External Scaffolding for Rapid Use of Arterio-Venous Fistula

- CONFIDENTIAL -

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Abstract
An arterio-venous (AV) fistula is a surgical connection made between an artery and a vein that serves as a vascular access for patients who require hemodialysis. The goal of this project is to identify a material that can be utilized as scaffolding for the AV fistula which take 1-3 months to mature. We tested the ability of four materials (alginate, Pluronic F-127, polyethylene glycol diacrylate (PEG-DA) and fibrin gel) to adhere to tissue and withstand this testing. An adhesion test measured how much force was required to separate tissue connected with each material. The maximum forces for alginate, fibrin gel, and PEG-DA were 0.044 N, 2.177 N, and 0.410 N, respectively. Pluronic F-127 adhesion was not sufficient to with-stand this testing. A puncture test was performed by puncturing a vessel with each material polymerized on its surface. Both alginate and Pluronic F-127 were semi-solid and easily removed from the vessel. Fibrin gel and PEG-DA withstood puncture by minimizing vessel collapse and retained adhesion to the vessel tissue. Based on these results, fibrin gel was selected to be further investigated throughout the remainder of the project.

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Problem Statement

Many patients who require hemodialysis have an arterio-venous (AV) fistula inserted to allow blood vessels to be accessed. While these vascular access points are considered the "life-lines" of these patients, surgeries for AV fistula implants only have a 45% success rate [16]. Moreover, among the patients who have successful AV fistulas, vein collapse may occur during hemodialysis due blood being rapidly drawn from the vessel [23]. The maturation period of AV fistula is also one to three months; yet, these patients require immediate fistula utilization. The goal of our project is to modify an existing material that will enhance the AV fistula. This requires strengthening and tethering of the veins and shortening of the fistula maturation period.

Introduction

Renal Failure

Renal, or kidney, failure occurs when the body is no longer able to cleanse waste product, resulting in abnormal levels of fluid and waste in the body [18]. In addition to cleansing waste products, kidneys are also responsible for cleaning blood and producing hormones that keep bones strong and the blood healthy [18]. End-stage Renal Disease (ESRD) can be caused by many factors including diabetes, hypertension, glomerulonephritis and chronic kidney disease [16]. If someone suffers from renal failure, treatment is necessary to perform the function of the failed kidney [18]. Most patients undergo hemodialysis to help manage this condition because few kidneys are available for transplantation [26]. These patients are situated in a clinic so that blood flows out of one access point, typically in the arm, through a hemodialysis machine where it is filtered several ounces at a time to remove fluid and waste (Figure 1; [18]). The clean blood

is then returned to the body through another access point. Patients must receive this treatment 3 to 5 times per week and each treatment takes about four hours [18].

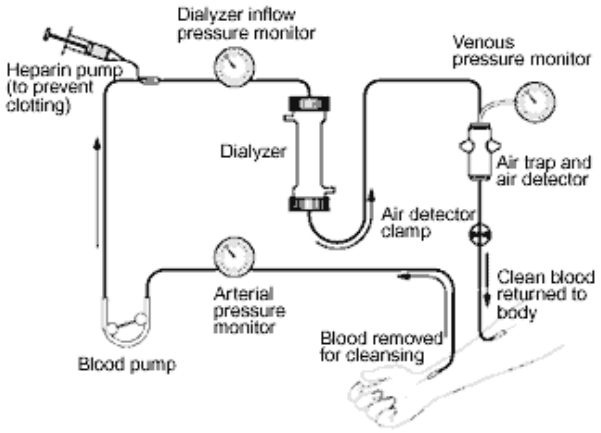

Figure 1: In the set up for hemodialysis treatment, a patient is situated so that blood flows out of one access point through a hemodialysis machine. Clean blood returns to the body through another access point [18].

The ideal vascular access for hemodialysis provides adequate blood flow so that dialysis is maximized every time a patient receives treatment [29]. The three most common types of vascular access are catheters, arterio-venous (AV) grafts and AV fistulas [16].

Catheter

The first method is a central venous catheter which can be inserted into a major vein, such as the femoral, subclavian or jugular vein [23]. One end of the catheter is external to the body, and the other end remains in either the superior vena cava or the right atrium of the heart [23]. While catheters are easily accessed, they are the least successful of the three methods listed above because they run a high risk of infection [29].

Graft

To create a graft, a surgeon inserts a small piece of artificial vessel to bridge an artery and a vein forming a by-pass that can be punctured by hemodialysis needles [23]. A graft cannot be used until three weeks after its formation so the internal wall of the connection can stabilize [29]. As foreign material is inserted in graft formation, they also run a high risk of infection and thrombosis, and therefore have a low success rate [29].

Arterio-Venous Fistula

The AV fistula is the preferred method for vascular access. Figure 2, shows a schematic representation of an AV fistula. There is a lower incidence of thrombosis and infection in patients with AV fistulas since no foreign material is inserted for fistula formation [29]. To create an AV fistula, a surgeon attaches a vein and an artery, most often in the non-dominant arm of a patient. This connection can take from 1 to 3 months to mature [29]. With this new blood flow path, most blood will bypass the high flow resistance of the downstream capillary bed, thereby producing an increase in the blood flow rate through the fistula [23]. Furthermore, although it is not possible to repeatedly puncture an artery, formation of the fistula "arterializes" the vein. The arterialized vein can be punctured repeatedly, and the high blood flow increases the efficiency of hemodialysis [23].

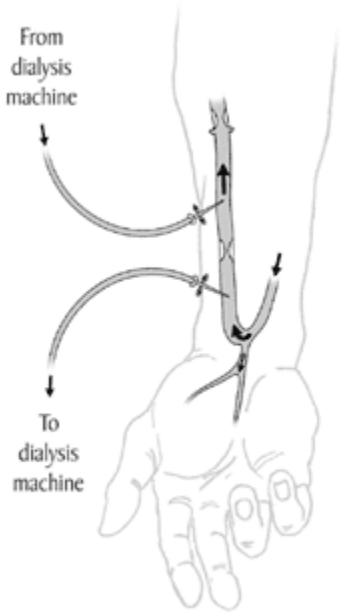

Figure 2: AV fistula serves as an access point for hemodialysis and is created by surgically attaching a vein and an artery [18].

Project Motivation

After 5 years of hemodialysis treatment, the average patient survival rate is about 31.9% [16]. Vascular access-related problems are responsible for 50% of hospitalizations of hemodialysis patients [29]. The motivation of our clients is to obtain a material to stabilize the AV fistula during its formation. This solution could allow more rapid use of this type of vascular access and increase the success rate of AV fistulas that are inserted in patients who rely on hemodialysis treatment.

Design Specifications

According to the criteria provided by our clients, the AV fistula scaffolding needs to be an injectable liquid that can be easily applied to a patient. After injection, the material needs to be polymerizable *in situ*. Ideally, the polymerization process will not require any heat or light treatment, such as UV radiation. The polymerized material must adhere and tether to the vein. It will serve as a coating around the fistula that strengthens and prevents vein collapse. The adhesive material should be able to withstand puncture and tension, since these external forces will be present when the patient undergoes hemodialysis. Lastly, this material must be biocompatible so the patient does not experience any additional discomfort.

Existing Products

Multiple patent searches using the U.S. Patent and Trademark Office website revealed most of the patents detailing products to improve dialysis modify either the graft or catheter method. One exception is Patent 6,609,014 which details an invention that attempts to increase the success of AV fistulas. This invention approaches this challenge in a different way than our

proposed solution, as it aims to inhibit or reduce thrombosis of blood vessels due to intimal hyperplasia with a low-dose photodynamic therapy, instead of attempting to stabilize the AV fistula [1]. Thus, based on these patent searches and multiple internet searches, we are confident we have a novel approach to the solution of AV fistula failure.

Investigated Materials

Alginate

Properties

The first material that we have been investing is alginate, which is extracted from the cell walls of brown algae [25]. It is a widely used encapsulation material due to its ability to cross-link and form hydrogels [7]. The premixed form of alginate is a bipolymer alginic acid sodium salt, and it is made by adding the sodium alginate into deionized water (2% w/w). However, alginate is a linear branching copolymer that forms coordinate bonding with polyvalent cations, i.e. ions with multiple positive charges. Without the addition of divalent cations, alginate is a linear unbranched copolymer of β-D-mannuronic acid (M) and α-L-guluronic acid (G) units [7]. When polyvalent cations are present (in this project Ca^{2+}), these cations serve as linkages between the G-units to form a hydrogel network [7]. The polymerization of alginate with Ca^{2+} occurs at room temperature, under continuous mixing of the two solutions.

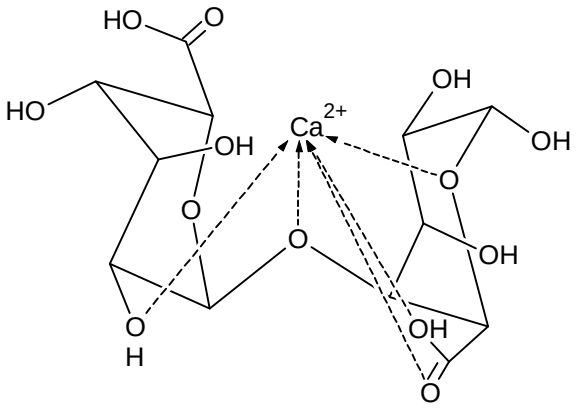

Figure 3: The chemical structure of alginate. Note that the oxygen anions from the unbranching polymer are attracted to the positive calcium cations.

Applications

The easy preparation and cross-linking of alginate give rise to the frequent employment of this material to prepare delivery systems such as beads, microspheres and film-coating in medical applications [7]. Alginate absorbs water quickly, so it is often used as an preservative in dehydrated products such as "slimming aids" [20]. It is also used for mold-making material, which makes it useful for dental filling and prosthetics. Since alginate is extracted from seaweed, it is commonly used in food applications and medicine. It could be used to thicken soups, make jellies, and make sodium alginate matrix tablets [7]. These applications indicate that alginate is biocompatible, thus it is also used for cell immobilization in research and encapsulation.

Degradation

The degradation of alginate hydrogel is very slow and not easily controlled. It undergoes a slow and unpredictable dissolution process *in vivo*. It is mainly due to the sensitivity of the gels towards calcium chelating compounds [3]. A previously reported experiment indicated that a controlled alginate degradation is by using partial periodate oxidation [3]. As alginate oxidizes

in the presence of sodium periodate, the rings of alginate break down and degradation occurs (Figure 3).

Advantages and Disadvantages

An advantage of utilizing alginate as the encapsulation material is its ability of immediate polymerization. It forms cross-linking with calcium cations when it comes into contact with calcium cation solution. This process does not require heating or any other form of energy input except stirring, which makes it a good encapsulation material. However, alginate's advantage leads to its disadvantage. The immediate polymerization of alginate makes it difficult to control the polymer shape at the site of injection. It does not fully encapsulate the desired vein surfaces before it gels. Furthermore, the mixing and stirring process disrupts the adhesion interface between the tissue and material.

Pluronic F-127

Properties

Another polymer that we investigated for its tissue adhesiveness was Pluronic F-127 (PF-127), or more generally, poloxamer 407 [10]. PF-127 is a triblock copolymer of repeating polyoxyethylene and polyoxypropylene subunits (Figure 4) [27]. Consequently, PF-127 is synthesized through condensation reactions between ethylene oxide and propylene oxide [27]. One of the most significant characteristics of PF-127 is that it is a thermoreversible polymer. Specifically, in aqueous solutions at low temperatures, a hydration layer surrounds PF-127 molecules thereby increasing solvation and hydrogen bonding [10]. But as the temperature rises, these hydrogen bonds break, resulting in the desolvation of the hydrophilic chains of the

copolymer [10]. Hydrophobic interactions among the polyoxypropylene domains take precedence leading to gel formation [10]. However, if the temperature cools, hydrogen bonds can again predominate, thereby solvating the chains back into solution [10]. Specifically, 20% (w/v) solutions and above have shown to undergo gellation at body temperature, or 37 °C [10, 19, 24, 27].

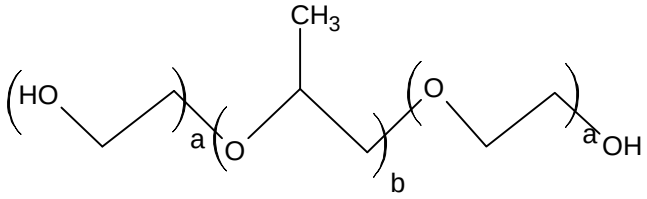

Figure 4*:* Chemical structure of Pluronic F-127 where the a-repeating subunits are polyoxyethylene domains and the b-repeating unit is a polyoxypropylene domain. Depiction was produced using ChemSketch software.

Applications

Due to its thermoreversible properties, PF-127 has been utilized in a variety of clinical and commercial applications. In fact, at high concentrations, PF-127 has been found to form aggregates consisting of a hydrophobic central core with hydrophilic polyoxyethylene chains facing the aqueous medium [10]. In this micelle-like configuration, PF-127 has been utilized as a carrier of low molecular mass pharmaceuticals and polypeptides through oral, topical, intranasal, rectal, ocular, and parenteral administration routes [10]. One of the first applications for which PF-127 was utilized was as a synthetic skin for burn treatments [27]. Solutions (20%) of PF-127 were prepared and poured onto the skin where the liquid polymerized to form a solid barrier. As the polymer is water-soluble, when the wound had healed or removal was desired, the layer could be simply washed away [27]. Additionally, PF-127 has been employed in a number of vascular applications, most notably as a temporary, material-based embolism during

vascular surgeries [19]. For this purpose, PF-127 is injected into the bloodstream to form a compact blockage. In this way, the material is used to impede flow or prevent dispersal of certain chemicals to the vascular beds [24].

Degradation

As a water-soluble polymer, even at physiologic temperatures PF-127 is subject to dissolution within the aqueous environment of the body as presented through the aforementioned applications. Specifically to its use as a material embolism, polymerized PF-127 has been found to degrade within 10-80 minutes after having been introduced into the blood stream [24]. Thus, even as a compact aggregate, mechanical perturbances within the aqueous environment have been found to degrade PF-127 through dissolution.

Advantages and Disadvantages

Given these properties, PF-127 presents multiple advantages and disadvantages for a foundation from which to design the material scaffold of interest. First, of the methods of polymerization considered, the thermogelling tendencies of PF-127 make it ideal for our application. For instance, if we could tailor a material to have a solution-gellation temperature very near 37 °C, the liquid solution could be mixed and stored in the clinical setting at room temperature and then upon injection into the body, would immediately polymerize at body temperature. This property presents the fastest and safest method of polymerization of the materials considered for the application. Additionally, PF-127 is a very simple polymer to make, as it only requires dissolving powdered PF-127 into cold deionized water. Thus, if the designed

material was to incorporate PF-127, this characteristic could prove advantageous for performing repetitive testing protocols and also if the material would be commercialized.

However, in comparison to some of the other polymers investigated, PF-127 is less stable in a polymerized form, thus presenting a significant disadvantage. Specifically, as the gellation of PF-127 takes place through hydrophobic interactions as opposed to stronger, more stable covalent cross-linking, the resulting polymer is only a semi-solid. While this characteristic may promote distention and dilation of the vein as it matures, it would not provide the initial support of the vessel wall required of the designed material. Thus, our investigation of the properties of PF-127 determined that while the polymer presents multiple advantages to our desired application, there are many disadvantages to its incorporation as well.

Poly(ethylene glycol) Diacrylate

Properties

Poly(ethylene glycol) diacrylate (PEG-DA) is a condensation polymer of ethylene oxide and water that is formed by a radical-initiated covalent crosslink of the polymer chains. Polymerization occurs in the presence of a photoinitiator, such as Irgacure-2959, and ultraviolet (UV) light [12]. The polymer is non-toxic, neutral, and nonirritating. PEG-DA viscosity is directed by the molecular weight of the polymer and ranges from liquid at less than 700 g/mol to a wax-like stabilizing solid at greater than 10,000 g/mol. PEG-DA also may be lipid or water soluble based on the chain length of the final polymer [15]. Thus, PEG-DA polymers can exhibit a wide range of physical properties to suit a variety of applications.

Applications

Due to the variation in solubility, PEG-DA has applications ranging from water-soluble lubricants, pharmaceuticals, and suppository bases to hydrophobic surfactants and detergents. PEG-DA is also commonly used as a crosslinking agent between polymer units and as a comonomer for the synthesis of plastics [32].

Degradation

The degradation properties of PEG-DA can be manipulated by adjusting the cross-linking, concentration, and copolymerization of the polymer [15]. In addition, PEG-DA stability is affected by the concentration of dissolved oxygen in the surrounding medium [22].

Advantages and Disadvantages

Advantages of PEG-DA include the ability to form a firm, strongly-adhesive polymer, be manipulated to exhibit a range of mechanical and physical properties, and be stored in the dark at -20 degrees Celsius for up to three months [30]. In addition, PEG-DA can be copolymerized with a variety of other molecules, allowing further manipulation of polymer's chemical nature. However, photoinitiation of the polymer would be difficult *in vivo* and would necessitate exposing patients to UV light radiation, which may cause oxidative DNA damage and cancer.

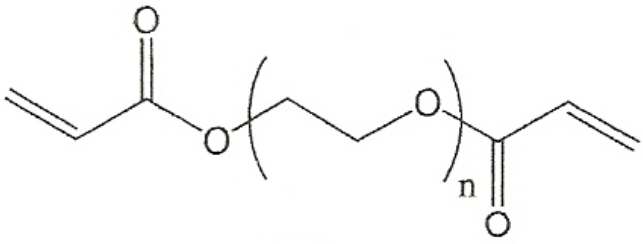

Figure 5: Molecular structure of PEG-DA (Masters).

Fibrin Gel

Properties

The last investigated material is fibrin gel (Figure 6). Thrombin initiates the polymerization of fibrin gel through the proteolytic cleavage of fibrinogen [2]. The active fibrin molecules then align noncovalently with each other in a half staggered fashion building up polymer strands [2]. Fibrin gel can be optimized by varying fibrinogen concentration, thrombin concentration and ionic strength. These modifications can generate gels with different appearance, mechanical properties and stability [11].

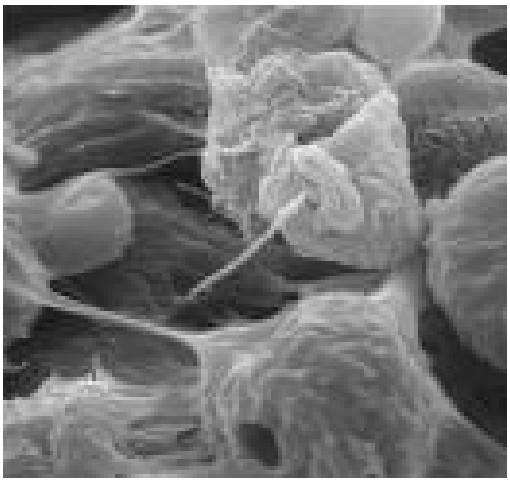

Figure 6: Photograph of fibrin (citation).

Application

In the field of tissue engineering, fibrin gel already has several applications. Peretti and colleagues demonstrated that when a formula of fibrin was injected into mice and swine models, it promoted cartilage formation [21]. Brown and his colleagues demonstrated that inflammatory cells, fibroblasts and endothelial cells will migrate into fibrin gel and organize it into connective tissue [4]. Fibrin gel has also been used as an adhesive agent, a vascular sealant in surgery and to transport cells [5, 11]. For example, Bruns and colleagues have been working on developing therapies for liver disease using fibrin gel to transplant cells. They seeded cells into a small amount of fibrin, which was injected directly in the liver of a rat model. By observing the explanted liver, they determined that culture in the fibrin gel allowed stable cell numbers and tissue formation [5].

Degradation

Fibrin gel is degraded by the fibrinolytic cascade [11]. In this process, the zymogen, plasminogen, is activated by plasma or tissue activators at fibrin's surface [8]. Plasmin is the active form that cleaves fibrin at multiple locations forming polymer fragments which are further degraded [8]. This is the same mechanism the body naturally uses to break down blood clots.

Advantages and Disadvantages

The body naturally uses thrombin and fibrinogen in the clotting cascade. Consequently, one advantage of fibrin gel is that it can be mediated by already existing biological processes. Additionally, fibrin gel can be produced from the patients own blood, and therefore is less likely to be recognized as a foreign body [13].

A disadvantage of fibrin gel is that it requires multiple polymerizing agents. Both thrombin and fibrinogen must be injected simultaneously for polymerization. This characteristic will become a challenge when designing the injection method of the material.

Testing

Adhesion Test

A series of tests was performed to determine how well each material meets the design criteria of adhering to the vein and of withstanding puncture with an 18G needle. To test adhesion, each material was polymerized between two sections of vessel tissue with approximately equal surface area. The tissue sections were fastened above and below, and tension was gradually increased until separation (Figure 7). The tension force was measured using a load cell and LabVIEW software, and a maximum force was recorded to relate the

adhesion strengths (Appendix B). The maximum forces recorded for alginate, fibrin gel, and PEG-DA were 0.044 N, 2.177 N, and 0.410 N, respectively. Pluronic F-127 does not appear on the plot because adhesion was not sufficient to withstand the testing protocol. The results of the adhesion test are plotted in Figure 8 and indicate that fibrin gel has outstanding adhesion strength among the materials tested.

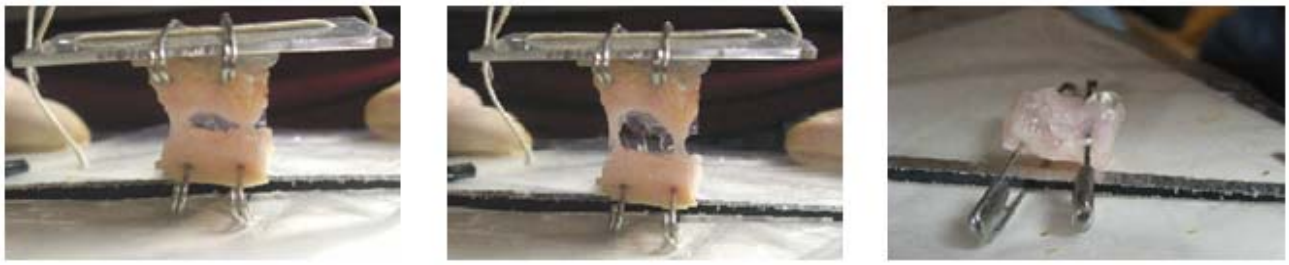

Figure 7: Adhesion test protocol of fastening the tissue sections, applying a gradually increasing tension force, and separating the tissue-material interface. Photos of fibrin gel.

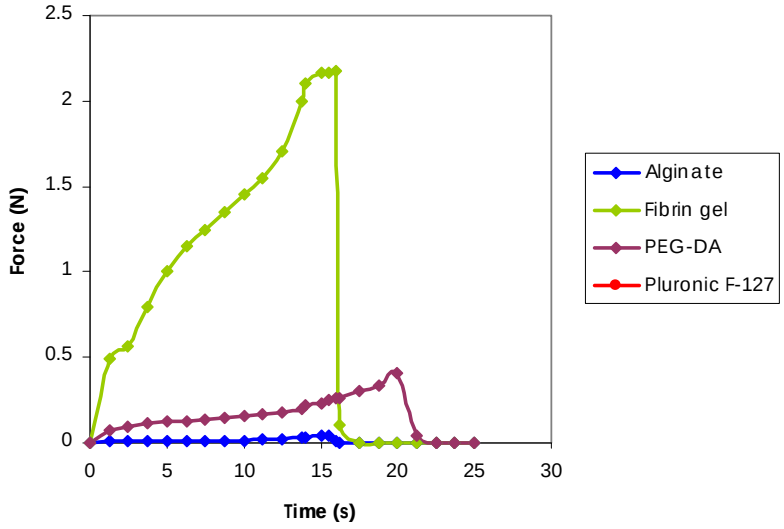

Figure 8: Relative strengths of the tissue-biomaterial adhesion for alginate, fibrin gel, PEG-DA, and Pluronic F-127 on bovine aorta tissue. Data obtained from a load cell and National Instruments LabVIEW.

Puncture Test

Ability to withstand puncture was tested by polymerizing each material on a vessel surface and puncturing each vessel with an 18G needle. Vessels were punctured slowly so the

tissue-material interface could be monitored throughout the test. The polymers of alginate and Pluronic F-127 responded to puncture by collapsing with the tissue and did not provide significant adhesion to the vessel surface (Figure 9). Both polymers were semi-solid and easily removed from the vessel. In contrast, the firm and thick polymers of fibrin gel and PEG-DA withstood puncture by minimizing vessel collapse and retained adhesion to the vessel tissue. The polymers were solid and polymerized in excess of what was necessary to coat the vessel, indicating that both materials may be candidates for tethering to tissues that surround vessels *in vivo.*

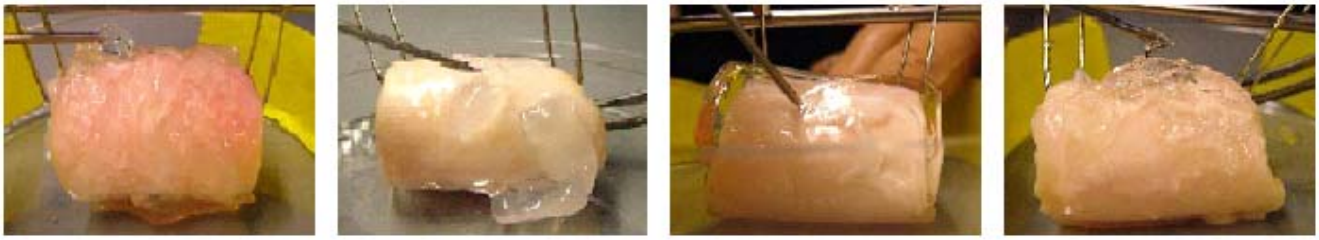

Figure 9: Response to puncture with an 18 G need of vessels coated in a thick layer of alginate, fibrin gel, PEG-DA, and Pluronic F-127.

Material Matrix

Having subjected the four polymers to *in vitro* tissue adhesion and puncture testing, we assessed their respective performances within a material matrix based upon the required material properties set forth by our clients (Appendix C). Specifically, each of the four materials was ranked for its adhesion to the tissue, the ease of its polymerization method, its elasticity in the context of its ability to allow distention and dilation of the maturing vessel, the quality with which it sealed after puncture, and whether the polymer was found to favorably degrade within the physiological environment. As the adhesion strength of the material is the most important characteristic for the overall success of the material, this criterion was ranked highest, with an

associated value of 0.50 out of 1.0. Method of polymerization and elasticity were each given a rank of 0.20 as they too are required of the optimal final product but are both factors that can be resolved if not already perfect for the application. Finally, self-sealing qualities and biodegradability were each given a value of 0.05 as the final material could be deemed successful without each of these characteristics but their incorporation would make the material ideal. With the criteria for ranking established, each polymer was assigned a value between 1 and 5 for the assessed performance in each category, with a 5 signifying that the material best met the indicated criterion.

Given its outstanding performance in each of the defined criteria, fibrin gel scored highest within the matrix with a value of 4.30 out of 5. Specifically, fibrin gel proved to possess the greatest tissue adhesion strength of the four polymers examined, earning it a score of 5 in this category. Also, the fact that fibrin gel can be polymerized simply by simultaneously injecting solutions of fibrinogen and thrombin without additional mixing, irradiation, or heat is a very favorable characteristic for the application, resulting in a score of 4 for the method of polymerization criterion. While both PF-127 and alginate proved to have greater elasticity than fibrin gel, the polymer proved adequate in allowing distention and dilation of the vessel during testing, and received a score of 3 for this property. Additionally, during the puncture test, fibrin gel was found to be very easy to puncture and closed around the site of puncture with removal of the needle, unlike alginate or PF-127 which both left the site of puncture exposed to the environment. Consequently, fibrin gel was given a score of 4 for this category. Finally, as fibrin is known to be degraded by the body through the enzymatic activity of the fibrinolytic cascade, we feel that this knowledge could be utilized to tailor our final material to degrade through similar mechanisms and the fact that fibrin gel holds this potential earned it a rank of 4 for the

category. Thus, for these reasons, we have decided to pursue the design of our final material using fibrin gel as a foundation.

Future Work

For the remainder of the semester we will be utilizing the knowledge gained through research and *in vitro* testing to optimize the properties of fibrin gel to best meet the criteria for the final AV fistula scaffold. Most notably, we must ensure that the final material, although composed from fibrinogen and thrombin, poses no risk to the patient with regard to thrombus formation or embolization. Additionally, we will modify the polymer to make it more pliable to changes within the vessel morphology without altering tissue adhesion strength or self-sealing capabilities. Finally, alterations allowing the fibrinogen and thrombin solutions to be mixed immediately prior to injection instead of requiring simultaneous injection would make polymerization of the polymer *in situ* much easier for the user and a more successful product overall.

Evaluation of all iterations made to the original fibrin gel formulation will be initially tested *in vitro* using the tissue adhesion strength and puncture testing protocols established during the first half of the semester. Then, materials that warrant more advanced testing will be examined for behavior using Dr. Chesler's pressurized flow chamber which offers a more indicative evaluation of the vasculature under physiological conditions. The final product will then undergo more sophisticated testing within a pig model system in which AV fistulas will be created using the femoral vessels. After the procedure, the material will be injected around the fistula as would occur in the actual application, with the fistula in the other limb left unsupported as a control. Observations of the required maturation period prior to use as well as any adverse

reactions would provide evidence for the overall success of the material as a support scaffold allowing for rapid use of AV fistulas. In this way, we hope to establish that the final material design meets the criteria outlined by our clients and provides a safe and effective means of supporting developing AV fistulas during the process of hemodialysis.

Ethical Considerations

The main ethical consideration we must consider when optimizing the characteristics of fibrin gel is safety. Fibrin gel has been shown to be biocompatible and, therefore can be safely injected outside the external surface of an AV fistula [11]. Animal tissue used in testing must be disposed of in an appropriate manner and proper protocols must be followed when performing tests of the material in a pig model system *in vivo*. Therefore, to successfully identify the appropriate material for an AV fistula scaffold, in addition to fulfilling all design criteria, we must evaluate the ethical consequences of our decisions that will result in a product that is safe for all involved in its application.

Conclusion

Vascular access points are the "Achilles heel of modern hemodialysis" [29]. Their failure can lead to death in patients who rely on their function. The AV fistula is currently the most reliable method used to access the blood vessels, as it has the least incidence of infection and thrombosis. Our goal is to identify a material that can support the AV fistula, allowing for an increase in success rate and more rapid use. The adhesion and ability to withstand puncture of alginate, Pluronic F-127, PEG-DA and fibrin glue were tested. As fibrin gel demonstrated the best results in both tests, we will work to optimize its properties as a potential AV fistula scaffolding material.

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Appendix A: Material Preparations

After multiple iterations, the following protocols describe the preparations utilized during final testing of the materials and produce polymers that we feel are indicative of their innate properties.

Alginate

1. Prepare a 2% (w/v) aqueous solution of alginic acid sodium salt. Rotate overnight to achieve complete dissolution [14, 31].

2. Mix alginic acid solution in 3:1 ratio with 100 mM $CaCl_2$ [33].

Pluronic F-127

1. Prepare a 70% (w/v) aqueous solution of powdered Pluronic F-127 [10, 19, 24]. Add to cold deionized water a few flakes at a time with stirring to allow gradual solvation [27].

PEG-DA

1. Place 10 grams of poly(ethylene glycol) (MW 8000) in 3-neck, round-bottom flask sitting in an ice bath.
2. Add 30 mL methylene chloride to flask.
3. Purge the flask with argon for 5 minutes.
4. Add 1.102 mL triethylamine (TEA).
5. In a funnel, combine 15 mL MeCl2 with 1 mL acryloyl chloride.
6. Very slowly, drip the acryloyl chloride solution into the PEG/MeCl2/TEA solution.
7. Let this drip for 45 to 60 minutes, then cap the flask and place on stir plate in ice overnight [6].
8. Precipitate the product in ethyl ether. Filter, then leave in hood overnight to evaporate ether.
9. Lyophilize product.
10. With dried product, create 20% (w/v) solution in PBS.
11. Add 1% (w/v) aqueous Irgacure-2959 photoinitiator to PEG-DA solution in 1:5 ratio [28].
12. Place solution under UV light to polymerize.

Fibrin Gel

1. Dissolve fibrinogen in 0.9% (w/v) saline at concentration of 0.11 g/mL.
2. Prepare thrombin solution of 5 mg/mL thrombin diluted 1:20 with 40 mM $CaCl_2$.
3. Mix fibrinogen and diluted thrombin solutions in 1:1 ratio. Gel forms within seconds [9, 11].

Appendix B: LabVIEW Plots of Adhesion Testing

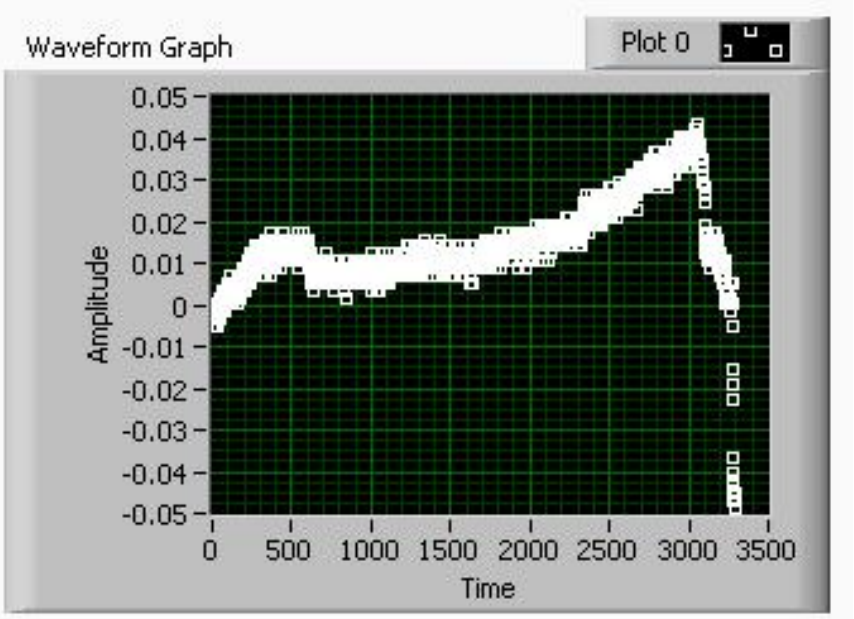

Figure 10: Plot of force applied to the tissue-alginate interface until separation occurred at the max value (0.044 N). Plot obtained from a load cell and National Instruments LabVIEW. Time displayed in iterations with 200 Hz data acquisition.

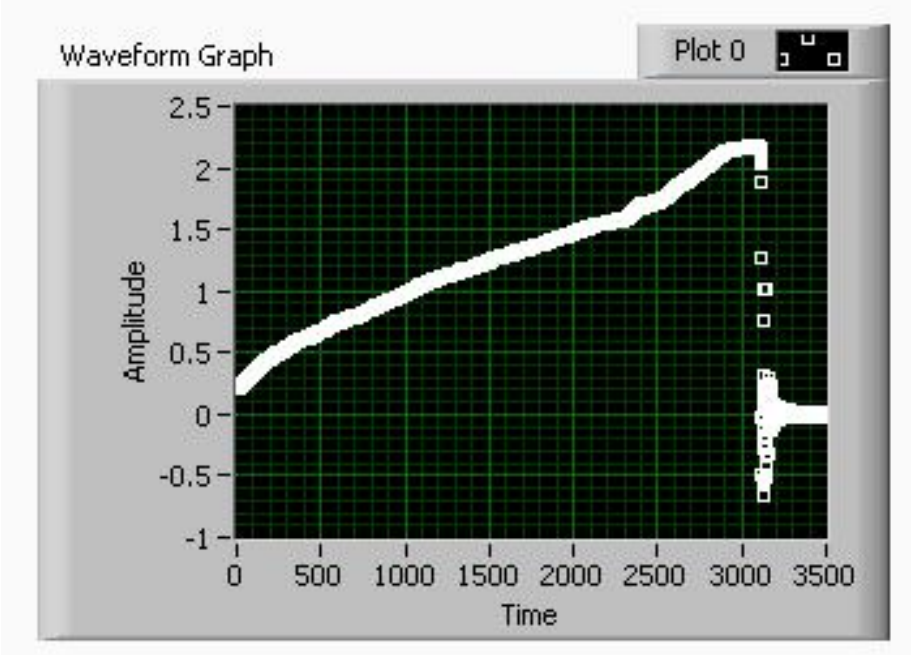

Figure 11: Plot of force applied to the tissue-fibrin gel interface until separation occurred at the max value (2.18 N). Plot obtained from a load cell and National Instruments LabVIEW. Time displayed in iterations with 200 Hz data acquisition.

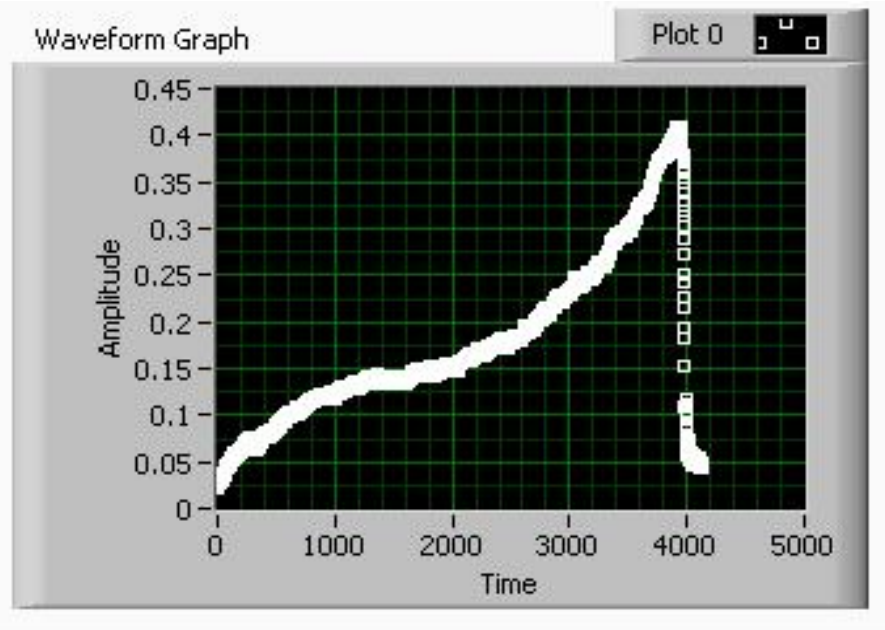

Figure 12: Plot of force applied to the tissue-PEG-DA interface until separation occurred at the max value (0.410 N). Plot obtained from a load cell and National Instruments LabVIEW. Time displayed in iterations with 200 Hz data acquisition.

Appendix C: Material Matrix

Criterion	Rank	Materials			
		Alginate	Fibrin Gel	PEG-DA	Pluronic F-127
Adhesiveness	0.50	2	5	4	1
Polymerization Method	0.20	2	4	1	5
Elasticity	0.20	4	3	3	4
Self-Sealing	0.05	1	4	4	1
Biodegradable	0.05	3	4	3	2
Totals	1.0	2.40	4.30	3.15	2.45

Appendix D: Product Design Specifications

External Scaffolding for Rapid Use of Arterio-venous Fistulas

Product Design Specification

Last Updated: February 13, 2008

Team Members:

- Kellen Sheedy, Leader
- Holly Liske, Communications
- Karen Chen, BWIG
- Laura Piechura, BSAC

Function:

Patients who undergo hemodialysis often require the placement of an arteriovenous (AV) fistula, a surgical connection between an artery and a vein typically in the arm. Currently, AV fistulas are successful only 45% of the time that they are surgically inserted. If the fistula does mature, it serves as an access point for the large hemodialysis needles. However, maturation can take 1-3 months and during this time the patient must rely on less ideal access points, such as a catheter, for hemodialysis. Our clients believe that external scaffolding to prevent fistula collapse during dialysis will enable more rapid use of AV fistulas in dialysis patients. We will work to design an external scaffolding of appropriate biomaterials that will adhere to the outer surface of the vein and tether it to the surrounding tissue, thereby supporting the vessel as it matures while making it immediately available for use in hemodialysis.

Client Requirements

- The designed material must adhere to the outer surface of the vasculature and tether to surrounding structures.
- The material must be injectable into the tissue surrounding the fistula.
- The material must be polymerizable *in situ.*
- The material must support the vessel during canulation without separation of the scaffolding and vascular wall.

Design Requirements:

I. Physical and Operational Characteristics

a. *Performance requirements:* To provide for more rapid use of arteriovenous fistulas, the designed biomaterial must adhere to the outer surface of the vascular connection to thicken the wall of the developing vein while allowing for natural maturation. To avoid additional surgery for the patient, the material should be injectable into the tissue surrounding the fistula and then polymerizable *in situ*. Once polymerized, the material should support the vessel without losing contact with the vasculature during insertion of the hemodialysis needles and self-sealing upon removal. Additionally, it would be ideal if the material biodegraded at a rate similar to the development of the arterialized vein.

b. *Safety:* This material is to be adhered outside the venous wall in the human arm, therefore, it is critical that the materials used in its design do not cause harm to the body. Testing in a pig model and FDA approval are necessary prior to use in humans.

c. *Accuracy and Reliability:* The material needs to be able to be localized to a specific area on the exterior of a vein. Ideally, the material will improve the success rate of AV fistulas from 45% to 100%.

d. *Life in Service:* Currently, arterio-venous (AV) fistulas take 1 to 3 months to mature. Ideally, the scaffold will biodegrade at the same rate the fistula develops and, therefore, will last between 1 to 3 months.

e. *Shelf Life:* The material needs to remain in a liquid form long enough for a surgeon to inject the material into the arm after connecting the vein and artery.

f. *Operating Environment*: The material will be made outside the body and will be injected into the human arm where it will polymerize and remain for the rest of its life in service.

g. *Ergonomics:* The material should have good fluidity so that it encapsulates the desired tissue (vein) before it polymerizes. This lowers the chance of creating the scaffold around the undesired region on the patient, which could potentially cause further problems. Moreover, the encapsulation material should not cause pressure or stress on the patient's operation area. The injected material should not be felt by the patient even when suppressing the scaffolding location.

h. *Size:* The size of the material after being applied to the patient should be exactly encapsulating the vein of the patient. This should be no more than 2mm in diameter for the vein on the forearm. The length of the material encapsulating the vein may vary from patient to patient due to arm length differences. The size of the pre-polymerized material container should be approximately 5~10ml.

i. *Weight:* After the material encapsulates the vein, the weight of this material should not be felt or sensed by the patient. Ideally, it will be a weightless membrane that wraps around the vein.

j. *Materials:* Material that does not adhere to a vessel, stiffens too quickly, has no elasticity, is not self-sealing, and/or has no strengthening properties should be avoided. A gel-like polymerizable material is preferred. Materials such as Pluronics F127, Alginate, and PEG-diacrylate are potentially suitable materials. Thermal gelling (gelling by body temperature) is also a characteristic to consider.

k. *Aesthetics:* The material itself does not have to be aesthetically pleasing, but the finished scaffold should not bulge out from the skin surface (the scaffold should not be visible from the external skin surface).

II. Production Characteristics

a. *Quantity*: Design of a single material is required for proof-of-concept. Material production must be conducive to widespread use in patient treatment.

b. *Target Product Cost*: Material production must be cost- and resource-efficient to allow for widespread use. Cost minimization would increase the accessibility of this treatment to hospitals and patients.

III. Miscellaneous

a. *Standards and Specifications*: FDA approval of this class III medical device would be required, as the material is intended to support life and failure would be life threatening. As a medical device, the material must be manufactured under a quality assurance program, be suitable for the intended use, be adequately packaged and properly labeled, and have establishment registration and device listing forms on file with the FDA.

b. *Customer*: Ease of use is required so that health professionals may effectively use the material.

c. *Patient-related concerns*: The material must not present danger, discomfort, or additional inconvenience neither when the material is surgically placed in the body nor when the patient is undergoing hemodialysis.

d. *Competition*: Numerous patents exist to improve the implementation of arterio-venous fistulas. However, these patents focus primarily on the design of a stent for implantation into the lumen of canulated veins. No other attempt to provide external biomaterial support to the canulated vein is known to exist. Thus, the approach of this project is believed to be novel.