2015

Burst Pressure Properties and Ex Vivo Analysis of Alginate-Based Hydrogels for Tissue Sealant Applications

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BURST PRESSURE PROPERTIES AND EX VIVO ANALYSIS OF ALGINATE-BASED HYDROGELS FOR TISSUE SEALANT APPLICATIONS

A Thesis Presented

by

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to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements
for the Degree of Master of Science
Specializing in Mechanical Engineering

October, 2015

Defense Date: August 25, 2015
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ABSTRACT

Lung diseases, cancers, and trauma can result in injury to the connective tissue lining the lung, i.e., the pleura. Pleural injuries lead to pneumothoraxes or pleural effusions, i.e., air or fluid leaking out of the lung respectively, and potential lung collapse - an immediately life threatening condition. While several bioengineered soft tissue sealants exist on the market, there is only one sealant FDA-approved for use in pulmonary surgery. In addition, very limited techniques are presented in the literature for characterizing the burst properties of hydrogel tissue sealants. For my thesis, I proposed to develop a protocol for characterizing the burst properties of hydrogel sealants using a novel burst pressure test chamber. I further proposed a novel combination of oxidation and methacrylation reactions of alginate for tissue sealant applications, with a particular focus on developing a pulmonary sealant. The proposed research objectives are: 1) To develop protocol for testing hydrogel sealants for soft tissue applications; 2) To verify alginate as a potential for tissue sealant applications; and 3) To optimize an alginate hydrogel sealant and perform *ex vivo* analysis for a pleural sealant application. Alginate materials with varying degrees of oxidation and methacrylation were synthesized and characterized. Oscillatory rheometry was used to characterize material properties such as viscosity, hydrogel gelation kinetics, and complex moduli. Burst pressure measurements properties and failure mechanisms, i.e. delamination or material failure, were collected for a liquid and dry-state application. Preliminary *ex vivo* mouse lung model testing demonstrated that methacrylated alginate hydrogels are able to withstand physiological pressures associated with breathing, and failure occurs within the hydrogel for adhesive alginate-based tissue sealants.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Prof. Rachael Oldinski, for her continuous guidance, time, and support throughout my graduate career. Thank you for always understanding the stresses of school and having my wellbeing in mind, but most of all for pushing me to do the best work possible. You are an inspiration for the entire EBRL.

A sincere thank you to Prof. Daniel Weiss for being invested in me since the beginning and for your guidance through my research pursuits and thesis. I would also like to thank the other members of my committee, Prof. Yves Dubief and Prof. Patrick Lee, for their time, feedback, and suggestions.

Many people have provided ceaseless assistance and support over the past two years. Thank you EBRL members, especially Spencer and Tianxin, for teaching me how to perform solid research, for your help along the way, and for always making the laboratory a fun place to be. Thank you to our collaborators in the Weiss lab, especially Darcy, Amy, and Franzi, who have been instrumental in my research. A special thanks to Keara, Nirav, and Minara for your help with *ex vivo* testing.

Lastly, there is no way to adequately express my unending gratitude for the love and support my parents and family have given me over the years. You have always been there for me, always encouraged me to do my best, and always loved me regardless of the results. Thank you.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER 1: MOTIVATION

1.1. Introduction

The lungs are a complex system of airways and alveoli, which permit for the gas exchange vital to life. The average person breathes 12-20 breathes per minute, which equates to roughly 21,000 cyclic loads per day or 550,000,000 cyclic loads over the life of the lungs.[1] During respiration, the diaphragm muscles helps expand the chest cavity, causing a pressure difference in the cavity and allowing for air flow into and expansion of the lungs.[2] As with any system that sees repeated cyclic loading within a confined space, minimized friction is critical for optimal function. This is accomplished in the chest cavity via the visceral and parietal pleurae, which line the surfaces of the lung and chest cavity respectively.[2] These pleurae secrete small amounts of pleural fluid, which forms a slippery coating between the thoracic cavity and the lungs, providing lubrication during respiration.[2] The pleura are also responsible for preventing fluids, such as air, lung surfactant, mucus, etc., from leaking out of the lungs and into the pleural cavity surrounding the lungs.[3]

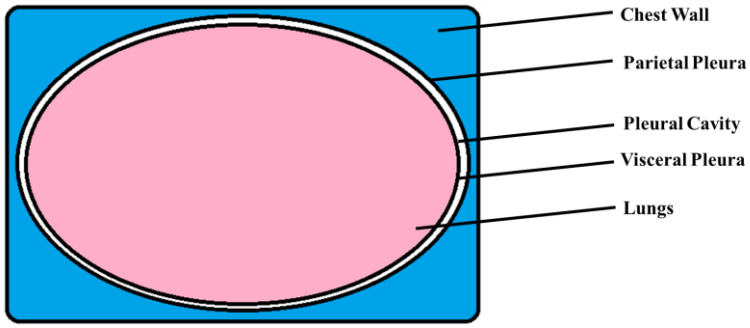

Figure 1. Simplified schematic showing lungs and pleurae within the chest

Each year, over one million people develop diseases which result in air (pneumothorax) or liquid (pleural effusions) leaking out of the lung and into the pleural space.[4] These pleural diseases can be the result of a variety of diseases, such as congestive heart failure, pneumonia, and lung cancer, or of physical trauma to the lung, such as a puncture or tear.[4] The resulting lung collapse can be immediately life threatening and requires immediate intervention. A thoracentesis must be performed to remove the trapped fluid and allow for the lung to expand.[5] This is done by placing a needle or tube in the pleural cavity to drain out the air or liquid. Depending on the cause of the leak, chronic leaking, also known as a bronchopleural fistula, may occur and repeated chest tube drainage may be necessary.[6, 7]

Several different methods have been developed to treat these chronic injuries in the lung pleura. These methods include pleurodesis (chemical or physical),[8-13] natural[14-25] and synthetic polymer sealants.[26-38] These treatments vary in physical characteristics and mechanical properties, which alters efficacy, duration of treatment, and invasiveness as pulmonary sealants.

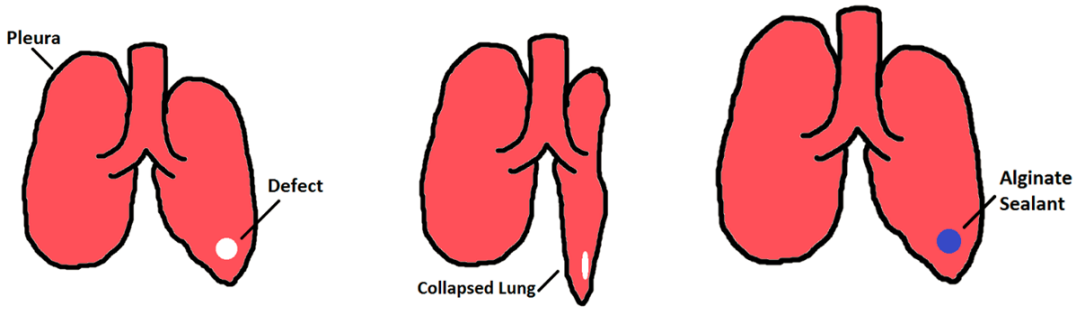

Figure 2: Cartoon schematic of proposed study

1.2. Goal of Study

Engineered tissue adhesives and surgical sealants are becoming more prevalent in surgical suites as current medical and robotic technologies improve, simplifying the procedures of complex internal surgeries. As these technologies advance, conventional methods of sealing tissues, such as staples and sutures, become more cumbersome and time-consuming.[39] Currently there are very few effective treatments for persistent pneumothoraxes and pleural effusions and only one FDA-approved pulmonary surgical sealant to prevent recurring leaks as the underlying tissue is repaired. The overall goal of this study is to investigate the potential use of alginate, an algae-derived biopolymer, as a surgical sealant in pulmonary applications. Various chemical modifications will be addressed for modifying the mechanical properties of alginate to serve as an artificial pleura biomaterial. Alginate hydrogel solutions will be mechanically characterized through oscillatory rheology. Protocols will be developed for characterizing the burst properties of the resultant alginate hydrogels on a custom-fabricated burst pressure device, modified from ASTM F2392-04R10, the standard test method for burst strength of surgical sealants.[40] Finally, *ex vivo* mouse model testing will be performed to test the efficacy of the alginate material as a pulmonary sealant.

CHAPTER 2: Background and Literature Review

2.1. Pleurodesis

In the 1990's pleurodesis thoracostomy was the current, and most commonly practiced treatment for pneumothorax and pleural effusion to prevent further leakage into the pleural cavity after the initial removal of fluid.[11] "The aim of pleurodesis is to achieve a symphysis between visceral and parietal pleural layers, in order to prevent accumulation of either air or fluid in the pleural space". [12] Physical pleurodesis is a surgical technique involving suturing or pleural abrasion procedures through the use of thoracotomy, which involves creating an incision into the pleural space and using advanced video-assisted thoracic surgery (VATS). VATS enables a small video camera to be put into the chest cavity, giving the surgeon more visibility during surgery and allowing for a much safer procedure.[13] Once inside the chest cavity, the hole can be sutured shut via a staple or pleural abrasion can be done. Pleural abrasion involves irritating the parietal pleura using a domestic nylon scouring pad or gauze. Due to this inflammation, the visceral and parietal pleurae become adhered to each other, sealing the leak site.[9] Chan et al studied 88 video-assisted thoracoscopic pleurodesis surgeries from 1992 to 1998, where the parietal pleura was abraded with a piece of Marlex mesh scratch pad.[41] Of those 88 there were only five recurrences (5.7%).[13] With most patients under general anesthesia the pain levels remained fairly low, 64 cases reported a 0 pain level (72.7%).[13]

Chemical pleurodesis consists of applying a chemical solution on the lung wall via a chest tube to cultivate an inflammatory response, adhering the pleurae and preventing leakage. Several different chemicals have been used to induce this

inflammation based on availability, cost, efficiency, effectiveness and safety.[11] A concentrated tetracycline solution, which causes an effective seal, was commonly used in early trials of chemical pleurodesis.[8] Parenteral tetracycline, however, is no longer commercially available in the U.S. due to a shortage, which led to a search for a substitute.[10, 11] Currently doxycycline, bleomycin, and talc remain as reliable and generally effective chemical agents and are of the most commonly used. [11]Doxycycline was an immediate replacement for Tetracycline, but was quickly replaced since multiple treatments were required for effectiveness, which is less than optimal.[11] Bleomycin, an antibiotic that binds DNA producing breakage and inhibits DNA synthesis, was shown to be much more efficient, requiring less treatments than that of doxycycline.[11] Bleomycin is widely used because of its efficiency and sclerosing properties for pleurodesis, however, some patients experience fatal alveolar injury due to the toxicity of the drug.[11] Zimmer et al. shows that bleomycin reduces pain and difficulty of breathing (dyspnea) but the overall success rate of treatment still remains around 70%.[11] Talc, a tri-layered magnesium sheet silicate that is readily available, efficient, and cost effective, is more commonly preferred in today's treatments because of a high success rate.[10] Talc is applied either using a dry heat exposure as a poudrage (powder or dusting) or as a slurry via thoracostomy. Both methods and compositions are effective in the control of malignant pleural effusion.[11] Not only does talc have high success rates, but the patient's difficulty breathing or dyspnea reduces the most compared with previous chemical treatments. Although chemical pleurodesis has proven to have high success rates, the procedure is intrusive and may have many negative short term effects, including fever, pain, infection, and respiratory failure.[10]

2.2. Natural Polymeric Tissue Sealant Approaches

Hydrogels have been used in a widening variety of biomedical applications. Due to their tunable mechanical properties through polymeric chemistry, much interest has been shown in developing specialized hydrogel biomaterials for specific clinical use. While the application of hydrogels as surgical sealants and wound treatment began developing in the 1960's, the use of hydrogels to treat pulmonary air leaks began in earnest in the late 1980's[14]. Early hydrogels used for pulmonary seals were composed of naturally-derived polymers (collagen, fibrin), but many other naturally- and synthetically-derived polymers have been investigated for surgical sealant applications as our understanding of hydrogels increases.[15, 16]

Starting in the late 1990's, fibrin began to be studied as a natural-based polymer sealant. Fibrin sealant is a haemostatic and wound support product consisting of blood coagulation factors fibrinogen, factor XIII, thrombin, aprotinin and calcium chloride. This mixture gives rise to the formation of a stable, cross-linked fibrin clot. Granulation tissue, new vascular tissue on the healing surface of a wound, can be seen 3 days after implementation and proliferation of collagen fibers after 4-7 days.[17] Studies have shown that the clotting time is dependent on the concentration of thrombin in the sealant. For thoracic and vascular surgical procedures it is common practice to perform suturing prior to applying the fibrin solution. In a single-blind study, i.e. a study where the researcher but not the subjects know the makeup of test and control groups, comparing suturing and suturing with fibrin sealant, the length of stay in the hospital was one day less in patients who received treatment for a variety of anastomoses with fibrin sealant than those who

did not. A reduction in the incidence of pulmonary (or bronchopleural) air leakage of 41% was associated with the use of fibrin sealant.[17] In 2002, Fabian et al. discovered a better application method for surgical applications using an aerosolized fibrin glue rather than the topical application. In a randomized, single-blind study, Fabian et al. discovered a 50% reduction of alveolar air leaks after pulmonary resection with the use of aerosolized fibrin glue (HemaMyst® System) and standard stapling procedures compared to just standard stapling procedures.[18] The preparation of glue components only requires roughly 5 minutes and another minute to apply the glue. Best of all it is relatively inexpensive ranging from $70 and $90 per cc.[18] The fibrin glue Tisseel®, is FDA approved and is used in many applications.[19] Despite this, fibrin glue has failed to demonstrate efficacy for pulmonary applications in several small clinical trials.[18]

Another sealant that is being used is chitosan, which is derived from chitin. Chitosan has many application is due to its biocompatibility, low toxicity, structure similarity to glycosaminoglycan's, and its ability to degrade with certain enzymes.[16] Ono et al. reported a bursting pressure of 51± 11 mmHg (69±15 cmH_2O).[20] This study suggests that modified chitosan can form a more effective seal in aortic, tracheal, and pulmonary applications, but is still being researched.

There are a few applications of multiple sealants being used. TachoComb®, discovered by Matsutani et al., is a fibrin-glue coated collagen fleece that is able to conform in shape to the pleural defect that it is applied to and withstand high pressures (greater than 40 cmH2O). TachoComb® is combined with overlapping the defect with the surrounding normal pleural tissue.[21] An issue with TachoComb® is when applying the material to a pleural defect it is difficult to keep it free of blood/fluid interaction

before implementing to the site. When the material comes in contact with blood it almost instantly becomes very sticky and difficult to manipulate. Nakajima et al. discovered an innovative design to deliver the TachoComb® via a rubber tube, then conveying it into the application site and applying it using standard laparoscopic forceps.[22] This allows the TachoComb® to remain free of blood/fluid before it reaches its destination and allows TachoComb® to be used in minimally invasive surgery. TachoComb® did not receive FDA approval due to the use of bovine thrombin and aprotinin.

One concern with fibrin is how it can possibly transmit blood-borne diseases. With this concern in mind, a new product has evolved called Integran. Integran is a sheet type absorbable topical collagen hemostat which contains no blood-derived products. On average Integran cut the number of air leaks by a third after application compared to TachoComb® .[23]

Another natural polymer-based sealant involves the reaction of bovine serum albumin (BSA) and glutaraldehyde, which covalently bond to form a flexible seal upon mixing.[24, 25] This reaction is used in the commercially available BioGlue®. The BSA and glutaraldehyde solutions come in a dual syringe applicator, which mixes the solution as they are applied. The FDA reported that BioGlue® grafts withheld pressures of 300 mmHg.[26] This far exceeds the normal physiological pulmonary pressure associated with respiration of 30 cmH_2O.[1, 3]

2.3. Synthetic Polymeric Tissue Sealant Approaches

Due to its biocompatibility, low toxicity, and FDA approval, poly(ethylene glycol) (PEG), also known as poly(ethylene oxide) (PEO) or poly(oxyethylene) (POE)

depending on the molecular weight, is the primary synthetic polymer used in repairing pulmonary and alveolar air leaks, although there is interest in a surgical sealant based on poly(lactic-co-glycolic acid) (PLGA).[16, 27-31] One of the first PEG-based pulmonary seals utilized a two-solution (primer-sealant) method with acrylate-capped poly(L-lactide) and poly(trimethylene carbonate) and a photoinitator, Eosin-Y, to form a sealant.[32-34] Upon exposure of the photoinitiator to blue-green light, the primer and sealant polymerize to form a crosslinked, flexible hydrogel network.[33, 42] Macchiarini et al. reported a polymerization time of 40 seconds.[32] While this sealant is able to withstand pressures exceeding peak pulmonary pressure, the application method requires intraoperative photoinitation, which can be complicated.[34] This PEG- based sealant is commercially available in Europe and Canada as FocalSeal® and was approved by the FDA in 2000.

To avoid photoinitation, several two-solution, PEG-based surgical sealants were developed which begin to polymerize after the two solutions are mixed.[28, 35, 41] The earlier of these sealants consisted of two basic components: PEG disuccinimidyl succinate (PEG-SS2), a PEG derivative, and human serum albumin (HSA), a protein naturally produced by the liver.[28] The two solutions are mixed at the time of use and delivered through a specialized dual-syringe system. Kobayashi et al. reported rapid chemical crosslinking between the PEG-SS2 and HSA, forming a hydrogel within 15 seconds that can withstand twice normal peak pulmonary pressure.[28] A major drawback to this hydrogel is that HSA is derived from blood plasma, which presents control and disease problems. Commercially available Progel® utilizes this PEG-SS2 HSA chemistry. A second PEG-based, two solution sealant consists of dilute PEG- hydrogen chloride and PEG- sodium phosphate/sodium carbonate solutions, which come in a preassembled, dual

syringe applicator[36]. Campbell et al. reported a polymerization time of less than three seconds after mixing, while Wallace et al. reported a burst strength five times greater than peak pulmonary pressure.[29, 36] This dual PEG-based-solution treatment is available in Europe, but not the United States, commercially as CoSeal®. A third two-solution surgical sealant utilizes PEG and trilysine, an essential amino acid, to form a hydrogel. Similar to CoSeal® and Progel® , the PEG and trilysine solutions are mixed upon application and crosslink within seconds to form a flexible hydrogel.[35] Pederson et al. reported a median bursting strength of 35 cmH_2O, which is ablove normal PPP. This technology was introduced as a surgical sealant in Europe as PleuraSeal®, but never obtained FDA approval and later underwent a voluntary recall.

A final synthetic polymer sealant currently being tested as a surgical sealant contrasts with the previously mentioned sealants due to a lack of liquid application, greatly decreasing the difficulty of application.[31] This patch sealant is composed of PLGA-Poly(VP-AAc-AAc(NHS)) and adheres to the lung after application under moderate pressure through rapidly formed amide bonds.[37] Shea reported a bursting strength of 140.6±58.2 cmH_2O.[38]

2.4 Alginate

Alginate is a naturally-derived polysaccharide polymer composed of repeating (1,4)-linked β-D-mannuronate (M) and α-L-guluronate (G) mers.[43] (Figure 2Figure 3) Alginate, derived from brown alga, is a popular polymer in the food, cosmetic, and bioengineering fields due to its low-cost, abundant source, and biocompatible nature.[16, 43, 44] As such, there are many commercially available alginates with a wide range of

characteristics and mechanical properties for use in varying applications. Alginate is popular among bioengineers due to its highly tunable physical properties and various crosslinking methods.[44-48] Composition, block length, and molecular weight are three characteristics which, when altered, will drastically change the mechanical properties of the resulting alginate hydrogel.[43] Alginate derived from various brown algae will have varying compositions of mannuronate and guluronate building blocks, which affects the overall structure of the polymer. The repetition of these building blocks to form consecutive G residues, consecutive M residues, and alternating M-G residues, and the length of these residues, also greatly affects the properties of these polymers in different applications.[43] For example, guluronate blocks drive intermolecular cross-linking via divalent cations. Increasing molecular weight changes the physical propertes of the resulting gel, i.e. increased loading, stiffness, and moduli, while also increasing viscosity. Longer chains are able to entangle and interact more readily than shorter chains, leading to these increased properties. Addition of functional side groups and modifications to the alginate backbone also provides an opportunity to tune properties for specific applications.[47, 48] These chemical modifications also allows alginate to be crosslinked in a variety of manners. Typically alginate can be ionically crosslinked, however the addition of functional side groups can allow for covalent, thermal, and improved physical crosslinking. The research presented below is based on Manugel GMB alginate (FMC Biopolymer), which has a high G block content and relatively high molecular weight (80-120 kDa). Manugel GMB was chosen because the high G:M ratio allows for effective ionic crosslinking via a calcium chloride reaction and the high molecular weight will promote physical entanglement, which may allow for increased elasticity in the resultant

hydrogel.

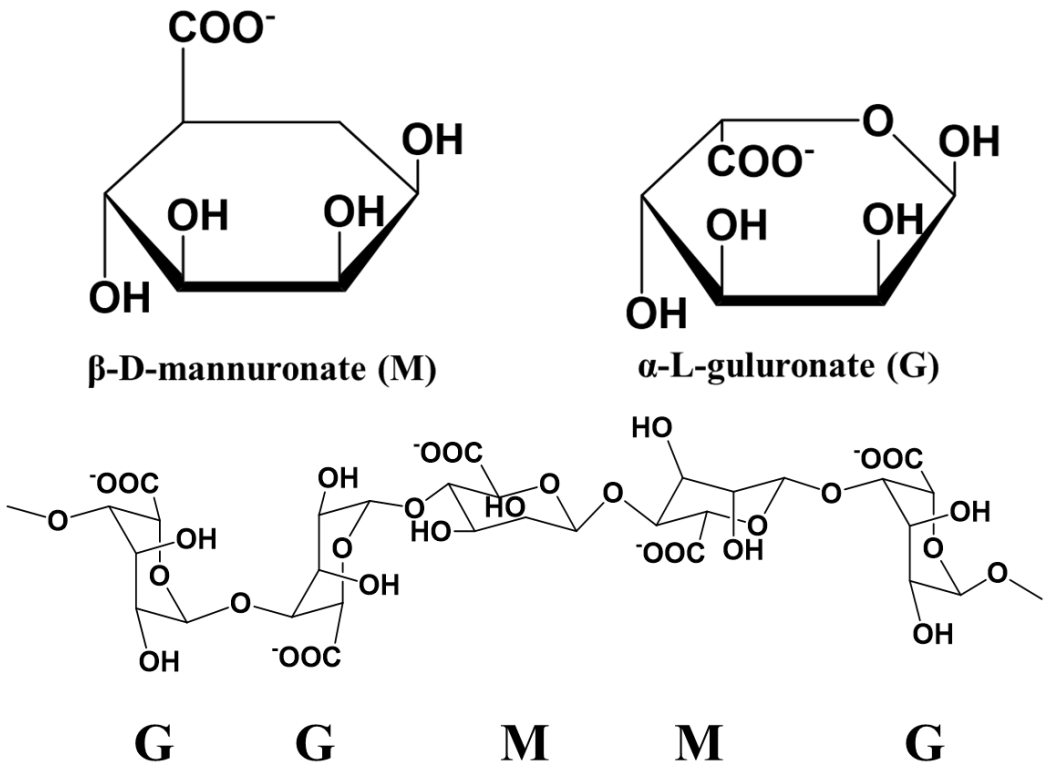

Figure 3: Top- Chemical structure of alginate repeat units; Bottom- alginate chain depicting short G-G, M-M, and M-G residues

2.4.1 Alginate Chemistry

Several groups have investigated chemical modification of alginate for a variety of bioengineering applications.[43, 45, 47] Jeon et al. reported methacrylating alginate via 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride and N-hydroxysuccinimide, resulting in a photocrosslinkable alginate for therapeutic materials.[47] Skardal et al. reported a means of methacrylating hyaluronan, another commonly used polysaccharide biopolymer, utilizing methacrylic anhydride in deionized water.[49] Lee et al. also explored other means of crosslinking alginate. This group was able to covalently crosslink alginate with poly(ethylene glycol)-diamines.[43] Besides

these means of covalently crosslinking alginate, there are several other means for alginate gelation, such as thermal cross-linking, though these means fall outside the scope of this study.[43] Another chemical modification that has been explored in alginate chemistry is oxidation. Several groups have used oxidation as a means of controlling the degradation properties, i.e. rate of degradation, particle size, etc., of alginate.[46, 48] Gomez et al. and Jeon et al. both present slightly varying methods of oxidation via sodium periodate, though many oxidizing agents exist. As this method seemed compatible with alginate, sodium periodate oxidation was chosen as the desired method.

2.5 Mechanical Testing of Tissue Sealants and Adhesives

A literature review results in few cases of mechanical burst pressure testing of soft hydrogel sealants. The American Society for Testing and Materials (ASTM) is the best source of information on testing procedures for a wide range of materials. ASTM F2392 Standard Test Method for Burst Strength of Surgical Sealants provided the basic design for a burst pressure device.[40] The protocol didn't allow for testing of hydrogel sealants with *in situ* photocrosslinking and was modified accordingly. Several other standards provided information on collecting adhesive strength, but the load application didn't reflect pulmonary conditions and were not pursued.[50-52]

CHAPTER 3: ALGINATE CHEMISTRY

3.1. Introduction

As was mentioned above, the mechanical and physical properties of alginate can be changed via chemical modification. This can be done through the addition of functional side groups or by modifying the backbone of the alginate itself. Care must be taken when modifying any polymer, as the chemical modifications can have unintended repercussions, such as chain scission. This study focuses on the addition of a functional methacrylate side group and open-chain adduct conformation to tune properties for a pulmonary sealant application.

3.2. Methacrylation Chemistry

In order to form an effective pulmonary hydrogel sealant, a safe and effective means of crosslinking alginate into a hydrogel must first be addressed. Commercially available sodium alginate can be crosslinked to form a hydrogel upon exposure to calcium chloride, forming ionic bonds between the alginate chains. Unfortunately, the resulting hydrogel is relatively weak, as the ionic bonds can be easily broken, and stiff, not allowing the sealant to flex with the lung. Alternatively, the addition of functional methacrylate side groups onto the alginate backbone allows for covalent bonding between chains, forming a more durable hydrogel. Several methods have been developed in order to methacrylate alginate to varying degrees. Control over the reaction is paramount, as the degree of methacrylation will influence the final properties of the hydrogel.

3.2.1. Aqueous Method

The first method investigated as a means of methacrylation was an aqueous-based reaction utilizing methacrylic anhydride (Sigma Aldrich). A 20-molar excess of methacrylic anhydride is added to a 1% (w/v) sodium alginate solution.[49] (Figure 4) The resulting solution is treated with sodium hydroxide to maintain a solution pH of 8.5 and allowed to stir for 12 hours. The solution is then further treated with sodium hydroxide to a pH of 7.0 and dialyzed with deionized water for 3 days, adjusting the pH periodically. The final solution is lyophilized and stored until testing and characterization. Since methacrylic anhydride is poorly soluble in water, a 20-molar excess is required to ensure the reaction occurs. This poor solubility and molar excess limits control over the methacrylation reaction and therefore alginate methacrylated in this manner has a theoretical modification of 100%, though full modification is seldom obtained. Sodium alginate can be replaced with oxidized sodium alginate if oxidized methacrylated alginate is desired. Proton nuclear magnetic resonance spectroscopy was utilized to determine the degree of methacrylation, or ratio of methacrylate groups per repeat unit of alginate. This was accomplished by calculating ratios of relative integrations of the methacrylate peaks and alginate methyl proton.

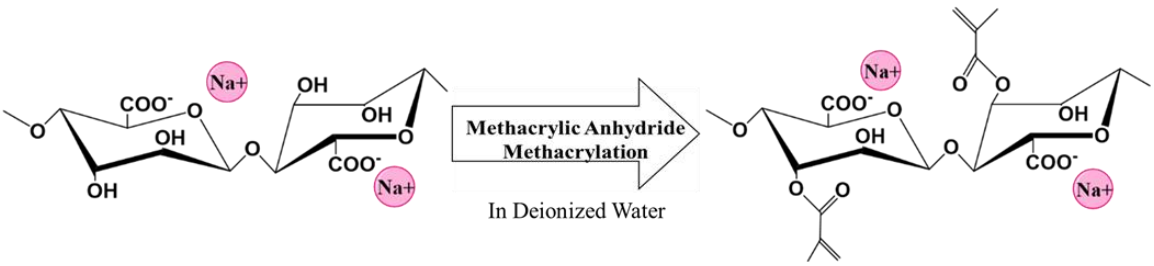

Figure 4: Chemical synthesis of methacrylated sodium alginate via aqueous chemistry. Methacrylation can occur at any of the hydroxide sites on any uronate ring.

3.2.2. Anhydrous Method

The second method investigated for methacrylation was a novel reaction utilizing anhydrous dimethyl sulfoxide (DMSO, Sigma Aldrich) and methacrylic anhydride. Since methacrylic anhydride is more readily soluble in dimethyl sulfosxide, a high molar excess is not required to ensure a proper methacrylation, allowing for more control over the reaction. While sodium alginate is soluble in water, it is not readily soluble in DMSO. In order to increase the solubility, an ion exchange is performed, replacing the sodium ion with a dodecyltrimethylammonium ion. (Figure 5) This can be accomplished by gradually mixing a 1% (w/v) alginate polymer solution in deionized water with an equal volume of a 2-3% solution of dodecyltrimethylammonium bromide (DTAB, Sigma Aldrich) in deionized water. Upon mixing, the sodium ion on the alginate backbone reacts with the bromide ion in the DTAB, forming sodium bromide. The remaining dodecyltrimethylammonium ions bind with the alginate backbone, forming a compound that is insoluble in water, and falls out of the solution as a precipitate. The supernatant (sodium bromide solution in deionized water) can be removed and the remaining precipitate can be rinsed in deionized water several times and lyophilized. The resulting dodecyltrimethylammonium alginate (Alg-DTA) polymer can be readily solubilized in DMSO for the methacrylation reaction. For methacrylation, an Alg-DTA solution in DMSO must be prepared under nitrogen gas flow to prevent any moisture in the air from disrupting the reaction, with a final 1% (w/v) concentration of alginate. Next, a 1:2 molar ratio of 4-dimethylaminopyridine (DMAP, Sigma Aldrich) to Alg-DTA is added to the solution. The DMAP acts as a catalyst and allows for full modification with

a lower molar excess. For a theoretical 100% modification, a 4:1 molar ratio of methacrylic anhydride to alginate is added under nitrogen flow and stirred overnight. Altering the molar ratio of methacrylic anhydride to alginate allows for greater control over the degree of methacrylation. After stirring, a molar equivalent of hydrochloric acid is added to neutralize any remaining DMAP. This solution is dialyzed against deionized water 1 day, followed by a 3 day dialysis against 0.8M sodium dibasic phosphate and a further 3 day dialysis against deionized water. During the first dialysis, the resulting methacrylated Alg-DTA polymer falls out of solution as the DMSO is replaced with the less solubilizing deionized water. Dialyzing against 0.8M sodium dibasic phosphate drives another ion exchance, replacing the dodecyltrimethylammonium ion with a sodium ion. This enables the methacrylated alginate polymer's solubility in water. The final dialysis in deionized water removes any excess sodium ions from the polymer. The polymer solution pH is periodically adjusted to 7.0. This final solution can be lyophilized to a dry form. Sodium alginate can be replaced with oxidized sodium alginate if oxidized methacrylated alginate is desired. Specifying the degree of methacrylation is desirable because altering the degree of methacrylation changes the structure of the resulting hydrogel network, directly impacting the mechanical properties of the hydrogel. For example, a lower degree of methacrylation would result in a less crosslinked network that may exhibit enhanced flexibility, allowing the hydrogel sealant to conform to the lung surface during respiration. Materials with high and low degrees of methacrylation will be prepared to see the effects on the resultant hydrogel. Proton nuclear magnetic resonance spectroscopy was utilized to determine the degree of methacrylation, or ratio of methacrylate groups per repeat unit of alginate. This was accomplished by calculating

ratios of relative integrations of the methacrylate peaks and alginate methyl proton.

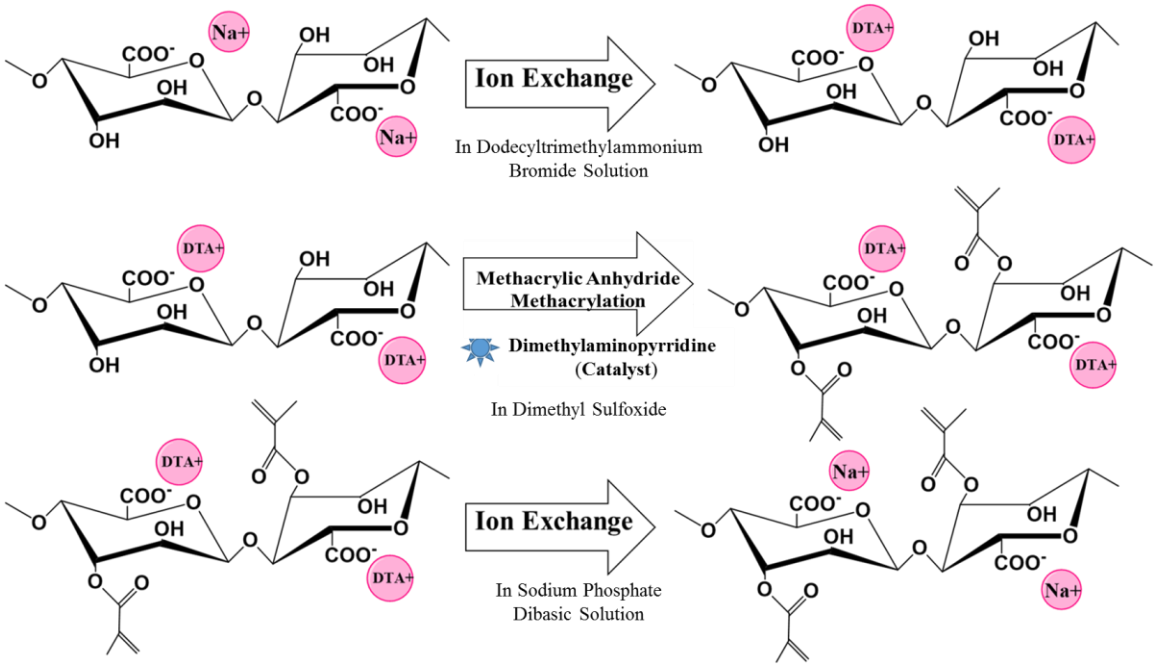

Figure 5: Chemical synthesis of methacrylated sodium alginate via Anhydrous chemistry. Methacrylation can occur at any of the hydroxide sites on any uronate ring.

3.3. Oxidation Chemistry

Besides providing an effective sealing barrier, any pulmonary sealant must also adhere to the lung surface and remain in place during respiration. Initial studies of methacrylated alginate indicated an effective, if brief, sealing of puncture lung tissue before patch delamination, i.e. separation from the lung surface without bursting. Alginate inherently lacks cell adhesivity, explaining the cause of delamination and presenting a new challenge to address. While there are many methods to promote cell and tissue adhesion, one of the simplest methods is oxidation chemistry. Sodium periodate (Sigma Aldrich) oxidation alters the conformation of the uronate residues to an open-chain adduct (Figure 66), increasing the valence of the residue and allowing for improved

interaction with proteins, such as collagen, in the lung tissue.[45, 48] For the oxidation reaction, 1-2% (w/v) alginate and 2-5% (w/v) sodium periodate solutions are prepared in deionized water in separate vessels. To achieve a theoretical full oxidation, a 1:1 mass ratio of alginate to sodium periodate is used. Altering this ratio allows for control over the degree of oxidation. The solutions are then mixed and allowed to stir for 24 hours at room temperature in a dark environment. The resulting oxidized sodium alginate solution is dialyzed against deionized water for 3 days and lyophilized to a dry form. Sodium alginate can be replaced with methacrylated sodium alginate if oxidized methacrylated alginate is desired. The oxidation reaction alters the structure of the uronate rings, preventing methacrylation of the hydroxide group formerly at that site. Proton nuclear magnetic resonance spectroscopy was utilized to determine the degree of methacrylation, or ratio of methacrylate groups per repeat unit of alginate. This was accomplished by calculating ratios of relative integrations alginate methyl proton and first methylene proton.[48]

Figure 6: Chemical synthesis of oxidized sodium alginate via sodium periodate oxidation chemistry. Oxidation reaction can alter the uronate ring for either guluronate or mannuronate.

CHAPTER 4: RHEOLOGICAL CHARACTERIZATION

4.1. Introduction

After chemical modification, the first step to understanding the characteristics and mechanical properties of the hydrogel precursors is through rheological testing. Rheology is often used to characterize the deformation and loading characteristics of non-Newtonian fluids, such as polymer solutions. All measurements were carried out on a rheometer (AR2000, TA Instruments) equipped with a Peltier cone-and-plate geometry (40-mm diameter, 1°59′47″, hard anodized aluminum) maintained at 25°C

4.2. Viscosity and Shear Stress

Chemical modification of alginate may result in degradation, i.e. chain scission, and reduction in the molecular weight of the alginate backbone, depending on the nature of the chemical reaction. It has been documented that methacrylation chemistry can be damaging to the alginate backbone and oxidation reactions have been used to partially degrade polymers in various applications, thus changes in the polymer structure are expected as a result of the modifications.[43, 48] These structural changes result in materials with varying mechanical properties, which were examined via rheology. Viscosity (Pa s) and shear stress (Pa) of the polymer solutions were measured at varied shear rates, 1-100 (1/s), at 1% radial strain over one minute and analyzed using analytical software (TA Data Analysis). The effect of shear rate on viscosity and shear stress values of 3% (w/v) solutions is depicted below. (Figure 77) A 3% solution was chosen as it allows for collection of reliable data while minimizing polymer use. Solution viscosities decreased exponentially with increasing shear rates for all solutions tested. Compared to non-

modified alginate solutions, the aqueous methacrylated alginate (Alg-MA) solution maintained relatively high viscosities and shear stresses. In contrast the methacrylated and oxidized alginate (Alg-MA-Ox) solutions exhibited greatly reduced viscosities and shear stresses when compared to alginate and Alg-MA solutions. This indicates the oxidation reaction resulted in degradation, or chain scission, of the polymer backbone, decreasing its stiffness, moduli, and viscosity. While both the 10 and 30 DOO Alg-MA-Ox were degraded, the 10 DOO sample had a slightly higher viscosity, indicating increasing DOO increases degradation of the polymer backbone.

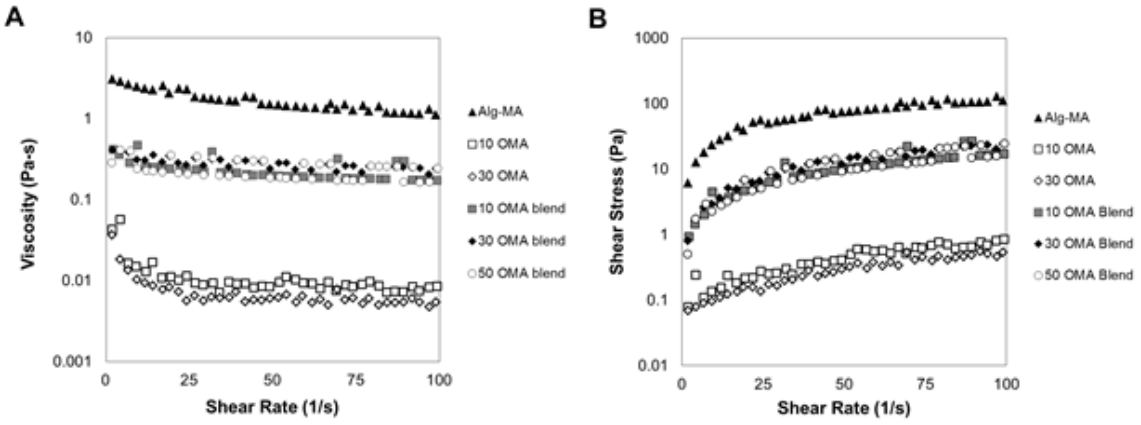

Figure 7: Rheological experiments were performed on 3% (w/v) Alg-MA and Alg-MA-Ox solutions and (1:1) blends of Alg-MA and Alg-MA-Ox. (A) Viscosity vs. shear rate plots and (B) shear stress vs. shear rate plots are shown, average values are reported. Aqueous methacrylation method.

To form an effective seal, a liquid tissue sealant must portray a viscosity suitable to maintain a film over the defect during the curing process. While oxidation is required to maintain adhesion to the pulmonary tissue, the necessary amount or degree of oxidation has not been fully studied. Therefore, Alg-MA was blended with Alg-MA-Ox to preserve the viscosity and material properties, while maintaining theoretical adhesion. Alg-MA and Alg-MA-Ox blends (1:1) were created from the same aqueous

formulation of Alg-MA and Alg-Ox-MA with varying degrees of oxidation (10, 30, and 50%). (Figure 7) The viscosity and shear stress values for the blended solutions lie between respective component values and were similar, indicating that the properties of the Alg-MA polymer are dominant for viscosity and shear stress.

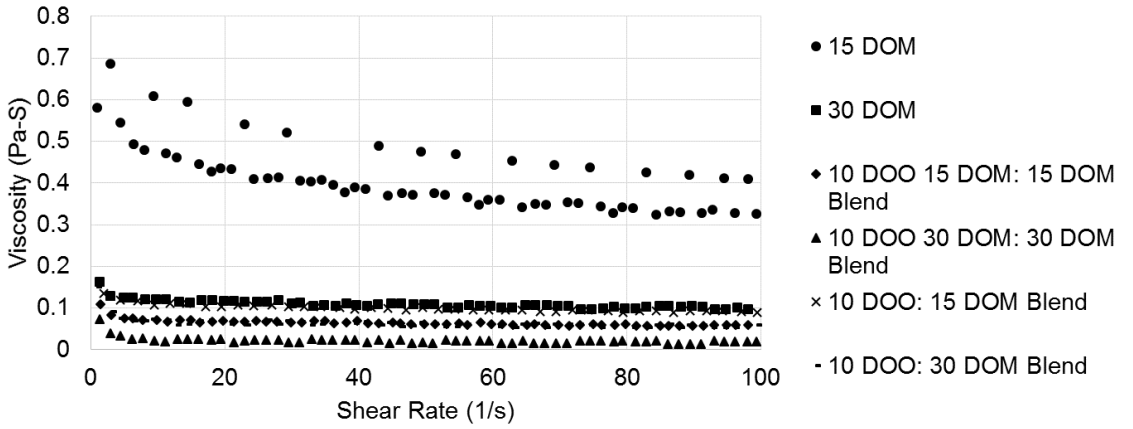

Figure 8: Rheological experiments were performed on 3% (w/v) Alg-MA, (1:1) blends of Alg-Ox-MA and Alg-MA, and (1:1) blends of Alg-Ox and Alg-MA. Viscosity vs. shear rate average values are reported. Anhydrous methacrylation utilized.

The effect of shear rate on viscosity for the latest generation of materials, those oxidized and then methacrylated (Alg-Ox-MA) via the anhydrous method, are depicted above. (Figure 88) Alginate was oxidized first to study an earlier hypothesis that oxidation prior to methacrylation would result in a hydrogel with greater elasticity. Alginate was oxidized to varying degrees (10, 30, or 50%) and subsequently methacrylated to varying degrees (15 or 30%). Unfortunately, the 30 and 50 DOO alginates proved too degraded for testing. This provided initial indication that oxidation prior to methacrylation may result in a further degraded polymer. Unmodified alginate was also methacrylated as a control. 3% (w/v) solutions of 15 DOM, 30 DOM, blended (1:1) 10 DOO 15 DOM: 15 DOM, blended (1:1) 10 DOO 30 DOM: 30 DOM, blended

(1:1) 10 DOO: 15 DOM, and blended (1:1) 10 DOO: 30 DOM were formed and fully characterized. Solution viscosities decreased exponentially with increasing shear rates for all solutions tested. Methacrylated alginates solution viscosity was relatively higher than the blended solution viscosities, indicating polymer degradation due to the oxidation. Solution viscosity also dropped due to the methacrylation reaction, evident by the decreased viscosity for the 30 DOM solution compared to the 15 DOM material. Both values were considerably lower than the solutions prepared via the aqueous method, indicating the anhydrous reaction may be harmful to the polymer backbone.

4.3. Photocrosslinking and Gelation

Oscillatory time sweeps were conducted at 10% radial strain and 1 Hz during exposure to green light (525 nm, custom $3^{7}/_{8}$ inch diameter LED ring, NFLS-G30X3-WHT, SuperbrightLEDs) over a period of 10 minutes (600 seconds). Data collection was initiated upon the start of light exposure. Shear storage (G′) and loss (G″) moduli were recorded; tan delta (ratio of G″ to G′) was analyzed using analytical software (TA Data Analysis). The gelation of Alg-MA and Alg-Ox-MA hydrogels was examined after shear sweep experiments. Aqueous Alg-MA and Alg-Ox-MA solutions, in the presence of photoinitiators, were crosslinked and formed into hydrogels upon exposure to green light. Eosin Y in 1-vinyl-2-pyrrolidinone was used as photosensitizer and catalyst, while triethanolamine served as an electron donor.[53-57] The gelation times of the hydrogel solutions were determined from oscillatory time sweep plots. (Figure 9 and Figure 10) The initiation of gelation, *i.e.*, gelation time, was determined to be at the inflection point of the delta vs. time curve. The Alg-Ox-MA solutions with degrees of oxidation at or above 30 failed to form a gel, *i.e.*, photocrosslink, under rheological testing (data not

shown). For the other Alg-MA and Alg-Ox-MA solutions and blends, there is a clear inflection in the delta curve, indicative of successful photo-initiation and subsequent crosslinking.

G′ is a better indicator for terminal crosslinking as it plateaus. (Figure 99 & Figure 1010) Upon inspection of the G′ curves, it appears a majority or crosslinking has been completed by 600 s. By this time, G′ has increased by several orders of magnitude and the rate of change has decreased substantially. Thus, an exposure time of 10 minutes (600 seconds) was used to form hydrogels for burst testing. These behaviors are evident in the solutions prepared via the aqueous methacrylation chemistry. Materials with lower degrees of methacrylation, i.e. those prepared via anhydrous methacrylation, did not fully plateau, although G′ is still orders of magnitude higher, indicating elastic hydrogel formation. Rheological values collected past the ten minute point can become unreliable as the sample dehydrates. This dehydration would also cause G′ to increase, preventing a plateau.

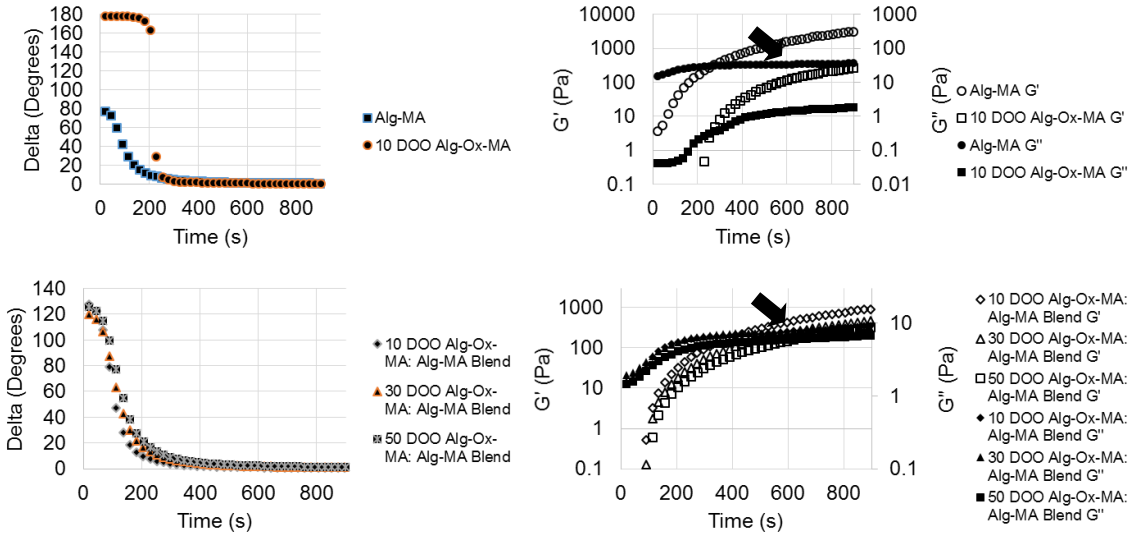

Figure 9: Rheological experiments were performed on 3% (w/v) Alg-MA and Alg-MA-Ox solutions and (1:1) blends of Alg-MA and Alg-MA-Ox. Top left depicts delta vs. time for Alg-MA, 10 DOO Alg-MA-Ox, and 30 DOO Alg-MA-Ox. Top right depicts complex moduli vs. time for Alg-MA, and 10 DOO Alg-MA-Ox. Bottom left depicts delta vs. time for (1:1) blends of Alg-MA and Alg-MA-Ox. Bottom right depicts complex moduli vs. time for (1:1) blends of Alg-MA and Alg-MA-Ox. Gelation time indicated by black arrow. Aqueous methacrylation utilized.

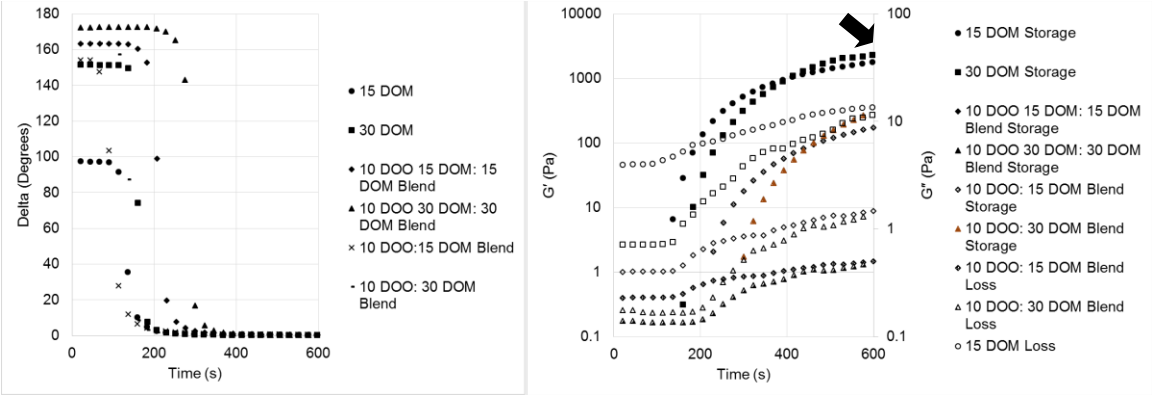

Figure 10: Rheological experiments were performed on 3% (w/v) Alg-MA, (1:1) blends of Alg-Ox-MA and Alg-MA, and (1:1) blends of Alg-Ox and Alg-MA. Left depicts delta vs. time. Right depicts complex moduli vs. time. Gelation time indicated by black arrow. Anhydrous methacrylation utilized.

CHAPTER 5: BURST PRESSURE TESTING

5.1. Introduction

Burst pressure values were determined following ASTM F2392-04R10, with minor modifications for our photo-crosslinking hydrogel. Data was measured via a custom built device designed according to the standard. The main pressure vessel was machined from polyether ether ketone (PEEK) to provide low cost fabrication and resistance to degradation regardless of the pressurizing fluid. The main vessel measures 2½ inches in diameter and $1^{7}/_{8}$ inches in height and is built in two pieces to allow for easy specimen loading between the sections. The top section is fastened by means of two ¼"-20 thread x 1½ inch length bolts permanently fixed in the lower section and held in place by two stainless steel thumbscrews. The lower section has a 7/16 inch diameter hole to allow fluid from a syringe pump to apply pressure to the substrate, while the top section has a 7/8 inch diameter hole, allowing the substrate to react under loading. A 1 inch diameter fluoroelastomer o-ring is placed between the sample and top section to provide an airtight seal, ensuring that the fluid pressure is applied to the specimen. (Figure 111)

A syringe pump (Harvard Apparatus PHD 2000 Infusion) was used to pressurize the system, which can be used in conjunction with a variety of physiologically relevant fluids. A 60 cc, 26.7 mm diameter syringe (ExelInt) was fixed into the syringe pump and connected to ¼ inch diameter plastic tubing via a male luer to tube hose adapter. The tubing attaches to the pressure vessel via a NPT port bored into the body. A pressure transducer (Omega PX-409-030AUSBH) also is connected to the pressure vessel through a NPT port, allowing for accurate, real-time data acquisition via a connected computer. (Figure 111) Failure mechanism (adhesive or material failure) was assessed via visual

inspection of the specimen and a digital microscope (Dino-Lite Pro AM413TA, Dino-Lite Digital Microscopes) during and after testing.

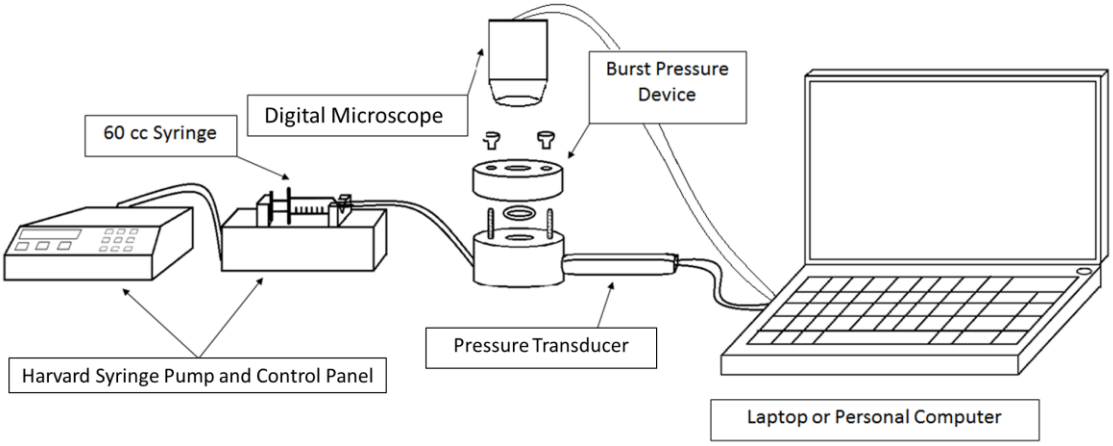

Figure 11: Schematic illustration of testing setup for mechanical burst pressure testing of alginate-based sealants.

5.2. Liquid Sealant Testing

A burst pressure testing protocol was developed and performed utilizing a liquid sealant application. All burst pressure tests were conducted at room temperature with compressed air at an infusion rate of 75ml/hr. Collagen testing substrates (collagen middles natural protein, The Sausage Maker Inc.) were rehydrated for five minutes in deionized water. Prior to testing, the substrate is fixed into the burst pressure device and pressurized to ensure an intact substrate and proper seal. The substrates were next punctured with a 3mm diameter biopsy punch and retested to ensure a through-thickness defect was formed. Punctured substrates were clamped between a glass slide and a Teflon mold with a 15mm diameter hole centered on the collagen puncture. Hydrogel pre-cursor solution (0.5mL) was deposited into the mold, forming a film over the puncture. The mold is then placed on a reflective surface, such as aluminum foil, encircled with a

custom, $3^{7}/_{8}$in diameter LED ring (525nm, NFLS-G30X3-WHT LEDs, SuperbrightLEDs), covered with a reflective material, and photocrosslinked for ten minutes.

After crosslinking, the collagen substrates were unclamped and the Teflon mold was carefully removed so as not to disturb the hydrogel sealant. The collagen substrates are then removed carefully from the slide to ensure the sealant is not stressed. The substrate is positioned in the burst pressure device such that the defect and hydrogel sealant are centered in the device. The fluroelastomer o-ring is centered around the sealant with no overlapping and top section of the device is clamped in place. The syringe pump is enabled and the sealant is tested until failure. Close visual inspection and notes of the testing were made in order to determine the mode of failure. Utilization of a digital microscope and video recording software greatly enhanced the ability to precisely characterize the mode of failure, as the sealant tends to fail in a sudden manner. Figure 122, below, depicts the modes of failure experienced while testing the liquid sealant.

Figure 12: Cartoon schematic and images of failure modes.

Results from burst pressure testing are shown in Figure 133, with a physiologically relevant pulmonary pressure plotted for reference, and summarized in Table 1. While the Alg-MA hydrogel withstood the greatest individual pressure, it also had the highest standard deviation amongst the groups. In order to understand the burst pressure results fully, the mode of failure must be accounted for. Two modes of failure, delamination and material, were observed during testing. Delamination occurs when the hydrogel patch separates from the substrate with no visible sign of damage. Material failure, conversely, occurs when the area directly above the puncture fails, resulting in damage to the patch. The Alg-MA patches failed exclusively through delamination, while the Alg-MA-Ox patches experienced material failure, with the exception of a single 10 DOO Alg-MA-Ox:Alg-MA blend trail. Since alginate is inherently non-adhesive, the wide variation in burst pressure in Alg-MA could be explained by the mode of failure. The Alg-MA-Ox and blended materials are able to adhere to the substrate due to the increased substrate interaction resulting from the open-adduct conformation and adhere

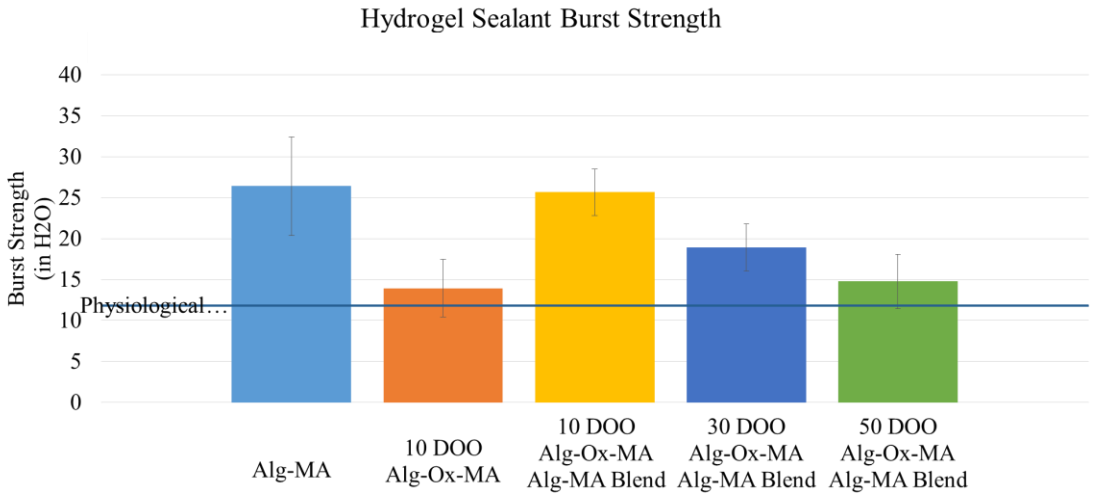

Figure 13: Burst pressure for successful liquid hydrogel sealant (aqueous reaction). Standard error mean is depicted. The physiological line represents 30cmH_2O, a widely used maximum threshold for respiration before pulmonary damage can occur

until material failure occurs. The only case of delamination outside the Alg-MA group occurred in the blend with the lowest theoretical degree of oxidation. This lower oxidation provides fewer interactions with the substrate, possibly enabling delamination.

Table 1: Summary of Liquid Sealant Burst Pressure Testing

Group	Degree of Methacrylation (%)	Degree of Oxidation (%)	Burst Pressure (in H_2O)	Mode of Failure
Alg-MA	77	n/a	26.42 ± 12.01	Delamination
Alg-MA-Ox	70	10	13.96 ± 7.02	Material
Alg-MA-Ox Alg-MA Blend	73.52	5	25.69 ± 5.69	Delamination (1) Material
Alg-MA-Ox Alg-MA Blend	70.5	12.5	18.93 ± 5.70	Material
Alg-MA-Ox Alg-MA Blend	54	19.8	14.79 ± 6.61	Material

5.3. Patch Sealant Testing

One of the greatest issues with the liquid sealant application is keeping the hydrogel in place over the defect during gelation. This problem was overcome in earlier testing by using a Teflon mold, but this solution would prove nearly impossible in lung tissue applications. Therefore, a solid hydrogel patch was devised to overcome this issue. The dry patch could be applied to the lung surface, hydrated with fluids present on the tissue surface, and photocrosslinked *in situ*. 15 DOM, 30 DOM, blended (1:1) 10 DOO 15 DOM: 15 DOM, blended (1:1) 10 DOO 30 DOM: 30 DOM, blended (1:1) 10 DOO: 15 DOM, and blended (1:1) 10 DOO: 30 DOM solutions were mixed with

photoinitiators to achieve viscous solutions. The resulting solutions were then spun onto glass slides and lyophilized to form dry, thin films. The resulting films were removed from the slide, punched into 8mm diameter patches, and stored until testing. Once the photoinitiator is introduced to the system, all fabrication and handling of the material must occur in a dark environment and under red light to prevent gelation from occurring prior to application.

A new protocol was developed to characterize the burst properties of the resulting patches. Burst pressure testing was performed in an environmental incubator maintained at 37° C with compressed air at an infusion rate of 75ml/hr. Collagen substrates (collagen middles natural protein, The Sausage Maker Inc.) were rehydrated for five minutes in deionized water. Prior to testing, the substrate is fixed into the burst pressure device and pressurized to ensure an intact substrate and proper seal. The substrate was next punctured with a 1.5mm diameter biopsy punch and inspected to ensure a through-thickness defect was formed. The substrate surface is placed on a glass slide and wetted to allow the patch to begin hydrating upon application. Since the patches were thinner than expected, two 8mm diameter patches are placed above the defect and wetted with deionized water from an eyedropper. Enough water was applied for the patches to become fully hydrated without becoming supersaturated, which could lead to disintegration of the patches. The substrate is then covered with the Teflon mold to prevent dehydration as a result of close proximity to LEDs during gelation. The glass slide is placed on a reflective surface, such as aluminum foil, encircled with a custom, $3^{7}/_{8}$in diameter LED ring (525nm, NFLS-G30X3-WHT LEDs, SuperbrightLEDs), covered with a reflective material, and photocrosslinked for ten minutes.

After crosslinking, the collagen substrates are removed evenly from the slide to ensure the sealant is not stressed. The substrate is positioned in the burst pressure device such that the defect and hydrogel sealant are centered in the device. The fluroelastomer o-ring is centered around the sealant with no overlapping and top section of the device is clamped in place. The syringe pump is enabled and the sealant is tested until failure. A digital microscope and video recording software is used to precisely characterize the mode of failure, as the sealant tends to fail in a sudden manner.

Results from burst pressure tests for the solid application are depicted in Figure 144 and summarized in Table 2. Both Alg-MA groups saw relatively high burst pressures, but also have high deviations. The only blended material with significant burst pressures is the 10 DOO:30 DOM blended material. Alg-Ox-MA: Alg-MA (1:1) blends experienced very low burst pressures compared to the Alg-MA groups, which suggests the material was highly degraded. Oxidation is known to degrade polymer chains and alters the conformation of the alginate uronate residue such that the functional methacrylate side group is unable to bind. This potentially limited the efficiency of the methacrylation reaction and resulted in alginates with lower degrees of methacrylation than expected. This lower degree of methacrylation would result in a less crosslinked polymer network and ultimately result in a weaker sealant. As was the case with the liquid application, methacrylated alginates failed exclusively through delamination, while the groups blended with oxidized alginate experienced exclusively material failure before delamination could occur. This data also suggests that a simple blend of oxidized and methacrylated alginate shows potential as a surgical sealant. Further study of these blends will need to be performed to determine the extent of polymer interaction between the two

materials and whether separation could occur in time. Since the methacrylation method, degree of methacrylation, and order of oxidation and methacrylation all changed between the generations of hydrogel sealants, it is not possible to declare either the liquid or dry-state application method superior at this time.

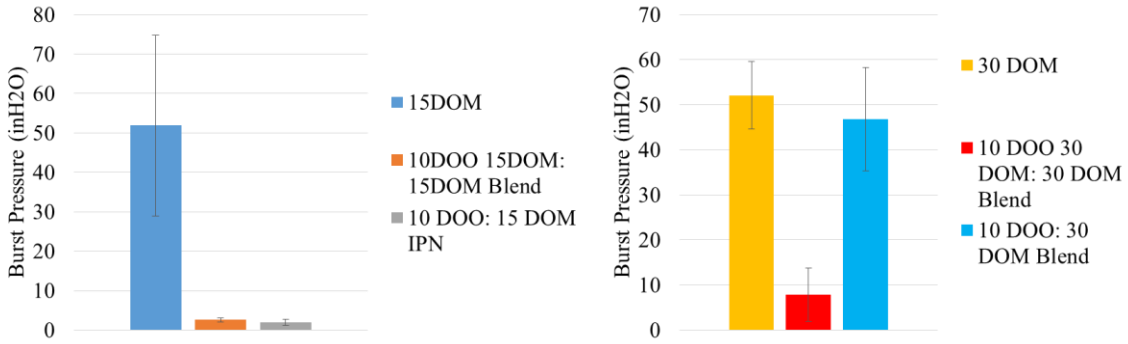

Figure 14: Burst pressure for solid hydrogel patch sealant. Standard error mean is depicted. Left depicted groups with 15 DOM materials. Right depicts groups with 30 DOM materials.

Table 2: Summary of Solid Patch Sealant Testing

Group	Theoretical DOM (%)	Theoretical DOO (%)	Burst Pressure (in H_2O)	Mode of Failure
Alg-MA	15	n/a	51.94 ± 22.94	Delamination
Alg-MA	30	n/a	52.12 ± 7.42	Delamination
Alg-Ox-MA Alg-MA Blend	15	5	2.56 ± 0.56	Material
Alg-Ox-MA Alg-MA Blend	30	5	7.81 ± 5.90	Material
Alg-Ox Alg-Ma Blend	7.5	5	1.98 ± 0.79	Material
Alg-Ox Alg Ma Blend	15	5	46.83 ± 11.46	Material

CHAPTER 6: EX VIVO LUNG TESTING

6.1. Introduction

Initial mechanical performance of the hydrogel sealants were studied via *ex vivo* mouse lung models. The amount of time and resources going into these *ex vivo* studies, however, was not sustainable for high throughput studies or for developing an optimized material. Therefore we transitioned away from the mouse lung model for early iterations of materials, instead developing an in-laboratory characterization that allowed for rapid testing. While testing on the burst pressure device provides a quick and easy way to gather the burst pressure of a hydrogel sealant, the testing conditions do not match physiological pulmonary conditions. In order to prove an effective pulmonary sealant, ex vivo mouse lung testing must be performed to more closely simulate physiological application. Lungs are promptly excised from euthanized mice and cannulated. Once excised, the lungs are placed in phosphate buffer solution and mechanically tested on a ventilator. Unfortunately, later iterations of the alginate hydrogel sealant were not assessed via the mouse lung model.

6.2 Excision

In order to perform successful *ex vivo* testing, an intact lung must first be excised. Mice are euthanized using by lethal administration of sodium pentobarbital according to accepted AAALAC and institutional standards and under existing IACUC protocol (CHRMS 15-008). The mouse is then stretched and pinned onto a rubber surgical mat. An incision is made in the neck and the skin is pulled to the side, exposing the neck muscles. These muscles are carefully cut to expose the trachea. An incision is made in the trachea so that a cannula can be inserted and tied in place via suture thread.

An incision is then made below the ribs and connected to the neck. The skin covering the chest can then be pulled to the side, exposing the abdomen and rib cage. Cutting through the abdomen and pulling back the liver will expose the diaphragm, which can be punctured to deflate the lungs. Next, the ribs and connective tissue lining the lungs is removed with care to ensure that the lungs remain intact. After the connective tissue is removed, the lungs can be removed from the chest cavity and placed in phosphate buffer solution for testing.

6.3 Mechanical Testing

Once excised, the lungs can then be tested on either a Minivent or Flexivent ventilator. The Minivent gives the user to ventilate the lungs with control over the tidal volume and stroke rate. Lungs are connected to the Minivent via the cannula and ventilated to ensure the lung was properly excised with no puncture. Successfully excised lungs are then punctured and re-ventilated to confirm a leak as a result of the defect. Excised lungs are then sealed with the alginate hydrogel sealant and allowed to crosslink. Finally, the lungs are ventilated at 2 cm positive end-expiratory pressure over a range of tidal volumes until the sealant fails. The maximum sealant conditions are recorded and the tissue and sealant are observed to determine mode of failure. The Flexivent give the user the ability to perform more complicated ventilation techniques via a computer program. Sensors are also used to measure the volume and pressure during ventilation. Lungs tested on the Flexivent are subjected to similar initial testing to validate an uninjured lung and subsequently confirm the puncture leak. The results from early *ex vivo* testing on the MiniVent and FlexiVent are summarized below in Table 3 and Table 4, respectively.

Table 3. Summary of Initial MiniVent Testing

Material	Application Mode	Stroke Rate (strokes/min)	Tidal Volume at Failure (μL)	Failure Mode
Alg-MA	Dry-State	180	325	Delamination
Alg-MA	Dry-State	180	250	Delamination
Alg-MA	Dry-State	180	350	Delamination
Alg-MA	Liquid	180	330	Delamination

Table 4. Summary of Initial FlexiVent Testing

Material	Application Mode	Pressure at Failure (cmH_2O)	Failure Mode
Alg-MA	Dry-State	25	Delamination
Alg-MA	Dry-State	30	Delamination
Alg-MA	Liquid	25	Delamination

Initial testing on *ex vivo* mouse lungs indicated that alginate-based hydrogels were able to mechanically withstand physiologically relevant conditions before delaminating. The repetitive delamination failures showed the need for improved adherence to the tissue substrate, which alginate inherently lacks. While both MiniVent and FlexiVent testing suggests the alginate hydrogels can serve as pulmonary sealants, all sealants delaminated shortly after loading. All methacrylated alginate tested via MiniVent and Flexivent ventilation were synthesized via the aqueous methacrylation method.

CHAPTER 7: CONCLUSIONS

7.1 Summary of Work

Each year thousands of individuals suffer from pleural defects or lung damage as a result of trauma, cancer, or various other pulmonary diseases, resulting in fluid flow into the pleural cavity. While short term solutions, such as thoracentesis or chest tube drainage, are effective at draining the fluid from the pleural cavity, current treatment methods for the long-term prevention of recurring leaks is still lacking. Several treatment options do exist, but these treatments involve the chemical or physical irritation of the pleural lining to inflame the tissue and adhere the two pleurae or synthetic polymer sealants in conjunction with conventional methods, such as sutures or staples. Alginate, a natural biopolymer derived from brown algae, was investigated as a potential alternative tissue sealant for pulmonary surgical applications. Additionally, in-laboratory protocol was developed for mechanical characterization of material burst pressure and failure analysis.

Alginate hydrogels were synthesized using high molecular weight, commercially available Manugel GMB alginate. The alginate was chemically modified via the addition of functional methacrylate side groups onto the polysaccharide backbone, i.e. methacrylation, and the exposing of functional aldehyde groups through alteration of the alginate uronate structure, i.e. oxidation. Methacrylation of alginate allows for covalent crosslinking, which is preferred over the weaker divalent cation ionic crosslinking of unmodified alginate. Though unmodified alginate inherently lacks cell-adhesivity, oxidation enables adhesion through the interaction of the exposed aldehyde group and tissue proteins, such as collagen. Several synthesis methods were utilized,

including aqueous and anhydrous methacrylation and sodium periodate oxidation. The reaction order was also altered to investigate the hypothesis that oxidation prior to methacrylation would result in a more elastic hydrogel. The oxidation reaction, and to a lesser extent the anhydrous methacrylation reaction, results in high degrees of polymer degradation via chain scission, limiting the application of oxidized methacrylated alginate in its purest form. Therefore (1:1) blends of methacrylated alginate and oxidized methacrylated alginate or oxidized alginate were studied as well.

Rheological testing of the hydrogel precursor solutions indicate that lower degrees of oxidation result in less polymer degradation, with an upper limit of 10% oxidized alginate indicated from testing. Oxidation before methacrylation degrades the polymer, which is then more susceptible to further degradation during the methacrylation reaction. Also, oxidation alters the alginate uronate rings, preventing methacrylation from occurring as efficiently by eliminating potential binding sites for the methacrylate functional group. While the initial hypothesis was that oxidation prior to methacrylation might increase elasticity in the hydrogel network, the resulting degradation was too great. Methacrylation prior to oxidation will achieve higher degrees of methacrylation and lower degrees of degradation prior to oxidation. Low degrees of oxidation will also prevent high degrees of degradation while also allowing improved tissue adhesion.

A burst pressure device was designed, fabricated, and implemented for mechanical analysis of various alginate hydrogels and blended solutions. Protocol was developed to test both a liquid and solid patch application method, allowing a wider range of materials to be analyzed in laboratory. Several hydrogel solutions achieved burst pressures of or exceeding relevant physiological pressures and exhibited bulk material

failure opposed to not adhesive failure. These results are promising for further analysis of visible-light crosslinked alginate-based sealants for pulmonary tissue repair.

7.2. Future Directions

Despite the promise of alginate hydrogels as surgical sealants for pulmonary applications, continuing research is needed to develop a sealant for clinical use. Degree of methacrylation and oxidation need to be fine-tuned to determine optimized chemical modification of alginate for enhanced mechanical sealant properties while obtaining effective tissue adhesion. An upper degree of oxidation has been defined in this study, therefore lower degrees of oxidation should be investigated for improved adhesion with minimal degradation. It is still unclear if lower degrees of methacrylation is superior to higher degrees for a surgical sealant application. Hydrogels with higher degrees of methacrylation or more densely crosslinked, leading to higher mechanical moduli, but also increased stiffness. A successful surgical sealant must balance mechanical strength, elasticity, and adhesion. Further investigation into the methacrylation reaction will allow for a balance between mechanical strength and elasticity. Lastly, while this research focused around the development of an in-laboratory means of mechanically characterizing the burst strength and failure mode of hydrogels, *ex vivo* mouse lung model testing must be performed on promising alginate hydrogel systems. In-laboratory characterization allows for high throughput, rapid testing of hydrogels to determine potentially successful systems, however this characterization method doesn't simulate the complex physiological environment in the pulmonary system. *Ex vivo* testing more closely simulates physiological conditions and will allow for the selection of a final alginate-based pulmonary surgical sealant.

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APPENDIX A: Standard Operating Procedures

A.1 Alginate Partial Oxidation

Goal: To perform partial oxidation chemical modification of the alginate polysaccharide polymer.

Materials:

- Alginate (FMC BioPolymer)
- Sodium Periodate, $NaIO_4$ (Sigma Aldrich)
- Deionised water

Methods:

1. Prepare 1-2% w/v polymer solution in deionized water
2. In separate vessel, prepare 2-5% w/v $NaIO_4$ solution in deionized water for desired degree of oxidation
    a. Theoretical DOO equivalent to Alginate:$NaIO_4$ ratio
    e.g. 10 DOO→ 1 g Alginate:0.1g $NaIO_4$
3. Mix $NaIO_4$ solution into alginate solution and allow to stir for 24 hrs at room temperature in dark space
4. Dialyze against deionized water for 3 days
5. Freeze at -80$^{\circ C}$ and lyophilize

Note: Oxidation reaction can be stopped by addition of molar equivalent of ethylene glycol to $NaIO_4$

Reference:

Bouhadir, Kamal H., et al. "Degradation of partially oxidized alginate and its potential application for tissue engineering." *Biotechnology progress* 17.5 (2001): 945-950.

Jeon, Oju, et al. "The effect of oxidation on the degradation of photocrosslinkable alginate hydrogels." *Biomaterials* 33.13 (2012): 3503-3514.

A.2 Burst Pressure Testing- Liquid Application

Goal: To quantify the burst pressure of a liquid hydrogel sealant on a collagen substrate utilizing a custom fabricated burst pressure device and to perform failure analysis.

Materials:

- Hydrogel Precursor Solution and crosslinking system
- Syringe Pump (Harvard Apparaus PHD 200 Infusion)
- 60 cc syringe (ExelInt, 26.7mm diameter)
- Burst Pressure Device- BPD (custom fabricated)
- Pressure Transducer (Omega PX406030AUSBH)
- Computer or Laptop
- Digital Microscope (DinoLite Pro AM413TA)
- Collagen Substrate (Collagen Middles Natural Protein, The Sausage Maker Inc)
- 3mm biopsy punch
- 2x3" glass slide
- Teflon Mold (2x3" with central 15mm diameter hole)
- Project clips
- Deionized water

Methods:

1) Prepare hydrogel precursor solution to desired concentration
2) Set up BPD.
    a. Program Harvard Pump to prescribed values:
        i. Syringe: 60cc, 26.7mm diameter
        ii. Infusion Rate: 75ml/hr
        iii. Mode: Volume Infusion
    b. Place syringe on pump
    c. Connect BPD to infusion pump via the luer-lock connector
        i. Ensure an airtight seal. Parafilm can be wrapped around connection to help form seal
    d. Connect pressure transducer to BPD via NPT port
        i. Ensure airtight seal. Teflon tape can be used in threading to help form seal
    e. Connect pressure transducer to laptop and open up data acquisition software
        i. Sampling rate can be adjusted to your desire, but 10Hz seems suitable
        ii. Adjust units accordingly. inH2O is useful for surgical sealant applications as there is no cmH2O setting
3) Prepare collagen substrates
    a. Cut collagen to form 3cm squares

    b. Just prior to testing, rehydrate in deionized water for 5 minutes
4) Ensure the system is airtight and the collagen substrate is intact by testing unpunctured substrate in BPD
    a. Place collagen over hole in lower half of BPD
    b. Place o-ring over collagen substrate
    c. Place top half of BPD over substrate and O-ring
    d. Connect two halves with two hand screws
    e. Begin data acquisition software and engage syringe pump
    f. After confirming pressure is building in system (via acquisition software), disengage syringe pump and data acquisition and remove substrate from BPD
5) Punch 3mm hole in center of collagen substrate
    a. Confirm through thickness defect via testing on BPD or visual inspection
6) Clamp substrate between glass slide and Teflon mold such that puncture defect is centered in the mold
7) Pour 0.5mL of hydrogel precursor solution into Teflon mold and crosslink in place
    a. Previous studies should be performed to determine necessary form and duration of crosslinking
8) Unclamp mold and carefully remove Teflon top to minimize stress to sealant
9) Slide collagen substrate from glass slide to BPD to minimize stress to the sealant
10) Fix substrate into BDP so that puncture and sealant are centered
11) Place digital microscope over BPD and connect to computer
12) Begin video and data acquisition and engage pump
    a. Allow pressure to build in device until failure (sealant is broken and unable to withstand pressure)
13) Disengage syringe pump and data/video acquisition
    a. Save data file as excel to recreate pressure curves
    b. Video can be viewed to determine mode of failure
        i. Delamination- sealant separates from substrate with no visible damage
        ii. Material Failure- patch fails directly above puncture but remains adhered
14) Remove sample from BPD for examination and disposal

Note: This test can be performed on laboratory benchtop at room temperature or can be relocated into an environmental incubator for testing in a humid environment at 37 celsius.

Reference:

ASTM F2392-04(2015), Standard Test Method for Burst Strength of Surgical Sealants, ASTM International, West Conshohocken, PA, 2015, www.astm.org

A.3 Burst Pressure Testing- Dry State Application

Goals: To quantify the burst pressure of a dry-state hydrogel sealant on a collagen substrate utilizing a custom fabricated burst pressure device and to perform failure analysis.

Materials:

- Hydrogel Precursor Patch and crosslinking system
- Syringe Pump (Harvard Apparaus PHD 200 Infusion)
- 60 cc syringe (ExelInt, 26.7mm diameter)
- Burst Pressure Device- BPD (custom fabricated)
- Pressure Transducer (Omega PX406030AUSBH)
- Computer or Laptop
- Digital Microscope (DinoLite Pro AM413TA)
- Collagen Substrate (Collagen Middles Natural Protein, The Sausage Maker Inc)
- 8mm Biopsy Punch
- 1.5mm Biopsy Punch
- 2x3" Glass Slide
- Teflon Mold (2x3" with central 15mm diameter hole)
- Mist Bottle
- Eye Dropper
- Deionized Water

Methods:

1. Punch prepared hydrogel precursor patch using 8mm biopsy punch
2. Set up BPD.
    a. Program Harvard Pump to prescribed values:
        i. Syringe: 60cc, 26.7mm diameter
        ii. Infusion Rate: 75ml/hr
        iii. Mode: Volume Infusion
    b. Place syringe on pump
    c. Connect BPD to infusion pump via the luer-lock connector
        i. Ensure an airtight seal. Parafilm can be wrapped around connection to help form seal
    d. Connect pressure transducer to BPD via NPT port
        i. Ensure airtight seal. Teflon tape can be used in threading to help form seal
    e. Connect pressure transducer to laptop and open up data acquisition software
        i. Sampling rate can be adjusted to your desire, but 10Hz seems suitable

ii. Adjust units accordingly. inH2O is useful for surgical sealant applications as there is no cmH2O setting

3. Prepare collagen substrates
    a. Cut collagen to form 3cm squares
    b. Just prior to testing, rehydrate in deionized water for 5 minutes
4. Ensure the system is airtight and the collagen substrate is intact by testing unpunctured substrate in BPD
    a. Place collagen over hole in lower half of BPD
    b. Place o-ring over collagen substrate
    c. Place top half of BPD over substrate and O-ring
    d. Connect two halves with two hand screws
    e. Begin data acquisition software and engage syringe pump
    f. After confirming pressure is building in system (via acquisition software), disengage syringe pump and data acquisition and remove substrate from BPD
5. Punch 1.5mm hole in center of collagen substrate
    a. Confirm through thickness defect via testing on BPD or visual inspection
6. Lightly wet (mist bottle) collagen surface and place on glass slide
7. Apply 2 patches over defect and rehydrate with deionized water using eye dropper
    a. Lightly apply pressure to patch to ensure solid contact with substrate without damaging patch, which can be brittle and subject to cracking
    b. Do not supersaturate hydrogel to avoid dissolution/dissolving
    c. 1-2 drops of water per patch seems to suffice, but this amount should be adjusted for patch thickness, composition, etc.
8. Cover remainder of substrate with Teflon mold to help prevent dehydration of substrate during crosslinking
9. Crosslink patch in place
    a. Previous studies should be performed to determine necessary form and duration of crosslinking
    b. Remove Teflon mold after crosslinking
10. Slide collagen substrate from glass slide to BPD to minimize stress to the sealant
11. Fix substrate into BDP so that puncture and sealant are centered
12. Place digital microscope over BPD and connect to computer
13. Begin video and data acquisition and engage pump
    a. Allow pressure to build in device until failure (sealant is broken and unable to withstand pressure)
14. Disengage syringe pump and data/video acquisition
    a. Save data file as excel to recreate pressure curves
    b. Video can be viewed to determine mode of failure
        i. Delamination- sealant separates from substrate with no visible damage
        ii. Material Failure- patch fails directly above puncture but remains adhered

15. Remove sample from BPD for examination and disposal

Note: This test can be performed on laboratory benchtop at room temperature or can be relocated into an environmental incubator for testing in a humid environment at 37 celsius.

Reference:

ASTM F2392-04(2015), Standard Test Method for Burst Strength of Surgical Sealants, ASTM International, West Conshohocken, PA, 2015, www.astm.org