

Chemical Dissolution of Abdominal Adhesions

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Abstract

Abdominal adhesions are common byproducts of abdominal surgeries and can oftentimes result in serious complications. The goal of this design project is to decrease the reformation of abdominal adhesions in surgical patients through a supplemental procedure to a minimally invasive surgical method. The team's design solution involves administration of a fibrinolytic agent, plasmin, to inhibit the adhesion formation process. A PEG-DA hydrogel crosslinked *in vivo* will be used to administer plasmin to adhesion sites during laparoscopic surgeries. Testing revealed plasmin's ability to degrade fibrin gels in an *ex vivo* environment. Future work will focus on refining testing conditions to more accurately reflect body levels of plasmin and fibrin as well as characterize plasmin release from the PEG-DA hydrogel.

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I. Introduction

A common byproduct of abdominal surgeries is the formation of adhesions. Adhesions are bands of scar-like tissue that form in between internal tissues that are not meant to be linked. An example of an adhesion can be seen in **Figure 1** below. They occur after 67-100% of invasive abdominal surgeries [1]. Adhesions are not always a problem for patients, however 15-18% of patients who develop adhesions experience further complications from them. Some of these problems include small bowel obstructions, female infertility, and severe pain [1]. These complications can become serious and necessitate adhesion removal.

When patients suffer from problems like small bowel obstructions, multiple actions are taken to attempt to get rid of the obstruction without surgery. For example, the patient may undergo aggressive intravenous fluid therapy and correction of electrolyte imbalance. CT scans and physical examinations are required to continuously monitor the progress of the obstruction [2]. If the preliminary treatments fail, the patient will be admitted for a laparotomy in which the surgeon will enter the abdominal cavity laparoscopically to sever the problematic adhesions with surgical scissors. This relieves the tension in the abdomen and treats the small bowel obstruction [3]. This laparoscopic removal technique resolves the immediate problems caused by the adhesions, but it does not prevent reformation of further adhesions due to the laparotomy itself. This problem of recurrent abdominal adhesions is most often seen in the elderly population that has undergone many invasive abdominal surgeries. Stopping the recurrence of these adhesions is the problem that the team is seeking to address.

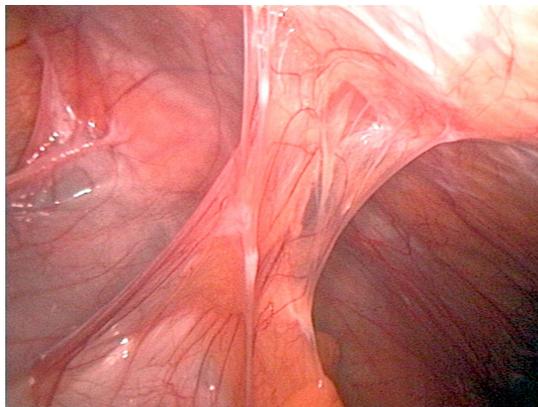


Figure 1. This is a photo of a mature adhesion. The dark red lines are vasculature and the light pink is the extracellular matrix (ECM).

Competing Designs

There are many preventative technologies that are currently in clinical trials that attempt to prevent the formation of adhesions after abdominal surgeries. However, these preventative measures aim to prevent the formation of original adhesions as well as recurrent adhesions.

Simvastatin is a strong fibrinolytic agent in human mesothelial cells that can be used to prevent adhesion formation. There have been clinical trials to determine if oral administration is effective in preventing adhesion formation. It was concluded that if taken orally, this agent is ineffective for intra-abdominal adhesion formation. However, in a study conducted on rats this agent was administered intraperitoneally. This served as an effective preventative method for postoperative intra-abdominal adhesion development [4].

Nitric Oxide Synthase is a typical inflammatory agent used to reduce fibrin development, which is the beginning stage of a developing adhesion. This agent has been analyzed as a preventative method. After testing, it was dictated that the expression of iNOS, the gene affected by nitric oxide, is delayed. Therefore the development of adhesions is restricted [5].

Finally, anti-adhesion film is a chemical application placed onto the area that is at risk for adhesion development. Chemicals on a physical film adhere to the ECM of native tissues. The substrate blocks the collagen and does not allow it to connect with new developing ECM. This prevents the growth of new adhesions because the fibrin is unable to lay to develop into the ECM [6].

Problem Statement

Original Problem Statement:

Abdominal adhesions are common in many patients with past surgical histories. In elderly patients especially these adhesions can become painful and cause further complications such as small bowel obstructions. The only viable non-preventative method for removing adhesions is surgery to mechanically cut them out. However, this invasive technique can lead to more adhesion formation. Therefore, the team has been tasked with developing an alternative non-invasive solution for adhesion removal.

Problem Statement Redefinition:

After determining that chemical dissolution of mature adhesions offers no advantages over the mechanical severing of them, the problem was re-defined. The new problem now focused on

supplementing the current adhesion removal surgery with a preventative method in order to decrease recurrent adhesion formation. As such, the project shifted away from dissolving mature adhesions to preventing the recurrence of further adhesions.

II. Background

Adhesions that result from abdominal surgeries usually result from poor handling of internal organs, drying out of tissues, and contact with foreign materials during surgery [3]. Adhesions are the result of an inflammatory process that occurs at the site of surgery or trauma. Following the pathway shown below in **Figure 2**, adhesions are initially made up of fibrin. The formation of fibrin occurs within the first 72 hours. After the formation of fibrin, collagen synthesis begins in the adhesion. Collagen synthesis continues until all the fibrin has been turned over into collagen. At this point the adhesion is primarily made up of a collagenous ECM. Once the collagenous ECM has been formed the adhesion can either continue to mature via the integration of vasculature, or it can be degraded by matrix metalloproteinases (MMPs) to allow normal healing of tissues.

In adhesion fibroblasts, the mRNA expressions of MMPs and tissue inhibitors of matrix metalloproteinases (TIMPs) are both upregulated. This suggests that the adhesion is consistently developing and degrading its ECM [7].

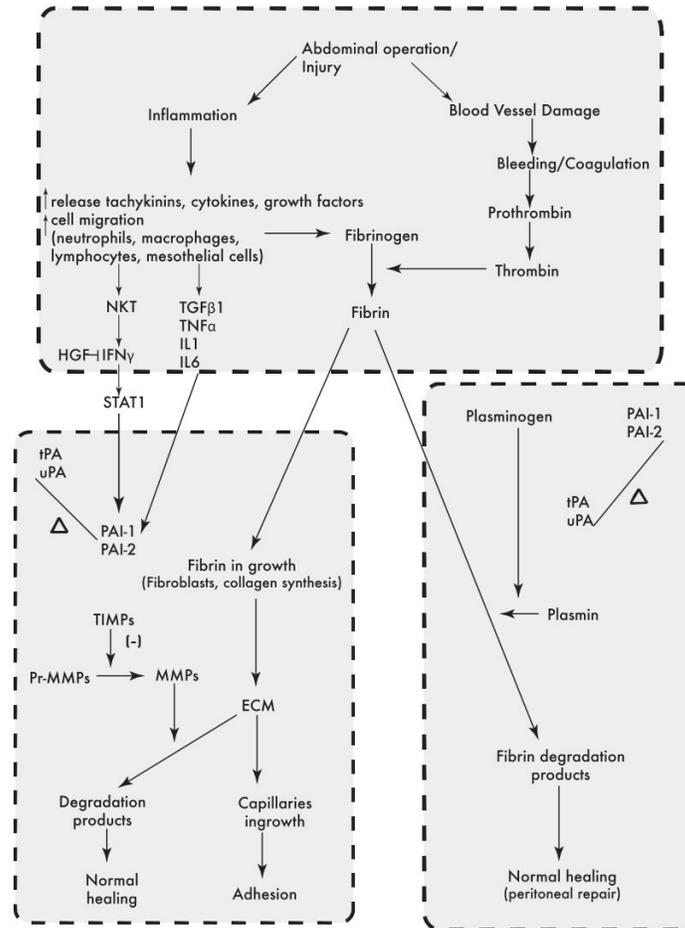


Figure 2. This diagram shows the cascade of healing as a result of an abdominal surgery. In this case, the focus is on the initial fibrinous tissue that is quickly turned over into a collagenous ECM [7].

Fibrin is normally present in the body in the inactive form, fibrinogen. Fibrinogen is highly concentrated in the blood where it waits until needed to form a clot. When the signal arrives, fibrinogen is converted to fibrin, which then assembles into an extended network of fibers [8].

Plasmin, on the other hand, is a fibrinolytic agent that directly degrades fibrin. Plasminogen circulates in the blood plasma and is activated by both plasma-derived and tissue-derived activating proteases to form plasmin. As seen in **Figure 3**, plasmin is able to act on both fibrinogen, breaking it down into fibrinogen degradation products, and on fibrin [9].

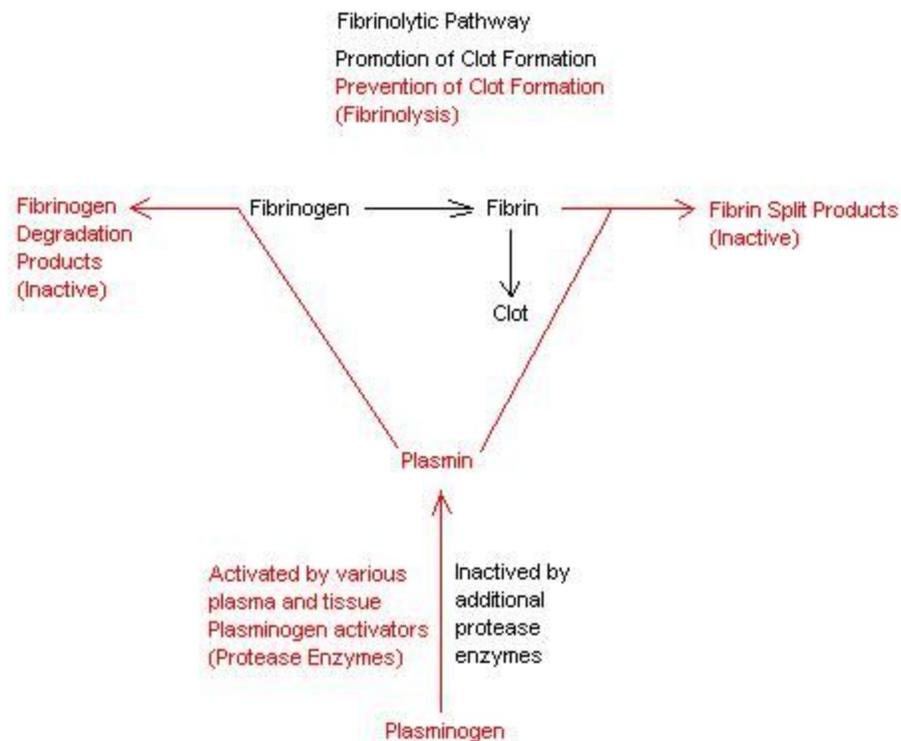


Figure 3. Diagram illustrating the fibrinolytic pathway [9].

Client Information

The client is Dr. Philip Bain, who is a practicing internist at the Dean Clinic in Madison, WI. Dr. Bain has had many patients come into his office complaining of severe bowel pain. Often these cases are diagnosed as small bowel obstructions from abdominal adhesions and require surgical removal.

Design Specifications

The main specifications of the design pertain to safety, biocompatibility, and ease of use. The product must be FDA approved and sterile in compliance with FDA Articles 820.25, 70, 50, 72 [PDS 1]. The product must also be biocompatible with the human body, and blood homeostasis must be maintained. For example, the pH must not go outside of the 7.35 - 7.45 range and the level of fibrinogen must remain within 200 - 400 mg/dL [PDS 2]. Also, the product must be easy for surgeons to use and it must be compatible with current surgical techniques. It must be easily implemented into their operating setting. A more complete list of design specifications can be found in Appendix A.

III. Preliminary Designs

Selection of Original Chemical Agent

There are three main components of a mature adhesion that can be targeted--cells, vasculature, and the ECM. In order to oust a mature adhesion, the structure must be degraded; thus the ECM will be the target of choice since it is the structural component of adhesions. If the cells or vasculature of an adhesion are targeted, the adhesion would die but its structure would still remain in the body. As a result, the chemical agent of choice is a collagenase, or an enzyme that acts to naturally degrade collagen. More specifically, MMPs will be used. These are synthesized by the epithelial adhesion cells in a latent form that becomes active after interaction with a pro-MMP [10]. They function by preferentially degrading the proteins in the ECM.

Selection of Original Delivery Method

After selecting an appropriate chemical agent, a delivery system must be chosen to effectively administer this agent to the adhesion sites. The following three design ideas represent the team's ideas for delivery methods.

Design Idea 1: Hydrogel

Design idea 1 consists of exogenous MMP delivery through a hydrogel application (**Figure 4**). The main idea is to wrap a hydrogel around the adhesion via a laparoscopic procedure. The hydrogel will contain MMPs that will selectively diffuse through only one side of the hydrogel, and this will be the face that is in contact with the adhesion. The hydrogel will span approximately 75% the length of the total adhesion, and it will have dimensions on the scale of millimeters. Since each adhesion differs in size, the originally fabricated hydrogel may need to be trimmed by the surgeon upon implantation.

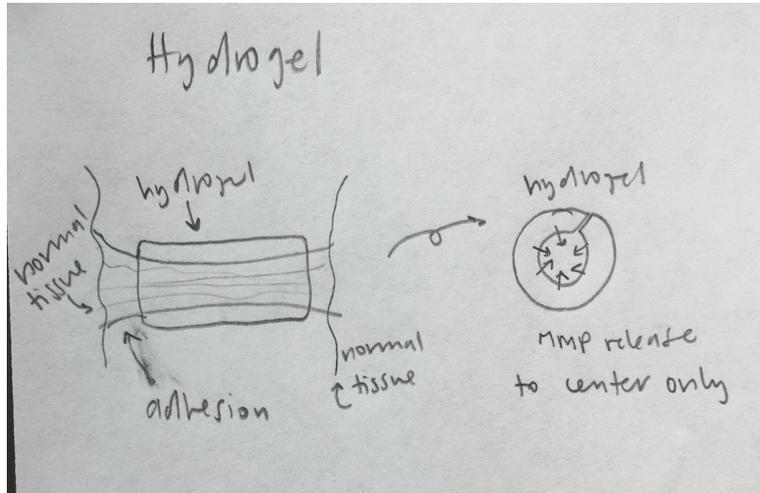


Figure 4. Design idea 1 uses a hydrogel that wraps around the adhesion and selectively diffuses MMPs to the adhesion. Its exact dimensions will vary depending upon the specific adhesion targeted, but it will be on the scale of millimeters.

Design Idea 2: Chemical Scalpel

Design idea 2 incorporates exogenous MMPs in a scalpel-like instrument with which the surgeon can cut the adhesion by releasing MMPs at the end of the instrument (**Figure 5**). The instrument will contain a compartment with MMPs and an ejection tip. The release of MMPs will be controlled by the surgeon; the surgeon will most likely release MMPs at multiple sites on the adhesion to completely sever the adhesion. The ejection tip will apply the MMPs straight from the MMP compartment to the adhesion. This design idea also uses a laparoscopic procedure to deliver the MMPs.

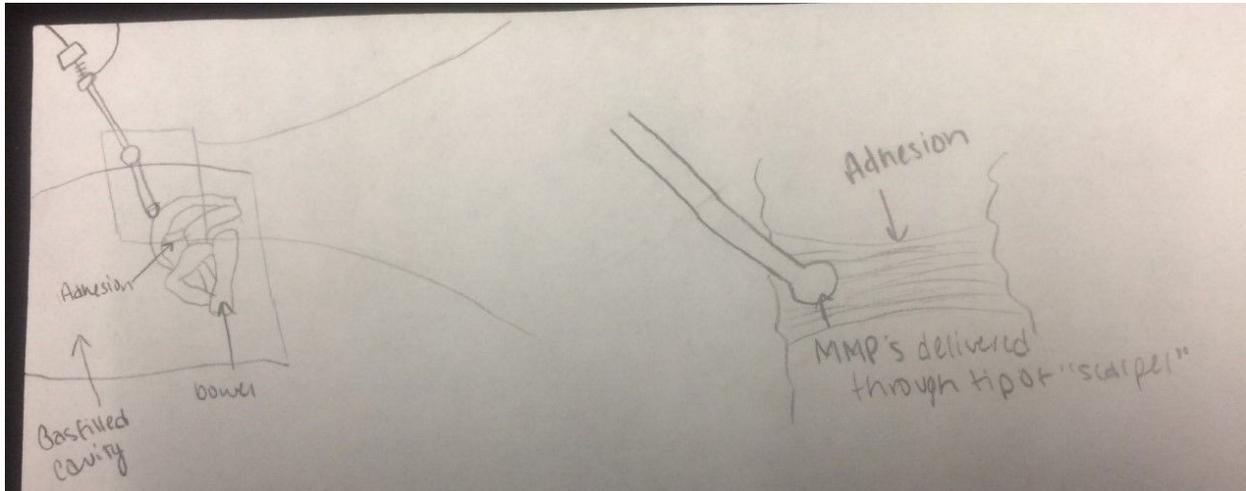


Figure 5. Design idea 2 uses a scalpel-like instrument to deliver MMPs to the adhesion. MMPs will be delivered in multiple areas of the adhesion, and delivery is controlled by the operating surgeon.

Design Idea 3: Gene Therapy

Design idea 3 utilizes gene therapy to overexpress endogenous MMPs in the human body. Many cellular signalling pathways have been attributed to the regulation of MMPs, and the idea behind this design is to manipulate the second messengers involved in these cascades. Cytokines and growth factors stimulate the MAPK and FAK signalling cascades that ultimately lead to an increase in the transcription of AP-1 and PEA3. These transcription factors are responsible for the translation of various MMPs [11]. This design is intended to target a specific gene that will disrupt the homeostasis of MMP/TIMP regulation at the adhesion by overexpressing a gene that produces MMPs. With an increase of MMPs at the adhesion site, the body can naturally degrade the adhesion without the need of a laparoscopic procedure or any other invasive procedure.

IV. Preliminary Design Evaluation

A design matrix was used to evaluate the preliminary design ideas based off of relevant criteria (Table 1).

Criteria	Weight	Design 1: Hydrogel		Design 2: Chemical Scalpel		Design 3: Gene Therapy	
Safety	(30)	4/5	24	3/5	18	2/5	12
Performance	(25)	4/5	20	3/5	15	5/5	25
Simplicity (Ease of Use, Risk of Failure)	(20)	3/5	12	4/5	16	1/5	4
Cost	(15)	4/5	12	3/5	9	1/5	3
Fabrication	(10)	4/5	8	3/5	6	1/5	2
Total	(100)		70		64		46

Table 1. A design matrix was used to evaluate and rank the three design ideas. The hydrogel design scored highest in safety, cost, and fabrication, thus leading the team to choose it as the winning design.

The hydrogel application was selected as the proposed final design because it ranked highest overall as well as in the individual design criteria of safety, cost, and fabrication. The hydrogel ranked highest in the safety category for multiple reasons. The hydrogel would be both biodegradable and biocompatible, thus lowering associated risks of induced toxicity that may come with implantation. The hydrogel also would regulate the release of the MMPs and provide a physical barrier to the rest of the body that would restrict MMPs from escaping the adhesion site. This would reduce the risk of MMP attack on healthy tissues and improve the safety of the MMP release. The other two designs contain less of a barrier aspect than the hydrogel, and the chemical scalpel design ranked lower than the hydrogel in safety for this reason. On the other hand, gene therapy ranked lowest for safety because it is extremely experimental and a current topic of research. As a result, It is difficult to predict its effects on the body since most of its research has been done on cell cultures *ex vivo* [11].

The hydrogel design also scored highest in cost. Gene therapy is extremely experimental and requires intensive research, thus rendering it costly and timely. The chemical scalpel requires fabrication of a specialized scalpel-like instrument that would be able to both store and release MMPs in a controlled manner. Since hydrogels are relatively cheap and widely available, the hydrogel design scored highest in the cost criteria.

Finally, the hydrogel application scored highest in fabrication because hydrogels are common products that can be easily fabricated in labs. The fabrication of the chemical scalpel would require a unique design and very specific fabrication method. Again, since the gene therapy design is extremely experimental, fabrication, or development of this design, will be extremely difficult and timely.

Original Proposed Final Design:

As a result of the overall high ranking of the hydrogel, the team has decided to pursue this as the proposed final design. The ideal hydrogel would be biocompatible, malleable, and easily able to wrap around adhesions. The hydrogel would also have an MMP coating that would be in contact with the adhesion. When the hydrogel is wrapped around the adhesion, the MMPs would diffuse into the adhesion and sever it. Ideally the hydrogel would be biodegradable and able to degrade harmlessly inside the patient after the adhesion is severed.

VI. Final Design

After reshifting the problem statement from degrading mature adhesions to preventing the recurrence of further adhesions, the original proposed final design of a hydrogel changed to a preventative method to be implemented during adhesion removal surgeries. The new final design utilizes a fibrinolytic agent to prevent the deposition of fibrin at adhesion sites in order to stop the formation of recurrent adhesions. The fibrinolytic agent will be delivered through an injectable hydrogel that will be UV crosslinked *in vivo*. The hydrogel and fibrinolytic agent will be administered after severing existing adhesions during laparotomies.

Materials

Hydrogel

Based on the design criteria of ease of fabrication and use, safety and physical adhesion prevention effectiveness, PEG-DA was chosen as the injectable hydrogel medium for the fibrinolytic agent. Poly(ethylene glycol) diacrylate (PEG-DA) is a hydrogel consisting of repeating ether units with acrylate groups at each end [12]. The hydrogel comes in the form of a powder and can easily be dissolved in a buffer and crosslinked via UV light to form a gel (see Appendix B). The gel can be crosslinked *in vivo*, however it can be difficult to limit tissue UV exposure [13] (see future work). Secondly, the gel is biocompatible in that it doesn't cause any "allergic or rejective reactions" and it resists protein adhesion [14]. Finally, PEGDA also has properties that resist cellular adhesion which allows it to be used as a physical barrier to adhesion formation [12].

Simple modeling of diffusion of the drug through the hydrogel can be modeled by the diffusion equation in Eq. 1:

$$\frac{\partial c}{\partial t} = \nabla \cdot (D \nabla c) \quad \text{Eq. 1}$$

This model is the simplest representation of diffusion through a hydrogel. Modeling of the actual diffusion through a hydrogel would involve taking into account both the swelling and contracting of the gel as well as the pore size and size of the drug itself. This gets extremely complicated quickly and involves infinite sums of the complex error function [15]. Neglecting the pore and molecule size and assuming the diffusion coefficient is constant ($D = 0$), the diffusion can be approximated to be released proportionally to \sqrt{t} [15].

Fibrinolytic Agent

There are multiple methods for fibrinolysis, namely, preventing or limiting the inflammatory response which exudes thrombin and fibrinogen; dissolving fully formed fibrin; or inhibiting the cells that produce fibrin [16]. The easiest method is modifying the dissolution of formed fibrin to dissolve it as it's forming, essentially preventing its formation. Drugs that upregulate the dissolution of fibrin are called fibrinolytics. Two agents that were considered are found in the fibrinolytic system in the body: Tissue type Plasminogen Activator (t-PA) and Plasmin. T-PA is the protein that activates plasminogen into plasmin, while plasmin is the agent that directly breaks down fibrin [16]. Although t-PAs (Alteplase) are currently approved for systemic administration (to dissolve blood clots in stroke patients), they can cause bleeding in remote areas of the body. However, plasmin has been found to prevent postoperative adhesions with no adverse side effects [16,17]. The mechanism behind this is that plasmin has a very fast acting inhibitor ($\alpha 2$ -antiplasmin), so while the t-PA will activate plasmin all over the body, application of plasmin directly will cause it to bind to any fibrin and degrade it while the antiplasmin will quickly neutralize plasmin before it can spread to where it's not needed [17]. Although plasmin is not currently FDA approved, the safety factor of not causing bleeding in other areas of the body is more important than current FDA approval, although plasmin has been getting more attention as a safe fibrinolytic [17,18].

Methods

Injection protocol

While there are still uncertainties due to inconclusive results in testing such as PEG concentration (see testing, results, and future work), the final prototype will consist of a solution of PEG gel in PBS (not the plasmin, since it needs to be frozen). The surgeon's preparatory team will combine the plasmin (held as lyophilized powder) with the prepared solution. The surgeon will proceed with the laparoscopic adhesiolysis as normal, but before they close up, they will use one probe for applying the solution, and another probe for UV crosslinking of the gel (**Figure 6**). The surgeon will apply the solution both on top of the spots where the adhesions were cut and

around the holes where the incisions were made to prevent *de novo* adhesions. Finally the surgeon will close up the patient as normal.

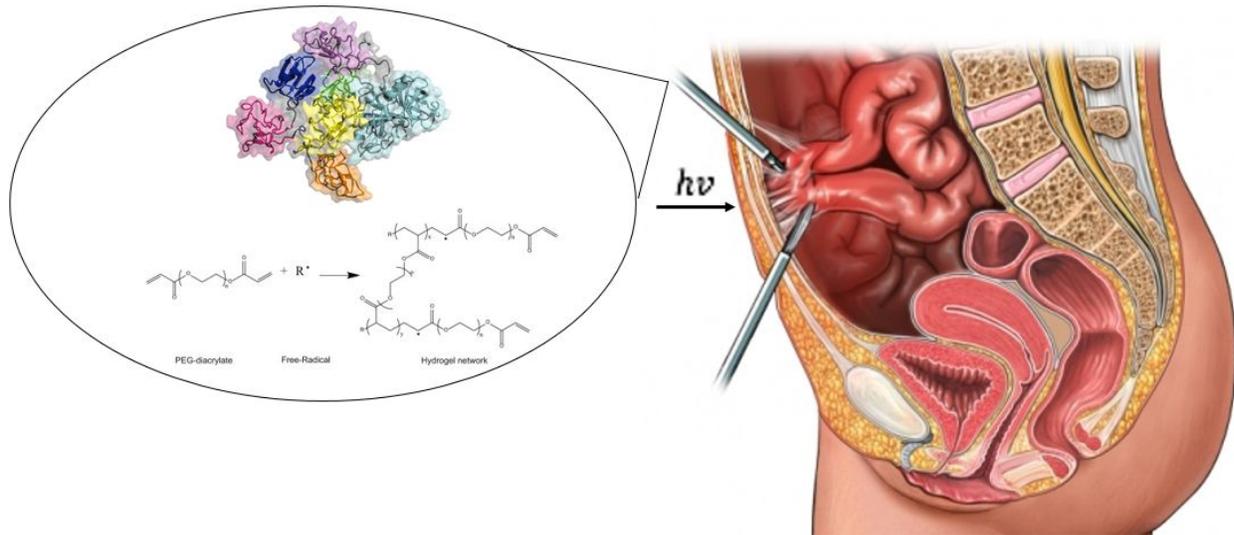


Figure 6: Surgeon will apply solution of PEG-DA and Plasmin and crosslink via UV light immediately post adhesiolysis and before closing up the patient.

VII. Testing

Two parts of our final design were investigated through preliminary testing--hydrogel drug release and fibrin degradation.

Hydrogel Drug Release:

This testing was carried out by crosslinking a fluorescent agent, Fluorescein isothiocyanate–dextran (FITC-D 70), into 10, 15, and 20 % by mass PEG-DA gels. The fluorescent molecule was chosen to model our agent, plasmin (83 kDa), due to its similar molecular weight at 70 kDa and its accessibility. The testing was carried out by placing 50 μ L gels into a 96-well plate in 200 μ L of PBS solution to be incubated for 48 hours. 25 μ L of supernatant was taken off at $t=24$ and 48 hours to be measured for fluorescence. See the complete protocol in Appendix B.

Fibrin Degradation:

Fibrin degradation by plasmin was assessed by placing varying amounts of plasmin-- 0.012, 0.09, 0.06, 0.03, or 0.0012 units--on 100 μ L fibrin gels. These gels were incubated for 30 minutes and the degraded fibrin and extraneous buffer was pipetted off of the gels prior to weighing them. See the complete protocol in Appendix B.

VIII. Results

Hydrogel Drug Release:

Our hydrogel drug release data was inconclusive after measuring zero fluorescence at both the 24 and 48 hour time points. Our titration of FITC-D that was measured as a control offered sensical data that has been attached in Appendix D.

Fibrin Degradation:

Fibrin degradation testing yielded average gel masses of each plasmin treatment that were statistically significant from the control (highest concentration vs. control: $p=0$. Lowest concentration vs control: $p<3e-4$. See Appendix E for methods of calculation). Data is visualized below in **Figure 7**.

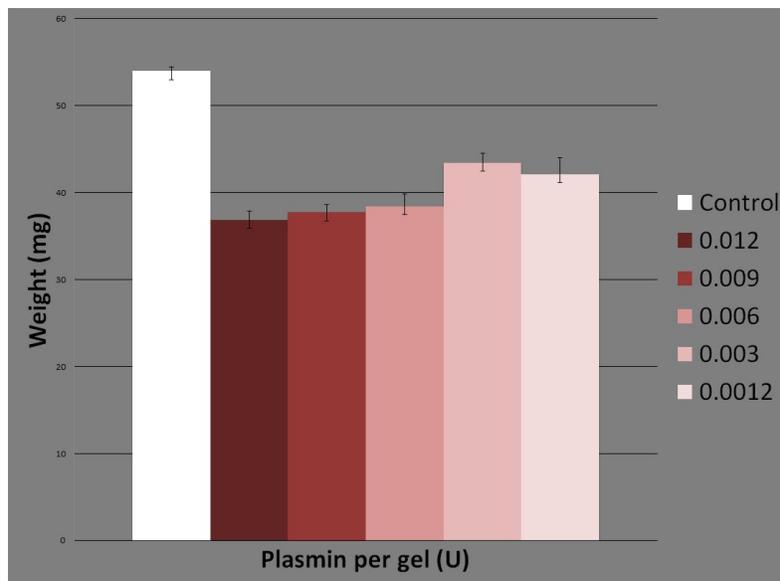


Figure 7. Average fibrin gel mass after 30 minute plasmin incubation. Error bars represent standard error. Data was analyzed through a series of t-tests that showed that each plasmin treatment group had been significantly degraded when compared to the control, but not between treatment groups.

The data shown above was also analyzed by calculating a percent degradation based off of the control gels. Percent degradation was found by taking $100 - ((\text{ave mass treatment} / \text{ave control}) * 100)$. **Table 2** below shows the percent degradation values fall between 20 and 30% degraded over a 30 minute incubation period.

Plasmin Treatment (U)	Percent Degradation (%)
0.012	31.72
0.009	30.15
0.006	28.82
0.003	19.54
0.0012	21.97

Table 2. This table shows the percent degradation of the fibrin gels relative to an applied plasmin treatment.

IX. Discussion

Discussion of Results

Hydrogel Drug Release:

The implications of our hydrogel testing are inconclusive due to the zero fluorescence measured at both time points. Some possible sources of error could be the photobleaching of the fluorescent molecule, the pore size of the PEG-DA gel used, or the non-uniform shape of the gels that were formed. First of all, the PEG-DA/FITC-D mixture was crosslinked in UV light for 2 minutes; it could be that during those two minutes the FITC-D was photobleached, meaning it would not emit fluorescence following UV exposure. Second, it could be that the pore size of the PEG-DA gel was not large enough for the FITC-D to fit within it, and as such, the molecule was never crosslinked into the gel. Finally, the gels formed in rings on the bottom of the wells, rather than in uniform discs as desired. These abnormal shapes could influence drug release.

All of these concerns, as well as making changes to the percentages of PEG-DA gel and concentrations of FITC-D could be addressed in future work in order to improve upon the testing that has been done thus far.

Fibrin Degradation:

Fibrin degradation testing showed that at the levels of plasmin added, between 0.012 U and 0.0012 U per 100 uL fibrin gel for 30 minutes, there was between 20 and 30 % degradation. With the testing mentioned above, no significance was found between the plasmin treated groups, despite a visible trend. This is an important benchmark in order to begin determining the exact amount of plasmin that would need to be released in order to prevent adhesion formation.

One problem with the design of this experiment is that it does not completely model the system, since in this case the fibrin is fully formed. As such, the amount of plasmin needed to degrade fully formed fibrin could reasonably be expected to be lower to prevent fibrin formation. This question of scale is a very important aspect of future research that will need to be addressed to provide a fully defined product.

Discussion of Final Design

From this semester's testing, we can draw no definitive conclusion about our design. The design provides a potential solution to the issue of recurrent abdominal adhesions, however further testing would be needed to investigate the true practicality of our design proposal. Based off our testing we can withdraw that the if we put in between 0.012 U and 0.0012 U per 100 uL fibrin gel, we will see degradation of 30% when directly degrading fibrin. This would give us an appropriate amount to crosslink into our hydrogel for release. This testing was directly conducted without diffusion. In order to understand the effectivity as a result of delayed release of the active agent fibrin.

The final design effectivity is also inconclusive because we do not have an accurate model of the hydrogel release. Without this we do not know how much fibrin will be released from the hydrogel at a given time. Without this information it is unknown if the team's current model will be able to disrupt the cycle of adhesion formation. It would be necessary to conduct further testing in order to confirm or reject the function of our final design.

Discussion of Ethical and Social Implications

Due to the inconclusivity of our final design it is important to consider how relevant continuing to evaluate this design for practicality. In terms of social impact, evaluating this problem will be very important in the coming years. As more and more of the population who has experienced significant abdominal surgery ages, their risk for adhesions and their associated complications increases. Therefore it is important to consider the aging population, and how important an effective method to treat these adhesions, and prevent the cycle of recurrent adhesions, is for practicing physicians now and in the future.

X. Conclusions

Abdominal Adhesions that lead to complications are very common in patients who have had extensive abdominal surgeries. Recurrence of excessive of adhesions are leading to the development of small bowel obstruction, female infertility, and increased complications in future abdominal surgeries. Due to the complexity of this issue, the development of a device to deliver a chemical agent will require further testing and consideration in both surgical procedure and

practicality in order to create an effective solution. This is a need that is rapidly growing in the coming years, and should be a focus in the coming years as the medical field grows in its preventative practices.

Future Work

Upon evaluation of our results from testing, it was concluded that reevaluation of our initial testing was needed. In order to further improve the hydrogel testing to measure release kinetics, it would be necessary to pick a better agent to model plasmin. We were unable to measure the Dextran Agent we added to the PEG-DA hydrogel due to the potential of photobleaching. Because of this risk of photobleaching it would be appropriate to choose a different fluorescent agent, or another type of agent, to model plasmin more effectively. In order to choose this agent it will be necessary to take into consideration not only the molecular weight, but also the way the molecule is incorporated into the PEG-DA gel and the Stokes radius.

The team would also reevaluate how to accurately measure the concentration of the supernatant left in the well after the hydrogel diffusion. When pipetting up the remaining supernatant, it is common that the hydrogel would become disturbed. This would not only alter the concentration of the remaining supernatant, but also disrupt and lead to error within our testing. In order to prevent this, the team would need to develop further methodology for removing this supernatant to measure the concentration with accuracy, without disturbing the hydrogel.

In addition to these modifications to the original testing, the following would need to be tested in order to rule out any potential alternative active agents. While plasmin is a fibrinolytic agent that directly reduces fibrin, when considering something as complex as the body, it is necessary to consider the entire cascade by which fibrin is formed. By attacking a higher point within the cascade (such as tissue plasminogen activator), it is possible to target fibrin before it even begins to form. Moving forward it would be useful to investigate this further and develop a better understanding of these other agents to choose the most appropriate agent for preventing fibrin formation, and eventual maturation. This testing would become much more complex in the ability to model in-vivo environments, and therefore would need not only significant monetary resources, but access to sophisticated testing equipment.

One aspect of testing that was not tested is the ability to test our fibrinolytic agent on forming fibrin, and not pre-developed fibrin. The team's testing this semester focused on testing plasmin, to see its impact on fibrin. However, when put in-vivo and used in practice, the plasmin will be actively degrading fibrin as it forms. In order to understand the true efficacy of our design, this testing would need to be conducted. One limitation this semester was our ability to model this ex-vivo. With budgetary restraints, as well as access to limited equipment, our testing was able to

determine a rate at which fibrin can be degraded by plasmin, but not a rate at which plasmin can prevent fibrin formation. This would be an important factor to consider if this project is continued.

Lastly, one thing that needs to have significant testing is the impact in vivo gelling would have inside the body. While it is currently not very well understood, gelling in situ can be very difficult. As is discussed in current research, in order to optimize in situ gelling, it is necessary to take into consider the complexity of the body and the functionality of the hydrogel after gelling. When gelling in situ you must limit exposure by having the crosslinking period be as short as possible. Another factor to consider is the potential damage done by UV light probe when working in the body. These factors as well as several others would need to be considered and an analysis of the benefits versus potential complications would need to be evaluated. [19]

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XII. Appendix

A. Expenses and Materials

Given a budget of \$200, the team made several purchases used for testing various aspects of the final design.

Purchase	Quantity	Price in US dollars	Part #	Purchased From
Plasmin, 150 µg lyophilized powder	1	84.30	P1867-150UG	sigmaaldrich.com
Fibrinogen, human plasma 100 mg	1	59.00	341576-100MG	emdmillipore.com
Thrombin, human plasma 100 units	1	59.00	605190-100U	emdmillipore.com
Fluorescein-isothiocyanate dextran (1.3 mg)	1	free	n/a	Andrew Dias, Murphy Lab
Poly(ethylene glycol)-diacrylate (PEGDA) (.071 g)	1	free	n/a	Andrew Dias, Murphy Lab
Phosphate buffered saline (PBS)	1	free	n/a	Andrew Dias, Murphy Lab
Tris buffered saline (TBS)	1	free	n/a	Department of Biomedical Engineering

B. Protocols

Fibrin Gel Formation and Testing:

Creating Fibrin gel [20]:

1. Prepare TBS
 - a. Dissolve 6.05 g Tris and 8.76 g NaCl in 800 mL of diH₂O.
 - b. Adjust pH to 7.6 with 1 M HCl.
2. Prepare stock thrombin at concentration of 6 U/mL in TBS buffer solvent
 - a. Given we have 100 U, 16.666 mL of TBS buffer
3. Prepare fibrinogen at concentration of 30 mg/mL
 - a. Given we have 100mg, 3.33 mL of TBS
4. Prepare CaCl₂ at concentration of 2.5 mM
 - a. .0011 g of CaCl₂ in 4 mL of TBS
5. All three are mixed in a 1:1:1 ratio to make an aliquot of a 100 uL in the lid of a 96-well plate
 - a. 33.3 uL of thrombin + 33.3 uL of fibrinogen + 33.3 uL of CaCl₂
6. Make aliquots in lids of 96-well plates
7. Incubate in 37 degree C for 10 minutes

Plasmin Preparation:

1. Dissolve 150 ug (around .3 U) of plasmin in 250 uL of TBS
2. Prepare dilutions of plasmin according to **Table 3** below

Dilution	Concentration	uL of Plasmin	uL of TBS Buffer
1	.0012 U/uL	NA (see above)	NA (see above)
2	.0009 U/uL	150 of Dilution 1	50
3	.0006 U/uL	100 of Dilution 2	50
4	.0003 U/uL	50 of Dilution 3	50

5	.00012 U/uL	10 of Dilution 1	90
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Table 3. Table of plasmin dilutions used in fibrin degradation testing.

Testing of Fibrin gel with Plasmin:

1. Prepare 8 fibrin gel aliquots for 6 categories according to layout shown below
2. Administer 10 uL of agent to each gel by dotting it on the center of the gel
 - a. Columns 1+2: Control (TBS)
 - b. Columns 3+4: 0.012 U Plasmin
 - c. Columns 5+6: 0.009 U Plasmin
 - d. Columns 7+8: 0.006 U Plasmin
 - e. Columns 9+10: 0.003 U Plasmin
 - f. Columns 11+12: 0.0012 U Plasmin

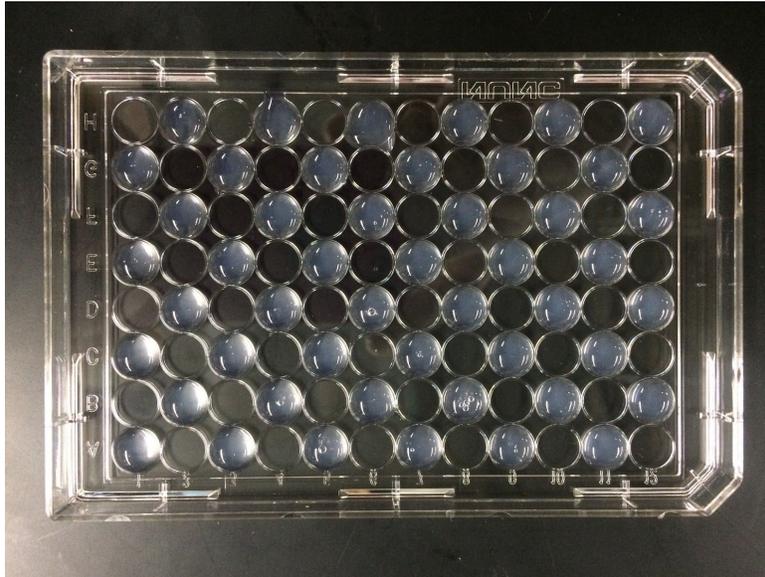


Figure 8. Image of fibrin gels on 96 well plate lid. Gels were spaced kiddy-corner to prevent merging of neighboring gels.

3. Let incubate at 37 degree C for 30 minutes
4. Pipette off excess liquid (degraded fibrin and extra plasmin)
5. Weigh gels

Hydrogel Formation and Testing:

Hydrogel Formation

1. Create PEG gels at concentrations of 10%, 15%, 20%
 - a. Mix PEG powder to 20%, 30%, and 40% concentrated by weight in 80 uL groupings
 - i. .0152 g PEG-DA for 20%, .02388 g for 30%, .03184 g for 40%
 - ii. Samples were spun and sonicated in order to completely dissolve
 - b. PEG-DA solutions were then combined with equal parts photoinitiator to produce 10%, 15%, 20% PEG-DA
 - c. Add “drug”-- fluorescence element to each experimental grouping (1.518 ug FITC-D to 60 uL aliquots of experimental PEG concentrations)
 - d. Pipet aliquots of 10 uL into 96 well plate
 - i. A: 1-5 are experimental 10% PEG, 7-8 are control 10% PEG
 - ii. B: empty
 - iii. C: 1-5 are experimental 15% PEG, 7-8 are control 15% PEG
 - iv. D: empty
 - v. E: 1-3 are experimental 20% PEG, 7 is control 20% PEG
 - e. Place in 37 degree C incubator
 - f. Drug Release Kinetics
 - i. After t=24, t=48, remove 25 uL supernatant, add to 96 well plate
 - ii. Add 25 uL PBS to supernatants in 96 well plate
 - iii. Create serial dilutions of stock (0.759 uM dextran solution), add to 96 well plate (see **Table 4.**)
 - iv. Run 96 well plate in fluorescence reader
 - v. Replace 25 uL supernatant with 25 uL PBS in hydrogel 96 well plate
 - vi. Replace in incubator at 37 degrees C

Volume (uL)	1	2	3	4	5	6
PBS	0	25	37.5	43.75	46.875	98.4375
Stock dextran (0.759 uM)	50	25	12.5	6.25	3.125	1.5625
Total	50	50	50	50	50	100

Table 4. Serial dilution volumes for fluorescent testing of dextran agent.

C. Product Design Specifications

Chemical Dissolution of Abdominal Adhesions Product Design Specifications

Hanna Barton, Raven Brenneke, Julia Handel, Katie Hohenwalter, Nate Richman

Function: Our design is to prevent formation of recurrent adhesions in patients undergoing abdominal surgeries. The team's methodology for approaching this problem is to find a way to stop the cycle of recurrent adhesions.

Client Requirements:

- Must be able to remove/prevent adhesions
- Meet the unmet need in the market for a way to prevent/remove adhesions within a patient having bowel obstructions
- Must not be more invasive than current adhesion removal laparotomies

Design Requirements:

1. Physical and Operational Characteristics

a. *Performance requirements:* The product must prevent recurrence of adhesions after abdominal surgeries. It should reduce adhesion reformation so only 5% of adhesion patients reform adhesions after removal.

b. *Safety:* The product must be FDA approved and completely sterile when being used in the operating room. The sterility of the product must comply with Articles 820.25, 70, 50, 72 of the FDA [21]. The product must also be biocompatible with the human body, and blood homeostasis must be maintained. For example, the pH must not go outside of the 7.35 - 7.45 range and the level of fibrinogen must remain within 200 - 400 mg/dL [22].

c. *Accuracy and Reliability:* This product must be able to target a localized region without seeping into other parts of the body. It must also be able to prevent the adhesion without subsequent major surgery for device removal once the solution has acted on the adhesion. No more than 2% of the introduced solution should seep out of the area containing the adhesion.

d. *Life in Service:* The solution should ideally be fast acting after it is cross linked internally. It would ideally release the active agent over a period of 72 hours to dissolve the forming adhesion. The active agent should not be excessively activated and should not be active for more than a week in vivo.

e. *Shelf Life:* The individual chemicals should last for multiple years in appropriate storage. The injectable hydrogel and fibrinolytic agent itself should last multiple hours prior to implementation in the body.

f. *Operating Environment:* The product is to be administered in an operating/procedure room where all FDA sterility standards apply [21].

g. *Ergonomics:* The product must be user friendly for those in an operating/procedure room. The product must follow OSHA's Sections 1910.103, 1910.106 through 1910.111, and 1910.119, 1910.120, and 1910.122 through 1910.126, which declare the standards for hazardous materials [23].

h. *Size*: The hydrogel/plasmin solution must be on the scale of milliliters so that it can be ejected into the body during a laparoscopic procedure. The UV probe used to crosslink the hydrogel *in vivo* must be between 3-10mm since current laparoscopic tools are on this scale. The design must be compatible with current surgical tools.

i. *Weight*: The injected hydrogel must not exceed a few grams since it will remain in the body for an extended period of time. Any hydrogel that is too heavy may create unnecessary stress on surrounding tissues.

j. *Materials*: The product will be composed of an injectable hydrogel PEG-DA solution, plasmin, a UV light probe, and a syringe-like device to inject the solution.

k. *Aesthetics, Appearance, and Finish*: This product is intended to dissolve adhesions within the body, and thus will not be seen once implanted in the patient. As a result, its appearance and finish are not of great concern.

2. Production Characteristics

a. *Quantity*: Since the team's target customers are patients with unique complications, the product will most likely be produced on a relatively small scale with the possibility of individualization.

b. *Target Product Cost*: The product should not cost substantially more for the patient or hospital than the current surgery used to remove mature adhesions. Specific material and procedural costs will depend greatly upon the final design chosen.

3. Miscellaneous

a. *Standards and Specifications*: The product must comply with all hospital and FDA regulations regarding sterility for critical items [21]. It must also reduce patient discomfort as compared to the patient's comfort levels prior to the operation.

b. *Customer*: The intended customers are patients who are undergoing abdominal adhesiolysis. They are most likely to be older patients who had abdominal surgeries before preventative methods were implemented. The direct customer will be the hospitals, so future marketing of this product must be geared towards them.

c. *Patient-related concerns*: The patient should experience minimal discomfort and no further adhesion development as a result of the treatment. The patient should not have any additional discomfort other than the discomfort associated with laparoscopy. The patient should also subjectively report increased comfort postop.

d. *Competition*: Although there are numerous products focusing on adhesion prevention, our design must make use of naturally occurring proteins in the body to prevent the creation of future abdominal adhesions.

D. Hydrogel/Fluorescence Data

Pilot Study

Goal: Test varying concentrations of fluorescent molecule and different amounts.

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.795	3.795										
B	0.759	0.759										
C	0.0759	0.0759										
D	0.00795	0.00795										
E	0.000759	0.000759										
F												
G												
H												

Figure 9: Schematic of 96 well plate with corresponding concentrations of fluorescing molecule solutions in ug/uL.
All solutions in column 1 contained 100 uL while all solutions in column 2 contained 50 uL.

Software Version 2.09.1

Experiment File Path: T:\ActiveProjects\Andrew Dias\BME design project\12-02-16 pilot assay.xpt

Protocol File Path:

Plate Number Plate 1

Date 12/2/2016

Time 3:05:23 PM

Reader Type: Synergy HTX

Reader Serial Number: Unknown

Reading Type Reader

Procedure Details

Plate Type 96 WELL PLATE

Eject plate on completion

Read Fluorescence Endpoint

Full Plate

Filter Set 1

Excitation: 485/20, Emission: 528/20

Optics: Top, Gain: 35

Light Source: Tungsten, Standard Dynamic Range

Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 10

Read Height: 1 mm

Results

Actual Temperature: 23.3

	1	2	3	4	5	6
A	26105	17019	0	0	0	0
B	9797	4971	0	0	0	0
C	1066	457	0	0	0	0
D	112	45	0	0	0	0
E	9	3	0	0	0	1
F	0	0	0	0	0	0
G	0	0	1	0	0	0
H	0	0	0	0	0	0

Figure 10: Image of fluorescence data from pilot study corresponding to plate setup in **Figure 9**..

24 Hours

Goal: Create a plate of fluorescent molecule and hydrogel supernatant after 24 hours of 37 degree incubation to compare the fluorescing of the two.

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.795	3.795			S1			10%		15%		20%

B	0.759	0.759			S2			10%		15%		20%
C	0.0759	0.0759			S3			10%		15%		20%
D	0.00795	0.00795			S4			10%		15%		
E	0.000759	0.000759			S5			10%		15%		
F					S6							
G								10%C		15%C		20%C
H								10%C		15%C		

Figure 9: Schematic of 96 well plate with corresponding concentrations of fluorescing molecule solutions and hydrogel solutions after 24 hours. Columns 1 and 2 correspond to concentrations specified in **Figure 9** plated at time 0 hours. All solutions in column 1 contained 100 uL while all solutions in column 2 contained 50 uL. Solutions in column 5 corresponded to serial dilutions of stock fluorescing molecule solutions prepared at time 24 hours in accordance with volumes in **Table 4**. Columns 8, 10, and 12 contain the supernatants of the various crosslinking groups. Rows G and H contain controls of the respective groups.

Software Version 2.09.1

Experiment File Path: T:\ActiveProjects\Andrew Dias\BME design project\12-03-16 24h.xpt

Protocol File Path:

Plate Number Plate 1

Date 12/3/2016

Time 5:00:17 PM

Reader Type: Synergy HTX

Reader Serial Number: 160524D

Reading Type Reader

Procedure Details

Plate Type 96 WELL PLATE

Eject plate on completion

Read Fluorescence Endpoint

Full Plate

Filter Set 1

Excitation: 485/20, Emission: 528/20

Optics: Top, Gain: 35

Light Source: Tungsten, Standard Dynamic Range

Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 10

Read Height: 1 mm

Results

Actual Temperature: 23

	1	2	3	4	5	6
A	24114	14528	0	0	4644	0
B	8955	4265	0	0	2276	0
C	944	402	0	0	989	0
D	100	37	0	0	411	0
E	8	4	0	1	152	0
F	0	0	1	0	53	0
G	0	1	0	0	0	0
H	0	0	0	0	0	1

Figure 10: Image of fluorescence data after 24 hours. All grid squares with relevant data are shown. The grid squares not shown in this image did not contain any significant values and read “0” fluorescence.

48 Hours

Goal: Create a plate of fluorescent molecule and hydrogel supernatant after 48 hours of 37 degree incubation to compare the fluorescing of the two.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1		10%		15%		20%					

B	S2		10%		15%		20%					
C	S3		10%		15%		20%					
D	S4		10%		15%							
E	S5		10%		15%							
F	S6											
G			10%		15%		20%					
H			10%		15%							

Figure 11: Schematic of 96 well plate with corresponding concentrations of fluorescing molecule solutions and hydrogel solutions after 24 hours. Columns 1 solutions corresponded to serial dilutions of stock fluorescing molecule solutions prepared at time 48 hours in accordance with volumes in **Table 4**. Columns 3, 5, and 7 contain the supernatants of the various crosslinking groups. Rows G and H contain controls of the respective groups.

Software Version 2.09.1

Experiment File Path: T:\ActiveProjects\Andrew Dias\BME design project\12-03-16 48h.xpt

Protocol File Path:

Plate Number Plate 1

Date 12/4/2016

Time 4:33:33 PM

Reader Type: Synergy HTX

Reader Serial Number: Unknown

Reading Type Reader

Procedure Details

Plate Type 96 WELL PLATE

Eject plate on completion

Read Fluorescence Endpoint

Full Plate

Filter Set 1

Excitation: 485/20, Emission: 528/20

Optics: Top, Gain: 35

Light Source: Tungsten, Standard Dynamic Range

Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 10

Read Height: 1 mm

Results

Actual Temperature: 23

	1	2	3	4	5	6
A	5155	0	0	0	0	0
B	2318	0	0	0	0	0
C	1064	0	0	0	0	0
D	211	0	0	0	0	0
E	395	0	0	0	0	0
F	62	0	0	1	0	0
G	0	0	0	1	0	0
H	0	0	1	0	0	0

Figure 12: Image of fluorescence data after 48 hours. All grid squares with relevant data are shown. The grid squares not shown in this image did not contain any significant values and read “0” fluorescence.

E. Statistical Methods: Bootstrap

Code from matlab used to produce p-val given two independent populations with unknown variances and small sample size: Bootstrap method.

```
1 function [ pval ] = bootstrap2pop(dat1,dat2,numBoot)
2 %bootstrap Computes bootstrap CI
3 % dat1=first population
4 % dat2= second population
5 % numBoot= number of simulations to run
6 % returns p-value
7 - truemean = mean(dat1)-mean(dat2);
8 - n1 = length(dat1);
9 - n2 =length(dat2);
10 - tObs = abs((mean(dat1)-mean(dat2))/(sqrt(var(dat1)/n1+var(dat2)/n2)));
11 - low = [];
12 - high = [];
13 - for i=1:numBoot
14 -
15 -     samp1 = datasample(dat1,n1);
16 -     samp2 = datasample(dat2,n2);
17 -     samp1mean = mean(samp1);
18 -     samp2mean = mean(samp2);
19 -     samp1var = var(samp1);
20 -     samp2var = var(samp2);
21 -     thisObs = (samp1mean-samp2mean-truemean)/(sqrt((samp1var/n1)+(samp2var/n2)));
22 -     %fprintf('%f\n',thisObs);
23 -     if thisObs<(-tObs)
24 -         low = [low thisObs];
25 -     end
26 -     if thisObs>tObs
27 -         high = [high thisObs];
28 -     end
29 - end
30 - pval = (length(low)+length(high))/numBoot;
31 - end
```

Figure 13: Image of MATLAB code used to calculate p-values for fibrin gel testing.