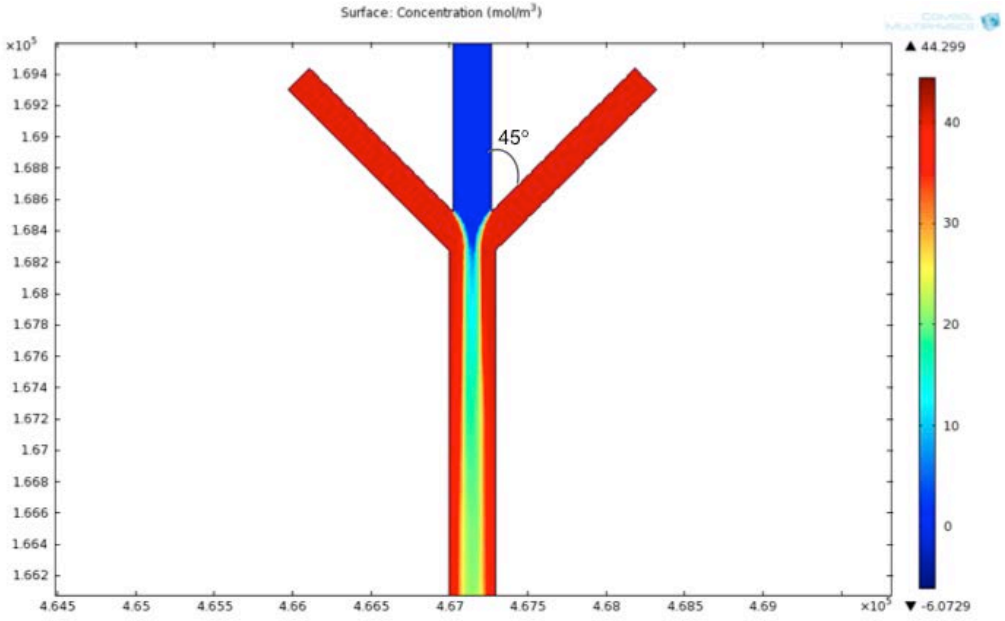


Figure 5.12: COMSOL Simulation of mixing at 45° and 40mM Inlet Concentration

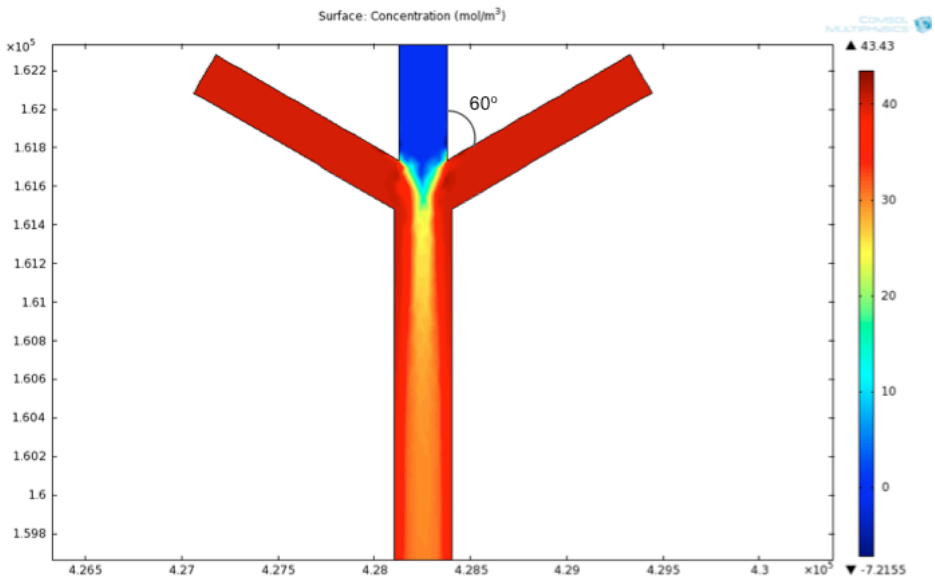
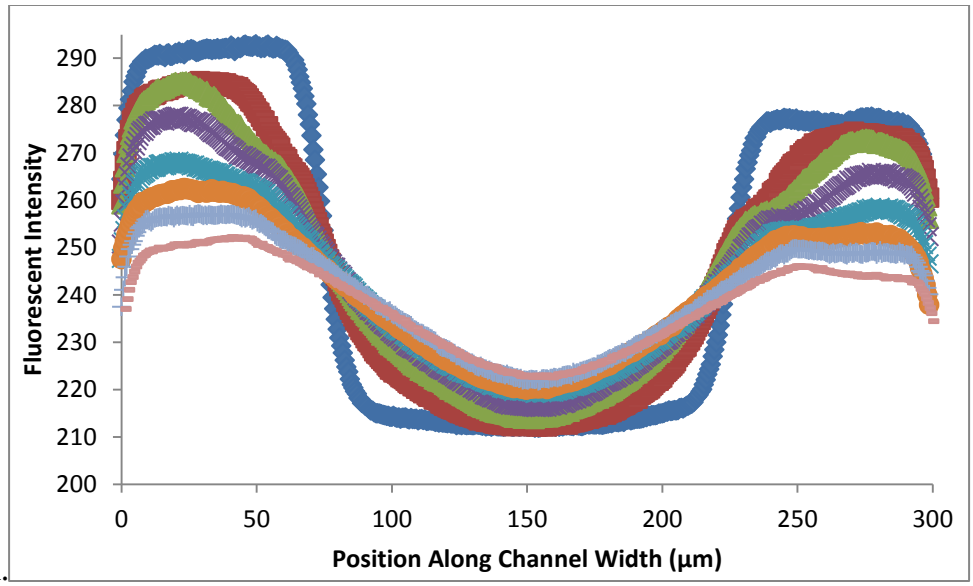


Figure 5.13: COMSOL Simulation of mixing at 60° and 40mM Inlet Concentration

5.2.3 Lab Tests and Results

The first test for on-chip recalcification was run using a fluorescent dye in the buffer in lieu of calcium, since it would be easiest to confirm functionality in preliminary experiments. The diffusivity of the fluorescent marker was slightly lower than that of calcium, so diffusion would most likely be understated. Additionally, the viscosity of the buffer was not adjusted to that of blood in this experiment, so for this experiment the viscosity of blood is 3 cP and the viscosity of the buffer is 1 cP. Figures 5.14a and 5.14b show the fluorescent intensity as a function of position along the channel. The earliest time points are in darker colored lines, and the latest time points are lighter. The images in Figure 5.14 are from studies using blood and buffer flow rates of 1.91 μL/sec. Both 20° and 60° angles of incidence between blood and buffer were tested. It is clear from Figure 5.14 that the fluorescent dye reaches a more even concentration over time with the smaller angle of incidence.

a.



b.

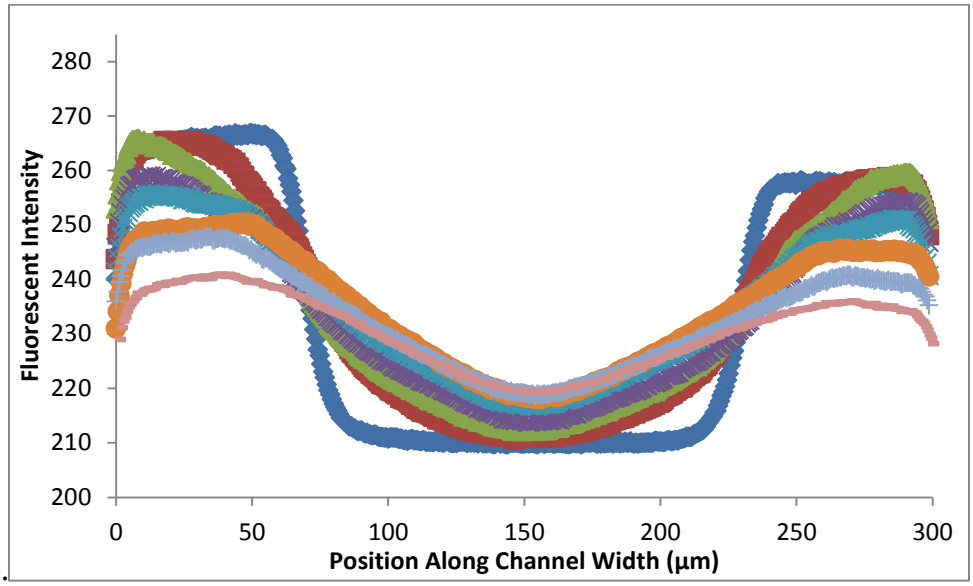


Figure 5.14 a) Fluorescent intensity versus position along the microfluidic channel width over time for a 60° angle of incidence between buffer and blood. b) Fluorescent intensity versus position along the microfluidic channel width over time for a 20° angle of incidence between buffer and blood. For both images, changes in position along the channel are shown by fading of the lines, ie., the smaller distances are shown darkest and the later times are the lightest in shading.

Figure 5.15 plots the percent of inlet concentration against the distance in millimeters along the channel for different ratios of blood to calcium buffer, with a constant blood flow rate of 1.91 µL/sec. The values for percent of inlet concentration were determined at the center of the channel using level of fluorescence. It is clear that as the ratio of blood to buffer decreases,

the center of the channel reaches a larger percent of the inlet concentration in a shorter distance-- i.e., a shorter time. Since in the Microfluidic Multi-Surface Coagulation Assay chip, calcium will replace the fluorescent marker, it is expected that in the next experiment centerline concentration will increase to the same levels in a shorter distance.

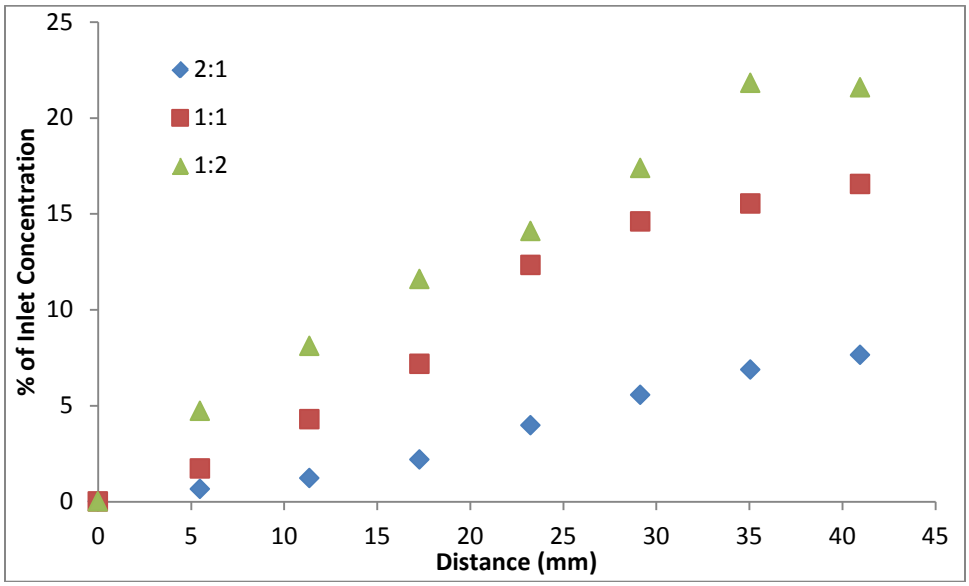


Figure 5.15 Percent of inlet concentration of fluorescent marker at the center of the channel versus distance along the channel. Ratios denote the ratio between blood flow rate and fluorescent buffer flow rate, with a constant blood flow rate of 1.91 μL/min and at a 20° angle of incidence between the blood and the buffer.

The width of blood in the channel varied with the ratio of blood to buffer, and similar trends are see between this blood width and the ratio. As the width of blood in the channel decreases, the rate at which the centerline concentration as a percentage of the inlet buffer concentration changes goes down. This change is plotted in Figure 5.16, and both 20° (small) and 60° (large) angles of incidence are shown.

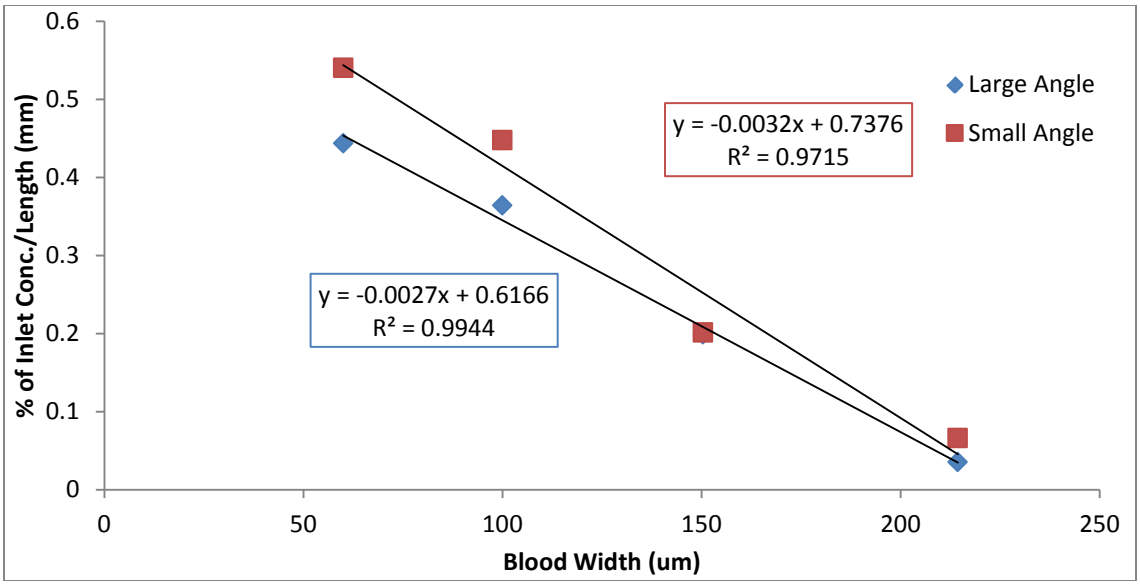


Figure 5.16 Percent of inlet concentration of fluorescent marker per length in millimeters versus width of blood in channels. As blood width in the channel decreases, the rate at which the fluorescent marker diffuses into the blood decreases. Channel width for all measurements is 300 μm.

Instead of calcium in these preliminary experiments, fluorescein isothiocyanate (FITC) was mixed into the buffer and its fluorescence along the channel monitored at different lengths along the channel to determine efficacy of the design. Figure 5.17 displays the results, with each line representing a different length for diffusion of FITC. The times for the data points, determined from a flow rate of 4.09 mm/s, range from 0 through 10 seconds, in intervals of approximately 1.5 seconds.

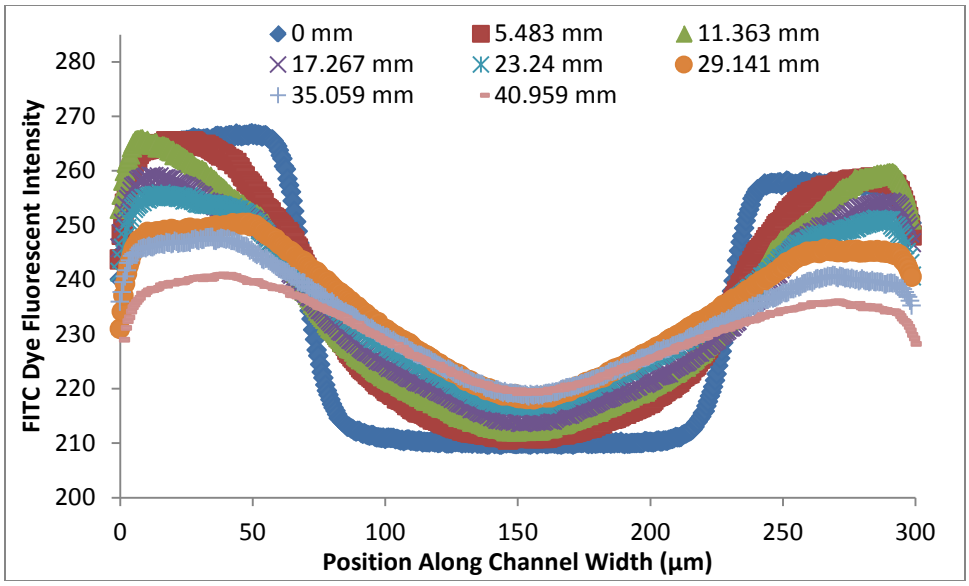
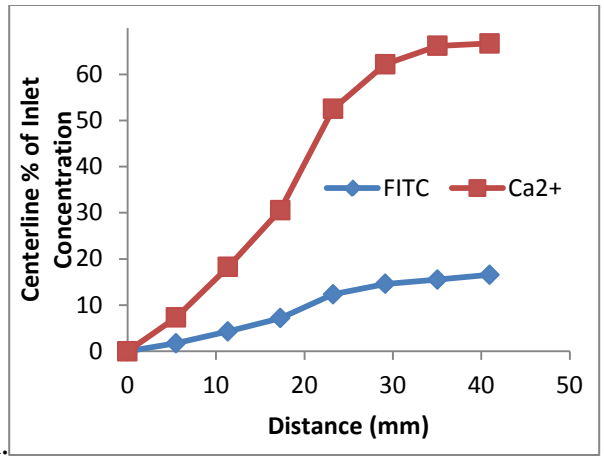


Figure 5.17 Fluorescent intensity versus position along the microfluidic channel for different diffusion distances for the 45° angle of incidence between buffer and blood.

Figure 5.18(a) adjusts the diffusion of FITC to model and predict the diffusion of calcium within the Microfluidic Multi-Surface Coagulation Assay. The diffusion was adjusted for different values in 0

diffusivity, since the diffusivity of calcium is much higher than that of FITC. Figure 5.18 (b) predicts the centerline concentration of calcium assuming a 30 mM calcium buffer. According to the model, the centerline concentration will reach the desired minimum of 10 mM in a distance of about 20 mm. The results are consistent with the analytical methods for contact length, with the adjustment of buffer concentration to more closely mimic a 'constant' concentration at the interface.

a. 

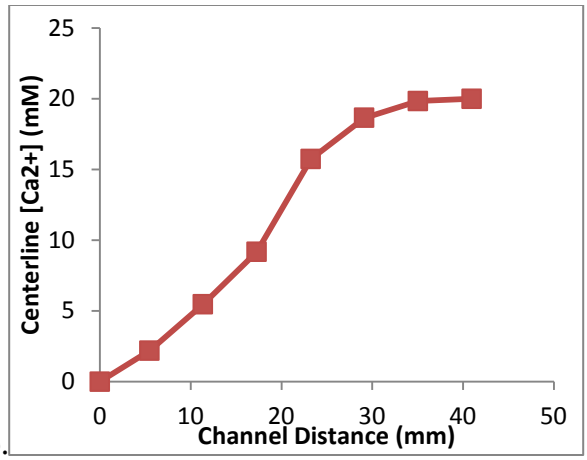
b. 

Figure 5.18 (a) Centerline percent of inlet concentration of FITC or Ca^{2+} versus distance from the point of intersection between buffer and blood. (b) Prediction for centerline concentration of Ca^{2+} in mM versus distance along the channel, determined using a 30 mM calcium buffer for reference.

To determine the optimal length for which the blood should be in contact with the calcium buffer, another experiment was done. The buffer was mixed with methyl cellulose to increase the viscosity to that of blood (3 cP). Blood was flowed through the channels with an angle of intersection of 45°, and 8 different microfluidic devices were created with collagen strips placed at different distances from the point of intersection. Figure 5.19 a and b show the platelet and fibrin fluorescent intensity, respectively, over time in minutes for different calcium diffusion distances to collagen surfaces using a 30 mM calcium buffer. Figure 5.19 c and d show the platelet and fibrin fluorescent intensity, respectively, over time in minutes for for different diffustion distances to collagen surfaces using a 0 mM calcium buffer-- the control. While the response for the 30 mM buffer is highest for the 35.237 mm diffusion distance, it is not necessary to achieve this level of fluorescence or, in the case of the Microfluidic Multi-Surface Coagulation Assay, calcium concentration. Based on our simulations and subsequent lab testing, our design uses a 2 cm diffusion distance between the point of intersection and the clotting surfaces. The control confirms that the clotting response is due to the calcium diffusing into the blood over the diffusing distance.

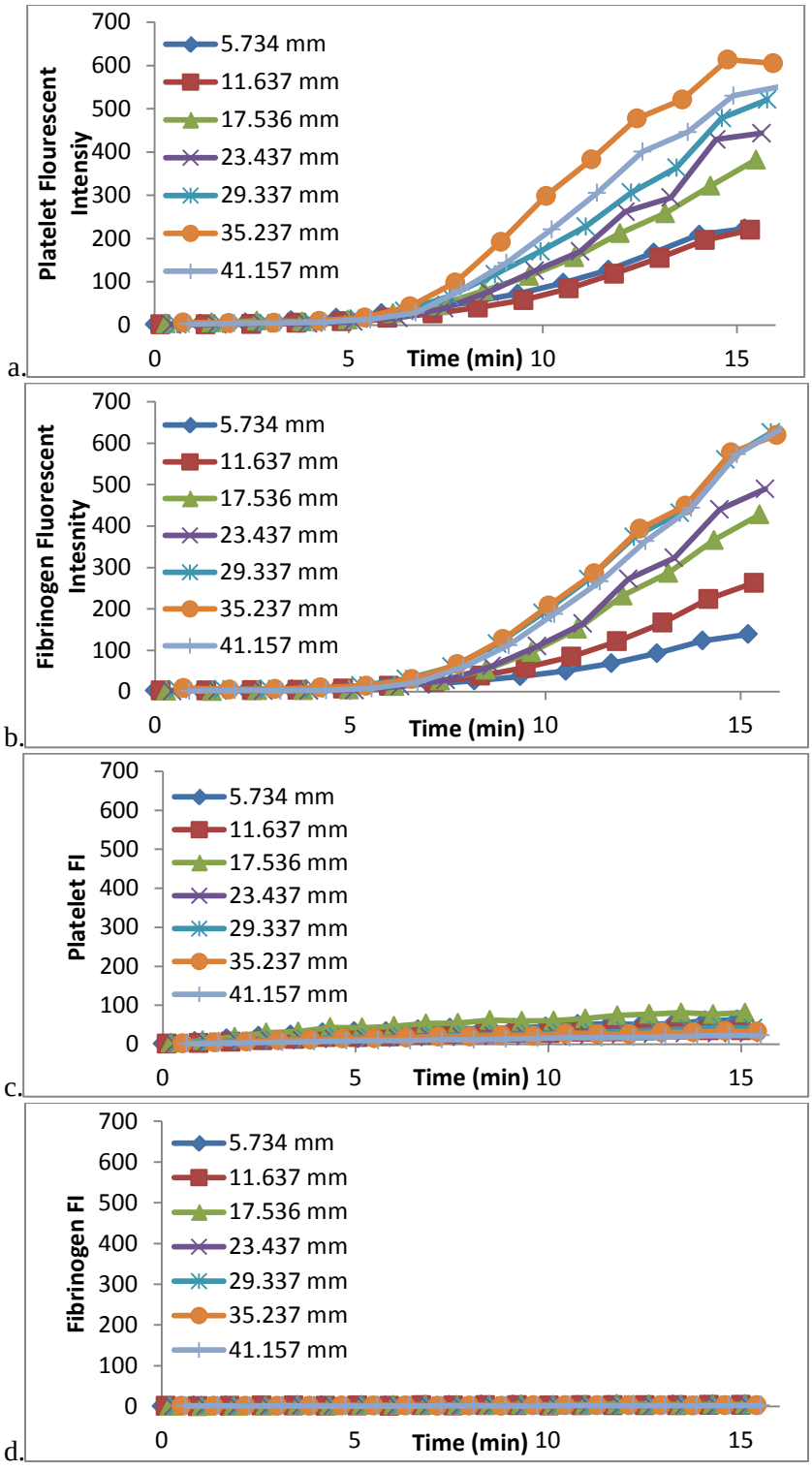


Figure 5.19 (a) Platelet fluorescent intensity versus time of flow in minutes of blood over collagen surfaces at various lengths along microfluidic channels using 30 mM calcium buffer. (b) Fibrinogen fluorescent intensity versus time of flow in minutes of blood over collagen surfaces at various lengths along microfluidic channels using 30 mM calcium buffer. (c) Platelet fluorescent intensity versus time of flow in minutes of blood over collagen surfaces at various lengths along microfluidic channels using 0 mM calcium buffer. (d) Fibrinogen fluorescent intensity versus time of flow in minutes of blood over collagen surfaces at various lengths along microfluidic channels using 0 mM calcium buffer.

Figure 5.20 plots the time in seconds for 25% of maximum fluorescent intensity (red and blue lines) and the calcium centerline concentration in mM prediction versus the distance from the point of intersection along the channel in millimeters. The minimum time to 25% max fluorescence is apparent around 35 mm along the channel. Again, the maximum level of fluorescence is not required in the actual device and the data for 20 mm along the channel is adequate for the Microfluidic Multi-Surface Coagulation Assay.

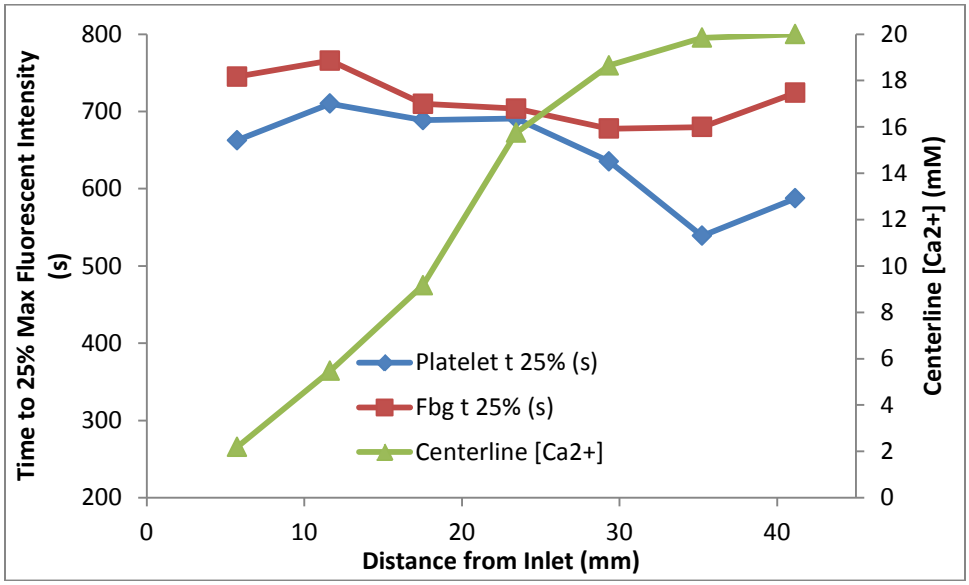


Figure 5.20 Time to 25% of maximum fluorescent intensity in seconds (red and blue) and centerline Ca^{2+} concentration in mM (green) versus distance from the point of intersection in mm for platelets (blue), fibrinogen (red), and calcium prediction from Figure 5.14 (green).

Figure 5.21 displays an image from the computer in the lab showing side by side the fluorescence of fibrinogen and platelets on the collagen surface for 45° (left) and 20° (right) angles of incidence between blood and calcium buffer. The twenty degree angle of incidence provided a more vivid clotting event on the collagen surface, indicating better calcium diffusion which leads to a more fluorescent clot.

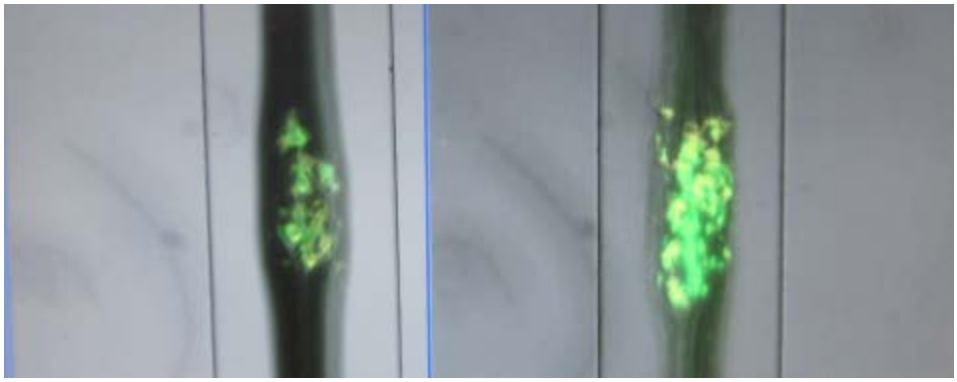


Figure 5.21 Platelet and fibrinogen accumulation on collagen surface for 45° (left) and 20° (right) angles of incidence between calcium buffer an citrated blood. It is clear that the 20° angle of incidence promotes better diffusion of calcium into blood as more fluorescence (clotting) is visible.

Figure 5.22 a displays an image of the intersection between the blood and the Ca^{2+} buffer for a 20° angle of intersection. Figure 5.22b shows the downstream clot on a collagen and tissue factor surface after 10 minutes of flow and 22.3 mm downstream from the intersection. High levels of fluorescently marked platelets and fibrinogen have accumulated on the clotting surface by this point, which would not have occured without the calcium buffer, as demonstrated in Figure 5.23. Figure 5.23 shows the control 0 mM calcium buffer on the left hand side with the 30 mM buffer on the right hand side. No significant clotting occured in the control, indicating that the cause of the platelets and fibrin accumulation on the clotting surface is due to the presence of calcium in the buffer.

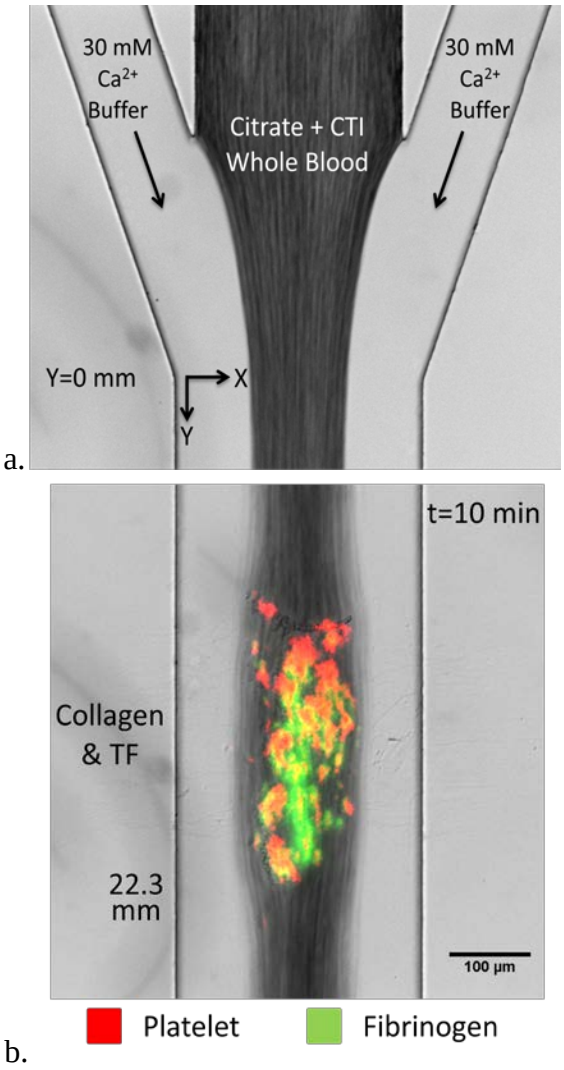


Figure 5.22 a) Magnified image of intersection between citrated blood sample and calcium buffer at a 20° angle of incidence. b) Image of clotting surface (collagen and tissue factor) downstream of the intersection after ten minutes of flow. Clotting surface is placed 22.3 mm from the intersection. Since clotting occurs, calcium diffusion by sheath flow was a success.

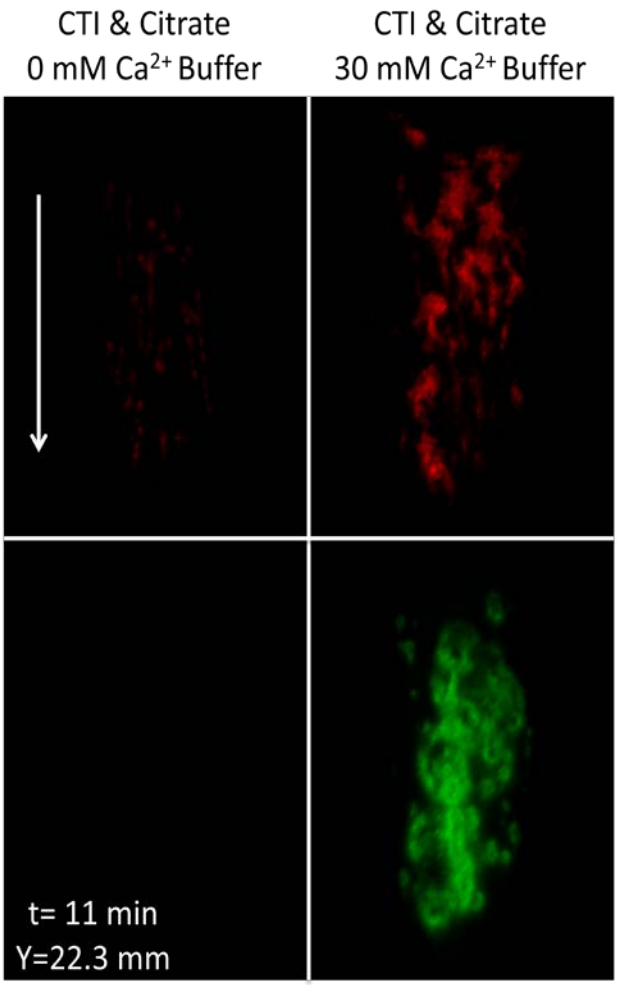


Figure 5.23 Accumulation of platelets (red) and fibrinogen (green) after 11 minutes of flow over collagen clotting surface 22.3 mm downstream of the location of incidence between the calcium buffer and citrated blood with CTI, a Factor XII inhibitor. Blood was sheathed with a 0 mM (left) or a 30 mM (right) calcium buffer with a 20° angle of incidence between the streams. It is clear that the calcium is the source of the high platelet and fibrinogen levels on the right hand side,l since the 0mM Ca^{2+} control results in no coagulation on the collagen surface.

To further demonstrate the success of our model and its functionality, Figure 5.24 provides a visualization of the diffusion within the buffer-sheathed blood. Instead of calcium, Texas Red Dye is mixed into the buffer in the sheath stream to allow observation of the diffusion in the channel. Snapshots of regions within the flow streams are shown at the point of intersection and at distances of 23.4 and 41.1 mm from this point of intersection. The result mimics that of the diffusion modeled in COMSOL and shown in Figures 5.11-13. Based on our

experiments, we can say with confidence that this method for on-chip recalcification of citrated blood will work in the context of our device.

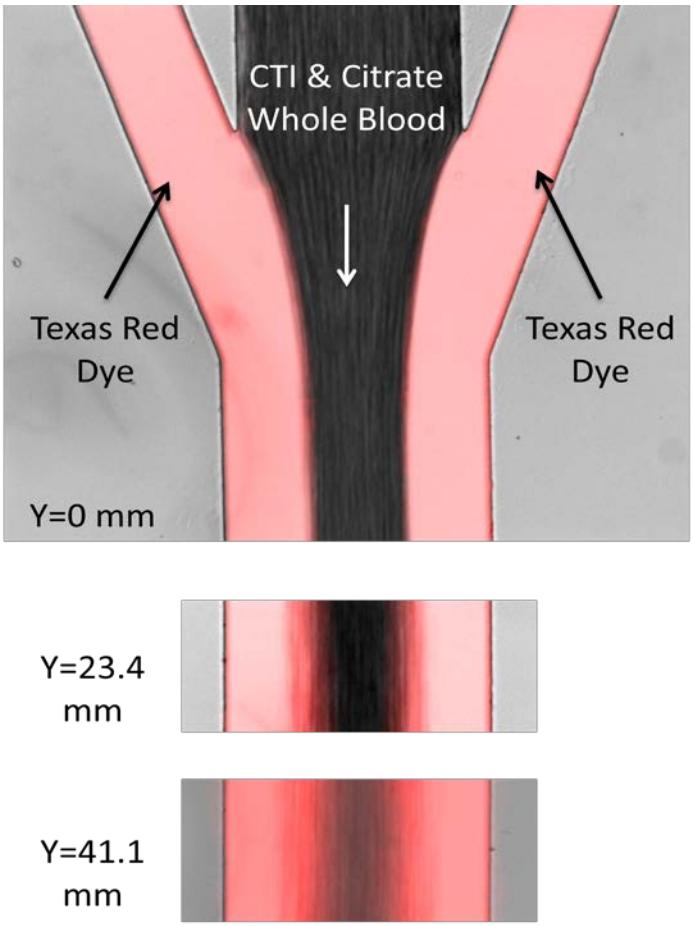


Figure 5.24 Visualization of diffusion of Texas Red Dye into a stream of citrated whole blood with CTI. The diffusion of the red dye appears to follow similar patterns as seen in the COMSOL simulation, Figures 5.11-13. At a distance of 23.4 mm, dye appears to have reached the center of the stream. By a distance of 41.1 mm, dye concentration is high throughout the stream of blood. Since the diffusivity of this Texas Red Dye is lower than the calcium in the buffer, calcium diffusion is expected to occur more quickly within the channel.

5.3 Device Design

When considering the design of the device, each component is first considered separately and then they are considered in the scope of the entire design. The focus of this section will be on the design of the cartridge and its components as well as the composition of the clotting surfaces. For the cartridge, the number of pipettes and the locations of the reagent reservoirs were considered with respect to how the device will operate inside the processing unit. For the chip, the optimal clotting surfaces are determined through experimentation. The exact design of the microfluidic chip that is inserted into the cartridge of the device will be detailed in Chapter 6.

5.3.1 Chip Holder (Cartridge)

The cartridge is the plastic portion of the device that contains the pipette tips used by the processing unit and both the calcium buffer solution and the dye solution. It is also the place where the physician or technician will deposit the blood sample into the device in order to analyze it. Inside of the processing unit, a liquid handler mixes the blood with the dye and also deposits both the blood-dye mixture and the calcium buffer into the appropriate wells. Initially we began our design of the cartridge with the idea of having a robotic liquid handler that would only need to move in two directions. But this approach led to a cartridge that was too long with a lot of wasted space. The current design uses space the most efficiently, but requires the robotic liquid handler to move along all three directional axes. Besides providing the reagents and the pipette tips, the cartridge also offers structural support for the chip of the device and is where the physician or technician will primarily handle the device so as to prevent disturbance of the more sensitive microfluidic component.

5.3.2 Tips on Cartridge

The number of tips inside of the cartridge depends on the number of liquid handling steps involved with the analysis. The first liquid handling step involves mixing the blood with the dye and in the same motion, depositing the mixture onto the microfluidic chip. The second liquid handling step is the deposition of the calcium buffer into the wells surrounding the blood channels. Since only two liquid handling steps are required, it is possible to have a cartridge with only two pipette tips loaded. However, because pipette tips are relatively inexpensive, a total of four pipette tips are loaded into the device in case one of the liquid handling steps is not successfully completed.

5.3.3 Fluorescent Antibody, Calcium Buffer

Within the 'dye' well will be a mixture of fluorescent antibodies that target platelets and fibrin. As platelets and fibrin accumulate on the clotting surface during flow, these antibodies will accumulate on the proteins. Light at the excitation wavelength of the respective fluorescent antibodies will be shone on the surfaces in quick succession. The level of fluorescence will be determined and the extent of platelet and fibrin accumulation on the surface will be measured, and finally the extent of coagulation can be determined.

The calcium buffer for recalcification will be 30 mM, allowing excess in calcium to increase its diffusion into the blood. The buffer itself will be mixed into HEPES-buffered saline, a mixture of sodium hydroxide, sodium phosphate dibasic dihydrate, HEPES, and ultrapure water. This can be purchased premixed from companies such as Sigma Aldrich, but is relatively simple and inexpensive to make from its components.

5.3.4 Diagram

Figure 5.25 shows the cartridge in relation to the chip of the device. The chip is mounted onto the cartridge during the manufacturing of the device.

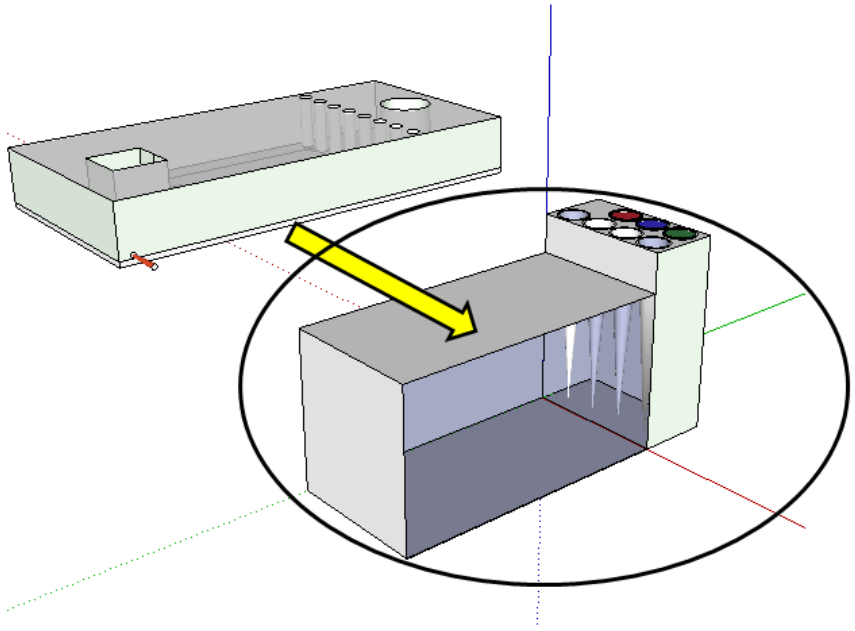


Figure 5.25 3D model of the mounting of the Microfluidic Multi-Surface Coagulation Assay onto the cartridge

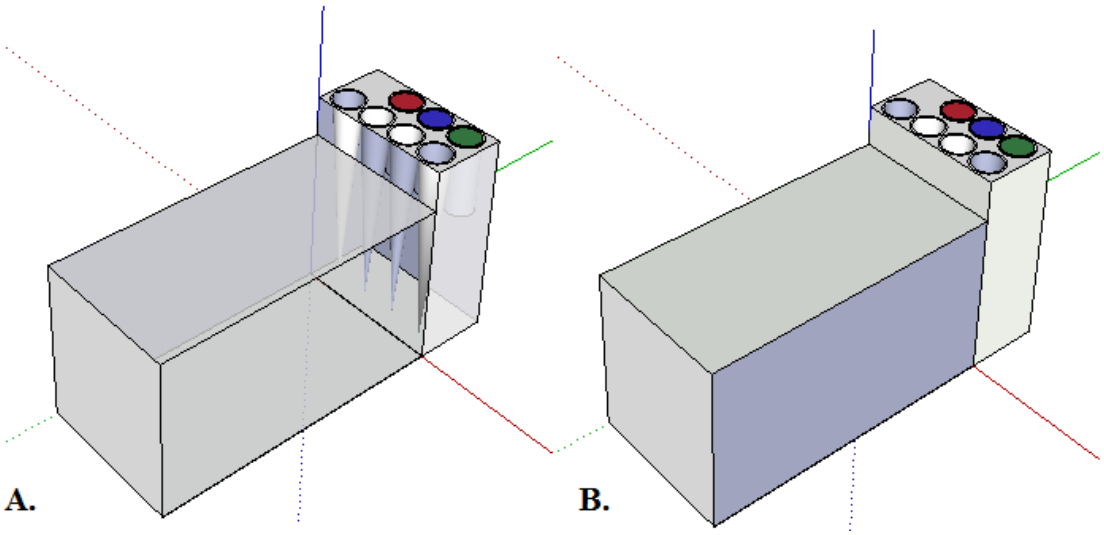


Figure 5.26 3-D model of the Microfluidic Multi-Surface Coagulation Assay cartridge created using Google SketchUp. A. The walls are transparent to allow a view of the pipette tips and the reservoirs extending into the cartridge. B. The cartridge as it would look to a consumer after the citrated blood has been added

Figure 5.26 shows only the cartridge portion, both as it will be seen by the physician or technician using the device and with some surfaces slightly transparent so that the depth of the reagent reservoirs and the loaded pipette tips can be seen. The figure emphasizes the simplicity of the cartridge design.

5.3.5 Clotting Surfaces

There will be four clotting surfaces on the device, 3 of which are different. The first and simplest surface will be a collagen surface, which is described by Colace[2] and is summarized and reproduced in Appendix 13.4. The second will emulate the extrinsic clotting pathway: a collagen-tissue factor surface also described by Colace[2] and again summarized and reproduced in Appendix 13.4. The third distinct surface will be a kaolin-collagen surface to activate the intrinsic clotting pathway, which previously has not been done.

The first attempt at a collagen-kaolin surface was performed in the laboratory on February 1, 2013 with the help of Tom Colace. Kaolin is a nonpolar clay mineral ($Al_2Si_2O_5(OH)_4$) and in when purchased as a powder, the size is not consistent and special consideration is taken when using kaolin as a reagent for our device. A kaolin mixture was created by adding 200 mg powdered kaolin to 5 mL HBS, stirring, and centrifuging briefly. The solution was allowed to settle overnight, and several distinct layers formed, shown in Figure 5.27. Since the top and least cloudy layer contained what should be the smallest particles, this is what was used in the protocol.

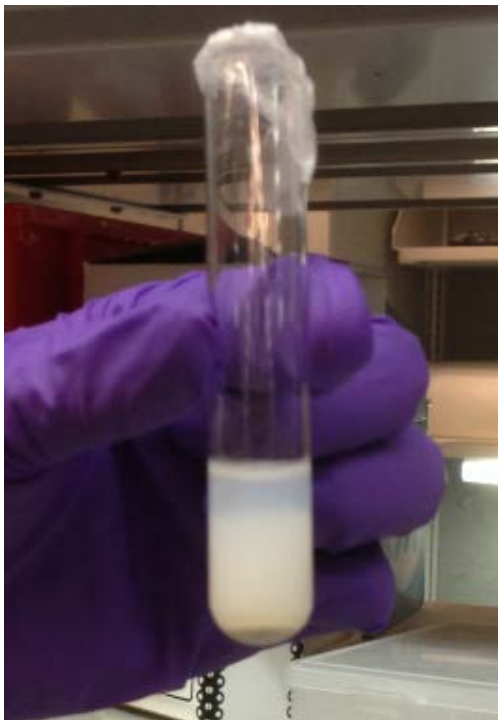


Figure 5.27: Kaolin mixed with water, centrifuged briefly, and allowed to settle overnight. Three distinct bands appeared within the solution, with the heavier particles at the bottom. For the kaolin-collagen surface, the top layer was used.

Within a microfluidic chip, collagen was diffused over the surface. Excess collagen solution was removed. A 0.5% BSA (bovine serum albumen) in HBS (HEPES-buffered saline) solution was diffused over the collagen and removed. Next, kaolin from the top layer of the tube was diffused over the collagen. This surface was observed under a microscope and is displayed in Figure 5.28. Figure 5.28a is a collagen-perfused surface, while figure 5.28b is a collagen-kaolin surface. The collagen particles in figure 5.28b appear to be 'rougher,' suggesting that each collagen fibril is coated in the kaolin. Watching the microscope display in real time revealed kaolin diffused over the surface in a display of Brownian motion.

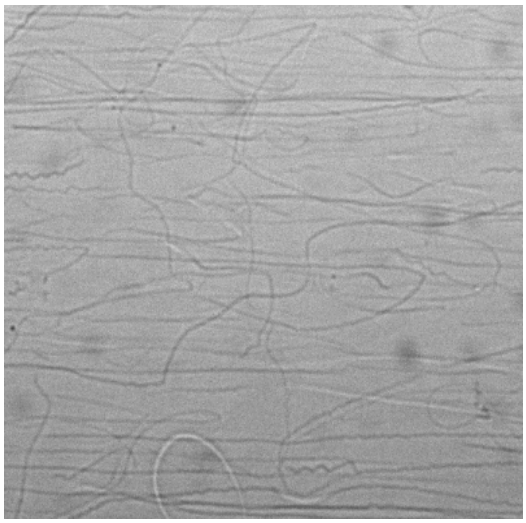
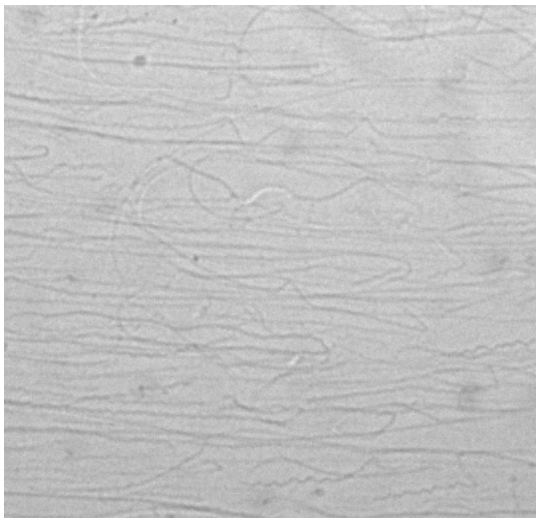
a.  b. 

Figure 5.28: a) Collagen surface, no kaolin. b) Collagen surface after perfusion of kaolin. The collagen-kaolin surface appears 'rougher' than the collagen surface alone. This suggests that the collagen particles are coated in kaolin.

Blood was collected from a donor and coagulation was inhibited immediately upon collection using a 4 μg/mL corn trypsin inhibitor (CTI), a Factor XII inhibitor. Fluorescent antibodies against platelets and fibrin were added to the blood and gently mixed. Immediately before perfusion over the surface, a solution of calcium chloride in HBS was added to the blood for recalcification. Blood was added to the wells on the microfluidic chip and pulled over the surface by pulling with a syringe pump to achieve a shear rate of 100 s^{-1} and a flow rate of 1 μL/second.

The results were not indicative of success, since fewer platelets appeared to accumulate on the kaolin-collagen surface when compared with the collagen surface, Figure 5.29. The results are compiled in Figure 5.30, which plots fluorescent intensity as a function of time. Thus the two graphs can be used to determine the extent and time of onset of coagulation. The addition of kaolin to the collagen surface appears to have had no effect on the coagulation time on the chip. Additional experiments would be required.

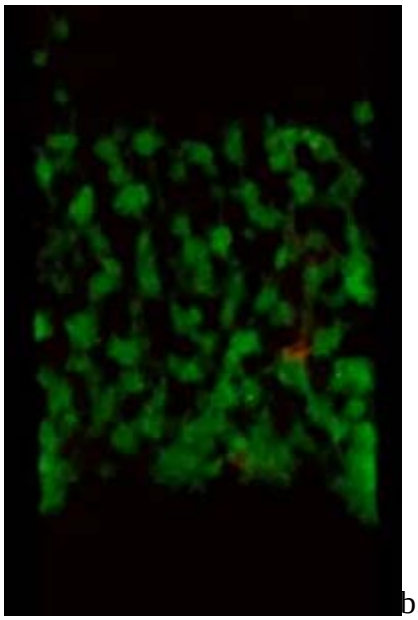
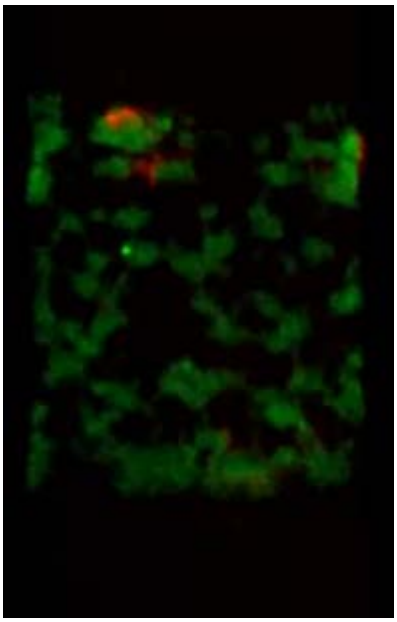
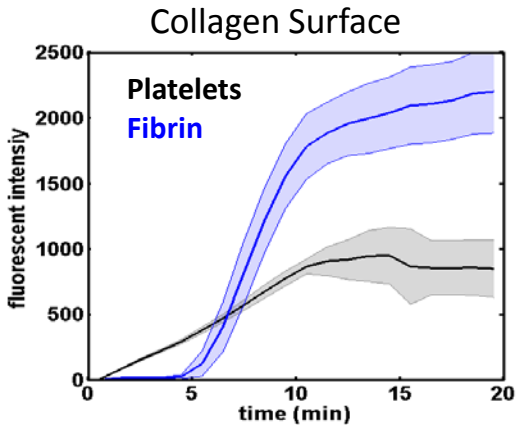
a.  b. 

Figure 5.29 Early platelet accumulation on the collagen surface (a) and the kaolin-collagen surface (b). Platelets are fluorescing in green. A more sparse accumulation of platelets is observed on the Kaolin-Collagen Surface. Images are taken from a compilation video of the on-chip coagulation event, which have been uploaded and can be viewed on Youtube[3,4].

a. 

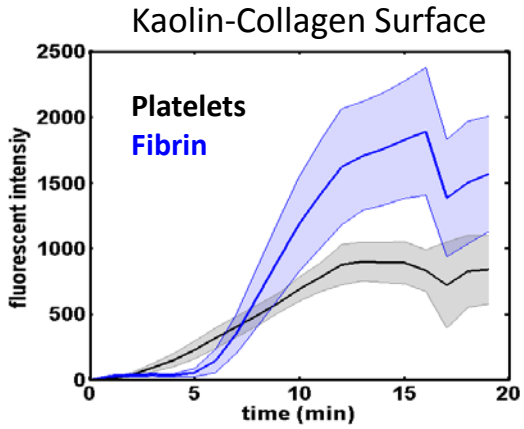
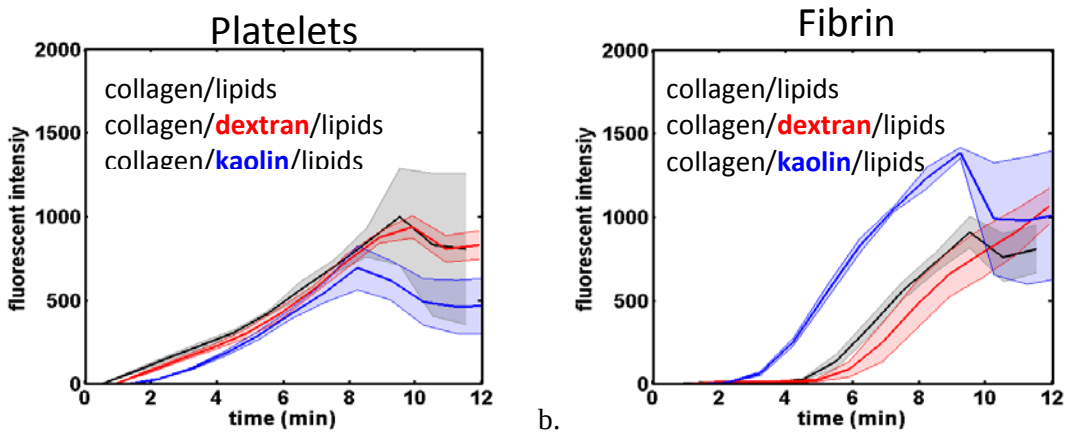
b. 

Figure 5.30: Accumulation of platelets and fibrin over time as blood diffused over both the collagen (a) and kaolin-collagen (b) surface. Platelets are displayed by the black line with error bars defined by the standard deviation among 8 surfaces. Fibrin is displayed by the blue line with error bars defined by the standard deviation among 8 surfaces. Platelet levels appear lower in the kaolin-collagen surface than the collagen surface. Fibrin begins to accumulate around the same time for both surfaces, around five minutes.

Additional consideration was made to determine the next steps in the creation of a collagen-kaolin surface that would promote coagulation better and 'faster.' The idea initially was considered since liposomes weakly adsorb to kaolin particles[21], and liposomes had previously been used to target tissue factor to the collagen surface[2]. It is also known that lipids help to promote coagulation[22].

The next experiment in lab tested kaolin-collagen-lipid surfaces, dextran sulfate-collagen-lipid surfaces, and collagen-lipid surfaces. Kaolin was prepared as described above. Before perfusion over the surface, liposomes with a composition of 80% phosphatidylcholine and 20% phosphatidylserine were added to the mixed and centrifuged kaolin in a 1:10 ratio. The mixture was perfused over a collagen surface and allowed to incubate for thirty minutes before perfusion with .5% BSA in HBS. Dextran sulfate was mixed to form a dilute mixture, mixed in the same ratio with the same lipid ratio, perfused over a collagen surface, allowed to incubate for thirty minutes, and then the channel was perfused with BSA. Similar techniques were applied for the control, collagen-lipid surface, except HBS was used in place of the kaolin or dextran sulfate mixture.

Figure 5.31 portrays the results of this experiment. All three surfaces are detailed, with the accumulation of platelets (a) and fibrin (b) indicated by the fluorescent intensity versus time for the duration of the coagulation event. It is readily observable that fibrin began to accumulate at the collagen-kaolin-lipid surface at a time of 3 minutes. This is about half the time the other two surfaces took to begin fibrin accumulation, and suggests a successful experiment. Figure 5.32 is a snapshot of the fluorescence at t=5 minutes, which highlights that the fibrin accumulation on the surface is 20 times greater for the collagen/kaolin/lipid surface than it is for the collagen/lipid surface. It also suggests that platelet accumulation is comparable on all three surfaces.



a. b.

Figure 5.31 Accumulation of platelets (a) and fibrin (b) on collagen/lipid surface (black), collagen/dextran/lipid surface (red), and collagen/kaolin/lipid surface (blue).While platelets appearslightly repelled by the collagen/kaolin/lipid surface, fibrin accumulation began in almost half the time of the other two surfaces for the surface with kaolin.

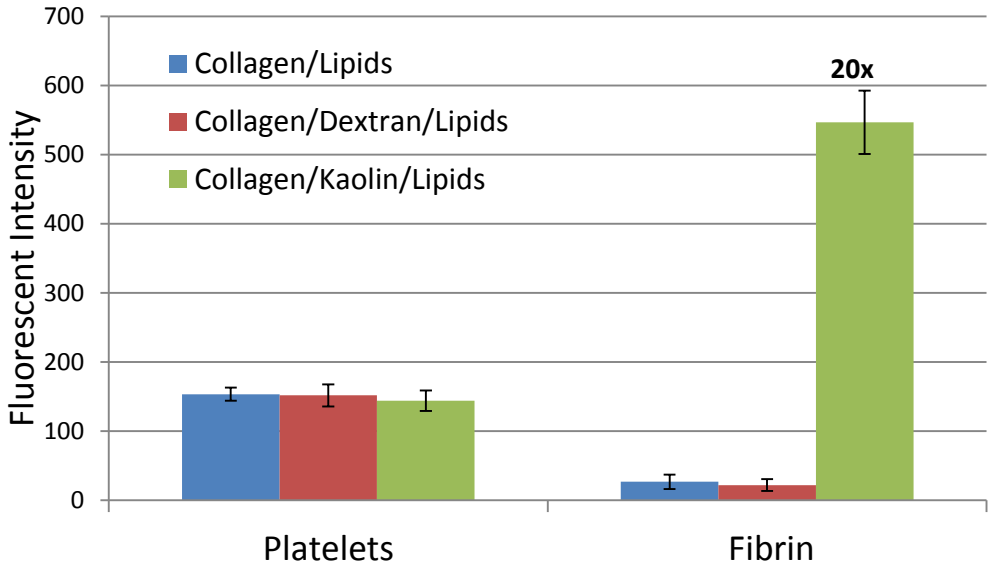


Figure 5.32 Fluorescent intensity of surfaces at t=5 minutes.Fluorescent intensity increases as platelets or fibrin accumulation increases. While platelet accumulation appears similar on all three surfaces, fibrin accumulation on the collagen/kaolin/lipid surface is twenty times greater than on the collagen/lipid surface after five minutes of flow.

This early clotting event is vastly useful in the Microfluidic Multi-Surface Coagulation Assay Chip, since measurements for all three surfaces can be used to determine the extent of clotting at a time as early as after 5 minutes of flow. Devices or blood assays currently on the market take much longer to measure coagulation time.

Chapter 6: Microfluidic Device Design Principles

6.1 Introduction

Highly precise fabrication techniques are required to manufacture channels that are only up to 300 micrometers in width. Additionally, because fluid is difficult to characterize at such a small length scale, the microfluidic device must be made with material that will not interact with the reagents that pass through it. As mentioned in Chapter 5, the Microfluidic Multi-Surface Coagulation Assay device will have two components – a cartridge and a chip. The cartridge is made of plastic and is used for the storage of dye and buffer solutions as well as pipette tips. The chip, in turn is comprised of a glass slide bonded to the molded elastomer poly(dimethylsiloxane), or PDMS. The PDMS layer contains the pattern of the microfluidic channels and the glass layer contains the four clotting surfaces. To pattern the PDMS, soft lithography was chosen as the method for replica molding and rapid prototyping. Soft lithography requires a master mold which is in turn constructed through photolithography. To deposit the clotting surfaces onto the glass chip, a method of microdispensing is necessary due to the small amount deposited. Precision printing using ink-jet technology was chosen due to the speed, precision, and digital nature of the technology that makes it suited for automated control in a manufacturing setting.

6.2 PDMS

Fabrication of microfluidic devices using polymers is relatively simple and reduces the cost, complexity, and time of manufacturing. PDMS is a widely used elastomer in microfluidics due to its many beneficial properties. The structure of PDMS consists of repeating $–OSi(CH_3)_2–$ units as shown in Figure 6.1[25]. Depending on the number of repeated units, the viscosity of PDMS can be altered. PDMS has the ability to allow for features with dimensions as small as 0.1 micron to be accurately fabricated, allowing for detailed designs of the channels within our device. Moreover, PDMS is nontoxic as well as thermally and electrically insulating and non-

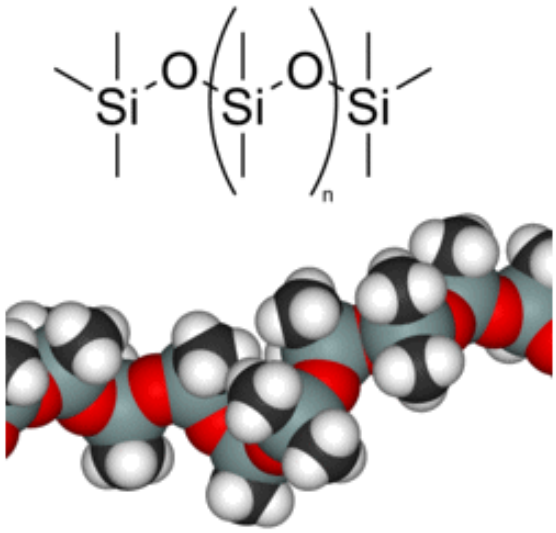


Figure 6.1 The atomic and molecular structure of PDMS, composed of repeating polymer music (2).

reactive with the reagents used in the Microfluidic Multi-Surface Coagulation Assay. PDMS is inert, meaning it is nor reactive toward most reagents. However, PDMS can be oxidized through exposure to plasma, which is a crucial step in the chip manufacturing process. In addition, the Microfluidic Multi-Surface Coagulation Assay tests for fibrin and platelet presence in blood with light, and PDMS permits for optical detection from between 240 to 1100 nm, allowing flexibility in the design of our device. PDMS is also widely available for purchase in bulk and is relatively inexpensive and the manufacturing procedure can be conducted without an expensive

clean room. Finally, PDMS can be irreversibly sealed to another surface if both are Si-based materials and have been oxidized by plasma before contact, making it ideal to be sealed to the glass chip[26].

PDMS is supplied in two components, a base and a curing agent, and the first step in creating a PDMS microfluidic device is to mix the two which results in the silicon hydride groups in the curing agent reacting with the vinyl groups in the base, causing polymerization and forming the desired elastomeric solid. The components are mixed, usually in a 10:1 ratio of base : curing agent and subsequently poured over a master, which is made from photolithography. Patterning of the PDMS with the desired microfluidic structures occurs when the liquid PDMS pre-polymer then conforms to the shape of the mold and replicates it with fidelity within 10 nanometers after curing through a process known as soft lithography. PDMS has low surface free energy and its elasticity also allows it to be removed from the master without damaging either the mold or itself[24].

6.3 Photolithography

In order to manufacture PDMS with the desired design of the microfluidic device, a master mold needs to be made with the reverse pattern of the mold. The master mold is made using photolithography – a method commonly utilized in the field of electronics. Through photolithography, geometric shapes are transferred from a mask to the surface of a silicon wafer. The steps of photolithography include: spin coating, soft bake, exposure, post exposure bake (or hard bake), and development. A light sensitive substrate, or photoresist, is deposited on a photomask and exposed to UV light. Both positive and negative photoresists can be used. For positive resists, exposure to UV light causes the structure of the resist to change so that it is

more soluble in the developer and thus positive photoresists are added to the photomask wherever the underlying material needs to be removed. Negative photoresists are exactly opposite, exposure to UV light initiates cross-linking in the photoresist and thus it does not dissolve [27]. Figure 6.2 shows the pathway of both positive and negative photoresists. The Microfluidic Multi-Surface Coagulation Assay utilizes a negative photoresists in the development of the master mold.

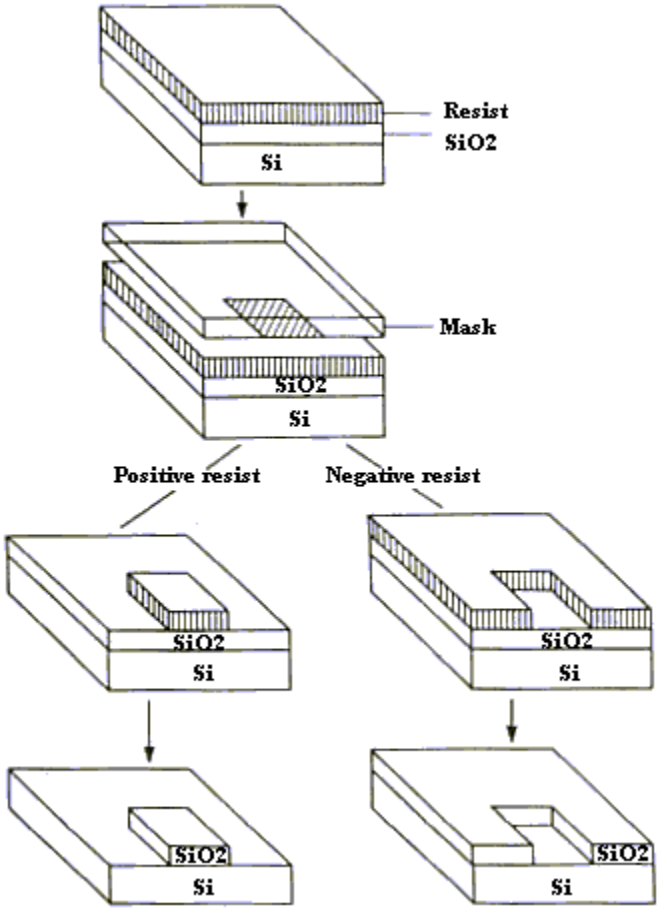


Figure 6.2 The process of photolithography to make a master mold using a photosensitive resist and exposing it to light (4).

For our device, a photomask designed using SolidWorks, a computer-aided drawing (CAD) program is laid on top of a silicon wafer. Since a negatively charged photoresist is used, the mask contains the inverse of the desired pattern to be transferred. The photoresist used to create the experimental molds is the KMPR ® 1000 by MicroChem. The photoresist is deposited

onto the silicon and spin coating is used to deposit the layer evenly onto the wafer with the speed and time of spinning dependent on the desired height of the mold. The spinning starts slowly, around 500 rpm and increases up to 3000 rmp. Next, a soft bake step occurs to slightly harden the surface and the length of baking time is also dependent on the thickness of the layer. In the case of the Microfluidic Multi-Surface Coagulation Assay, 60 microns was the target thickness and the soft bake time is approximately 20 minutes at 100^0C[29].

The next step in photolithography is exposure, where the photoresist is exposed to high intensity ultraviolet light. Alignment of the photomask and the silicon wafer is critical for the success of this step. Three primary exposure methods are contact, proximity, and projection and Figure 6.3 illustrates the relative positions of the light source, the optical system, the mask, the photoresist and the silicon wafer for each method. Contact lithography was chosen because high

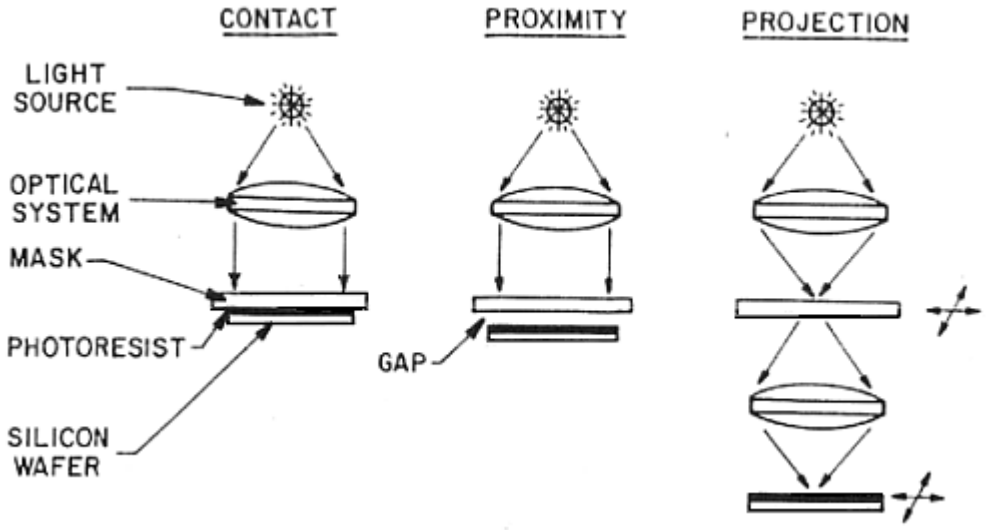


Figure 6.3 Representation of the three different types of photo lithography: contact, proximity, and projection (4).

resolution is possible due to the contact between the photomask and the photoresist. During contact lithography, cross-linking occurs in the negatively charged photoresist everywhere the

light shines through the mask. Full contact is vital because the light could shine beneath the mask if it is not achieved, and the imprint will be compromised. As with the spin coating and soft bake steps, the exposure energy is related to the thickness of layer.

Directly after the exposure step is the post exposure bake where the photomask is removed and the silicon wafer is baked for 4 minutes at 100^0C. The final bake is necessary to harden the photoresist and improve its adhesion to the silicon surface. The final step is development, where the un-polymerized areas of photoresist are removed from the wafer, leaving only the desired pattern behind. And aqueous alkaline developer, tetramethylammonium hydroxide (TMAH), removes the untreated areas upon immersion and strong agitation. The develop time is around 8 minutes for our desired thickness and it is during this step that the patterns of the mold become visible. After development, the master mold can be used once it has been rinsed and dried. With proper care and handling, a single master mold can be the basis for hundreds of PDMS molds from soft lithography[29]. In Figure 6.4, an example of successfully deposited photoresist on a silicon wafer is shown.

There are some limitations and precautions that must be considered with photolithography. This process cannot be used to create precision to the nanometer scale because it is limited by the wavelength of the UV light used. Additionally, more complex patterns such as pyramidal shapes or concaves within the thickness of the photoresist are more difficult to achieve. Other important considerations that could affect the quality of the mold include the humidity of the environment, bubbles forming in the resist solution and the ambient

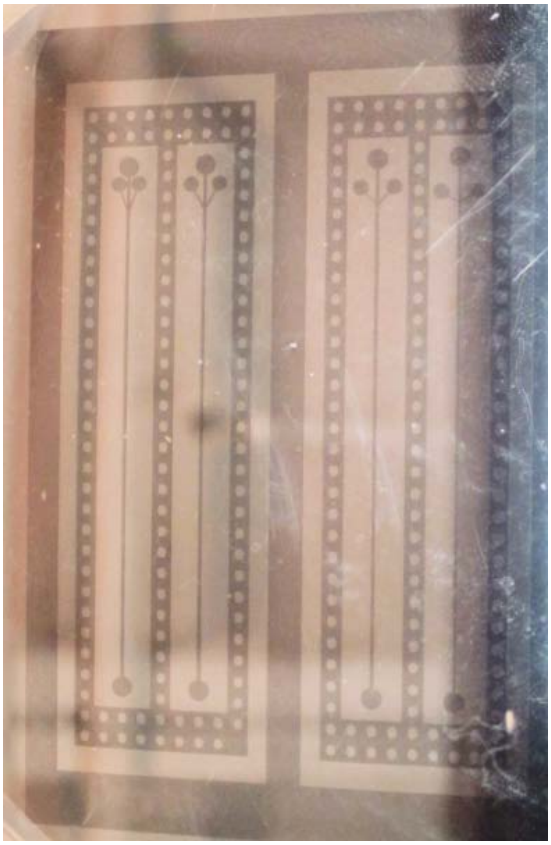


Figure 6.4 Images of the microfluidic PDSM patters that were used for research behind the scenes. Picture quality is due to the reflective nature of the mold.

light that could affect the sample. It is best to work in a clean room when creating a master mold and filtering out lights that could affect the sample can prevent failed molds. After the successful creation of a master mold, the next step in manufacturing the chip is to create a PDMS replica from the mold.

6.4 Soft Lithography

In order to reach our manufacturing goal of 1 million devices per year, soft lithography was chosen as the method for rapid replica molding. Numerous methods of pattern transfer from master mold including contact printing and embossing. Replica molding was chosen for its advantages in mass production. Replica molding is the duplication of information such as shape, morphology and structure that is present in a master mold. Soft lithography offers a wide range of usable materials and has the advantage of being able to duplicate three-dimensional patterns

in one step. Highly complex topologies in the master mold can be accurately replicated onto numerous copies in a process that is simple and inexpensive. The elastomer used in this process is PDMS, which, in addition to the beneficial properties stated earlier, also has the ability to conform to the surface of a substrate over a relatively large area, and the elastic characteristic of PDMS allows it to be easily removed from the mold, even from complex patterns. PDMS is also durable, and thus ideal for manufactured products which may not be used immediately[31].

The replica is made by first pouring the PDMS, which consists of the mixture of base and curing agent, over the master mold. Subsequently, the PDMS solidifies under exposure to high temperatures and conforms to the shape of the mold. After enough time has passed to solidify the PDMS, it is peeled from the master to reveal the final pattern of the microfluidic device. When the PDMS is fixed to a glass slide, it creates the microfluidic channels, and together the two components create the microfluidic device. Figure 6.5 shows the steps of the soft lithography process on an extremely simplified model.

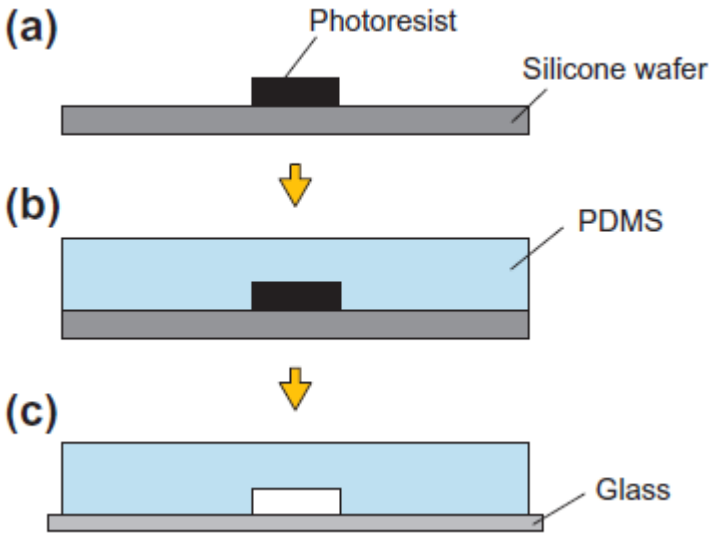


Figure 6.5 A simplified version of the PDMS manufacturing steps of pouring PDMS over photoresist, thermal curing, and then adhesion to the glass plate (7).

The main advantage of replica molding soft lithography is that many PDMS replicas can be constructed in parallel, which is extremely beneficial in a manufacturing setting. Features smaller than 100 nm have been accurately replicated, which is much more detailed than our microchip design. Figure 6.6 shows an example of a PDMS layer created from them master

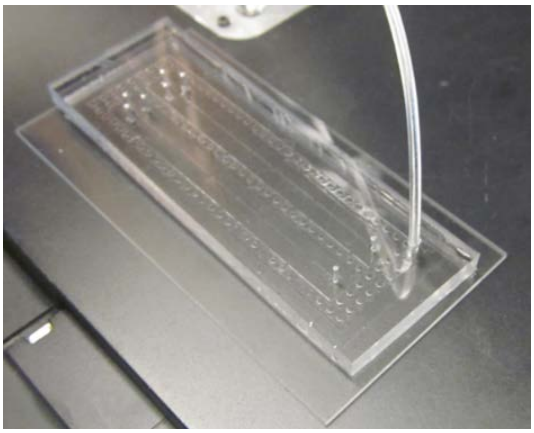


Figure 6.6 A lab-grade microfluidic device that is attached to the glass slide beneath it

mold that has been mounted onto a glass plate and is now ready for laboratory use. Soft Lithography also has a number of properties that must be addressed and considered. First, PDMS shrinks by around 1% after curing, and experimental testing is necessary to ensure repeatability of theoretical models. Also if the aspect ratio (length to height) ratio of the PDMS features is too small or too large, then pairing or sagging can occur. These conditions can be seen in Figure 6.7. Laboratory testing can ensure that our designs produce repeatable, durable, and accurate replicas despite these potential disadvantages[31]. With the successful fabrication of the PDMS layer of the chip, the next step is to consider the deposition of the clotting surfaces onto the glass slide.

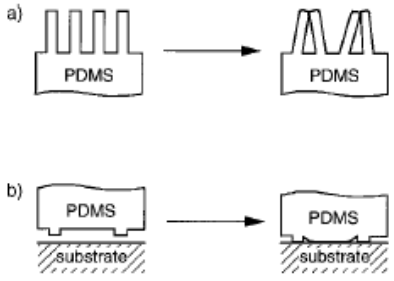


Figure 6.7 Possible disadvantages of PDMS include a) sagging, and b) shrinking (4).

6.5 Clotting Surfaces – Ink-Jet Fluid Handling

The design of the Microfluidic Multi-Surface Coagulation Assay calls for four clotting surfaces to be deposited onto the surface of the glass chip. Since only 3 surfaces are planned to be tested, the fourth surface will be a replica of one of the surface and be available for future tests. The three tested surfaces are: collagen, collagen with kaolin and lipid, and collagen with tissue factor. The collagen acts as a substrate for platelet deposition while the two other surfaces simulate the intrinsic and extrinsic clotting pathway. Over the years, miniaturization of electronic and photonic devices has increased the productivity of biomedical devices, but the physics of fluid dispensing at such small quantities can limit the degree to which miniaturization

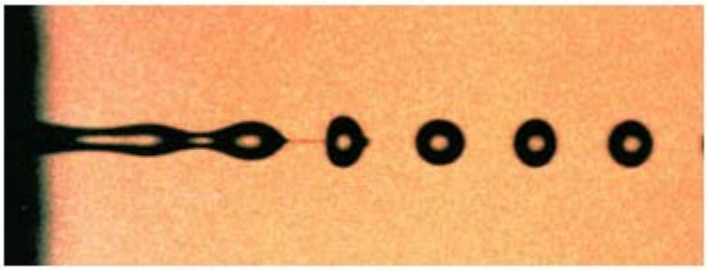


Figure 6.8 Continuous dispensing with ink-jet printing that results in multiple droplets of equal sides[9].

can occur. The microdispensing technique chosen for our device is ink-jet fluid handling. Widely used in printing today, ink-jet fluid handling has been increasingly used for microdispensing and precision printing for adhesives, liquid solder, optical and electrical polymers, and biomedical materials such as diagnostic reagents, proteins and DNA[33]. Ink-jet fluid handling dispenses fluid in two manners: continuous and drop-on-demand. Continuous dispensing, fluid constantly flows through an orifice with an electrochemical device that creates pressure oscillations that break the fluid into droplets of equal size. The droplet breaks from the stream in the presence of a charging field and thus acquires and electrostatic charge. Another electrostatic field, known as the deflection field, directs the drops to their desired locations. Figure 6.8 shows an image from a continuously dispensing system. Drop-on-demand, or

demand mode, a volumetric change in the fluid occurs when a voltage pulse is applied to a piezoelectric material that is either directly or indirectly coupled to the fluid. The resulting volumetric change causes a pressure or velocity

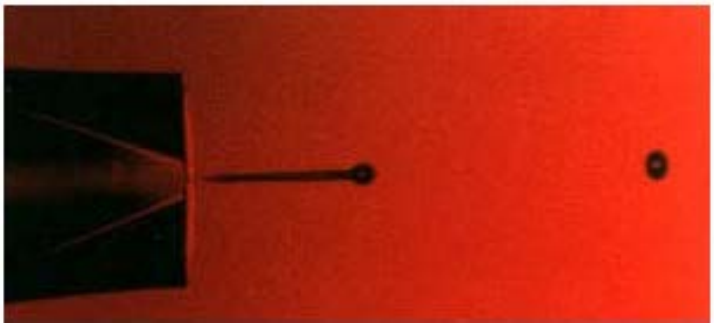


Figure 6.9 Demand mode ink-jet printing where only a small amount is needed with great accuracy [9].

change in the fluid and leads to the production of a single drop from the opening. Figure 6.9 shows images from a drop-on-demand system. Since a drop will only appear when voltage is applied, demand mode is also more easily adjusted for our specifications. The ink-jet handling system has the capability of delivering droplets 80-100 times per second[32].

6.6 Adhesion of Glass to PDMS – Plasma Oxidizer

In order to complete the chip, the PDMS layer that has been patterned with the microfluidic channels must be bound to the glass slide after the four clotting surfaces had been deposited. PDMS has the ability to bond both reversibly and irreversibly to a surface. For our device, irreversible sealing is necessary. For irreversible sealing, the surface must be exposed to oxygen plasma[34]. When oxidized, the PDMS and glass form covalent O-Si-O bonds. The seals are watertight and can be formed without high temperatures or other adhesives. In many microfluidic devices, only the PDMS receives oxygen plasma treatment, and not the secondary surface. But in the case of our device, if the PDMS undergoes oxygen plasma treatment and is placed over the clotting surfaces, then the surfaces might be affected. So since PDMS can be irreversibly sealed to another surface if both are Si-based materials and the surface has been

oxidized by plasma before contact, the glass slide will undergo oxygen plasma treatment first before the surfaces are deposited[26].

Chapter 7: Manufacturing

7.1 Introduction

The Microfluidic Multi-Surface Coagulation Assay will mainly be manufactured within our own facilities. The device is made of two components – the cartridge and the chip. Additionally, the processing unit structure into which a disposable device can be inserted and analyzed must also be sold to customers. The plastic cartridge is a relatively simple design and will be manufactured separately. The processing unit will also be outsourced due to our limited understanding of the electronics and electrical engineering necessary to design a fully functional processing unit. The idea of outsourcing was not considered for the chip component of the device due to the importance of protecting the intellectual property by maintaining complete control over its fabrication. The device fabrication and the loading of reagents into the cartridge as well as the final sealing and packaging steps will all take place within our manufacturing facility.

Initially, the manufacturing will proceed with manual labor during the prototype phase and then move onto automated processes with human operators. In our manufacturing facility, there will be three main pathways that converge into the completed device. First there is the chip, which in its own fabrication contains two manufacturing lines: the PDMS layer and the glass slide. The PDMS layer must be molded and the clotting surfaces must be deposited on the glass slide. Then the PDMS layer and the glass slide must be bonded together. The cartridge is its own separate path where calcium buffer and dye are deposited and pipet tips are loaded into the plastic shell. Finally, the chip and the cartridge are put together and packaged as the final

device. The success of the device depends on the speed and accuracy of our manufacturing, especially to keep up with our goal of 1 million devices per year.

7.2 Manufacturing Overview

At 10 percent market penetration, the goal is to produce 1 million chips per year. Certain parameters and assumptions about the manufacturing facility must be made in order to determine the number of chips that need to be manufactured in one work day in order to meet the market capture goal. The first assumption is that the manufacturing facility will operate for 8 hours a day for 330 days per year. Equation 7.1 shows that the number of chips that must be manufactured in one day is approximately 3,031.

$$\frac{1{,}000{,}000\ devices\ per\ year}{330\ days\ per\ year} = 3030.333\ devices\ per\ day \qquad 7.1$$

After working with our industry consultant, the next assumption we made is that 1 percent of the chips made will be used for quality assurance testing. This number was set due to the large number of chips manufactured in one day as well as the projected reliability of the manufacturing process. Since the chips that are manufactured are transparent, quality inspection could simply be a visual test and those chips that are deemed suitable could still be counted towards the total chips needed to be produced in one day. However, we decided to follow a more conservative approach and assume that all chips that are needed for quality assurance testing will not be counted towards the 3,030 chip goal. As a result, in order to produce 3,030 chips for sale, a total of 3,061 chips must be manufactured (see Equation 7.2).

$$3030.33\ devices\ per\ day\ \times (1 + 0.01\ quality\ assurance) = 3060.66\ devices\ per\ day \qquad 7.2$$

Finally, the possibility of failures and defects must be taken into account when outlining a manufacturing plan. According to our industry consultant, a 1 percent failure rate, or 99 percent success rate would be an appropriate assumption. Taking these estimations into account, 3,092 chips must be manufactured in one day in order to reach the goal of 3,031 chips manufactured for sales, Equation 7.3. This number is further broken down into six batches of 516 chips that will be manufactured throughout the day. The exact methodology of the breakdown is discussed later in this chapter.

$$3036.66\ devices\ per\ day\ \times (1 + 0.01\ failure) = 3092.21\ devices\ per\ day \quad 7.3$$

This division results in the manufacturing of a few excess devices but the number was chosen so that each batch contains the same number of chips and the manufacturing process is consistent. The exact methodology of the breakdown is discussed later in this chapter. This manufacturing process is able to successfully accommodate batches of 516 chips and fit six batches into an 8 hour work day.

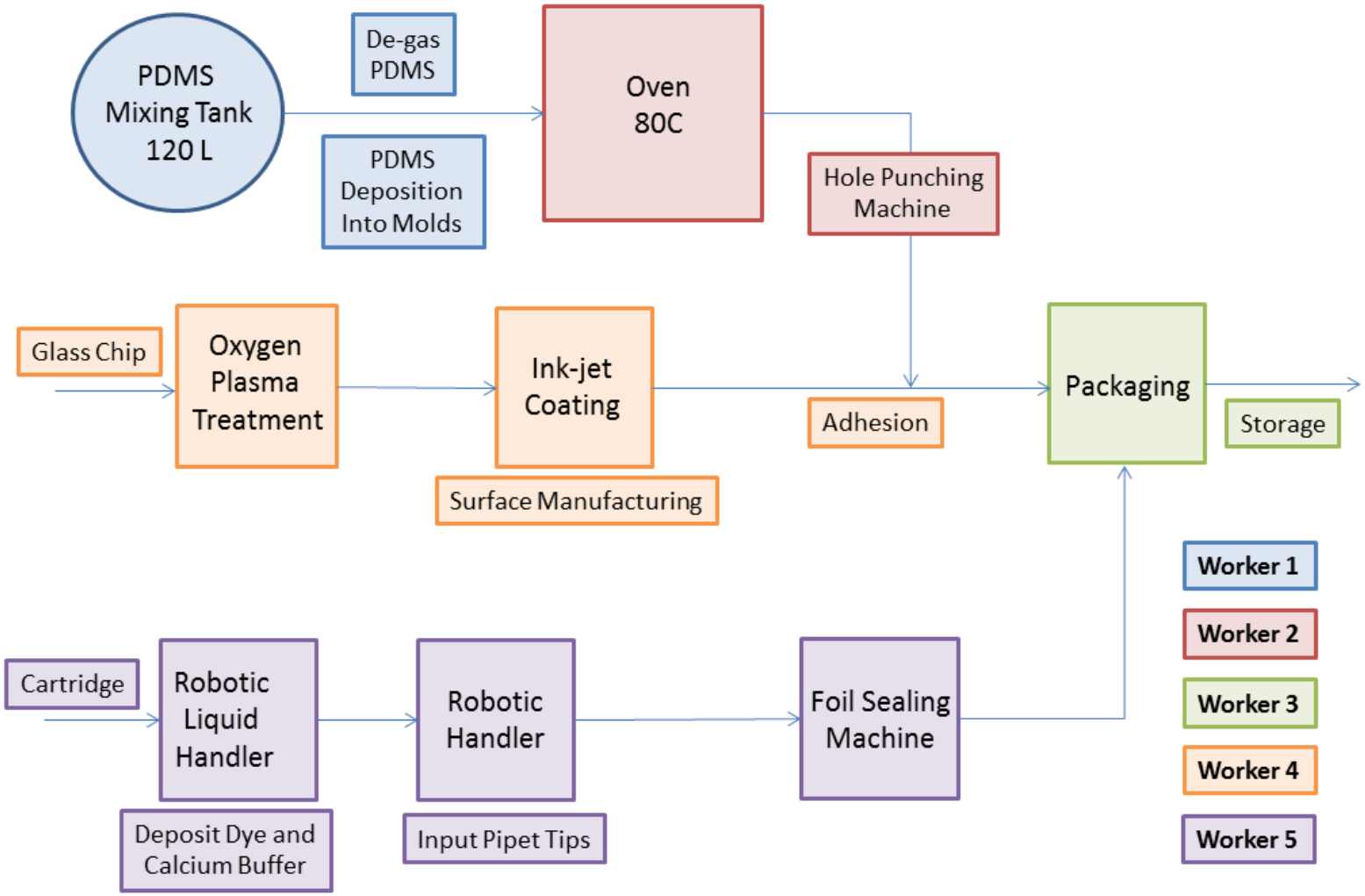


Figure 7.1 Manufacturing process of the μMCA shown as 'unit operations' and color coded to indicate the number of factory workers needed.

Finally, the actual manufacturing plan must be determined, and in order to accurately gauge the cost of manufacturing, the roles and numbers of employees were considered. Figure 7.1 provides a simplified model of the steps in the manufacturing facility, as well as an overview of the equipment required. The details of the equipment selection as well as the timing of the manufacturing day will be presented in subsequent sections of this chapter. Figure 7.1 also shows the breakdown of labor on the manufacturing floor. Since the process is automated, a single operator can oversee several machines within the manufacturing line, and thus it was determined that 5 workers would be needed to oversee the manufacturing. Further employment was also considered and the final roster of necessary employees is shown in Table 7.1. A breakdown of the wages and compensations that were used to determine the total labor cost of manufacturing can be found in Appendix 13.5.

Job	**Number**	**Wage (total)**
Manufacturing Workers	5	$274,600.00
Quality Assurance	1	$92,780.00
Maintenance	1	$41,110.00
Supervisor	1	$114,490.00
Engineer	1	$92,260.00
Sales/Marketing	1	$84,510.00
Field Reps	3	$287,250.00
Research and Development	3	$312,180.00
Clinical Trial Manager	1	$96,380.00
	Total	**$1,395,560.00**
Total with Compensation/Benefits (1.5x)		**$2,093,340.00**

Table 7.1 Labor expenses and compensation for the manufacturing process of the µMCA with employment data found from the Bureau of Labor Statistics

7.3 Fabrication of Microfluidic Chip

The microfluidic chip is where the chemical interaction between the blood sample and the clotting surfaces occurs. The microfluidic channels are patterned onto the PDMS layer. As mentioned in Chapter 6, PDMS was chosen as the elastomer for the microfluidic device due to its numerous beneficial properties, from its optical transparency to the targeted wavelengths to its chemical stability and low level of interaction with reagents.

During a typical manufacturing day, the PDMS solution is prepared first. The two components of PDMS, the base and the curing agent, must be first mixed. A 10:1 ratio of base to curing agent is used and after mixing at low rpm for 10 minutes, the PDMS is ready to be used in manufacturing. Since approximately 35mL of PDMS is used for each device, a 120 tank is used to store the PDMS in the factory. The excess space is for further manufacturing needs or future expansion. In Chapter 5, soft lithography was detailed and will serve as the method for rapid production of the PDMS layer. The soft lithography will be based off of a master mold made using photolithography. Only a few of these masters need to be made. The channels each have a height of 60 μm and the lengths of the channels vary from hundreds of microns to centimeters, which all fit within the parameter for photolithography. Once the master molds are created using a negative photoresist, a PDMS ‘stamp’ can be created. A stamp is a PDMS layer that contains the inverse of the final pattern and these stamps will in turn be used for the actual manufacturing processes. In order to achieve the goal of manufacturing 3,091 devices in one day, hundreds of these PDMS stamps can be used in parallel to mold the PDMS for the actual devices. PDMS stamps can be used to create accurate patterns for greater than 10 uses, so it is assumed that, at most, new stamps would have to be created once per week[36]. After the PDMS

is poured into the mold, it undergoes thermal curing by heat treatment in an oven for 1 hour in order to create structurally stable layers.

The oven that the molds are placed in must be large enough to accommodate all of the molds and maintain a temperature of 80^0C. At any time, there will be a maximum of 516 PDMS molds inside the oven given our current manufacturing pace. A suitable oven was found in a previous senior design project by Abbot, Lee, Kohli and O'Brien. The curing oven is produced by the Wisconsin Oven Corporation and its inside area is approximated to be 60 ft^2. In order to accommodate 516 PDMS molds, less than 20 ft^2 is required (specific sizing calculations can be found in Appendix 13.6). Purchasing a large oven allows for the possibility of larger batch sizes in the future as production expands. The curing oven is priced at $22,400, which was deemed reasonable by our industry consultants and thus is used in our cost estimation.

Following the removal of the PDMS molds from the oven, the newly-cured PDMS layers are separated from the molds. This process is assumed to be trivial and will not require any chemical or thermal aid because one of the favorable properties of PDMS is its ease of detachment. Next, in order to create the larger reservoirs for both the deposition of the blood, dye, and the waste reservoir in the device, a hole-punching machine will be utilized. The hole-punch machine is estimated to cost $20,000. After this step, the PDMS layer is complete and ready for bonding with the glass slides coated with clotting surfaces[37].

The second manufacturing component of the chip is the coagulation surfaces on the glass slide. Because of the sensitive nature of the surfaces that are deposited on the glass slide, it was decided that the oxygen plasma treatment of the glass slide will occur before the surfaces are deposited. The glass slides are laboratory-grade, and the cost of the oxygen plasma

treatment machine is estimated to be $45,000[38] and in order to successful treat all 516 slides is estimated to be 30 minutes. After oxidation, the four clotting surfaces are deposited onto the slide using ink-jet printing technology, described in Chapter 5. After consulting with David Wallace of MicroFab Technologies, Inc, it was decided that a full ink-jet printing system would be purchased with four separate print heads for the four surfaces. The full quotation can be seen in Appendix 13.7, and the total cost of the JL4-xl printing system with the PH-46 print heads is estimated to be $68,550. The full process of depositing the surfaces onto the surfaces is estimated to take 1.5 hours based on our laboratory experiments.

After the surfaces have been deposited on the oxidized glass slide, an adhesion process must occur to bind the PDMS to the layer to the glass slide. A favorable property of PDMS is demonstrated in this step as the only step required for adhesion is contact between the PDMS layer and the glass slide for 30 minutes[39]. After the adhesion process is complete, the chip is ready to be packaged along with the cartridge.

7.4 Cartridge Assembly and Packaging

Though the cartridge body will not be manufactured in our facility, reagents such as the dye and buffer need to be added to the cartridge before packaging and pipette tips also need to be delivered. A robotic liquid handling system was chosen to deliver the reagents to the cartridge. The robotic liquid handling system is estimated to cost $60,000. It is assumed that it will take 2 seconds to deposit the reagents into each cartridge body so 30 minutes have been allotted to completing the reagent deposition in all 516 cartridges. The delivery of pipette tips into the cartridge is also automated and the delivery of four standard-sized pipettes into the cartridge is estimated to take 2 seconds as well, meaning another 30 minutes is allotted for this

step, and the cost is estimated to be $30,000. The final step in the cartridge manufacturing process is the foil sealing of reagents. The seal prevents contamination of the reagents and the physicians or technicians will remove the seal in order to deposit their blood sample into the cartridge. After looking at a range of foil-sealing machines, it was estimated that foil-sealing will cost $7,500 and need 1 hour to completely seal all of the cartridges.

Finally, when all the components of the device are complete, the final step is packaging. Our device will be individually sealed and packed into customized boxes. After consulting with Imperial Printing & Paper Box MFG, it was determined that the yearly cost for packaging is $23,971 with a one-time designing fee of $590, the full quote can be found in Appendix 13.8. After the chips have been packaged, they are placed in a large -80°C freezer for storage until they are removed for shipment or quality assurance testing. This last step is estimated to take 30 minutes, which puts the total time for manufacturing one 516-device batch at approximately 3 hours, since many of the steps occur in parallel.

7.5 Manufacturing Timeline

At approximately 10 percent market capture, 1 million devices need to be manufactured in 1 year. According to our assumed operation conditions of 330 days a year and 8 hours a day, 3,090 chips must be manufactured in one day, accounting for defects and quality testing as mentioned in the previous sections. In order to determine the number of batches needed and the number of devices per batch, a breakdown of the manufacturing of one batch is needed. Figure 7.2 shows the breakdown of the manufacturing. The PDMS manufacturing steps include the de-gassing of the PDMS mixture and the thermal curing in the oven. Both processes are 1 hour so the total time required is 2 hours. The treatment of the glass slide requires 30 minutes and the

deposition of the clotting surface onto the slide needs 1.5 hours so the total process also takes 2 hours. Since these two are independent processes, they can occur in parallel and after 2 hours both will be ready for the adhesion process, which takes 30 minutes, meaning that the chip of the device is complete after 2.5 hours of manufacturing. The cartridge manufacturing also occurs separately, with both the reagent and pipette deposition taking 30 minutes for a total of 1 hour. The foil-sealing process also takes 1 hour so the cartridges are prepared after 2 hours. The packaging step takes place at the 2.5 hour mark and takes 30 minutes, rounding off the manufacturing process at a total rate of 3 hours per batch.

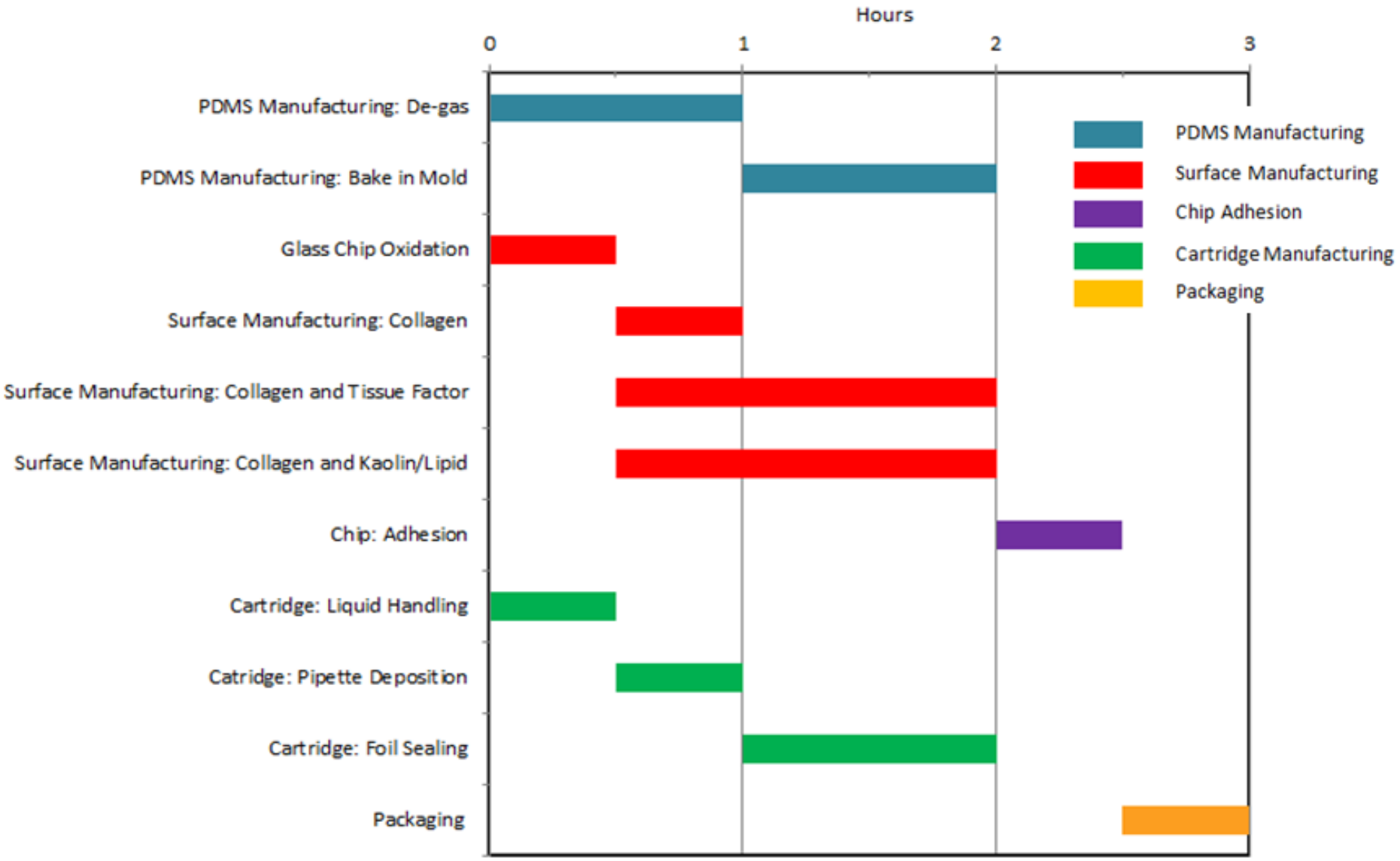


Figure 7.2 Timeline for manufacturing a single batch of μMCA showing the overlapping steps that can occur at once.

In one 8-hour workday, all 3,090 chips must be manufactured. The manufacturing process can be thought of as a series of batch-like reactions because many of the process can only handle one batch at a time. After analyzing the timeline of producing one batch, the limit of this manufacturing process is the curing oven. A new batch of PDMS molds can only be put into the oven once the curing process of the previous batch has been completed. This means that the manufacturing process of the next batch cannot start until one hour after the previous batch. Assigning the beginning of the work day as hour 0, and taking into account the three hours that are required for a batch to be complete, the last batch must begin the process at hour 5. So a total of 6 batches can be completed in one day. Equation 7.4 can then be used to calculate the number of chips that need to be in each batch.

$$\frac{3{,}092.21\ devices\ per\ day}{6\ batches\ per\ day} = 515.368\ devices\ per\ batch\ \cong 516\ devices\ per\ batch \qquad 7.4$$

Six batches per day of 516 devices per batch is the final breakdown of the manufacturing plan. The overlapping of multiple batches allows the manufacturing to be complete in one working day. Figure some just shows a diagram of the manufacturing breakdown with each of the steps of the chip and cartridge manufacturing collapsed into one continues time.

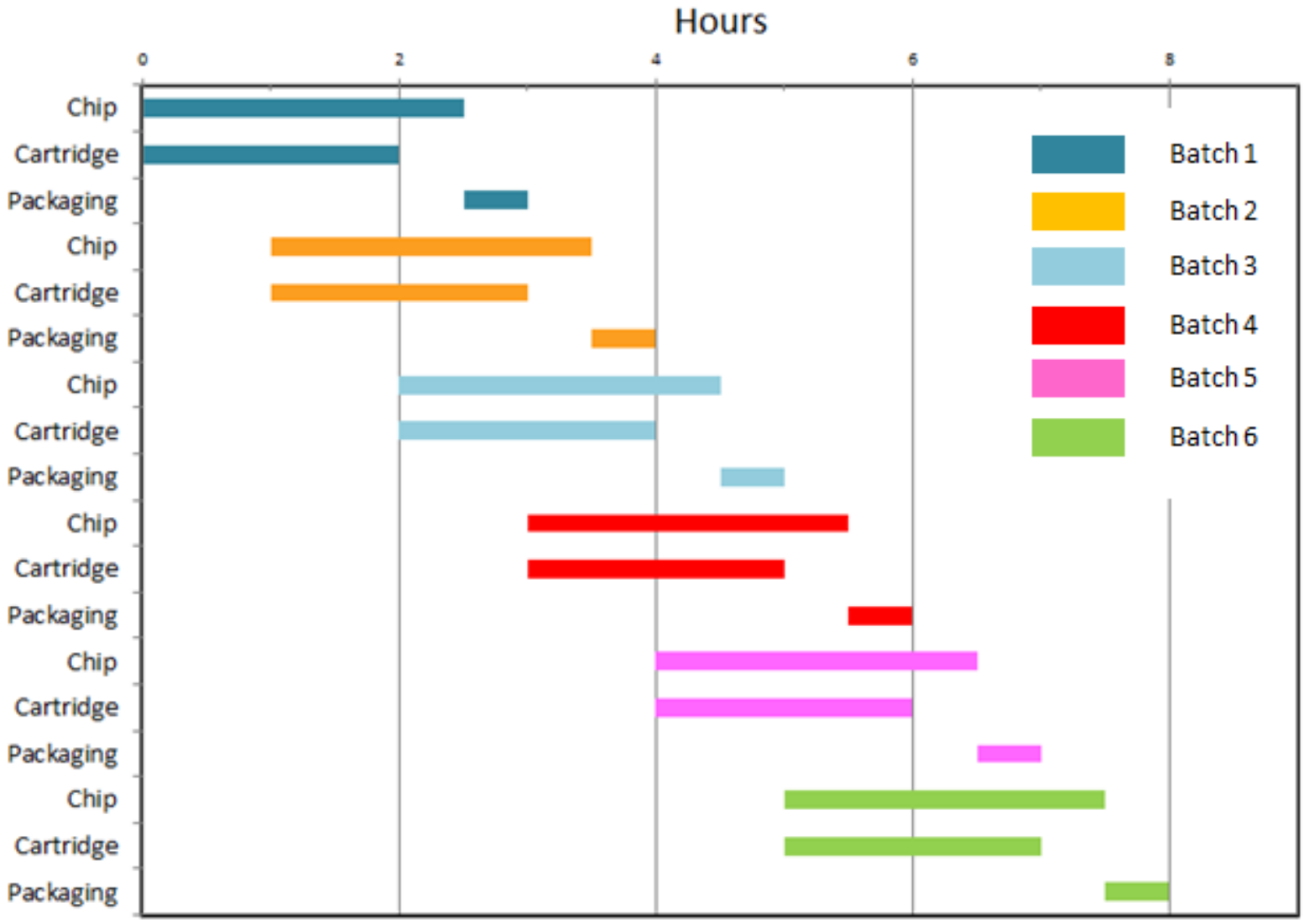


Figure 7.3 Timeline for an entire workday for manufacturing devices. Each color corresponds to a separate batch.

Chapter 8: The µMCA Development Timeline

8.1 Introduction

Upon completion of the design of The Microfluidic Multi-Surface Coagulation Assay, we project that the device can be market ready in one year. The time is divided between FDA approval as well as Clinical Trials and prototyping.

8.2 Product Development and Prototyping

Results in lab provide us with confidence that clinical trials will go well and ensure more rapid and useful testing than other devices or tests currently in the market. Additionally, during the phase of clinical trials, it can be confirmed that specific design features such as the applied pressure drop are optimal for measurement of coagulation. If necessary, during this one year timeframe, features such as the pressure drop can be modified for optimal measurements.

The clinical trials are also important since these data will be used as a standard for assessing patient blood samples once the µMCA is in the marketplace. The average fluorescence values from healthy patients will be considered ‘normal’ or ‘average,’ and deviations from this value will indicate coagulation dysfunction and can be used to determine treatment options. We will standardize the device output information so that physicians are able to receive consistent and accurate diagnostic data. Since the output will be a number and can easily be compared to ‘normal,’ the µMCA will be appealing to not only doctors but insurance companies who value standardized approaches to medicine.

8.3 FDA Regulations and Approval

The Microfluidic Multi-Surface Coagulation Assay will be treated as a medical device in the US market since its use will affect patient treatment in a hospital setting or possibly affect the outcome of clinical trials. The FDA classifies medical devices as Class I, II, or III depending on the level of control that must be associated with device production and regulation in the marketplace[42]. Class I devices require the least controls and do not pose risk to patients or users. Examples of Class I devices include bandages and medical examination gloves[42]. Class II devices require special controls beyond the basic ones required for Class I devices, and typically require some degree of equivalence to a device already legally in the market. Class III devices require the most stringent controls, since their uses often support life or could cause life threatening results for patients[42]. Class III devices require Premarket Approval which confirms safety and efficacy of these devices[42].

Based on the FDA website, The Microfluidic Multi-Surface Coagulation Assay will be classified as a Type II device. The device is most likely classified as a "Multipurpose system for in vitro coagulation studies," defined in the Code of Federal Regulations Title 21 Volume 8[43]. To ensure correct device classification, a prototype as well as manufacturing protocols and design specifications must be sent to the FDA.

8.4 Clinical Trials

In order to achieve approval of The Microfluidic Multi-Surface Coagulation Assay, rigorous clinical trials must take place to prove safety and efficacy of the product. An Investigational Device Exemption (IDE) must be filed before clinical trials begin. This process entails approval by an institutional review board (IRB), informed consent from all patients,

labeling denoting that the product is for investigational use only, observation of the study, and thorough records and reports[45].

For all trials, patients will provide one blood sample a week for four weeks. Each sample will be tested 3 times using The Microfluidic Multi-Surface Coagulation Assay to ensure self-consistency of results. Additionally, the blood will be tested using the competitor, the INNOVANCE PFA-200 System by Siemens, as well as the traditional activated partial thromboplastin time (aPTT) test to compare results and applicability of the results.

We estimate that the Clinical Trials as outlined will cost $3MM. When combined with the estimated $500K for FDA approval including legal advice, the cost to bring the device to market totals $3.5MM.

8.4.1 Phase I

One hundred patients will participate during Phase I clinical trials, which will span 1 month. The purpose of this stage is to prove initial effectiveness, and to avoid major capital losses a small number of patients will be tested. Each patient in this stage is healthy, thus establishing a basis for comparison to unhealthy patients or patients being treated for a clotting abnormality. The data will be compiled and analyzed. Ultimately this stage proves repeatability of tests and a basis for further tests.

8.4.2 Phase II

Three hundred patients will participate during Phase II clinical trials, including healthy patients as well as patients with clotting abnormalities and patients on anticoagulants and promoters of coagulation. Phase 2 will span 3 months and ultimately enable comparisons between the healthy patient base readings and patients with clotting disabilities.

8.4.3 Phase III

1000 patients will participate in Phase III clinical trials. The goal of phase III is the goal of the entire process: proof that the device is market ready. A large number of patients will participate with the same regular interval testing. Six months are allotted for Phase III clinical testing.

8.5 Preparations for the Marketplace

Subsequent to the completion of clinical trials and while waiting for FDA approval to come through, or during months 11 and 12, preparations will be made for manufacturing. Steps to achieve this include renting a factory space, establishing relationships with vendors for reagents, and preordering equipment for manufacture. Following the steps outlined here will bring the device to market within 1 year of the completion of design.

Chapter 9: Financial Analysis

9.1 Introduction

The Financial Analysis of this project is focused on the costs and revenues in the development and production of the chip. Other forms of revenue such as technical support contracts and sales of the processing units are not considered in this analysis. This section will discuss market shares, costs, and operating assumptions and then discuss product viability in the market.

9.2 Market Share and Sales Projection

The rest of 2013 has been allocated to further development of this product. Another year has been allocated for the FDA trials and filing discussed in Chapter 8. As we wait for approval the manufacturing site will be found, leased, and fitted with the necessary machinery. This is another year; therefore production begins the year 2016. The price for each chip is assumed to be the pre-discount cost of $100/chip. An inflation rate of 2.4% was used and was calculated from an average of the inflation rates for the years 2003-2013[46]. It has been assumed that we would capture 5% of the market in the first year of production. This number would increase to 7.5% in the second year of production, and finally reaching an asymptote at 10% in the third year of production. Calculations can be found in the Appendix Section 13.3. The expected revenues for the ten years after filing are shown in Table 9.1. Expanding the market will add additional costs due to increased production needs. These costs are not reflected in the further financial analysis but are however taken into consideration in Section 9.8, which talks about expansion.

Year	Revenues	% Market Penetration
2014	0	0
2015	0	0
2016	$107.3	5
2017	$110.0	7.5
2018	$168.8	10
2019	$230.5	10
2020	$295.1	10
2021	$302.2	10
2022	$309.4	10
2023	$316.9	10

Table 9.1: Total Projected Revenues with base case assumptions. Revenues in Millions

9.3 Costs Sheets

Manufacturing the Microfluidic Multi-Surface Coagulation Assay includes various costs, such as the cost of cartridge and the processing unit, laboratory space, and clinical trials. The costs associated with the production of the cartridge and processing unit are compiled below.

The costs associated with manufacturing the cartridge, including shift operators, facilities, equipment, raw materials, etc. can be found in Appendix 13.3 in the Financial Analysis Tables. For the manufacture of the cartridge it is estimated that we need approximately 10,000 ft^2 for manufacturing space, lab space, and office space. The cost of renting a 10,000 ft^2 office space in the Philadelphia area is approximately $150,000/yr or $15/$ft^2$/yr [46]. Extrapolating for the added costs of retrofitting existing office space for manufacture and laboratories, we estimate that renting a facility for our purposes would cost $500,000/yr. Other expenses included in the $500,000/yr include facilities items such as a cafeteria, a gym, break rooms, etc.

Since the product is targeted for use as a medical device, clinical trials need to be performed to prove efficacy as discussed in Chapter 8. As mentioned in Chapter 8, the estimated cost of FDA and Clinical trials is $3.5M.

9.3.1 Costing the Chip

The cost of the materials in the chip itself was determined in a variety of ways. The prices of specialized materials, such as tissue factor or antibodies against collagen type 1, were determined from various source websites. The costs for more generic, lab-grade reagents were determined from sources such as Sigma Aldrich. These sources are not tailored for bulk ordering of reagents, but for the scale of individual research laboratories, so the prices are slightly overstated. However, it is assumed that this overstatement is negligible since costs are already so low due to the small volumes per chip, and the chip will be sold at such a high markup from the material costs. Table 9.2 summarizes the costing analysis, with a net cost of materials per chip around $1.16. For the projected 10^6 chips per year goal, the materials for all chips in a year will cost $1.6 million.

Item	Price Per Unit (dollars)	Unit		Price		How much		Cost per device (Dollars)	Source	Date Priced
Calcium Chloride	215	2.5	kg	86	per kg	0.137	mg/device	1.18E-05	Sigma Aldrich	2/14/2013
Magnesium Chloride	214	1	kg	214	per kg	4.71E-02	mg/device	1.01E-05	Sigma Aldrich	2/14/2013
Zinc Chloride	5720	1	kg	5720	per kg	6.74E-05	g/device	3.85E-04	Sigma Aldrich	2/14/2013
Collagen	220	1	mL	220	per mL	0.9	ul/device	0.198	ChronoLog	2/14/2013
Sodium Chloride	184	5	kg	36.8	per kg	5.79E-05	g/device	2.13E-06	Sigma Aldrich	2/14/2013
Sodium Hydroxide	146	1	kg	146	per kg	2.93E-06	g/device	4.28E-07	Sigma Aldrich	2/14/2013
sodium phosphate dibasic dihydrate	106.5	1	kg	107	per kg	1.96E-06	g/device	2.08E-07	Sigma Aldrich	2/14/2013
HEPES	499	1	kg	499	per kg	8.69E-05	g/device	4.34E-05	Sigma Aldrich	2/14/2013
Distilled Water	NEGLECT	FACTORY	COSTS							2/14/2013
Tissue Factor	12322	1	mg	12322	per mg	4.03E-06	mg/device	4.97E-05	American Diagnostica (former)	2/14/2013
BSA	5000	10	kg	500	per kg	5.14E-05	g/device	2.57E-05	Santa Cruz Biotechnology, inc	2/14/2013
Kaolin	152	2.5	kg	60.8	per kg	1.20E-07	kg/device	7.30E-06	Sigma Aldrich	2/16/2013
Heart PC (L-α-phosphatidylcholine (Heart, Bovine))	2060	1000	g	2.06	per mg	1.24E-03	mg/device	2.56E-03	Avanti Polar Lipids	2/16/2013
Brain PS (L-α-phosphatidylserine (Brain, Porcine))	1280	500	mg	2.56	per mg	3.21E-03	mg/device	8.21E-03	Avanti Polar Lipids	2/16/2013
Biotin PE	2660	10	mg	266	per mg	4.60E-05	mg/device	0.0122	Avanti Polar Lipids	2/16/2013
Anti-Collagen I antibody (biotin)	362	100	ug	3.62	per ug	0.12	ug/device	0.434	Abcam	2/16/2013
Streptavidin	591	10	mg	59.1	per mg	0.0003	mg/device	0.0177	Sigma Aldrich	2/16/2013
Sigmacote	96.9	100	ml	0.969	per ml	5.4	ul/device	0.00523	Sigma Aldrich	2/16/2013
Triton X-100	155	3785	ml	0.0409	per ml	0.002	ml/device	8.19E-08	Sigma Aldrich	2/16/2013
Bio Beads SM-2	425	100	g	4.25	per g	0.8	mg/device	0.00340	Bio-Rad	2/16/2013
PDMS	2	1	kg	2	per kg	0.0338	kg/device	0.0676	Alibaba	2/17/2013
Plastic Cartridge	0.05	1	unit	0.05	per unit	1	unit/device	0.05	assume	2/17/2013
Pipette Tips	193	3840	tips	0.0503	per tip	5	tips/device	0.251	Sigma Aldrich	2/17/2013
glass slides (1"x3")	86.4	1440	slides	0.06	per slide	1	slide/device	0.06	Alibaba	2/19/2013
KMPR 1050	4138	4	L	1.03	per mL	1	mL/cast	0.05	MicroChem	2/19/2013
							Total Cost	1.16		

Table 9.2: Summary of cost and amount of reagent used per device. Prices were determined from sources such as Sigma Aldrich or Alibaba or more specialized vendors for reagents such as tissue factor. Dates that costs were determined are displayed. According to the costing analysis, each chip will cost $1.16 in materials alone.

9.3.2 Costing the Processing Unit

The final design and construction of the Microfluidic Multi-Surface Coagulation Assay processing unit will be contracted to an outside company. To judge the cost of construction of a processing unit, it was treated as fabricated equipment. As defined by Seider et al., fabricated equipment are devices that are manufactured from its individual parts and put together in the lab[12]. The contracted company would purchase the individual parts from distributors and combine them within a casing. The list of parts and the costs of the parts is shown in Table 9.2. The cost to manufacture the devices was assumed to be 1.0x the total cost of individual internal parts. The total cost of parts was $10,750 and the total cost to produce a processing unit (including markup) is $21,500.

In pricing the processing unit, the prices of the competitors discussed in Chapter 3 were considered. We also considered our company's sales model in pricing the unit. The main profit driver for the company comes from the sales of the chips, which the customer will need to continually purchase. Taking these concerns into account, the sticker price for the processing unit is $30,000. As with the competition, this price is subject to change based on the contract negotiated between the customer and the sales department.

Processing Unit Component	Number of Components	Estimated Cost per Unit
Liquid Handler	1	$1000
Syringe Pump	1	$1000
Mobile Tray Handler	1	$4000
LEDs	2	$500
CCD Camera	1	$1000
Injection Molded Case	1	$1000
Computer + Software	1	$750
	Parts Total	$10,750
Manufacturing	Estimated Cost is 1.0*Parts Total	$10,750
	Total Cost	$21,500

Table 9.3: Costing of components for the Processing Unit.

9.4 Operating Assumptions

Sales, General, and Administration Costs are overhead for the business. As shown in Appendix 13.3, this calculated amount is a percentage of revenues. The cost of performing clinical trials and filing FDA approval were accounted for in the "miscellaneous" fixed costs. It is also important to note that the financial analysis used percentages and hourly wages to calculate the overall costs of employees in the manufacturing facility. To bring the estimate as close as possible to that shown in Chapter 7, the weighted average of the employee's hourly salaries was used. We are also working under the assumption that all customers will pay the same amount for the device. The projected market lifetime for this product is ten years. We expect new technologies will enter the market and produce new devices.

9.5 Inventory, Working Capital and PP&E

Inventory, Working Capital, and Plant, Property, and Equipment costs can all be found in the Appendix section 13.3. Inventory costs were calculated based on the number of chips required per year (one million). The working capital is determined based on current liabilities and current assets. Current assets are the total cash reserves, the value of the inventory, and the accounts receivable. We are planning on having 30 days of cash reserves as well as 30 days of accounts receivable. Having 30 days of cash reserves is equivalent to having 8.33% (1/12 of a year) of the cost of manufacturing. Similarly the 30 days of accounts receivable means having 8.33% of the annual sales of the product. We also plan on holding seven days (1.92% of annual sales) of chip inventory. The current liabilities are the accounts payable is the money owed to vendors. We are planning on keeping 30 days of accounts payable, which amounts to 9.1% of raw material costs. The plant, property, and equipment costs refer to the purchasing and

maintenance capital expenditures. Since the plant is being leased, this cost only refers to the price and maintenance of manufacturing equipment.[12]

9.6 Income Statement and Cash Flow

The income statement shows the net income created by the Microfluidic Multi-Surface Coagulation Assay after taking into account cost of goods sold, indirect costs, and taxes. The cost of goods sold is a compilation of the direct costs associated with the production of goods. The indirect costs are the costs not directly related to the manufacture of the device like facility, administration, and other fixed and variable costs. The tax for the device is what is paid to the government and is found at a rate of 37%.[12]

The cash flow is another important feature of the economic analysis. The cash flow allows investors to observe the cash going into the company and leaving the company. It takes into account all costs including depreciation. The cash flow is defined as "the net passage of money into or out of a company due to an investment." [12]

9.7 Valuation, Returns, Payback Period

The company was valuated as having a 2013 NPV of $176,195,600 and an IRR of 247%. These values were found using the Discounted Cash Flow model. The net present value is calculated for 10 years of production. The cash flow over the lifetime of the company was calculated and these values were discounted to the present value. These values are under the assumption that we would sell our cartridges at $100 per unit. With the different costs involved in the set up to production, it is important to look at how long it would take for the investors to realize profit on their investment. This time until their investment is repaid is known as the

Payback Period. Looking at Appendix 13.3, the payback period for the Microfluidic Multi-Surface Coagulation Assay occurs within the first year of production.

9.8 Sensitivity Analysis

A sensitivity analysis was performed to see how the IRR changed as an effect of sales price and variable costs. Sensitivity analyses show how a calculated value like the IRR is affected by parameters that can be controlled. In this study we look at how different chip prices and different variable costs affect the IRR. Chip price and variable costs were chosen because they are values that the company decides on depending on how the market is fairing. The base price was set at $100/unit and the base variable cost was calculated to be $17.8 M/yr. As mentioned before, the IRR at the base values is 247%. The two variables were allowed to deviate +/- 50%. The best case scenario is a price of $150 and very minimal variable costs which would allow investors to realize a 293% IRR. The worst case scenario for this analysis occurs at a sales price of $50 with very high variable costs, which results in an IRR of 112.7%. These numbers are summarized in Table 9.3, below.

Sensitivity Analyses

Product Price		Variable Costs										
		$8,896,892	$10,676,270	$12,455,648	$14,235,027	$16,014,405	***$17,793,783***	$19,573,162	$21,352,540	$23,131,918	$24,911,297	$26,690,675
		0.5	0.6	0.7	0.8	0.9	1	1.1	1.2	1.3	1.4	1.5
$50.00	0.5	175.09%	169.46%	163.73%	157.87%	151.89%	145.77%	139.51%	133.09%	126.50%	119.73%	112.76%
$60.00	0.6	198.04%	193.03%	187.95%	182.77%	177.51%	172.16%	166.72%	161.17%	155.51%	149.74%	143.84%
$70.00	0.7	217.99%	213.43%	208.82%	204.14%	199.40%	194.60%	189.72%	184.77%	179.74%	174.63%	169.44%
$80.00	0.8	235.66%	231.46%	227.22%	222.92%	218.58%	214.18%	209.73%	205.23%	200.66%	196.04%	191.36%
$90.00	0.9	251.56%	247.65%	243.70%	239.71%	235.68%	231.60%	227.49%	223.33%	219.13%	214.88%	210.58%
$100.00	1	266.02%	262.34%	258.63%	254.89%	251.12%	247.32%	243.47%	239.60%	235.69%	231.74%	227.75%
$110.00	1.1	279.27%	275.80%	272.30%	268.77%	265.22%	261.63%	258.02%	254.38%	250.70%	247.00%	243.27%
$120.00	1.2	291.52%	288.22%	284.90%	281.55%	278.18%	274.79%	271.37%	267.92%	264.46%	260.96%	257.44%
$130.00	1.3	302.90%	299.75%	296.59%	293.40%	290.19%	286.96%	283.71%	280.43%	277.14%	273.82%	270.48%
$140.00	1.4	313.53%	310.52%	307.48%	304.43%	301.37%	298.28%	295.18%	292.05%	288.91%	285.75%	282.57%
$150.00	1.5	323.50%	320.60%	317.69%	314.76%	311.82%	308.86%	305.89%	302.90%	299.89%	296.87%	293.83%

Table 9.3: Sensitivity Analysis testing the effect of Product Price and Variable Costs on IRR

9.9 Expansion

As stated in Section 9.1, the market share captured by the Microfluidic Multi-Surface Coagulation Assay is projected to increase over the first five years. This would introduce an expansion cost. There are multiple ways to expand the facilities. The first method would be to construct a second process line. Another method would be to introduce a second shift for the manufacture of the devices. This would double the number of devices produced by the manufacturing facility. Since we are estimating that market expansion will end at around 25%, we would be able to run three shorter shifts to accommodate demand as well as manufacturing inefficiencies such as downtime between shifts.

9.10 Summary

In summary the overall financial analysis shows that the Microfluidic Multi-Surface Coagulation Assay has the potential for profit generation. With an IRR, ROI, and NPV of 247%, 442.4%, and $176,195,600 respectively this project has strong economic feasibility. The data and calculations for these values can be found in the Appendix, Section 13.3.

Chapter 10: Recommendations and Conclusions

The Microfluidic Multi-Surface Coagulation Assay utilizes microfluidic technology to create a faster and more efficient product to detect blood coagulation. It will allow healthcare professionals to achieve more detailed information from a single sample in less time than any of our competitors. There are still ways that the µMCA can be improved to fulfill its potential and adapt as the market grows and changes.

An advantage of our device is its potential for flexibility. The four clotting surfaces can be changed with relative ease in the manufacturing process and placed at any point along the 4 cm length of the flow channels. Additionally, different reagents and dyes can be added to the cartridge so blood clotting under completely different conditions can be tested without changing the basic architecture of our device. This greatly widens the potential application for this product. For example, magnesium and zinc buffer solutions can also be mixed with citrated whole blood to initiate clotting but has not been readily studied. In order to successfully customize the device, the diffusion of these new buffers must be studied to determine the length along the channel to place the clotting surfaces. Furthermore, with customizations, although the behavior of the flows can be modeled, they will still need to be tested with prototypes to ensure that the device performs the tests with accuracy and precision.

Both testing of the product and profits can also be improved if the manufacturing of the processing unit were to occur in-house. In the current plan, the manufacturing of the processing unit is outsourced to an electronic equipment supplier. If instead, the processing units were incorporated into our manufacturing division, it would make it easier to make alterations to its design and make the units more available to test new designs of the device, instead of relying on an outside supplier.

As stated in Chapter 3, the United States encompasses approximately 40 percent of the market for blood-related products. After our target market penetration has been achieved, we can adjust our marketing strategy and business plan in order to make the Microfluidic Multi-Surface Coagulation Assay available to the international market. This would require testing to ensure that the product meets the standards of other countries and further understanding of the current state of the market for testing of blood coagulation.

Chapter 11: Acknowledgements

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Chapter 13: Appendix

13.1 MSDS Reports

PDFs of material safety data sheets are excluded from this report for brevity, but are available upon request by emailing Elizabeth Cummings, e.m.cummings2@gmail.com. Chemicals used throughout the report and experiments are listed below, along with sources. PDFs for individual chemicals and reagents are available on the provider's website.

Bovine Serum Albumen: Santa Cruz Biotechnology, Inc.; Santa Cruz, CA

Calcium Chloride: Fisher Scientific; Fair Lawn, NJ

Collagen: American Biochemical and Pharmaceutical Ltd.; Surry, UK

HEPES Buffered Saline: Sigma Aldrich; St. Louis, MO

Kaolin: Sigma Aldrich; St. Louis, MO

KMPR 1050: MicroChem; Newton, MA

L-α-phosphatidylcholine (Heart, Bovine): Avanti Polar Lipids; Alabaster, AL

L-α-phosphatidylserine (Brain, Porcine) (sodium salt): Avanti Polar Lipids; Alabaster, AL

1-oleoyl-2-(12-biotinyl(aminododecanoyl))-sn-*glycero-3-phosphoethanolamine*:Avanti Polar Lipids; Alabaster, AL

Magnesium Chloride: Fisher Scientific; Fair Lawn, NJ

Methyl Cellulose: Sigma Aldrich; St. Louis, MO

PDMS: Acros Organics BVBA; Geel, Belgium

Rabbit Polyclonal to Collagen I (Biotin): Abcam Inc.; Cambridge, MA

Recombinant Human Tissue Factor Apoprotein: American Diagnostica Inc.; Stamford, CT

Sigmacote®: Sigma Aldrich; St. Louis, MO

Streptavidin from Streptomyces Avidinii: Sigma Aldrich; St. Louis, MO

Triton™ X-100: Sigma Aldrich; St. Louis, MO

Zinc Chloride: Fisher Scientific; Fair Lawn, NJ

13.2 Market Analysis

The breakdown of the target markets for the Microfluidic Multi-Surface Coagulation Assay can be separated into four categories. First are victims of strokes and heart attacks. Second are people who are in need of anti-clotting drugs, and the third category is clinical trials, and though clinical trials can range from hundreds to tens of thousands of participants, a conservative estimate of 200 patients per trial was used. The final category is trauma cases including emergency room visits for injuries and motor vehicle accidents. Not all patients in any of these categories will need the Microfluidic Multi-Surface Coagulation Assay, so 20 percent of the total was used in our calculations. With these calculations, the total market size is estimated to be 11.4 million, and we decided to use 10 million as a more conservative estimate. Table 13.1 shows the numerical breakdown used to achieve approximately 10 million total market size for the Microfluidic Multi-Surface Coagulation Assay.

	Number of Individuals per Year
Heart Attack	935,000
Stroke	795,000
Anti-Clotting Drugs	5,800,000
Clinical Trials	379,000
Emergency Room - Injury	45,200,000
Emergency Room - Motor Vehicle Accident	4,047,000
Total	**57,156,000**
20% of Total	**11,431,200**

Table 13.1 Breakdown of the markets for the Microfluidic Multi-Surface Coagulation Assay

13.3 Financial Analysis

13.3.1 Summary of Inputs and Chronology of Events for $100 chip

General Information

Process Title: **µMCA Manufacture Process**
Product: **µMCA**
Plant Site Location: **Mid-Atlantic Region**
Site Factor: **1.00**
Operating Hours per Year: **2640**
Operating Days Per Year: **110**
Operating Factor: **0.3014**

Product Information

This Process will Yield

387 Unit of µMCA per hour
9,288 Unit of µMCA per day
1,021,680 Unit of µMCA per year

Price **$100.00 /Unit**

Chronology

Year	Action	Distribution of Permanent Investment	Production Capacity	Depreciation 5 year MACRS	Product Price
2013	Design		0.0%		
2014	Construction	100%	0.0%		
2015	Construction	0%	0.0%		
2016	Production	0%	49.0%	20.00%	$100.00
2017	Production	0%	73.5%	32.00%	$102.40
2018	Production		98.0%	19.20%	$104.86
2019	Production		98.0%	11.52%	$107.37
2020	Production		98.0%	11.52%	$109.95
2021	Production		98.0%	5.76%	$112.59
2022	Production		98.0%		$115.29
2023	Production		98.0%		$118.06
2024	Production		98.0%		$120.89
2025	Production		98.0%		$123.79

Equipment Costs

Equipment Description		Bare Module Cost
Oven x2	Process Machinery	$89,600
Liquid Handling Robot x4	Process Machinery	$360,000
Robot Handler x2	Process Machinery	$90,000
Oxygen Plasma Machine x2	Process Machinery	$180,000
Foil Sealing Machine x2	Process Machinery	$22,500
Ink-Jet Printer x2	Process Machinery	$280,000
Packaging x2	Process Machinery	$95,884
Foundry Machine x2	Process Machinery	$80,000
Total		***$1,197,984***

Raw Materials

	Raw Material:	Unit:	Required Ratio:	Cost of Raw Material:	
1	All Raw Materials	Unit:	1 Unit: per Unit of µMCA	$1.162	per Unit:
	Total Weighted Average:			$1.162	per Unit of µMCA

Utilities

	Utility:	Unit:	Required Ratio	Utility Cost	
1	High Pressure Steam	lb	0 lb per Unit of µMCA	$0.000E+0 0	per lb
2	Low Pressure Steam	lb	0 lb per Unit of µMCA	$0.000E+0 0	per lb
3	Process Water	gal	0 gal per Unit of µMCA	$0.000E+0 0	per gal
4	Cooling Water	lb	0 lb per Unit of µMCA	$0.000E+0 0	per lb
5	Electricity	kWh	0.07 kWh per Unit of µMCA	$0.060	per kWh
	Total Weighted Average:			$4.200E-03	per Unit of µMCA

13.3.2 Variable Cost

Utilities

	Utility:	Unit:	Required Ratio		Utility Cost
1	High Pressure Steam	lb	0	lb per Unit of µMCA	$0.000E+00 per lb
2	Low Pressure Steam	lb	0	lb per Unit of µMCA	$0.000E+00 per lb
3	Process Water	gal	0	gal per Unit of µMCA	$0.000E+00 per gal
4	Cooling Water	lb	0	lb per Unit of µMCA	$0.000E+00 per lb
5	Electricity	kWh	0.07	kWh per Unit of µMCA	$0.060 per kWh
	Total Weighted Average:				$4.200E-03 per Unit of µMCA

Variable Costs

General Expenses:

Selling / Transfer Expenses:	**3.00% of Sales**
Direct Research:	**8.00% of Sales**
Allocated Research:	**2.00% of Sales**
Administrative Expense:	**2.00% of Sales**
Management Incentive Compensation:	**1.25% of Sales**

Working Capital

Accounts Receivable	☐	**30**	**Days**
Cash Reserves (excluding Raw Materials)	☐	**30**	**Days**
Accounts Payable	☐	**30**	**Days**
µMCA Inventory	☐	**7**	**Days**
Raw Materials	☐	**7**	**Days**

Total Permanent Investment

Cost of Site Preparations:	**5.00% of Total Bare Module Costs**
Cost of Service Facilities:	**5.00% of Total Bare Module Costs**
Allocated Costs for utility plants and related facilities:	**$0**
Cost of Contingencies and Contractor Fees:	**18.00% of Direct Permanent Investment**
Cost of Land:	**0.00% of Total Depreciable Capital**
Cost of Royalties:	**$0**
Cost of Plant Start-Up:	**10.00% of Total Depreciable Capital**

13.3.3 Fixed Costs

Fixed Costs

Operations

Operators per Shift:	**8 (assuming 1 shifts)**
Direct Wages and Benefits:	**$30 /operator hour**
Direct Salaries and Benefits:	**15% of Direct Wages and Benefits**
Operating Supplies and Services:	**6% of Direct Wages and Benefits**
Technical Assistance to Manufacturing:	**$0.00 per year, for each Operator per Shift**
Control Laboratory:	**$0.00 per year, for each Operator per Shift**

Maintenance

Wages and Benefits:	**15.00 % of Total Depreciable Capital**
Salaries and Benefits:	**25% of Maintenance Wages and Benefits**
Materials and Services:	**100% of Maintenance Wages and Benefits**
Maintenance Overhead:	**5% of Maintenance Wages and Benefits**

Operating Overhead

General Plant Overhead:	**7.10% of Maintenance and Operations Wages and Benefits**
Mechanical Department Services:	**2.40% of Maintenance and Operations Wages and Benefits**
Employee Relations Department:	**5.90% of Maintenance and Operations Wages and Benefits**
Business Services:	**7.40% of Maintenance and Operations Wages and Benefits**

Property Taxes and Insurance

Property Taxes and Insurance:	**2% of Total Depreciable Capital**

Straight Line Depreciation

Direct Plant:	8.00 %	**of Total Depreciable Capital, less 1.18 times the Allocated Costs for Utility Plants and Related Facilities**
Allocated Plant:	6.00 %	**of 1.18 times the Allocated Costs for Utility Plants and Related Facilities**

Other Annual Expenses

Rental Fees (Office and Laboratory Space):	**$500,000**
Licensing Fees:	**$0**
Miscellaneous:	**$1,000,000**

Depletion Allowance

Annual Depletion Allowance:	**$0**

13.3.4 Fixed Cost Summary

Fixed Cost Summary

Operations			
	Direct Wages and Benefits	$	499,200
	Direct Salaries and Benefits	$	74,880
	Operating Supplies and Services	$	29,952
	Technical Assistance to Manufacturing	$	-
	Control Laboratory	$	-
	Total Operations	**$**	**604,032**
Maintenance			
	Wages and Benefits	$	233,247
	Salaries and Benefits	$	58,312
	Materials and Services	$	233,247
	Maintenance Overhead	$	11,662
	Total Maintenance	**$**	**536,469**
Operating Overhead			
	General Plant Overhead:	$	61,460
	Mechanical Department Services:	$	20,775
	Employee Relations Department:	$	51,073
	Business Services:	$	64,057
	Total Operating Overhead	**$**	**197,366**

Property Taxes and Insurance

Property Taxes and Insurance:	$	31,100

Other Annual Expenses

Rental Fees (Office and Laboratory Space):	$	500,000
Licensing Fees:	$	-
Miscellaneous:	$	1,000,000
Total Other Annual Expenses	**$**	**1,500,000**

Total Fixed Costs	**$**	**2,868,967**

13.3.5 Investment Summary

Investment Summary				
Bare Module Costs				
Fabricated Equipment	$	-		
Process Machinery	$	1,197,984		
Spares	$	-		
Storage	$	-		
Other Equipment	$	-		
Catalysts	$	-		
Computers, Software, Etc.	$	-		
Total Bare Module Costs:			**$**	**1,197,984**
Direct Permanent Investment				
Cost of Site Preparations:	$	59,899		
Cost of Service Facilities:	$	59,899		
Allocated Costs for utility plants and related facilities:	$	-		
Direct Permanent Investment			**$**	**1,317,782**
Total Depreciable Capital				
Cost of Contingencies & Contractor Fees	$	237,201		
Total Depreciable Capital			**$**	**1,554,983**
Total Permanent Investment				
Cost of Land:	$	-		
Cost of Royalties:	$	-		
Cost of Plant Start-Up:	$	155,498		
Total Permanent Investment - Unadjusted			$	1,710,482
Site Factor				1.00
Total Permanent Investment			**$**	**1,710,482**

13.3.6 Working Capital

Working Capital

	2015	2016	2017
Accounts Receivable	$ 4,114,711	$ 2,057,356	$ 2,057,356
Cash Reserves	$ 55,307	$ 27,653	$ 27,653
Accounts Payable	$ (47,986)	$ (23,993)	$ (23,993)
µMCA Inventory	$ 960,099	$ 480,050	$ 480,050
Raw Materials	$ 11,156	$ 5,578	$ 5,578
Total	**$ 5,093,288**	**$ 2,546,644**	**$ 2,546,644**
Present Value at 15%	*$ 3,851,257*	*$ 1,674,460*	*$ 1,456,052*

Total Capital Investment **$ 8,692,250**

13.3.7 Cash Flow

Cash Flow Summary

Percentage of Design Capacity	Product Unit Price	Sales	Capital Costs	Working Capital	Var Costs	Fixed Costs	Depreciation	Depletion Allowance	Taxible Income	Taxes	Net Earnings	Cash Flow	Cumulative Net Present Value at 15%
0%		-	-	-	-	-	-	-	-	-	-	-	-
0%		-	(1,710,500)	-	-	-	-	-	-	-	-	(1,710,500)	(1,487,400)
0%		-	-	(5,093,300)	-	-	-	-	-	-	-	(5,093,300)	(5,338,600)
49%	$100.00	50,062,300	-	(2,546,600)	(8,719,000)	(2,869,000)	(311,000)	-	38,163,400	(14,120,500)	24,042,900	21,807,300	9,000,000
74%	$102.40	76,895,700	-	(2,546,600)	(13,392,300)	(2,937,800)	(497,600)	-	60,068,000	(22,225,200)	37,842,800	35,793,800	29,465,200
98%	$104.86	104,988,300	-	-	(18,285,000)	(3,008,300)	(298,600)	-	83,396,400	(30,856,700)	52,539,800	52,838,300	55,735,200
98%	$107.37	107,508,000	-	-	(18,723,800)	(3,080,500)	(179,100)	-	85,524,500	(31,644,100)	53,880,500	54,059,600	79,106,700
98%	$109.95	110,088,200	-	-	(19,173,200)	(3,154,500)	(179,100)	-	87,581,400	(32,405,100)	55,176,300	55,355,400	99,916,800
98%	$112.59	112,730,300	-	-	(19,633,300)	(3,230,200)	(89,600)	-	89,777,200	(33,217,600)	56,559,700	56,649,200	118,435,600
98%	$115.29	115,435,900	-	-	(20,104,500)	(3,307,700)	-	-	92,023,600	(34,048,700)	57,974,900	57,974,900	134,915,600
98%	$118.06	118,206,300	-	-	(20,587,000)	(3,387,100)	-	-	94,232,200	(34,865,900)	59,366,300	59,366,300	149,590,100
98%	$120.89	121,043,300	-	-	(21,081,100)	(3,468,400)	-	-	96,493,800	(35,702,700)	60,791,100	60,791,100	162,656,700
98%	$123.79	123,948,300	-	10,186,600	(21,587,100)	(3,551,600)	-	-	98,809,600	(36,559,600)	62,250,100	72,436,600	176,195,600

13.3.8 Profitability Measures

Profitability Measures	
The Internal Rate of Return (IRR) for this project is	247.32%
The Net Present Value (NPV) of this project in 2013 is	$ 176,195,600

ROI Analysis (Third Production Year)

Annual Sales	**104,988,295**
Annual Costs	**(21,293,301)**
Depreciation	**(136,839)**
Income Tax	**(30,916,517)**
Net Earnings	**52,641,638**
Total Capital Investment	**11,897,057**
ROI	**442.48%**

13.3.9 Sensitivity Analysis

Sensitivity Analyses

		Variable Costs										
		$8,896,892	$10,676,270	$12,455,648	$14,235,027	$16,014,405	***$17,793,783***	$19,573,162	$21,352,540	$23,131,918	$24,911,297	$26,690,675
		0.5	0.6	0.7	0.8	0.9	1	1.1	1.2	1.3	1.4	1.5
Product Price	$50.00	0.5	175.09%	169.46%	163.73%	157.87%	151.89%	145.77%	139.51%	133.09%	126.50%	119.73%	112.76%
	$60.00	0.6	198.04%	193.03%	187.95%	182.77%	177.51%	172.16%	166.72%	161.17%	155.51%	149.74%	143.84%
	$70.00	0.7	217.99%	213.43%	208.82%	204.14%	199.40%	194.60%	189.72%	184.77%	179.74%	174.63%	169.44%
	$80.00	0.8	235.66%	231.46%	227.22%	222.92%	218.58%	214.18%	209.73%	205.23%	200.66%	196.04%	191.36%
	$90.00	0.9	251.56%	247.65%	243.70%	239.71%	235.68%	231.60%	227.49%	223.33%	219.13%	214.88%	210.58%
	$100.00	1	266.02%	262.34%	258.63%	254.89%	251.12%	247.32%	243.47%	239.60%	235.69%	231.74%	227.75%
	$110.00	1.1	279.27%	275.80%	272.30%	268.77%	265.22%	261.63%	258.02%	254.38%	250.70%	247.00%	243.27%
	$120.00	1.2	291.52%	288.22%	284.90%	281.55%	278.18%	274.79%	271.37%	267.92%	264.46%	260.96%	257.44%
	$130.00	1.3	302.90%	299.75%	296.59%	293.40%	290.19%	286.96%	283.71%	280.43%	277.14%	273.82%	270.48%
	$140.00	1.4	313.53%	310.52%	307.48%	304.43%	301.37%	298.28%	295.18%	292.05%	288.91%	285.75%	282.57%
	$150.00	1.5	323.50%	320.60%	317.69%	314.76%	311.82%	308.86%	305.89%	302.90%	299.89%	296.87%	293.83%

13.3.10 Summary of Inputs and Chronology of Events for $30 chip

General Information

Process Title: **µMCA Manufacture Process**
Product: **µMCA**
Plant Site Location: **Mid-Atlantic Region**
Site Factor: **1.00**
Operating Hours per Year: **2640**
Operating Days Per Year: **110**
Operating Factor: **0.3014**

Product Information

This Process will Yield

387 Unit of µMCA per hour
9,288 Unit of µMCA per day
1,021,680 Unit of µMCA per year

Price **$30.00 /Unit**

Chronology

Year	Action	Distribution of Permanent Investment	Production Capacity	Depreciation 5 year MACRS	Product Price
2013	Design		0.0%		
2014	Construction	100%	0.0%		
2015	Construction	0%	0.0%		
2016	Production	0%	49.0%	20.00%	$30.00
2017	Production	0%	73.5%	32.00%	$30.72
2018	Production		98.0%	19.20%	$31.46
2019	Production		98.0%	11.52%	$32.21
2020	Production		98.0%	11.52%	$32.99
2021	Production		98.0%	5.76%	$33.78
2022	Production		98.0%		$34.59
2023	Production		98.0%		$35.42
2024	Production		98.0%		$36.27
2025	Production		98.0%		$37.14

13.3.11 Variable Cost Summary for $30 Chip

Variable Cost Summary				
Variable Costs at 100% Capacity:				
General Expenses				
Selling / Transfer Expenses:			$	919,512
Direct Research:			$	2,452,032
Allocated Research:			$	613,008
Administrative Expense:			$	613,008
Management Incentive Compensation:			$	383,130
Total General Expenses			$	4,980,690
Raw Materials	$1.162000	per Unit of µMCA		$1,187,192
Byproducts	$0.000000	per Unit of µMCA		$0
Utilities	$0.004200	per Unit of µMCA		$4,291
Total Variable Costs			**$**	**6,172,173**

13.3.12 Profitability Measures for $30 Chip

Profitability Measures	
The Internal Rate of Return (IRR) for this project is	137.61%
The Net Present Value (NPV) of this project in 2013 is	$ 44,924,000

ROI Analysis (Third Production Year)

Annual Sales	**31,496,488**
Annual Costs	**(9,350,882)**
Depreciation	**(136,839)**
Income Tax	**(8,143,244)**
Net Earnings	**13,865,523**
Total Capital Investment	**4,792,322**
ROI	**289.33%**

13.3.13 Profitability Measures for $100 Chip 2x Expansion

Profitability Measures

The Internal Rate of Return (IRR) for this project is 312.04%

The Net Present Value (NPV) of this project in 2013 is $ 359,143,900

ROI Analysis (Third Production Year)

Annual Sales	**209,976,589**
Annual Costs	**(40,348,894)**
Depreciation	**(136,839)**
Income Tax	**(62,711,617)**
Net Earnings	**106,779,240**
Total Capital Investment	**22,032,561**
ROI	**484.64%**

13.3.14 Variable Cost Summary for $100 Chip 2x Expansion

Variable Cost Summary

Variable Costs at 100% Capacity:

General Expenses

Selling / Transfer Expenses:			$	6,130,080
Direct Research:			$	16,346,880
Allocated Research:			$	4,086,720
Administrative Expense:			$	4,086,720
Management Incentive Compensation:			$	2,554,200
Total General Expenses			$	33,204,600
Raw Materials	$1.162000	per Unit of µMCA		$2,374,384
Byproducts	$0.000000	per Unit of µMCA		$0
Utilities	$0.004200	per Unit of µMCA		$8,582
Total Variable Costs			**$**	**35,587,566**

13.3.15 Fixed Cost Summary for $100 Chip 2x Expansion

Fixed Cost Summary			
Operations			
	Direct Wages and Benefits	$	998,400
	Direct Salaries and Benefits	$	149,760
	Operating Supplies and Services	$	59,904
	Technical Assistance to Manufacturing	$	-
	Control Laboratory	$	-
	Total Operations	**$**	**1,208,064**
Maintenance			
	Wages and Benefits	$	233,247
	Salaries and Benefits	$	58,312
	Materials and Services	$	233,247
	Maintenance Overhead	$	11,662
	Total Maintenance	**$**	**536,469**
Operating Overhead			
	General Plant Overhead:	$	102,220
	Mechanical Department Services:	$	34,553
	Employee Relations Department:	$	84,943
	Business Services:	$	106,539
	Total Operating Overhead	**$**	**328,256**
Property Taxes and Insurance			
	Property Taxes and Insurance:	$	31,100
Other Annual Expenses			
	Rental Fees (Office and Laboratory Space):	$	500,000
	Licensing Fees:	$	-
	Miscellaneous:	$	1,000,000
	Total Other Annual Expenses	**$**	**1,500,000**
Total Fixed Costs		**$**	**3,603,889**

13.3.16 Market Projections

	0	1	2	3	4	5	6	7	8	9	10
Year	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
% Market Share	0%	0%	0%	5%	7.5%	10%	10%	10%	10%	10%	10%
Total Number of Cartridge Sold	0	0	0	500000	750000	1000000	1000000	1000000	1000000	1000000	1000000
Price per Cartridge w/o Inflation	100	100	100	100	100	100	100	100	100	100	100
Inflation	2.4%	2.4%	2.4%	2.4%	2.4%	2.4%	2.4%	2.4%	2.4%	2.4%	2.4%
Price per Cartridge with Inflation	100	102.4	104.8576	107.37	109.95	112.59	115.29	118.06	120.89	123.79	126.77
Total Cartridge Revenue	$0	$0	$0	$53,687,091	$82,463,372	$112,589,991	$115,292,150	$118,059,162	$120,892,582	$123,794,004	$126,765,060

13.4 Lab Protocols

13.4.1 Collagen Surfaces

Making a Collagen Surface [1]

Reagents

- Sigmacote (Sigma)
- Equine Type I Collagen Fibrils (Chrono-Par, Chrono-Log, Havertown, PA) (1 mg/mL)
- .5% bovine serum albumen in HEPES-buffered saline solution

Instructions

1. Treat surface with Sigmacote[2]
   - Cover clean and dry surface with Sigmacote
   - Allow to dry in a hood, and rinse with water
2. Allow Collagen Solution (1 mg/mL) to rest on glass surface for 30 minutes.
3. Wash surface with .5% BSA in HBS.

13.4.2 Tissue Factor Liposomes

Making Tissue Factor Liposomes[47]

Reagents

- Phosphatidylcholine (PC); Avanti Polar Lipids, Alabaster, AL
- Phosphatidylserine (PS); Avanti Polar Lipids, Alabaster, AL
- Biotinylated phosphatidylethanolamine; Avanti Polar Lipids, Alabaster, AL
- 4 mM Triton X-100 in HBS; Fisher Scientific, Fair Lawn, NJ
- Bio-Beads SM-2; BioRad Laboratories
- Purified Recombinant Tissue Factor (1.3 mg/mL)[3]; American Diagnostica Inc, Stamford, CT
- HBS

Instructions

1. Mix 79% PC, 20% PS and 1% biotinylated phosphatidylethanolamine[1].
2. Dry in glass tubes under nitrogen and place in high vacuum for one hour (to remove residual chloroform)
3. Resuspend in HBS, vortex thoroughly
4. Wash Bio-Beads SM-2 in methanol[4]
    - Add 200 mL of methanol to 30g Bio-Beads SM-2, stir for 15 min.
    - Collect beads on sintered glass funnel, wash with 500 mL of methanol.
    - ((Immediately wash with 1000 mL water, wash in chromatography column with another 2000mL water.)) Store in HBS until required.
    - Immediately before using, place beads in weigh dish, remove excess liquid by aspiration, and weigh beads. Do not let beads dry prior to use.
5. Dissolve a total of 2.6 μmol phospholipid (1.59 mg pC, .43 mg PS, .023 mg biotinylated phosphatidylethanolamine) in 993.8 μL of 3 mM Triton X-100 in HBS. Add 6.2 μL recombinant tissue factor (to achieve a 1:10000 protein to lipid ratio)
6. To remove detergent, add 50 mg Bio-Beads SM-2 to above solution in an Eppendorf tube. Mix gently for 90 min at room temperature at 20 rpm on a tube rotator.
7. Add 350 mg Bio-Beads SM-2, rotate for an additional 90 minutes.
8. Allow beads to settle by gravity for 2 min.
9. Remove supernatant which contains the rTF liposomes and store at -80 °C until use.

13.4.3 Tissue-Factor Collagen Surfaces

Making a Tissue-Factor Collagen Surface[2]

Reagents

- Collagen Surface
- Biotinylated polyclonal antibody against collagen type 1 (40 μg/mL); Abcam, Cambridge, MA
- .5% bovine serum albumen in HEPES-buffered saline solution
- Streptavidin (100 μg/mL); Sigma
- Tissue-Factor Liposomes

Instructions

1. Perfuse collagen surface with biotinylated polyclonal antibody against collagen type 1 (40 μg/mL). Allow to rest for 5 minutes
2. Wash surface with BSA.
3. Fill channel with streptavidin immediately. Allow to rest for 5 minutes
4. Fill channel with TF-liposomes. Allow to incubate for 1 hour.
5. Rinse with BSA.
6. Fill devices with BSA for at least 30 minutes before use with blood.[2]

13.4.4 Kaolin-Liposome Mixture

Mixing Liposomes with Kaolin

Reagents

- Kaolin; Sigma Aldrich
- Phosphatidylcholine (PC); Avanti Polar Lipids, Alabaster, AL
- Phosphatidylserine (PS); Avanti Polar Lipids, Alabaster, AL
- Biotinylated phosphatidylethanolamine; Avanti Polar Lipids, Alabaster, AL
- HBS

Instructions

1. Mix 80% PC, 20% PS^2.
2. Dry in glass tubes under nitrogen and place in high vacuum for one hour (to remove residual chloroform)
3. Resuspend in HBS, vortex thoroughly.
4. Make a .1125M suspension of kaolin by mixing 25 mg of kaolin to a total volume of 1 mL in HBS. Make sure there are no pockets of unsuspended kaolin in the tube.
5. Add 800 μL of the kaolin solution to 200 μL of liposome mixture.
6. Vortex this solution thoroughly.

13.4.5 Kaolin-Liposome-Collagen Surface

Making a Kaolin-Collagen Surface

Reagents

- Collagen Surface
- .5% bovine serum albumen in HEPES-buffered saline solution
- Kaolin-Liposome solution, described above

Instructions

1. Perfuse collagen surface with kaolin-liposome solution. Allow to incubate for 30 minutes.
2. Rinse surface with BSA. Surface is ready to use

13.5 Labor Costs

Job	Detailed Job	Number	Wage (hourly)	Wage (annual)	Wage (total)
Manufacturing Workers	Chemical Plant and System Operator	5	$26.40	$54,920.00	$274,600.00
Quality Assurance	Chemical Engineer in pharma manufacturing	1	$44.61	$92,780.00	$92,780.00
Maintenance	Maintenance Workers, Machinery	1	$19.77	$41,110.00	$41,110.00
Supervisor	General and Operations Manager	1	$55.04	$114,490.00	$114,490.00
Engineer	Engineers, all other	1	$44.36	$92,260.00	$92,260.00
Sales/Marketing	advertising sales agents	1	$40.63	$84,510.00	$84,510.00
Field Reps	Sales reps, scientific product	3	$46.04	$95,750.00	$287,250.00
Research and Development	Chemical Engineer in R&D	3	$50.03	$104,060.00	$312,180.00
Clinical Trial Manager	Medical Scientist	1	$46.38	$96,380.00	$96,380.00
				Total	**$1,395,560.00**
				Total with Compensation/Benefits (1.5x)	**$2,093,340.00**

Table 13.2 Breakdown of Labor Cots

13.6 Manufacturing Batch Sizing

Total Devices	1000000	
Total Days of Operation	330	
Devices Per Day	3030.303	
1% quality testing	3060.912	30.60912
1% failure	3091.83	30.9183
Number of Batches a Day	6	
Number of Devices per Batch	515.3051	
Length	8	cm
Width	4	cm
Area	32	cm^2
Total Area per Batch	16489.76	cm^2
	1.648976	m^2
	17.74942	ft^2

Table 13.3 Sizing of a single batch of devices

13.7 Ink Jet Printing Quote

QUOTATION

MicroFab TECHNOLOGIES • INC.

Quotation Number: DW032513
Date: March 25, 2013
Period Valid: 90 days
Prepared by: David Wallace, ext. 12,
david.wallace@microfab.com

For: Melissa Wang
University of Pennsylvania
School of Engineering and Applied Sciences
wmelissa@seas.upenn.edu
513.374.7776

Jetlab® 4xl with two fluid printhead: one piezo and one valve-jet.

Item	Qty	Part no.	Description	Unit Price	Total Price
1	1	JL4-xl	jetlab® 4 printing system, 210x260mm substrate size, base price. Specify 120 or 240 volts. Included in all jetlab® 4 models: • Integrated JetDrive™ III controller with Level 02 firmware; burst, and continuous mode operation; 12 point arbitrary waveform; • CCD camera & optics and synchronous LED illumination for drop observation; • Ethernet, RS-232 port, USB port, CDRW; • Windows based system control program; • JetLab® system control program; • Print-on-the-Fly (both axes simultaneously) and Point-to-Point printing modes; • Raster & vector printing modes; 1. Complex print job definition through scripting; • Software based alignment and rotation correction; and • Single channel of pneumatic controls.	$ 51,000	$ 51,000
2	1	PH-46-AT SPECIAL	Four Fluid Printhead assembly for low temperature MJ style devices and integrated heater to maintain devices (only) at up to 50ºC. Base unit with no fluid channels. Add up to two C-05a fluid channels and/or up to two VJET fluid channels.	$ 2,500	$ 2,500
3	1	C-05a	Fluid channel for PH-46 and PH-46 H printhead: tubing, fittings, fluid block, and polypropylene reservoir.	$ 600	$ 600
4	2	MJ-AT-01-050	Threaded fitting microdispensing device; 50µm diameter orifice. For C-05a.	$ 800	$ 1,600
5	1	VJET-x	Fluid channel for PH-46 and PH-46H SPECIAL printhead: high speed solenoid valve, tubing, fittings, fluid block, drive circuit, orifice, and reservoir.	$ 2,000	$ 2,000

Quotation Number: DW032513
Date: March 25, 2013
Page 2

6	1	VS-4	Vertical optics assembly for substrate fiducial recognition and print inspection.	$ 3,000	$ 3,000
7	1	ZS-01	Motorized Z-stage option.	$ 1,500	$ 1,500
8	1	PL-V-4	Vacuum platen.	$ 1,250	$ 1,250
9	1	CP-01	Pneumatics control channels, *integrated*.	$ 1,200	$ 1,200
10	1	MX-J	Eight channel output, eight channel input relay board for multi-plexed PH-46 printhead and auxiliary control input / output. *Integrated*.	$ 900	$ 900
11	1	INS	On-site installation, training, and process development assis-tance, two days. Travel cost included.	$ 3,000	$ 3,000
			Total		**$ 68,550**

Delivery: Ship 8-12 weeks ARO.
Shipping: Prepaid and added.
Terms: 30 days net.
Notes: *Conditional Sale and License*: This sale is conditioned on the acceptance of the following limited use license. MicroFab grants to the purchaser a use license to use its current and future patent portfolio by employing the Jetlab system purchased from MicroFab. This license is provided solely for the benefit of the purchaser and its affiliated companies. MicroFab does not warrant that all uses of the system are covered by MicroFab's intellectual property, or that the user is indemnified from infringement of the intellectual property of third parties.

MicroFab TECHNOLOGIES • INC.
an ink-jet innovation company
1104 Summit Ave. Ste. 110 Plano, TX 75074 +1-972-578-8076 (p) +1-972-423-2438 (f) www.microfab.com

13.8 Packaging Quote

Imperial
PRINTING & PAPER BOX MFG. CO.
DBA ABDALIAN CARTON CO.
1622 W. 130th STREET • GARDENA, CA 90249
TEL: (310) 323-7300 • FAX: (310) 323-5423
e-mail: info@imperialbox.net web site: www.imperialbox.net

DATE: March 27, 2013

TO: University of Pennsylvania TEL: (513) 374-7776
ATTN: Melissa Wang EMAIL: wmelissa@seas.upenn.edu

FROM: Henny B. Zuiderwijk **QUOTE #: 18709-I**

QUOTATION FORM 3.2a

Description & Specifications	Quantity	Price/M
TTAB size 8 x 8 x 2 ¼ on .020PT CCNB, Printed 4/C+AQ	50,000	260.22/M
Die Cut, Fold, Glue and Pack	100,000	239.71/M

Plate & Proof Charges $240
One time Die Charge approx. $350

Thank you for the opportunity to quote you on your packing needs. Quantities are plus or minus 10%. Prices are FOB our plant, Gardena CA. This quotation is good for 15 days.

Reviewed by: John Setareh

REVISION LEVEL: F APPROVAL DATE: 06/16/11 APPROVED BY: GS