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42611 Engineering Biomaterials, Fall 2021
Carnegie Mellon University
2021 Dec 14
Final Project: NIH-Style R21 Proposal on Biomaterials-Based Technologies

Synergistic Optimal Blood Clotting Resistance for Hemodialysis via an Experimentally Determined Three-Component System of Filter Material, Protein Resistant Coating, and Anticoagulant

Assignment Summary: Students will form groups (3 students per group) and identify an existing medical need that could be addressed by a new biomaterials-based technology. The student groups will perform background literature reviews to identify gaps in existing technology. The students will then propose a new invention and an associated hypothesis-based research project that (if completed) will validate (or not!) the merits of the proposed material/technology. The students will communicate their research, proposed innovation, and research plan in the form of an NIH-style R21 grant proposal.

Synergistic Optimal Blood Clotting Resistance for Hemodialysis via an Experimentally Determined Three-Component System of Filter Material, Protein Resistant Coating, and Anticoagulant

2. SPECIFIC AIMS

Approximately 37 million Americans, more than 1 in 7 adults in the United States, are affected by Chronic Kidney Disease (CKD), with an estimated 2 in 1,000 Americans living with end-stage kidney disease (ESKD) or End-Stage Renal Disease (ESRD).[1][2] Of the patients with ESKD in the United States who receive treatment, only 29% will receive a kidney transplant, and 71% are on dialysis.[3] The goal of dialysis is to remove waste toxins and excess water from patients. Hemodialysis is the most common technique to filter blood *ex vivo* and accounts for around 87.6% of dialysis in the United States.[4] Hemodialysis patients are at high risk for blood clotting and "cardiovascular events as well as an altered coagulation with both increased thrombotic and bleeding risks".[5][6][7] Patients with CKD are more than twice as likely to have an ischemic stroke caused by blood clotting compared to patients who do not have chronic kidney disease.[6][7] An extracorporeal membrane oxygenation (ECMO) machine uses a process similar to hemodialysis to filter blood.[8] In 2021, research demonstrated the use of ECMO, with a "3,4-dihydroxyphenylalanine (DOPA)-linked PCB coating (DOPA-PCB)" and clotting factor FXII900 infusion, provided the optimal prevention of blood clotting in a rabbit model.[8] The PCB coating and FXII900 infusion demonstrated a "markedly reduced … coagulation and tissue bleeding times versus the clinical standard of heparin anticoagulation".[8] Since the 2021 ECMO rabbit model research did not include an evaluation of hemodialysis, it is reasonable to conclude that a PCB coating and FXII900 infusion applied to hemodialysis will also reduce blood clotting.[8] **These researchers were unable to find any literature discussing the clinical use of a hemodialysis filter with a PCB coating and FXII900 infusion treatment.** A synergistic effect is where a combined effect is greater than the sum of their separate effects. **We hypothesize that there exists a synergistic effect of the optimal combination of filter material, protein resistant coating (e.g. PCB coating), and anticoagulation medicine (e.g. FXII900 infusion) which greatly increases protein resistance on surfaces and decreases blood coagulation, which is unknown to science and medicine at this time.** Therefore, to establish the system and test our hypothesis of finding the optimal combination, we propose the following specific aims:

Specific Aim 1. Catalog. We aim to catalog the existing different filter materials, protein resistant coatings, and anticoagulation medicine used in Hemodialysis, Peritoneal Dialysis, and ECMO to experimentally determine the greatest reduction of coagulation as a baseline control. Then, experimentally determine the existing highest performing reduction of coagulation combination with the addition of FXII900 infusion.

Specific Aim 2. Efficacy Measurement. We aim to utilize blood coagulation measurement techniques in-vitro to verify the synergistic effect of the optimal combination of filter materials, protein resistant coatings, anticoagulation medicine, and FXII900 infusion to compare the results. We plan to use the following blood coagulation measurement techniques to correlate effects: Optical (SPR), Electro-mechanical (QCM), Photoacoustic detection, and Electrochemical (Amperometric impedance analysis).[9]

Specific Aim 3. Biological Model Testing. We aim to design and test an in-vivo hemodialysis animal model to study properties and catalog the synergistic effect based on their anticoagulatory properties. We plan to test the synergistic effect of the optimal combination of filter materials, protein resistant coatings, anticoagulation medicine, and FXII900 infusion to compare the results using rabbit blood due to the "model's highly procoagulant nature", sheep blood, in a hemodialysis extracorporeal circuit.[8]

3. RESEARCH STRATEGY

(A). Background and Significance

The **specific medical problem** that this document will address is blood clotting related to hemodialysis and protein-resistant surfaces. More than 1 in 7 adults in the United States are affected by Chronic Kidney Disease (CKD), and an estimated 2 in 1,000 Americans are living with end-stage kidney disease (ESKD).[1][2] Of the ESKD patients in the United States who receive treatment, 29% receive a kidney transplant, and 71% are on dialysis.[3] Once a patient gets on to the kidney transplant waiting list, the median wait time for a kidney transplant is 4.1 years.[2] In 2020, only 22,817 kidney transplants were completed in the United States, while as of August 2021, there are 90,201 people on the kidney transplant waiting list.[2] Therefore dialysis is a vitally important treatment for patients with ESKD.

Dialysis supplements the damaged or diseased normal organ functions of filtration. The dialysis process filters select body fluids via an extracorporeal circuit (ECC).[8] Unfiltered body fluid is transported outside of the body where it is processed in a machine with a highly specialized filter which removes the majority but not all of the body's waste products, and the filtered body fluid is returned to the body. The body fluid can be peritoneal in the case of peritoneal dialysis or whole blood in the case of hemodialysis. There are three main types of dialysis: in-center hemodialysis, home hemodialysis, and peritoneal dialysis.[10] Extracorporeal membrane oxygenation (ECMO) is a similar process, which represents a “treatment that uses a pump to circulate blood through an artificial lung back into the bloodstream”.[12] ECMO is usually reserved for intensive emergency care. This document discusses the challenges of blood clotting as it relates to hemodialysis and protein-resistant antifouling surfaces.

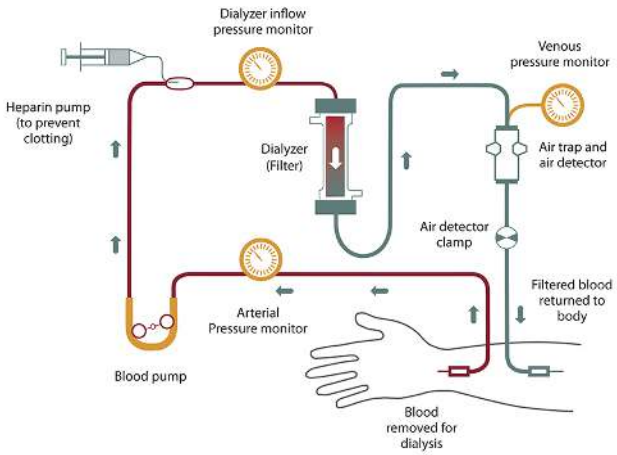

Figure 1. Hemodialysis process.[11]

Preventing blood clotting in the dialysis extracorporeal circuit is necessary to allow routine outpatient hemodialysis to be carried out in satellite dialysis centers or at home.[13] Blood clotting occurs when blood comes in contact with exogenous surfaces and “could lead to thrombocyte adhesion and activation, which results in triggering plasmatic coagulation and/or activation of the complement system”.[14] Heparin, the naturally occurring compound found in the liver and other tissues, inhibits blood clotting. During the hemodialysis process, blood is removed from the body by a blood pump and a separate Heparin pump delivers Heparin into the pre-filtered blood to prevent blood clotting.[15] (See Figure 1.) The solution of blood and Heparin is processed by a hemodialysis filter e.g. dialyzer, which blocks waste products from being reintroduced into the bloodstream. Waste products are carried away in the dialysis solution (dialysate). Small amounts of Heparin inadvertently pass through the filter and travel with the filtered blood as it is reintroduced back into the body completing the circuit.[16] Since some Heparin passes through the filter during hemodialysis, patients face a higher risk of bleeding, with “clotting rates that can be as high as 39%”.[16] Due to this, decreasing or eliminating Heparin use during hemodialysis would be ideal, hence the importance of optimizing anticoagulant surfaces.[16]

The **prospective significance** of solving this problem is decreased complications caused by blood clotting related to hemodialysis. These researchers acknowledge that we would be remiss to not at least acknowledge the role of tubing, connectors, pump impellers, blood component sensors, check valves, and other parts of the extracorporeal circuit and their role in blood clot initiation and nucleation points. However, for this document, we will focus on the hemodialysis filters. Finding the synergistic effect of the optimal combination of filter material, protein resistant coating (e.g. PCB coating), and anticoagulation medicine (e.g.

FXII900 infusion), which greatly increases protein resistance on surfaces and decreases blood coagulation, is unknown to science and medicine at this time. Once found we could greatly increase life expectancy by optimistically eliminating blood clotting complications caused by artificial surfaces.

(B). Innovation

<u>The current state of the art that is used is</u>: As of December 10, 2021, a search via Google.com for keywords hemodialysis “FXII900” or dialysis “FXII900” returned 14 results, and a search via scholar.google.com hemodialysis “FXII900” or dialysis “FXII900” returned 3 results, none of which indicate that FXII900 is currently being used in hemodialysis treatment. ***These researchers were unable to find any literature discussing the clinical use of a hemodialysis filter with a PCB coating and FXII900 infusion treatment.***

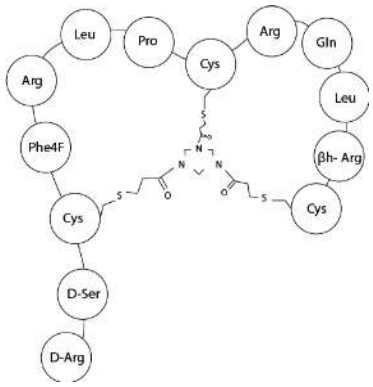

Figure 2. The Molecular Structure of FXII900. [8] was referenced for this **new image**.

<u>Potential Shortcomings of Existing Technologies</u>: Higher levels of blood clotting.

<u>Competitive Advantages Over Existing Technologies</u>: A “PCB coating + FXII900 infusion” combination consists of a “polycarboxybetaine coating and factor XII inhibitor” which “reduces clot formation while preserving normal tissue coagulation” during ECMO.[8] The combination of PCB coating and PXII900 infusion has been demonstrated with ECMO in rabbits due to the “model’s highly procoagulant nature” to be “markedly reduced … coagulation and tissue bleeding times versus the clinical standard of heparin anticoagulation”.[8]

(C). Proposed Research Approach

<u>Hypothesis and Rationale</u>: In 2021, research demonstrated the use of ECMO, with a “3,4-dihydroxyphenylalanine (DOPA)-linked PCB coating (DOPA-PCB)” and clotting factor FXII900 infusion, provided the optimal prevention of blood clotting in a rabbit model.[8] Rabbits were selected due to the “model’s highly procoagulant nature” and the PCB coating and FXII900 infusion demonstrated a “markedly reduced … coagulation and tissue bleeding times versus the clinical standard of heparin anticoagulation”.[8] Since the 2021 ECMO rabbit model research did not include an evaluation of hemodialysis, it is reasonable to conclude that a PCB coating and FXII900 infusion applied to hemodialysis will also reduce blood clotting. **These researchers were unable to find any literature discussing the clinical use of a hemodialysis filter with a PCB coating and FXII900 infusion treatment.** A synergistic effect is where a combined effect is greater than the sum of their separate effects. **<u>We hypothesize that there exists a synergistic effect of the optimal combination of filter material, protein resistant coating (e.g. PCB coating), and anticoagulation medicine (e.g. FXII900 infusion) which greatly increases protein resistance on surfaces and decreases blood coagulation, which is unknown to science and medicine at this time.</u>** The **<u>rationale</u>** is that because FXII900 infusion was first used in 2020, then demonstrated in rabbit animal model using ECMO in 2021, and not yet used with hemodialysis, there may exist a synergistic effect of the optimal combination of filter materials, protein resistant coatings (e.g. PCB coating), and anticoagulation medicine (e.g. FXII900 infusion) which greatly reduces blood coagulation.[8][17] **This would thus <u>generate new scientific knowledge</u> and <u>demonstrate the superiority of this proposed technology advancement.</u>**

<u>Specific Aim 1. Catalog.</u> We aim to catalog the existing different filter materials, protein resistant coatings, and anticoagulation medicine used in hemodialysis, Peritoneal Dialysis, and ECMO to experimentally determine the greatest reduction of coagulation as a baseline control. Then, experimentally determine the existing highest performing reduction of coagulation combination with the addition of FXII900 infusion.

<u>Rationale</u>: The human race could already possess but not yet know or utilize the optimal combination of filter materials, protein resistant coatings (e.g. PCB coating), and anticoagulation medicine (e.g. FXII900 infusion), which greatly reduces blood coagulation. However, the synergistic effect of the optimal combination is currently unknown to science and medicine at this time. Therefore while we have a list below generated from literature, these researchers have determined that it is not yet exhaustive. Based on scanning electron microscope (SEM) images like the one in Figure 3, observable effects of the optimal combination of filter material, protein resistant coating, and anticoagulation medicine should allow experimentation to identify component combinations based on effectiveness.

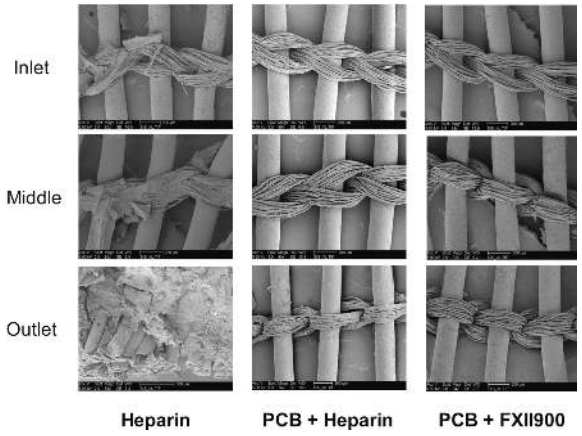

Figure 3. An example of a scanning electron microscope (SEM) image of the fiber bundle inlet, middle, and outlet for Heparin, PCB + Heparin, and PCB + FXII900.[8]

Known Filter Materials: <u>(Total 14)</u> 2-Methyl-2-Propenoic Acid Methyl Ester Homopolymer (Polymethylmethacrylate, Pmma), Acetylcellulose (Cellulose Acetate), Acrylonitrile (Polyacrylonitrile), Cellophane (Cellulose Regenerated by the Deacetyl Method), Dicopper Chloride Trihydroxide (Cellulose Rayon Regenerated by the Cuprammonium Method, Toxic), Ethenol Polymer With Ethene (Ethylene Vinyl Alcohol, EVAL, Copolymer), Polyacrylonitrile (PAN), Polyamide Cellulose Triacetate (CTA), Polyester Polymer Alloy (PEPA), Polyethersulfone (PES), Polyethersulfone Polyarylate (PEPA) Blend Polymer, Polymethylmethacrylate (PMMA), Polysulfone (PS).[8][18][19]

Known Filter Materials: <u>(Total 14)</u> 2-Methyl-2-Propenoic Acid Methyl Ester Homopolymer (Polymethylmethacrylate, Pmma), Acetylcellulose (Cellulose Acetate), Acrylonitrile (Polyacrylonitrile), Cellophane (Cellulose Regenerated by the Deacetyl Method), Dicopper Chloride Trihydroxide (Cellulose Rayon Regenerated by the Cuprammonium Method, Toxic), Ethenol Polymer With Ethene (Ethylene Vinyl Alcohol, EVAL, Copolymer), Polyacrylonitrile (PAN), Polyamide Cellulose Triacetate (CTA), Polyester Polymer Alloy (PEPA), Polyethersulfone (PES), Polyethersulfone Polyarylate (PEPA) Blend Polymer, Polymethylmethacrylate (PMMA), Polysulfone (PS).[8][18][19]

Known Protein Resistant Coatings: <u>(Total 9)</u> 3,4-dihydroxyphenylalanine (DOPA)-linked PCB coating (DOPA-PCB), Bicyclononyne (BCN), Bisphenol A (BPA) product SEC-1 (Toyobo), candle soot (included because of negligible cost and completeness), Phosphorylcholine (PC), Poly(ethylene glycol) (PEG), polycarboxybetaine (PCB), polysulfobetaine (PSB).[8][20][21][22]

Known Anticoagulation Medicine: <u>(Total 18)</u> Macrocyclic peptide inhibitor of activated FXII (FXIIa) with sub-nanomolar activity (FXII900), Argatroban, Citrate, Coumadin Jantoven (warfarin), Dalteparin, Danaparoid, Eliquis (apixaban), Hep-Lock U/P Hep-Lock HepFlush-10 (Heparin), Hirudin Lepirudin, Lovenox (Enoxaparin), Low Heparin (with maintenance dose), Low-molecular-weight Heparin (LMWH), Nadroparin, Pradaxa (dabigatran), Tinzaparin, Unfractionated Heparin (UFH) Standard Heparin, Very low Heparin (without loading or maintenance dose), Xarelto (rivaroxaban).[8][17][23][24]

Detailed Experiments:

Methods: Hemodialysis filters or dialyzers are made from different materials including “cellulose triacetate (CTA), ethylene vinyl alcohol (EVAL), polyacrylonitrile (PAN), polyester polymer alloy (PEPA), polyethersulfone (PES), polymethylmethacrylate (PMMA), and polysulfone (PS)”, which have different impacts on the patient life expectancy i.e. mortality.[19] The first commercial protein-resistant Dialyzer filter material with a surface coating used “polyethylene glycol (PEG, Medtronic Trillium and Balance Biosurface) and Poly-1-methylethylacrylate (PMEA, Terumo X-Coating)”.[8] It was found that “PEG decomposes in the presence of oxygen and transition metal ions in whole blood ... and is thus not suitable for long-term use.” [8] Furthermore “Zwitterionic polymer coatings such as PCB, polysulfobetaine (PSB) and phosphorylcholine (PC) are more promising due to their greater ability to inhibit protein adsorption”.[8]

Therefore, these researchers have determined that cataloging all the existing known filter materials, protein resistant coatings, and anticoagulation medicine used in hemodialysis, peritoneal dialysis, and ECMO is of utmost importance to know what individual components should be combined to compare.

Prospective Techniques: Use of scanning electron microscope for rapid combination identification. Generation of a material and chemical science property database.

Outcome and Risk Mitigation:

Data that will be Generated: Generation of a material and chemical science property database.

Potential Risk Elements of this Specific Aim: Normal laboratory procedures for dealing with unknown yet anticipated interactions of chemicals. This process will take a long time and a lot of money to investigate. It is also possible that while not published in literature because the material or coating is protected by proprietary biomedical intellectual property the combination has already been found. Also, we may not be able to acquire some of the materials or chemicals used in the hemodialysis filters because the manufactures will not supply them to research to protect proprietary biomedical intellectual property.

Specific Aim 2. Efficacy Measurement. We aim to utilize blood coagulation measurement techniques in-vitro to verify the synergistic effect of the optimal combination of filter materials, protein resistant coatings, anticoagulation medicine, and FXII900 infusion to compare the results.

Rationale: An *in vitro* model is used to test the efficacy of the optimal combination of filter materials, protein resistant coatings, anticoagulation medicine, and the FXII900 fusion. The *in vitro* model is chosen for two reasons: 1) the model can be generated with controlled variables, which allow for the combination to be tested in a system without any distractions, and 2) *in vitro* models can be developed and replicated in a smaller aspect compared to *in vivo* models, which means the model is less time-consuming and cheaper to be made. The *in vitro* model will be used as the first line of efficacy and toxicity testing, and any viable combinations will be further analyzed with *in vivo* models.

Detailed Experiments:

Methods: To test the toxicity and cell viability of the filter combination, an MTT assay will be used.[25] “MTT is a tetrazolium dye which is converted into formazan by metabolically active cells.”[25] Hematopoietic cells will be cultured and used to develop the *in vitro* model.[25][26] After culturing, an MTT labeling agent will be added to each cell-containing well.[25] The incubated cell cultures will be divided into sets of triplicate samples: a triplicate of control samples which will be treated with standard filters, and a triplicate of filters treated with the optimal combination. The control samples will be used to generate baseline results of blood coagulation when an unaltered filter is used, and an unaltered filter will be applied to the triplicate control cell culture to test for blood coagulation. The combination samples will be made by incubating each of the cell culture samples with the combination that is being tested.[27][28] Dimethyl sulfoxide (DMSO) will be added to

the samples and ultraviolet measurements of the dyed samples will be measured to identify if any of the tested combinations are causing an increased amount of cell death.[26][28][29][30]

To test the risk of blood clotting, we must analyze the filter combinations for their effectiveness at blocking anticoagulants from passing back into the blood. To test filter combination efficacy *in vitro*, a simple filter model can be created. The filter combination can be isolated over unaltered dialysate.[31][32] A second sample of dialysate will be made by adding a specific quantity of anticoagulant to the initial dialysate.[31] The anticoagulant-infused dialysate will be added to the system containing the pure dialysate and filter combination. A baseline sample will be created using an unchanged filter. The test can be run either using a duration-specific method (compare the quantity of anticoagulant diffused by the samples over a specific period time) or a diffusion duration method (allow for complete diffusion of the baseline sample and the altered sample and compare the amount of time required for diffusion between the two samples).[32]

Prospective Techniques: We plan to use the following blood coagulation measurement techniques to correlate effects: Optical (**SPR**), Electro-mechanical (**QCM**), Photoacoustic detection, and Electrochemical (Amperometric impedance analysis).[9]

Outcome and Risk Mitigation:

Data that will be Generated: The toxicity and cell viability study (MTT assay) should give a better perspective as to whether any of the derived combinations are harmful to the hematocytes, which the filter combination would actively be in contact with when implemented for dialysis. The efficacy test will be used as a first attempt to determine whether the novel filter combination is more effective in blocking anticoagulant transfer with filtered blood.

Potential Risk Elements of this Specific Aim: Chemicals used in the testing procedures. Material safety is used in the filter material samples. Zoonotic blood pathogens from animals e.g. Hantavirus diseases, Rat-bite fever, Yellow fever, etc.[33] Human blood pathogens e.g. hepatitis B virus, hepatitis C virus, human immunodeficiency virus, etc.

Specific Aim 3. Biological Model Testing. We aim to design and test an in-vivo hemodialysis animal model to study properties and catalog the synergistic effect based on their anticoagulatory properties. We plan to test the synergistic effect of the optimal combination of filter materials, protein resistant coatings, anticoagulation medicine, and FXII900 infusion to compare the results using rabbit blood due to the "model's highly procoagulant nature", sheep blood, in a hemodialysis extracorporeal circuit.[8]

Rationale: It is vital to understand the *in vivo* implications of using a new polymer formulation onto the filtrate to ensure no adverse effects occur. Functional classification and network analysis performed using bioinformatics tools shed light on the involvement of adsorbed proteins into important molecular processes, such as lipid transport and metabolism, cell growth differentiation and communication, and the coagulation cascade.[34] The in vivo model recreates the processes involved in the filtration of blood, and we will be able to study the performance of the PCB + FXII900 combination.

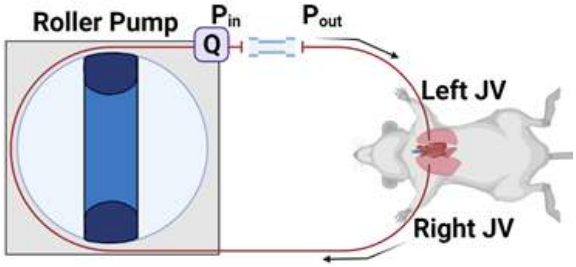

Figure 4. Schematic diagram of potential rabbit animal model venovenous extracorporeal circuit. [8] was referenced for this **new image**.

Detailed Experiments:

Methods: Animal model testing will be similar to Figure 4. The blood will exit the Right Jugular Vein (JV) pass through a roller pump (selected to decrease confound variables potentially caused by fouling due to blood clotting), where $\mathbf{P_{IN}}$ represents the pressure inlet to the combination being tested, then $\mathbf{P_{OUT}}$ represents the pressure outlet and blood being returned into the Left Jugular Vein (JV). Depending on the factors such as

weight, histophysiology - the models are subjected to varying concentrations of saline, and anticoagulants. The adequacy of the anticoagulation can be monitored by measuring the activated coagulation time.[35] The blood is collated through catheters to the dialyzers, flowing over the surface of dialyzers coated with the proposed formulation PCB + FXII900. During hemodialysis the effective flow rates, ultrafiltration rates are recorded to ascertain the performance of the dialyzer.

Prospective Techniques: Test the synergistic effect of the optimal combination of filter materials, protein resistant coatings, anticoagulation medicine, and FXII900 infusion to compare the results using rabbit blood due to the “model’s highly procoagulant nature”, sheep blood, primate blood, then human blood in a Hemodialysis, Peritoneal Dialysis, and ECMO extracorporeal circuit.[8] While we are focusing on hemodialysis, study replication of Peritoneal Dialysis and ECMO has value for future clinical use. The use of methods like mass spectroscopy has been conducted previously to study the adsorption of particles to membranes.[36] To study the coagulation of blood on the membranes, we expect to recreate similar test environments to observe the mass of molecules settled on the surface of these membranes. By the study of the mass of adsorbed molecules, it is possible to extrapolate information such as anticoagulatory effects of the polymer utilized.[34]

To study the urea reduction rate, and overall filtration efficacy spectrophotometry will be utilized.[37] To study blood morphology before and after the study we will be using an automated hematology analyzer. It is also important to record values of the electrolyte concentrations in the blood after a course of hemodialysis to ensure that there is no filtration of essential minerals from the bloodstream, causing future complications. The performance of the dialyzer material can be recorded in terms of retention of blood constituents, and filtration of the impurities.

Accordingly, the measure of filtered out impurities, in tandem with the anticoagulatory properties of the novel constituents in the dialyzer materials and coatings, can be ascertained.[38] In the past, anticoagulatory properties of dialyzers have been tested with proteomic technology investigations; which can be used to record values of blood protein retention, which will give a direct measure of the performance of the dialyzer.[34]

Outcome and Risk Mitigation:

Data that will be Generated: We aim to record the values of the membrane’s ability to augment native kidney function, such as maintaining a water balance, ionic solute balance, and excretion of small molecule, and middle molecules. Apart from the blood rheology components of recording, data from mass spectroscopy will be used to obtain information on the amount of coagulation that has occurred onto the dialyzer. This data will be used to study the synergistic effects of the novel dialyzer materials.

Potential Risk Elements of this Specific Aim: Chemicals used in the testing procedures. Material safety is used in the filter material samples. Zoonotic blood pathogens from animals e.g. Hantavirus diseases, Rat-bite fever, Yellow fever, etc.[33] Human blood pathogens e.g. hepatitis B virus, hepatitis C virus, human immunodeficiency virus, etc.

(D). Outcome, Impact, and Next Steps

Summary of Anticipated Outcome and Impact of this Research Effort: This innovation will advance the field of material science, medicine, hepatology, and nephrology by creating knowledge that currently does not exist. In addition, it has the potential to drastically reduce blood clotting in hemodialysis, a vital treatment for end-stage kidney disease. It is also possible that this technology can be applied to many other aspects of medicine.

The Prospective Next Steps for this Technology: If after we have completed all the Specific Aims 1 through 3, then it will be ready for submission to the FDA approval process for human testing.

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Acknowledgments: Figure 4: Created with BioRender.com.

NOTE: This document was created on 2021 Dec 14 during the COVID-19 Pandemic, for Dr. Christopher Bettinger, Carnegie Mellon University, Fall 2021, Graduate Level 42611 A ENGINEERING BIOMATERIALS. **Key grading points are highlighted in bold.**