Absorbable Hydrodissection Fluid

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ABSTRACT

Radiofrequency (RF) ablation and cryoablation are two minimally invasive techniques for the treatment of malignant tumors of the liver, lungs, and kidneys. Hydrodissection is used during ablation procedures to protect surrounding tissues from the extreme effects of the ablation procedure. Current solutions, such as saline and D5W (5% dextrose in water), are adequate for protection; however, these fluids tend to migrate throughout the peritoneal cavity.

Dr. Chris Brace, Dr. James Hinshaw, and Dr. Meghan Lubner proposed the development of a more viscous hydrodissection fluid to prevent fluid migration and barrier degradation within the peritoneal cavity. Three design alternatives were developed and a design matrix was used to determine the best of the three alternatives. Five categories were evaluated: biocompatibility, viscosity, cost of materials, ergonomics, and temperature range.

The poloxamer 407 (Lutrol F-127) solution was most favorable for future development and was pursued. Poloxamer solutions with varying concentration were synthesized and the gelation temperature was tested for each concentration. A 19.0% (w/v) poloxamer solution was designed to gel at 32^{o}C to meet design specifications and was used for all testing. Imaging of poloxamer and D5W was conducted with ultrasound and CT scans. An iodinated contrast medium (Iohexal) was added to both solutions to increase contrast during CT scanning. To determine electrical conductivity, impedance testing was performed with an RF ablation machine. The viscosity of poloxamer was found using a capillary viscometer across a range of temperatures.

It was found that as the concentration of poloxamer increases, the gelation temperature decreases. A 19.0% poloxamer solution had the lowest viscosity at approximately 15^{o}C. The poloxamer solution did not inhibit imaging with CT and ultrasound scans. The poloxamer solution had a high impedance value suggesting it will work well as an electrical insulator. Due to its high viscosity at room temperature it is recommended that the poloxamer solution be cooled prior to injection within the peritoneal cavity. A 19.0% poloxamer solution is expected to prevent fluid migration and barrier degradation, while providing adequate protection to surrounding tissue during ablation procedures. Animal testing and clinical trials are required to determine efficacy of the design. Pending results, a 19.0% poloxamer solution would outperform currently used hydrodissection fluids and be a top quality product in the current market.

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PROBLEM MOTIVATION

Hepatic cancer (cancer of the liver) is one of the deadliest diseases with over 500,000 new cases estimated annually worldwide [1]. Readily accepted methods for treatment of this disease are radiofrequency (RF) ablation and cryoablation[2]. Patient complications when using these procedures vary in severity, but most are minimal and result from unintended burning of adjacent organs or tissues with the ablation probe [3-4]. Damage to the diaphragm may result in slight pain while breathing, while intestinal damage can result in death [4]. Hydrodissection fluids aim to limit complications that may result from the ablation procedure.

During thermal ablation procedures, the hydrodissection fluid is injected between the target ablation site and the surrounding tissues to create a suitable physical, thermal, and electrical barrier for protection. Currently used liquids, like 5% dextrose in water (D5W) and saline, satisfy most of these requirements and have been relatively successful. However, they lack the necessary viscosity to prevent unintended fluid migration and quick absorption which result in barrier degradation. Because of this, a large amount of liquid (>1L) is typically required for adequate protection. This can lead to post-procedural complications such as bloating, which must be minimized. Therefore, our clients, Dr. Chris Brace, Dr. James Hinshaw, and Dr. Meghan Lubner have proposed that we design and fabricate a fluid that retains all the favorable qualities of D5W, such as thermal/electrical insulation and biocompatibility, while alleviating its faults.

BACKGROUND

Cancer treatments for tumors of the heart, lungs, liver, and kidneys include chemotherapy, radiation therapy, and surgical removal. Aside from these, many minimally invasive procedures have become increasingly accepted for treatment of malignant tumors over the past 15 years; these include: RF ablation, microwave ablation, laser ablation, cryoablation,

ethanol ablation, and chemoembolization. Results of these procedures have surpassed those of chemo- and radiation therapy [3]. Hydrodissection performed during RF ablation and cryoablation were of primary concern in the design process.

Radiofrequency ablation is a relatively simple procedure. As seen in the setup shown in Figure 1, the RF ablation probe is inserted into the tumor. Radiofrequency AC electrical current is then applied through the electrode causing a temperature increase in the target tissue. This results in the ablation, or destruction, of the tumor.

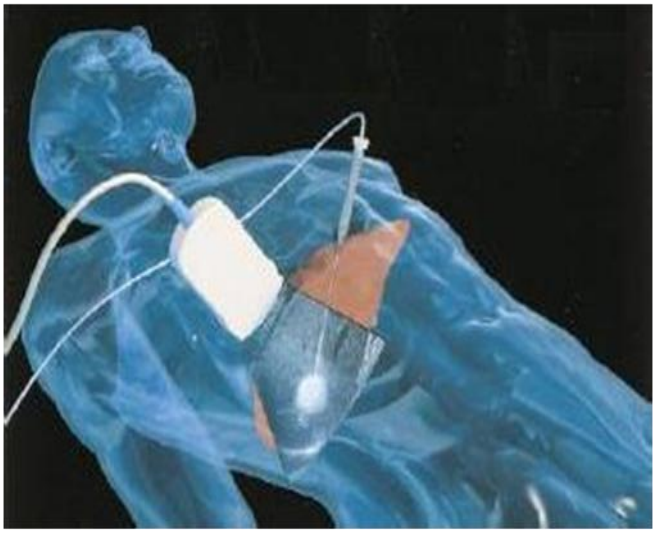


Figure 1 – RF ablation operational setup. A RF electrode is inserted into the tumor using imaging methods for guidance. Image from [3].

The three main methods of RF ablation are surgical, percutaneous, and laparoscopic. Using surgical methods is the most invasive, and involves opening the patient for precise probe placement. General anesthesia is required for surgical RF ablation. In the laparoscopic method, an incision is made in the skin, through which a laparoscope is inserted. This device is then used to accurately place the needle-like RF electrode(s).

Percutaneous RF ablation is the most common clinical method [5]. This is similar to the laparoscopic method, except only the RF electrode is passed through the skin. A variety of different imaging techniques may be used to accurately place the electrode, these include: ultrasound, computed tomography scan (CT scan), and magnetic resonance imaging (MRI) [5].

Only local anesthesia is required for percutaneous and laparoscopic RF ablation; because of this, most RF ablations are outpatient procedures.

Cryoablation is the oldest of the thermal ablation techniques [6]. In comparison with RF ablation, cryoablation uses extremely cold temperatures to kill harmful tissue. Cryoablation can also be performed surgically, percutaneously, or laparoscopically using a cryoprobe. A cryoprobe is a hollow tube that circulates cold fluid; this can be seen in Figure 2. An advantage of cryoablation over RF ablation is that multiple cryoprobes can work simultaneously to form differently shaped ice balls. These ice balls are easier to see using imaging equipment, and can therefore treat larger tumors than RF ablation [3].

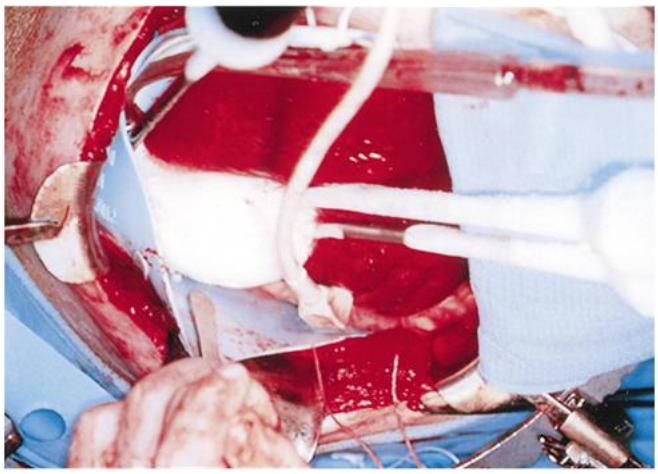


Figure 2 – An ice ball is formed during cryoablation to destroy harmful tissue. Image from [3].

Cryoablation and RF ablation methods have yielded favorable patient results. Both have successful resection more than 85% of the time with complete ablation and no reoccurrence of tumors in 52-67% of patients [3]. Cryoablation generally offers better control to the doctor during the procedure, and is able to treat larger tumors (> 3 cm) than other ablation techniques. Because of this, tumor recurrence for cryoablation is approximately 13%, whereas tumor recurrence for RF ablation can be upwards of 30%. Aside from this, RF ablation generally has fewer patient complications because it is a less invasive procedure [3]. Fewer than 5% of patients are seriously injured although ablation related deaths have been reported [3, 5, 7].

CURRENT TECHNOLOGY

A crucial factor in the success of the operation and the survival of the patient is protection of the surrounding, non-cancerous tissue. Cryoablation and RF ablation do not inherently differentiate between healthy and unhealthy tissue; it is up to medical personnel to localize tissue damage to the tumors. To help with this, a layer of protective fluid is injected into the patient around the target area in a process known as hydrodissection. This fluid layer separates the target and surrounding tissue creating a barrier protecting untargeted tissue from the effects of the ablation procedure. There are three current options used for this: saline, D5W, and carbon dioxide (CO_2) [2].

Saline

Saline is sterilized salt water that is isotonic to body tissue (0.91% NaCl) and is readily available for a variety of medical applications including: intravenous infusion, cleansing wounds, nasal irrigation, and treating dehydration. Saline is cheap and can be easily injected percutaneously to the site of ablation. Since saline is mostly water, it has a high specific heat and shields well from extreme temperature changes [8]. The intraperitoneal (IP) pressure of the body cavity can push the non-viscous saline away from the target tissue; because of this, large amounts (>1 L) are often necessary to obtain adequate tissue displacement (1-2 cm) [9]. Unfortunately, saline is an ionic solution and therefore conducts electricity in RF ablation; this increases damage to surrounding tissue [2].

CO_2

Carbon dioxide may be administered in two ways: via a gas-filled balloon, or via insufflation (injection of gas into the body cavity) [10-11]. Unfortunately, both of these methods are more invasive than a saline or D5W injection. Also, CO_2 needs to be handled very carefully

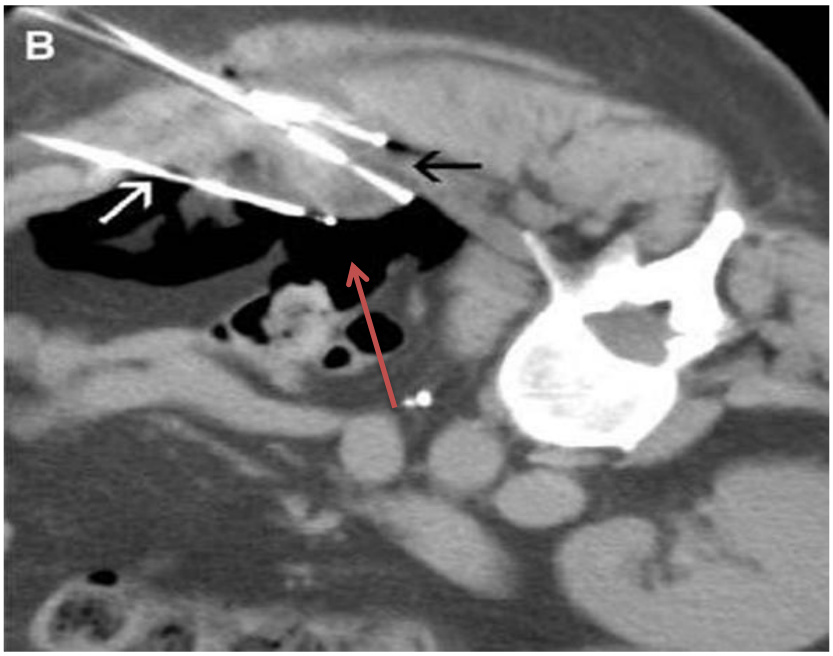


Figure 3 –The shows imaging problems of CO2 with CT scans. The white arrow points to the RF electrode, the black arrow points to a thermocouple, and the red arrow points to the CO_2 gas which inhibits imaging during CT scans. Image from [10].

within the body cavity since it could cause a fatal air embolism [10]. Gas can also be difficult to control within the peritoneal cavity. This results in the use of several gas bags or large amounts of CO_2 (>1 L) [2]. CO_2 is an efficient insulator; however, it blocks imaging, an effect that can be clearly seen in Figure 3.

5% Dextrose in Water (D5W)

The most commonly used hydrodissection fluid, D5W, is a sterilized isotonic solution of dextrose and water that is commonly used as IV fluid. It is both cheap and plentiful in the hospital environment, and can be easily introduced to the target area by percutaneous injection. D5W is relatively non-invasive, though it suffers from many of the same setbacks as saline. Again, large volumes (>1 L) may be required to adequately protect tissue due to the low viscosity of the solution and the pressure of the body cavity [2]. The main advantage of D5W

over saline is that it is not electrically conductive. This reduces unwanted tissue damage by as much as 35% compared to saline. The effectiveness of D5W can be seen in Figure 4 [9].

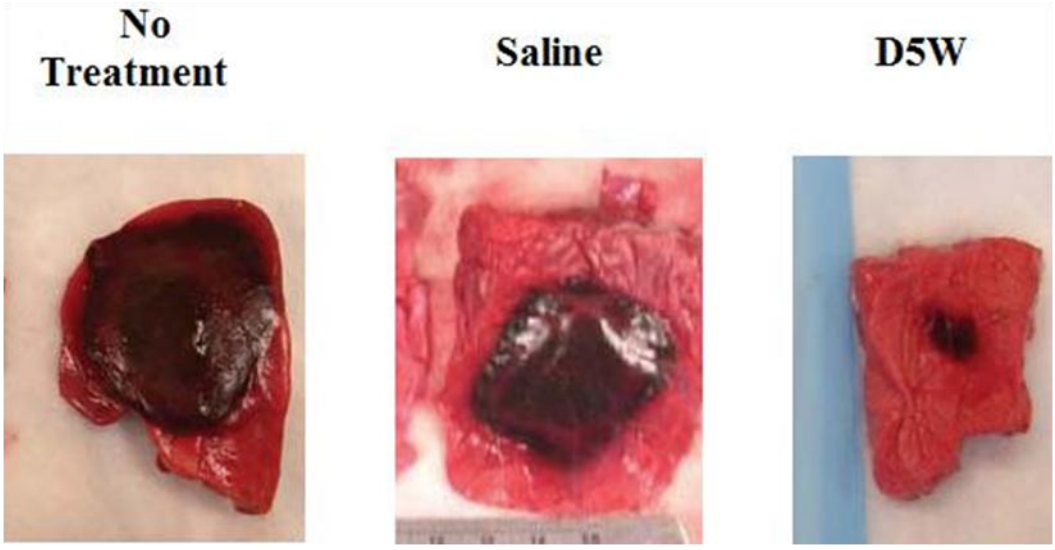


Figure 4 – Swine lung lesions resulting from RF ablation treatment. D5W minimizes unwanted tissue damage most efficiently. Image from [9].

DESIGN REQUIREMENTS

The clients require that the new product be equal in favorable characteristics of D5W and saline, the two most commonly used hydrodissection fluids, while incorporating additional characteristics that are ideal for hydrodissection. Table 1 shows the favorable characteristics of both D5W and saline along with an additional column of characteristics an ideal hydrodissection fluid would have. The design would have to include these characteristics for the product to be competitive on the market.

D5W		Saline		Ideal Hydrodissection Fluid
Pro	Con	Pro	Con	Additional requirements
• Electrical Insulator • Thermal Insulator • Biocompatible	• Fluid migration • Barrier degradation	• Thermal Insulator • Biocompatible	• Electrically Conductive • Fluid migration • Barrier degradation	• Increased Viscosity • Decreased fluid migration • Decreased barrier degradation

Table 1 – A list of characteristics attained by current technology, saline and D5W, and additional qualities necessary for an ideal hydrodissection fluid.

Since patient safety is of the utmost importance, the first necessary requirement is biocompatibility. The design is intended for use on human subjects and must meet the requirements of the FDA (Food and Drug Administration). The product is to be injected into the body cavity and should accurately function within the body's environmental thresholds. The fluid must be completely biodegradable or bioabsorbable and cause no immune response. During breakdown and absorption, the product should be easily excreted from the human body. The final design should optimize physician ease of use, as well as patient safety and comfort both during and post treatment.

To effectively protect tissues adjacent to the target organ the product must be both a thermal and electrical insulator. During RF ablation, a current is applied directly to the target site, heating tissue to temperatures exceeding 60^{o}C [12]. Because of the extreme temperatures involved, ineffective insulation surrounding the target organ could result in patient complications and tissue death. Because of this, the product must be completely reliable and accurate.

The design must be ergonomically efficient for effective procedural use. To maintain a minimally invasive treatment, the product must be easily injectable through a 20 gauge needle

for initial fluid placement. Guidance of the ablation applicator is done through ultrasound imaging, CT scans, or MRIs. For this reason the product must be ultrasound transparent and easily distinguishable from surrounding tissue when using computed tomography; the product should not inhibit imaging during the ablation procedure.

To outperform current methods of hydrodissection, the product must not migrate throughout the peritoneal cavity. Current methods sometimes require over a liter of fluid to achieve adequate tissue displacement. Once product placement has occurred, the fluid should remain there until degradation or absorption is complete; this is expected to occur in 12-24 hours. The product must maintain at least a 1cm displacement of tissue throughout the ablation procedure which normally lasts 1-3 hours.

The product is to be sterilized and packaged in single use, 250 ml IV bags. The target cost of the product is less than $200. Saline and D5W are significantly cheaper than this; however, with less fluid volume needed for adequate protection, the product's benefits will outweigh the cost increase. A complete list of product design specifications can be found in Appendix A.

DESIGN ALTERNATIVES

Poly(ethylene glycol)

Poly(ethylene glycol) (PEG) consists of long polymer chains made of repeated $–CH_2-CH_2O-$ units, see Figure 5 [13]. Polymers with different numbers of these repeated units have different molecular weights. High molecular weight polymers tend to be solid at room temperature while low molecular weight

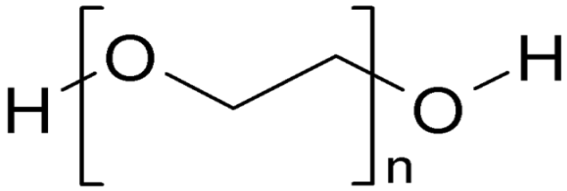


Figure 5 - PEG polymer structure. A higher number of repeating *n* units results in a higher molecular weight. Image adapted from [13].

polymers tend to be liquid. The FDA considers PEG a biologically inert substance [14].

The higher molecular weight PEGs are used commercially and industrially as thickening agents in skin creams, lubricants, laxatives, and toothpaste. A study was done at the University of Georgia in the 1970's to demonstrate the effectiveness of PEG as a thickening agent, see Figure 6 [15]. This study added PEG-6000 to water and the results showed a substantial increase

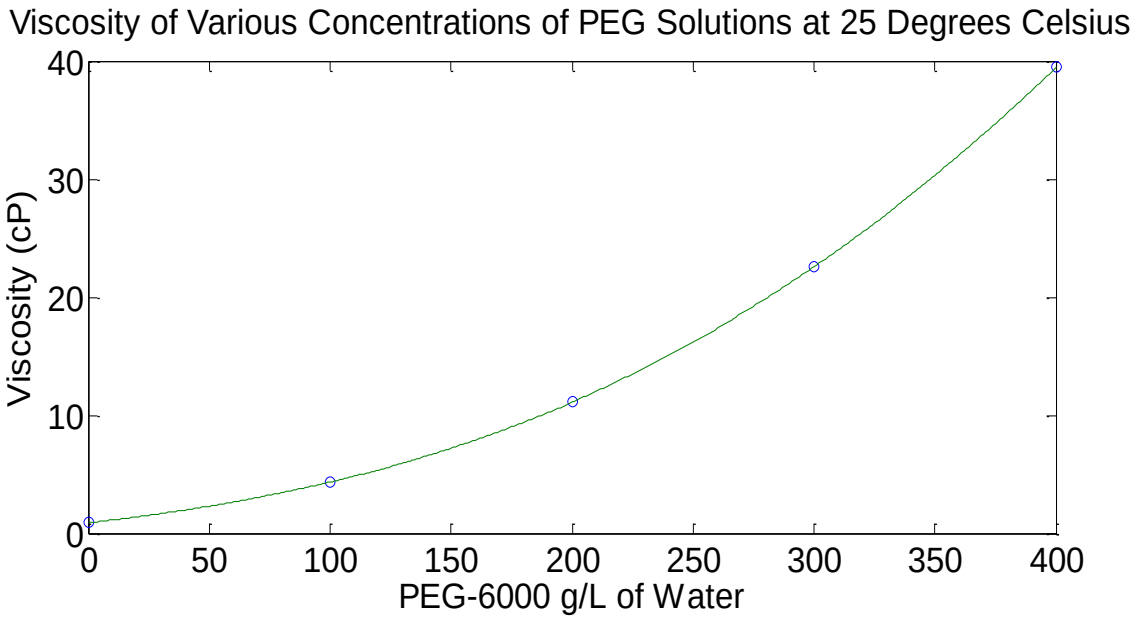


Figure 6 - Viscosity of various PEG-6000 solutions at 25°C. An increase in PEG resulted in a much higher viscosity. Image adapted from [15].

in viscosity. In all temperature scenarios the increase in viscosity was directly attributed to an increase in the mass to volume ratio of PEG-6000 and water. So the incorporation of this polymer into a D5W solution would fix the migration and viscosity problems associated with D5W, while retaining all of its superior characteristics. This approach would also provide a substantially more stable barrier for protection while using less volume.

A drawback of PEG is that it would have to be injected as a viscous solution. A PEG solution may be difficult to push through a 20 gauge needle which makes this option less viable. In order for it to be injected through the needle, it may be necessary to decrease the amount of

PEG in solution which may not make the solution viscous enough to prevent fluid migration. The solution would have to be tailored to optimize tissue protection, while be ergonomically efficient for physicians.

Sodium Alginate

Sodium alginate is a natural polymer that comes from seaweed. It is inert in the human body, and allows for natural degradation. Alginate hydrogels are commonly used as scaffolds for tissue growth, drug delivery vehicles, and wound dressings [16].

Sodium alginate in solution forms a hydrogel when mixed with sufficient divalent cations. During gelation, Ca^{2+} ionically cross-links the carboxylate groups of alginate. Gel formation is instantaneous upon contact with a solution sufficient in calcium ions [17]. After forming a gel, the hydrogel would be sufficiently viscous to deter migration within the body cavity. After an ablation procedure, the hydrogel will degrade naturally over time. Degradation rates for sodium alginate hydrogels vary based on the concentration of sodium alginate. Typical alginate hydrogels degrade uncontrollably, leaving many large strands which are hard for the body to breakdown. To counter this degradation, partially oxidized alginate can be used.

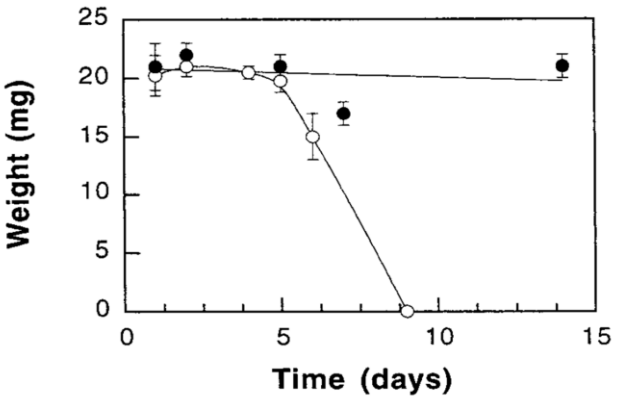


Figure 7 - Change of dry weight of partially oxidized (white dots) and alginate (black dots) as a function of degradation time. Image from [16].

Partially oxidized alginate is made when a solution of alginate is mixed with sodium periodate which is then precipitated using ethyl alcohol [17]. As seen in Figure 7, partially oxidized alginate hydrogel degrades in approximately one week, whereas the typical

alginate hydrogel takes over a month to fully degrade. Also influencing this rate is the molecular weight of the hydrogel; larger weights take longer to degrade [17].

Despite the definite benefits of alginate hydrogels, there are a few potential problems with its widespread use. Due to the ionic nature of the cross-linking, the gel may create an electric current in the body during RF ablation. Additionally, injecting the alginate could prove problematic. Either the alginate gel will require two injections, one for each the alginate and calcium ion solution, or a slow gelling process would need to be attempted. One such process involves $CaSO_4$ powder mixed into the alginate solution. The powder slowly releases Ca^{2+} ions to form the gel [16]. A third possibility is having one needle but three syringes as seen in Figure 8. The first injection consists of $CaCl_2$, followed by an injection of D5W to clear calcium ions

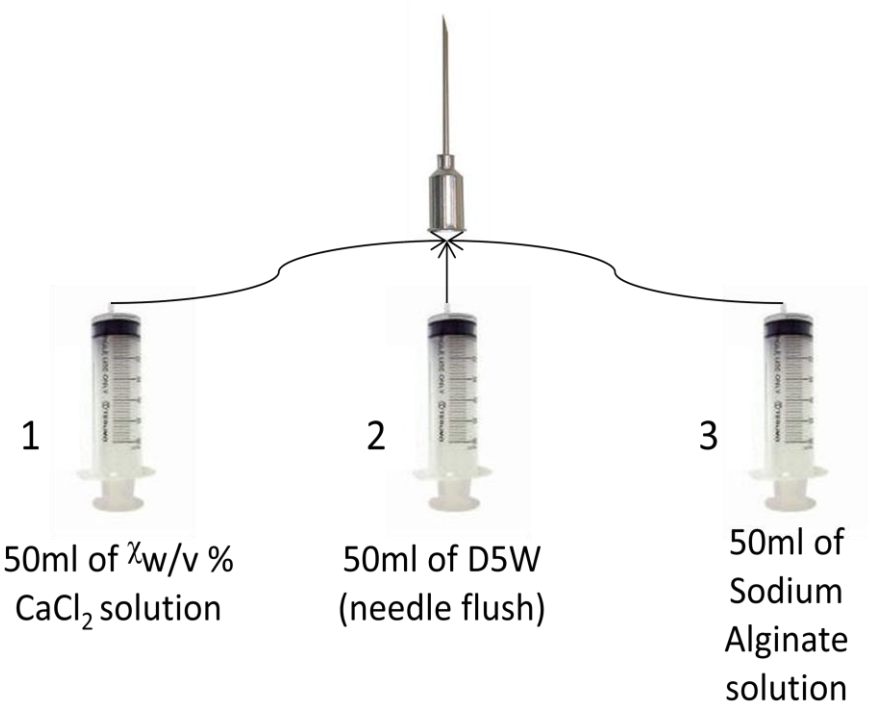


Figure 8 - A possible injection method accomplished using one needle and three different syringes. Images adapted from [41-42].

from the needle. Finally, the injection of sodium alginate will cause the gel to form in the body.

Poloxamer

A poloxamer is a polymer containing both hydrophobic and hydrophilic groups [18]. The triblock copolymer consists of a number of poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) or PEO-PPO-PEO blocks; a figure of the polymer block can be viewed in Figure 9 [16, 19]. The number of blocks in each poloxamer gives it unique characteristics. Poloxamers are non-ionic and are considered bioabsorbable when the polymer has a molecular weight less than 13 kDa [16].

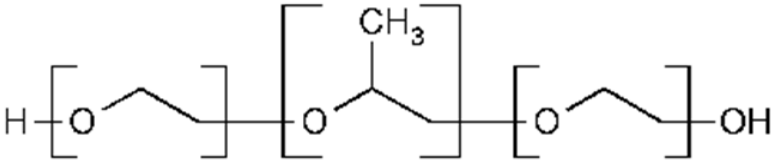


Figure 9 – The triblock structure of poloxamer. The number of units in a poloxamer gives the poloxamer its name and special characteristics. Image adapted from [19].

Poloxamer 407 (Lutrol F 127; BASF) has the unique property of thermoreversibility when mixed with deionized water. This thermoreversible solution to gel phase change occurs when micelles form as temperature increases amongst hydrophobic (PPO) and hydrophilic (PPE) groups. These micelles become organized and form structure [16, 20]. This micellization process can be viewed in Figure 10. The temperature at which this takes place is named the gelation

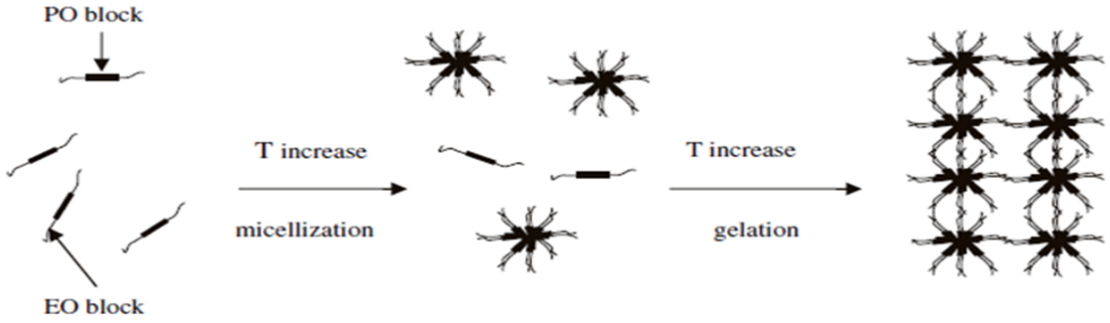


Figure 10 – Micellization process for poloxamer units. As temperature increases the units in solution begin to form micelles which then produce an organized structure. Image adapted from [20].

temperature. This gelation temperature must be experimentally determined and varies depending

on the concentration of poloxamer in solution [16, 18, 20]. The gelation temperature would be altered to 32^{o}C due to client preference and further optimized to increase viscosity once injected.

The poloxamer gel is often unattractive as a biomaterial because of its rapid erosion and low mechanical strength; however, these properties should not affect this design [16]. Rapid erosion of the gel would expedite the excretion of the fluid and lessen the likelihood of residue deposits. The low mechanical strength is not of concern since the patient is relatively still throughout the procedure. Poloxamer solutions are non-ionic and it is expected to work well as both a thermal and electrical insulator.

The key characteristic, thermoreversibility, would allow the product to be injected into the patient as a solution which would then gel at body temperature. A visual representation of the phase change from solution to gel can be seen in Figure 11. Due to the solution to gel transition of poloxamer, the viscosity of the product would greatly increase once injected into the peritoneal cavity. This would be ideal for a hydrodissection fluid because the product could be injected as a solution through a 20 gauge needle and would create a gel at once warmed to 32^{o}C (body temperature is ~37^{o}C). The viscosity of the poloxamer solution increases with an increase in temperature or concentration until the gelation temperature is reached.

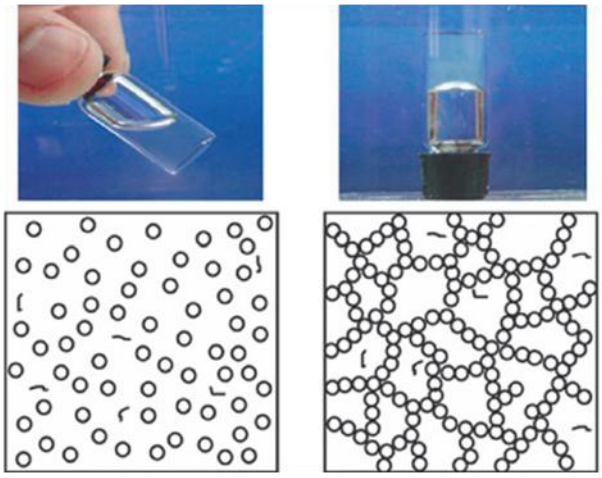


Figure 11 – A noticeable phase change occurs as micelles become organized and the solution becomes a gel. Adapted from [16]

Once gelled, a temperature is eventually reached where the poloxamer begins to precipitate out of solution [16]. This is expected to slightly affect the efficacy of this design. However, only the edge nearest the ablated tissue would be affected by extreme temperatures. It is expected that the tissue furthest from the ablation site (tissue to be protected) would still be adequately protected by the poloxamer gel. In vivo imaging to determine the extent of gelation and tissue displacement during an ablation procedure would verify this. If the gel were to break down during an ablation procedure, a solution similar to D5W (currently used) would result, and the same extent of protection currently available to patients would be provided.

With the viscosity increase, it is expected that the migration of fluid within the body cavity would be greatly reduced during the ablation procedure. With a gel formation at the site of ablation, it is hypothesized that a fluid volume of less than 250ml would be required for effective tissue displacement.

DESIGN MATRIX

A design matrix was used to assess which design option would be best to pursue for the remainder of the project. The five categories chosen were biocompatibility, viscosity, cost of materials, ergonomics, and temperature range. These were based on the design specifications desired by the client. The client ranked each attribute according to importance, and from this, point values were assigned. Thermal and electrical resistance was not included in the design matrix, as it is an inherent property of each design option we are investigating. If any option did not have this property, it would not be valid. Poloxamer 407 was pursued based on the results of the design matrix; these results of the design matrix can be seen in Table 2.

	Poly(ethylene glycol)	Poloxamer 407	Sodium Alginate
Biocompatibility (30 pts)	30	25	20
Viscosity (20 pts)	15	20	20
Cost of Materials (10 pts)	10	10	5
Ergonomics (15 pts)	5	15	5
Temperature Range (25 pts)	25	20	25
Total	85	**90**	75

Table 2 – Design Matrix – Five categories were chosen based on client preference to evaluate each design alternatives.

Biocompatibility

Biocompatibility is the ability for a material that is introduced into a biological environment to perform its intended function without eliciting any undesirable effects [21]. This category was allocated thirty points in the design matrix because having a product that works effectively but causes harm to the user would be futile.

Poly(ethylene glycol) is an FDA approved material [22]. It is biologically inert and passes through the body mostly unaffected [14]. Because of this unique property, it has been used in many medical products, the most common being laxatives. It has also been added in skin creams and lubricants because of its thickening ability. PEG was given full points in this category because of its use in everyday materials and the fact that it is FDA approved.

While alginate is extremely biocompatible in the human body, it is flawed. Due to the gel being formed by an ionic solution, there will be ions left over after gelation. These ions may cause electrical conductivity to increase which can damage other organs in the body. Sodium alginate also has a long degradation time compared to the other alternatives [17]. Because of this, sodium alginate was given 20 out of 30 points.

Poloxamer was giving a value of 25 out of 30. It was graded less than PEG for biocompatibility because the poloxamer gel is bioabsorbable, not biodegradable. The gel would be broken down, processed through the kidneys, and excreted in the urine. Poloxamer was given a higher value than alginate because it is non-ionic.

Viscosity

The major problem with current methods is their lack of viscosity which results in unintended migration. Designing a product to minimize migration would infer a viscous material so viscosity was given one fifth of the total points.

One of PEG's commercially and industrially favorable properties is its ability to act as a thickening agent. This is evident in its addition to some skin creams, lubricants and toothpaste. Although PEG could be a highly viscous solution, the degree of viscosity is limited by the necessity to inject the fluid through a 20 gauge needle. Because of this PEG was given only fifteen of the possible twenty points. On the other hand, poloxamer and sodium alginate are able to form a viscoelastic gel in vivo which is why they received the highest point value for this category.

Ergonomics

In order to be used during the ablation procedure the product must be ergonomically efficient for medical personnel. This category was given a max point value of fifteen. The use of the poloxamer solution would make no change in the current hydrodissection procedure, and less total fluid volume would be required for effective hydrodissection. Therefore, the poloxamer solution was given the maximum point value for this category. Unlike poloxamer, PEG would have to be injected as a viscous solution. This may be difficult to push through a 20 gauge needle

and accurately place on the ablation site which is why PEG was given five out of fifteen possible points. Sodium alginate lost points here due to the difficulties involved in injecting the solution. The multiple injections or multiple syringes make the gel more cumbersome to use efficiently.

Temperature Range

The operating temperature range of the gel was very important to our clients, which warranted the heavy weight of the category. Sodium alginate received full points in this category because the gelation is not based on temperature. The gel is cross-linked by ionic calcium and will hold together regardless of temperature in the body. PEG is commercially used as a thickening agent and this property will allow it to retain its viscosity at temperatures typically associated with RF ablation. For this reason it was given full points in this category. Because the viscosity of the gel varies with temperature, poloxamer was given the least point value out of the three alternatives. This temperature dependent characteristic gives medical personnel little control over gel function once injected into the peritoneal cavity.

Cost

In order for our fluid to be competitive in the current market, it must be relatively inexpensive. Our clients reported that a product with favorable properties would receive widespread use if it cost less than $200. Due to the high cost of pharmaceutical grade alginate, which is necessary for use in the body, sodium alginate lost points in this category. However, the cost of poloxamer and PEG would be comparable with a unit cost of approximately $10, which is well within the limits of the client for the target product cost.

POLOXAMER TESTING

Gelation Temperature

Testing of poloxamer 407 was done in order to determine the relationship between gelation temperature and concentrations of poloxamer 407 (w/v %). Lutrol F 127 (Poloxamer 407) was received from BASF as a testing sample for the design. Clients suggested the gelation temperature for the design to be 32^{o}C.

The poloxamer solutions were prepared in cold, filtered, deionized water. The water was stored at 4^{o}C prior to solution synthesis and placed on ice during formulation to aid in the dissolution of poloxamer. Filtered water was used to make sure no contaminants were present; minor contamination could alter physical/chemical characteristics of the solution. The appropriate amount of poloxamer 407 for 120 mL of water was measured on an analytical balance. The poloxamer was slowly added to the 500 mL beaker containing 120 ml of water while being stirred on a hot plate at a rate varying from 500-1000 rpm with a magnetic stir bar. At higher concentrations (20, 22.5 w/v %) the solution began to gel prior to full incorporation of poloxamer. In this case, a 1 liter beaker with ice was used to cool the smaller beaker while stirring continued; this reduced the temperature and viscosity of the solution, thereby allowing for adequate stirring. The protocol for poloxamer fluid synthesis can be found in Appendix B.

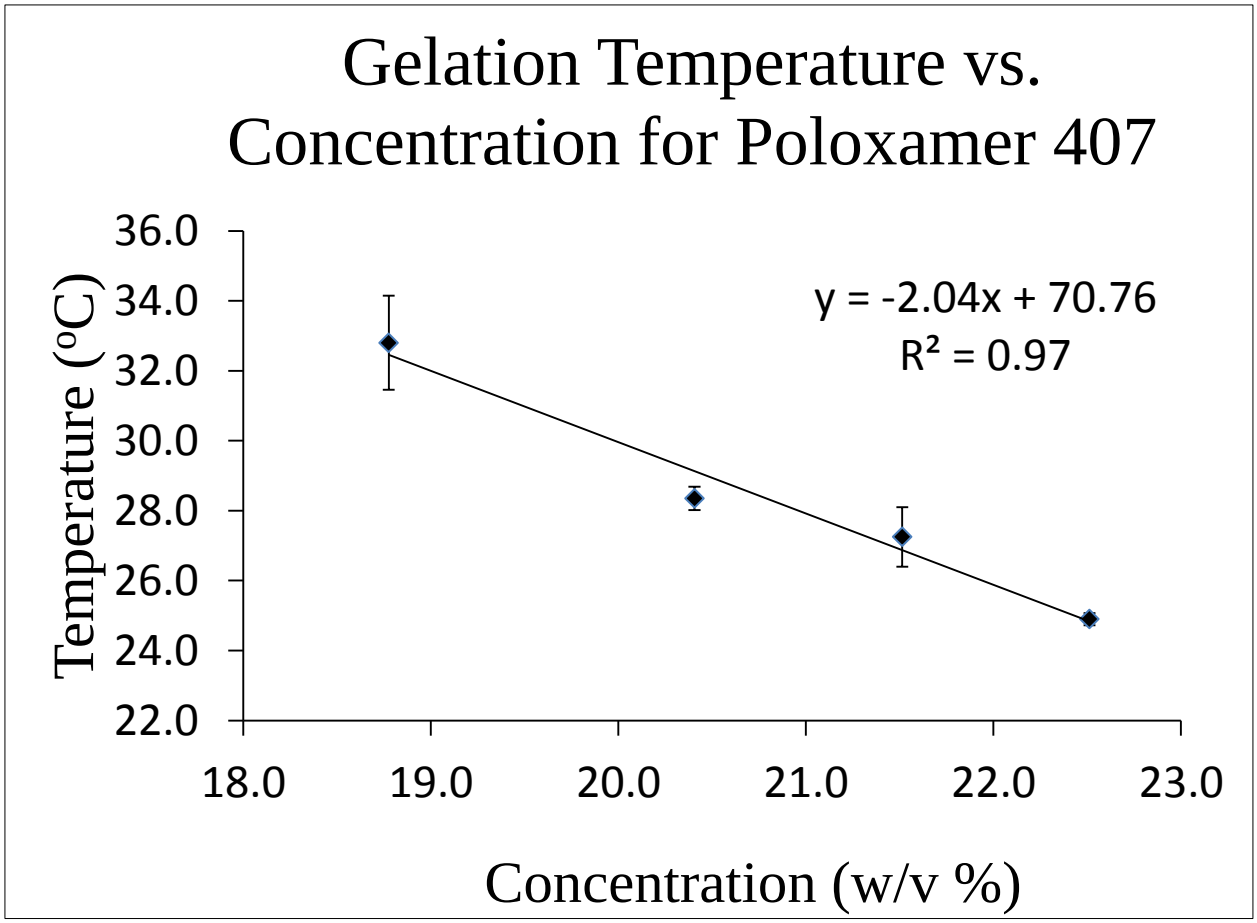


Figure 12 – A plot of gelation temperature versus concentration of poloxamer.

To determine the gelation temperature approximately 40 mL of poloxamer solution was added to a 250mL beaker and placed on ice. Once cooled, this was place in a 1000 mL beaker with 150-200 mL of warmed deionized water (34-37^{o}C). This was done to evenly heat the poloxamer solution. The stir rate was placed on the slowest setting (~60 rpm). The solution to gel phase change, gelation temperature, was considered the time point when the stir bar could no longer freely move in solution. At this time the temperature was recorded; multiple trials were conducted for each condition. A plot of concentration versus gelation temperature is shown in Figure 12. A linear correlation between the gelation temperature (T) and concentration of poloxamer (C) was found to be T = -2.04C + 70.7. From this equation, a 19.0% poloxamer

solution must be used to attain the specified gelation temperature of 32^{o}C. This would allow the gel to be injected into the body as a solution which would then gel once in place. A visual representation of the solution when gelled can be seen in Figure 13.

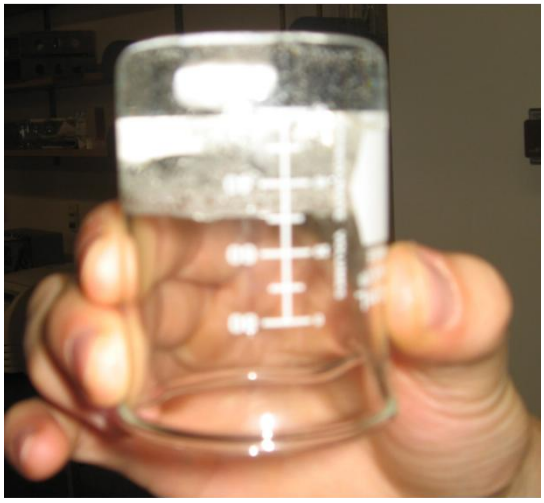


Figure 13 – A 20.0 w/v % poloxamer gel past gelation temperature produces in the Tissue Engineering Lab at the University of Wisconsin-Madison.

Imaging

One of the requirements of the design is to have the fluid be transparent on ultrasound. . To test this, an ultrasound of each solution (D5W and poloxamer) was performed. About 50 mL of each solution was placed into a flat container. This solution was then subject to ultrasound using a SonixTOUCH Ultrasound System. Dextrose in water had a very clear ultrasound, seeing only the echo from the bottom of the container. The poloxamer solution appeared very similar to D5W; however, once gelled, the

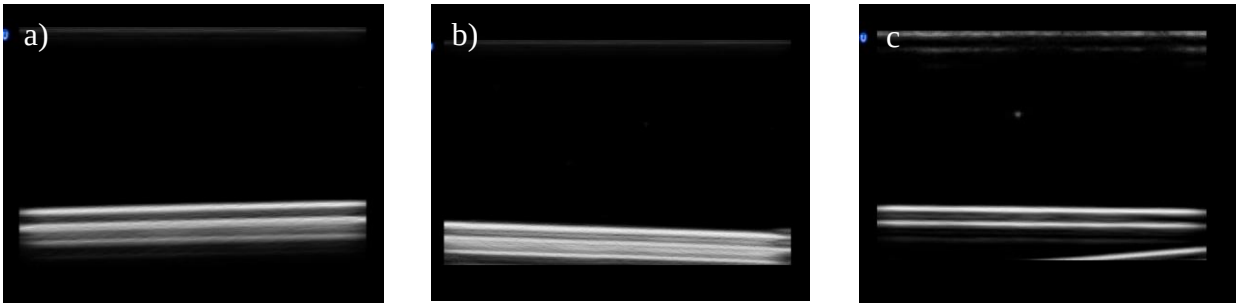


Figure 14. Ultrasound images showing the transparency of (a) poloxamer solution, (b) D5W, and (c) poloxamer gel on an ultrasound.

poloxamer had an additional echo on the top of the gel. This is likely due to air between the gel and transducer. The differences between the solutions and gel can be seen in Figure 14. The images suggest that poloxamer will minimally obscure ultrasounds in the body and will not impair the physician's ability to perform the ablation procedure.

A second imaging requirement is that the design be clearly seen on a CT scan. The poloxamer was tested against the most commonly used alternative, D5W. Approximately 50 mL of D5W and 19.0% poloxamer were placed within 50 mL centrifuge tubes (two tubes for poloxamer). One tube of poloxamer was allowed to gel in warm water. The tubes were then imaged in a CT scanner. The resulting ROIs (Region of Interest), a measure of contrast for an image, were compared and can be view in Table 3. The ROI of liver tissue is approximately 70 [23].

	D5W	**19.0% Poloxamer**	**Gel – 19.0% Poloxamer**
ROI	8.9 ± 2.9	14.1 ± 2.5	14.7 ± 2.2
ROI w/ Iohexal	220.6 ± 4.3	106.4 ± 2.3	N/A

Table 3 – Region of Interest (ROI) results from CT Scanning on D5W and poloxamer (solution and gelled).

Initially, all three of the samples had very low ROIs. An iodine contrast solution is often used in ablation procedures to help distinguish tissue from the injected fluid. Iohexol was added to both solutions (~1:50 dilution); the ROI for D5W and the poloxamer solution were then much larger. According to clients, an ROI of approximately 150 is ideal for imaging during ablation procedures; it is expected with slightly more Iohexol the poloxamer solution will meet this criteria. Additionally, the poloxamer solution was then gelled after the addition of Iohexol; this was done to be sure the iodination did not inhibit the gelation of poloxamer. Due to the fact that iodination of the poloxamer solution works well for CT scanning, poloxamer will satisfy this design requirement and will not inhibit procedural imaging.

Viscosity

The main goal of this design project was to improve the viscosity of the solution while maintaining all the benefits of D5W. The use of poloxamer 407 allows for this desired increase in viscosity while also adding a thermoreversible property to the solution.

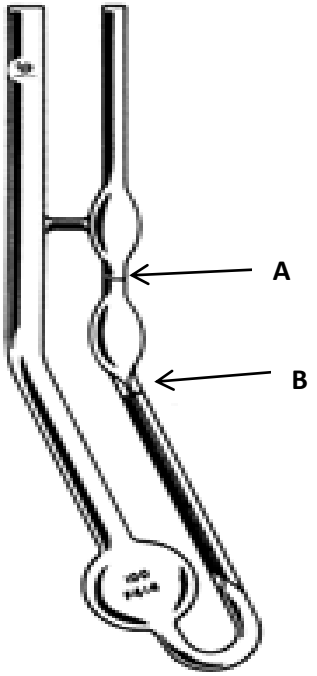


Figure 15 - A Cannon-Fenske size 200 viscometer which was used to measure kinematic viscosity in this experiment. The time it took the solutions to travel from point A to point B was measured. Image from [43]

Kinematic viscosity testing was accomplished using a Cannon-Fenske capillary viscometer size 200. A Fischer Scientific Isotemp 1016D water bath machine was used to maintain a constant temperature throughout all trials. The viscometer was initially cleaned using deionized water, followed by an acetone wash and was then set aside to air dry. Triplicates of each condition (aside from 21.0^{o}C) were performed; 6.5mL of 19% poloxamer solution was measured using a graduated cylinder and then added to the viscometer. A pipet bulb was then used to draw the fluid up approximately half a centimeter above the top marking of the upper glass bulb, labeled point A in Figure 15. The pipet bulb was removed and a stop watch was used to record the time it took the fluid to travel from the top mark A, to the bottom mark B in Figure 15. This was repeated for each temperature trial. The results of the viscosity testing can be seen in Figure 16. The times recorded for each trial were converted to seconds and were then converted to centistokes (cSt) by using the conversion constant (0.1) for the viscometer; $x\ cSt = 0.1 * y\ seconds$ [24-26].

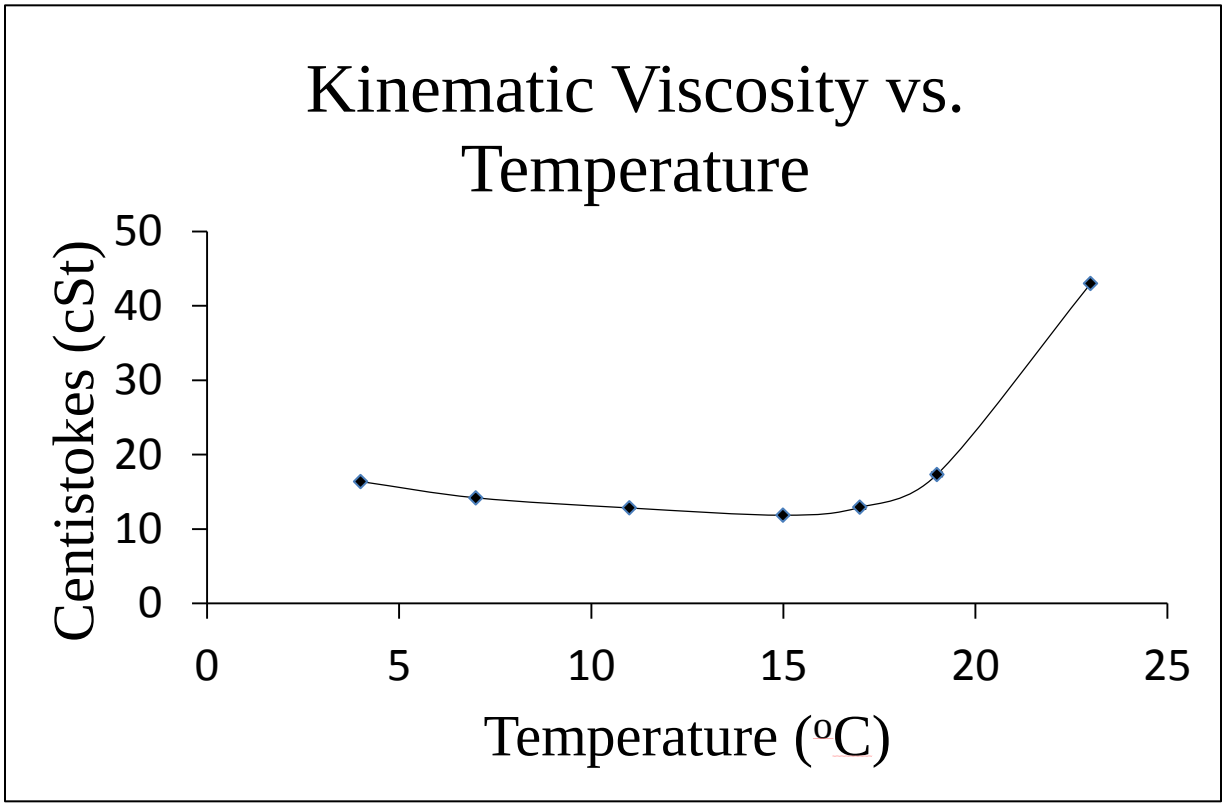


Figure 16: Viscosity versus temperature for 19% w/v Poloxamer 407 solution. Initially the solution follows a typical fluid pattern of lower viscosity with increasing temperature; however, after fifteen degrees Celsius the micelles begin to conglomerate causing a rapid increase in viscosity.

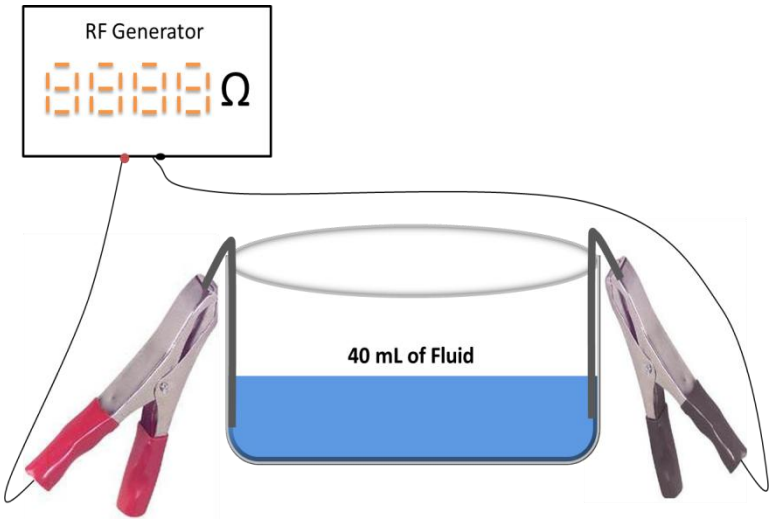
The lowest observed viscosity was recorded at 15°C. This may seem counterintuitive since the poloxamer solution should continue to get less viscous at lower temperatures as a result of less micelle formation. However, since the solution is mostly water, like water the viscosity initially decreases; the fluid is more viscous at lower temperatures and gets less viscous as the fluid warms [41]. At approximately 15°C micelles form due to dehydration of the hydrophobic groups and start forming structures, thereby increasing the viscosity.

The viscometer available for testing was calibrated for kinematic viscosity ranging from 20-100 cSt; since the results reported are outside this range the test should be conducted in the future with a size 150 viscometer to more accurately determine the kinematic viscosity values [24-26].

The centistokes value is a measure of kinetic viscosity not the absolute viscosity. The absolute viscosity is in units of Pascals. The relationship between the two is $v = \mu/\rho$ where v is the kinetic viscosity, μ is the absolute viscosity, and ρ is the density [27]. The density of the poloxamer solution will be difficult to determine due to the minute changes in volume with temperature. Laboratory instruments that provide such volumetric accuracy were unavailable.

Impedance

A requirement of the poloxamer solution is to be electrically insulating. To determine this, the impedance of the solution was tested and compared with D5W and saline. The set-up can be viewed in Figure 17. Aluminum tape was applied to opposite sides of the inside of a small plastic beaker (~200 mL). An RF ablation machine (ValleyLab Cool-Tip) was used to generate radiofrequency current and give a direct impedance reading. Alligator clips were first applied to the same piece of aluminum tape and an impedance reading was taken; this was considered the blank. Between testing trials the beaker was washed with deionized water and dried with a paper towel. The results of the impedance test can be viewed in Table 4. Poloxamer solution,



Sample	Impedance (Ω)
Blank	40
Saline	88
D5W	High (>1000)
19.0% Poloxamer (solution)	High (>1000)
19.0% Poloxamer (gelled)	High (>1000)

Figure 17 & Table 4 – Left shows a schematic of the set-up used for impedance testing. Right is a table of testing results. Impedance rating of high means the impedance was greater than 1000Ω.

poloxamer gel, and D5W all possessed high impedance (> 1000 Ω). This suggests that poloxamer, both in a solution and gel, would act like an electrical insulator and protect surrounding tissue from damage during ablation procedures.

Ease of Injection

To test the ease of injection of the 19.0% poloxamer solution, a Kent Scientific Genie Plus 3012 syringe pump was used; a syringe (Terumo, 60 mL) diameter and volumetric flow rate were set to constant values. The machine then depressed the syringe at the set volumetric flow rate, forcing the fluid out the needle (21 gauge, 23 cm long, 0.514 mm diameter). The fluid was collected in a small beaker to preserve the fluid for additional testing. Using a force transducer with this setup, the force required to push the poloxamer solution out of this needle at different temperatures could be determined. Viscosity testing suggests the temperature requiring the least amount of force will be around 15^{o} C.

Unfortunately, a force transducer was not available for testing. Another more theoretical method was considered, employing the equation:

$$F/A_1 = \tfrac{1}{2}\,\rho\left((A_1/A_2)^2 - 1\right) v_1^{\,2} \qquad (1)$$

Where A_1 is the cross sectional area of the syringe, A_2 is the cross sectional area of the needle, ρ is the density of the fluid, and v_1 is the linear velocity of the plunger as it is depressed. However, this method fails to account for additional resistance due to needle length.

When the syringe pump was set to a volumetric flow rate of 5mL/min, the motor of the device began to stain due to the significant resistance created by the poloxamer solution. Common laboratory syringe pumps are able to exert a force of 40 lbs (~180 N); it is expected that the force necessary to push the poloxamer solution through the 21 gauge needle was greater

than this [24]. An MTS Sintech Universal Testing Machine capable of exerting a much greater force will be used for force determination in future testing.

The poloxamer solution was considered to be significantly more difficult to push through the syringe and needle compared to D5W. Two current options to deal with this difficulty are: (1) to decrease the viscosity of the solution while maintaining its favorable gelling characteristics, or (2) to find or design a device that allows the user to apply greater pressure while maintaining effective control of the syringe. The latter appears to the most promising; there are already many manufacturers that make syringe guns able to inject viscous fluids [28-30].

FUTURE WORKS

FDA Approval/Clinical Testing

Prior to clinical testing, accurate, reliable, and reproducible data must be obtained during animal testing. Once the efficacy of the design has been established, the poloxamer solution/gel must be thoroughly tested for toxicity. Poloxamer 407 has been FDA approved for many applications (i.e. ophthalmic delivery, topical application, etc.); however, there has yet to be an approval for injection into the peritoneal cavity (IP injection).

Toxicity testing must be conducted on two species (other than human) with a complete systematic overview [32]. Regulations outlined in the FDA handbooks "Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs" and "Guidance for Industry: CGMP (Current Good Manufacturing Practice) for Phase 1 Investigation Drugs" will be followed to ensure the proper analysis of immune response and that all ethical matters are taken into consideration.

Pending toxicity results, the final step prior to manufacturing the product is clinical trials. All human subjects will be informed of the relative risks and benefits of clinical tests. The ethical principles listed below will be maintained throughout clinical trials.

> The National Commission for the Protection of Human Subjects in Biomedical and Behavioral Research's Three Ethical Principles [33]:
>
> **Beneficence:** Maximizing good outcomes for science, humanity, and individual research participants, while avoiding or minimizing unnecessary risk, harm, or wrong.
>
> **Respect:** Protecting the autonomy of autonomous persons and treating all, including the nonautonomous, with courtesy and respect.
>
> **Justice:** Ensuring reasonable, nonexplorative, and carefully considered procedures and their fair administration, with fair distribution of costs and benefits among person and groups.

Human subjects which are candidates for RF ablation procedures will be used for clinical trials. A clinical study comparing the efficacy of the 19.0% poloxamer solution versus currently used hydrodissection fluids (i.e. D5W and saline) would then be conducted to determine the possible advantages of the poloxamer solution. Patients would be monitored for 14-28 days post-surgery.

Toxicity

Although not FDA approved for IP injection, poloxamer 407 lacks any inherent myotoxicity following single or multiple intramuscular injections; toxicity was comparable to that of saline or peanut oil [34]. Poloxamer 407 is also well tolerated when administered subcutaneously [35]. The FDA lists poloxamer 407 as an inactive ingredient for inhalation, oral solutions, suspensions, ophthalmics, topical formulations, and intra-venous (IV) injections [36]. It is classified as non-hazardous by OSHA [37]. Poloxmaer 407 typically exhibits a pH of 6.0 – 7.5 in aqueous solutions, which is similar to the human body [37].

Poloxamer 407 did not result in either morbidity or mortality when administered via IP injection to mice and rats for 1 year [42]. However, there are some complications involved with IP injection of poloxamer 407. When injected within the intraperitoneal cavity, poloxamer 407 can induce alterations in lipid metabolism by inducing hyper-triglyceridemia and hyper-cholesterolemia. The necessary IP dose to induce these hyper-lipidemic conditions is 0.5-1.0 g/kg body weight; renal toxicity is 5.0 g/kg. There is a preferential uptake of poloxamer 407 in hepatic tissue compared to renal tissue that may account for alterations in lipid metabolism [20].

Animal Testing

In order to determine the effectiveness of the design, animal testing will be conducted. The animal tests will be conducted following the policies of the Animal Welfare Act and Laboratory Animal Welfare (OLAW). Swine will be used as test subjects and a similar procedure reported by Brace, et al. will be followed [9]. Protocol number M01814 will be followed for swine testing. A comparison of efficacy will be made of the poloxamer solution and D5W. The swine will be sedated, injected with either D5W or poloxamer and then subjected to RF ablation or cryoablation treatments of the liver. The swine will be monitored for several days post treatment and then euthanized to determine the efficiency of the solutions. We expect the poloxamer to outperform the D5W in both maximizing protection of surrounding tissues and minimizing unwanted barrier degradation.

Additives

The 19.0 % poloxamer solution was difficult to push through a 21 gauge needle when cooled (~15^{o}C). To overcome this barrier a secondary agent could be incorporated into the solution to decrease the gelation temperature and increase the mechanical strength of the gel.

This in turn could possibly decrease the viscosity of the solution making it easier for injection. Poly(ethylene glycol) is a promising candidate as a possible additive.

Poly(ethylene glycol) 400 is very inert and would not react with the poloxamer. It has been shown that the solution to gel transition temperature of poloxamer 407 solutions decrease with the addition of PEG 400 while the viscosity of the poloxamer gel increases [38]. This infers the possibility that addition of PEG 400 would decrease the viscosity of the 19.0% poloxamer solution because less poloxamer would be necessary in the solution for a gel with identical properties. The gelation temperature would still be maintained a 32^{o}C since the PEG 400 would decrease the higher gelation temperatures associated with a lower concentrated poloxamer 407 solution. This is expected to occur due to the extremely hydrophilic PEG 400 chains interacting with free water molecules in solution. This results in less of an opportunity for poloxamer to bond with water molecules, thus increasing the probability of micelles reacting with themselves. As a result, gelation temperature is lowered [38]. PEG has also been a proven viscosity reducer in detergent solutions [39].

Additionally, poloxamer 188 could be incorporated into the gel to increase bioadhesive properties in vivo. Poloxamer 188 (Lutrol F-68) was received from BASF as a gift for design purposes. With a slightly smaller molecular weight compared to poloxamer 407, poloxamer 188 has different properties. Poloxamer 188 has been shown to have bioadhesive properties resulting in adherence to tissue [40]. This is expected to increase the bioadhesion capabilities of the gel in vivo further preventing the migration during ablation procedures.

General

All chemical supplies for the design were provided by BASF as samples. For the final design prototype it is expected that approximately 50 grams of poloxamer 407 will be necessary

($120 for 1kg of Poloxamer 407; Sigma Aldrich); this would result in a final product costing less than $10/unit. The project cost for the semester is under $50 for laboratory supplies and $50 for poster presentation.

Currently the product is being reviewed by WARF (Wisconsin Alumni Research Foundation). Pending the WARF disclosure meeting, the decision to pursue the patent application is made by WARF. Companies that may be interested in the patent include ablation device companies (i.e. Valley Lab, Boston Scientific, etc.) and material research companies (i.e. Johnson & Johnson, Proctor & Gamble, etc.).

REFERENCES

[1] M. Deutsch, "Is primary biliary cirrhosis a risk factor for hepatic and extrahepatic malignancies?."

[2] C. Brace*, et al.*, "Electrical isolation during radiofrequency ablation: 5% dextrose in water provides better protection than saline," 2008, pp. 5021-5024.

[3] G. Dodd*, et al.*, "Minimally Invasive Treatment of Malignant Hepatic Tumors: At the Threshold of a Major Breakthrough1," *RadioGraphics,* vol. 20, p. 9, 2000.

[4] J. Hinshaw*, et al.*, "Radiofrequency ablation of peripheral liver tumors: intraperitoneal 5% dextrose in water decreases postprocedural pain," *American Journal of Roentgenology,* vol. 186, p. S306, 2006.

[5] RadiologyInfo.org, "Radiofrequency Ablation of Liver Tumors," vol. 2010, RadiologyInfo.org, Ed., ed, 2010.

[6] I. Cooper, "Cryogenic surgery," *New England Journal of Medicine,* vol. 268, pp. 743-749, 1963.

[7] D. Mahvi and F. Lee Jr, "Radiofrequency ablation of hepatic malignancies: Is heat better than cold?," *Annals of Surgery,* vol. 230, p. 9, 1999.

[8] J. Morales*, et al.*, "Cold saline irrigation of the renal pelvis during Radiofrequency Ablation of a central renal neoplasm: a case report," *Journal of Medical Case Reports,* vol. 2, p. 40, 2008.

[9] P. Laeseke*, et al.*, "Unintended thermal injuries from radiofrequency ablation: protection with 5% dextrose in water," *American journal of roentgenology,* vol. 186, p. S249, 2006.

[10] X. Buy*, et al.*, "Thermal protection during percutaneous thermal ablation procedures: interest of carbon dioxide dissection and temperature monitoring," *Cardiovascular and interventional radiology,* vol. 32, pp. 529-534, 2009.

[11] A. Kam*, et al.*, "Thermal protection during percutaneous thermal ablation of renal cell carcinoma," *Journal of vascular and interventional radiology: JVIR,* vol. 15, p. 753, 2004.

[12] B. B. B. Shafi. (2009, December 2010). *Percutaneous Radiofrequency Ablation of Liver Tumors: Treatment & Medication*. Available: http://emedicine.medscape.com/article/1390475-treatment

[13] web.mit.edu. October 14). Available: http://web.mit.edu/course/3/3.082/www/team2_s02/materials.html

[14] S. Zalipsky, "Chemistry of polyethylene glycol conjugates with biologically active molecules," *Advanced Drug Delivery Reviews,* vol. 16, pp. 157-182, 1995.

[15] B. Michel and M. Kaufmann, "The osmotic potential of polyethylene glycol 6000," *Plant physiology,* vol. 51, p. 914, 1973.

[16] L. Yu and J. Ding, "Injectable hydrogels as unique biomedical materials," *Chemical Society Reviews,* vol. 37, pp. 1473-1481, 2008.

[17] K. Y. L. Kamal H. Bouhadir, Eben Alsberg, Kelly L. Damm, Kenneth W. Anderson, David J. Mooney. (2001, 10/5/2010). Degradation of Partially Oxidized Alginate and Its Potential Application for Tissue Engineering. *Biotechnology Program 17(5),* 945-950. Available: http://onlinelibrary.wiley.com.ezproxy.library.wisc.edu/doi/10.1002/jcp.1041520225/abstract

[18] S. Singh-Joy and V. McLain, "Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as used in cosmetics," *International journal of toxicology,* vol. 27, p. 93, 2008.

[19] C. Sheeham. October 9). *Poloxamer*. Available: http://www.uspbpep.com/usp28/v28230/usp28nf23s0_m66210.htm

[20] G. Dumortier*, et al.*, "A review of poloxamer 407 pharmaceutical and pharmacological characteristics," *Pharmaceutical research,* vol. 23, pp. 2709-2728, 2006.

[21] Merriam-Webster. October 15). Available: http://www.merriam-webster.com/dictionary/biocompatibility
[22] F. Veronese and G. Pasut, "PEGylation, successful approach to drug delivery," *Drug Discovery Today,* vol. 10, pp. 1451-1458, 2005.
[23] D. C. Brace, "Client Meeting," Meeting with Dr. Brace at WIMR. ed, 2010.
[24] Cole-Palmer. (2010, *Cannon-Fenske Routine Viscometers*. Available: http://www.coleparmer.com/catalog/product_view.asp?sku=9893455
[25] P. S. Limited. (2010, *Cannon-Fenske Routine Viscometer*. Available: http://www.paragon-sci.com/cannonfenske-routine-viscometer-p-890.html?pSsid=4ab3a6e0cced000a9b4efbcc18e88414
[26] C. Viscometer. (2010, *Capillary Viscometer: Providing the latest in capillary viscometer information and products*. Available: www.capillaryviscometer.info
[27] T. E. Toolbox. *Dynamic, Absolute and Kinetic Viscosity*. Available: http://www.engineeringtoolbox.com/dynamic-absolute-kinematic-viscosity-d_412.html
[28] (2010, 12/03/2010). *Syrine Gun*. Available: http://www.dispensinglink.com/syringe_gun.htm
[29] (2010, 12/03/2010). *Aspiration Biopsy Syringe Gun*. Available: http://www.sthelens.com.cn/english/inrad-2.htm
[30] (2010, 12/03/2010). *Manual Syringe Gun*. Available: http://www.findtheneedle.co.uk/products/2388675-manual-syringe-gun.asp?tab=jobs
[31] C. DeBenedectis*, et al.*, "Utility of Iodinated Contrast Medium in Hydrodissection Fluid when Performing Renal Tumor Ablation," *Journal of Vascular and Interventional Radiology,* 2010.
[32] U. S. D. o. H. a. H. Services. (2002, 30 November). *Guidance for industry: Immunotoxicology evaluation of investigational new drugs*. Available: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079239.pdf
[33] J. Sieber, "Ethical considerations in planning and conducting research on human subjects," *Academic Medicine,* vol. 68, p. S9, 1993.
[34] Y. Liu*, et al.*, "Controlled delivery of recombinant hirudin based on thermo-sensitive Pluronic® F127 hydrogel for subcutaneous administration: In vitro and in vivo characterization," *Journal of Controlled Release,* vol. 117, pp. 387-395, 2007.
[35] I. Schmolka, "Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns," *Journal of Biomedical Materials Research,* vol. 6, pp. 571-582, 1972.
[36] R. Rowe*, et al.*, *Pharmaceutical Handbook of Pharmaceutical Excipients*, 5th ed. Washington, USA: London UK and American Pharmaceutical Association, 2005.
[37] "Material Safety Data Sheet: Lutrol F 127 NF Prill," BASF, Ed., 2.2 ed, 2009.
[38] E. Ricci*, et al.*, "Rheological characterization of Poloxamer 407 lidocaine hydrochloride gels," *European Journal of Pharmaceutical Sciences,* vol. 17, pp. 161-167, 2002.
[39] D. Machin and J. van de Pas, "Liquid detergent compositions containing a PEG viscosity reducing polymer," ed: Google Patents, 1992.
[40] R. Lee*, et al.*, "Surfactant-induced sealing of electropermeabilized skeletal muscle membranes in vivo," *Proceedings of the National Academy of Sciences of the United States of America,* vol. 89, p. 4524, 1992.
[41] T. E. Toolbox. *Water - Dynamic and Kinematic Viscosity*. Available: http://www.engineeringtoolbox.com/water-dynamic-kinematic-viscosity-d_596.html
[42] T. Johnston*, et al.*, "Effects on splenic, hepatic, hematological, and growth parameters following high-dose poloxamer 407 administration to rats," *International Journal of Pharmaceutics,* vol. 100, pp. 279-284, 1993.

APPENDIX A

ABSORBABLE HYDRODISSECTION FLUID

PRODUCT DESIGN SPECIFICATIONS

6 December 2010

Team Members: *Anthony Sprangers, Alex Johnson, Patrick Cassidy, and Sean Heyrman*

Advisor: *Dr. John Puccinelli*

Hydrodissection is used to protect adjacent organs during percutaneous thermal ablation. Current techniques involve the injection of 5% dextrose with water (D5W) or saline. Although effective in protecting surrounding tissue, these solutions tend to migrate into the body cavity. To remain effective, large amounts of fluid are necessary (approximately one liter). The goal of this project is to develop a solution/gel that encompasses favorable aspects of the current solutions: easy to introduce, ultrasound transparent, visible on CT/MRI, biocompatible, absorbable, thermally and electrically insulating, and relatively low cost; but also puts a stop to solution migration into the body cavity.

Client Requirements:

- The designed fluid must prevent the migration of solution within the body cavity during hydrodissection and ablation.
- The designed fluid must be comparable with the current favorable characteristics of D5W. These include:
    - Easy to introduce/inject – The product must be able to be introduced through 20 gauge needle (0.6 mm inner diameter).
    - Ultrasound transparent and visible on CT/MRI – The product should not reduce tumor visibility or imaging capabilities.
    - Biocompatible/absorbable – The product must be well tolerated by the body cavity and leave no post treatment residue.
    - Thermal /electrical insulator – In order for the product to effectively protect adjacent tissue, it must be a thermal and electrical insulator.
    - Comparable cost – The current cost of D5W is minimal, approximately five dollars per one liter unit.

Design Requirements:

1. Physical and Operational Characteristics

A. Performance requirements: The product must contain all favorable characteristics of current hydrodissection methods: ease of injection, biocompatibility, thermal and electrical insulator, and reasonable cost. In addition, it must prevent the migration of fluid into the peritoneal cavity.

B. Safety: Since the fluid is to be introduced into the body cavity, the final design must be non-toxic, biocompatible, and hypoallergenic.

C. Accuracy and Reliability: Failure of the product could result in serious complications to the patient; therefore, the product must be completely reliable. The accuracy of fluid retention time is imperative to the effectiveness of the treatment. Efficient hydrodissection must persist for at least one hour.

D. Life in Service: This product is to be used for hydrodissection during radiofrequency ablation lasting approximately one hour. Prior to treatment, the fluid will be stored in a 250 ml IV bag.

E. Shelf Life: The fluid is to be packaged in 250 ml IV bags and must have at least a one year shelf life; this is necessary to be competitive with currently used products.

F. Operating Environment: The product is designed to be injected into the body cavity and should function predictably within the body's normal thresholds: approximately 7.3 pH, 35-37^{o}C, and should be isotonic to the peritoneal fluid.

G. Ergonomics: The final design must be comparable to D5W for ease of injection. The ability of the fluid to be introduced through a 20 gauge needle is necessary for patient safety.

H. Size: A single effective treatment should require less than one IV bag, 250mL of fluid.

I. Weight: Weight requirements are not applicable for this product.

J. *Materials:* All the materials used in this design must meet the standards of the Food and Drug Administration (FDA), as it is designed for use on human subjects.

K. Aesthetics, Appearance, and Finish: Requirements for the design necessitate distinction between the fluid and tumor during procedural imaging.

2. Production Characteristics

A. Quantity: A volume of 250mL or less should be sufficient for one treatment.

B. Target Product Cost: Less than $200 per unit. Minimizing the cost is essential to market success of this product. Ideally the unit price would be comparable to D5W.

3. Miscellaneous

A. *Standards and Specifications:* The final product will require the approval of the Food and Drug Administration for use in the human body.

B. Customer: Prospective customers of this product would require effective hydrodissection, ease of use, reasonable cost, and biocompatibility. The primary customers are medical personnel performing hydrodissection procedures, this product will be an alternative to current hydrodissection techniques during patient consults.

C. Patient-related concerns: Patient safety is the first concern; the prevention of non-targeted tissue damage is essential. Additionally, patient comfort should be maximized during and after treatment.

D. Competition: D5W is most commonly used in hydrodissection procedures and fulfills most requirements for an ideal hydrodissection fluid. Also, 0.9% saline is used for hydrodissection; however, because of the ionic characteristic of saline, it is less common than D5W.

APPENDIX B

POLOXAMER FLUID SYNTHESIS - PROTOCOL

Purpose:

To develop a poloxamer solution that will effectively form a gel at body temperature, approximately 37^{o}C. Because the gel becomes more viscous as temperature increases, a solution that gels between 32-34^{o}C would be ideal. This fluid is being synthesized for future testing. The hopes of this design are for medical application as a fluid for hydrodissection during tumor ablation procedures.

Materials:

- Beakers (100ml, 500ml, 1L)
- Pipette helper
- Pipette helper tips (25ml)
- Parafilm
- 50ml Centrifuge tubes
- Poloxamer - Lutrol F 127 (BASF) Article #: 51632903
- Ultra-pure deionized water
- Stir/hot plate
- Magnetic stir bars
- Analytical balance
- Weight boats
- Spatula

Procedure:

1. Obtain 1 L of deionized water and cool to 4^{o}C.
    a. This is necessary to synthesize fluids with high concentration of poloxamer because at room temperature the fluids begin the sol-gel transition.
2. Determine the amount of poloxamer solution to be synthesized and the sought concentration.
    a. For example, we want 120ml of 20 w/v % poloxamer solution.
3. Place 120ml of deionized water in a 500ml beaker and place on a stir plate.
4. Stir the water with a magnetic stir bar at a speed with the range of 500-1000 rpm.
5. Weight out required amount of poloxamer.
    a. Ex.

$$\frac{\textbf{Figure}}{20\ \text{w/v\%}} = 100\% \quad 120\text{ml}$$

6. Slowly pour the poloxamer into the deionized water.
7. Mix until all poloxamer is in solution.
8. This could take several hours.
    a. If necessary place parafilm over the top of the beaker and leave overnight.
    b. To help poloxamer uptake at high concentration, place beaker with poloxamer solution inside a larger 1L beaker and surround the 500ml beaker with ice. This will cool the poloxamer solution which will decrease the viscosity and allow for better mixing.
9. Poloxamer solution can be stored at room temperature or 4^{o}C.