AWARD NUMBER: W81XWH-19-1-0313

TITLE: Clinical Development of a Novel Pleural and Tracheal Sealant

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CONTRACTING ORGANIZATION: University of Vermont

REPORT DATE: JULY 2020

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JULY 2020	ANNUAL	7/1/19-6/30/20

4. TITLE AND SUBTITLE
Clinical Development of a Novel Pleural and Tracheal Sealant

5a. CONTRACT NUMBER
W81XWH-19-1-0313

5b. GRANT NUMBER
PR181641

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Vermont State and Agricultural College

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

A variety of lung diseases such as emphysema, infections, and lung cancers as well as lung injury from trauma, including battlefield trauma, and complications of respirator life support of critically ill patients in intensive care units can result in lung collapse that can be immediately life-threatening or result in chronic leaking of air or fluid out of the lung. These remain challenging medical problems for which few good options are currently available and result in significant morbidity, mortality, hospital stays, health care costs, and other complications. New options are thus desperately needed. We are developing a novel approach to provide an easy-to-apply lung sealant which can repair lung leaks. The current report details progress made since the inception of DOD grant support.

15. SUBJECT TERMS
Lung, lung health, respiratory health, lung disease, pneumothorax, pleural sealant

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	45	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. Introduction

A variety of lung diseases such as emphysema, infections, and lung cancers as well as lung injury from trauma, including battlefield trauma, and complications of respirator life support of critically ill patients in intensive care units can result in lung collapse that can be immediately life-threatening or result in chronic leaking of air or fluid out of the lung. These remain challenging medical problems for which few good options are currently available and result in significant morbidity, mortality, hospital stays, health care costs, and other complications. New options are thus desperately needed. We are developing a novel approach to provide an easy-to-apply lung sealant which can repair lung leaks. This initially involved use of a chemically modified form of alginate, a naturally occurring seaweed derivative, increasingly being explored for a variety of biomedical applications. Particular attributes include easy availability, low cost, easy use, biodegradability, and lack of significant toxicity. In previous studies, in part supported by a Peer Reviewed Medical Research Program Discovery Award, "Development of a Novel Alginate-Based Pleural Sealant" (W81XWH-15-1-0107, Principal Investigator Daniel J. Weiss MD PhD, 2014-2017), we had identified several promising compounds, notably dopamine-conjugated methacrylated alginate (ALG-MA-DA), that are being further evaluated as part of the current DOD support. This includes extensive materials characterization as well as initial evaluations in small (rodent) and large (pig) *ex vivo* lung models as well as initial *in vivo* evaluations of several compounds in a non-survival surgery rat lung injury model. The goal of the current studies is to continue both materials characterizations, including sterilization and storage attributes, continue small animal (rat) survival studies, and to extend the studies into large animal (pig) models of lung injuries. We are also extending these studies, through collaborators at the University of Connecticut, into small and large animal models of tracheal injuries. The overall goal is to provide a firm platform for initial discussions with the FDA about new INDs for a clinical investigation.

2. Keywords

Lung, lung health, lung disease, pneumothorax, pleura, pleural sealant, alginate

3. Accomplishments

a) What were the major goals of the project? Listed from the Statement of Work

Sites: 1) University of Vermont and State Agricultural College
85 South Prospect Street
Burlington, VT 05405

2) University of Connecticut
Department of Pediatrics
CT Children's Surgical Research Laboratory
263 Farmington Avenue, Farmington, CT 06030-1319

3) Akina, Inc.
3495 Kent Avenue
West Lafayette, IN 47906

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Partnering PI: Christine Finck MD UConn (CF)
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Post-Doctoral Associates: Ishna Sharma MD UConn (IS)
Laboratory Technicians: Evan Hoffman UVM (EH), Todd Jensen UConn (TJ), Nirav Daphthary (ND)
Animal Technicians: Stephen Bell UVM (SB), Sheila Russell UVM (SR)
Thoracic Surgical Consultant: Bruce Leavitt MD UVM (BL)

Specific Aim 1(specified in proposal) **To optimize manufacturing, sterilization, preservation, and storage conditions of pre-formed ALG-MA-DA patches**	**Timeline** 1-36	**Sites 1,3**
Major Task 1: Standardize synthesis and characterization of ALG-MA-DA patches	Months	
Subtask 1: Develop large scale synthesis approach for ALG-MA-DA patches	1-6	JG
Subtask 2: Standardize characterization of ALG-MA-DA patches: NRM, FTIR	1-12	JG
Milestone(s) Achieved: Standardized large scale ALG-MA-DA patch synthesis with controllable, reliable, and reproducible degrees of methacrylation and dopamine conjugation.	By Month 12	JG
Major Task 2: Develop quality control approaches for rheologic and mechanical characterization of ALG-MA-DA patches.	Months	
Subtask 1: Burst pressure and analyze cohesion and adhesion assessments on collagen substrates.	1-12	JG
Subtask 2: Degradation of standardized patches Subtask 3: Cytotoxicity of standardized patches	1-12	JG JG, DW, EH
Milestone(s) Achieved: Reliable and reproducible degradation and lack of cytotoxicity.	1-24	JG, DW, EH
Major Task 3: Define optimal sterilization, and storage conditions.	Months	
Subtask 1: Define optimal sterilization approach for optimized patch	1-24	JG
Subtask 2: Clarify need for addition of preservatives to optimized patch Subtask 3: Define optimal long term storage (packaging, temperature, humidity, etc) conditions	1-36 1-36	JG
Milestone(s) Achieved: Optimal sterilization and storage conditions	By month 36	JG

Specific Aim 2(specified in proposal) **To define long term efficacy and safety in longitudinal small (rat) and large (pig) models of adult pleural injury and of adult and pediatric tracheal injuries.**	**Timeline** 1-36	**Sites 1,2**
Major Task 1: Assess longitudinal efficacy and safety in adult pleural and tracheal injury models	Months	
Subtask 1: Small animal (rat). Includes post-operative behavioral observation, chest radiographs (CT), lung mechanics evaluation, histologic and toxicologic evaluations.	1-36	DW, BL, EH, SB, SR, CF, TJ, IS
Subtask 2: Large animal (pig). Includes post-operative behavioral observation, chest radiographs (CT), lung mechanics evaluation and toxicologic evaluations.	6-36	DW, BL, EH, SB, SR, CF, TJ, IS
Milestone(s) Achieved: Define longitudinal efficacy and safety in adult rat pleural injury models	By month 36	DW, BL, EH, SB, SR, CF, TJ, IS, ND
Milestones Achieved Local IRB/IACUC and HRPO/ACURO approvals	Will be obtained prior to institution of animal studies	DW, CF
Major Task 2: Assess longitudinal efficacy and safety in pediatric tracheal injury models	Months	
Subtask 1: Small animal (rat). Includes post-operative behavioral observation, chest radiographs (CT), lung mechanics evaluation, histologic and toxicologic evaluations.	1-36	CF, TJ, IS
Subtask 2: Large animal (pig). Includes post-operative behavioral observation, chest radiographs (CT), lung mechanics evaluation and toxicologic evaluations	6-36	DW, BL, EH, SB, SR, CF, TJ, IS
Milestone(s) Achieved: Demonstration of ALG-MA-DA patch longitudinal efficacy and safety in pediatric tracheal injury models	By month 36	DW, BL, EH, SB, SR, CF, TJ, IS

Specific Aim 3(specified in proposal) **To assess short term efficacy in a pleural injury model in ex vivo ventilated normal and diseased (COPD/emphysema) human lungs**	Timeline 1-36	Site 1
Major Task 1: Assess short term ALG-MA-DA patch efficacy in ex vivo ventilated human autopsy lungs	Months	
Subtask 1: Normal lungs	1-36	DW, EH
Subtask 2: COPD lungs Milestone(s) Achieved: Demonstration of patch adherence and absence of air leak over a 24 hour period	By month 36	DW, EH

What was accomplished under these goals?

1) Major activities

We have made significant progress towards milestones for all three Major Tasks in **Specific Aim 1**, detailed in the relevant sections below. This has been limited recently given restrictions on laboratory research at the participating sites in the setting of the COVD-19 pandemic. Due to ongoing modifications and optimization of the pleural sealant materials being studied, as well as COVID-19 limitations, studies for **Specific Aim 2** and **3** have not yet been initiated at any of the participating sites.

2) Specific objectives

The major objective of the proposal is to develop a pleural sealant that will have optimized mechanical and biological properties, coupled with low cost, ease-of use, appropriate storage, and other logistical considerations. Based on promising data at the time of proposal submission, the proposal is focused on dopamine-conjugated methacrylated alginate (ALG-MA-DA). Continued study of the ALG-MA-DA formulations in Specific Aim 1 has focused on optimizing manufacturing and quality control with focus on eventual large scale manufacturing. These studies have identified a number of issues including but not restricted to control of manufacturing conditions (oxidation, pH), optimization of the degree of dopamine conjugation, identification of alternative more effective cross-linking reagants (Major Task 1). We have continued with burst pressure evaluations of the ALG-MA-DA and in particular have been comparing to the only currently available lung sealant, Progel™ (Major Task 2). We have also developed a novel oscillatory burst pressure technique that is more applicable for tissue sealants for dynamic tissues such as lung compared to current ASTM static burst pressure testing (Major Task 2). We have also generated initial data on storage and preservation conditions for the ALG-MA-DA patches (Major Task 3).

3) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)

Specific Aim 1
Major Task 1: Standardize synthesis and characterization of ALG-MA-DA patches
Subtask 1: Develop large scale synthesis approach for ALG-MA-DA patches

Synthetic Approach

The original manufacturing method derived as part of development of ALG-MA-DA was experimental in nature and contained several elements which were not readily amenable to standardization and scale up. Efforts have been undertaken to develop more consistent reaction methods which enable both manufacture of larger scale of components as well as batch-to-batch standardization. A few of these efforts will be highlighted below. These have predominantly taken place at Akina Inc but some also at UVM.

ALG-MA
The initial laboratory reaction methodology could be summarized as follows:

1. Prepare a 1% w/v solution of desired molecular weight (mw) sodium alginate in water
2. Place reaction vessel in an ice bath
3. Stir solution via magnetic stir-bar
4. Dropwise, add a 20 molar excess of Methacrylic anhydride (relative to the number of alcohol groups on the alginate's sodium mannuronic/guluronic acid monomers) to the alginate solution.
5. Maintain reaction between pH 7-8 via the dropwise addition of 5N NaOH for 24 hours
6. Dialyze against water for at least 5 days
7. Lyophilize.

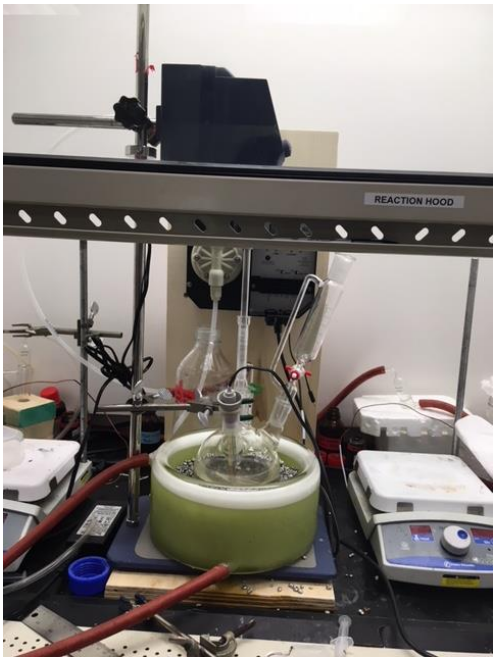
In practice there are several technical issues with this methodology which complicate translation to large scale manufacturing. These include:

- Use of an ice-bath (which thaws over time) providing inconsistent temperature control
- Manual monitoring of pH by a dedicated person over an extended period of time
- Use of a pH balancing agent (NaOH) which is established to have the ability to break down the alginate polymer

Each of these issues was approached systematically as follows:

Temperature
The temperature control by ice-water bath was replaced with a cooling system comprised of water-circulating chiller (Neslab RTE-7, Thermo) filled with a 50:50 mixture of purified water and commercial antifreeze agent to enable sub-zero Celsius operations. Initial batches were cooled by wrapping copper tubing in a coil around the round-bottom flask. This was determined to have relatively poor contact and transfer and was adapted to consisted of a jacketed cooling aluminum bead bath fed by a water-circulating chiller set to 5 ºC instead (**Fig 1**). Based on reactions performed using this device this has been determined to provide suitable temperature control.

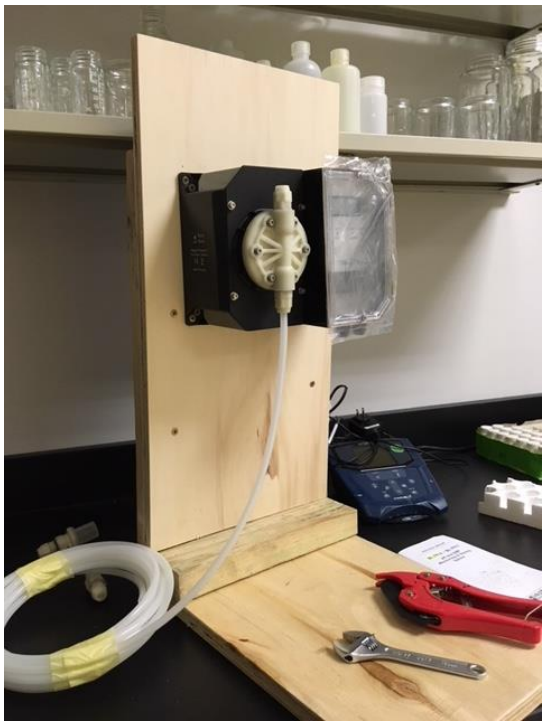
Figure 1. Round-Bottom Flask assembly loaded into a water-jacketed aluminum bead bath for ALG-Ma reaction.



Manual pH Control

For long-term pH control, it is not economically viable outside of an academic lab to have a dedicated person for control of pH. Initial work in pH control focused on controlling the rate of addition of the basifying agent so as to provide for a pH adjustment that matched the speed of the addition. This was initially done using 4.2 M NaOH loaded into a 60-ml syringe pump set to a pump rate of 1 mL/h. The next day the pH was measured (typically ~ 5-6) and the syringe was typically reloaded with additional NaOH to provide for additional basification to raise the pH up to ~6-7. This was, however, determined to be too unreliable for the sustained basification. The timed delay system was replaced with a pH responsive system comprised of a Hanna Instruments pH Controller and Pump (Model # BL7916) set to maintain the pH at 8 automatically.

Figure 2. pH responsive controller mounted onto bracket to position it for the reaction



Hydrolytic Agent

The sodium hydroxide (NaOH) used in development is a powerful basifying agent which can break down the alginate if applied in excess quantity due to its ability to catalyze hydrolytic reactions (Niemelä, K., & Sjöström, E. (1985). Alkaline degradation of alginates to carboxylic acids. Carbohydrate research, 144(2), 241-249. https://www.sciencedirect.com/science/article/pii/S0008621500906724). Notably, several batches made using NaOH were observed to have lower viscosity, potentially due to lower mw, relative to the original alginate material. In order to preserve material integrity, Sodium Carbonate (Na_2CO_3) was used at a concentration of 4M. Note that the pKb of NaOH is -0.56 while sodium carbonate is 3.67 as it is a weaker base. After this switch, reduced viscosity of ALG-MA batches was no longer observed. Additionally, sodium carbonate has the added benefit of being both dibasic as well as converting into a volatile acid (carbonic acid) which decomposes both cooling the reaction as well as removing the acid from the reacting solution. For these reasons, sodium carbonate is a superior buffering agent to use relative to the more caustic sodium hydroxide.

Based on these changes, and a few other minor modifications, the current ALG-MA synthetic methodology as of the writing of this report is typified as described for an example lot below.

Alginate-MA (Lot# 181113AHT-A)

Weighed out 5.0011g Alginate (BioPolymer lot #G9402001) and added 500 mL DiH2O under stirrer for ~2 hours. Prepared 500mL 2M Na2CO3 by adding 106.0216g Na2CO3 (Fisher lot #174819) stirred until dissolved for ~ ½ hour. Alginate solution transferred to a 4-neck 1000mL (clean/dried in 100ºC oven) RBF and placed in a jacketed cooling bath which was placed on a ring stand with an overhead stirrer and glass stir rod with plastic paddle and placed in middle neck of RBF. This in turn was placed on top of wooden stand with pH pump (Hanna instruments BL 7916) attached. Dosing tube (inlet) on pH pump in Na_2CO_3 2M solution. Outlet tube connected to a stopper attachment arm which fit into another neck of the RBF. A dripper arm with 15 mL methacrylic anhydride (Sigma-Aldrich lot #STGB9534) was connected to another neck of the RBF and added to alginate solution dropwise. A pH probe (attached to the pH pump) was placed in the 4th neck of the RBF (with rubber adapter and O-ring around the probe). The pH pump was set to 8, so as to add Na_2CO_3 when pH dropped below 8. Pump turned on after methacrylic anhydride had been added. Let stir overnight at ~250rpm with pH pump on to control pH. Final pH was 7.97. The solution was then poured into 12-14 kDa dialysis tubing and placed in a 4L beaker with ~3 ½ L Argon sparged DiH2O, and left to stir overnight. Water bath was changed out every day for 2 more days. Product placed in HarvestRight freeze drier overnight. Final yield was 6.61g.

ALG-MA-DA

The resultant ALG-MA material was reacted with Dopamine (DA) coupling via a carbodiimide pathway initially described as follows:

Alginate-Methacrylate-Dopamine

1. Prepare a 1% w/v methacrylated sodium alginate solution in 50mM MES buffer.
2. Add a 4x Molar equivalent of EDC and a 4x molar equivalent NHS to 1% ALG solution.
3. Adjust pH of solution to 6 [1N HCl dropwise while stirring] and stir for 45 minutes.
4. Prepare a 4x molar equivalent solution of Dopamine HCl in 50mM MES and adjust pH to 4 using NaOH. (Ex: for 1 gram of Sodium Alginate add 3.51 grams Dopamine HCl)
5. Add the pH 4 Dopamine solution to the ALG/EDC/NHS solution. Adjust pH of combined solution to 4 and allow reaction to proceed for 2 hours.
6. After 2 hours, adjust the pH to 6 (using NaOH) and allow the reaction to continue for 12 hours.
7. Dialyze against water @pH 7. Can alternatively precipitate alginate in 100% ethanol and thoroughly wash/vacuum filter 3 times.
8. Freeze @ -80C (at least 1 hour), then Lyophilize and store powder (-80 + dark for long term)

The original reaction was performed as described in the following example lot

Alginate-MA-DOPA (Lot# 181101AHT-A)

Prepared a 1% w/v Alg-MA solution by dissolving 1.0015g Alginate-MA (Lot# 181018AHT-A) in 100 mL MES buffer 50 mM, pH 6.0 (Bioworld lot #18101004). Stirred to dissolve, then added 4X molar equivalent of EDC, 1.131 g (Sigma-Aldrich lot #SLBN8074V) plus 1.743 g (Sigma Aldrich lot # SLBQ8691V) and 4X molar equivalent NHS, 2.130 g (Sigma-Aldrich lot #MKBS3566V) stirred to dissolve. Stirred for 45 min, then 4X molar equivalent Dopamine HCl (Sigma-Aldrich lot #BGBX5420) solution in 50mM MES buffer (3.5159 g in 100mL argon sparged MES buffer and argon sparged after dopamine addition to reduce pinkness) was added dropwise to the Alg-MA solution and allowed to stir for 2 hours. The RBF was filled with argon then allowed to react overnight, covered with foil, stirring at 180 rpm at room temperature. The solution was a dark green at this point. The solution was then poured into 12-14kDa Spectra/Por dialysis tubing (Spectrum labs #132680) and placed in a 4L DiH_2O batch overnight. The water bath was changed out every day for 2 more days. The entire batch was then placed in the Harvest Right freeze dryer to dry. The final weight was 1.43g. This batch was then washed with 200 proof Ethanol and allowed to stir for one hour (wash solution subsequently dried and dissolved in D_2O for NMR which can be found in results section), filtered and then dried in a deep vacuum chamber. Variations of this general reaction were attempted several times, however the resultant material visibly oxidized to a black color due to oxygen exposure each time.

Given the oxidation issue, the dopamine was tested for stability as follows:

Dopamine Solution Aging Test

A small portion of the generated dopamine solution made as part of (~10% w/v in sparged pH 5.5 buffer) was transferred into a cuvette and covered with a teflon cap but otherwise no particular protections were provided for oxygen protection. At predetermined time points, the solution was scanned for UV/Vis absorbance against a blank of a pH 5.5 phosphate buffer. Figure 3 below shows overlays of select time points.

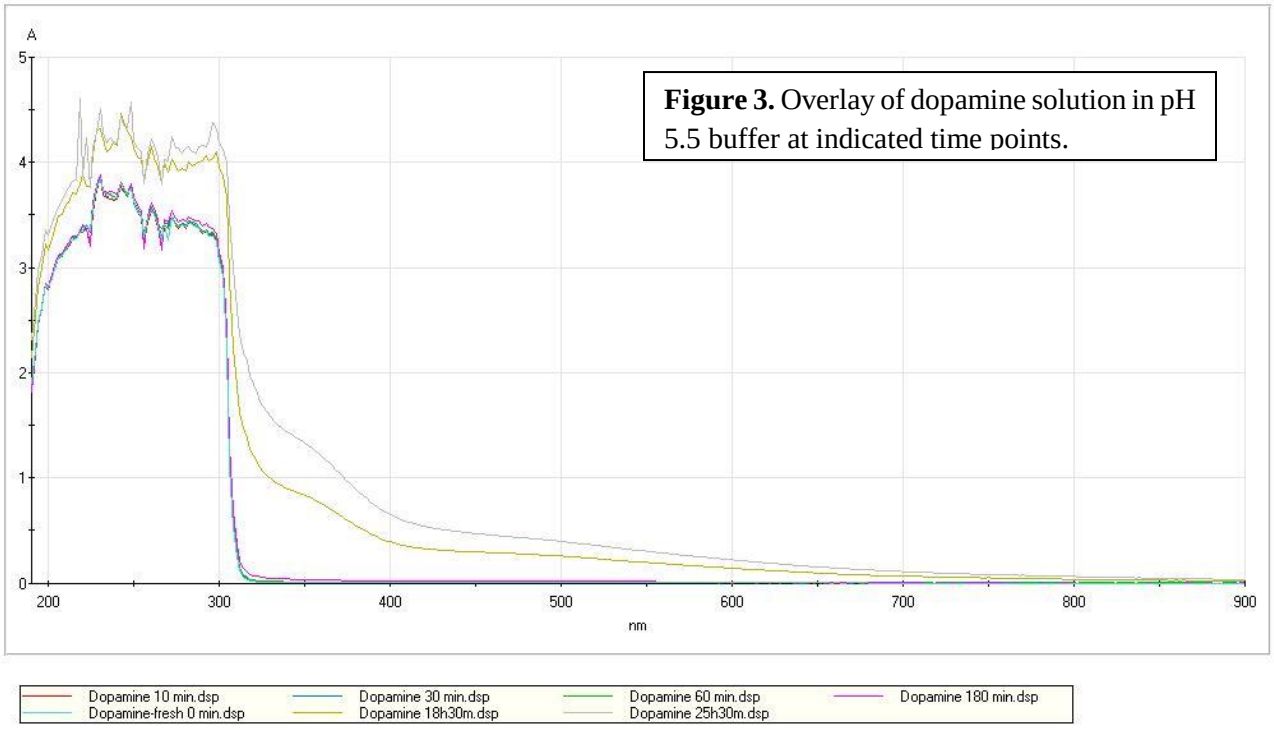


Figure 3. Overlay of dopamine solution in pH 5.5 buffer at indicated time points.

Minimal change was observed between the fresh solution and up to 3 hours. However, upon sitting overnight, the solution darkened to a brown color and displayed higher absorbance in the 300-400 nm wavelength region which increased slightly over the next 7 hours. This transition occurred with the material sitting in the dark indicating that dopamine solutions in fully sparged buffer are only stable for up to 3 hours.

Due to its particular susceptibility to oxidation, DA provided only randomly successful batches during these initial test reactions. Several modifications were thus required to the reaction method as detailed below (**Figure 4**).

Initial synthesis modifications: Alginate-MA-DOPA (Lot# 181127JSG-A)

Prepared 0.01M phosphate buffer and pH adjusted to 5.51 as previously described. Buffer solution degassed by stirring under reduced pressure (KNF labaport vacuum pump) for 10 minutes with argon back-flush followed by argon sparging in glove box for 1 hour with stirring at 500 RPM. Connected aluminum-foil wrapped 2-neck 1-Liter round-bottom flask (RBF) to vacuum pump and argon source by 3-way stopcock with glass stopper on alternate neck. Vacuum flushed for ~2-5 minutes followed by argon flush for ~2-5 minutes of the empty RBF twice before placing 1.398 gram solid Alginate-MA (181113AHT) into the RBF. The solid ALG-MA was vacuum purged followed by argon flush three times (**Fig 4A**). At the last flush, the argon flow was set to ~60-100 cc/min to maintain continuous flush of argon through the RBF. The phosphate buffer was measured out using a specially designed assembly of a 1-hole cap threaded through with a short length of PTFE tubing connected to a 60 ml syringe (**Fig 4B,C**) this was then introduced using a needle through a thin nitrile septa into the RBF (**Fig 4D**). This was done multiple times to deliver 140 ml of buffer into the RBF and the solution was stirred to dissolve the Alg-Ma. After dissolution of Alg-Ma, the stopcock was replaced with a thermometer adapter and a 9 inch Pasteur pipette was attached to the argon outlet to provide for continuous sparging of the Alg-Ma solution at 50 cc/min of argon with ventilation out via 18Ga needle stuck in septa. The solution was subsequently sparged in this manner throughout the entirety of the reaction. A light-protected dropper-arm was attached to facilitate transfer of the dopamine solution (**Fig 4E**).

Separately, the argon glove-box was flushed out with argon until the oxygen level reached ~1-2% (measured by Apogee Instruments MO-200). To a clean, dry amber bottle added 6.0 mg of BHT (Aldrich cat# B1378), argon flushed the bottle and transferred in to glovebox along with stir-bar, light-protected 60 ml syringe, and remaining pH 5.5 phosphate buffer. Resparged the pH 5.5 phosphate buffer for an additional hour to reduce oxygen content and continued argon flush. Rechecked oxygen content in box is 0.5 – 0.7%. Working inside glove-box, transferred entire contents of freshly-opened Dopamine-HCl 5 gram bottle (Aldrich Cat# H8502-5G, actual mass transferred based on weighing bottle full and empty was 5.95 g) into argon-flushed amber bottle containing 6 mg of BHT. Used syringe to add in 60 ml of argon-sparged pH 5.5 phosphate buffer to the amber bottle and stirred inside argon glove box to dissolve the Dopamine and BHT (**Fig 4F**). After dissolution, pulled Dopamine solution into light-protected syringe through one-hole lid and PTFE tubing (**Fig 4G**). Sealed off with leur cap and transferred out of glove-box to fume hood (**Fig 4H**). Note total volume of solution was slightly more than what syringe could hold and 3 ml of solution remained in excess. Transferred 3 ml of solution into quartz cuvette for UV-Vis analysis of dopamine degradation against pH 5.5 phosphate buffer blank.

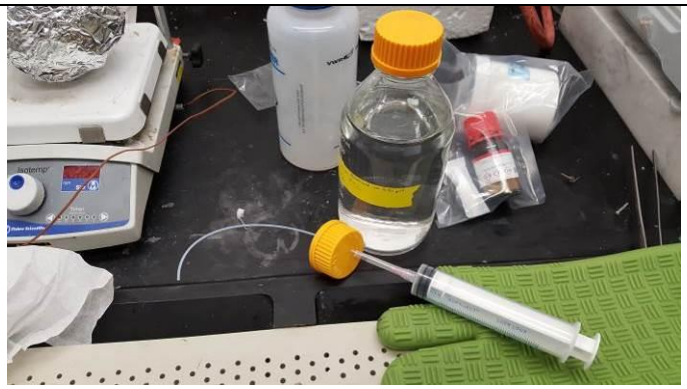
Into ALG-Ma solution in RBF (still argon-sparging at 50 cc/min) added 2.972g NHS and 4.004g EDC-HCl (freshly opened) via funned under continuous sparge. Reattached dropper arm and left with continouos argon sparge during EDC-NHS reaction for 45 minutes. After this, injected 60 ml of dopamine solution into light-protected dropper arm via syringe through nitrile septa. Added to ALG-MA-NHS reaction solution dropwise under continuous stirring at 500 RPM and with 50 cc/min argon sparge. Reacted at room temperature overnight with continuous argon sparge and stirring.

Separately, 3 liters of 200 proof ethanol (Decon Laboratories) magnetically stirred at 300 RPM and sparged with argon bubbling through a fisher-brand 5ml serological pipette for 1 hour prior to being transferred into refrigerator overnight to cool.

The following day, attached a splitter to the argon outlet port and piped one end to 0.86 mm polyethylene tubing fed down into chilled ethanol through one-hole cap for continuous sparging while other outlet fitted to continue sparging of reaction solution (**Fig 4J**). Used light-protected syringe fed through nitrile septa into PTFE tubing to carefully remove the reaction solution from the RBF and then directly injected the solution into the sparging ethanol bottle with continuous magnetic stirring. This process repeated until level of solution was below the reach of the PTFE tubing at which point a 10 ml Eppendorf pipette was used to remove the remainder of the solution and transfer it over into the ethanol bath under continuous argon flush (**Fig 4K**). White, fluffy precipitate observed to form within the ethanol bath. Initially ethanol was separated from the precipitate by fitting the bottle with a hole-drilled cap with filter paper (Whatmann type 1 cut to fit) with positive displacement provided by flushing argon in at a rate of 50 cc/min through the back of the bottle via syringe needle stuck into it (**Fig 4L, M**). By this method, roughly 1.5 L of ethanol was filtered away. Afterwards, the remainder of the ethanol was removed by rapid filtration through coffee filter in Buchner funnel to collect the Alg-MA-Dopa product which was observed to be white in color (Fig 3N). Dried material overnight over dessicant (Drierite) under moderate vacuum, weighed product (2.06 g). Transferred into drying jar containing oxygen absorber packets (FreshUs®) and silica desiccant to dry under moderate vacuum (KNF labaport pump).

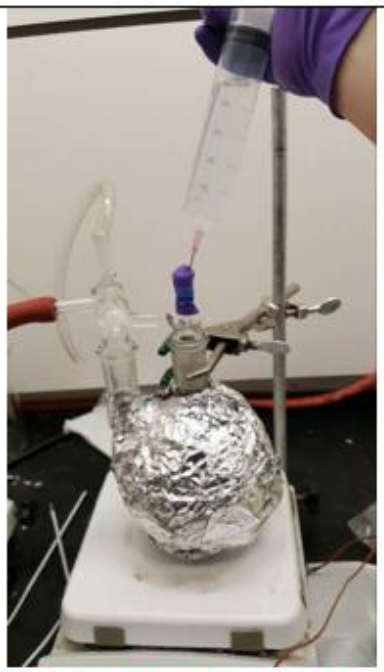
Figure 4: Modified ALG-MA-DA synthesis
Image method series reaction Alginate-MA-DOPA (Lot# 181127JSG-A)

A. Vacuum/argon flush - preparation



B. Buffer addition assembly.

C. Buffer addition process.

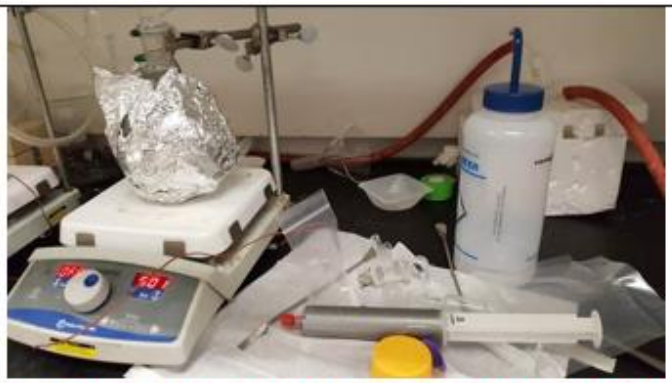


D. Buffer addition into RBF through septa.

E. Reaction glassware setup with continouos sparge

F. stirring Dopamine-BHT solution in glove-box.

G. Transferring dopamine solution into light-protected syringe.



H. Dopamine solution sealed in light-protected syringe for transfer.

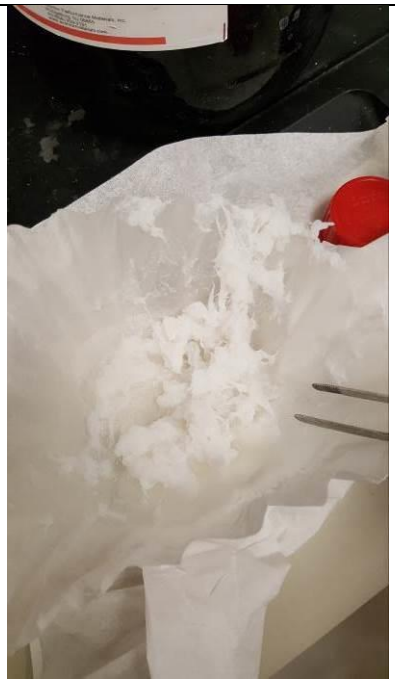
I. Air-free injection of dopamine solution to dropper arm.

J. Sparging of precipitate and reaction solution

K. Light-protected injection into argon-sparged ethanol bath.

L. Filter-cap composition.

M. Cap-filter filtration under positive argon pressure.



N. Collected Alg-Ma-DOPA filtrate

Summary of Cogent Methodologic Changes

- ALG-MA buffer used is simple phosphate buffer with less potential for side reaction
- All solutions thoroughly sparged/degassed prior to use
- Dopamine solution preparation handled entirely under argon atmosphere
- Dialysis purification in the absence of oxygen exposure impractical, replaced with alcohol precipitation
- Air-free techniques applied to ever step of the reaction

Although this method did provide for successful manufacture of the ALG-MA-DA, the overall method is complicated and labor intensive, requires an excessive amount of argon gas for solution preparation, and other steps which could be better performed in more enclosed systems that would require less flushing. Additionally, the quantity of dopamine added was determined to be more than necessary. As such, the reaction was modified to provide to provide for direct sparge-preparation of a lower concentration dopamine solution in the side-arm of the reaction flask along with rubber-septa control of oxygen exposure.

Revised synthesis modifications: Alginate-MA-Dopamine (Lot# 200618AHT-A121)
Into 2-neck RBF with stirbar and stopcock, wrapped in aluminum foil, measured 0.78 g Alg-MA (200127AHT-A121). Placed a septum onto 2nd neck of RBF, vacuum purged/argon flushed several times. Added 78 mL KH_2PO_4 (described above) by 60 mL syringe through septum. Stirred to dissolve about 1 ½ hours. Removed septum while argon flowing, quickly added in 2.243 g EDC (SA #SLCC4199) and 1.66 g NHS (SA #MKCJ1481). Stirred to react EDC/NHS solution with AlgMA for about 45 min. Prepared side-arm dripper with rubber septa and needle pierced through it. Attached end of 20 guage tubing the length of the dripper arm plus 2". Fed tubing down into dripper arm for sparging and dissolving dopamine. In the argon filled glove box, weighed out 1.369 g dopamine (SA #BCBX5420) into the dripper arm. Replaced septa and carried to reaction hood. Attached to center neck of reaction flask. Side neck with septa and needle tubed to an oil-bubbler gas trap. Removed oil-bubble trap during vacuum flushing of dripper arm with dopamine 3 times. Reattached needle and oil-bubble trap. Argon set to flow at about 60 cc/min. Added about 20 mL KH_2PO_4. Allowed to argon sparge/stir/dissolve for about ½ hour. Some dopamine still in neck (stop-cock) of dropper arm which was dislodged when opened and closed several times. Then allowed to drip and react overnight under argon flow.
Placed argon sparged ethanol (~2L) into refrigerator overnight to cool.

On ethanol bottle placed cap with hole drilled in it to feed cannula needle (16 ga) and tubing (18 ga) attached to argon source. Placed other end of cannula into septum on reaction RBF with AlgMA-DA solution and needle from argon source. Flushed ethanol with argon and then closed stopcock on reaction flask to push the AlglMA-DA up and over into the sparged ethanol bottle. Capped off ethanol and precipitate and transferred to glove box with argon continuously flowing. Filtered ethanol/precipitate through coffee filter and Buchner funnel. Collected precipitate off of coffee filter and placed in small tared containers to be placed in freeze drier (about 4 hours for filtering). Placed containers into Harvest Right freeze drier over the weekend to dry. Yield = 1.43 g. Argon flushed containers and placed in freezer for storage.

The resultant 200618AHT batch yielded a whitish fluffy material which was analyzed by FTIR and HNMR. The FTIR peaks corresponded to expected locations indicating hydroxyl, amine, and alkyl moieties. The NMR analyzed against calcium formate internal standard indicated a content of 32 μmoles methacrylate/g ALG-MA-DA and 577 umoles of dopamine/g ALG-MA-DA. NMR and FTIR characterizations are further discussed in Subtask 2 below. Other materials characterizations with this formulation are discussed in Major Tasks 2 and 3.

Methacrylate Calculations

		NMR							
	Sample	peak 8.4 ppm (CaF2)	peakd 5.7 ppm (MA)	ratio	Mol CF2	mole MA	mg sampl	mole MA/mg	**uM MA/g ALG-MA-DOPA**
Methacrylate	ALG-MA-DOPA (Lot200618AHT-A)	0.74	0.16	0.432432	7.67972E-07	3.32096E-07	10.3	3.22E-08	**32**
Dopa	NMR	peak 8.4 ppm (CaF2)	peakd 7.0 ppm (DOPA-3H)			mole DOPA	mg sampl	mole DOPA/m	**uM DOPA/g ALG-MA-DOPA**
	ALG-MA-DOPA (Lot200618AHT-A) (akina500-5914)	0.74	8.59	7.738739	7.67972E-07	5.94313E-06	10.3	5.77003E-07	**577**

In addition to ALG-MA and ALG-MA-DA, we had also developed an alternative material for further investigation as a pleural sealant, dopamine-conjugated, methcrylated gelatin (GEL-MA-DA). The lessons learned above for ALG-MA-DA were applied towards making a gelatin equivalent was also manufactured according to the method detailed in example lots described here:

Gelatin-methacrylate (GEL-MA) (Lot# 190919AHT-A)

Argon flushed 2-neck, 250 ml foil-wrapped round bottom flask equipped with oval stir bar. Add in 100 ml of phosphate buffered saline (PBS, Aldrich P4417). Heated with stirring at 550 RPM to 60 °C under argon flush. Added in 10.003g gelatin (Aldrich cat# SKCB3384). Stirred at 60 °C for 1 hr then cooled to 40 °C. Added into dropper arm 10 ml of methacrylic anhydride (Aldrich cat# 276685-100ml, lot# STBH5178, opened 7-1-19) by Serological pipette. Opened dripper arm to slowly add in MA dropwise. Left to react overnight at 40 °C. Heated ~2L DIH_2O to 40ºC. Poured contents of RBF into 3500 MWCO dialysis tubing and placed in 40 ºC DIH_2O. Water bath refreshed after about 4 hours and left to dialyze over the weekend. Water bath subsequently replaced two more times. Contents of dialysis tubing poured onto a freeze drier tray and placed in Harvest Right freeze drier overnight to dry. Product was a white, fluffy, styrofoam-like material. Placed in tared container and into a refrigerator. Yield was 8.25 g.

GEL-MA-DA (191120AHT-A)

Weighed out 0.6854 g KH2PO4 (Fisher lot #174819) and added 500 mL argon sparged DIH2O. Stirred to dissolve. Added 0.1M NaOH dropwise to pH solution to 5.51. Into 2-neck RBF with stirbar and stopcock, measured 2.02 g GelMA (190919AHT-A). Placed a septum onto 2nd neck of RBF, vacuum purged/argon flushed several times. Added 200 mL KH2PO4 by syringe through septum. Stirred to dissolve in 30ºC heated aluminum bead bath overnight. In a separate 250 mL RBF with oval stirbar, attached a rubber septum. Used needle to vacuum purge ~ 2 min and backflush with argon, several times. Removed septum, quickly added in 5.745 g EDC (SA #SLCC4199) and 4.271 g NHS (SA #MKBS3566V). Reattached septum and vacuum purged/argon back flushed three times. Injected ~60 mL KH2PO4 solution and stirred to dissolve. Attached double-tipped cannula between NHS/EDC solution RBF and the main reaction RBF with GelMa solution in it. Used argon pressure to push NHS/EDC solution up and over into the GelMA solution RBF, about 20 minutes.

Stirred to react EDC/NHS solution with GelMA for about 45 min. In a separate 250 mL RBF with oval stirbar, wrapped with aluminum foil, attached a rubber septum. Used needle to vacuum purge/argon back flush. Removed septum and quickly added ~12 mg BHT (SA lot#BCCB4438) and ~7 g Dopamine HCl (SA lot #BCBX5420). Reattached septum and vacuum purged/argon back flushed solids three times. Injected ~60 mL of KH2PO4 solution and stirred to dissolve ~30 min. Attached double-tipped cannula between Dopamine/BHT solution RBF and main reaction RBF with GelMA (EDC/NHS) in it. Used argon pressure to push Dopamine solution up and over into GelMA/NHS solution RBF. Reacted overnight under slow argon flush. Placed argon flushed Ethanol (~3L) into refrigerator overnight to cool.

On ethanol bottle placed cap with hole drilled in it to feed cannula needle and tubing (18 ga) attached to argon source. Placed other end of cannula into septum on reaction RBF with GelMA-DA solution and needle from argon source. Flushed ethanol with argon and then closed stopcock on reaction flask to push the GelMA-DA up and over into the sparged ethanol bottle. This took several hours and di not finished by the end of the work day. Sealed off flask and placed ethanol bottle (with precipitate-argon flushed again) in refrigerator overnight.
Finished flowing GelMA-DA solution from RBF through cannula needle to ethanol with pressure from argon. Vacuum filtered ethanol/ppt through coffee filter in Buchner funnel. As filtering slowed down, scrapped off any ppt and placed in argon filled tared container and argon flushed again until opened again. Went through several filters as ppt slowed down filtering and needed to keep it from exposure to air as short as possible. After last of the ppt was filtered and placed in container, argon filled one more time before placing in Harvest Right freeze drier overnight.

Overall Summary for Major Task 1: Subtask 1

Through the course of working on Subtask 1, several features of the reaction chemistry were elucidated. The systematic application of methacrylate to the alginate requires careful pH control through the use of a less-caustic basifying agent in order to both achieve good degree of methacrylation as well as minimalize any degradation of the alginate backbone. The formation of ALG-MA by this methodology is robust and routinely reproducible.

Dopamine in liquid state is extraordinarily oxygen sensitive and requires protection beyond the normal practices of typical laboratory conditions. Performance of the dopamine conjugation requires absolute air (oxygen)-free conditions in order to minimize the rapid and spontaneous oxidation of this reagent. To feasibly achieve this, several modifications were made to the general method in terms of how certain steps are performed to focus on steps which are more amenable to air-free reaction measures. Under the right conditions, the formation of ALG-MA-DA is reproducible however the degree of care required in the reaction makes the process onerous and subsequent steps will focus on simplifying the methodology so that manufacture, particularly large-scale manufacture, can be routine and efficient.

Recent work has focused on the dopamine conjugation methodology. The applied dopamine reagent is in 4X molar excess. In normal chemistry the only motivating factor to reduce reagent usage is typically cost, however, given dopamine's capacity to self-polymerize, oxidize, and participate in other reactions, it may improve the quality of the material to not use more than the requisite minimum amount of dopamine in these conjugation reactions. Current efforts are focused on minimizing the dopamine for conjugation to only the absolute minimum quantity necessary.

Subtask 2: Standardize characterization of ALG-MA-DA patches: NRM, FTIR

Characterization of ALG-MA-DA by NMR and comparable methods is a critical tool for ensuing batch to batch consistency and reproducibility. Characteristic spectra are depicted in the below schematic (**Figure 5**).

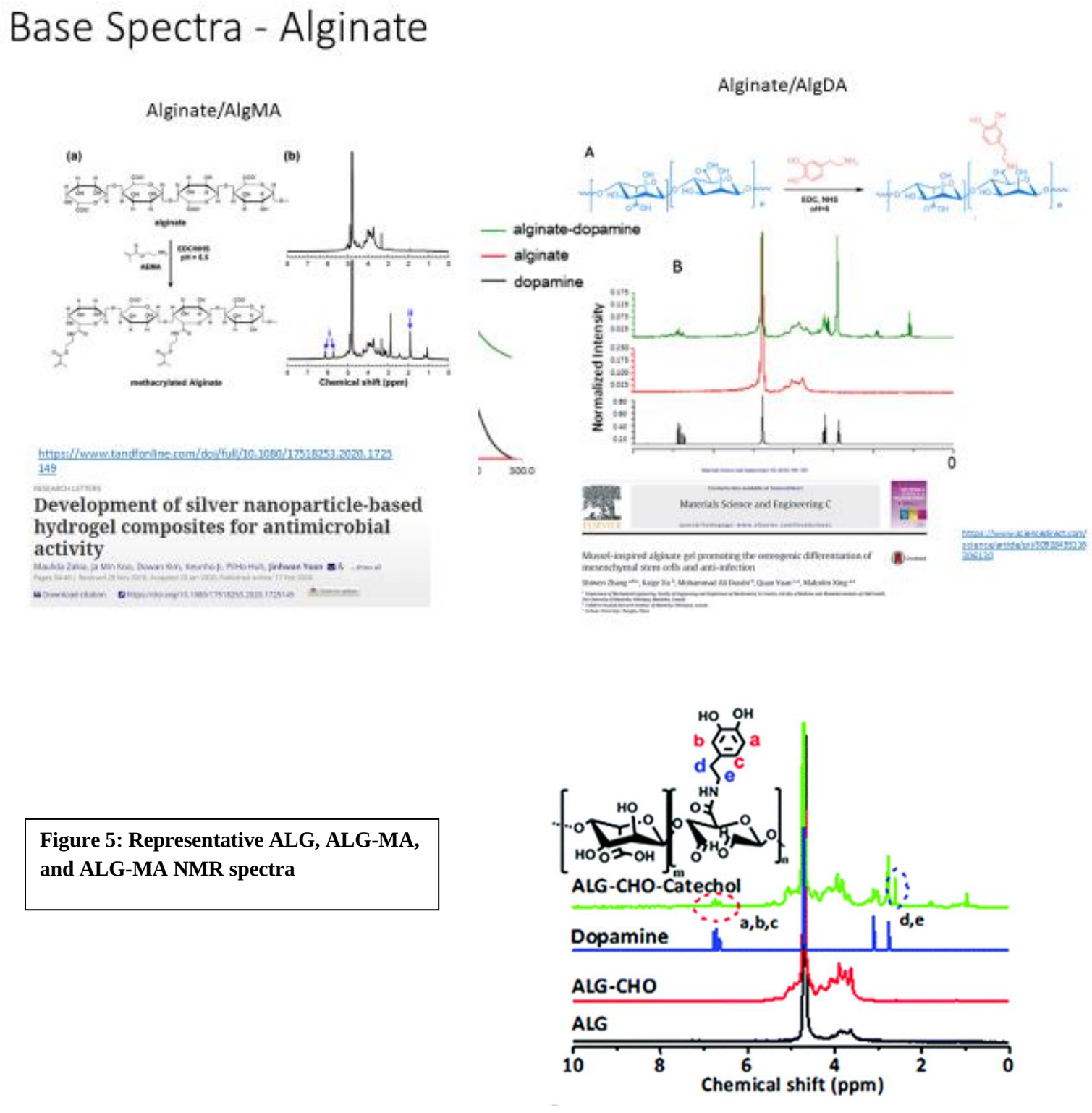


Figure 5: Representative ALG, ALG-MA, and ALG-MA NMR spectra

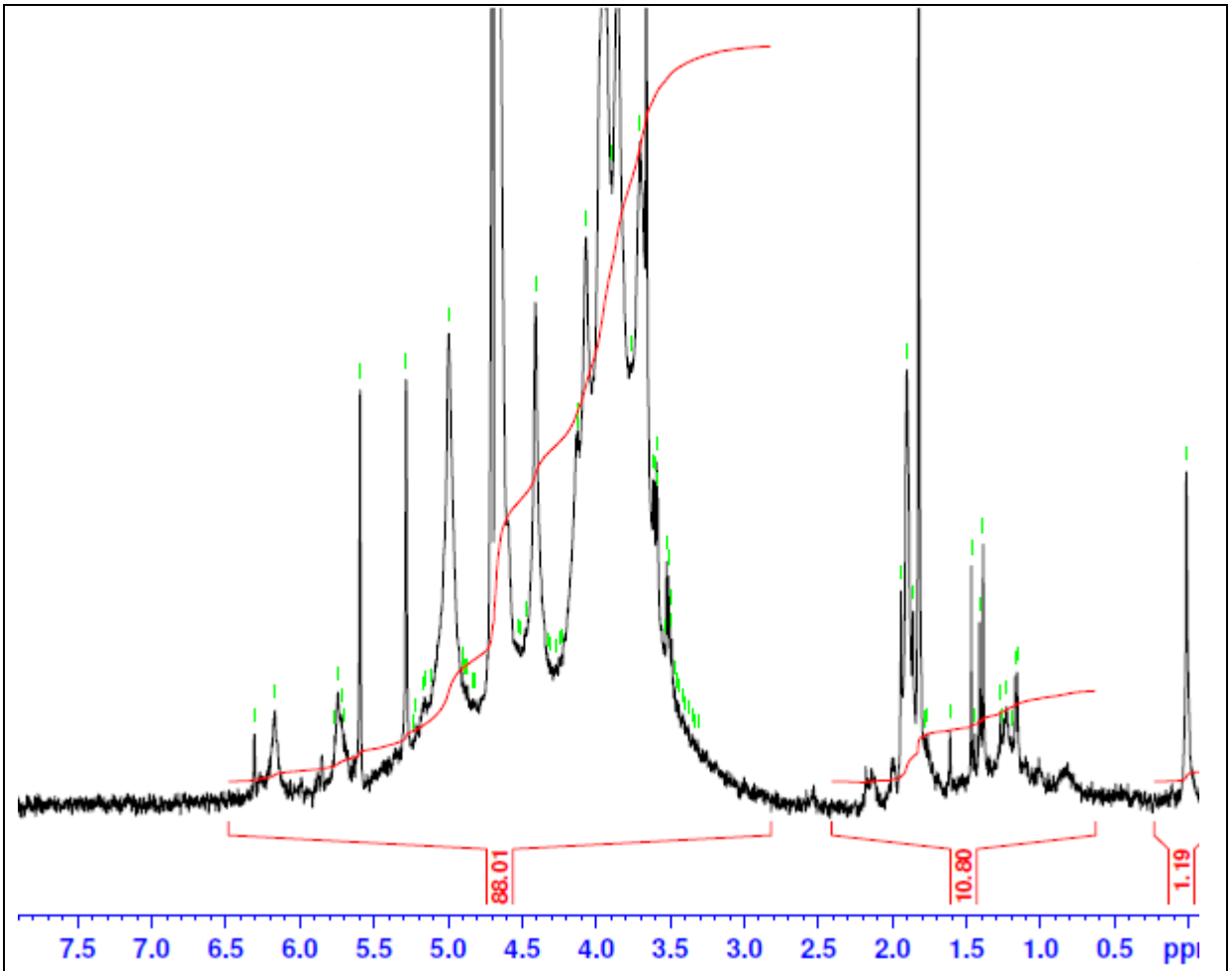
The initial NMR methodology utilized provided for analysis of the batches via HNMR. An example of this method is shown below:

NMR

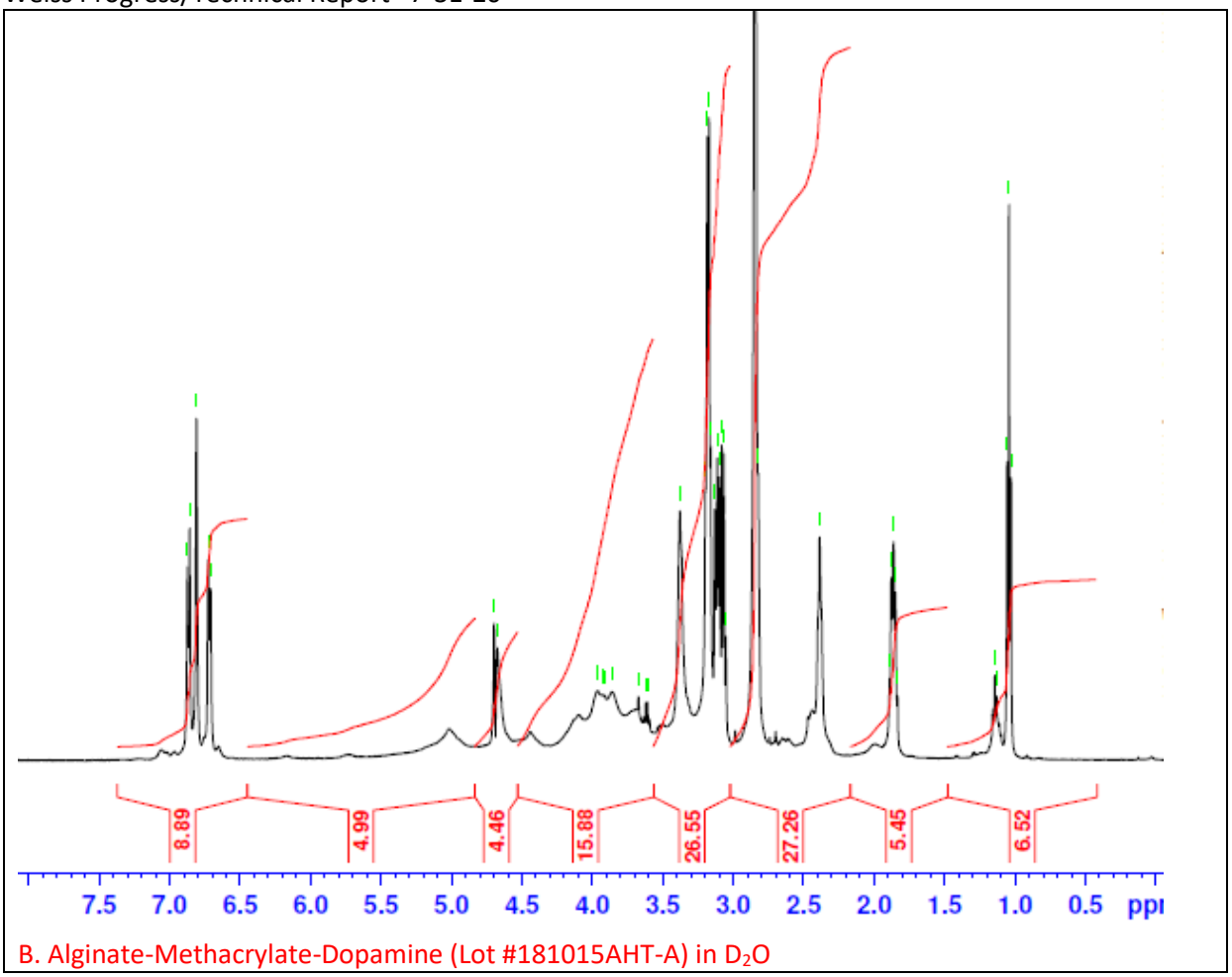
Samples were dissolved ~ 5-10 mg in 0.8 ml of D_2O and transferred into a NMR tube. The NMR spectrum was collected from indicated solution by Purdue Interdepartmental NMR Facility (PINMRF http://www.pinmrf.purdue.edu/).

Example resultant spectra from some test batches are displayed here (**Figure 6**). In this case it is for representative sample ALG-MA and ALG-MA-DA batches.

Figure 6. Representative NMR for Akina produced ALG-MA (A) and ALG-MA-DA (B)



A. Alginate-MA (Lot# 180921AHT-A) in D_2O



B. Alginate-Methacrylate-Dopamine (Lot #181015AHT-A) in D_2O

Based on the literature (**Figure 5**), methacrylate vinyl units demonstrate NMR peaks at ~ 5.5 ppm and 6.1 ppm as shown in example below for methyl methacrylate while dopamine displays measurable peaks around 6.8 ppm as shown for loose dopamine in reference data below.

Although peaks corresponding to these moieties have been generally reproducibly observed within successive ALG-MA and ALG-MA-DA batches, the prevalence of broad peak overlap and variability in peak area within the general polysaccharide region makes quantifying or comparing the data difficult. For this reason, a calcium formate internal standard was spiked into the NMR samples to provide for a comparison peak to evaluate the quantity of each peak component. This was done as follows as an example method.

NMR – Internal Standard Method

An internal standard stock solution was prepared by dissolving 10.4 mg calcium formate (Aldrich cat# 03826-1G) in with 10.408 g of D_2O (Cambridge Isotopes). Tests were performed using this solution with addition of 0.10 µl of stock solution to prepared samples of indicated mass (~ 5-10 mg) diluted up to 0.8 ml using D_2O. These samples were transferred into a NMR tube (Wilmad glass). The NMR spectrum was collected from indicated solution by Purdue Interdepartmental NMR Facility (PINMRF http://www.pinmrf.purdue.edu/).

The resultant spectrum from some examples of these is shown in **Figure 7** below. In many cases the default NMR spectra provided by PINMRF combined multiple peaks together for a single integration so these were separated and integrated individually using ACD/Spectrus software. Raw data and additional spectra are available on request.

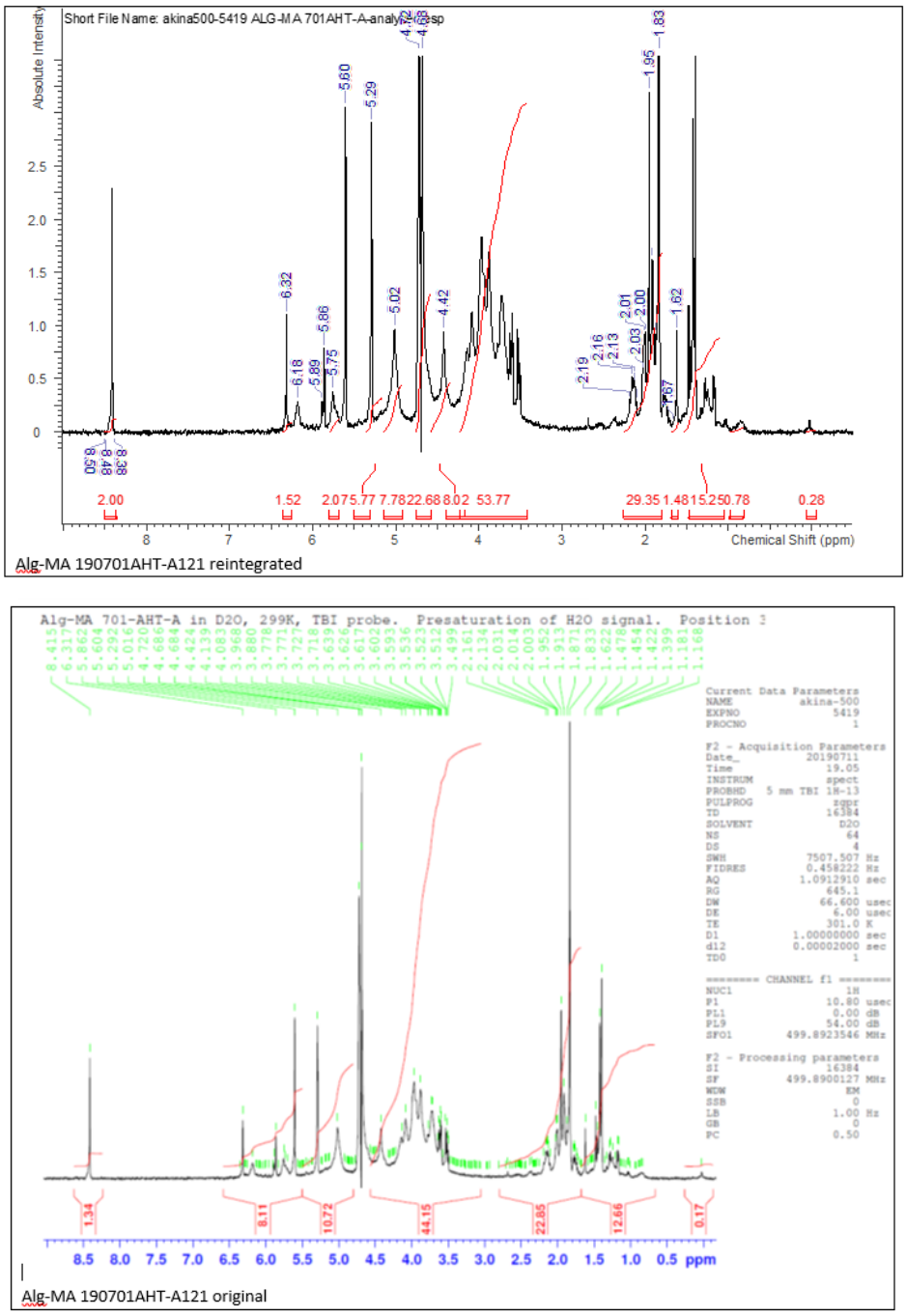


Figure 7. Examples of NMR spectra including calcium formate internal standard (peak at 8.4 ppm).

By comparing the peak intensity at ~ 8.4 ppm (calcium formate, **2H**) and the methacrylate peak at 5.7 ppm (**1H**) the quantity of methacrylate can be determined as follows:

Mc(g) = (Vs(ml) x Cs(mg/ml))/1000
Pm/(Pc/2) x (Mc(g)/130.113) = mole MA
mole MA/mg sample * 1000000000 = micromoles MA/g Alg-MA

Where Pm is peak integration of methacrylate peak at 5.7 ppm, Pc is peak integration of calcium formate at ~ 8 ppm. Mc(g) is mass of calcium formate in grams calculated using volume of stock "Vs(ml)"and concentration of stock "Cs(mg/ml)".

Similarly, the value for dopamine was determined according to the peaks for 3H of the benzyl chain from 6.4 – 6.8 ppm The only change for this calculation was dividing the peak integration for the DOPA signal. Here "Pd" is the dopamine peak around 6.5 ppm. (Pd/3)/(Pc/2) x (Mc(g)/130.113) = mole MA

The other calculations are the same as performed for methacrylate determination.

So, for the representative examples in **Figure 7** and other similarly synthesized ALG-MA and ALG-MA-DA batches, the methacrylate and dopamine content was evaluated as follows:

Table 1. Methacrylation and Dopamine (micromole MA/g sample) of indicated samples.

Sample	Methacrylation (micromole MA/g sample)	Dopamine (micromole DA/g sample)
Alg-MA (190701AHT-A121)	215	N/A
Alg-MA-DA 190724AHT-AV	394	4768
Alg-MA-DA 190724AHT-AFD	606	8131

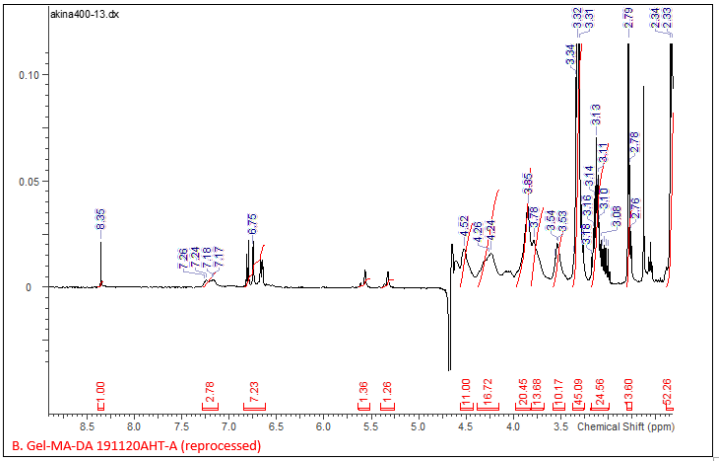
Gel-MA-DA characterization by NMR

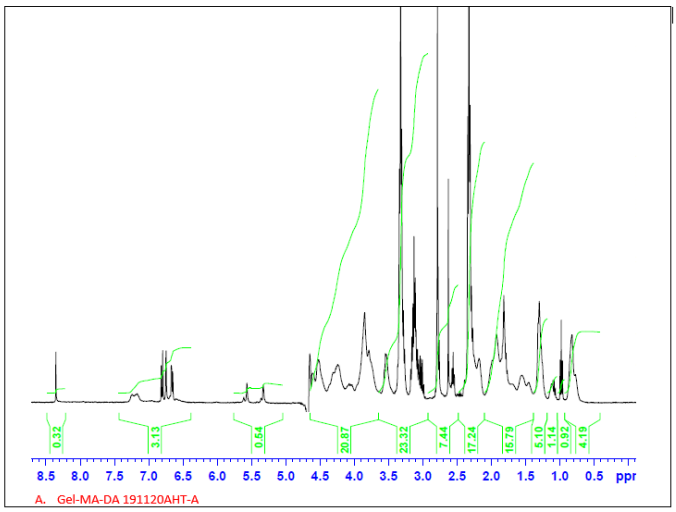
In addition to ALG-MA-DA, the manufactured GEL-MA-DA was also characterized. This data is from the previously described Gel-Ma-Da (Lot# 191120AHT-A) described in section "Subtask 1: Develop large scale synthesis approach for ALG-MA-DA patches" above.

GEL-MA-DA NMR Characterization

A sample of the batch was dissolved in D_2O and tested by HNMR. The resultant spectrum is shown in **Figure 8** below. If necessary, spectral reprocessing was performed using ACD/Spectrus (2015) software. Raw data is available for these spectra upon request.

Figure 8. GEL-MA-DA HNMR spectra





As with ALG-MA-DA, By comparing the peak intensity at ~ 8.4 ppm (calcium formate, **2H**) and the methacrylate peak at 5.7 ppm (**1H**) the quantity of methacrylate in GEL-MA-DA can be determined as follows:

Mc(g) = (Vs(ml) x Cs(mg/ml))/1000
Pm/(Pc/2) x (Mc(g)/130.113) = mole MA
mole MA/mg sample * 1000000000 = micromoles MA/g Alg-MA

Where Pm is peak integration of methacrylate peak at 5.7 ppm, Pc is peak integration of calcium formate at ~ 8 ppm. Mc(g) is mass of calcium formate in grams calculated using volume of stock "Vs(ml)"and concentration of stock "Cs(mg/ml)".

Similarly, the value for dopamine was determined according to the peaks for 3H of the benzyl chain from 6.4 – 6.8 ppm (Fig 2). The only change for this calculation was dividing the peak integration for the DOPA signal. Here "Pd" is the dopamine peak around 6.5 ppm. (Pd/3)/(Pc/2) x (Mc(g)/130.113) = mole MA

The other calculations are same as performed for methacrylate determination.

Table 2. Methacrylation and Dopamine (micromole MA/g sample) of indicated samples.

NMR Calculations								
Sample	peak 8.4 ppm (CaF2)	peaks 5.7 ppm (MA)	ratio	Mol CF2	mole MA	mg sample	mole MA/mg	**uM MA/g Gel-MA-DOPA**
Gel-MA-DA (Lot# 191120AHT-A)	1	1.36	2.72	7.68E-07	2.09E-06	5.00	4.2E-07	**418**
NMR	peak 8.4 ppm (CaF2)	peaks 6.4-6.8 ppm (DOPA-3H)			mole DOPA	mg sample	mole DOPA/mg	**uM DOPA/g Gel-MA-DOPA**
Gel-MA-DA (Lot# 191120AHT-A)	1	7.23	4.82	7.68E-07	3.70E-06	5.00	7.4E-07	**740**

FTIR

In addition to NMR, FTIR analysis has been performed on the samples. FTIR is commonly applied as a routine analytical technique for the determination of chemical moieties in a sample as a qualitative process of chemical identification. This was done using a sample compressed in a KBr salt-pellet and placed in Nicollet Avatar 320 spectrometer and analyzed in transmission mode. **Figure 9** below shows resultant spectra from example products.

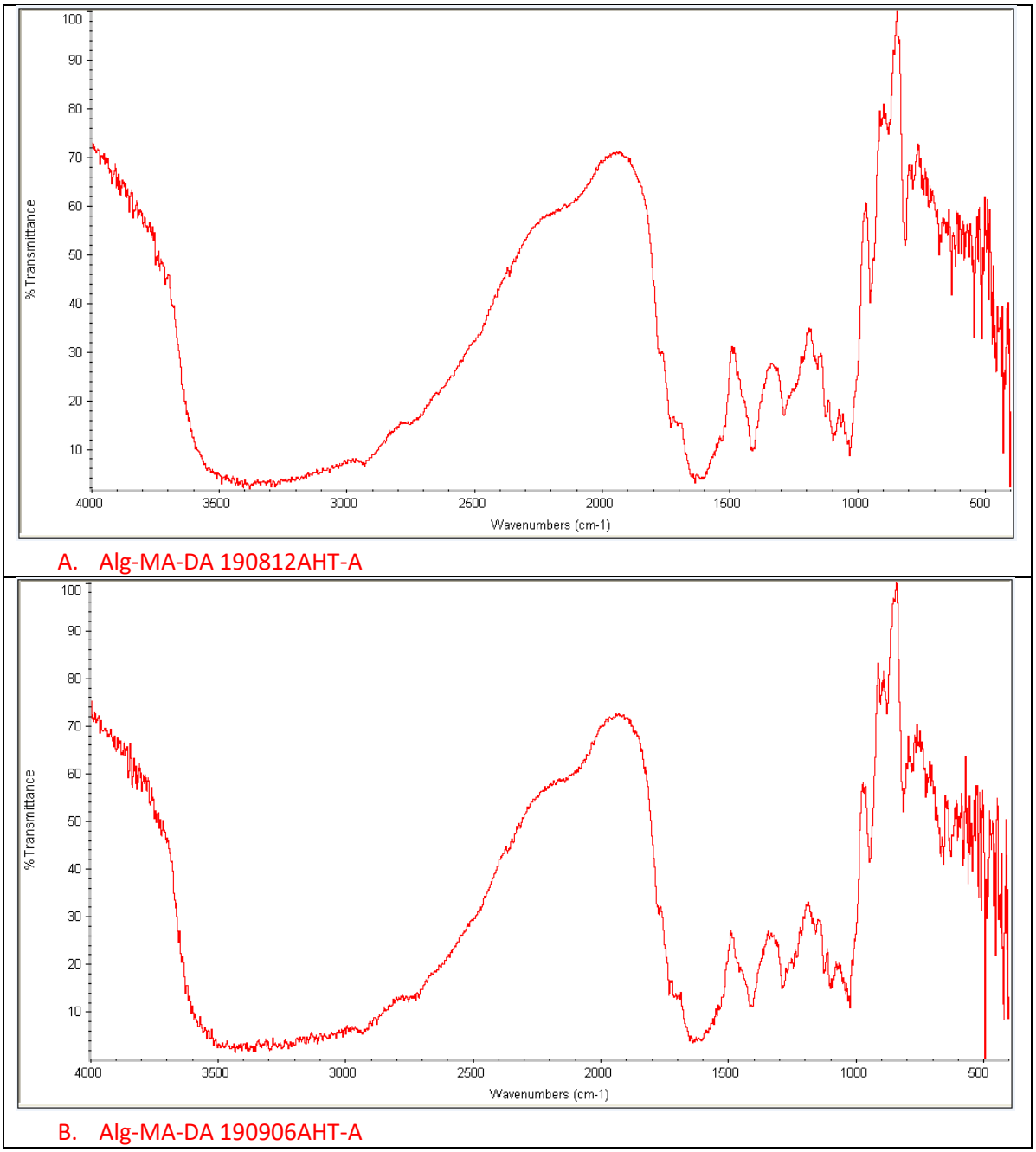
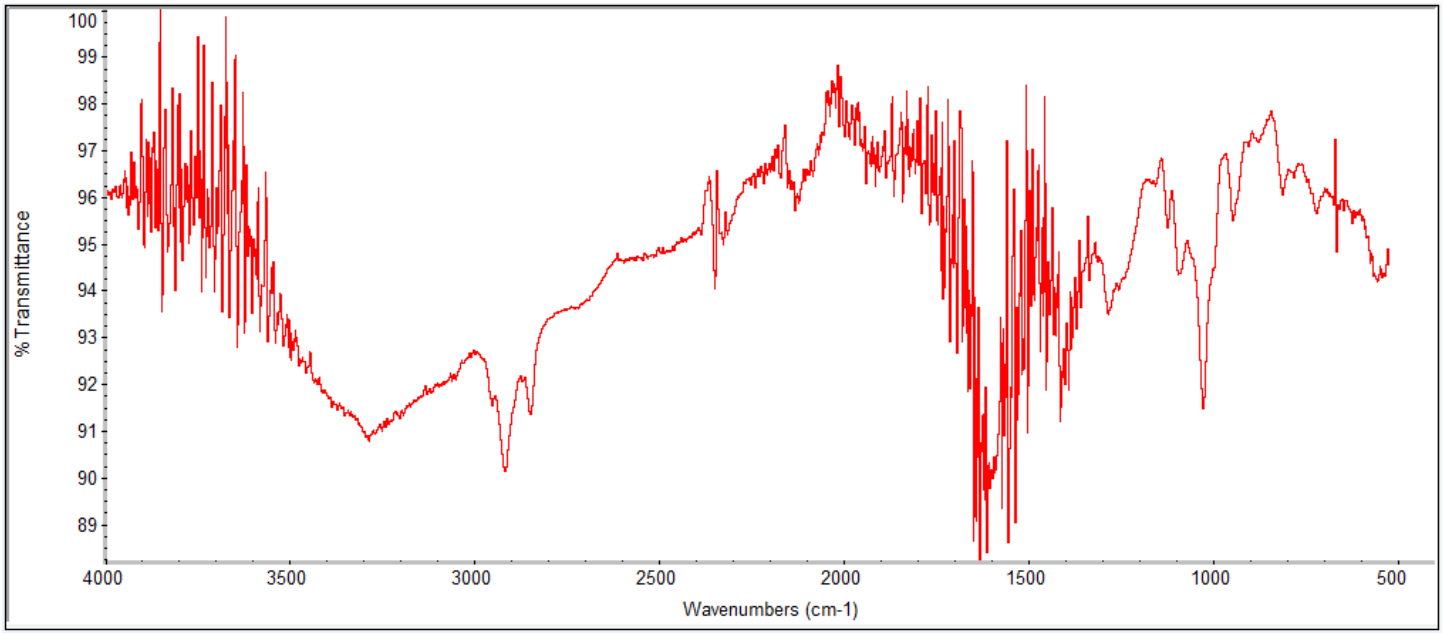


Figure 9. FTIR results of indicated samples

The data was compared to peak assignments from public domain sources (https://webspectra.chem.ucla.edu/irtable.html, https://orgchemboulder.com/Spectroscopy/specttutor/irchart.pdf) strong peak at 3000-3500 correlates to hydroxyl and amine stretching from a multitude of hydroxyl (O-H) and some amine (N-H) bonds. Peak at 1600-1700 corresponds to carbonyl stretching (C=O) from esters and amides. Peak around 1400 ppm corresponds to C-H bending (alkane), 1300-1200 ppm to C-O stretching, and peak around 1000-1200 to C-N stretch. It should be noted that several of these peak regions overlap and so the assignments are not conclusive. That being said, the absorption peaks observed in the sample correspond to expected moieities in the ALG-MA-Da product.

FTIR was also analyzed using a Nicolete Avatar model 380 with diamond ATR module. For this module, the sample was simply compressed against the sampling window with no particular preparation steps applied.

Figure 10: Alg-MA-DA 200618AHT-A FTIR collected by ATR.



The relatively reduced absorbance overall provided further definition to the peaks in the 2800-3000 range which correlate to general alkyl (C-C, C-H) spectra. Otherwise, the FTIR spectra are in general agreement with one another regardless of collection method.

Between the two techniques, HNMR provides a great deal of more information both about the qualitative chemical nature and quantitative properties of the material. In this case, HNMR is a superior method for routine evaluation of the produced batches. FTIR may be applied as a supplemental information technique but HNMR will primarily be used for the characterization of these batches.

Overall Summary for Major Task 1: Subtask 2

Through the course of working on Subtask 1, several features of the hNMR and also FTIR analytical methods were refined. These will continue to be powerful tools for continuing materials evaluations.

Crosslinking and Gelation

In addition to synthesis and NMR/FLIR characterization, we incorporated further studies on ALG-MA-DA gelation into Major Task 1. This became necessary when it was observed that several batches of ALG-MA-DA produced at Akina failed to gel appropriately when subsequently analyzed at UVM. This resulted in need to explore other cross-linking agents in place of the original Eosin-Y/TEOA and we have now identified LAP as a suitable and reproducibly successful cross-linking agent.

Previous gel cross-linking approach: In brief, a 4% methacrylated polymer solution in DiH_2O was generated and placed on shaker to dissolve. A 0.5% Eosin Y (Aldrich MKBW0030V) in 1-Vinyl-2-Pyrrolidinone (1V2P) (Aldrich MKCC6000) was generated and dissolved on the shaker. A 5M triethanolamine (initiator, TEOA) (Aldrich MKCC8886) in DiH_2O solution was created and dissolved with shaking. To test, 5µL of Eosin Y/1V2P solution and 50 µL TEOA solution was added to 2 ml of the 4% w/v alginate-methacrylate solution and mixed thoroughly with the vortexer. Parafilm was wrapped around both ends of a microscope slide to create space between the top and bottom slides. Alginate-methacrylate solution mixed with photoinitiator (100 µL) was pipetted onto the space between these slides. The slide was then exposed to visible green light of wavelength 510 nm until cross-linked.

This photocrosslinking approach using EY/1NVP + TEOA, had previously been demonstrated to be effective and repeatable when preparing gels/patches from Methacrylatred Alginate (AlgMa) or Methacrylated Gelatin (GelMa). However, during our initial studies using AlG-MA-DA, we discovered that addition of the Eosin-y/TEOA photocrosslinker was causing fairly rapid oxidation of the solution during the preparation of gels and patches. After further investigation we determined that addition of the EY/1NVP + TEOA photocrosslinker caused a moderate increase in the pH of the AlgMaDa solution from 7.0 to ~ 9 – 10. Higher pH is known to increase the favorability of oxidation reactions and we suspect that this was a major contributor to premature oxidation.

To address this challenge we examined additional candidate photocrosslinking agents and found that LAP (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate, source: Allevi) had no significant effects on the pH of the ALG-MA-DA when dissolved into solution, and no obvious oxidation was observed during mixing. When preparing gels using this new formulation, the solution remained clear when exposed to room air for short periods of time (5 – 10 minutes) (**Figure 11**). This new formulation was successfully photocrosslinked using a 385 - 405 nm UV/blue light source. More importantly, no color change was identified to indicate oxidation during the gelation process. The resulting gels were frozen and lyophilized to prepare patches for further testing. **Figure 11** further demonstrates the comparison between the use of these two photoinitiator systems at various stages of the patch preparation and application process. The results highlight a clear different in the resulting patches and suggest that use of LAP negates the challenge of premature oxidation. Further studies comparing the effectiveness of formulations with the goal of improving adhesiveness are discussed below as part of Major Task 2.

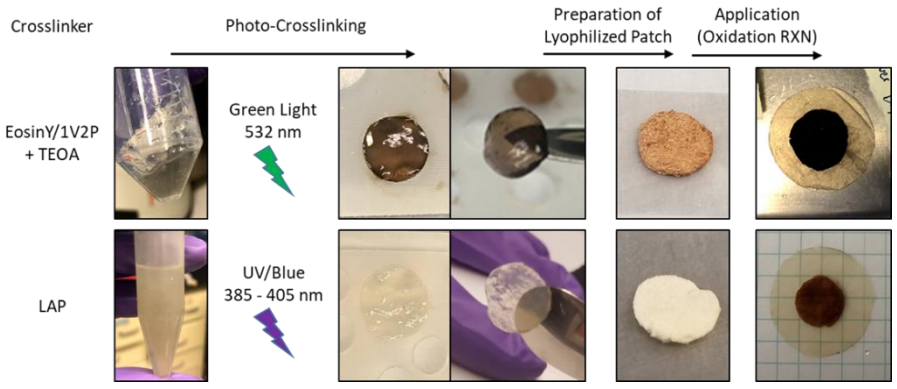


Figure 11. Step-by-Step Summary of the Patch Preparation Process Demonstrating the Qualitative Comparisons between the original (Eosin Y/1V2P/TEOA) and candidate (LAP) photo-crosslinking systems

Specific Aim 1, Major Task 1: Significant Milestones Achieved:

A) Progress towards large scale ALG-MA-DA patch synthesis with controllable, reliable, and reproducible degrees of methacrylation and dopamine conjugation.

- Routine, robust and simplified synthesis of ALG-MA precursor via externally controlled pH using a non-caustic basifying agent.
- Successful synthesis of ALG-MA-DA using air-free techniques to limit dopamine oxidation
- Initiated dopamine quantity optimization and other methodological changes to make ALG-MA-DA synthesis routine and simple

B) Identification of a more effective cross-linking agent (LAP) than the previously utilized Eosin-Y/TEOA.

- Successful change to a different photo-initiator that has reliably and reproducibly allowed effective patch formation from improved ALG-MA-DA materials

Major Task 2: Develop quality control approaches for rheologic and mechanical characterization of ALG-MA-DA patches.

Subtask 1: Burst pressure and analyze cohesion and adhesion assessments on collagen substrates.
Subtask 2: Degradation of standardized patches
Subtask 3: Cytotoxicity of standardized patches

Subtask 1: Burst Pressure

A) Technical advances

As part of the original proposal, we had previously designed a custom modified burst pressure test apparatus was constructed that allowed testing under submerged conditions. This more accurately reflects biological conditions for pleural and tracheal sealants than standard ASTM testing conditions. We have subsequently developed an oscillatory syringe pump-driven burst pressure device that can mimic pressure, frequency, and amplitudes that a sealant will encounter if placed on a functioning lung (**Figure 12**).

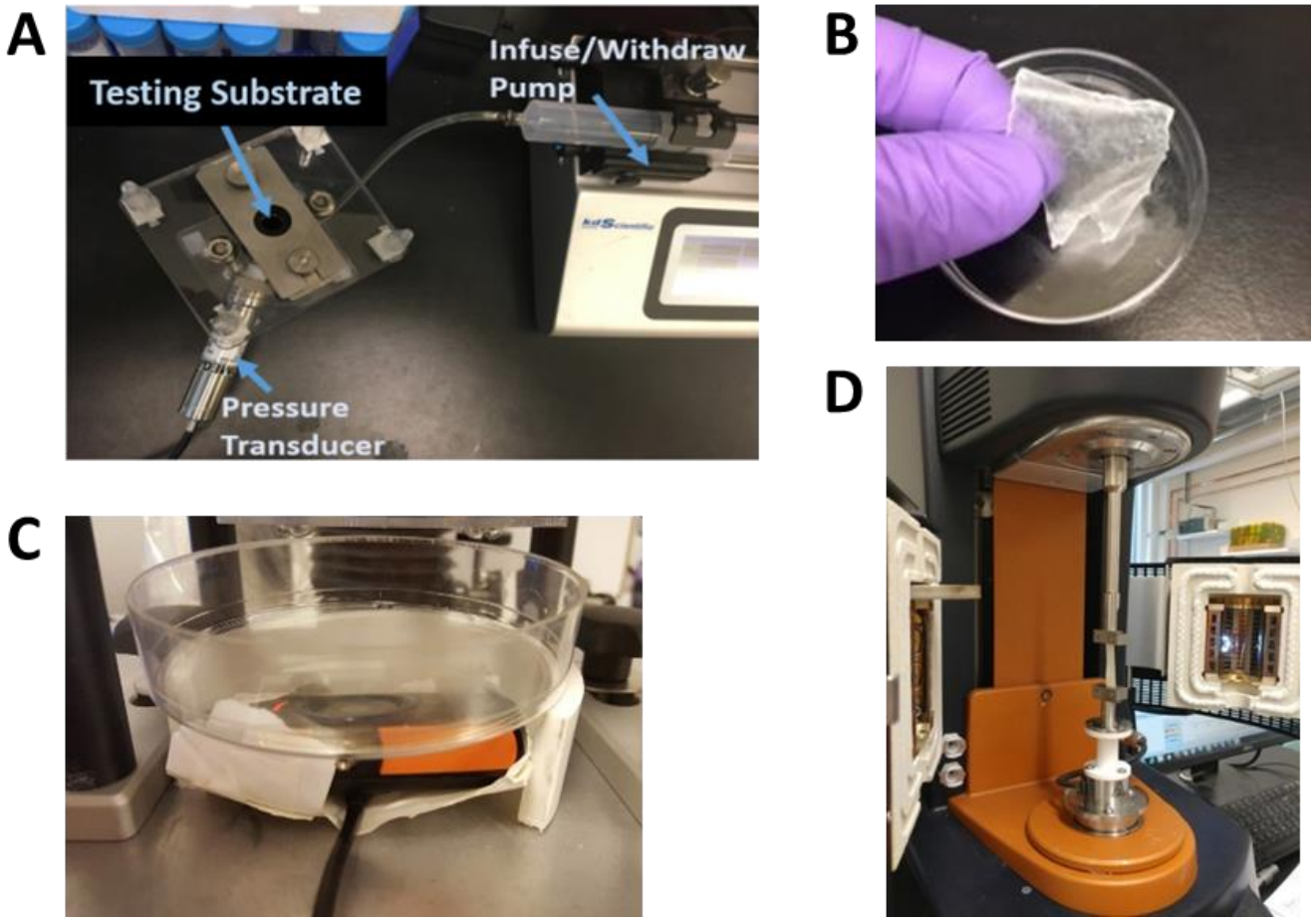


Figure 12: A) Overview of syringe pump driven burst pressure testing.

B) Representative decellularized pig lung pleura.

C) Camera and splash-shield for imaging condition. Splash-shield can be filled with water, saline or acidic/basic solutions and raised to allow for wet testing.

D) Advanced Rheometer device with which to correlate burst pressure assessments

The underlying rationale for developing a tunable oscillatory burst pressure strategy is that this will better further mimic the relevant physiologic environment a pleural sealant will encounter. To assess this, an easily testable protocol algorithm was developed as follows:

Ramping Oscillatory Pressure Testing: A circular defect of 2 mm in diameter is made in a substrate made of collagen or decellularized pleura, substrate is attached to a pressure chamber on a custom burst pressure device, sealant is applied to the substrate defect as a pre-formed patch (5 mm diameter) or as a liquid (100 uL) and polymerized. The chamber is cyclically pressurized for 5 cycles at 5 cmH2O before pressure is increased by a cyclic step of 5 cmH20 until material failure (e.g. 5 cmH2O for 5 cycles, then 10 cmH2O for 5 cycles, then 15 cmH2O for 5 cycles, etc. until material delamination/failure).

Initial comparisons of dynamic and standard static burst pressure testing strategies demonstrates significant relevant similarities and important differences for a representative test sealant (gelatin methacroyl) demonstrating the value of dynamic material testing in generating more physiologically relevant values. Preliminary data demonstrates that sealants which can withstand relevant physiologic pressures of up to 3kPa under current ramping pressurization strategies fail at sub-physiologic pressures when subjected to minimal cyclic pressures (**Figure 13**). Dynamic testing leads to lower burst pressure values than static ramping approaches which is a potential explanation why sealants fail in the clinic based on too high predicted values from current ASTM testing approaches.

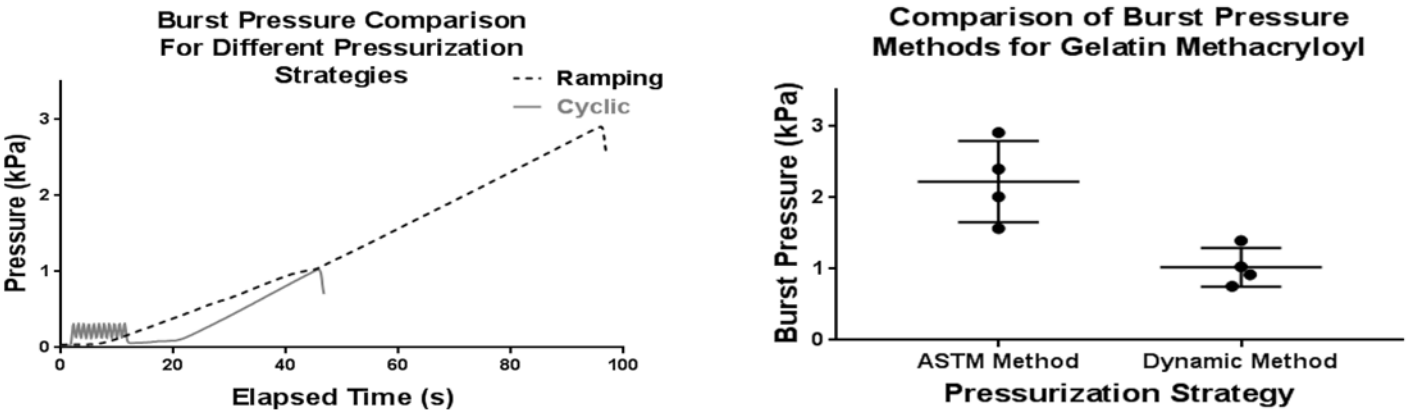
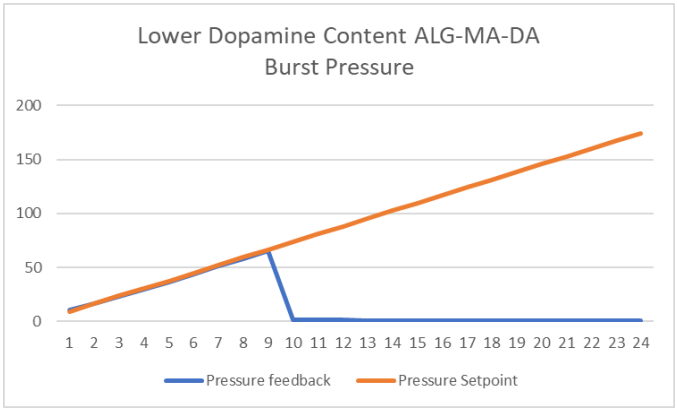


Figure 13: A 15%w/v gelatin methacryoyl solution with 0.1%w/v eosin y photoinitiator was subjected to either a ramping pressure of 1 mL/min [Ramping] until failure, or 10 cycles of pressurization to 300 pascals followed by a ramping pressure of 1mL/min until failure. Representative pressurization curves are shown to the left while means of 4 trials per strategy are shown at the right.

Oxidation and Sealant Adhesion to standard ASTM sausage casing (collagen) for burst pressure testing
The original oxiding agent utilized is 0.1% sodium metaperiodate. This was based on use with the Eosin Y/TEOA cross-linking agent which has some intrinsic cross-linking activity. Now that the cross-linker has been changed to LAP, which has no appreciable effect on dopamine oxidation, further refinement of the concentration and timing of metaperiodate application is being systematically evaluated. 1% w/v appears to cause significant and functional oxidation and ALG-MA-DA patch within approximately 5-10 minutes but this is undergoing further refinement as is the amount to be added relative to patch area.

B) Comparative burst pressure assessments: Representative examples

Utilizing the advances in both materials (Major Task 1) and in technical advances, we are systematically evaluating burst pressures for the ALG-MA-DA patches produced under the different conditions, for example low vs high dopamine content, and further comparing these to results obtained from comparable testing of Progel. Some representative figures are depicted below (**Figure 14**).



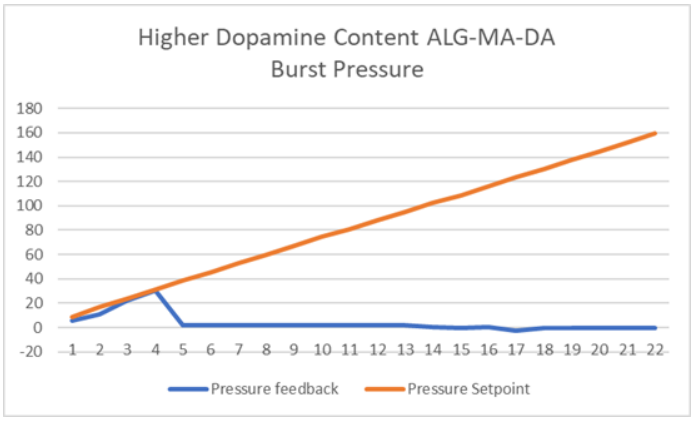
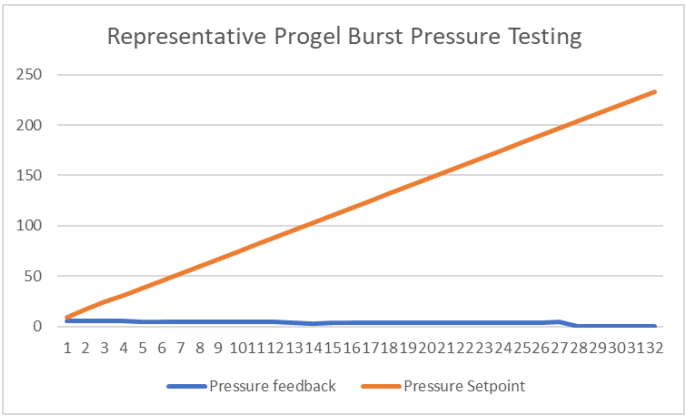


Figure 14 Comparative burst pressure assessments of low vs high dopamine content ALG-MA-Da vs Progel. Pressures are indicated on the Y-axis in cm H_2O and time in seconds on the X-axes. Pressure setpoint (orange line) indicates applied ramped pressures and pressure feedback (blue line) indicates measured pressures. The divergent point is the burst pressure.



The results depicted demonstrated that dopamine content can significantly affect ALG-MA-DA burst pressure in physiologic pressure ranges a patch may clinically encounter in unjured or diseased lungs. An ongoing goal for this aim is to continue to systematically define ALG-MA-DA materials and patch synthesis approaches that result in burst pressure appropriate for pleural application.

C) Tensile testing

Adhesion

Another important materials property is tensile strength and durability. Following initial successful screening utilizing the standard test method for strength properties of tissue adhesives in lap-shear by tension loading according to ASTM F2255-05, we have encountered variable results with the different materials. These have been mostly due to slipping and tearing at the clamp site. A variety of trouble shooting approaches have been systematically explored including embedding the end of the ALG-MA-DA patch test strips in cloth, sand paper, or polypropylene mesh. Sandpaper has proven most effective to date but we have also had to redesign the test mold itself. These are ongoing studies.

D) New mold methods

For materials testing and subsequent application to pleural and tracheal surfaces, optimization of the patch mold has been further systematically explored. We have found that by 3D printing an inverse design silicone mold allows better construction of ALG-MA-DA patches in the desired shapes and dimensions. Silicone provides a highly functional non-adherent, cleanable material. A representative mold and patch are depicted in **Figure 15**.

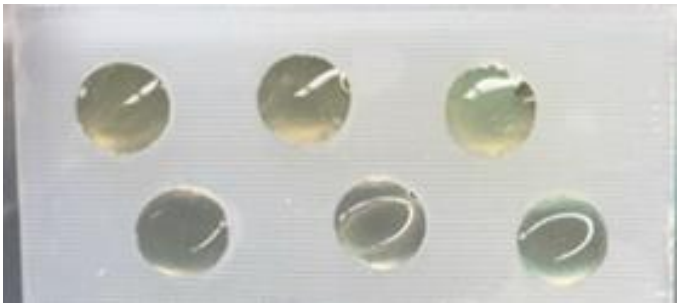
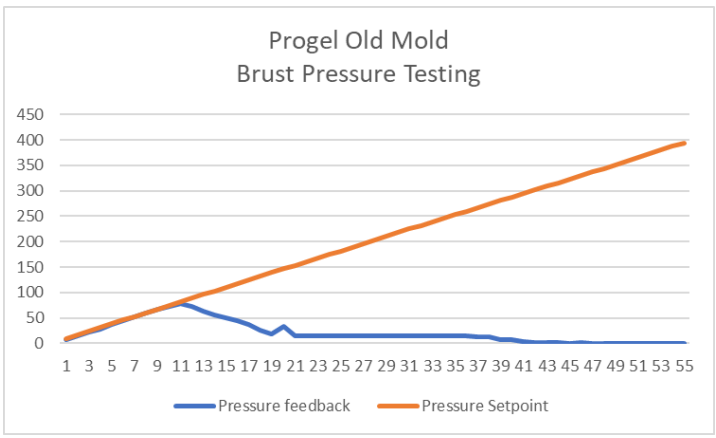


Figure 15: Representative new 3D-printed silicone mold with ALG-MA-DA patches.

This has allowed improved performance of materials utilizing the new vs old molds. A representative burst pressure study is depicted utilizing Progel as the test material (**Figure 16**).



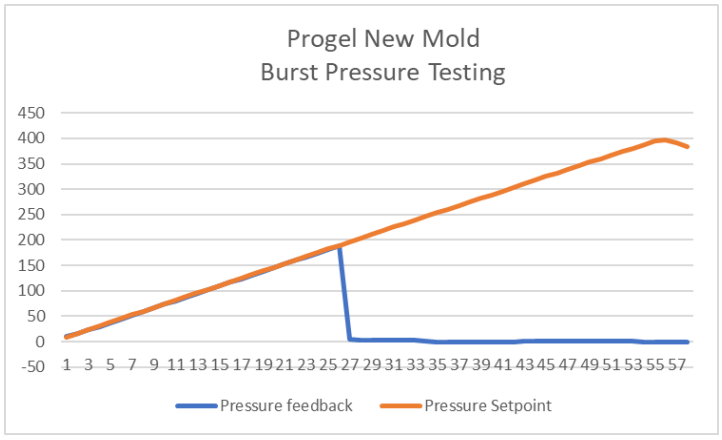


Figure 16: Comparative burst pressure assessments of low vs high dopamine content ALG-MA-Da vs Progel. Pressures are indicated on the Y-axis in cmH_2O and time in seconds on the X-axes. Pressure setpoint (orange line) indicates applied ramped pressures and pressure feedback (blue line) indicates measured pressures. The divergent point is the burst pressure.

Ongoing burst pressure assessments are being done to comparatively assess materials produced with the new (vs old) molds with goal of optimizing the mold utilized for patch formation.

Major Task 2: Subtask 1 Milestone(s) Achieved:

a. Development of a novel oscillatory burst pressure testing system
b. Progress in optimizing tensile testing approaches
c. Easily replicated and tunable patch molds for use in both materials testing (burst pressure, tensile) as well as in subsequent patch applications to pleural surfaces
d. Modifications in protocols for utilizing the oxidizing agent during patch application
e. Systematic comparative burst pressure evaluations of ALG-MA-DA materials produced utilizing the above improvements and also continuing comparisons vs Progel™

Major Task 2
Subtask 2: Degradation of standardized patches
Subtask 3: Cytotoxicity of standardized patches

We have held off on pursuing each of these subtasks until the materials and materials testing described above has been optimized. All of the methodologies for achieving the goals are established in Dr. Weiss' laboratory at UVM.

Major Task 3: Define optimal sterilization, and storage conditions.
Subtask 1: Define optimal sterilization approach for optimized patch

Sterilization Effect
For viable production of clinical material, the generated formulation must be either aseptically handled or terminally sterilized. Due to the cost of asceptic manufacture, terminal sterilization is preferred. A test sterilization was performed using an Anderson ethylene oxide sterilizer on a standard 12-hour cycle on an Anprolene model AN-74i using manufacturer recommended liner bag, gas kit, and dosimeter to confirm successful sterilization cycle.

A sample of ALG-MA-DA was tested by HNMR before and after ETO exposure. These samples were tested by NMR and spectra are demonstrated below (**Figure 17**, other spectra available on request):

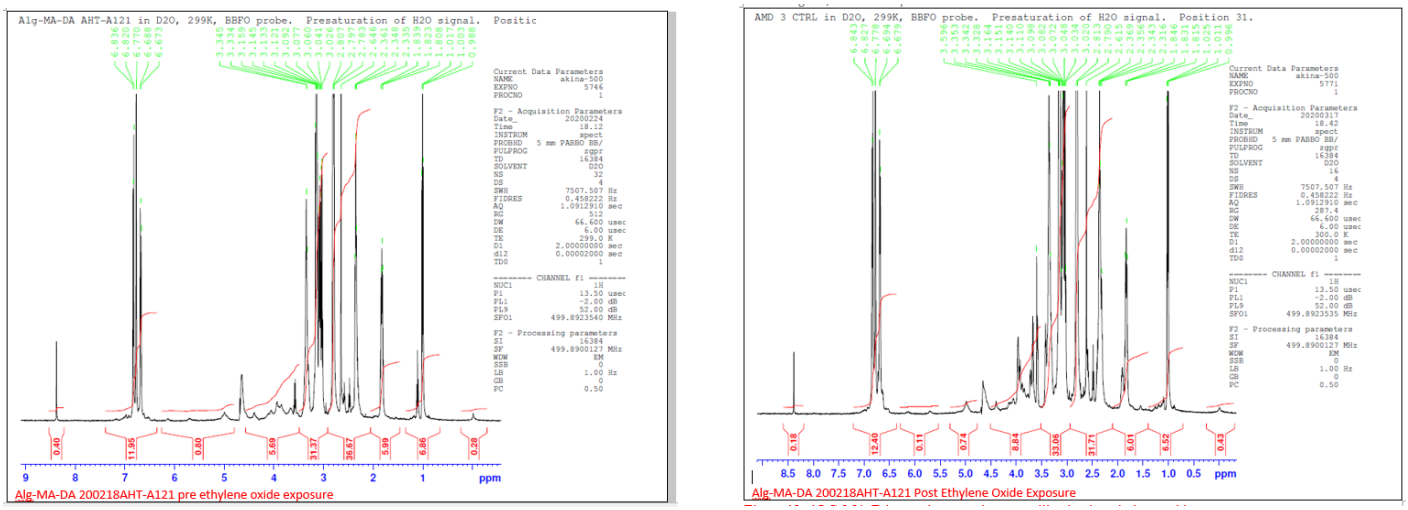


Figure 17. ALG-MA-DA sample pre (L) and post ® sterilization by ethylene oxide.

These spectral results generally indicate that the material has the ability to survive the process of sterilization by ethylene oxide with minimal chemical degradation or interference. Testing has not been performed yet on other sterilization techniques such as autoclave or gamma irradiation however the known harshness of these techniques likely rules them out as sterilization methods anyhow and so future work with sterilization will focus on the more gently ethylene oxide based methodology.

Subtask 2: Clarify need for addition of preservatives to optimized patch

Subtask 3: Define optimal long term storage (packaging, temperature, humidity, etc) conditions

Storage Stability
The ability of the material to possess a functional shelf-life is necessary for use clinically. Storage stability was tested under a variety of conditions as described below. Note that the acronym letters will be used to briefly reference each condition in this report.

FD: Forced Degradation
Southwest Science Shaking incubator set to 40 °C/ 0RPM (not shaking) with sealed container that has hygrometer and bottle of saturated sodium chloride in it. Periodically check the hygrometer and make sure that the needle is between 70-80 and if humidity drops extra water is added to the saturated sodium chloride to maintain.

LD: Light Degradation
Light provided by Tropic sun 5500K Daylight 18 inch 15 watt T8 styled bulb purchased from Zoo med laboratories, Inc. Light exposure performed in a Standard 10 gallon glass aquarium (20 1/4 inch x 10 1/2 inch x 12 9/16 inch, Aqeuon Central Aquatics item # 09010). Vials are placed in bottom of the dry aquarium and illuminated from above by the light source indicated above mounted in the aquarium's stock bulb housing. Vials are spaced apart from one another so as to not shadow each other.

RD: Room temperature degradation
Placed on upper shelf in Akina's laboratory. Exposed at periodic intervals (Mon – Fri, 8AM-5PM) to fluorescent lights. Temperature generally maintained at typical room temperature (25 ± 5°C) by conventional HVAC system.

FT: Samples placed in -20 °C freezer.

Storage testing was done in two stages.
The first storage test used the following conditions for stability of Alg-MA-DA Lot #'s 190812AHT-A121 and 190906AHT-A121 with 1% of listed preservative in a forced degradation chamber at 40ºC/75%RH for one month.

Packaging: Condition →	FD (Forced 40°C/75%RH)	Alg-MA-DA Lot # 190812AHT Wt (mg)	1% Preservative added (µL)	Alg-MA-DA Lot # 190906AHT Wt (mg)	1% Preservative added (µL)	Gel-MA-DA Lot # 191120AHT Wt (mg)	1% Preservative added (µL)
None (closed clear-glass vial in normal air) ("N")	Sample 1 month	13.7	N/A	11.8	N/A	11.3	N/A
Dark (closed, amber-glass vial in normal air) ("D")	Sample 1 month	11.5	N/A	11.2	N/A	9.3	N/A
Argon-Dark (closed, amber-glass vial, argon-flush) ("AD")	Sample 1 month	10.3	N/A	9.6	N/A	9.8	N/A

("AD-CYS") (closed, amber-glass vial in argon with Cysteine)	Sample 1 month	9.7	9.7	10.3	10.3	10.4	10.4
("AD-EDTA") (closed, amber-glass vial in argon with EDTA)	Sample 1 month	9.5	9.5	10.5	10.5	9.8	9.8
("AD-CA") (closed, amber-glass vial in argon with Citric acid)	Sample 1 month	9.7	9.7	9.4	9.4	9.4	9.4
("AD-AsA") (closed, amber-glass vial in argon with Ascorbic acid)	Sample 1 month	9.1	9.1	9.7	9.7	9.1	9.1

Table 3. NMR Spectra. Key: N-None (closed clear-glass vial in normal air); D-Dark (closed, amber-glass vial in normal air); AD-Argon-Dark (closed, amber-glass vial, argon-flush); AD-Cys (closed, amber-glass vial in argon with Cysteine); AD-EDTA (closed, amber-glass vial in argon with EDTA); AD-CA (closed, amber-glass vial in argon with Citric acid); AD-ASA (closed, amber-glass vial in argon with Ascorbic Acid)

The following are representative NMR spectra of the above different conditions.

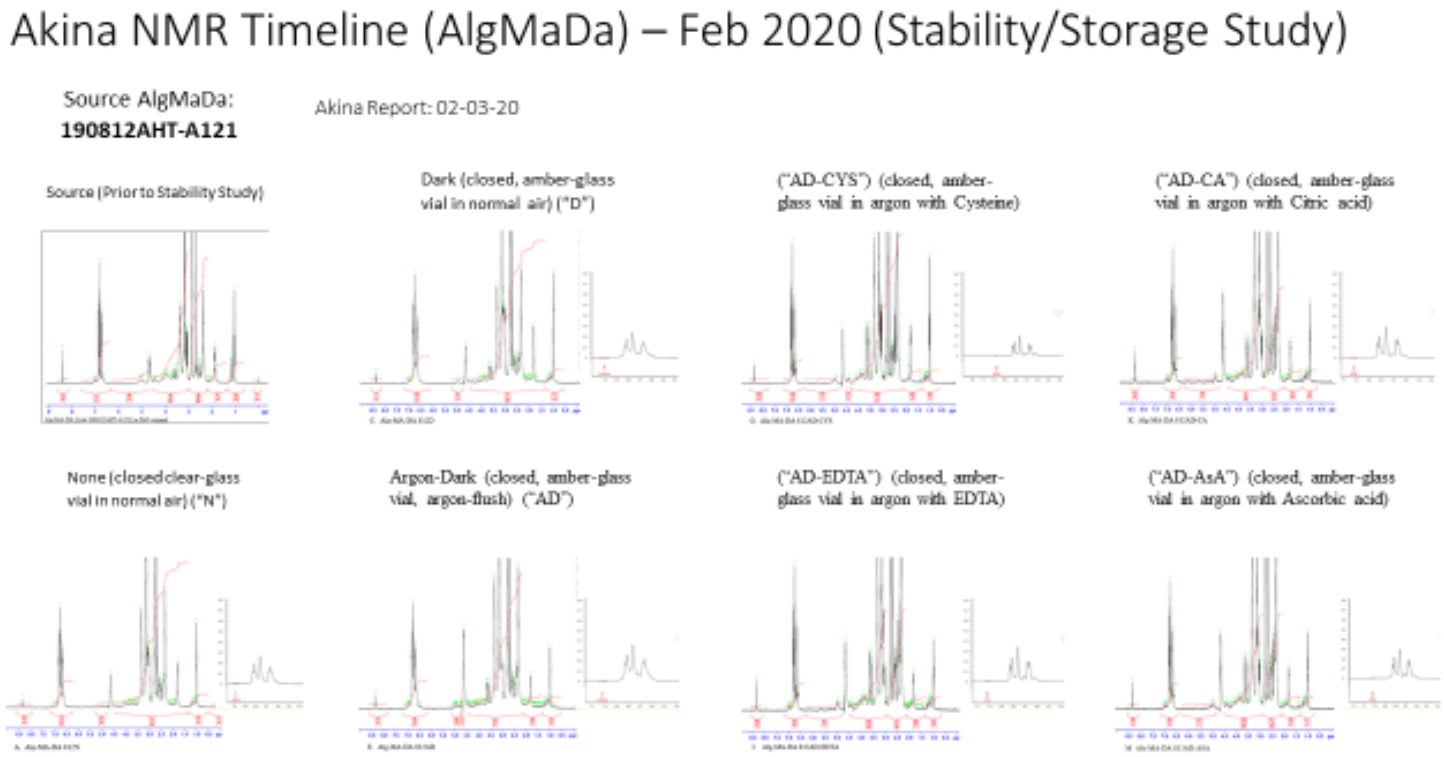


Figure 18: Compilation of NMR spectra obtained from ALG-MA-DA under the different storage conditions

The above storage stability studies indicated that the dopamine and methacrylate are quickly damaged under 40ºC conditions as well as significant self-reaction. Most samples self-crosslinked to become insoluble. NMR on the soluble portions indicated that the use of argon and dark-sided glassware slightly reduces damage under some conditions but the effect is not consistent. The use of preservatives actually made the storage conditions worse and introduced other problems as some materials presented a change in color (green). Raw data and additional spectra are available on request.

To continue the research, another storage study was performed. This time the material was ETO-sterilized prior to the storage stability and the preservative agents were more thoroughly mixed in with the ALG-MA-DA followed by freeze drying to ensure more thorough incorporation. A higher concentration of preservative agents was used relative to the ALG-MA-DA and the degradation time was reduced.

Took solid ALG-MA-DA, dissolve 100 mg into 10 ml of argon-sparged deionized water in a foil-wrapped argon flush scint vial. Weighed out solid ALG-MA-DA 200218AHT-A121, dissolved 100 mg into 10 ml of argon-sparged deionized water in a foil-wrapped argon flush scint vial. Into a series of ten small (2-dram style) amber-glass bottles, added the following:
#1 - 5 (nothing, control)
#6 – 1.5 mg Butylated hydroxytoluene (BHT)
#7 - 1.1 mg Cysteine
#8 – 1.3 mg ascorbic acid
#9 - 1.5 mg citric acid
#10 - 1.4 mg EDTA

Table 4: HNMR determined Methacrylate and Dopamine content of ALG-MA-DA stored under forced-degradation (40 ºC/75% RH) for 2 weeks vacuum sealed with indicated preservative agent.		
Sample	Methacrylate (uM/g ALG-MA-DA)	Dopamine (uM/g ALG-MA-DA)
Control #3 (no additive)	89	3327
BHT	63	3686
Cysteine	0	2098
Ascorbic Acid	0	6665
Citric Acid	41	2351
EDTA	137	2651

In brief summary, unlike the prior testing, the ALG-MA-DA samples did not spontaneously crosslink. However, the addition of preservatives was at a much higher concentration and the shelf-life test drastically shortened. These results do generally indicate the potential for longer-term storage however highlight the importance of careful packaging. The preservative agents did not provide substantial improvements in material stability relative to vacuum-packed control indicating that these materials, under the tested conditions, may not provide much advantage. Additionally, the process may be simplified by simply converting the material to the patch first and then storing the patch instead of the ALG-MA-DA precursor. This is the subject of current investigations.

Specific Aim 1: Major Task 3: Milestone(s) Achieved: Optimal sterilization and storage conditions

- Confirmed capacity of ALG-MA-DA to be sterilized using commercially available and conventional ethylene oxide gas exposure.
- Storage stability testing under accelerated degradation conditions evaluated. Confirmed need for air and light protection.
- Tested use of preservatives/antioxidants in accelerated conditions. Initial tests do not support substantial advantages to using these materials.
- Recognition that the best approach may to be initial production of the patches rather than long-term storage of the precursor materials.

Specific Aim 2
To define long term efficacy and safety in longitudinal small (rat) and large (pig) models of adult pleural injury and of adult and pediatric tracheal injuries.

Specific Aim 3
To assess short term efficacy in a pleural injury model in ex vivo ventilated normal and diseased (COPD/emphysema) human lungs

Studies for **Specific Aim 2** and **3** have not yet been initiated. These are anticipated to begin over the coming year.

What opportunities for training and professional development has the project provided?
Nothing to report.

How were the results disseminated to communities of interest?
Nothing to report as yet. Several technical manuscripts describing the evolving technologies being developed, including the oscillatory burst pressure device, are in progress as is a manuscript describing materials and materials characterization of the ALG-MA-DA.

What do you plan to do during the next reporting period to accomplish the goals?
As detailed above, we will systematically continue all of the major tasks in Specific Aim 1. The methodologic and materials advances achieved so far give us confidence that we will be able to meet major milestones with specific goal of developing a large scale manufacturing process capable of producing reliable high quality ALG-MA for use in Specific Aims 2 and 3. In addition the cytotoxicity and degradation studies (Subtasks 2 and 3 in Specific Aim 1, Major task 2) will be initiated with anticipated completion in the original timeline.

We anticipate initiating studies in **Specific Aim 2** in Fall 2020. This will include those being performed at the University of Connecticut with the collaborators there. Once these are successfully underway and efficacy of the ALG-MA-DA sealant is demonstrated, we will then move on to the studies in **Specific Aim 3**.

Other achievements
We have made significant advances in a range of technical issues associated with the pleural sealant development. This includes a new oscillatory burst pressure approach that we will eventually look to file for IP. As detailed in the above report, a number of scientific/technical manuscripts are in preparation detailing the results and findings of the studies to date. These result will also be submitted for presentations at relevant national/international scientific meetings (ATS, BMES, and others).

Stated goals not met
Although we have made significant progress in all major tasks of **Specific Aim 1**, there is still further optimization of the ALG-MA-DA material to be accomplished for this aim. The specific plans for doing so have been articulated throughout the above progress sections. Similarly we anticipate initiating studies in **Specific Aim 2** in Fall 2020 with those of **Specific Aim 3** to follow.

4) Impact
What was the impact on the development of the principal discipline(s) of the project?
We have made significant and promising progress in the overall goal of developing an effective ALG-MA-DA pleural sealant. This involves developing new technical approaches techniques and exploration of different application methods. We are optimistic that these will result in a clinically applicable product that can be further investigated in clinical trials.

What was the impact on other disciplines?
Nothing to report as yet

What was the impact on technology transfer?
Nothing to report as yet

What was the impact on society beyond science and technology?
Nothing to report as yet

5) Changes/Problems

Changes in approach and reasons for change

The expanding scope of investigations to incorporate technical advancements, functional modifications, and experimental approaches, detailed above, are all logical extensions of the original proposal and remain completely within the spirit and scope of the proposal.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing significant to report

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

6) Products

Nothing to report as yet under any category

7) Participants and Other Collaborating Organizations

PI: Daniel J. Weiss MD PhD UVM
Partnering PI: Christine Finck MD UConn
Partnering Contracted Vendor: John Garner PhD Akina Inc.
Post-Doctoral Associates: Ishna Sharma MD UConn
Laboratory Technicians: Evan Hoffman UVM, Todd Jensen UConn, Nirav Daphthary
Animal Technicians: Stephen Bell UVM, Sheila Russell UVM
Thoracic Surgical Consultant: Bruce Leavitt MD UVM

No change in effort for any of the above listed study participants

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
The PI, Dr. Daniel Weiss has received several additional grants since the grant submission. These are listed below. None are relevant for the current DOD proposal.
New grants since last submission

1) R13 HL149436 (DJ Weiss, PI) 07/04/2019-06/30/2020 2%
NIH/NHLBI $30,000 annual direct costs
The principle goals of the proposed meeting, "Stem Cells, Cell Therapies, and Bioengineering in Lung Biology and Diseases" are to convene the relevant experts and up and coming junior investigators to share and debate recent developments in this rapidly moving and exciting field with a specific goal to formulate basic, translational, and clinical research directions.

Specific Aims:
1) To convene national and international experts in stem cells, lung biology and bioengineering to discuss and debate recent advances in the roles of stem cells and cell therapies in lung biology and lung diseases
2) To address critical issues in this field, including the identity and role of endogenous lung progenitor cells, translational studies, and clinical cell therapy approaches for lung diseases
3) To address breaking advances in the field including embryonic stem cells, induced pluripotent stem cells, stem cells and lung cancer, and bioengineering approaches to lung regeneration
4) To provide a forum for junior investigators, graduate students, postdoctoral associates, and pulmonary research fellows to participate and present data in oral or poster format
5) To discuss the alarming rate of growth of unregulated and unproven stem cell and cell therapies in both the US and abroad and how to best combat this growing problem
6) To craft a series of recommendations for the NHLBI, FDA, and other relevant organizations to utilize for guiding basic and translational research in stem cells and cell therapy approaches in lung diseases. These same guidelines will also be designed to help investigators pursue NIH and other funding opportunities.

Name & Address of funding agency's procuring Contracting/Grants Officer:
Grant Management Specialist: Alyse Burton
Address: NIH/NHLBI
BG RKL1 RM 201-J
6705 ROCKLEDGE DR
BETHESDA MD 20817

2) SPARK UVM (DJ Weiss/Adam Jakus, Dimensions Inc.) 07/01/2018-06/30/2020 no measurable effort
Development of novel lung bioinks $45,000 total direct
Project goals are to test the mechanical and biochemical properties of lung-specific ECM-derived materials and evaluate optimized lung ECM-origin gels and patches in pre-clinical models of lung injury.

Specific Aims:
Specific Aim 1: To test the mechanical and biochemical properties of lung-specific ECM-derived materials.
Specific Aim 2: Evaluate optimized lung ECM-origin gels and patches in pre-clinical models of lung injury.

Name & Address of funding agency's procuring Contracting/Grants Officer:
Dan Harvey
Director of Operations to the Vice President for Research
Address: 85 South Prospect Street
330 Waterman Building, University of Vermont
Burlington, VT 05405

3) WEISS18P0 (Weiss) 04/01/2019-03/31/2021 15%
Cystic Fibrosis Foundation $125,000 annual direct costs
Role of Glycoproteins in Lung Recellularization
The goals for this proposal are to systematically analyze key matrikine binding and activation patterns to specific individual HS, CS and DS in decellularized human lungs, to systematically determine matrikine dependent and independent effects of individual HS, CS and DS chains on representative lung cell growth and differentiation

and to determine matrikine-dependent and independent effects of systematic repletion of decellularized normal or diseased human lung ECM with key HS, CS and/or DS functional groups on cell growth and differentiation.

Specific Aims:
1: Systematically analyze key matrikine binding and activation patterns to specific individual HS, CS and DS in decellularized human lungs
2: Systematically determine matrikine dependent and independent effects of individual HS, CS and DS chains on representative lung cell growth and differentiation
3: Determine matrikine-dependent and independent effects of systematic repletion of decellularized normal or diseased human lung ECM with key HS, CS and/or DS functional groups on cell growth and differentiation.

Name & Address of funding agency's procuring Contracting/Grants Officer:
CFF Grants and Contracts Office
Cystic Fibrosis Foundation
Office of Grants and Contracts
4550 Montgomery Avenue, suite 1100
N. Bethesda, MD 20814

4) S10 OD026976 (Weiss) 08/01/2019-07/31/2020 no effort requested
NIH $122,058 annual direct costs
Zeta View TWIN Laser Nanoparticle Tracking Analyzer
The goal of this proposal is to obtain a state-of-the-art ZetaView TWIN Laser Nanoparticle Tracking Analyzer (NTA) device at the University of Vermont (UVM).

Specific Aims:

1) Evaluate the accumulation, penetration depth, and efficacy of MSNs within the lung during an asthmatic response.
2) Test the ability of anti-IL-6-MSN to reduce an asthmatic response in vivo.

Name & Address of funding agency's procuring Contracting/Grants Officer:
Grants Management Specialist: Donna M James

Address: NIH/Office of the Director
BG RKL1 RM 202-J
6705 ROCKLEDGE DR
BETHESDA MD 20817

5) VLC Pilot Project 2019 (Weiss/van der Velden) 04/01/2020-03/31/2021 1%Institutional source $25,000 annual direct costs
Effects of human lung derived hydrogel organoid culture on lung progenitor cell behaviors
The goals of the project are to determine composition and differential effects of bulk lung or regional specific (airway vs alveolar) human lung dECM hydrogels on growth and functional behaviors of iAEC2 alveolospheres and the effects of combinatorial addition of relevant GAGs (heparan, chondroitin, and dermatan sulfates) to dECM hydrogels on growth and functional behaviors of iAEC2 alveolospheres.

Specific Aims:
1) To determine composition and differential effects of bulk lung or regional specific (airway vs alveolar) human lung dECM hydrogels on growth and functional behaviors of iAEC2 alveolospheres.
2) To determine the effects of combinatorial addition of relevant GAGs (heparan, chondroitin, and dermatan sulfates) to dECM hydrogels on growth and functional behaviors of iAEC2 alveolospheres.

Name & Address of funding agency's procuring Contracting/Grants Officer:
Carolynn Hatin
Pulmonary Business Manager
Address: 89 Beaumont Ave
Given Building D213, University of Vermont
Burlington, VT 05405

What other organizations were involved as partners?

A. Organization Name: Akina Inc.

Location of Organization: West Lafayette IN

Partner's contribution to the project (identify one or more)

Financial support: N/A

In-kind support: Akina manufactures and tests materials used for evaluation as pleural sealants

Facilities: Akina Inc. facilities are utilized for manufacture and materials evaluations of compounds to be tested at UVM in lung injury models

Collaboration: Akina Inc. personnel, led by John Garner, work closely and extensively with Dr. Weiss and his team at UVM

Personnel exchanges: N/A

Other: N/A

B. Organization Name: University of Connecticut (UConn)

Location of Organization: Farmington CT

Partner's contribution to the project (identify one or more)

Financial support: N/A

In-kind support: N/A

Facilities: Full laboratory and animal facility resources for planned animal testing

Collaboration: Collaborators at UConn will perform some of the studies in **Specific Aim 2**

Personnel exchanges: N/A

Other: N/A

8) Special Reporting Requirements
Collaborative awards: N/A

Quad Chart

PR181641 - "Clinical Development of a Novel Pleural and Tracheal Sealant"

PI: Daniel J. Weiss MD PhD University of Vermont College of Medicine

Budget: $1,724,027 **Topic Area: Respiratory Health** **Mechanism: PRMRP Expansion Award**

Research Area(s): Respiratory Health: Pleural Sealants **Award Status:** Open; POP:

Study Goals:

The overall goal is to develop a novel, effective, and easy to use modified alginate-based pleural sealant for use in traumatic and other lung injuries

Specific Aims:

(1) To optimize manufacturing, sterilization, preservation, and storage conditions of pre-formed ALG-MA-DA patches
(2) To define long term efficacy and safety in longitudinal small (rat) and large (pig) models of adult pleural injury and of adult and pediatric tracheal injuries
(3) To assess short term efficacy in a pleural injury model in ex vivo ventilated normal and diseased (COPD/emphysema) human lungs

Key Accomplishments for Year 1:

Detailed systematic evaluation of ALG-MA-DA large scale manufacturing

A) Progress towards large scale ALG-MA-DA patch synthesis with controllable, reliable, and reproducible degrees of methacrylation and dopamine conjugation.
B) Identification of a more effective cross-linking agent (LAP)
C) Development of a novel oscillatory burst pressure testing system
D) Progress in optimizing tensile testing approaches
E) Easily replicated and tunable patch molds for use in both materials testing (burst pressure, tensile) as well as in subsequent patch applications to pleural surfaces
F) Modifications in protocols for utilizing the oxidizing agent during patch application
G) Systematic comparative burst pressure evaluations of ALG-MA-DA materials produced utilizing the above improvements and also continuing comparisons vs ProgelTM

Key Outcomes for Year 1:

1. Progress towards reliable and reproducible large scale sealant manufacturing

9) Appendices: N/A