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Hollow Fiber Membranes for Artificial Lung Applications

An Undergraduate Honors College Thesis

in the

Ralph E. Martin Department of Chemical Engineering

College of Engineering

University of Arkansas

Fayetteville, AR

by

Lauren Elizabeth Reed

Abstract

Artificial lungs are in use, but difficult issues remain in the field of membrane development related to fouling issues. Currently there are external artificial lungs circulating blood outside the body, taking out the carbon dioxide, and inserting oxygenated blood back into the body. An example of this type of machine is the ExtraCorporeal Membrane Oxygenation (ECMO) machine currently used in hospitals. The ECMO takes over the functions for both the lungs and the heart but is only available for short term use by patients with respiratory failure due to infections [1]. The fibers in the machine develop fouling due to the fibers' small surface areas coupled with their long term exposure to proteins in the blood. These factors continuously decrease the gas transfer abilities of the fibers until the machine is no longer effective at exchanging gases with the blood. The goal of this research is to create materials to use in a lung that can function within the body until an actual lung becomes available using hollow fiber membranes with proteins attached to prevent fouling. A fouling study was performed on 17.8% polysulfone hollow fiber membranes with polydopamine and peptoid attached. Unmodified, polydopamine modified, and polydopamine and peptoid modified fibers were placed in a diffusion chamber with Bovine Serum Albumin (BSA) flowing on the outside of the fibers and oxygen flowing on the inside. Evapoporometry was run on the fibers to determine the pore size distribution of the fibers before and after the run. The evapoporometry of the fibers shows that the pores for the fibers after being in the chamber fouling for 72 hours were smaller overall and was easier to distinguish for the higher concentration BSA solution. The oxygen concentration of the BSA was also measured while the BSA was run outside of the fibers in the chamber. The fibers were considered to be completely fouled when no more oxygen was able to diffuse into the BSA. The fouling was shown to take longer to occur in the protein coated fibers than in the unmodified fibers. The fouling of BSA on the PSF fibers was characterized by different methods including FTIR, SEM images, and tensile strength.

Introduction

Artificial lungs are in use, but difficult issues remain in the field of membrane development related to fouling issues. Currently there are external artificial lungs circulating blood outside the body, taking out the carbon dioxide, and inserting oxygenated blood back into the body. An example of this type of machine is the ExtraCorporeal Membrane Oxygenation (ECMO) machine currently used in hospitals. The ECMO takes over the functions for both the lungs and the heart but is only available for short term use by patients with respiratory failure due to infections [1]. The fibers in the machine develop fouling due to the fibers' small surface areas coupled with their long term exposure to proteins in the blood. These factors continuously decrease the gas transfer abilities of the fibers until the machine is no longer effective at exchanging gases with the blood.

This research is an attempt to develop membranes and membrane characteristics that would ultimately lead to a lung that can be inserted in place of the actual failing lung within the body cavity. The goal of this research is to create a lung that can function within the body until an actual lung becomes available. The materials used for this lung are polysulfone hollow fiber membranes, commonly used in industry, coated in peptoid biomaterials to prevent biofouling of the pores in the membrane. The pores of the fibers will be used to help transfer carbon dioxide and oxygen to and from the blood within the lung. The size of the pores within the fibers must be maintained while in the body so the gas can pass through the pores. The fibers must be modified so they are compatible in blood and will not foul in the body. The fibers must also be flexible and durable, able to expand and contract like a lung does and withstand the forces acting on a lung. The flexibility and durability of the Hollow Fiber Membranes (HFMs) is tested in this research along with the ultimate strength. Previous research has been conducted on developing an artificial lung with hollow fiber membranes, but the fibers have been weak and easily gained a buildup of biofouling when exposed to blood [2].

Polysulfone (PSF) is used for the hollow fiber membranes in biomedical membrane applications as well as in this research due to its high porosity and high chemical, physical, and thermal stability [5-7, 10]. Fouling of the polysulfone occurs when proteins and other materials adsorb onto the PSF fiber's surface and within its pores while exposed to blood or similar fluids [3-6, 10]. To minimize fouling, the surface of fibers should be hydrophobic, electrically neutral, contain hydrogen bond acceptors, and not have hydrogen bond donors [8-10]. To create the surface characteristics that will inhibit biofouling on the outer surface of the fibers and maintain durability of the fibers for their long exposure to blood within the body, the PSF fibers in this research are modified with peptoid containing the 2-methoxyethyl (NMEG5) side chain which decreases the hydrophobicity and hydrogen bond donors of the fibers [10]. After the fibers have been modified with this peptoid, fouling studies are performed on the fibers to determine if the peptoid does decrease the amount of fouling of the HFMs while still maintaining the high gas exchange and integrity of the fibers. The goal for my research is to characterize the fouling of Bovine Serum Albumine (BSA) on modified hollow fiber membranes (HFMs) in order to specifically characterize what is fouling the membranes when the fibers are exposed to multicomponent fluids such as blood.

Background

In order to characterize the fouling of the fibers, the PSF fibers must first be modified and then fouled with BSA in a chamber. This previous work of modifying and fouling the fibers was done by other students in the group. The modification of the fiber is the attachment of the NMEG5 peptoid. In order to attach the peptoid to the PSF fibers, polydopamine (PDA) must first be attached. The PDA is adsorbed onto the surface of the HFM and creates reactive sites for the NMEG5 to then attach. Figure 2 shows the peptoid attachment process via PDA where the polysulfone fibers are labeled PSU. The PDA is adsorbed and undergoes oxidation creating the quinone and catechol groups. The catechol group is then able to react with the NMEG peptoid to attach the peptoid to the fiber surface [10]. PSF fibers, PSF fibers with PDA attached, and PSF fibers with PDA and peptoid attached were exposed to different concentrations of BSA in phosphate buffered saline (PBS) and water in order to characterize the fouling.

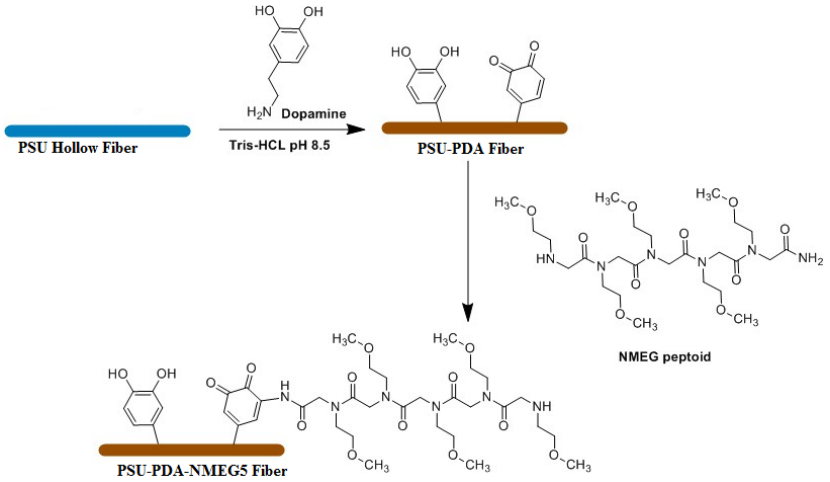

Figure 2 *Schematic of hollow fiber coating process. Polysulfone (PSU) hollow fibers are immersed in dopamine (0.5 mg/ml in TRIS-HCl, pH. 8.5) for 3 hours at room temperature. PSU-PDA fibers are immersed in NMEG5 (0.5 mg/mL in PBS) for 24 hours at T=60ºC [10]*

The initial fouling study was a static run where the unmodified, modified with PDA, and modified with PDA and NMEG5 fibers were soaked in the 5 mg/ml BSA in PBS and water solution. In the static run, the fibers were soaked in BSA for 24 hours. Figure 3 shows the results for the absorption of BSA onto the fibers with PSF fibers labeled PSU. The more BSA absorbed, the more fouling occurs on the fibers. PSF-PDA-NMEG5 fibers absorbed the least amount of BSA. The PDA coated fibers even had less fouling than the original unmodified fibers. Both of the proteins added to the fiber diminish the amount of fouling occurring on the fiber's surface.

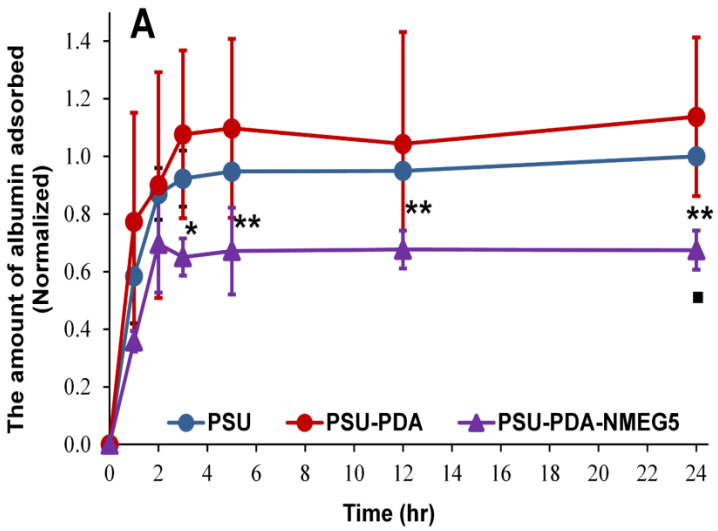

Figure 3 *PSU (closed circle), PSU-PDA (open circle), and PSU-PDA-NMEG5 (open triangle) fibers. Data are expressed as the mean ± standard deviation of three independent measurements. *p < 0.05, **p < 0.01, and ***p < 0.001 for PSU-PDA-NMEG5 vs PSU and ■p < 0.05, ■■p < 0.01, and ■■■p < 0.001 for PSU-PDA-NMEG5 vs PSU-PDA [10]*

In the dynamic run, the BSA, PBS, and water solution is pumped through a chamber on the outside of the fibers while oxygen is pumped through the fibers as seen in Figure 4. The chamber contains 5 hollow fibers in a fiber bundle. The oxygen concentration of the BSA solution is measured using the oxygen probe to determine the gas transfer coefficient of the fibers. The BSA solution is purged with nitrogen periodically to maintain accurate readings of the oxygen concentration. Figure 5 has a few of the results for the fibers fouled in the dynamic system over 24 hours. The modified fibers had little change in the oxygen transfer coefficient compared to the unmodified fibers. After these fibers were modified and fouled, the fibers were able to then be characterized to determine the amount of fouling on the fibers.

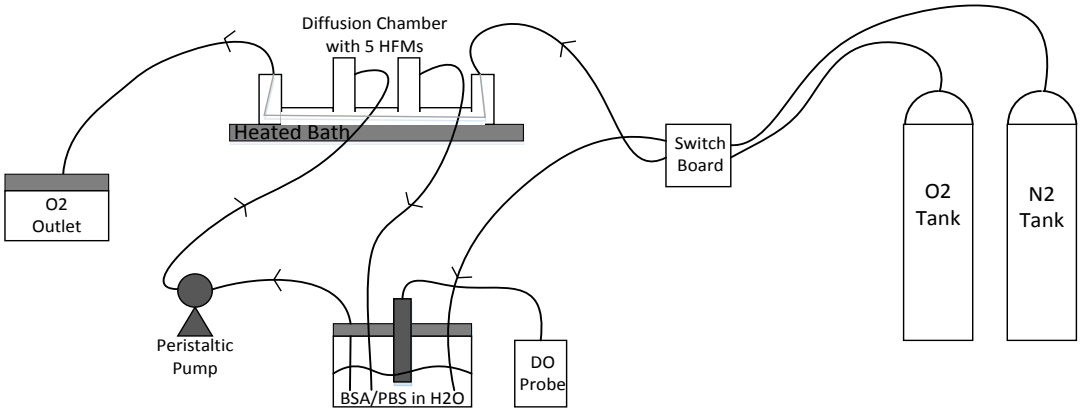

Figure 4 *Gas exchange set up for the dynamic biofouling studies measuring the oxygen transfer coefficient*

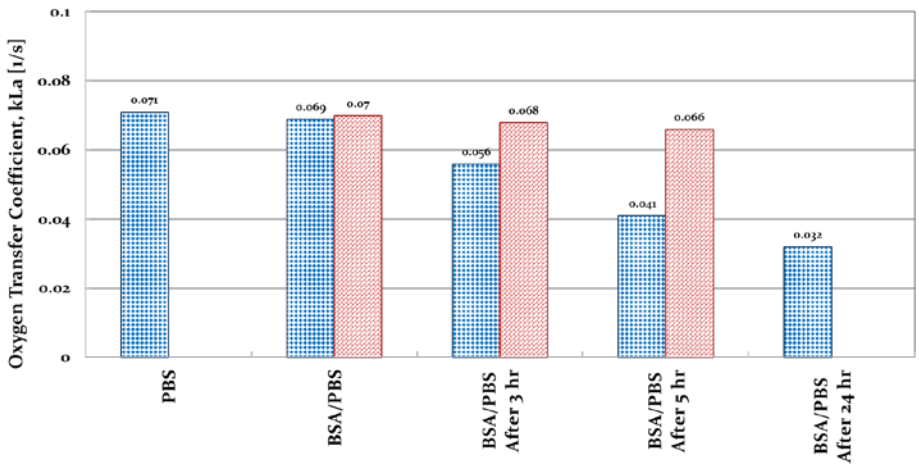

Figure 5 *Oxygen transfer coefficient versus time for the dynamic run of a gas exchange chamber with 5 mg/ml BSA/PBS absorption on unmodified PSF fibers and PSF fibers modified with PDA after 3, 5, and 24 hour runs*

Methods

Hollow Fiber Membrane Fabrication

For these experiments, the hollow fibers of varying concentrations must be created. The fibers are created using a conventional hollow fiber membrane spinning device consisting of a water bath and spinneret shown in Figure 6. The 0.8 mm inner and 1.6 mm outer diameter spinneret has pressurized nitrogen gas attached that pushes the dope and bore solutions through the spinneret. The dope solution consists of a certain percentage of polysulfone in N-Methylpyrrilidone (NMP) solution and makes up the outer layer of the fiber. The optimum dope solution concentration for this application was determined to be 17.8% (v/v) for its balance of strength and elasticity necessary for an artificial lung determined in previous tensile strength testing. The bore solution is made up of 15% (v/v) NMP in water and is what is responsible for hollowing out the polysulfone and creating the pores in the fibers. Once the solutions are pushed through the spinneret, they hit the water bath and a phase inversion occurs causing the liquid polysulfone to solidify into hollow fiber membranes that are then pulled under dowels and rolled up on a wheel. Varying the initial nitrogen pressures, air gap above the water, rate of spinning uptake wheel, concentration of hollow fibers, etc. can vary the pore size and elasticity of the fibers. The air gap chosen for the fibers tested was an 8cm gap. The uptake wheel speed was 2 m/min.

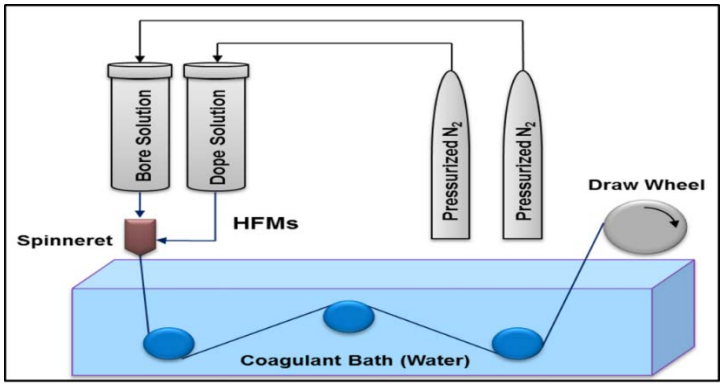

Figure 6 *Hollow fiber membrane spinning apparatus*

Evapoporometry

The Evapoporometry method was developed and patented by Krantz et al. Evapoporometry relates the diameter of pores to the rate of evaporation of isopropyl alcohol from the pores. The vapor pressure depression of the pores increases as the pore diameter decreases [11]. Evapoporometry uses a derivation of the Kelvin equation that describes the vapor pressure depression of a wetting liquid contained in small pores. Evapoporometry assumes that liquid draining from certain pores are saturated and the pores smaller than them are supersaturated so that the liquid in the largest pores will evaporate first [12]. The Kelvin equation is as follows:

$$\frac{P'}{P} = -\frac{2\gamma V}{RTr\cos\theta}$$

where P is the normal vapor pressure, γ is the surface tension, P' is the instantaneous vapor pressure, V is the liquid molar volume, R is the gas constant, θ is the contact angle, T is the absolute temperature and r is the principle radii of curvature of the liquid-gas interface [12,13]. Irving Langmuir's equation relates vapor pressure to evaporation rate and is given by the equation:

$$W = (P_v - P_P)\sqrt{\frac{m}{2\pi RT}}$$

where W is the evaporation rate, P_v is the vapor pressure of the liquid, P_P is the partial pressure of the vapor, m is the mass of each molecule of the vapor when assuming that the partial pressure of the vapor is negligible [13]. Initially, evaporation occurs from the free standing liquid layer on top of the fibers. This evaporation rate should be relatively constant as long as the temperature of the lab remains constant because the conditions for the evaporation rate will not change for the liquid. Once the free standing liquid has completely evaporated, the evaporation from the pores will begin and the evaporation rate will decrease as the pores the liquid is evaporating from get smaller. The instantaneous evaporation rate can be related to the radius of the pores by the derived equation:

$$r = -\frac{2\sigma V}{RT\cos\theta \ln\left(\frac{W'}{W^{\circ}}\right)}$$

where W' is the instantaneous evaporation rate and W^O is the evaporation rate from the free standing liquid [13].The proteins added to modify the fibers could change the radius of the pores and would be able to be detected by evapoporometry due to the difference in evaporation rates.

For the evapoporometry, hollow fibers are glued flat into a plexi glass container completely covering the bottom. Isopropyl alcohol is added to cover the fibers. The fibers that are covered in IPA are put on a scale connected to a computer that logs the change in mass over a time period for the hollow fiber and evaporating IPA system and a separate computer with a thermocouple that logs the change in temperature of the lab during the experiment. The system is left until all of the IPA evaporates from the fibers. Size of the pores can then be determined using a derivation of the Kelvin equation. The scale and test cell can be seen in Figure 7.

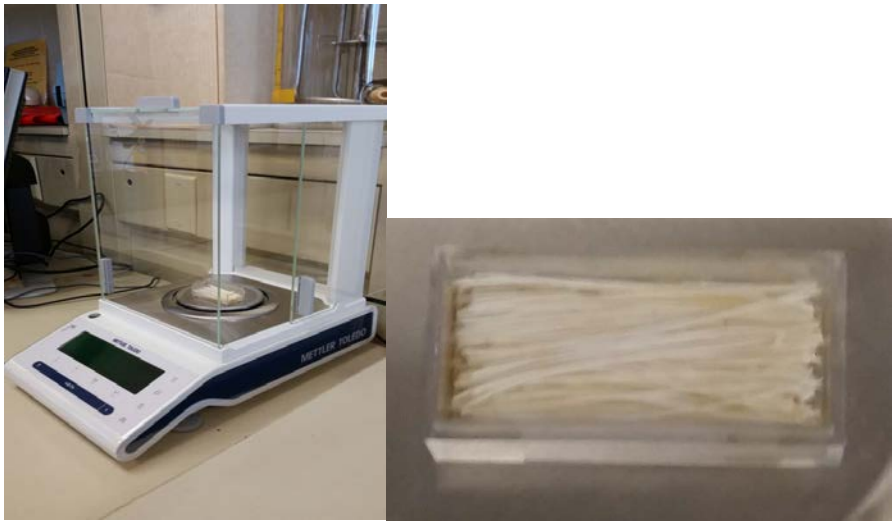

Figure 7 *Images of the Evapoporometry Setup*

Tensile Strength Testing

Tensile strength is the measurement of how much force per unit area a fiber can withstand before it completely loses all of its elasticity. Ultimate tensile strength is the amount of force per given area it takes to break the fibers in two. The force gauge used records the amount of force applied to the fibers while the test stand records the distance traveled by the force gauge while pulling apart the fiber. The force gauge and test stand continuously log data on a computer while the fibers are stretched. The effect the added proteins from the modification and the fouling of the fibers have on the tensile strength of the fibers will be measured and compared to published data on the tensile strength of the tissues in the lung and other materials used in artificial lungs.

For tensile strength, a force gauge and a travel stand are used to measure the load of the fibers over a given distance. The fibers are clamped and pulled taught on the test stand. The force gauge slowly moves up, stretching the fibers until they break. From this data, the ultimate strength and tensile modulus can be determined to compare the strength and elasticity of the fibers. The fibers will be tested before and after the fibers have been modified and before and after they have fouled to determine how the elasticity and strength of the fibers change.

Results

FTIR_ATR

The polysulfone fibers were analyzed using a Fourier Transform Infrared Spectroscopy (FTIR) to determine if the fouling could be seen and characterized by this method. Five types of fibers were tested 3 times each. Unmodified polysulfone fibers were analyzed along with PDA modified PSF fibers, PSF fibers fouled in 35 mg/ml BSA in PBS, PDA modified PSF fibers in 5 mg/ml BSA in PBS, and PDA modified PSF fibers in 35 mg/ml BSA in PBS. The results are in Figure 8. The peaks for polysulfone can be seen at 1580 cm^{-1} and 1480 cm^{-1} for the double bonds in the aromatic ring, at 1236 cm^{-1} for the aromatic ether, and at 1150 cm^{-1} for the sulfonyl group. These peaks are seen in all five samples. The PDA cannot be differentiated from the polysulfone by this method because they have similar peaks. The BSA, though, can be seen in peaks 1650 cm^{-1} and 1540 cm^{-1} for the amide bands the BSA contains. The peaks are specifically for the C=O of the peptide of the amide I band and the N-H bending and C-N stretching of the amide II band. The peaks also grow with the concentration of BSA. The fibers fouled in 35 mg/ml BSA in PBS have higher peaks than the fibers fouled in 5 mg/ml BSA in PBS. The BSA foulant can be distinguished from the PSF and PDA peaks.

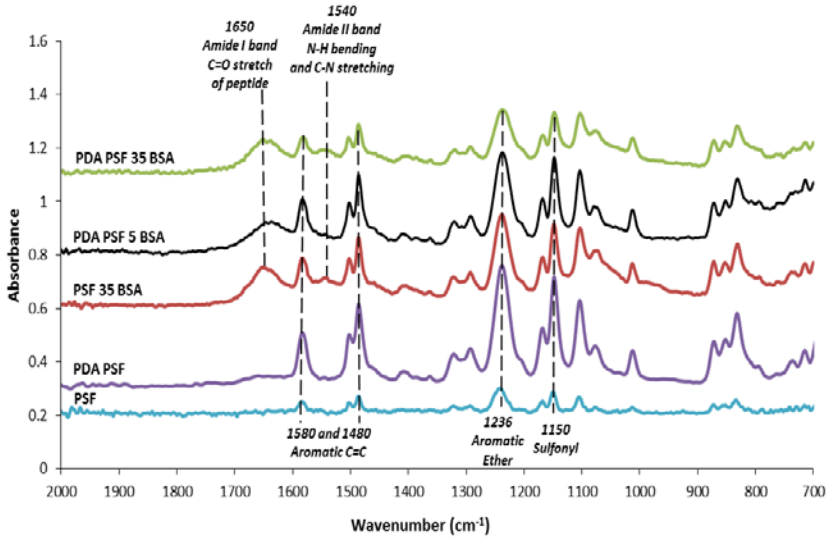

Figure 8 *FTIR analysis of PSF fibers, PSF PDA modified fibers, PSF fibers and PSF, PDA modified fibers both fouled in 35 mg/ml BSA/PBS in water, PSF, PDA modified fibers fouled in 5 mg/ml BSA/PBS in water*

SEM Images

Scanning electron microscope (SEM) Images were also taken to see if the fouling of the pores could physically be seen. Figure 9 contains the images of the fibers analyzed. The images were obtained by first, dipping the fibers into liquid nitrogen and then snapping the fibers apart as to not distort or stretch the pores seen in the cross sections. The two left images, images 1 and 2, are of unmodified polysulfone fibers before fouling. Images 3 and 4 are of the polysulfone fibers fouled in 5 mg/ml BSA in PBS after 72 hours. Image 1 and 2 are the image of the whole fiber cross section and the image 2 and 4 are of the fibers' walls so the pores can be better seen. The pores on the outside of the fibers marked by the brackets in image 2 are no longer seen in the wall of the fiber indicated by the bracket in image 4;

therefore, the fouling can be seen in the SEM images. The fouling would most likely be better seen with the fibers fouled in 35 mg/ml BSA in PBS. Using these images, it was also determined that the fibers are not uniform though out. For the next fibers spun, the water bath will be heated in an attempt to more evenly distribute the pores throughout the fibers.

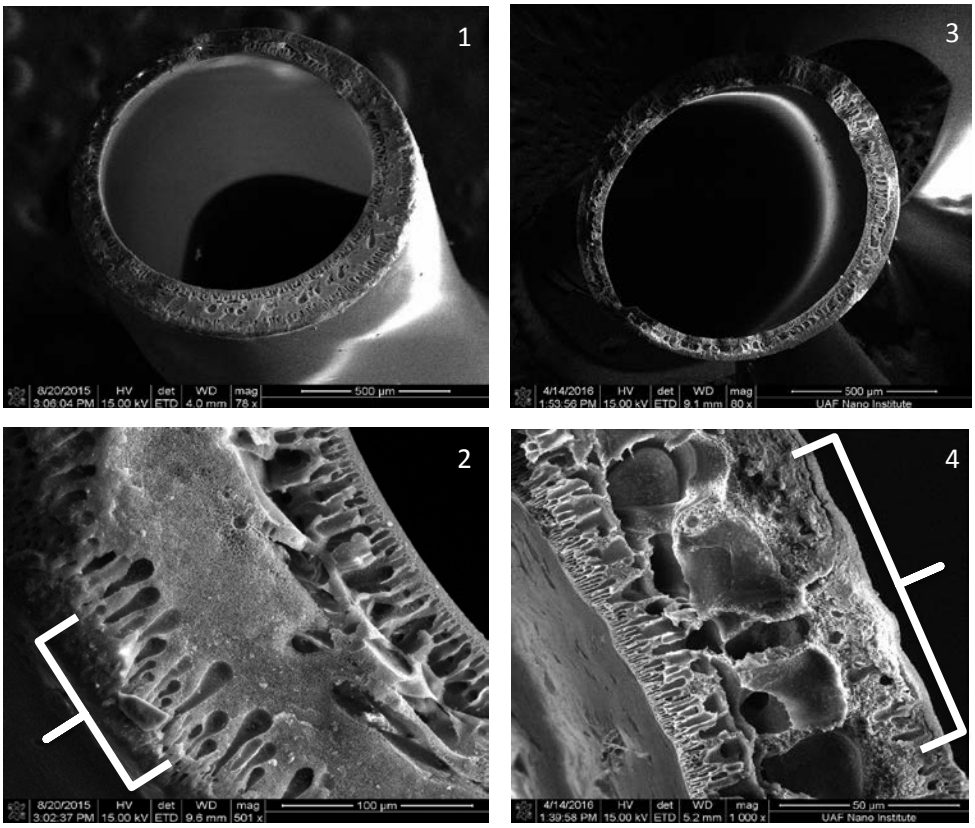

Figure 9 *SEM images comparing unmodified PSF fibers (images 1 and 2) and PSF fibers fouled in 5 mg/ml BSA/PBS in water for 72 hours (images 3 and 4)*

Evapoporometry

Evapoporometry was run for 6 different fiber types: PSF fibers, PSF fibers in 5 mg/ml BSA, PSF fibers in 35 mg/ml BSA, PDA modified PSF fibers, PDA modified PSF fibers in5 mg/ml BSA in PBS, and NMEG-5 peptoid PDA modified PSF fibers to determine how the pore sizes change with the modification of the fibers and the fouling of the fibers. The pore size distributions were compared for all of the fibers as seen in Figure 10. The average pore diameter was between 5 and 8.05 nm for all of the fibers tested. The PSF unmodified fiber before fouling had the highest average pore diameter of 8.04 nm. The modified fibers did not significantly affect the pore size. The PSF fibers in 35 mg/ml BSA in PBS had the highest number of smaller pores of less than 3nm. The fouling of the fibers with 5 mg/ml BSA in PBS could not be distinguished from the fibers before fouling but after the concentration of the BSA was raised to 35 mg/ml BSA in PBS, the change in pore size distribution could be distinguished. There were more of the smaller pores, fewer midsize pores, and the larger pores remained. The smaller to midsize pores around 3-13 nm adsorb proteins when the fibers are exposed to BSA creating more of the smaller pores but the larger pores are not affected.

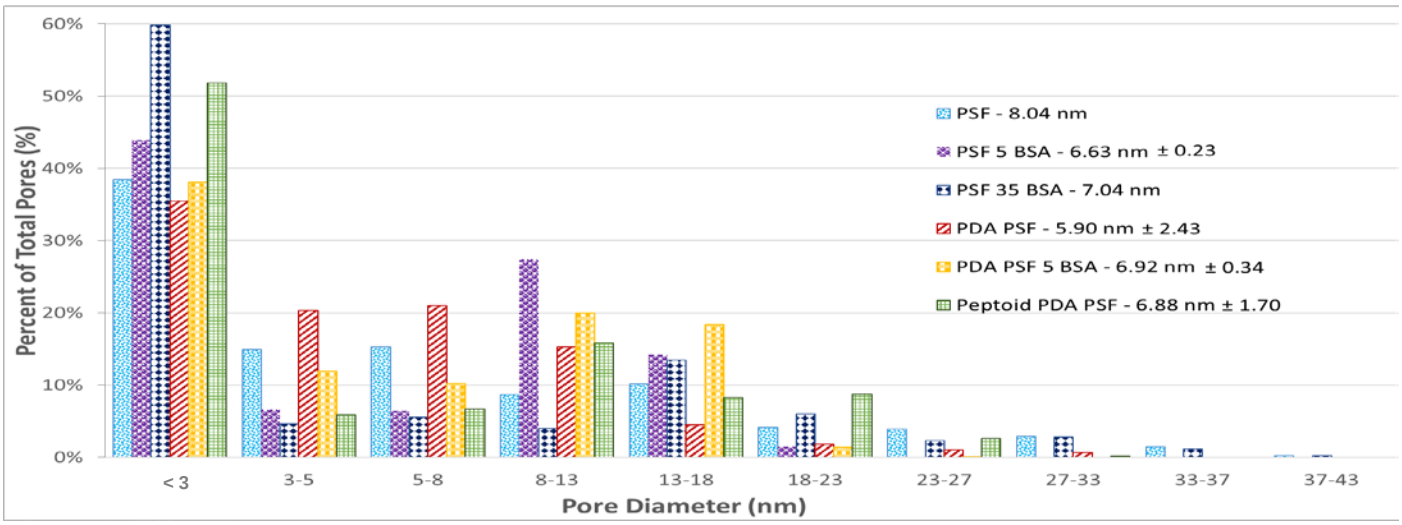

Figure 10 *Pore size distribution comparison of PSF fibers, PSF and PDA modified fibers, and PSF, PDA, and peptoid modified fibers before and after fouling*

Strength and Elasticity

The fibers were also tested for their strength and elasticity and compared to ensure that the fibers do not become more brittle over the long exposure to blood within the body. Figure 11 shows the ultimate strength in A and the Tensile Modulus in B for PSF fibers, PSF fibers in 35 mg/ml BSA in PBS, PDA modified fibers, PDA modified fibers in 35 mg/ml BSA in PBS, PDA modified fibers in 5 mg/ml BSA in PBS, NMEG-5 peptoid coated PDA modified fibers, and NMEG-5 peptoid coated PDA modified fibers in 35 mg/ml BSA in PBS. The figures show that the modified fibers stay the same or become stronger compared to the unmodified fibers as seen in Figure 11 A. With the exception of the peptoid coated fibers, the fibers seem to also become more elastic in nature too from Figure 11 B. This could be due to the fact that the BSA is filling/ attaching inside the pores. The fibers after fouling also generally become stronger and more elastic so the fibers becoming brittle in the chamber when exposed to blood is not an issue.

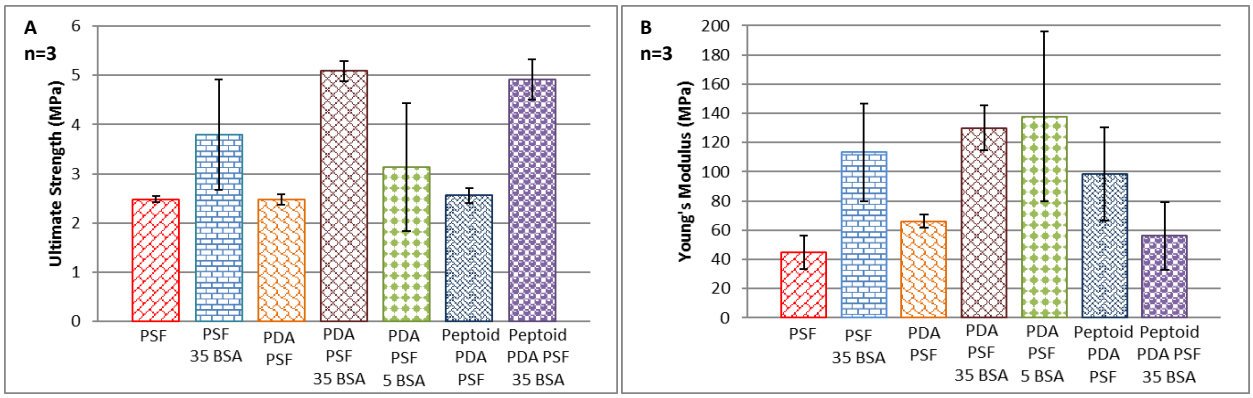

Figure 11 A) *Ultimate strength, the stress at which the fiber breaks, for different unmodified, modified, and fouled fibers.* ***B)*** *Young's Modulus, the slope of the stress strain curve as the fiber is stretched, to compare elasticity of the fibers. The unmodified and modified, fouled fibers were from two different HFM batches*

Conclusions

The BSA foulant could be distinguished from the unmodified and modified PSF fibers with the characterization methods used. With the pore size distribution, the BSA clogs the midsize pores, showing more of the smaller pores, the same amount of larger pores, and fewer midsize pores. Smaller pores and thus more fouling can be seen with the PSF fibers fouled in 35 mg/ml BSA as opposed to the PDA PSF and PSF fibers fouled in 5 mg/ml BSA. For the FTIR data, there are growing peaks at 1650 and 1540 cm^{-1} after the fibers are run through the chambers showing the amide I and II band of the BSA which distinguish the FTIR from just the PSF and PSF, PDA modified fibers. The fouling could be seen in the SEM images especially on the walls of the fibers. According to the Ultimate Strength, the fibers seemed to gain strength after being run through 35 mg/mL BSA/PBS in water. With the exception of the peptoid coated fibers, the fouled fibers also seemed to become more elastic in their nature.

The PDA does not seem to significantly affect the pore size distribution of the fibers after it is attached. The NMEG5 peptoid attachment affects the pore size more than the PDA but not enough to remove all of the pores for gas transfer within the fiber. From the previous research, the protein modifications also help the fibers have less fouling when exposed to BSA. Hopefully the peptoid will continue to have non-fouling characteristics and allow the fibers to last even longer when exposed to blood. The methods chosen to test the fibers do well with characterizing the fouling of BSA. The 35 mg/ml BSA in PBS was easier to distinguish from the unmodified fibers and is more representative of the concentration in human blood.

Future Works

Now that there are ways to characterize the fouling of the BSA on the fibers, the determined properties need to be applied to the gas exchange fouling studies for the peptoid coated fibers. The peptoid coated fibers need to be characterized before and after fouling with evapoporometry, SEM, FTIR, and stress vs strain. Other foulants within blood also need to be characterized in order to distinguish them from the BSA so that what is fouling the fibers will be able to be determined in a multicomponent system like blood. Also, new papers by the Krantz group have been published and should be applied to improve the evapoporometry test set-up. The characterizations of fouling determined need to then be applied to the analysis of the NMEG5 peptoid coated fibers before and after fouling in multicomponent systems such as blood. After these experiments, it can then be determined if the peptoid attachment will help lower fouling of the hollow fiber membranes when exposed to blood and will be a good material for use in an artificial lung. The current results suggest that will be the case.

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