Hydrophilic Poly(sulfobetaine methacrylate) Films on Tygon Tubing to reduce Surface Protein Adhesion

By

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Acknowledgments

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Introduction

Background

The favorable mechanical, optical, and chemical properties of Tygon® have led to the broad use of this bulk material as tubing since its invention in the 1930s[1]. Notable features of this thermoplastic include optical transparency, flexibility without breakage, and compatibility with aqueous media. These characteristics have resulted in its wide-ranging applications in areas of medicine and biotechnology, in many research laboratories, and in the chemical, petrochemical, food, beverage, and pharmaceutical industries.

Despite its favorable bulk properties, the hydrophobic surface properties of Tygon can lead to unwanted results. An example is when Tygon is used as tubing in a recirculatory "circuit" to transport blood between a patient and medical equipment, especially in an extracorporeal membrane oxygenation machine (ECMO), a device used to circulate blood and deliver oxygen into a patient's bloodstream. Currently, Tygon, when used for extended time periods in ECMO circuits, causes the blood to coagulate within the tube. In response to this undesirable behavior, it is necessary to deliver some form of anti-coagulate, such as heparin. This creates a delicate balance of over-heparinization, which could cause the patient to bleed out, in contrast to over-coagulation, which could prevent blood flow to the patient's brain and other vital organs. This M.S. thesis project aims to take the first step in solving this delicate battle many physicians face daily to treat critically ill patients.

The primary focus of this thesis will be the chemical modification of the surface of the Tygon tubing. The trademarked name Tygon® has become synonymous with quality tubing in a wide range of sizes and compositions. Typical Tygon tubing is patent protected by the French multi-national corporation Saint-Gobain. The tubing is made of silicone, polyvinyl chloride, polyurethane, fluoropolymers, thermoplastic elastomers, and other trade secret materials. Because of the wide applications the tubings are used for, the chemical composition of the Tygon, especially of the surface, can vary extensively. Obtaining reliable data about the composition of the select Tygon tubing is challenging. One aspect that makes Tygon so affordable is its ability to be extruded into different-sized diameters and thicknesses for many different applications. In the medical field, Tygon medical grade S-50-HL tubing is claimed to be non-toxic, non-pyrogenic, and non-hemolytic. The Tygon is also able to withstand the cyclical forces used when fluid is pumped through peristatic pumps in many different medical devices.

The focus of the problem that our team is exploring is the formation of blood coagulations in the ECMO circuit for patients under longer-term critical care. The extracorporeal membrane oxygenation machine, or ECMO, is often referred to as the bypass machine as it creates an alternate circuit to the circulatory system of the human body. The ECMO machine is used during the treatment of heart malformations, heart attacks, severe hypothermia, cardiogenic shock, acute respiratory distress syndrome, defects of the lungs or diaphragm, respiratory failure, severe thoracic trauma, and during surgery for heart or lung bypass[2]. During the COVID-19 pandemic, the long-term use of ECMO machines provided oxygenation to patients whose lungs had failed due to the disease[3]. One medical group collaborated and studied long-term COVID patients who were on the machine for over 90 days[3]. The use of ECMO is primarily a last resort to provide critical care to patients that have no other options. The machine bypasses the patient's heart and

lungs, ultimately allowing the tissue to recover from damage without having to continually function.

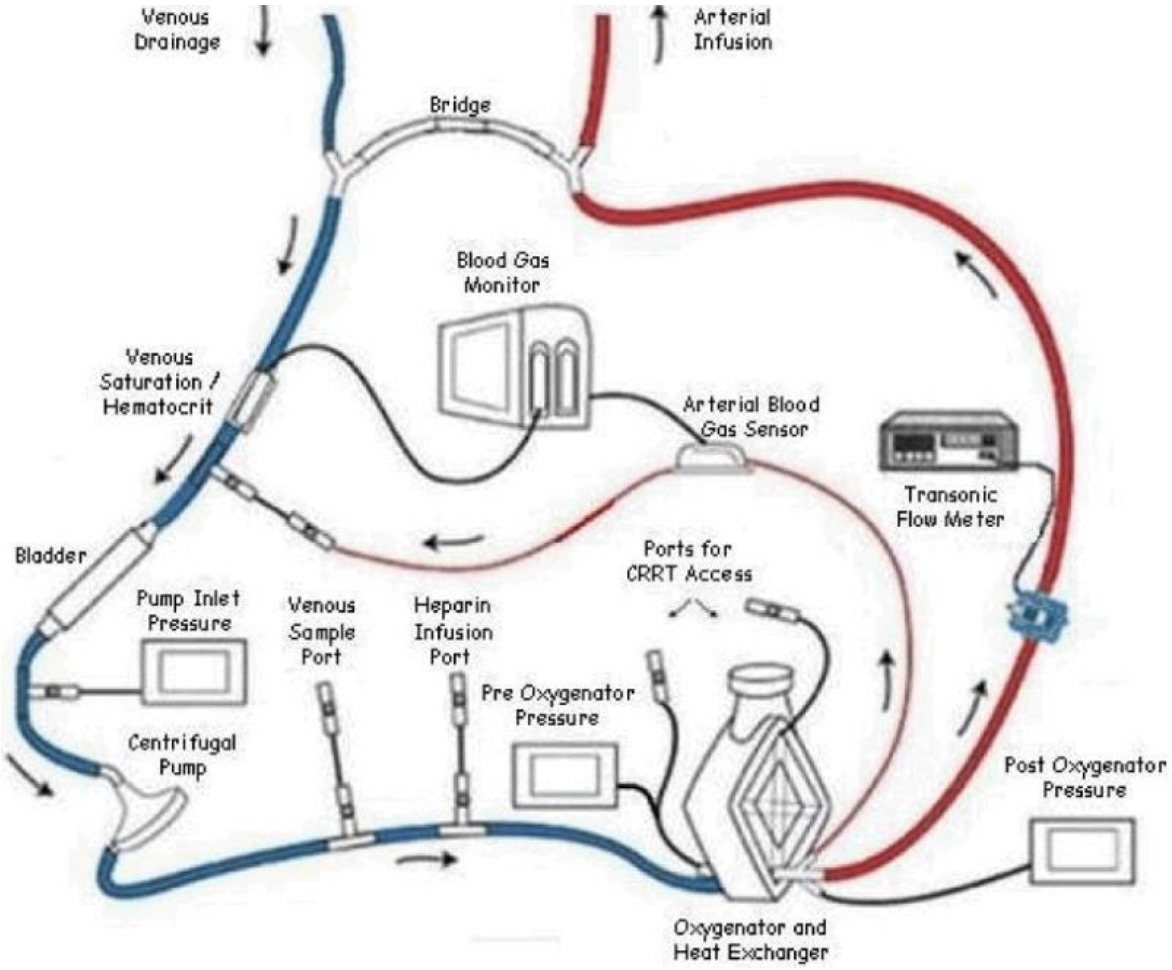

Figure 1[4]: Standard ECMO circuit. Venous blood drains from the patient, goes through a venous saturation sensor and a bladder, and is then pumped to the oxygenator/heat exchanger device. The oxygenated blood passes the ECMO circuit bridge before infusing back into the patient into the arterial (VA) or venous (VV) system. There are multiple infusion and access ports as well as pressure and flow monitors along the way. Adapted from (4).

The implementation of the ECMO circuit is associated with many risks. Operation of the machine requires the inlet and outlet flows of the device to be implanted into major arteries and veins in the body, such as the femoral artery, femoral vein, internal jugular vein, right atrium, or

the aorta[5]. These major blood vessels must withstand high pressures to prevent rupture and reduce the chance of blood escape. Enough coagulation potential is needed in the system to successfully form a clot around the insertion of the cannulas but not enough to impact the flow potential of the system. Likewise, the system needs to have enough anti-coagulant to prevent the clogging of the system by extensive clotting, but not enough to break the clot 'seals' formed around the cannula. This is the delicate balance the physicians must maintain to allow the machine and technique to work towards a successful outcome. Even a minor shift in the scale toward over-coagulation or over-heparinization could be detrimental to the patient. This battle is time-consuming for doctors and hospitals, costly for insurance companies, and potentially deadly to the critically ill.

Blood, the fluid that is so vital to many biological functions, might be the most important solution in the biological field. The evolution of this colloidal suspension allows organisms to transport nutrients, oxygen, waste, and other material vital for biological subsistence as well as regulating the body's concentration of components and temperature. Blood is primarily separated into two distinct fluids, plasma, composed primarily of water that suspends the specific proteins required for coagulation to occur, and the formed elements, which contain the platelets, erythrocytes (red) and leukocytes (white). Working in conjunction with the immune system, the circulatory system can identify damaged or unfamiliar locations and begin the coagulation process.

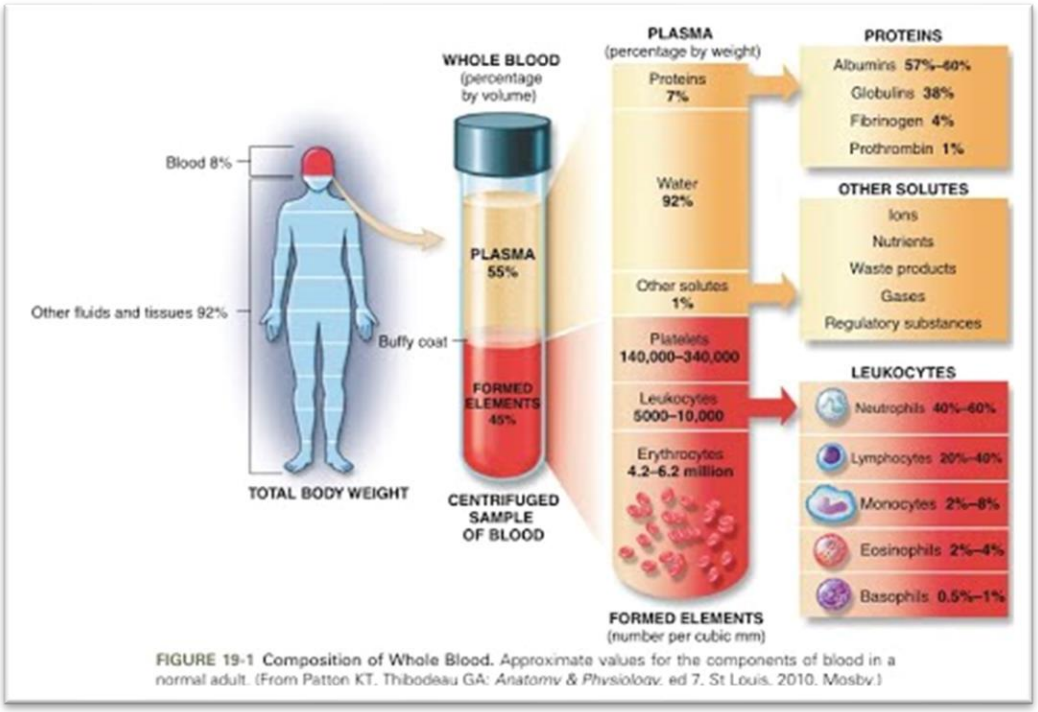

FIGURE 19-1 Composition of Whole Blood. Approximate values for the components of blood in a normal adult. (From Patton KT, Thibodeau GA: *Anatomy & Physiology*, ed 7, St Louis, 2010, Mosby.)

Figure 2[6]: Breakdown of the Components and Aspects of Blood. The composition and relative percentages of each component from their respective grouping are given. Approximate values are given from a normal human adult. Adapted from (6)

Coagulation is a complex cascade of enzymatic reactions that lead to a blood clot forming. Understanding the coagulation cascade is crucial for this project to change the way ECMO circuits are administered to patients. There are two main pathways for coagulation to occur. The more common pathway, the extrinsic pathway, is initiated when tissue factors are released from damaged blood vessels or surrounding tissues. This pathway allows the blood to coagulate around any injury the body suffers to prevent blood loss. The second pathway is the intrinsic pathway, which is activated by protein responses to an unfamiliar surface. This pathway protects the body from foreign bodies that enter the bloodstream. In more modern aspects, this pathway is responsible for the coagulation and biofouling that occurs on most modern biomedical devices. As

seen in Figure 3, the cascade responsible for the clotting of blood is intricate and cyclical. The non-isochronal cascade increases in intensity until a sufficient coagulation occurs.

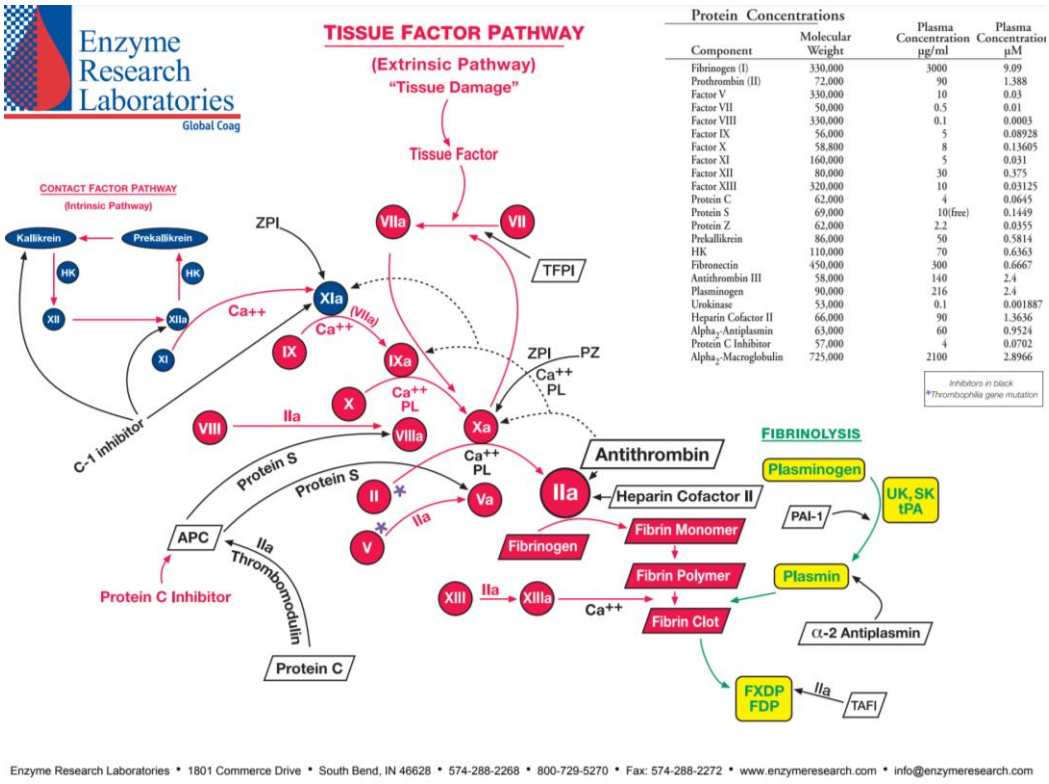

Figure 3[7]: Coagulation Cascade with pathways labeled. Adapted from (7)

With the implementation of the ECMO circuit, the intrinsic pathway is the larger hurdle when dealing with the problem of hemocoagulation. As blood is circulated and oxygenated, it comes into contact with many different unique non-biocompatible surfaces. The amount of surface contact differs for each machine and patient, but even pediatric ECMO units contain at least several square meters of surfaces excluding the oxygenation unit. This large amount of surface contact creates a large gradient of contact proteins to begin the process of blood coagulation, intensifying as the ECMO unit continues to operate over longer times without replacement. Many patients using ECMO circuits have a wide array of hemostatic disorders and cardiorespiratory changes[8].

The longer that patients remain on ECMO support, the worse the biofouling occurs in the tubing and oxygenator of the circuit. In a 2010 Extracorporeal Life Support Organization (ESLO) Extracorporeal Life Support(ECLS) international registry report, 8-17% of patients experience moderate to severe thrombosis, and 7-34% of patients deal with uncontrolled bleeding[9]. The need of physicians to balance the critical scale of unanticipated coagulation vs over-heparinization is demanding and this thesis hopes to provide the first steps towards a solution.

Literature Review

Many medical papers have been written on the now well understood mechanism of hemostasis, but there is very little information about the specific effect Tygon tubing has on the coagulation of blood. Saint Gobain's patent protects the rights of their products to be infringed upon, and the company does not reveal such proprietary information. Having the ability to combine the medical knowledge of the past decade with the engineering practices currently developing will hopefully provide a meaningful solution to this systemic problem.

Because of the limited information on the chemical makeup of Tygon tubing, previous literature is crucial to finding a starting point. In their 2018 publication in *Langmuir*, Jannat and Yang chemically modified Tygon to formulate bioassays[10]. They tested the exposure of Tygon tubing to a variety of chemical solvents to determine those that altered the physical characteristics of the tubing. Their team sought to develop a chemical modification to the polyvinyl chloride tubing without the use of a plasma treatment step, reasoning that plasma treatments are difficult to control, the results of the treatment vary, and the reactive groups on the surface of the substrate are short lived. Their research, along with polymer compatibility charts, indicates that Tygon reacts extremely poorly with extended exposure to most organic solvents, which creates the challenge of

chemically modifying the tube surface. After their experimentation, they determined that exposure of Tygon to a solution of 5% (3-aminopropyl)triehoxysilane or APTES, was able to provide a surface of long-lived free amines. They continued to develop their bioassays, but the reactive surface of free amines is a good anchor point for any further chemical modification.

Another tangentially related topic is the chemical modification of polyurethane catheter medical devices. Nagaoka and Akashi discuss the use of hydrophilic polymers to create low friction surfaces[12]. The team tested these polymer-coated catheters for biocompatibility and low surface friction coefficients. Hydrophilic polymers have been used before to modify medical devices, but concerns such at toxicity and bio reactiveness must be considered for new approaches.

Previous work done in the Laibinis-Jennings Laboratory examined different types of polymers to create nonfouling monolayer surfaces. In the dissertation of Bradley Baker, several different zwitterionic monomers were used to develop thin film hydrophilic surfaces[11]. In his thesis, Baker presented his novel procedure for producing precisely controlled thin films to impart fouling resistance to the surface of silicon wafers using ARGET ATRP, which stands for activators regenerated by electron transfer atom transfer radical polymerization. ARGET ATRP is a way of forming a carbon-carbon bond with a regenerating transition metal catalyst to form more uniform polymer chains.

Topic

Our team, in combination with medical professionals at Vanderbilt University Medical Center, have developed a novel procedure for chemically modifying the surface of Tygon tubing to develop an extremely hydrophilic surface. This hydrophilic surface is hypothesized to repel the surface binding proteins in blood that initiate the coagulation cascade. The polymer film is designed to inhibit the intrinsic pathway, which will ultimately provide a longer usable life for the ECMO circuit, allowing physicians to better manage a slower coagulation process and reducing complications for patients who receive ECMO care.

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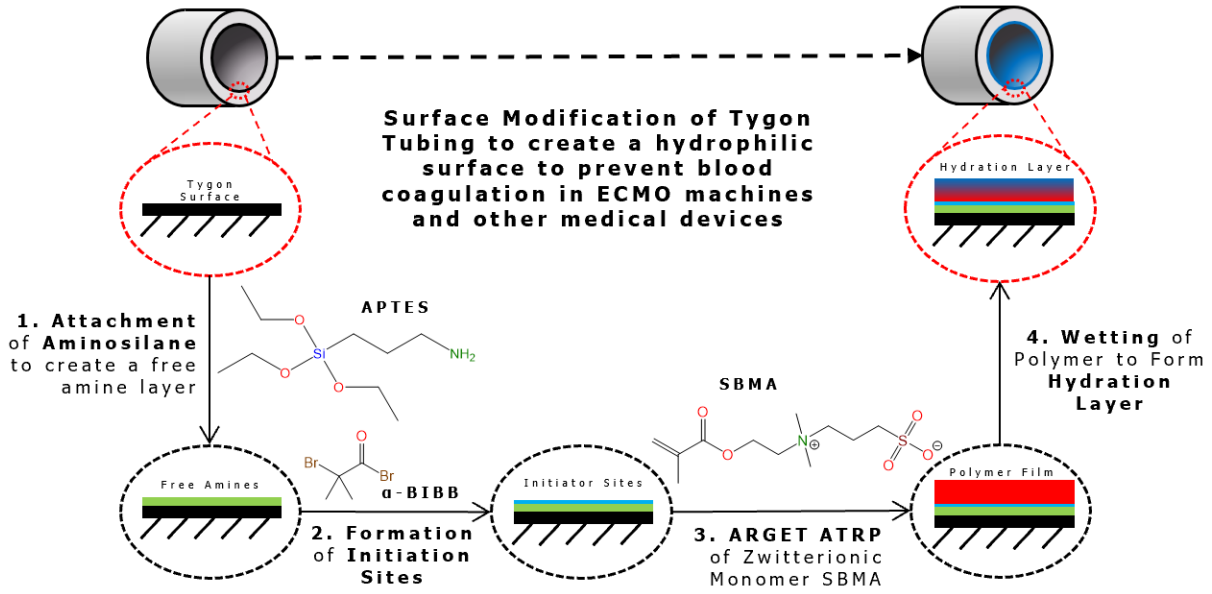

Figure 4: Schematic for development of hydrophilic polymer surfaces on Tygon Tubing

As previously discussed, the reproducible process developed here will combine steps from past literature to construct hydrophilic films on the surface of Tygon. As seen in Figure 4, an

unmodified Tygon tube can be modified in three simple steps. Our process uses a solution of APTES to provide an anchor of free amines on the surface of the Tygon. We then expose the APTES-modified tubing to a vapor of α-bromoisobutyryl bromide to covalently anchor initiator sites for our monomer. The modified tubing is then reacted with the zwitterionic monomer SBMA to create a zwitterionic poly(sulfobetaine methacrylate) polymer. This polymer is designed to capture and hold water molecules to create a hydration layer, similar to a hydrogel. The blood contact proteins approaching the surface will primarily detect water molecules, and thereby, will not begin the cascading effect.

Chapter II

Materials and Methods

Materials

Terumo-CVS 36" Quick Prime Line (Unmodified Tygon Tubing), Terumo-CVS 1/4X3/32 65D 2' X-Coated (Modified Tygon Coating 1), Medtronic 6C78R Custom Pack 3/8 Raceway (Modified Tygon Coating 2), and Medtronic Intersept PVC tubing 1/4X1/16" (Modified Tygon Coating 3) were supplied by Dr. Justin Godown of the Vanderbilt University Medical Center. (3-Aminopropyl) trimethoxysilane (APTMS; 97% purity), (3-aminopropyl) triethoxysilane (APTES; 98% purity), 10-undecenoyl chloride (97% purity), alpha-bromoisobutyryl bromide (98% purity), [2-(methacryloyoxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide (SBMA; 97% purity), copper (II) bromide ($CuBr_2$; 99% purity), tris(2-pyridylmethyl) amine (TPMA; 98% purity), and methanol (99.9% purity) were obtained from Sigma-Aldrich and used as received. Toluene (99.8% purity) was obtained from Fisher Scientific. Deionized water (10 MΩ) was purified by using a Millipore Elix filtration system.

Synthesis and Safety

Chemical synthesis was conducted by the described stepwise processes to form the investigated polymer coatings by solution-phase or vapor-phase reactions on the polymeric materials. No chemical reactions were performed to synthesize molecular compounds. All chemical solutions were formed by mixing and/or diluting prepared stock solutions based on mass weights and volumes to yield specified compositions.

All relevant safety protocols were followed in the process of this project. All lab work required, at a minimum, long sleeves, eye protection, and nitrile gloves. All handling and use of volatile chemicals were performed in a working certified fume hood.

The aminosilanes used in this project are class 3 hazards by the MSDS health hazard score and were handled in a fume hood. Work not done in a fume hood can lead to irritation or damage of the body's mucous membranes and upper respiratory tract with the target organs of aminosilanes being the nerves, liver, and kidney[13]. The initiator used in this process, α-bibb, is also volatile. It has an MSDS health hazard score of 3 and is classified as a skin corrosive/irritant that can cause severe chemical burns and eye damage[14]. Because both the aminosilane and initiator react with water vapor, proper storage of the bulk chemicals is essential. All work should be and was completed in a fume hood with special precautions taken for these volatile chemicals.

Tygon Surface Characterization

Unmodified Tygon tubing to be used in this investigation along with three commercially available Tygon tubings with surface coatings for blood contact were analyzed to prepare a baseline for later modification testing. Characterization of these tubing used water contact angle measurements and infrared spectroscopy to determine their hydrophilicity and the chemical information about their composition. Centimeter-long tubing samples were sliced from the provided Tygon lengths using either scissors, a scalpel, or an Exacto knife. A perpendicular clean edge of the samples was ideal but not necessary, as only the inner and outer surfaces--i.e., those parallel to the direction to the flow through the tubing--were relevant and probed. Contact angle measurements were performed using a NRL CA Goniometer (Ramé-Hart) and made with deionized water droplets applied to the probe surface by a syringe. Tygon tubing samples were placed on the stage on their curved side to see through the tubing center. The tubing samples were

viewed looking through the tube, then rotated 90˚ clockwise to present a flat surface at the apex of the tubing. The syringe needle was positioned above the apex of the tubing and one droplet was dispensed. Contact angles were measured on both sides of the droplet with care being taken to ensure the droplet remained on the apex of the tubing. For inner surface contact angles, the tubing samples were cut and spread apart on a hard surface and bound using clips to prevent re-roiling. Contact angle measurements were performed on the flattest part of the unrolled samples.

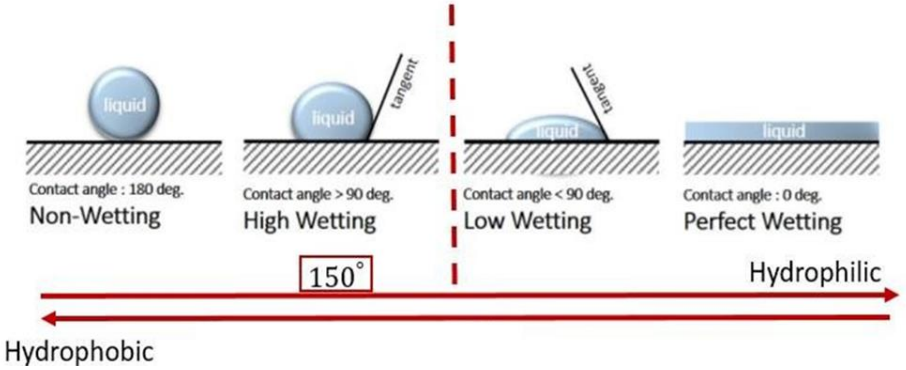

Figure 5: Schematic illustration of contact angles for various wetting behaviors. Angles over 90° are considered hydrophobic, while angles below 90° are considered hydrophilic, with angles less than 15° considered extremely hydrophilic.

To assess the chemical composition of thin films, samples were analyzed using a Nicolet 6700 FT-IR spectrometer (Thermo Scientific). The spectrometer lens and sample tray were first cleaned using a Kimwipe wet with water then with a Kimwipe wet with ethanol. After cleaning and ethanol evaporation, Tygon samples were placed under the sample holder and scans were taken for the absorbance of the sample. Spectra were recorded over wavelengths from 600-3500 cm^{-1}. Typically, 256 scans were collected for each sample to reduce background noise in the spectra. For the Tygon samples, the standard background was of room air, with the machine correcting for detection of CO_2 and H_2O. Each spectrum was analyzed using software on the

spectrometer to find major peaks which were labeled with their corresponding wavelength. The spectrum of unmodified Tygon was often subtracted from the spectrum of coated Tygon to more easily distinguish the key peaks due to the coating material.

APTMS-modified Tygon Surface

Following a similar protocol used by Jannat and Yang[10], an aminosilane was used to absorb into the Tygon surface and provide anchoring sites for further chemical modification[10]. In their work, they used APTES as the amine-rich reactive silane in their chemical modification. Our laboratory had APTMS, a structurally similar amine-rich reactive silane, available from prior work which we first tested to modify the Tygon surface.

First, tube samples of ~1 cm length were sliced from the bulk unmodified Tygon tubing. The samples were then sonicated in deionized water for at least 10 min to remove any contaminants and thoroughly dried. A solution of APTMS was made using 1 mL of aminosilane to 19 mL of deionized water to create a 5% solution of APTMS. The Tygon samples were immediately immersed in the 5% APTMS solution, the vial was capped and then swirled slightly to promote dispersion in solution and even coverage of the APTMS on the Tygon surface. Following the literature procedure of Jannat and Yang[3], the sealed samples were placed in a 50 ˚C water bath for 1-3 hrs. After the APTMS treatment, the samples were removed from solution, rinsed with deionized water, and placed in a dry, clean vial. The samples were then placed in a vacuum oven at 100 ˚C for 30 min to remove any excess water and promote cross-linking between the aminosilane molecules in the Tygon surface. After drying, the samples were sealed in vials to cool, and then characterized to determine their wettability by contact angle measurements and their surface composition using IR spectroscopy. Most APTMS-modified Tygon samples were compared to Tygon controls that similarly underwent incubation at 50 °C albeit with no APTMS

in the contacting phase. I varied both times of exposure to the APTMS solution and temperature of the water bath to optimize the absorption process. Times tested included 1, 2, and 3 h of exposure, and temperatures tested included 50, 60, 70, and 80 ˚C.

APTMS-modified Glass Surface

Glass slides were modified with APTMS to give a more accurate IR spectra of only APTMS. Glass samples were first cut from glass microscope slides. The glass samples were rinsed with deionized water and sonicated for 10 min. The samples were then treated with piranha solution. The piranha solution was made using a 3:1 ratio of concentrated sulfuric acid to 30% hydrogen peroxide, adding the peroxide slowly to the acid as the reaction is extremely exothermic. The glass slides were immersed in the piranha solution for 30 min and then rinsed with deionized water. APTES was then drop casted onto the surface of the glass slide to bind to the free hydroxide groups. Samples were then dried in a vacuum oven for 30 min at 100˚C. Contact angles and IR spectra were then taken to characterize the surface of the APTMS-treated glass samples. This experiment was repeated with a 5% APTMS solution dropped onto the glass surface to better differentiate the peaks resulting from APTMS rather than the Tygon.

APTES-modified Tygon Surface

In this experiment, APTES was used instead of APTMS. More closely following the procedure of Jannat and Yang would theoretically give the Tygon better absorption of the aminosilane. Similar to the APTMS experiment, this experiment follows similar protocols.

First, tube samples of ~1 cm length were sliced from the bulk unmodified Tygon tubing. The samples were then sonicated in deionized water for at least 10 min to remove any contaminants and thoroughly dried. A solution of APTES was made using 1 mL of aminosilane to 19 mL of

deionized water to create a 5% solution of APTES. The Tygon samples were immediately immersed in the 5% APTES solution and the vial was agitated slightly to promote dispersion of the APTES on the Tygon surface. The vials were then capped to prevent escape of any volatile aminosilane molecules. Following literature, the sealed samples were placed in a 50˚C water bath for 1 hr. After the APTMS treatment, the samples were taken out of solution, rinsed with deionized water and placed in a dry, clean vial. The samples were then positioned in a vacuum oven at 95-100˚C for 30 minutes to remove any excess water and promote cross-linking between the aminosilane molecules in the Tygon surface. After drying, the samples were sealed in vials to cool then characterized for contact angles and surface composition using IR spectroscopy. APTES modified samples were compared to controls using only deionized water during the heated water bath exposure.

For optimization of the APTES modification protocol, several variables were changed. Temperature trials were conducted at 50˚C, 60 ˚C, 70 ˚C, and 80 ˚C to determine which had the best absorption into the Tygon. Timed trials were conducted using exposure times from 5 min to 3 hrs with 15-min increments. Concentration trials were conducted between 0 - 10% concentration by volume to determine which concentration gave the best absorption before crosslinking instead of crosslinking in solution.

Organic Solvent Compatibility Testing

Tygon does not react well with most organic solvents as shown by solvent compatibility tables[15]. An acyl chloride could be used to bind to the free amines on the surface of the APTES-modified Tygon to provide a better peak for identification. Common laboratory organic solvents were tested against Tygon to determine which, if any, was compatible with Tygon. Small Tygon samples were submerged in hexane, toluene, acetone, and dimethylformamide. Samples were

allowed to interact for 3 h and then rinsed with deionized water and dried in a vacuum oven at 100˚C for 20 min to remove excess organic solvent and water. Physical characteristics such as transparency, flexibility, and elasticity were crudely evaluated to compare solvent interactions.

Tygon was tested against methanol and a 50:50 water-methanol solution as well. Tygon samples were submerged overnight (16 hrs) to determine compatibility for the next polymerization step. Physical characteristics were measured after samples were removed and rinsed.

Acyl Chloride-modified Tygon Samples

The IR spectra for samples modified with APTES exhibited peaks that were difficult to distinguish from peaks due to the bulk Tygon. Having the free amines react with an acyl chloride to produce an amide peak would hopefully provide more insight. A 50 mM acyl chloride solution was prepared by using 10-undecenoyl chloride in 20 mL of toluene. APTES-modified samples were submerged in the acyl chloride solution for 1 hr at room temperature. After treatment, samples were rinsed with deionized water and then dried in the vacuum oven for 30 min at 100˚C. Contact angles and IR spectra were taken for these samples.

Rinsing methods were considered in later experiments. Instead of rinsing with only water, the acyl chloride-modified Tygon was rinsed with water and ethanol. Other protocols were also evaluated, such as drying out the toluene from the sample for 72 hrs or dropping 10 μL of 10-undecenoyl chloride on the APTES-modified Tygon without an organic solvent. All samples were characterized using contact angles and IR spectroscopy.

Thin Polymer Films on Silicon Wafer

After the aminosilane absorption process, a hydrophilic surface can be built upon the scaffolding provided by the high coverage of free amines. Based on the previous work done by the Laibinis lab, we decided to utilize the zwitterionic monomer SBMA to create a hydration layer that would act as a hydrophilic surface[16]. The initiator α-BIBB is used to bind to the APTES to form an amide linker group. SBMA then polymerizes from the initiator sites using activators regenerated by electron transfer (ARGET) atom transfer radical polymerization (ATRP). This method allows for a controlled linear polymerization from the bound initiator to form the hydration layer.

Thin glass slides or silicon wafers were cut into small manageable samples that were about 1-2 cm^2. The samples were then treated with piranha solution for 1 hr to provide a chemically clean and reactive surface. The piranha solution was made using a 7:3 ratio of concentrated sulfuric acid to 30% hydrogen peroxide, adding the peroxide slowly to the acid as the reaction is extremely exothermic. The glass or silicon slides were immersed in the piranha solution for 1 hr, then rinsed with deionized water and ethanol, and then dried using a nitrogen gas stream. The slides were then immersed in a 5% APTES solution for 1 hr at 60˚C. The APTES solution was made using 1 mL of APTES stock solution and 19 mL of deionized water. After 1 hr of submersion, the samples were rinsed with deionized water and dried in a vacuum oven at 100˚C for 30 min.

Once a free amine surface was prepared on the glass/silicon samples, the initiator was attached to the surface via amide bonds formed by reaction of the free amines with an acyl bromide group on the initiator. A 100 mM α-BIBB solution was made in toluene. Toluene was used here, as it had the best compatibility with Tygon out of the organic solvents tested. The glass/silicon samples were submerged in the initiator solution and allowed to react overnight (16 hrs). The

samples were then rinsed with toluene to remove any unbound initiator and then vacuum dried at 100˚C for 2 hrs to evaporate any residual toluene. Once the initiator was bound, we proceeded with the polymerization.

ARGET ATRP must be done in an anaerobic environment to prevent oxygen from oxidizing the catalyst used in the reaction. First, three solutions were prepared. Solution 1 was the catalyst and ligand solution containing 18 mM $CuBr_2$ and 86 mM TPMA in methanol. Solution 2 was a reducing solution made of 25 mg of ascorbic acid per milliliter of methanol. Solution 3 was a monomer solution consisting of made with 0.075g SBMA, 7.5mL H_2O, 7.5mL methanol, and 60μL of solution 1 (18 mM $CuBr_2$ and 86 mM TPMA) added before any further steps. Solution 3 was sealed in a vial with a septum and sparged with nitrogen gas for 30 min to deoxygenate the solution. After 30 min, 1.2 mL of ascorbic acid solution 2 was added with a syringe then sparged for 5 more min. The initiator bound samples were placed in vials and sealed. The samples were sparged for 30 min to remove any oxygen or bound oxygen from the sample. Once both sparging steps were complete, the monomer solution was transferred to the sample solution via syringe and then sparged for 2 min and resealed. The samples were exposed to the monomer solution for 1-4 hrs depending on the experiment and coverage/polymer thickness needed. The samples were then unsealed which stopped polymerization once the catalyst was oxidized. Samples were submerged in a 50:50 water/methanol solution to remove any unreacted monomer before being rinsed with methanol and then water. Samples were dried in nitrogen if needed.

Thick Polymer Films on Tygon

Tygon samples were cut from the bulk Tygon tubing in sizes deemed appropriate for the experiment, typically 1-2 cm long. Tygon samples were sonicated in deionized water for 15-30 min to remove any contaminants on the tubing. Samples were placed in a 5% APTES solution (1mL APTES, 19mL deionized water) for 2 hrs at 60˚C. After silanization, the samples were rinsed with deionized water and then dried in a vacuum oven at 100˚C for 30 min to promote crosslinking.

After silanization, the initiator was bound to the APTES-modified Tygon by exposing to a 100 mM α-BIBB solution for 2 hrs at room temperature. Enough initiator solution was used to conclude that the limiting factor in the exposure was time and not α-BIBB reactant. The samples were rinsed with Tygon and dried in a vacuum oven at 100˚C for 30 min. Later experiments employed initiator in the vapor phase to react on the Tygon without the use of the organic solvent, toluene. In the vapor reactions, 100-200 μL of α-BIBB was placed in a vial. APTES-modified Tygon samples were then suspended by a rubber band or needle to hang in the vial while the vial was sealed. The Tygon was exposed to the vapor initiator for 30 min before being dried with nitrogen gas.

ARGET ATRP must be done in an anaerobic environment to prevent oxygen from oxidizing the catalyst used in the reaction. First, three solutions were prepared. Solution 1 is the catalyst and ligand solution containing 18mMol $CuBr_2$, 86mMol TPMA solution in methanol. Solution 2 was a reducing solution made of 25mg of ascorbic acid per milliliter of methanol. Solution 3 was a monomer solution made with 0.075g SBMA, 7.5mL H_2O, 7.5mL methanol, and 60μL of solution 1 (18mM $CuBr_2$, 86mM TPMA solution) added before any further steps. Solution 3 was sealed in a vial with a septum, then sparged with nitrogen gas for 30 min to deoxygenate the solution. After 30 min, 1.2 mL of ascorbic acid solution 2 was added with a syringe then sparged

for 5 more min. The initiator bound samples were placed in vials and sealed. The samples were sparged for 30 min to remove any oxygen or bound oxygen from the sample. Once both sparging steps were complete, the monomer solution was transferred over to the sample solution via syringe then sparged for 2 min and resealed. The samples were exposed for 1-4 hrs depending on the experiment and coverage/polymer thickness needed. Once polymerization time had passed, the samples were unsealed which stopped polymerization once the catalyst was oxidized. Samples were submerged in a 50:50 water/methanol solution to remove any unreacted monomer before being rinsed with methanol and then water. Samples were dried in nitrogen if needed.

For thicker films and more even coverage, later experiments increased the amount of monomer used in solution 3. For the best results, the amount of monomer was increased from 0.075g to 0.375g of SBMA. This five times increase provided thick coverage of the polymer. Polymerization continued to take overnight (roughly 16 hours), with the rinsing method staying the same for thicker films. Polymer films had contact angles and IR spectra captured to characterize the surfaces.

Plasma Coagulation Testing

The goal of this project is to develop a surface chemical modification that prevents coagulation on the surface of Tygon. To evaluate the effectiveness of the polymer modification for this goal, I worked with Dr. Gailani, a professor of hematology oncology at Vanderbilt University Medical Center, and his team to employ the techniques used in their lab[17]. Human normal pooled plasma was used in order to test the activation of the intrinsic pathway to indicate a coagulation response that would be appropriate of whole blood. In the lab, plasma was added to a cuvette with PBS, calcium, and a standardized silicon bead. The cuvette was placed in a Stago stART machine that oscillated the plasma after calcium is added to begin the coagulation cascade.

Time of plasma coagulation was automatically recorded by the machine when the silicon bead stopped oscillating due to the thicker viscosity of the coagulated plasma. To test the modified Tygon samples, Tygon was placed on a 37˚C heating pad to mimic the body's temperature. About 200 µL of normal pooled plasma was added to the Tygon sample with the plasma staying in the tube through capillary action. After 10 min of incubation, 35µL samples of incubated plasma were removed and tested in the stART machine. Times for coagulation were compared to those for the coagulation of control plasma. Longer coagulation times in this experiment were undesirable because the proteins needed for coagulation were bound to the surface of the modified Tygon and not in the plasma samples being tested.

Western blotting was also performed on the sample by Dr. Maxim Litvak of the Gailani Lab. After plasma samples were removed for viscosity testing, the Tygon sample surfaces were washed with PBS buffer. The wash was collected and run in a gel electrophoresis unit to separate the bound surface proteins based on size. After electrophoresis, the gels were washed with incubating solution and protein specific fluorescent antibodies to visualize the protein bands. Images of the gels with marked protein bands show which proteins are bound to the surface and the darkness of the band indicates how much of the specific protein is bound. Results of these experiments are presented and discussed in Chapter 3.

Chapter III

Results and Discussion

The team on this project consists of both engineering professionals and medical professionals to create a new coating for medical-grade Tygon tubing. We decided to create a chemically modified surface to prevent hemocoagulation. Chemically modifying the surface of the coating would theoretically allow for stronger, longer-lasting surface modifications as compared to dissolvable coatings that are commercially available. Tygon is a clear polyvinyl chloride tubing used across the medical, pharmaceutical, industrial, and food industry fields. It is popular due to its transparency, flexibility, structural, and non-toxic properties. This project aims to develop a chemical process that will take unmodified Tygon tubing and develop a better surface.

We ultimately decided to mimic the body's natural surface to prevent hemocoagulation. The body is over 70% water. Our solution is to develop a superhydrophilic surface on the inner diameter of the Tygon tubing to trap water in a hydration layer. Any blood flowing through the Tygon tube will not recognize an exterior surface to trigger the intrinsic pathway of blood coagulation, because the surface recognition proteins needed to start the pathway will only see water. The process is shown below.

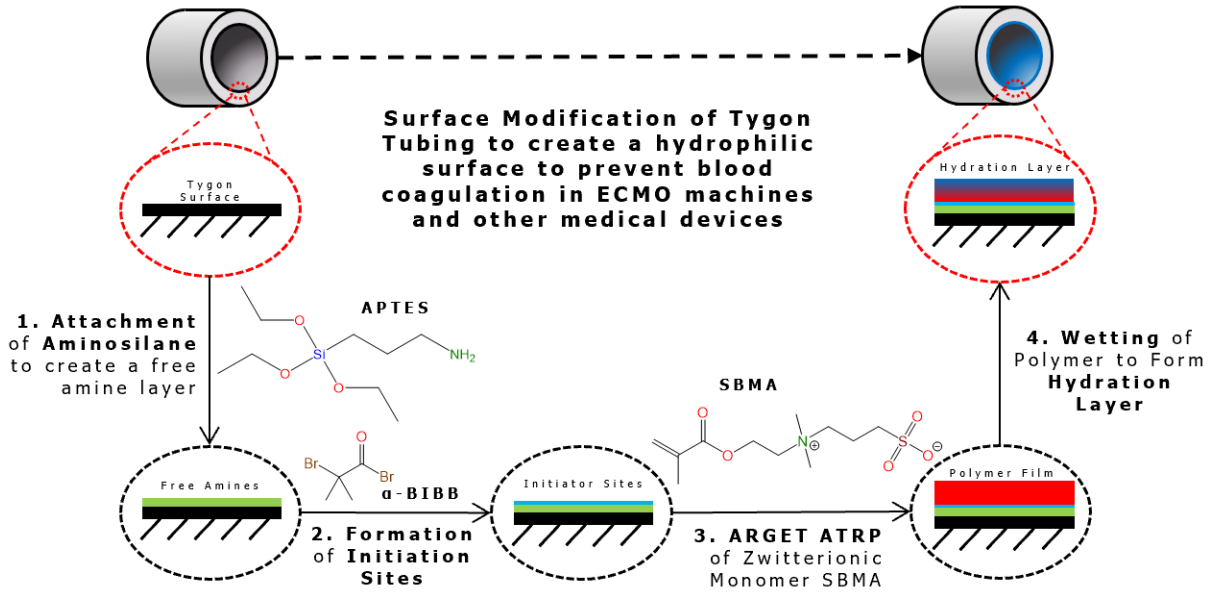

Figure 6: Schematic for development of hydrophilic polymer surfaces on Tygon Tubing to prevent hemocoagulation

As seen in Figure 6, the Tygon will be treated with an Aminosilane, either APTMS or APTES, to provide a surface of free amines upon which to bind an acyl bromide initiator. The acyl bromide will provide the initiation sites needed for the ARGET ATRP of the zwitterionic monomer SBMA. The resulting poly-zwitterionic polymer is designed to freely trap water to create a hydration layer to repel protein adhesion. This chapter will look at the key intermediary results in developing this process and the preliminary results of initial coagulation studies.

Characterization of Tygon Tubing

Several types of medical-grade Tygon tubing were donated by Dr. Justin Godown from the Vanderbilt Medical Center. The purpose of obtaining these types of modified tubings was to compare the results of our modification scheme to the commercially available coating to determine if a better, less thrombogenic surface could be created. The four tubings that were donated for this project include Terumo-CVS 36" Quick Prime Line (Unmodified Tygon Tubing), Terumo-CVS X-Coated (Coating 1), Medtronic 6C78R Custom Pack 3/8 (Coating 2), Medtronic Intersept PVC

tubing (Coating 3). All four tubings had, relatively, the same physical transparency and flexibility. Contact angles were taken of the four coatings as seen below. The difference in contact angles shows that regardless of visual characteristics, the surfaces are very different. Unmodified Tygon has the largest contact angle with water at about 93˚. The commercially available coatings ranged from hydrophobic to moderately hydrophilic, with all samples being more hydrophilic than unmodified Tygon.

Table 1: Surface contact angles of donated Tygon tubing with deionized water

Tygon Sample	Type of Surface	θ_{water} (˚)
Unmodified Tygon	polyvinyl polymer tubing	93
Terumo-CVS X-coated (Coating 1)	non-heparin based biopassive polymer	70
Medtronic 6C78R (Coating 2)	unknown commercial surface	88
Medtronic Inersept PVC (Coating 3)	End Point Attached heparin coating technology	54

IR spectra taken of the tubings showed very little difference in the bulk chemical composition, due to the small thickness of the commercial coating to the Tygon. IR spectroscopy of the modifications in this project is challenging due to the thin nature of the modifications. The machine can detect past the thickness of the coating into the bulk Tygon material. A sample of the IR spectra taken for unmodified Tygon can be seen below. Tygon is a patent-protected blend of many materials, resulting in the labeling of specific beaks being difficult to predict. The team used the unmodified Tygon spectra as a basis to compare the spectra of later modifications through spectral subtraction.

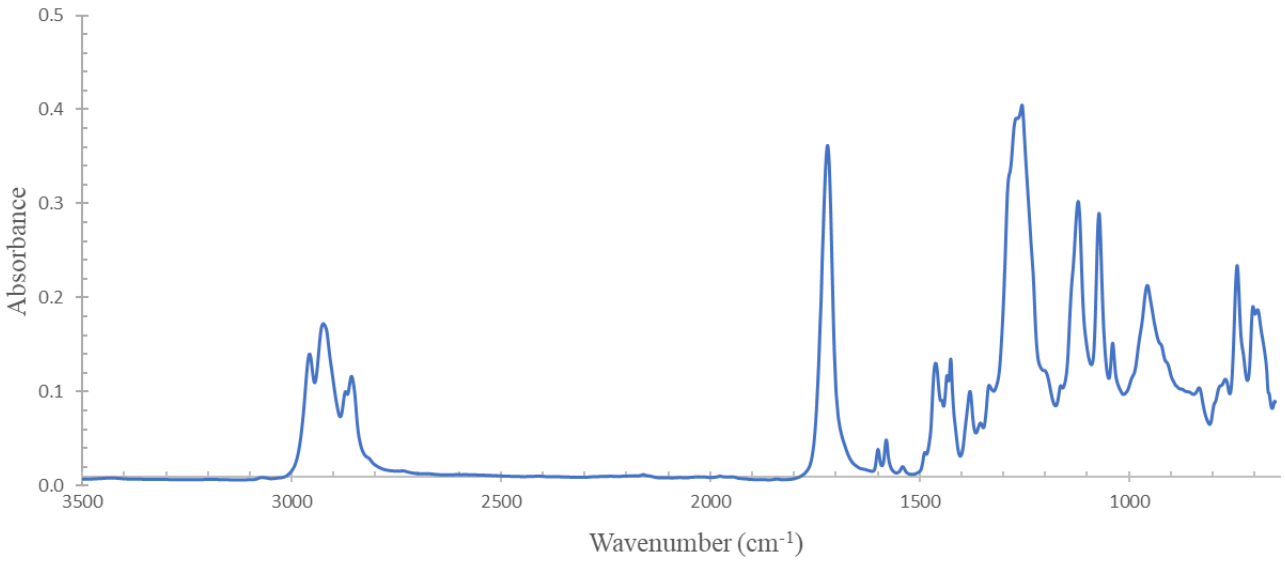

Figure 7: IR spectrum of Unmodified Tygon taken at a resolution of 256 scans with a background of air corrected for water vapor and carbon dioxide

Modification of Tygon Using APTMS

We first reacted the unmodified Tygon with the chemical (3-aminopropyl) trimethoxysilane, or APTMS, to provide a functional surface upon which to build further modifications. Tygon is incompatible with many organic solvents, so determining a way to functionalize the surface without altering the physical properties was challenging. We adapted the process used by Jannat and Yang to create a usable surface of free amines[10]. Their team found great success using APTES as a scaffold to further modify the surface of polyvinyl chloride tubing. We adopted a similar protocol but replaced APTES with APTMS, since it was available in our laboratory. After our first experiment, we found a decrease in contact angle of about 20˚. Major changes in the infrared spectra at approximately 2920 cm^{-1} and 1570 cm^{-1} showed large C-H stretching but a lack of a broad peak at 3000-3300 cm^{-1} showed a lack of free amines. The experiment was repeated at 60˚C, 70˚C, and 80˚C. The contact angles (Table 2) for tubing modified

at these higher temperatures varied widely and are not consistent with the trend of a good absorption process.

Table 2: Contact Angles of APTMS modified Tygon with Water after varying temperatures of treatment water bath

Temperature (˚C)	θwater (˚)
50	80
60	72
70	73
80	129

The widely variable contact angle is likely due to the volatility of the aminosilane at the increased temperature. With the widely variable contact angle and the unclear correlation between the IR spectra and the APTMS absorption, we decided to definitively determine the IR spectra of APTMS to compare to our collected spectra. The IR spectrum of Tygon has many undeterminable peaks, as seen in Figure 7, which is due to the many plasticizers and polymer additives incorporated in the polymer tubing. To definitively correlate what changes the APTMS made on the Tygon spectra, the experiment was repeated on functionalized silicon, an IR spectrum more easily distinguishable.

Modification of Glass/Silicon using APTMS

Glass slides were functionalized with APTMS to obtain a clean IR spectrum for the resulting film and compare it with that obtained by absorption of the aminosilane into the surface of the Tygon tubing. Figure 8 shows the IR spectrum of the film obtained by the reaction of APTMS on piranha-treated glass.

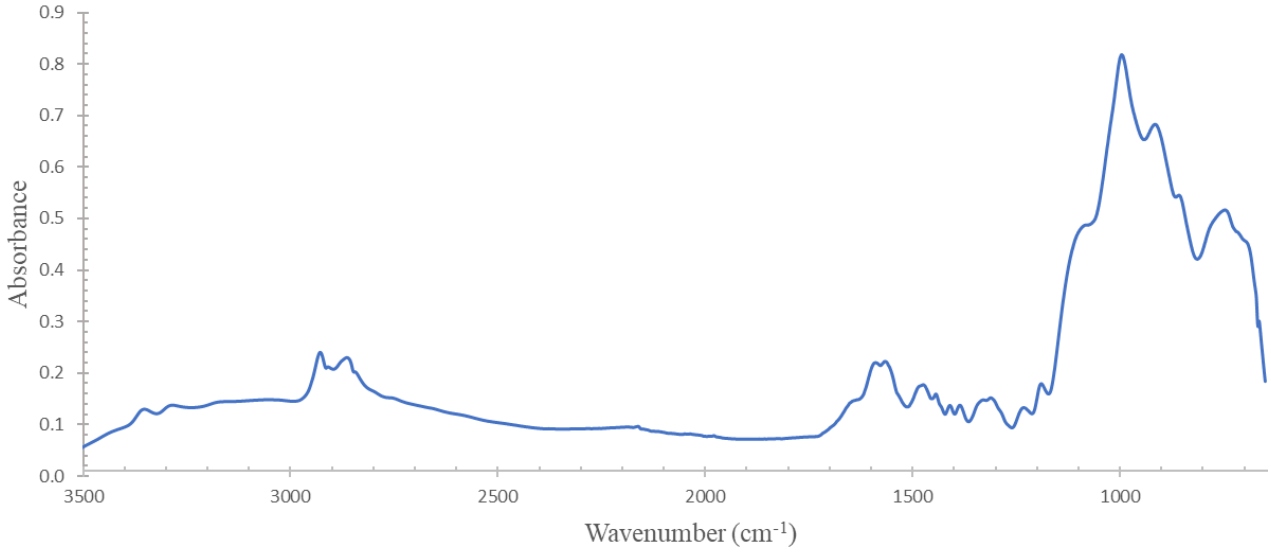

Figure 8: IR spectra of APTMS on functionalized glass

Peaks identifying APTMS can be seen at 3350 cm^{-1}, 2920 cm^{-1}, 2863 cm^{-1}, and 1566 cm^{-1}. These peaks result from the stretching bands of the free amines, methylene, and methyl groups formed during the crosslinking of the aminosilane. This spectrum shows that a large broad peak is expected to form about 3000-3300 cm-1 due to the free amines on the surface. On glass, the contact angle before and after the modification decreases from 40˚ to 14˚. This low of a contact angle confirms that the glass surface was altered to produce a hydrophilic amine-rich film. The experiment was repeated using a 5% APTMS solution dropped onto the functionalized surface of the glass. The IR spectra still showed large peaks at 3350cm^{-1}, 3171cm^{-1}, 2920cm^{-1}, 2860cm^{-1}, and 1590cm^{-1}. The contact angle for this experiment increased from 42˚ to 54˚, an angle more appropriate for the indication of free amines. The lack of strong indicator peaks in all our previous APTMS experiments and the smaller difference in contact angle led us to believe that it was not a suitable reactant for the process on Tygon. Whether this is caused by the volatility of APTMS compared to APTES or the size of the molecule and its ability to crosslink in the polymer is unknown. All further modifications were conducted with APTES.

Modification of Tygon Using APTES

After treatment of Tygon with APTMS yielded less than satisfactory results, we used APTES to more closely follow the initial modification done by Jannet and Yang[10], using the exact procedure used by the APTMS modification experiments. The theory guiding the switch of modifiers is that the extended ethane chains would promote better crosslinking inside the Tygon tubing. The increased crosslinking would provide a better-guided orientation for the amines to emerge from the Tygon tubing to provide a layer of higher coverage of free amines from which to build further chemical modifications.

The experiment was first replicated at the guided 50˚C. The resulting spectra were interesting. We expected peaks in the 3000-3300 cm^{-1} and 2900 cm^{-1} range to correspond with the N-H stretching in free amines and the C-H stretching in the crosslinked backbone. No big changes in the IR spectra from before to after the experiment could be seen. The spectra looked very similar as seen in Figure 11. The IR spectra of the APTES-modified Tygon can be seen in Figure 9 below which is very similar to the IR spectra of the unmodified Tygon. IR subtraction was used to determine the difference between the two spectra to determine how the experiment modified the Tygon. The IR spectrum taken of a control unmodified Tygon, which was only exposed to water and heat, was subtracted from the APTES-modified Tygon, which was exposed to 5% APTES in water and heat.

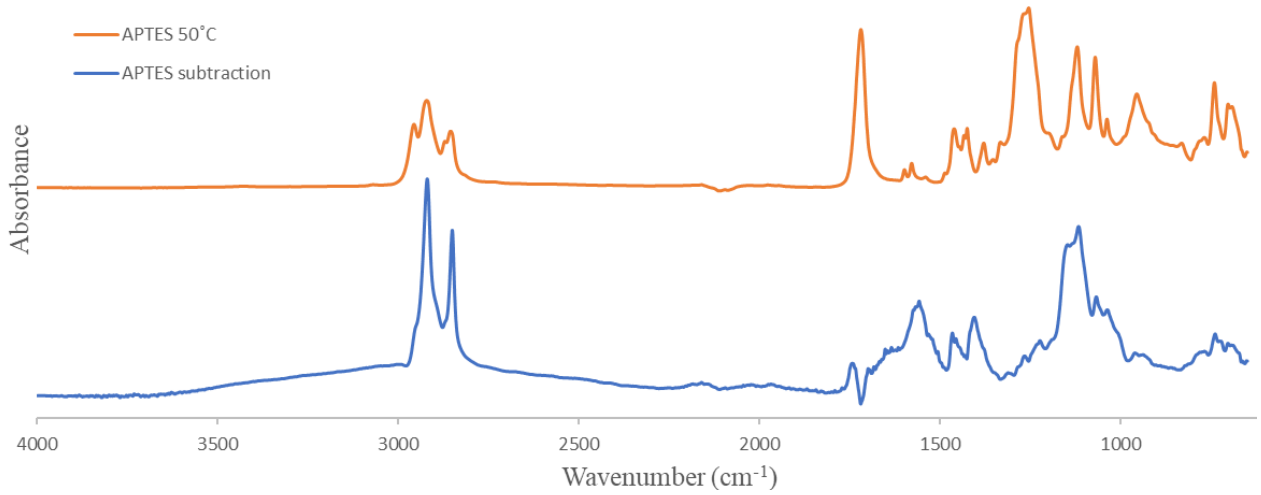

Figure 9: IR spectra of 5% APTES at 50˚C compared to the subtraction taken when compared to unmodified Tygon

Figure 9 shows the changes in the composition of the surface due to the modification of the Tygon with APTES. A large wide peak can be seen at 3000-3400 cm^{-1} for N-H stretching and a large sharp peak can be seen at 2920 cm^{-1} for C-H stretching. The contact angles of the tubing exposed to the aminosilane solution dropped from 95˚ to 63˚. This change to a more hydrophilic surface suggests that the modification produces a layer of free amines on the surface of the Tygon. The unreliability of the unsubtracted IR spectrum is due to the depth of penetration of the IR radiation beam. The spectrometer can detect the bulk layer of the Tygon, which is a majority of the depth being accessed, and as such, dilutes the signals given by the aminosilane-derived film absorbed into the surface of the Tygon. The use of IR subtraction helps with the elimination of the bulk Tygon peaks to better allow for the detection of the aminosilane in the experimental samples. Based on the decrease in contact angles and the increase in IR absorbance for several peaks in the subtracted spectrum of Figure 9, we determined that the modification process is reliable and easily repeatable to produce a reactive scaffold upon which to build further chemical modifications.

Optimization of APTES modification of Tygon

To optimize the absorption of the aminosilane into the Tygon surface, we examined APTES concentration in solution, as well as temperature and exposure time for assembly. To test the temperature of the absorption process, we tested the unmodified Tygon samples in a 5% APTES solution for 1 hr at temperatures of 50˚C, 60˚C, 70˚C, and 80˚C. We did not proceed above 80 ˚C due to the limitation of a water bath for heating and the volatility of the aminosilane. The contact angles (Table 3) for the films prepared at different temperatures all ranged between 61˚ and 67˚. The IR spectra of each temperature sample were taken and analyzed by subtracting the spectrum of the unmodified Tygon. The resulting subtracted spectrum was analyzed for local maxima. Two such maxima were found at 2920 cm^{-1}, which correlates to C-H stretching, and 1550 cm^{-1}, which correlates to N-H stretching in a free amine. The differences in absorption can be seen in Figure 10. Two different sets of trials were run to determine the reproducibility of the results. Although the maxima of each trial had different values, the trend of each trial had the same temperature maxima. The optimal temperature to produce the required result was determined to be 60˚C, based on the highest subtracted absorbance value calculated of any of the trials for both local maxima.

Table 3: Temperature of APTES treatment and the resultant sessile contact angle of the Tygon sample

Temperature (˚C)	θwater (˚)
50	63
60	62
70	61
80	67

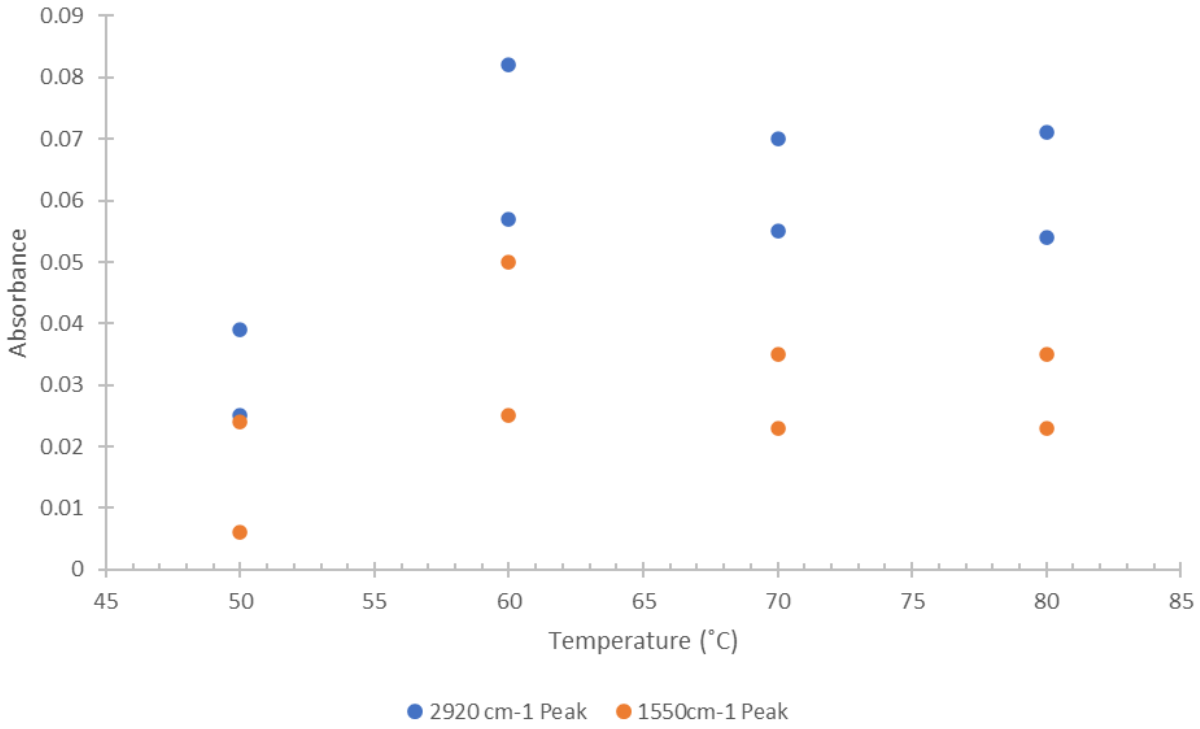

Figure 10: Peak height for characteristic peaks for various temperatures of aminosilane treatment

The absorption of APTES was shown to be most effective at 60 ˚C as that temperature increases the thermal energy of the system and promotes penetration of the aminosilane in the Tygon polymer. This is favorable as crosslinking in the solution will prevent the APTES molecule from forming a scaffold on the surface of the Tygon tubing. It seems higher temperatures either

denature the aminosilane or promote extraneous crosslinking in solution with is unproductive to developing a scaffold base. Following the results of this experiment, further optimization was conducted at 60 ˚C.

The next optimization we wanted to determine is at what time of exposure does the absorption of the APTES into the surface of Tygon have the greatest effect and is the most efficient. A greater absorption of APTES into the surface of the Tygon provides more free amines for reactions, but the time needed for the absorption must be considered. If too much time is needed for the reaction to commence, the process becomes inefficient and the development of a productive technique to modify the surface of Tygon becomes moot. Timed trials were conducted with the Tygon samples immersed in a 5% APTES solution at 60 ˚C for several different periods. Subtracted spectra were analyzed to determine which time period gave the most efficient absorption. The periods varied from 5 minutes to 2 hours as seen in Figure 11.

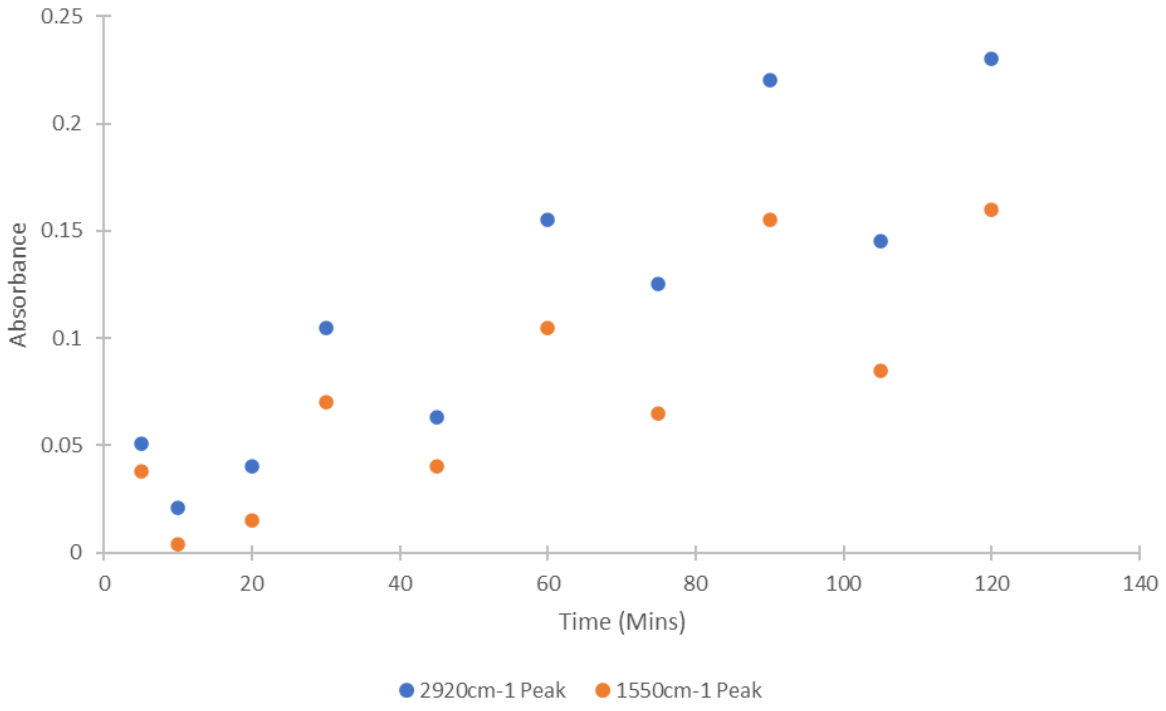

Figure 11: Peak height for characteristic peaks for various time periods of aminosilane treatment

Two different days were spent on the time trial of APTES. This can be seen in the difference between the two trends on the graph. The first day tested 5 minutes, 30 minutes, 1 hour, 90 minutes, and 2 hours, while the second day tested 10 minutes, 20 minutes, 45 minutes, 75 minutes, and 105 minutes. For each of the two days testing occurred, a trend appears for both the C-H stretching peak in blue and the N-H stretching peak in orange. The difference between the two days could vary due to something such as a human error in preparing the APTES solution, different atmospheric humidity conditions, or different times between the end of the experiment and the characterization of the surface. In either case, the trend line remains the same, the longer the treatment of aminosilane, the larger the effect on the surface of the tubing. As seen in the graph the trend line starts to asymptote towards the 2-hour mark, so further periods were not considered. Ultimately the team decided to move forward with a 2-hour treatment as it provided the highest effect without losing efficiency.

The last optimization that the project considered would be the concentration of aminosilane in the solution exposed to the Tygon. The thought was that too much aminosilane in the solution would cross-link outside of the Tygon surface and be unable to penetrate the surface of the Tygon. As seen in Figure 12, a simple experiment to determine the effectiveness of APTES concentration would be on the absorption of APTES into the surface of the Tygon.

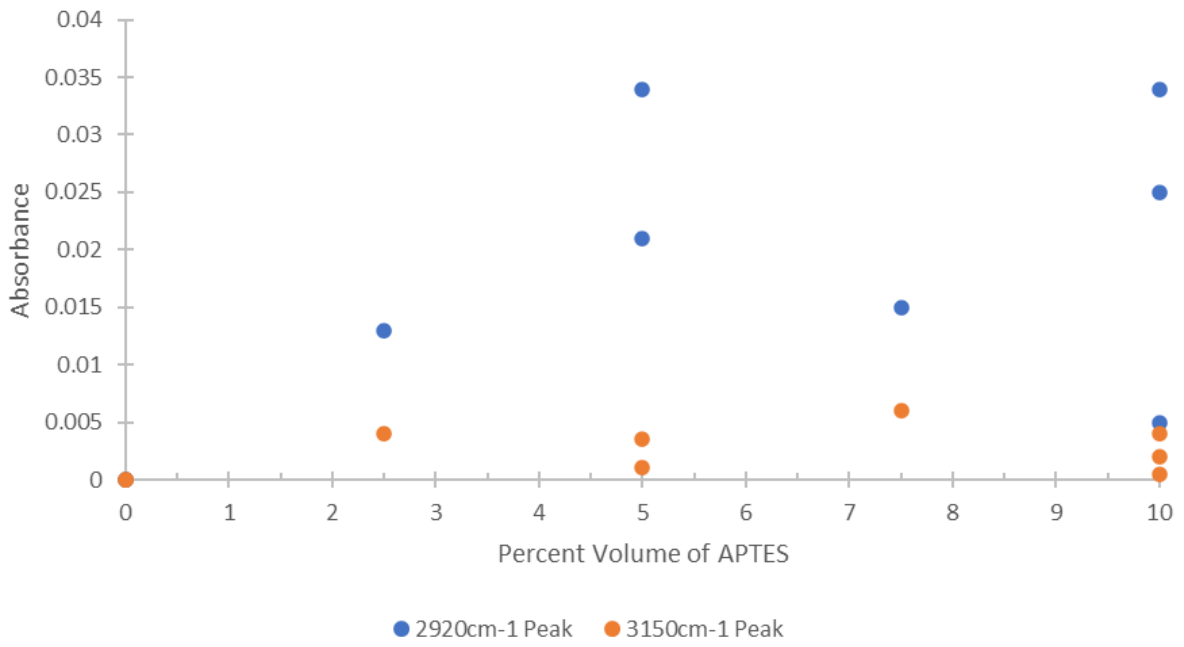

Figure 12: Concentration of APTES vs Subtraction Absorbance for APTES absorption into Tygon

For concentration trials, several concentrations were tested over several days. On the first day tested 0%, 5%, and 10%. On the second day tested 2.5%, 7.5%, and 10%. The data collected from these two days of trials showed two trends that followed each day. In the determination of which concentration to move forward with further experimentation, no clear difference between the increase in APTES concentration could be seen in the contact angles, all of which had a contact angle of 65˚-69˚. The stagnated trendlines guide us to believe that an increase of APTES in solution leads to more crosslinking in solution which led to a decrease in absorption. The small change in absorption peaks and contact angle led us to continue with a 5% APTES treatment to both absorb the aminosilane onto the surface of the Tygon, while not wasting excess material that would crosslink in the solution.

Moving forward with the process, all Tygon samples were treated to a 5% APTES solution for 2 hours at 60˚C. Based on the data gathered, the largest influencing factor on APTES absorption

is time, both concentration and temperature affected the absorption process, but longer periods of exposure led to larger absorption of the Aminosilane.

Compatibility of Tygon with Several Different Organic Solvents

Due to the difficulty of confirming the APTES monolayer using IR spectroscopy, the APTES-modified Tygon was further reacted with an acyl chloride to provide an amide IR peak to compare absorbance. An organic solvent was needed to create the acyl chloride solution to react with the APTES Tygon. Tygon is very incompatible with most organic solvents, so finding an organic solvent that did not degrade the Tygon tubing and could act as a solvent with an acyl chloride was imperative. Shown below in Table 4 are some common laboratory organic solvents that were tested for compatibility with Tygon after exposure for 3 hours.

Table 4: Compatibility of Tygon with Various Organic Solvents

Tygon Chemical Compatabiltiy		
Common Lab Organic Solvent	Compatible	Reasoning
Hexane	No	Polymer became rigid after prolonged exposure
Acetone	No	Polymer swelled in the solvent and loses transparency
Toluene	Yes	Polymer retained flexibility and transparency
Dimethylformamide	No	Polymer loses structural stability and became gel-like
Methanol	Yes	Polymer retained flexibility and transparency

Of the five reagents tested, only 2 were compatible with Tygon. When immersed in hexane, the Tygon became extremely rigid, and lost all flexibility, but remained transparent. When immersed in acetone, the Tygon swelled slightly and became opaque. The Tygon, after the acetone exposure, became much more flexible and lost its transparency. Toluene seemed to have little to

no impact on the Tygon for a 3-hour exposure limit. The Tygon sample remained transparent and flexible. After exposure to DMF, the Tygon dissolved into a gel-like substance and lost all structural stability. After literature review, Tygon was found to be dissolvable in DMF, THF, and DMAc[18]. These solvents were ruled out as Tygon is primarily made of polyvinyl chloride and this project requires the tubing maintains its structural stability. Methanol had little to no effect on the Tygon, even in exposures up to 100 hours. The team determined that Toluene would be an appropriate solvent for exposure times less than 3 hours, so we proceeded to try to use an acyl chloride to better identify to confirm the APTES absorption into the Tygon.

Modification of Tygon using an Acyl Chloride

To confirm the free amines present on the surface of the APTES-modified Tygon, the acyl chloride, 10-undecenol chloride, was reacted with the surface to create an amide complex that would be more easily identifiable on the infrared spectrum taken of the surface of the sample. This was done in two different ways. The first was to treat the APTES-modified Tygon to a 50mM solution of 10-undecenoyl chloride in toluene. For this experiment, infrared scans were taken before and after the acyl chloride exposure. A subtraction of the two spectra was taken to show the difference the treatment had on the surface, which can be seen below.

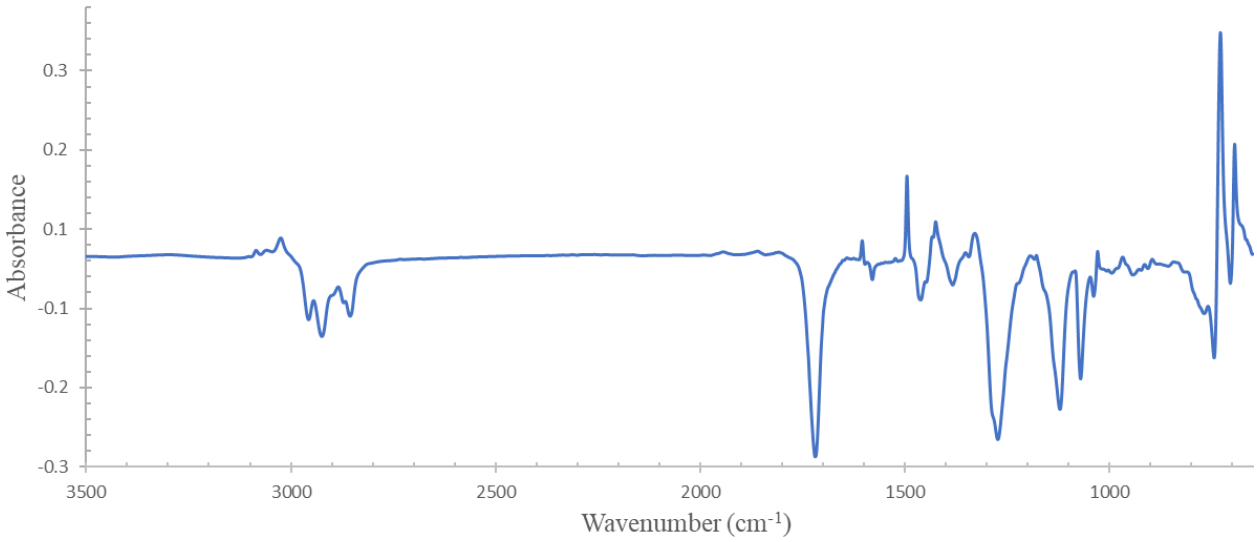

Figure 13: FT-IR subtraction of APTES-modified Tygon from UC/Toluene-modified Tygon

The main conclusion taken from the results of this experiment would be the major increase in the peak at approximately 730 cm^{-1}. This peak is most likely due to the absorption of toluene into the Tygon surface. Tygon has two major peaks in the 700 cm^{-1} region which when combined with the APTES-modified IR spectra, created the large increase in the peak seen at 728 cm^{-1}. The goal of this experiment was to see an amide peak approximately from 1600-1800 cm^{-1}. A small peak can be seen at 1604 cm^{-1}, which could be an amide complex, but more likely it is covered by the major loss at 1720 cm^{-1}. The peak at 1720 cm^{-1} is due to the various plasticizers found in Tygon, such as a phthalate ester, which could leach out of the Tygon tubing into the organic solvent. This major loss of a component on the surface of the tubing could be covering up the amide complex.

To confirm the presence of the amine, an experiment was run where the acyl chloride was dropped onto the surface of the APTES-modified Tygon without the organic solvent to complicate the IR spectra. A similar subtraction was completed with the IR spectrum of the APTES-modified Tygon subtracted from the UC drop modified Tygon. The subtraction can be seen below.

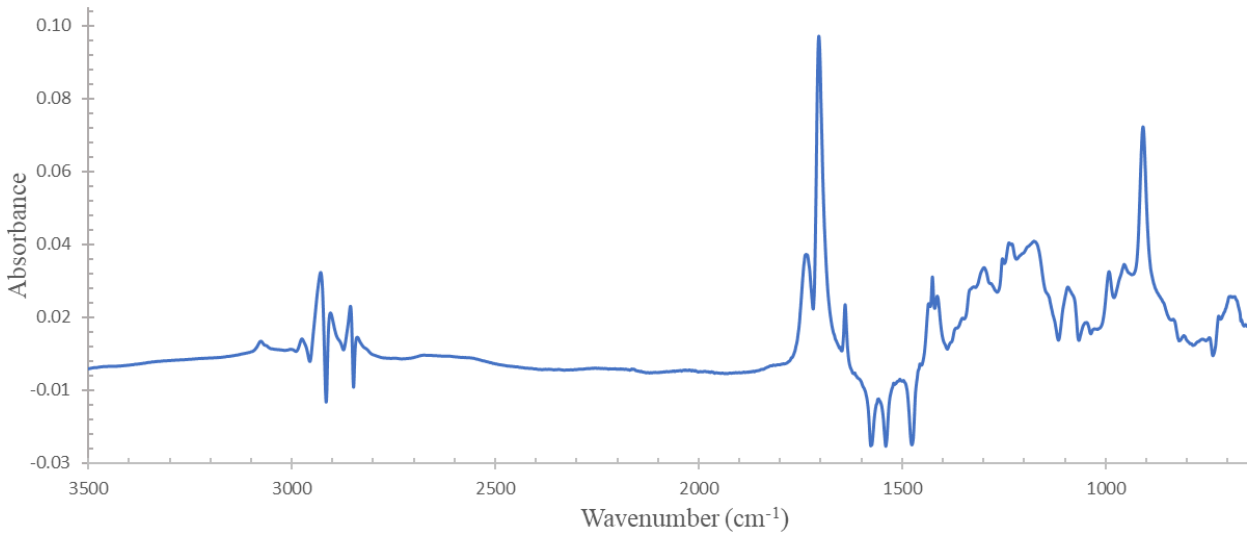

Figure 14: FT-IR subtraction of APTES-modified Tygon from UC-drop modified Tygon

As seen in the subtraction, without the addition of toluene into the experiment, the large peaks at 728 cm^{-1} never appear and the large plasticizer peaks at 1720 cm^{-1} remain in the spectrum. With the addition of 10-undecenoyl chloride peaks in the C-H stretching region of 2920 cm^{-1} are expected, which can be seen in the subtraction. A strong amide complex can be seen at 1640 cm^{-1}, which confirms the presence of the free amines on the APTES-modified surface. Now that the anchor layer of free amines is made and confirmed, the team works on building upon the free amines to create a hydrophilic surface.

Producing Thin Zwitterionic Polymer Films on Silicone surface

Work on hydrophilic polymeric surfaces has already been done by the Laibinis lab group in the past for molecularly thin films using ARGET ATRP. This form of polymerization, using radicalization, provides steady stepwise growth of a polymer chain. Using the SBMA monomer, the lab group previously developed a poly zwitterionic polymer that trapped water molecules to form a very thin hydration layer. To once again confirm that this process is achievable and controllable, ARGET ATRP of the zwitterionic monomer was conducted on functionalized glass

and silicon wafers. After several trials, a successful polymer coating was formed on the surface of a silicon wafer. The thin film was indistinguishable from the silicon wafer demonstrating the transparency of the polymer coating. After the formation of the thin film, the wafer was functionalized.

Table 5: Contact angles of water on silicon in process stages

Contact Surface	θ_{water} (˚)
Unmodified Silicon Wafer	66
APTES functionalized Silicon	36
Initiator Bound Silicon	42
Thin Polymer Film	<15

As seen in the table above, after polymerization, the surface becomes extremely hydrophilic. This is due to the water molecules interacting with the zwitterionic SBMA molecules to form a hydration layer. The polar water molecules form dipole-dipole interactions with functional chains of the polymer to increase the affinity of the water molecules' attraction to the chemically modified surface. A drastic change from 42˚, found after the α-BIBB initiator is bound to the surface, to less than 15˚, demonstrates the drastic hydrophilic nature of the thin polymer. The polymer surface was only measured at 15˚ as any contact angle more hydrophilic is extremely hard to measure.

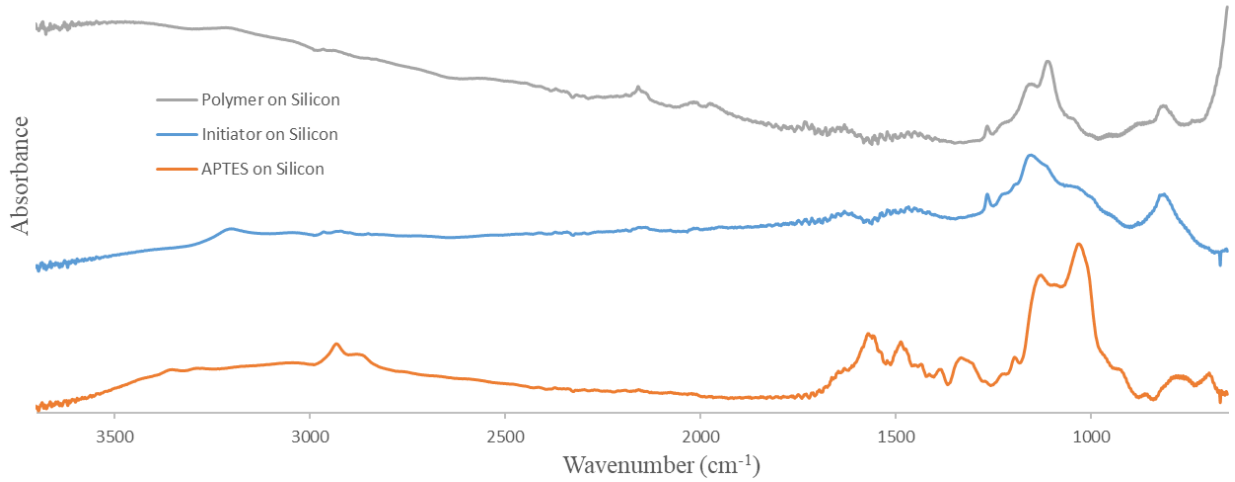

Figure 15: IR spectra of SBMA polymerization steps on silicon wafers

The IR spectra in Figure 15 compares the polymer film to the initiator-terminated film and a film of APTES. The spectra have been normalized and staggered to see peak changes. Peaks at 2920 cm^{-1} and 1570 cm^{-1} show functionalization of the silicon wafer with the aminosilane. Changes in the 3000- 3500 cm^{-1} region of the polymer spectrum are consistent with water molecules trapped in the polymer coating and the SBMA monomer. Although this spectrum is missing the signature double peaks of SBMA at 1170 cm^{-1} and 1040 cm^{-1}, it still contains a shifted large singular peak at 1120 cm^{-1}, which may be caused by the very thin layer of polymer formed not being enough to stop detection of the bulk silicon. With the uncertainty of the spectrum peak, the more definitive very low contact angle of the polymer modified silicon proved that the polymer was formed. Since evidence suggested the successful formation of the polymer on the silicon wafer, modification of the process to obtain absorption of APTES into Tygon was conducted to form a polymer on the surface on the Tygon tubing.

Producing Thick Zwitterionic Polymer Films on the Tygon Surface

Once the thin film was reproduced on the standardized silicon wafer and a super hydrophilic surface was achieved, a similar experiment was performed on the surface of the unmodified Tygon. The Tygon was modified using the aminosilane APTES to form a layer of free amines on the surface, followed by the acyl bromide α-BIBB to create initiator sites for the polymerization. A monomer solution of SBMA was then reacted to form a zwitterionic polymer layer and thereby achieve a hydrophilic surface on the Tygon tubing. Repeating the exact polymerization procedure on the surface of the Tygon surface resulted in a patchy polymer layer with only moderate hydrophilicity. The contact angle on the polymer-modified surface decreased to 40˚ from the 90˚ angle of the unmodified Tygon. Even with the large decrease, the polymer-modified angle was still not as low as expected or needed. From the IR spectrum, small changes were seen in the 3300-3500 cm^{-1} and 1000-1200 cm^{-1} region, but the bulk Tygon still dominated the IR spectrum with the typical Tygon peaks at 2920 cm^{-1}, 1720 cm^{-1}, and 1254 cm^{-1} similar to Figure 9. Further work still needs to be done on this specific polymerization process of SBMA.

To develop a usable polymer film on the surface of Tygon, the concentration of monomer was dramatically increased, as well as the polymerization time. The monomer used in the polymerization solution was increased from 0.075g to 0.75g. This new monomer concentration creates a 166mM solution of the monomer. The polymerization time was increased from 3 h to approximately 18 h. After introducing the new procedure, a thicker polymer was formed on the surface of the Tygon tubing. The resulting surfaces were characterized with goniometry and IR spectroscopy. The contact angle of the new surface was found to be less than 15˚, which signifies an extremely hydrophilic surface. A contact angle of less than 15˚ cannot be measured precisely

as the water droplet thins too much to provide discernable cusp; therefore the noted contact angle is 15˚.

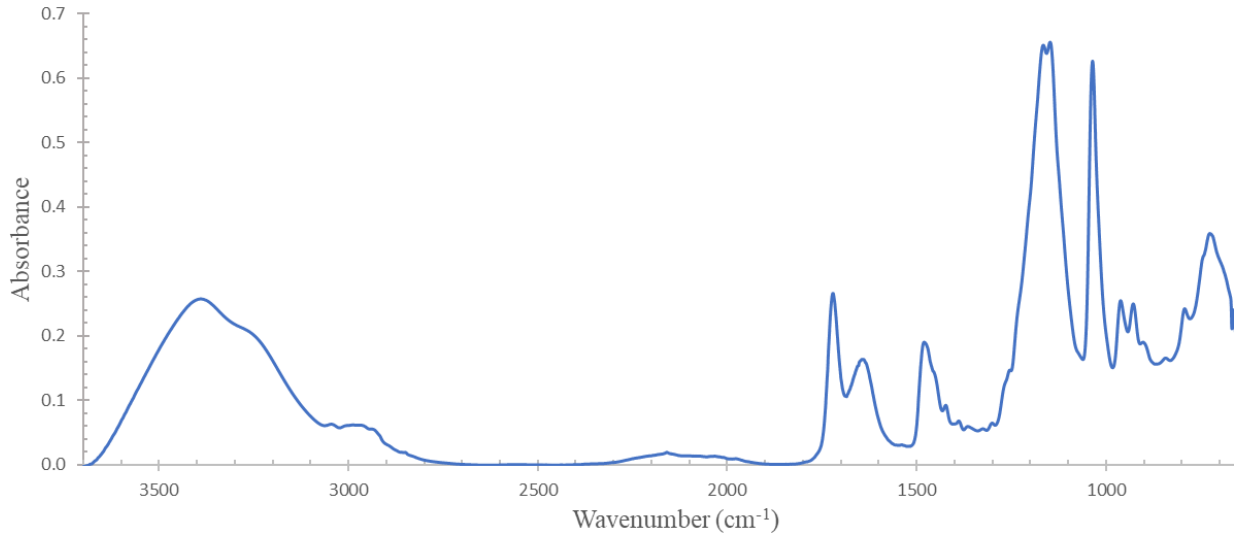

Figure 16: IR spectrum of zwitterionic polymer made through ARGET ATRP of SBMA monomer

The IR spectrum, in Figure 16, of the SBMA film shows large changes in the above and beyond the spectrum of Tygon, which was shown in Figure 7. As seen in the spectrum, large changes, from Figure 7, can be seen in areas with peaks at 3390 cm^{-1}, 1147 cm^{-1}, and 1035 cm^{-1}. The spectrum also matches the IR spectra of SBMA shown in literature and online[19]. The polymer surface was allowed to dry over the course of a week and exposed to room air. After a week, the polymer sample was re-characterized, and the IR spectrum remained the same. The contact angle increased to 20˚ but quickly decreased to less than 15˚, showing that the polymer was able to be rehydrated successfully after a period of storage.

To start the polymerization, the initiator must first be bound to the free amines. Previously this was done by creating an acyl bromide solution in toluene to react with the initiator in a liquid phase reaction. Due to concerns regarding the stability of Tygon in toluene, we sought a different approach. Due to the volatility of the initiator α-BIBB, a vapor-phase reaction was tested to see if

a similar result could be achieved without the use of the organic solvent toluene. APTES-modified samples were suspended over liquid α-BIBB in a sealed vial to allow for the vaporized acyl bromide to react with the free amines and form initiation sites on the Tygon. Polymerization using the previous successful method was done afterward. A successful hydrophilic polymer was formed after some trial and error, and a new protocol was established that allowed for the polymerization of SBMA to occur without the Tygon being exposed to toluene. After polymerization, polymer samples were characterized using contact angles for samples after 10, 20, or 30 min of vapor exposure.

Table 6: Time of vaper initiator exposure vs contact angle of the resulting SBMA polymerized surface

Time of Vapor Exposure (min)	θ_{water} (˚)
10	12-35
20	9-24
30	<15

As the time of exposure to the vapor initiator increases, the contact angle of the polymerized surface decreases. This decrease is due to the increased availability of initiation sites for polymerization resulting in a thicker, more uniform coverage of hydrophilic polymer.

After allowing the polymer to dry, the tacky, hydrated polymer dries into a hard plastic-like polymer. This dry polymer is prone to breakage if the polymer is too thick or if the Tygon tubing is flexed too much. In both the dry and hydrated state, the polymer maintains transparency and hydrophilicity, but in the dry state, it lacks the flexibility of Tygon. Because the Tygon is more flexible than the dry polymer, thicker coatings of the polymer can flake off in pieces. Optimization

of the polymer layer needs to be done to make the thinnest layer of polymer while maintaining the hydrophilic surface. Experiments were performed by changing the mass of the monomer added to the polymerization solution, ranging from the initial trials of 0.075g up to 0.375g. This range would show the transformation from the initiator-bound surface to the completely polymer-modified surface. Characterization of the trials with contact angles and IR spectra can be seen below.

Table 7: Surface modification vs contact angle of the surface to optimize monomer concentration

Contact Surface	**θwater (˚)**
APTES	58
Initiator	67
8mM (0.0375g)	41
17mM (0.075g)	31
33mM (0.15g)	24
50mM (0.225g)	18
66mM (0.3g)	15
83mM (0.375g)	<15

As the monomer concentration increased, the contact angle of the resultant surface decreased. After polymerization, the contact angle decreased from 67˚ after the initiator was bound to the surface, to about or below 15˚ for any monomer concentration of 66mM or higher. To provide a suitable surface with even coverage of the polymer, an 83mM concentration of monomer was used to standardize the process moving forward. This concentration of monomer allowed for a sufficiently thick layer to form on the Tygon to provide the lowest contact angle measurable, without being too thick. Further evidence of the transition from the Tygon surface to an SBMA polymer surface can be seen below.

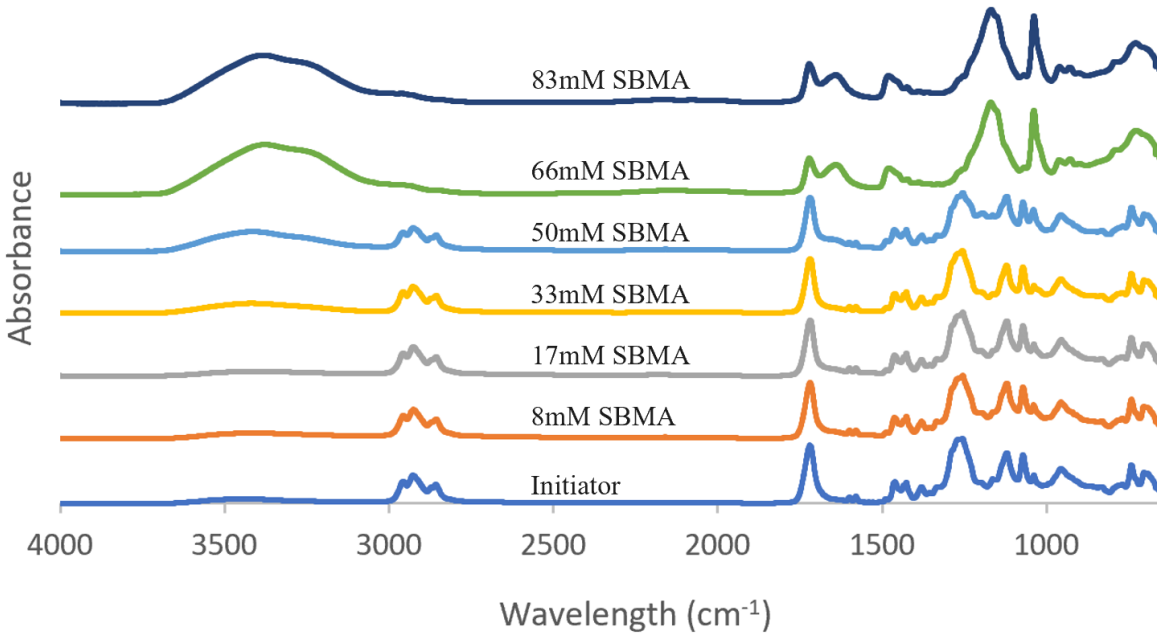

Figure 17: IR spectra of a polymer surface base on the amount of monomer used in polymerization solution based on a standard time of 18 hours

As shown in Figure 17, as the monomer concentration increases the IR spectra shifts from a typical Tygon-dominated spectrum to a hydrated SBMA polymer spectrum. The typical Tygon peaks, such as 2920 cm^{-1}, 1720 cm^{-1}, and 1255 cm^{-1}, are all present in the lower spectra with less monomer concentration. As a thicker polymer layer forms, those peaks gradually disappear and are replaced by those due to hydrated SBMA, including those at 3400 cm^{-1}, 1150 cm^{-1}, and 1035 cm^{-1}. The large double peaks at 1035-1150 cm^{-1} are typically attributed to the SBMA polymer[19], while the large broad peak at 3400 cm^{-1} is attributed to the hydration of the polymer. Large changes in the spectra can be seen between 50mM and 66mM. Most likely, at this monomer concentration, a thick enough polymer film is formed to prevent the FT-IR spectrometer from penetrating to the depth of the bulk Tygon, decreasing or eliminating the Tygon peaks on the spectrum. Overall, a more optimized polymer layer was formed and proven to maintain its hydrophilicity at a 83mM of

monomer concentration as compared to the monomer concentration of 166mM that formed an over-thick layer.

Preliminary Plasma Coagulation Testing

To test the effectiveness of the new chemical modification of Tygon, preliminary testing of the surfaces was done at the Vanderbilt Medical Center. Using normal pooled plasma, the initiation step of the intrinsic pathway can be determined. This initial step in the coagulation cascade is the only step that is of concern as it involves the activation of surface proteins. Normal human pooled plasma was used to identify which proteins bound to the surface and how fast these proteins activated to coagulate the plasma. In this section, coagulation will refer to the coagulation of the plasma used, not the coagulation of whole blood. The plasma used in the experiments lacks any cells or platelets and it is comprised of blood proteins, body minerals, water, and a calcium antagonist molecule to prevent free calcium from starting the coagulation cascade, which when activated turns the plasma tested into a gel-like substance.

In our testing of the polymer surface, a shorter coagulation time is preferable. As stated in the methods section, the plasma is exposed to the surface, then samples are tested in a viscosity testing machine. If the plasma coagulates faster, more surface-activating proteins are in the sample still and not bound to the polymer or tested surface. Once in the stART machine, calcium and a standardized silicon surface are added to start the coagulation cascade in a PBS or PTT reagent solution. The coagulation times of the first day of the study can be seen below. A plasma control was done to test the coagulation capacity of the plasma without exposure to a testing surface.

Table 8: Time required for plasma coagulation after exposure to the contact surface

Tygon Coagulation Testing	Plasma Control	Unmodified Tygon	APTES modified Tygon	New Polymer	Old Polymer
Sample 1 (PBS)	207	266.3	235.4	243.9	229.3
Sample 2 (PBS)	213.2	281.2	366.3	228.8	317
Sample 3 (PBS)		253	>600	227.5	517.4
Sample 4 (PTT)	31.5	32.2	33.7	51.5	124.1
Extended Contact		>600	>600	>600	

In the preliminary data, the plasma coagulation times were taken at 210 seconds after calcium was added. The plasma control data confirmed the plasma was not inhibited by the storage vessel and that this was the fastest coagulation time because the sample still contained all proteins. In samples with the PBS buffer, the coagulation time of the new hydrated polymer was comparable to or better than the coagulation of the plasma exposure to the unmodified Tygon, while the old, dried polymer was comparable to or worse than the unmodified Tygon. The samples exposed to PTT time, a colloidal suspension with an increased surface area for faster coagulation, had widely different results with PTT times increasing for the polymer. Some further study will need to be done on this reagent and the SBMA polymer, but for simple PBS buffer solution, the polymer samples have promising results.

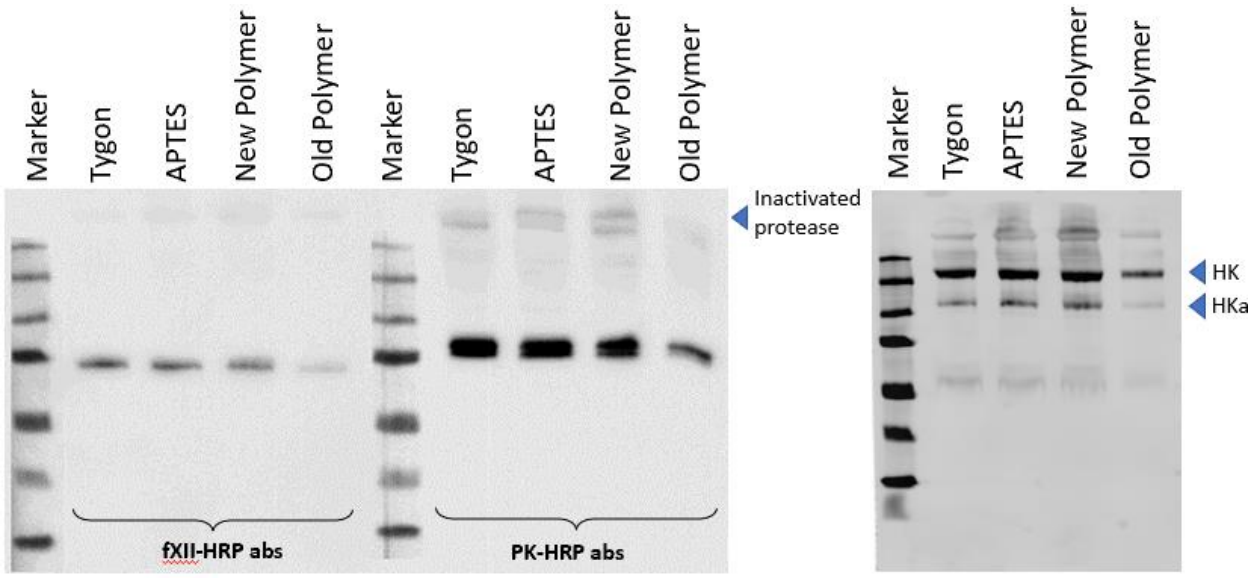

Figure 18: Western Blotting of sample surfaces with factor 12, prekallikrein, and separate blotting for high molecular weight kininogen

As seen in Figure 18, western blotting was done on the sample surfaces tested in the table above. The black bars on each blotting represent a fluorescent antibody bound to a specific protein. For the first image, specific antibodies were used to detect factor 12, a common surface factor in the intrinsic pathway. Of the four tested, the old polymer had the least bound proteins, followed by the new hydrated polymer. The second image shows prekallikrein, a precursor to the protease kallikrein, bound to the surface. Similarly, the old polymer has the lowest number of proteins followed by the new polymer. The third image shows the high molecular weight kininogen, a circulating plasma protein, bound to the surface. This one shows the least protein for the old polymer but a close tie for the other three samples. This experiment trends to the conclusion that the hydrated and dried polymer both repel proteins and can act as an anti-coagulant surface. Further study needs to be done on if the protein washed by the SDS solution used in the western blot collects all the proteins bound to the surface or only those weakly bound. That difference could change the result dramatically.

The second set of plasma coagulation results was studied to determine if the coagulation of plasma was being inhibited by a component of the polymer or a solvent used in its making. As seen in the figure below, a similar result was performed on the first day, with similar conflicting results.

Table 9: Time required for plasma coagulation for different polymer thicknesses

Plasma Coagulation Testing				
	Unmodified Tygon	APTES modified	Polymer 1	Polymer 2
PBS	212.9	489.7	168.5	156.8
PBS	263.6	547.8	143.4	157.7
PTT	29.8	41.5	71.5	73.3
PTT	31.1	40.7	69.8	74.1

Table 10: Plasma coagulation for unmodified Tygon over time

Unmodifed Tygon							
10mins		20mins		37mins*		55mins*	
PBS	212.9	PBS	231	PTT (1:0)	38.1	PBS (1:0)*	>999
PBS	263.6	PBS	404.5	PTT (1:1)	32.6	PBS (1:1)	520.1
PTT	29.8	PTT	36.2	PTT (1:0)	44.4	PTT (1:0)	38
PTT	31.1	PTT	35	PTT (1:1)	54.3	PTT (1:1)	37.6
*Fresh Plasma added to Exposed Plasma to test for Inhibition Factors on Tested Surface							
**Additional Phospolipids added to test for phospholipid inhibition							

Table 11: Plasma coagulation for standardized polymer (0.375 g SBMA) over time

Polymer 1					
10mins		25mins*		45mins*	
PBS	168.5	PBS (1:0)	112.4	PBS (1:0)	142.8
PBS	143.4	PBS (1:1)	116.1	PBS (1:1)	115.8
PTT	71.5	PTT (1:0)	62.1	PTT (1:0)	43.1
PTT	69.8	PTT (1:1)	59	PTT (1:1)	54.6
*Fresh Plasma added to Exposed Plasma to test for Inhibition Factors on Tested Surface					

Table 12: Plasma coagulation for thicker polymer (0.750 g SBMA) over time

Polymer 2					
10mins		20mins*		32mins*	
PBS	156.8	PBS (1:0)	133.1	PBS (1:0)	124.2
PBS	157.7	PBS (1:1)	121.3	PBS (1:1)	121.5
PTT	73.3	PTT (1:0)	63.8	PTT (1:0)	57.4
PTT	74.1	PTT (1:1)	64.4	PTT (1:1)	58.2
*Fresh Plasma added to Exposed Plasma to test for Inhibition Factors on Tested Surface					

Similarly to the first day of testing, the polymer results for the plasma mixed with PBS buffer solution decreased compared to the unmodified Tygon. Also, time increased for the plasma samples mixed with PTT reagent after exposure to the polymer surface, which is contraindicative as the PTT reagent should give similar results as the PBS buffer only faster as there is more surface area. More testing is needed to flesh out what component of the PTT reagent is causing this decrease in the coagulation time, but preliminary results from the PBS buffer treatment are encouraging that the polymer layer can act as an anti-coagulant surface by preventing protein adhesion.

In Table 10, the unmodified Tygon surface was tested over time, with new fresh plasma mixed with the surface exposed plasma to determine if there is an increase in the coagulation rate over time and if the decrease in coagulation is due to the depletion of proteins in the sample or the inhibition of the pathway. For unmodified Tygon, the rate gradually increases between 10 and 20 minutes. Also, when fresh plasma is added, the plasma samples coagulate much faster meaning there is no inhibition. In Table 11 and Table 12, the polymer surface was treated the same way. For the polymer, the coagulation rate seemed to increase which could be caused by the further hydration of the polymer which unbinds proteins after their initial surface interactions. When fresh

plasma is added, the plasma samples seem to decrease coagulation times slightly, leading to the possible conclusion that the polymer depletes the plasma proteins instead of inhibiting the cascade. Further research is needed in this field and continuing research is needed with this polymer surface interaction with whole human blood.

Based on the preliminary data provide by the lab at the Vanderbilt Medical Center, we believe that the SBMA polymer surface shows a tendency to create a hydration layer and prevent protein adhesion, and continuing research by the team on this subject will hopefully yield a positive result and develop a workable surface that can help many people.

Chapter IV

Conclusion

Extracorporeal membrane oxygenation (ECMO) machines are used for seriously ill patients who are afflicted by cardiac and pulmonary complications. This can include heart failure, heart transplants, heart defects, lung failure, severe respiratory disease, lung transplant, and many others. During prolonged operation, surface contact between human blood and the tubing in these machines can trigger an immune response, activating the intrinsic pathway for blood clot formation. This coagulation that occurs in ECMO machines impacts the performance of the machine and can jeopardizes the health and life of the critically ill patient.

Commercially available, coated Tygon tubing often used with these machines aims to prevent these obstructive clots from forming through the use of biocompatible layers or timed release of anti-coagulants. A recent trend is to use polymeric materials to change the hydrophilicity of the surface of the Tygon. This thesis detailed the development of a chemical process to create a superhydrophilic surface to repel surface-activating proteins to prevent hemocoagulation. Commercially available, unmodified Tygon was exposed to an aminosilane that absorbs into the polymer surface, crosslinks, and functionalizes the surface with a layer of free amines. The surface of free amines was then exposed to an acyl bromide to provide initiator sites for the subsequent polymerization step. This initiator-laden surface was then exposed to a solution of zwitterionic monomer to provide chain-wise growth of hydrophilic polymer chains via a surface-initiated polymerization process known as activators regenerated by electron transfer, atom transfer radical polymerization (ARGET ATRP). These polymer chains are designed to attract and hold ambient water molecules from the blood to form a hydration layer, which would repel blood proteins.

Optimization of the process was extensive with each step altering time, temperature, reactant concentrations, and reactant species.

Characterization of the resulting polymer revealed that a uniform, hydrophilic layer was formed along the surface of the Tygon tubing while maintaining the beneficial physical properties of Tygon, such as transparency and flexibility. After the process, the polymer modified Tygon showed a sessile contact angle of less than 15˚ with water. ATR-FTIR spectroscopy of the polymer modification showed changes in the area of distinct Tygon peaks, confirming a thick polymer coating was constructed. The spectrum of the polymer sample showed large peaks to announce the presence of trapped water confirming the idea of the hydration layer. Coagulation testing is currently being performed in collaboration with contributors from the Vanderbilt Medical Clinic to determine if the polymer layer is able to slow the cascade of the intrinsic pathway. Viscosity testing on the polymer modified samples had less protein interactions with normal pooled plasma while reacted with PBS, but not PTT reagent. The western blots taken after testing showed less protein bound to the polymer modified Tygon than the unmodified Tygon. Overall, this polymer modified Tygon was easy to make through a simple 3 step process that will hopefully provide a solution to this much needed problem in the future.

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