Chemical Dissolution of Abdominal Adhesions

BME 200/300

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Client: Dr. Philip Bain
Dean West Clinic

Advisor: Professor Kristyn Masters
University of Wisconsin-Madison Department of Biomedical Engineering

Team Members: Julia Handel (Team Leader), Katie Hohenwalter (BSAC), Hanna Barton (Communicator), Nate Richman (BPAG), Raven Brenneke (BWIG)

Abstract

Abdominal adhesions are a common result of abdominal surgeries, and on occasion can result in severe pain and small bowel obstructions. This is especially problematic in the aging generations that have undergone highly invasive procedures. Currently, when a patient presents with a small bowel obstruction due to adhesions, the standard procedure for removal involves a laparotomy where a surgeon manually cuts the adhesion. In theory, this should solve the problem; however the conduction of this removal surgery can lead to the formation of further adhesions, and ultimately, more bowel obstructions. The client is seeking a more effective, chemical solution to sever these adhesions. By attacking the collagenous extracellular matrix (ECM) with matrix metalloproteinases (MMPs), the adhesions can be degraded through natural physiological processes. Hydrogel technology will be used to deliver MMPs to adhesion sites. In the coming weeks, the team will design, fabricate, and test the efficacy of the proposed design solution.

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I. Introduction

A common, yet often understudied byproduct of abdominal surgeries is the formation of adhesions. Adhesions, or bands of scar tissue, form in between internal organs that are not meant to be linked. An example of an adhesion can be seen in Figure 1 below. Adhesions are invariably formed in all invasive abdominal surgeries and 67-100% of abdominal laparotomies performed [1]. In most patients, they do not cause any significant complications, and as such can remain in the body indefinitely. However in 15-18% of those patients with adhesions, they become problematic, causing small bowel obstructions, female infertility, severe pain, or other complications [1]. This can lead to the necessitation of adhesion removal.

Currently, when patients suffer from a small bowel obstruction, multiple actions are taken to attempt to get rid of the obstruction without surgery. The patient may undergo aggressive intravenous fluid therapy and correction of electrolyte imbalance. A nasogastric tube could be placed, which allows for decompression of the stomach and prevents aspiration. Continuous monitoring via CT scans and physical examinations is required to watch the progress of the obstruction [2].

If all of the preliminary treatments fail, the patient is finally admitted for a laparotomy in which the surgeon will enter the abdominal cavity laparoscopically to sever the problematic adhesions. This solution fixes the immediate small bowel obstruction, but in most cases, results again in subsequent adhesion formation. Recurrent small bowel obstructions due to problematic adhesions are seen especially in the elderly population that has undergone more invasive abdominal surgeries. This is the problem the team is seeking to address.

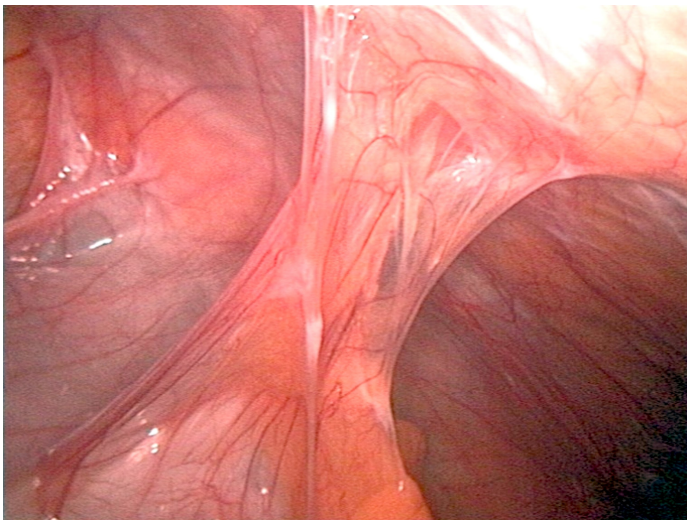

Figure 1. This image displays a visual of a mature adhesion. The dark red lines are vasculature while the light pink is the ECM.

Competing Designs

As mentioned above, the current adhesion removal procedure is a laparoscopic severing of the adhesion via mechanical means. In terms of mature adhesion removal, this is the only competing design. In this technique, the surgeon opens the abdominal cavity laparoscopically. Using laparoscopic scissors, the surgeon snips the adhesion in half, relieving the tension that is causing the small bowel obstruction [3].

There is also a lot of research being done on adhesion formation prevention--which focuses primarily on the initial makeup of an adhesion. This is not the focus of the project, but is important to understand.

Adhesions due to a surgery can come from a variety of sources including, poor handling of internal organs, drying out tissues, contact with foreign materials, and many other contaminants [3]. These are common problems in surgery, and sometimes are unavoidable, but there are many preventative technologies that are currently in clinical trials to combat the development of abdominal adhesions.

Simvastatin is a strong fibrinolytic agent in human mesothelial cells 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 one study conducted on rats, this agent was administered intraperitoneally, and 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. As fibrin often is the beginning of the development of an 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, expression is delayed. Therefore the development of adhesions is restricted [5].

On the other hand, the Anti-adhesion film is a chemical application onto the area that is at risk for adhesion development. By use of chemicals on a physical film that adhering to the extracellular matrix (ECM) of native tissues, the substrate comprises the collagen and doesn't 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 ECM [6].

Problem Statement

In many patients with past surgical histories abdominal adhesions are common. In elderly patients especially, these adhesions can become painful and cause further complications such as small bowel obstructions. Surgery is currently the only viable non-preventative method for removing adhesions. 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.

II. Background

Adhesions are a result of the inflammatory process that happens at the site of surgery or trauma. Following the pathway shown below in Figure 2, it is seen that adhesions are initially made up of fibrin. This adhesion formation happens within 72 hours. This time period is the target of preventative adhesion research.

After the formation, collagen synthesis begins in the adhesion. This collagen synthesis continues until all of the fibrin has been turned over and the adhesion is primarily made up of an collagenous extracellular matrix (ECM). Once the 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 form normal healing of the 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].

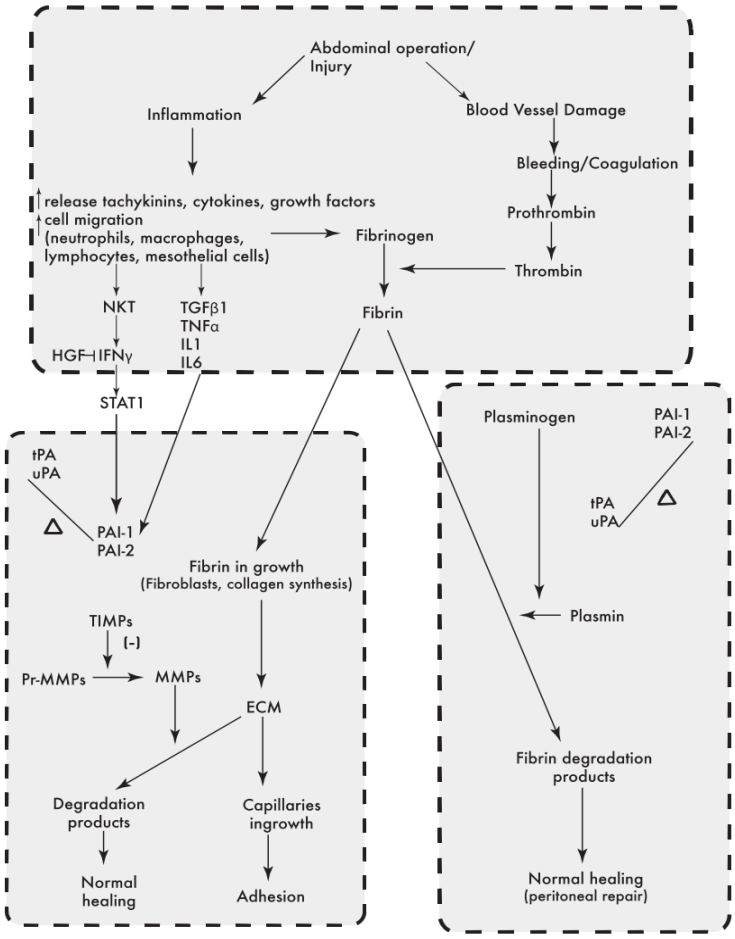

Figure 2. This diagram shows the cascade of healing as a result of an abdominal surgery. In this case, the focus is on the initial fibrinous tissue that is quickly turned over into a collagenous extracellular matrix [7].

Client Information

The client, Dr. Philip Bain is a practicing internist at the Dean clinic in Madison, WI. Dr. Bain has had many patients coming into his office complaining of severe bowel pain, which are often diagnosed as small bowel obstructions from abdominal adhesions.

Design Specifications

The main specifications of the design are that it must completely sever the adhesion and reduce its volume by more than 50% of its total volume. It must also degrade mature adhesions and cannot be a preventative solution. Also, 98% of the administered MMP must be contained to the site of the adhesion and should not seep into other tissues of the body. Finally, the delivery device and MMP must be viable for FDA approval in regards to sterility standards in hospital settings. A more complete list of design specifications can be found in Appendix A.

III. Preliminary Design

Selection of Chemical Agent

There are three main components of an adhesion that could be targeted--cells, vasculature, and the ECM. However, in order to effectively sever the adhesion, the ECM is the only viable target. This is because removing or diminishing cells and vasculature will not sever the physical connection that the ECM creates between tissues.

In order to remove the ECM that is our target, matrix metalloproteinases (MMPs) or collagenases will be used. These are synthesized by the epithelial adhesion cells in a latent form that becomes active after interaction with a pro-MMP [8]. They function by preferentially degrading the proteins in the ECM.

Selection of Delivery Method

Design Idea 1: Hydrogel

Design idea 1 consists of exogenous MMP delivery through a hydrogel application (Figure 3). 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.

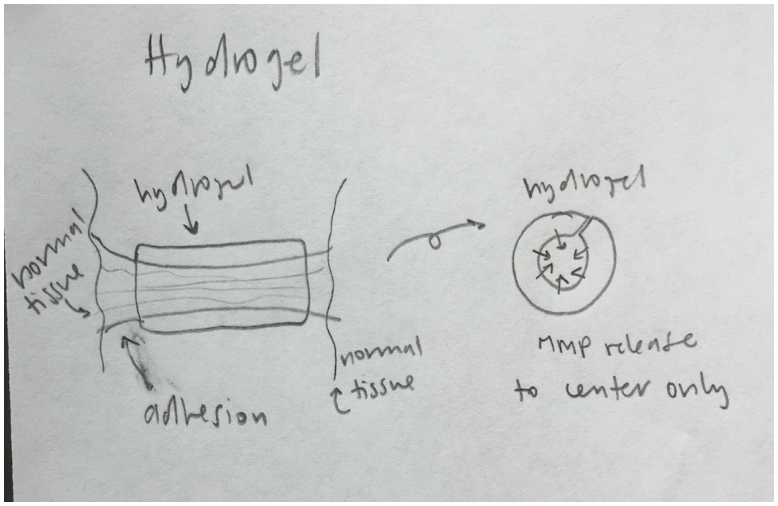

Figure 3. Design idea 1 uses a hydrogel that wraps around the adhesion and selectively diffuses MMPs to the adhesion. Its exact dimensions will vary depending upon the specific adhesion targeted, but it will be on the scale of millimeters.

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 4). 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.

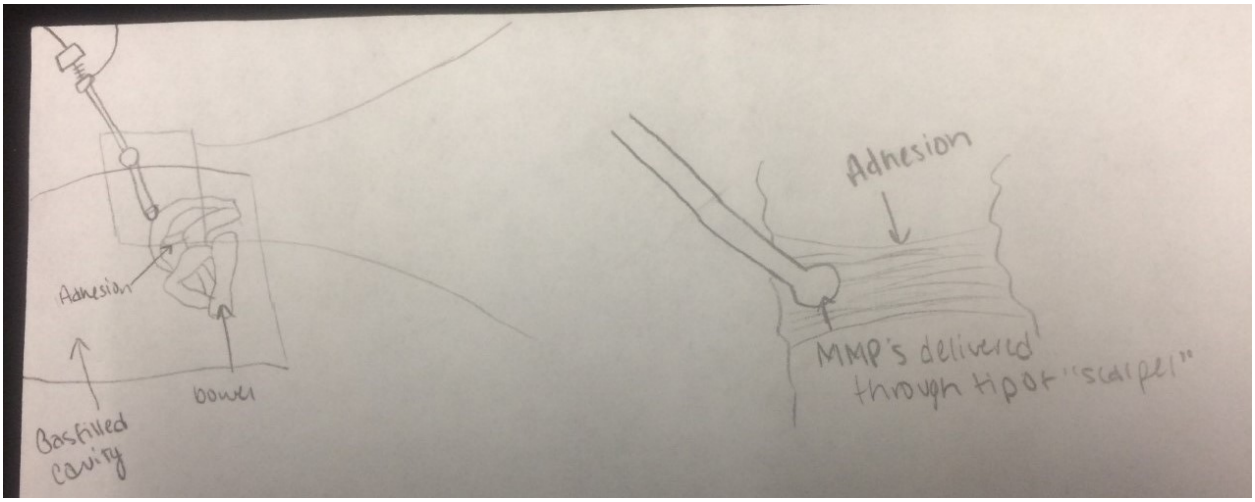

Figure 4. Design idea 2 uses a scalpel-like instrument to deliver MMPs to the adhesion. MMPs will be delivered in multiple areas of the adhesion, and delivery is controlled by the operating surgeon.

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 [9]. 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 our preliminary design ideas based off of relevant criteria (Figure 5).

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)		76		64		46

Figure 5. Displayed above is the design matrix used to evaluate and rank our three design ideas. The hydrogel design scored highest in safety, cost, and fabrication, thus rendering it a feasible design and leading the team to choose it as the winning design.

The hydrogel application was selected as the proposed final design. This design ranks highest in the criteria of safety, cost, and fabrication.

The hydrogel ranked highest in the safety category for multiple reasons. The hydrogel will be both biodegradable and biocompatible, thus lowering the risks of inducing toxicity upon implantation. The hydrogel also regulates the release of the MMPs and provides a physical barrier to the rest of the body that restricts MMPs from escaping the adhesion site. This reduces the risk of MMPs attacking healthy tissue, and improves the safety of the MMP release even though the MMPs are exogenous. Since the chemical scalpel design does not contain a barrier to prevent released MMPs from diffusing to healthy tissues, it ranked lower than the hydrogel in safety. Gene therapy was ranked lowest for safety because gene therapy is extremely experimental. It is difficult to predict its effects on other bodily processes since most research has been done on cell cultures in vitro and not in vivo. Specific tissue regulation of MMPs is still poorly understood and is currently under review, thus rendering its safety standards unreliable [9].

The hydrogel design also scored highest in cost and fabrication as compared to the other two designs. Gene therapy is extremely experimental and requires intensive research, thus rendering it costly and timely. The chemical scalpel requires fabrication of a scalpel-like instrument that contains a storage compartment for MMPs and a release mechanism for the MMPs. Since hydrogels are relatively cheap, the hydrogel design scored highest in the cost criteria.

Finally, the hydrogel application also scored highest in fabrication because hydrogels are common products that can be easily fabricated in labs. The fabrication of the chemical scalpel will require a unique design that will be able to incorporate MMP containment and a release

mechanism. Again, since the gene therapy design is extremely experimental, fabrication, or development of this design, will be extremely difficult and timely.

Proposed Final Design:

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

V. Fabrication

To fabricate the proposed final design, we will begin by choosing hydrogel components, which will allow MMPs to be contained and then diffused unilaterally. We will consider the hydrogels listed in Table 1 after more careful research on their properties. Once chosen, procedures already outlined in literature will be used to create this hydrogel. The chosen hydrogel will be fabricated and tested multiple times [10].

Drug delivery, pharmaceutical	poly(vinylpyrrolidone)	(Benamer et al., 2006; Rosiak et al., 1995)
	starch, poly(vinylpyrrolidone), poly(acrylic acid)	(Kumar et al., 2008; Spinelli et al., 2008)
	carboxymethyl cellulose, hydroxypropyl methyl cellulose	(Barbucci et al., 2004; Porsch & Wittgren, 2005)
	polyvinyl alcohol, acrylic acid, methacrylic acid	(Nho et al., 2005)
	chitosan, αβ-glycerophosphate	(Zhou et al., 2008)
	κ-carrageenan, acrylic acid, 2-acrylamido-2-methylpropanesulfonic acid	(Campo et al., 2009; Pourjavadi & Zohuriaan-Mehr, 2002)
	acrylic acid, carboxymethyl cellulose	(El-Naggar et al., 2006; Said et al., 2004)

Table 1. This table outlines numerous hydrogels that are currently in use for drug delivery purposes along with their sources [10].

VI. Testing and Future Results

In order to test the proposed final design's feasibility, a surgeon will be contacted to ensure that wrapping a hydrogel around an adhesion is practical. If this process is not possible due to the complexity of the adhesion structure, the hydrogel will be applied directly to one side of the adhesion. The hydrogel design will first be tested on collagen gels that have been fabricated in the biomaterials lab. This testing is intended to aid in determining the delivery efficacy of various hydrogels.

Another aspect of our design that needs to be quantified is how effective the delivery method is at isolating the MMP, and how compatible it is with laparoscopic tools. We will use the standard determined by the initial MMP testing to serve as our standard for containment, and the team will seek to limit that area to the delivery site. As for compatibility with laparoscopic tools, the team will consider the handling of the device in vivo, the ability to fit a laparoscopic scale, and the adaptability of tools to current devices.

VII. Discussion of Future Work

The team will be moving forward by selecting a hydrogel and beginning the fabrication process. The hydrogel must contain an appropriate MMP. In order to pick the most effective MMP for this design, a variety of aspects must be taken into consideration. The MMP must be collagen specific. In the case of this design, the MMP should effectively attack Collagen I and Collagen III [11]. The MMP should also have an appropriately short half-life. This will reduce the risk that part of the hydrogel will degrade healthy tissue if the placement is not 100% accurate (i.e. some of the hydrogel touches healthy tissue rather than the adhesion). Finally, the selected MMP should effectively degrade the extracellular matrix to reach the goal of dissolving 50% or more of the adhesion.

When the appropriate MMP is chosen, the team will need to conduct testing to determine the appropriate concentration of MMP to be placed in the hydrogel delivery system. Various concentrations will be tested on collagen gels fabricated in the biomaterials lab; these testing gels will be fabricated in accordance to the procedure outlined in Appendix B. From the degradation of collagen on these gels, the team will be able to gain a more appropriate allocation of MMP concentration and be able to quantify the degradation caused by the chosen MMP. Further testing will also need to be conducted to determine the effectiveness of the hydrogel on existing adhesions.

The team will be meeting with a surgeon from the University of Wisconsin-Madison Hospitals and Clinics, Dr. Matzke, who is familiar with abdominal adhesions. The meeting will aid the team in understanding how to produce a design that functions effectively in laparoscopic techniques.

VIII. Conclusions

Abdominal adhesions appear in elderly patients who have had previous abdominal procedures. As the population ages, more of these elderly patients report small bowel obstructions due to complications of these abdominal adhesions. Currently, the patient undergoes surgery to remove the adhesion by mechanically removing it. However, since this technique requires another abdominal procedure, there is further risk of subsequent adhesion development.

Due to increasing commonality of this problem, the team is tasked with creating an alternative solution to remove adhesions that decreases the risk of further adhesion development. Delivery of MMPs through a hydrogel application has been chosen as the proposed final design by the team. Following this choice of design, the team will now choose an appropriate MMP and establish both a fabrication procedure and testing protocol to develop this solution.

IX. References

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X. Appendix

A. PDS- Product Design Specifications

Chemical Dissolution of Abdominal Adhesions
Product Design Specifications

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

Function: To remove mature adhesions in patients who have received many surgeries and have resulting symptoms due to large adhesions. The solution must be less-invasive than current techniques.

Client Requirements:

- Must be able to remove adhesions after the adhesions are mature and well-developed (not a preventative measure)
- Must be non-invasive to reduce the risk of further adhesion development and other issues associated with large surgeries

Design Requirements:

1. Physical and Operational Characteristics

a. *Performance requirements*: The product is to assist in adhesion degradation or removal without the need for major surgical procedures. It should cause less of a risk of adhesion reformation than current removal methods, and it should reduce the overall adhesion volume by greater than 50%.

b. *Safety*: The product is to attack formed adhesions without negatively affecting functioning organs or causing adverse reactions in the body. This includes potential for chemical contamination to non-targeted tissues, induced toxicity to organs, and development of adverse side effects to patient or surgeon due to delivery method or MMP type. The product must maintain FDA standards through clinical testing for safety.

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 remove the adhesion without subsequent major surgery for device removal once the solution has acted on the adhesion. No more than 2% of the introduced MMP solution should seep out of the area containing the adhesion.

d. *Life in Service*: The solution should ideally be fast acting and with a short half-life to optimize its activity at the site of the adhesion before possible diffusion to other parts of the body. Half life should be as short as short as possible, while still allowing for destruction of collagen.

e. *Shelf Life:* The device itself should last for 1 year in appropriate storage, but the actual enzyme solution will be made within days to hours of administration.

f. *Operating Environment*: The product is to be administered in an operating/procedure room where all FDA sterility standards apply [2].

g. *Ergonomics*: The product must be user friendly for those in an operating/procedure room. According to 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.

h. *Size*: The product should be small enough to be conveniently inserted into the abdominal area of an adult patient without creating problems for neighboring organs. The design must also be able to be surgically implanted with laparoscopy techniques, and must not require a major surgery. Most laparoscopy tools are between 3-10mm so our tool will not exceed this range.

i. Weight: The device must not be too heavy that it will cause the patient uncomfort once implanted; it also should not cause unnecessary stress to the adhesion and surrounding tissue once implanted. The final weight of the design will be on the scale of grams, and no more than 20 grams maximum.

j. *Materials*: The materials currently include a collagenase solution (most likely a derivative of Collagenase Clostridium Histolyticum). The other materials are not yet known and will depend greatly upon the team's choice of delivery method. However, materials chosen must not cause adverse reactions to the body, and will ideally be bioabsorbable.

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 [2]. 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 have matured adhesions and resulting bowel obstructions. They are most likely to be older patients who had abdominal surgeries before preventative methods were implemented.

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, no major products target the removal of already-matured adhesions in the abdominal cavity. Our design may incorporate aspects of current preventative methods (suprafilms, surgical techniques), however, it must be able to degrade a mature adhesion.

PDS References:

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B. Collagen Gel Fabrication Protocol

Adapted from:
http://ibidi.com/fileadmin/support/application_notes/AN26_CollagenI_protocols.pdf

1.1 Material:

- Collagen I, bovine, pepsinized, 3 mg/ml (PureCol®, Advanced BioMatrix, 5005-B)
- 10 × MEM (Sigma, M0275), or
- 10 × DMEM (Sigma, D2429), or
- 10 × M199 (Sigma, M0650)
- RPMI 1640 (Sigma R8758), or
- DMEM (Sigma, D5796), or
- EC-Medium (Promocell, C-22010)
- NaOH in ultrapure H2O, 1M
- NaHCO3 7.5 % (Sigma, S8761)
- Sterile ultrapure water

1.2 Fabrication Protocol

1. Place all solutions at room temperature for half an hour before starting the experiment
2. Determine the final volume of collagen solution to be used (e.g. 300 µl) and the desired, final collagen concentration (e.g., 1.5 mg/ml) by using the table below.

3. Determine the final cell concentration in the gel. Multiply this concentration with a factor of 6 to calculate the required concentration. For example when using ibidi's µ- Slide Chemotaxis 3D, use 18 × 106 cells/ml to reach a final cell concentration of 3 × 106 cells/ml.
4. Prepare a sterile tube with sufficient volume capacity.
5. Mix the gel:
    a) Add all of the ingredients, as shown in the table below. The ingredients are listed in the order of pipetting.
    b) After adding the collagen, thoroughly mix the contents of the tube.
    c) If desired, add the prepared cell suspension to the mixture. If no cells are used, add 1 × medium.
    d) Thoroughly mix the contents of the tube.
6. Fill the gel into the culture dishes or slides within 5 minutes.
7. For gelation, place the gel in a cell culture incubator (37°C, 5 % CO_2) for 45 minutes.
8. The cells will continue to settle in the first few minutes. Therefore, to avoid having the cells settling on the bottom of the vessel, it may be best to incline the chamber vertically (note, this is only possible with chemotaxis chambers and channel slides).