Absorbable Hydrodissection Fluid

Final Report
May 4, 2011

Team Members
Patrick Cassidy: Team Leader
Sean Heyrman: Communicator
Anthony Sprangers: BSAC
Alex Johnson: BWIG

Clients
Dr. Chris Brace
Dr. J. Louis Hinshaw
Dr. Meghan Lubner

Advisor
Dr. John Puccinelli

ABSTRACT

Radiofrequency (RF) ablation and cryoablation are two minimally invasive techniques for treatment of malignant tumors of the liver, lungs, and kidneys. Hydrodissection is used to protect surrounding tissues from the extreme temperatures associated with these ablation techniques. 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, causing barrier degradation which may result in damage to healthy tissues. To prevent these complications, Dr. Chris Brace, Dr. J. Louis Hinshaw, and Dr. Meghan Lubner proposed the development of a more viscous hydrodissection fluid.

Poloxamer 407, when mixed with water, forms a thermoreversible gel. A 15.4 w/w% poloxamer 407 solution that would gel at 32^{o}C seemed a viable option. To keep the ablation procedure as minimally invasive as possible, the poloxamer solution needed to be easily injectable through a 21 gauge needle. Unfortunately, due to the high viscosity of the solution, this requirement was not met. Additionally, during preliminary surgical animal testing conducted on swine by Professor Brace, the animal's breathing slightly inhibited the gelation of the solution. Two areas of improvement were determined; these include the solution's viscosity and bioadhesion.

These characteristics can be affected with the addition of different compounds to the poloxamer solution. Candidates that favorably affected the solution were: benzoic acid, poloxamer 188, methylcellulose, and polyethylene-glycol (PEG) 400. Each of these additives were incorporated into poloxamer solutions of varying concentration and then tested to determine their effect on the gelation temperature and kinematic viscosity of the solution. Unfortunately, none of these additives were found to improve the existing prototype, as they induced unfavorable characteristics such as increased viscosity.

Our final design is a 15.4% poloxamer solution, as it retains the most favorable characteristics for a hydrodissection fluid compared to the potential additives. Cytotoxicity testing was also performed this semester using a staining live/dead assay on 3T3 fibroblasts to help determine the safety of the final design, which was found to be statistically similar to D5W, the current hydrodissection fluid standard.

Table of Contents

PROBLEM MOTIVATION

Hepatocellular carcinoma is the most common human solid malignancy worldwide with over one million new incidences occurring annually. Seventy to ninety percent of these liver tumors are not candidates for surgical resection so different methods of removal are necessary [1]. Other readily accepted methods for treatment of these tumors are radiofrequency (RF) ablation and cryoablation, which use extreme temperatures to cause tumor necrosis [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 and absorb quickly which results in unintended fluid migration and barrier degradation. Because of this, a large amount of liquid (> 1L) is typically required for adequate protection. This can lead to longer operation times and post-procedural complications such as bloating, which must be minimized. Therefore, Dr. Chris Brace, Dr. J. Louis Hinshaw, and Dr. Meghan Lubner have proposed the design of a fluid that retains all the favorable qualities of D5W, such as thermal/electrical insulation and biocompatibility, while alleviating its faults.

This was accomplished by developing a 16.0 w/w% poloxamer 407 solution in deionized water. This solution was tested in preliminary studies on swine and showed to prevent fluid

migration and barrier degradation; however, new problems arose. The main issue is that the poloxamer 407 solution is too viscous to be readily injected through the 19 - 20 gauge needle used in administering the hydrodissection fluid. In addition, animal breathing slightly retarded proper gelation in vivo. It is hypothesized that with an increase in solution adhesiveness, proper gelation will occur and the solution will perform better as a hydrodissection fluid. The goal of this is to optimize the previously design 16.0 w/w% poloxamer solution through the use of additives to alleviate the new problems: high viscosity and low bioadhesiveness.

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 prototype design.

Radiofrequency ablation is a relatively simple procedure. Figure 1 shows the RF ablation probe inserted into the tumor. The RF electrodes can be various shapes; each resulting in a unique ablation zone. A typical needle-like electrode has an ablation zone of about 3 cm, while an umbrella shaped electrode has a slightly larger ablation zone [5]. Radiofrequency, AC electrical current is applied through the electrode causing a temperature increase in the target tissue. This results in the ablation, or destruction, of the tumor.

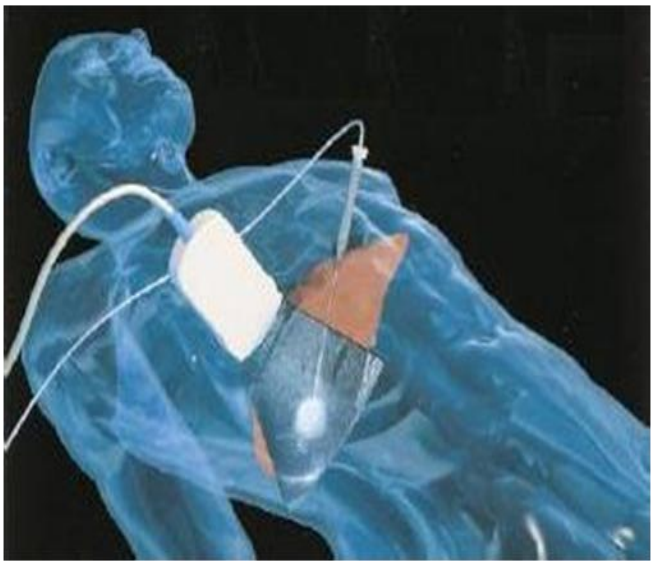

Figure 1 – *RF ablation operational setup. A RF electrode is inserted into the tumor using ultrasound or CT (not pictured) for guidance. Radiofrequency AC current is applied to the tumor which results in the high temperature necessary to kill the tumor. Image from [3].*

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 RF electrode(s) [3].

Percutaneous RF ablation is the most common clinical method [6]. 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) [6]. 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 [7]. 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 liquid nitrogen or argon; this can be seen in Figure 2 [8]. An advantage of cryoablation over RF ablation is that multiple cryoprobes can work simultaneously to form uniquely shaped ice balls. These ice balls are easier to see using imaging equipment, and can therefore treat larger tumors than RF ablation [3].

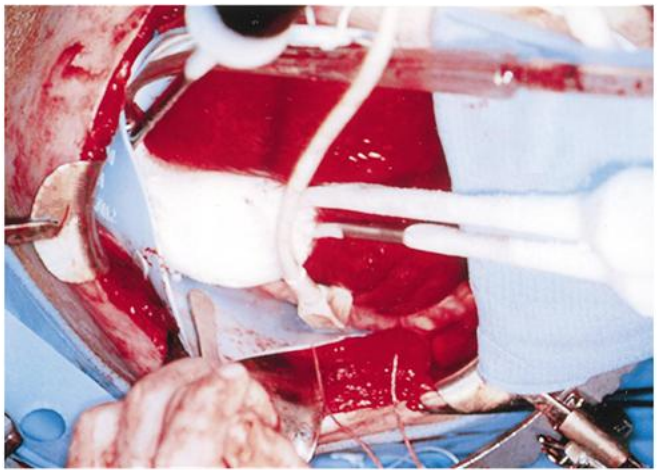

Figure 2 – *An ice ball is formed during cryoablation to destroy harmful tissue. Ice balls can be shaped using multiple probes to treat larger tumors; this results in higher variability in treatment candidates, an advantage over RF ablation. Image adapted from [3].*

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, approximately 0.5% of patients have ablation related deaths [1, 3, 6, 9].

CURRENT TECHNOLOGY

A crucial factor in the success of the ablation operation and the survival of the patient is the protection of the surrounding, non-cancerous tissue. Cryoablation and RF ablation do not inherently differentiate between healthy and unhealthy tissue, so 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 healthy 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 [10]. 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) [11]. Unfortunately, saline is an ionic solution and therefore conducts electricity in RF ablations which 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) [12-13]. Unfortunately, both of these methods are more invasive than a saline or D5W injection. Also, CO_2 needs to be handled very carefully

within the body cavity since it could cause a fatal air embolism [12]. 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.

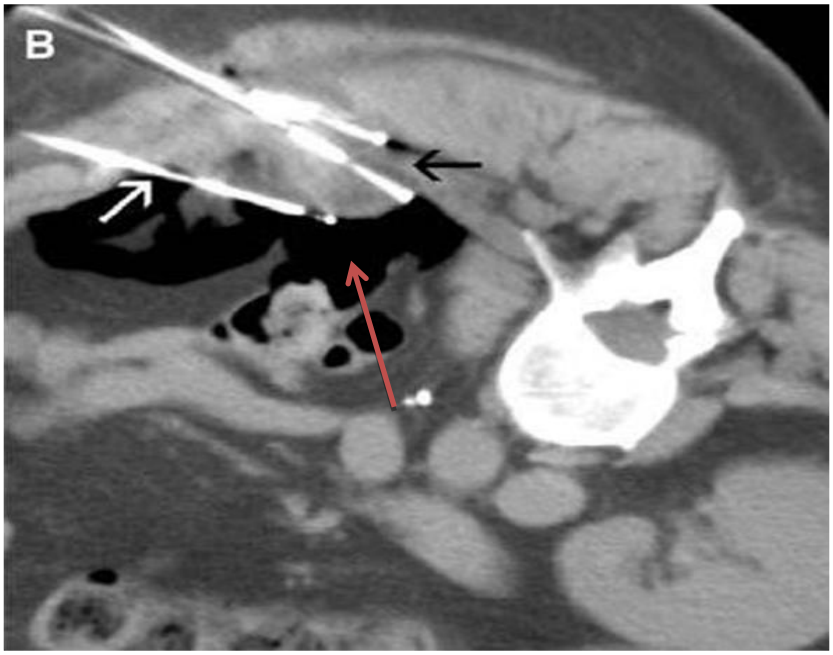

Figure 3 – *This CT scan image shows imaging problems of CO_2 with CT scans. The white arrow points to the RF electrode, the black arrow points to a thermocouple, and the red arrow points to the CO_2 gas which inhibits imaging during CT scans. Since imaging is an important part of ablation techniques, this effectively hinders the ablation procedure. Image adapted from [12].*

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. A D5W barrier thickness of 9 mm resulted in a healthy tissue temperature increase of only 3°C when measured from a 0.5 cm distance [2]. The effectiveness of D5W can be seen in Figure 4 [11].

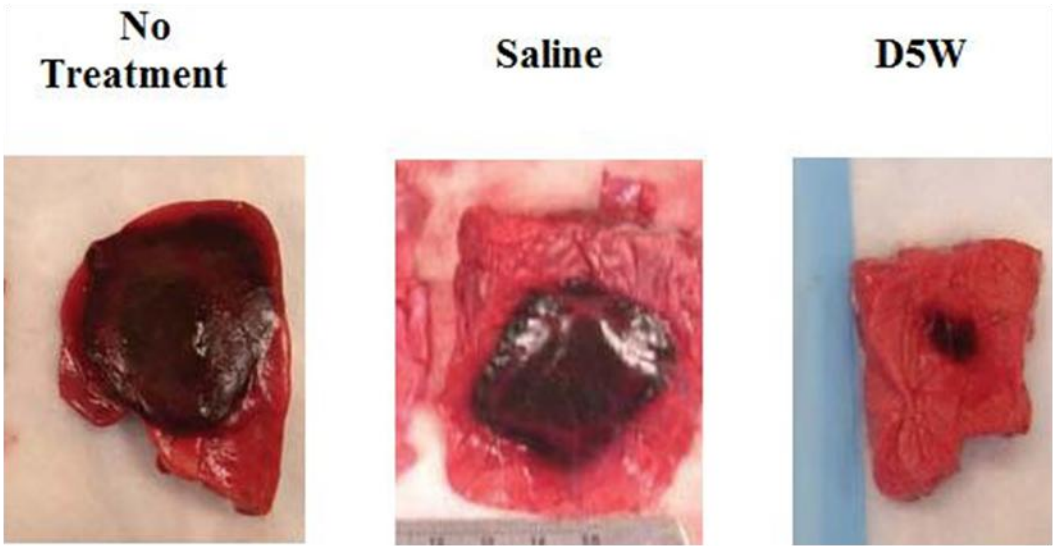

Figure 4 – *Swine lung lesions resulting from RF ablation treatment. D5W minimizes unwanted tissue damage most efficiently compared to saline and without any fluid. However, there is still unwanted damage on the lung from the RF procedure. Image adapted from [11].*

CURRENT DESIGN – POLOXAMER 407

To combat fluid migration and barrier degradation during ablation procedures, a 16.0 w/w% poloxamer solution was designed. Poloxamers are triblock copolymers containing both hydrophobic poly(propylene oxide) (PPO) and hydrophilic poly(ethylene oxide) (PEO) blocks arranged in a repeating triplet pattern PEO-PPO-PEO [14]. The structure of the polymer block can be viewed in Figure 5 [15-16]. The degree of polymerization (i.e. the number of units) for each block of the 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]. As a bioabsorbable substance, poloxamer chains are absorbed into the blood stream and passed from the body through the kidneys. The general process for this consists of the poloxamer being diffused from the blood into the nephrons of the kidneys. Diffusion of sugars and water back into

the blood occurs in the tubules which eventually make the urine very concentrated. The poloxamer is passed through these tubules leading to the bladder and finally is excreted in the urine [17]. This whole process is expected to take up to 3 days [18].

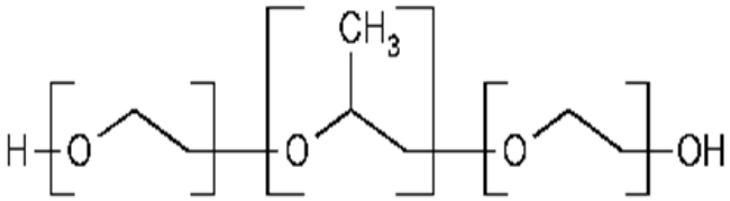

Figure 5 – *The triblock structure of poloxamer. The number of units in a poloxamer gives the poloxamer its name and special characteristics. The center, PPO block is hydrophobic and flanked by two hydrophilic PEO blocks. Image adapted from [15].*

Poloxamer 407 (Lutrol F-127; BASF) has the unique property of forming a thermoreversible gel when mixed with water. This thermoreversible solution to gel phase change occurs when micelles form. As temperature increases the hydrophobic PPO blocks become dehydrated and begin to clump together forming micelles; with increasing temperatures, more micelles form and the free hydrophilic PEO chains become entangled. This leads to a formation of an organized structure of micelles, which causes a phase change to occur. This phase change occurs as a sol-gel transition temperature when the solution becomes a viscoelastic gel [16, 18]. This micellization process can be viewed in Figure 6. The temperature at which this takes place is named the gelation temperature. This gelation temperature must be experimentally determined and varies depending on the concentration of poloxamer in solution [14, 16, 18].

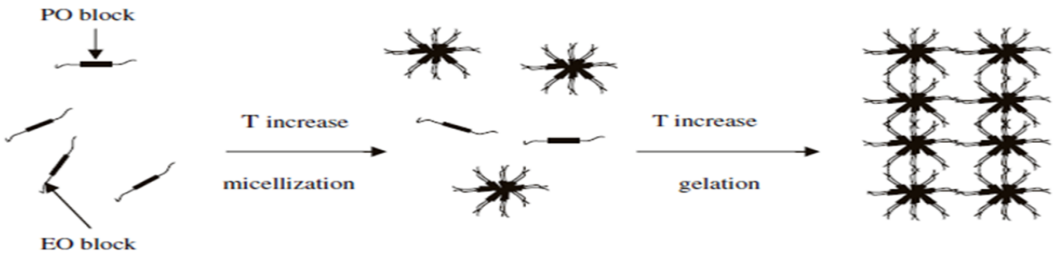

Figure 6 – *The micellization process of poloxamer. As temperature increases the core, hydrophobic blocks become dehydrated and bunch together; with increasing temperatures, more micelles form. Eventually a sol-gel transition temperature is reach and the free, hydrophilic branches become entangled causing the micelles to form an organized structure. This causes the solution to make a phase change and become a viscoelastic gel. Image adapted from [18].*

The poloxamer gel is sometimes unattractive as a biomaterial because of its rapid erosion and low mechanical strength; however, this 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. The breakdown of the poloxamer occurs in the body as the solution becomes dilute and the formed micelles are dismembered [19].

The key characteristic of the poloxamer 407 hydrodissection fluid is its thermoreversibility; this would allow it to be injected into the peritoneal cavity 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 7. Due to the solution to gel transition of poloxamer, the viscosity of the product would greatly increase once injected into the peritoneal cavity.

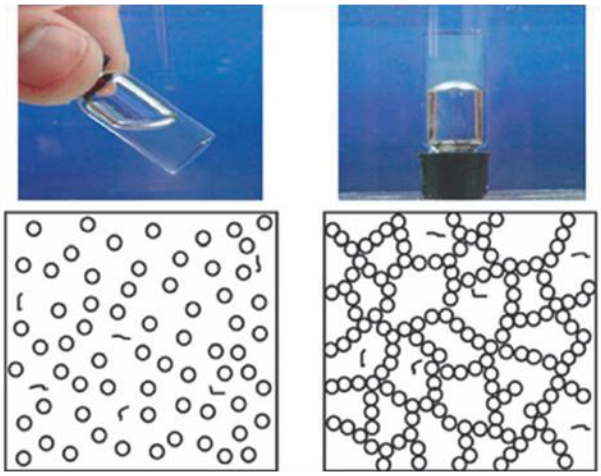

Figure 7 – *The left shows the poloxamer solution below the sol-gel transition temperature; at this temperature the poloxamer 407 hydrodissection fluid could be injected into the peritoneal cavity. At this point the solution would be brought to body temperature, 37^{o}C, and gelled between the target ablation site and adjacent tissue. This figure accurately portrays the phase change seen at the sol-gel transition temperature. Image adapted from [16].*

Once gelled, a temperature is eventually reached where the poloxamer begins to precipitate out of solution [16]. This temperature is called the gel melting temperature. This is expected to slightly affect the efficacy of the design; however, only the edge nearest the ablated tissue would be affected by extreme temperatures. The effects of this could be quantified using a tissue phantom to determine effectiveness as a thermal barrier. However, 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.

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 [20]. Poloxamer 407 is also well tolerated when administered subcutaneously [21]. The FDA lists poloxamer 407 as an inactive ingredient for inhalation, oral solutions,

suspensions, ophthalmics, topical formulations, and IV injections [22]. It is classified as non-hazardous by OSHA [23]. Poloxamer 407 typically exhibits a pH of 6.0 – 7.5 in aqueous solutions, which is similar to the human body [23].

Poloxamer 407 did not result in either morbidity or mortality when administered via IP injection to mice and rats for 1 year [24]. 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 [18]. Our solution would contain approximately 38.5 grams of poloxamer per 250 mL unit, suggesting a possible contraindication for patients under 80 kg.

DESIGN REQUIREMENTS

The clients require that the updated product incorporates the favorable characteristics of the current poloxamer solution, while being easier to inject into the body. Also, to combat the shear stress placed on the fluid by bodily movements, increased bioadhesion is also desired. Table 1 shows the favorable characteristics of both D5W, saline, and characteristics of an ideal hydrodissection fluid. The design would have to include these characteristics for the product to be competitive on the market.

	Pros	Cons
D5W	• Electrical insulator • Thermal insulator • Biocompatible	• Fluid migration • Barrier degradation
Saline	• Thermal insulator • Biocompatible	• Electrically conductive • Fluid migration • Barrier degradation
	Ideal Characteristics	
Ideal Hydrodissection Fluid	• Maintain favorable characteristics of D5W • Inhibit fluid migration and 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) as a Class III medical device [25]. The product is to be injected into the body cavity and should accurately function within the body's environmental thresholds. The fluid and additives 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.

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 [26]. 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. Currently, the product is difficult to push through a 21 gauge needle. 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 1 cm 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 $250. Saline and D5W are significantly cheaper than this (approximately $2-3) [27]; however, with less fluid volume needed for adequate protection, the product's benefits (prevention of fluid migration and barrier degradation) will outweigh the cost increase. A complete list of product design specifications can be found in Appendix A.

DESIGN ALTERNATIVES

Benzoic Acid

Benzoic acid has been studied to a limited extent as an additive in poloxamer drug delivery vehicles [28]. It has been shown to decrease the sol-gel transition temperature of poloxamer 407 gels; results reported by Gilbert et al. can be seen in Figure 8. The structure of benzoic acid is shown in Figure 9. The ester groups of benzoic acid bind with the hydrophilic PEO block of the poloxamer, which causes it to become dehydrated. This increases branch entanglement, thereby decreasing the gelation temperature of the solution.

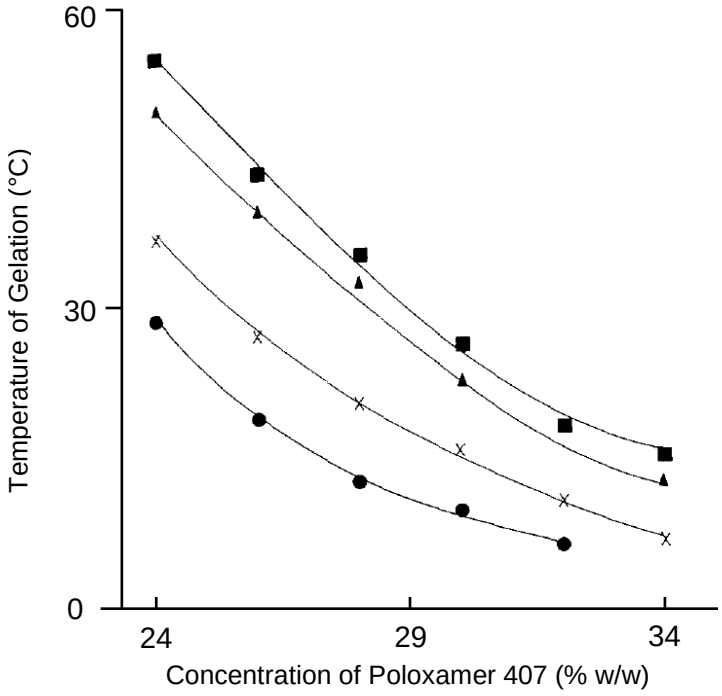

Figure 8 – *Key: Concentration of benzoic acid: (■) 0 % w/v, (▲) 0.4 % w/v, (X) 1.2 % w/v, (●) 2 % w/v. The concentration of benzoic acid is inversely proportional to the sol-gel transition temperature. Although the design poloxamer gel is 16.0%, it is expected that a concentration between 14.0-15.0 % can be used with an incorporation of benzoic acid. Because the viscosity of the poloxamer solution decrease with decreasing concentration, incorporation of benzoic acid would results in a decrease in solution viscosity. Image adapted from [28].*

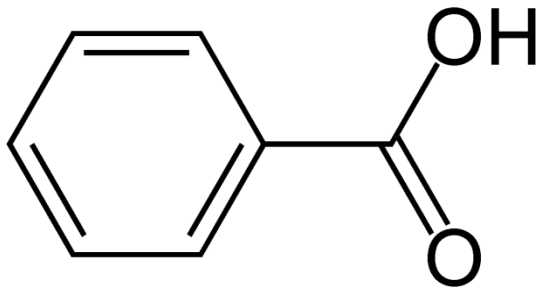

Figure 9 – *The chemical structure of benzoic acid. The ester groups of the chemical structure bond with the PEO block of the poloxamer copolymer causing dehydration of the normally hydrophilic branches. This increases branch entanglement and decreases the gelation temperature of the poloxamer 407 solution [29].*

The correlation seen between the concentration of benzoic acid and the gelation temperature suggests that the addition of benzoic acid would allow for the concentration of poloxamer 407 to be reduced while still maintaining the 32^{o}C sol-gel transition temperature required for design specifications. The decrease in poloxamer 407 concentration would lower the viscosity of the poloxamer solution facilitating injection into the peritoneal cavity. A flow diagram of benzoic acid's effect can be seen in Figure 10. The currently designed poloxamer solution is 16.0 w/w%; it is hypothesized that a 14-15 w/w% poloxamer solution could be used.

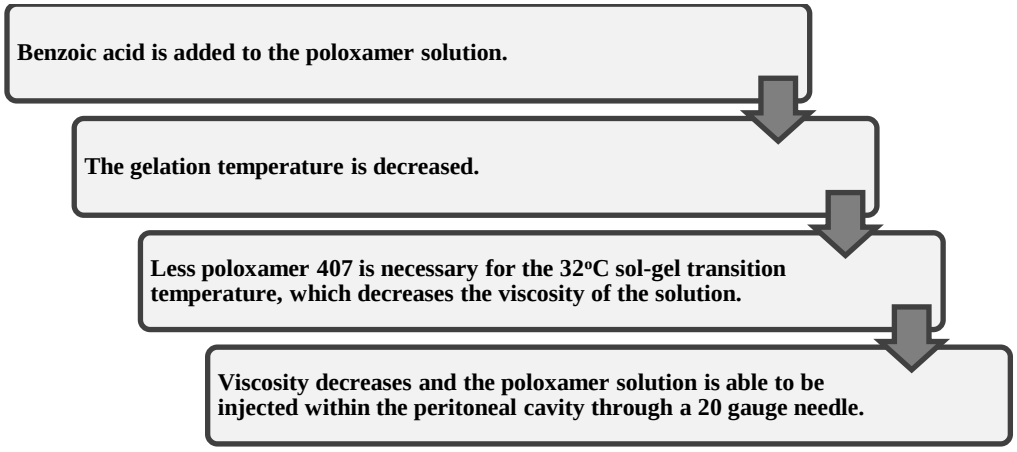

Figure 10 – *Flow diagram illustrating the effects of benzoic acid on the currently designed poloxamer 407 hydrodissection fluid. The incorporation of benzoic acid will alleviate the current issue with injection into the body cavity by reducing the amount of poloxamer in solution.*

Literature review did not suggest any other effects (i.e. change in thermal properties, degradation rates, electrical conductivity, etc.) benzoic acid could have on the poloxamer solution/gel. However, there is the possibility that gel characteristics may be affected. These would be addressed during testing.

Benzoic acid is a common additive in many day-to-day products. It is used in foods and oral solutions as a preservative, and included as an additive in medications administered topically, intravenously, intramuscularly, and rectally [30]. Benzoic acid is categorized by the FDA as GRAS (Generally Recognized as Safe) [31]. A low concentration, 0.5-2.0 w/w%, would

be used for the designed solution; the concentration would have to be tailored for gel optimization. It is expected that 1.25-5.00 grams would be adequate for a one unit (250 ml) of poloxamer solution; this would require an additional cost of $0.13-0.50 per packaged unit [32].

Poloxamer 188

Poloxamer 188 has a similar composition to poloxamer 407. It is the same triblock copolymer with a PEO-PPO-PEO structure. While poloxamer 407 has 202 PEO blocks and 56 PPO blocks, poloxamer 188 has 160 PEO blocks and 27 PPO blocks. These slight changes cause the properties of poloxamer 188 to be slightly different than poloxamer 407. Despite these differences, poloxamer 188 forms a thermorevesible gel when mixed with deionized water. However, poloxamer 188 has fewer PPO (hydrophobic) blocks; this reduces the gelation of poloxamer 188 solutions requiring concentrations greater than 20 w/w% for adequate gel formation [33]. This would not affect the design since the poloxamer mixture of 188 and 407 will gel at concentrations less than 20 w/w% poloxamer 188. Poloxamer 188, like other poloxamers, is a nonionic copolymer. With a molecular weight less than 13 kDa, poloxamer 188 will be readily absorbed by the body [16].

Addition of poloxamer 188 into a poloxamer 407 solution changes the gelation temperature. As more poloxamer 188 is added, the gelation temperature of the mixture increases to a maximum, then decreases as more poloxamer 188 is added. This occurs after a certain point, which changes with different concentrations of poloxamer 407 and 188, because poloxamer 188 becomes the main component of the micelles. Hence, with more poloxamer 188, there are more small branches (PEO blocks of poloxamer 188) for micelle entanglement than large branches (PEO blocks of poloxamer 407), this results in less entanglement and an increase in the sol-gel transition temperature. As poloxamer 188 concentration increases, the gelation temperature will decrease, similar to increasing the concentration of poloxamer 407 in solution. This relationship

can be seen in the graph in Figure 11 [34]. Since the gelation temperature of the poloxamer 407 solution increases with the addition of poloxamer 188, more poloxamer 407 must be added to lower the gelation temperature back to of the addition of poloxamer 188 on the design would be an increase in viscosity.

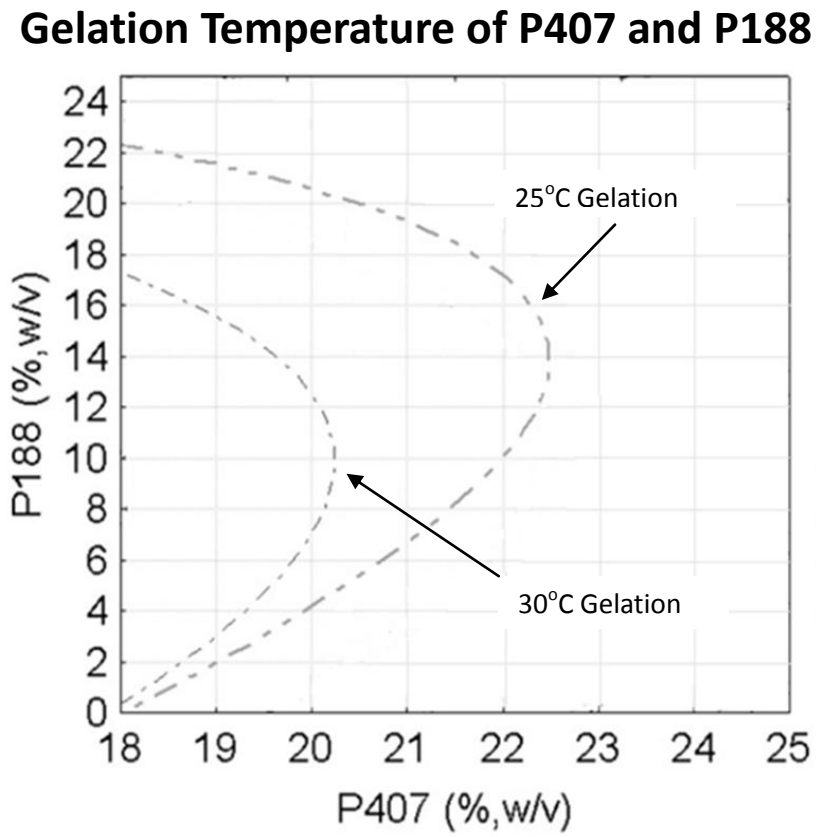

Figure 11 - *A table showing the effects of poloxamer 188 concentration on poloxamer 407 gelation temperature. Small amounts of poloxamer 188 increase the gelation temperature; however, as the poloxamer 188 concentration increases further, the gelation temperature decreases. Image from [34].*

Bioadhesiveness is a useful property of poloxamer 188. This increased bioadhesion results from the greater percentage of hydrophilic PEO chains that dominate the polymer's properties, and allow it to hydrogen bond to adjacent tissue [33-34]. In the body, the PEO blocks can hydrogen bond to ECM and cell surface proteins. Poloxamer 407 has a larger number of PEO blocks; however, because its relative proportions to PPO blocks are smaller, the poloxamer

is less bioadhesive [33]. Because poloxamer 188 increases bioadhesion of the fluid while increasing the viscosity, poloxamer 188 would have to be used in conjunction with a viscosity reducing additive.

Methylcellulose (MC)

Methylcellulose (MC) is a hydrophilic compound derived from cellulose, a polysaccharide consisting of many linked D-glucose units. Depending on the R groups attached to it, MC can be characterized as a variety of reagents; such as, hypromellose (HPMC), or hydroxyethyl cellulose (HEC), as detailed in Figure 12. Each has slightly different characteristics; but generally, these cellulose derivatives are non-toxic and not allergenic, though also not digestible [35].

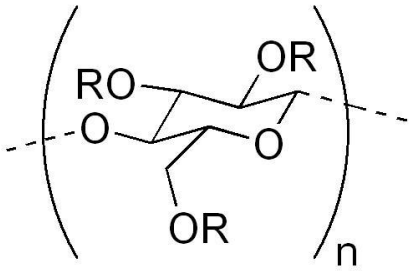

Figure 12 – *The general structure for cellulose products. R = H, CH_3 for methylcellulose and R = $CH_2CH(OH)CH_3$ for hydroxypropyl methylcellulose (hypromellose). The R chains mostly vary the molecular weight of the compound, which affects gel strength, but not gelation temperature. Studies have shown that methylcellulose has the greatest effect on viscosity and gel strength when mixed with poloxamer solutions. Image from [35].*

Typically, MC and its various forms are used as thickeners and emulsifiers, constipation treatments, lubricants, glues/binders, foam stabilizers, dough strengtheners, and long-term drug release gels [35-37]. This final application is the reason they have been used many times in conjunction with poloxamer gels for sustained drug release formulations [38].

Like poloxamer 407, MC forms a micellar, thermoreversible gel when mixed in sufficient quantities (>1 w/w%) with water [36, 39]. Also like poloxamer 407, the micelles form from

interactions between the hydrophobic parts of the polysaccharide. This property has helped increase poloxamer gel strength in other studies [38].

As Figure 13 shows, as the MC concentration in solution increases, the gelation temperature decreases. This property is also dependent on the number of methoxy group substitutions (recall R = H or CH_3), which also affects gel strength [36]. It should be noted that the dashed lines in the figure means that the incipient gelation temperature (IGT) is below the incipient precipitation temperature (IPT), and while it is possible to make these solutions, it is difficult to keep the MC from precipitating out [36]. Unfortunately, like poloxamer 407, MC also precipitates out of solution at higher temperatures. This usually occurs about 15-20°C above the IGT [36].

Another disadvantage to using MC is that cellulose is resistant to biodegradation. Tests show that it can take over 20 days to biodegrade by at least 96% [40]. In vivo studies have shown MC is a safe cerebral and ophthamlic tissue scaffold, even at high concentrations (8 w/w%), and that foreign body reaction was relatively mild; cellulose derivatives can be converted into biocompatible materials by physical and/or chemical transformation [41-43]. MC is FDA approved for ingestion, topical, and ophthalmic applications, as well as intramuscular injections [37, 44].

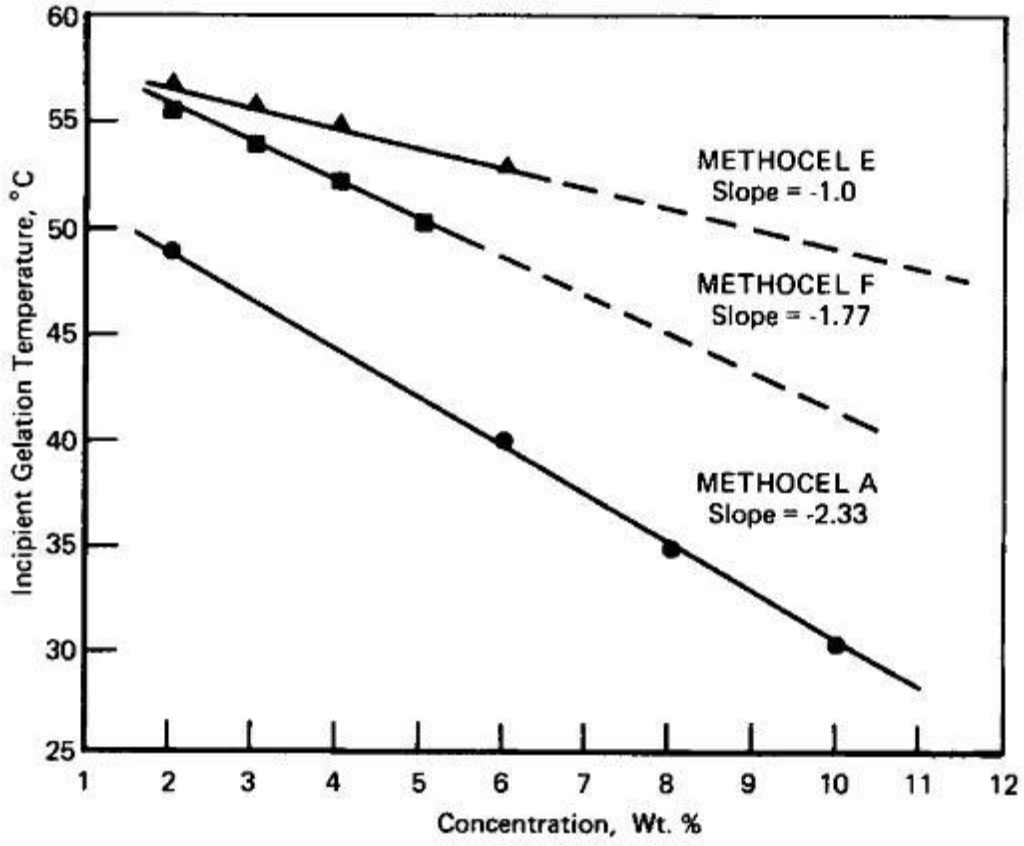

Figure 13 – *Gelation temperature vs. concentration for different MC polymers. Methocel is a trademarked name. Methocel A is methylcellulose, while Methocel F and E are hydroxypropyl methylcellulose. Dashed lines indicate solutions that are difficult or impossible to make. Higher concentrations of methylcellulose could form a gel at the same temperature as the poloxamer, which, although unnecessary, would increase gel strength more significantly as the methylcellulose could form a gel interacting with itself as well as with the poloxamer. Image from [36].*

The primary use for MC in this product would be to increase adhesion strength and reduce solution viscosity. Many studies use MC for precisely this purpose, with various controlled drug release gels. MC has been found to impart substantial mucoadhesive force to poloxamer solutions, without damaging mucosa or submucosa [38]. Additionally, MC has been shown to reduce the gelation temperature and increase the gel strength of poloxamer 407 solutions [24, 38]. With this information, MC would have a similar effect to benzoic acid on the viscosity of the solution. These solutions only require 1 - 2 w/w% of MC for this effect to be produced.

Polyethylene glycol 400

It is hypothesized that viscosity can be reduced by using polyethylene glycol 400 (PEG 400). This first stems from using less poloxamer 407 which is a much larger molecule than PEG 400. The decrease in amount of poloxamer in solution would naturally increase the gelation temperature and reduce the viscosity. PEG 400 is a low molecular weight, highly hydrophilic polymer [45-47]. Since the PEG 400 molecule is hydrophilic, it binds with free water molecules in the solution. This in turn results in a system having less water molecules to form bonds with the poloxamer. Thus, PEO chain entanglement occurs sooner and the gelation occurs at a lower temperature. This effect has been previously studied with poloxamer 407 solutions [46-48]. It has been shown that the addition of 5 w/w% PEG 400 decreases the gelation temperature of 25 w/w% poloxamer 407 solutions [46, 48]. This amount can also be increased if a lower viscosity is required.

This effect is the result of the PEG 400 decreasing the critical micelle concentration (cmc) of the poloxamer 407 solution. Cmc is the concentration above which micelles are spontaneously formed. Pandit and McIntyre demonstrated that the addition of 20 w/w% PEG decreased the solution's cmc drastically from 0.12 to 0.028 w/v% [48].

Besides affecting viscosity, the addition of PEG 400 has also been shown to increase the elastic modulus of the poloxamer gel [46]. This result can be seen in Figure 14.

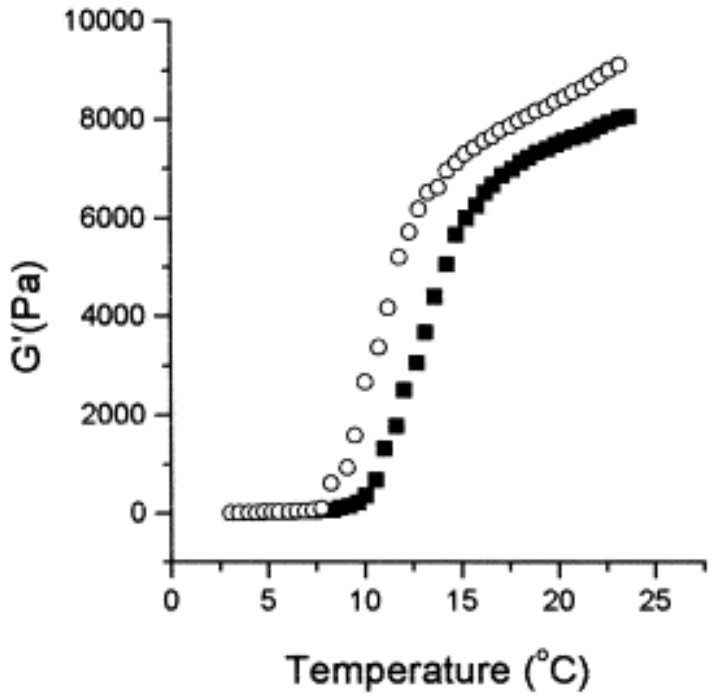

Figure 14 – *Graph showing the sol-gel transition temperature for poloxamer 407 25% and lidocaine 2% in solutions; effect of the addition of PEG 400. Elastic modulus, G' (Pa), as a function of temperature at a frequency of 1 rad s-1. Concentration of PEG 400: (■) - 0%, (o) - 5%. Graph from [46].*

Not only does PEG 400 decrease the gelation temperature of the poloxamer solution, but it also increases the gel melting temperature. A 20 w/w% addition of PEG 400 was shown to increase the gel melting temperature by 7 degrees Celsius [48]. This is important because this gives the gel a larger functional window by prolonging satisfactory tissue protection during high temperature RF ablation procedures.

PEG 400 seems to be a promising additive since it will decrease the viscosity of the solution, increase the elastic modulus of the gel, and increase the gel melting point all while maintaining the required 32°C gelation temperature.

DESIGN MATRIX

A design matrix was used to assess which additives were most ideal to incorporate into this product. The four additives were evaluated in four different categories: reduction of fluid viscosity, biocompatibility, bioadhesion, and cost. Importance of categories and the point values

assigned to them were based on the design specifications given by the client. The main problem with the previously designed poloxamer 407 solution was that it was too viscous to inject easily through a 21 gauge needle; therefore, reduction of fluid viscosity was allotted the most points in our design matrix. Biocompatibility was deemed the next most important category because a solution that prevents ablation damage, but causes unwanted physiological effects would be counterproductive. Bioadhesion was given slightly fewer points than biocompatibility because the previous solution was already somewhat bioadhesive. Additionally, it is unknown if greater bioadhesion would have a pronounced effect on the protection provided by the designed gel. Finally, cost was also a factor even though poloxamer 407 is already fairly inexpensive (< $10 per unit). Because patient safety is ultimately more important than a cheap product, it was only given 5 points.

The design matrix, shown in Table 2, breaks loosely into two categories: additives that reduce solution viscosity and additives that increase bioadhesion. However, methylcellulose has also been shown to lower the gelation temperature of poloxamer solutions, resulting in a reduced solution viscosity. Of all the additives, methylcellulose was found to be the best for accomplishing our design specifications because it had multiple beneficial effects on the poloxamer gel.

	Benzoic Acid	Poly(ethylene glycol) - 400	Methyl-cellulose	Poloxamer 188
Reduces Fluid Viscosity (40 pts)	40	40	40	0
Biocompatibility (30 pts)	15	30	15	23
Bioadhesion (25 pts)	0	0	25	25
Cost (5 pts)	5	5	4	5
Total	60	75	**84**	53

Table 2 – *Design Matrix – Four categories were chosen based on client preference to evaluate each design alternative. As can be seen, methylcellulose was found to be the best additive for the purpose of the project and will be further examined for the rest of the semester.*

Reduces Fluid Viscosity (40 pts)

The previous 16.0 w/w% solution developed was found to be too viscous to be easily injected through a 21 gauge needle, 23 cm in length. For this reason, the main goal of any additive is to reduce the viscosity of the poloxamer solution and this category was given the highest value of 40 points. This is accomplished by additives maintaining the gel strength, while reducing the poloxamer concentration of the solution.

Benzoic acid has previously been shown to decrease the gelation temperature when added at low concentrations (0.5-2.0 w/w%) to poloxamer 407 solutions. PEG 400 and methylcellulose have also been shown to have this effect when added at low concentrations 5 w/w% and 1-2 w/w%, respectively [24, 47]. These compounds reduce the amount of poloxamer 407 necessary to attain the 32^{o}C sol-gel transition temperature. With a lower poloxamer 407 concentration, the viscosity of the solution is lowered. Thus, the overall effect of benzoic acid, PEG 400, and methylcellulose as additives would result in a decrease in solution viscosity; for this reason, they were given 40 points.

Poloxamer 188 increases the gelation temperature of a poloxamer 407/188 solution. In order to maintain the 32^{o}C gelation temperature required by the clients, more poloxamer 407 must be added to the solution. This addition of poloxamer 407 will increase the viscosity of the solution. For this reason, poloxamer 188 was given 0 points.

Biocompatibility (30 pts)

Biocompatibility is the ability of a material that is introduced into a biological environment to perform its intended function without eliciting any undesirable effects. This category was given 30 points in the design matrix because an effective product that harms the patients would be futile.

PEG 400 has been used in ocular medications at 5 w/w% potency and is approved by the FDA for intramuscular and intravenous injection up to 20.3 w/w% [30]. PEG 400 was given full points in biocompatibility.

Benzoic acid is established as an additive in the category of GRAS by the FDA; because it is less biocompatible than PEG, it was given half the points for this category [31].

Methylcellulose does not cause allergic responses, is non-toxic, and is approved for ingestion, topical, and ophthalmic applications. However, MC is not broken down by the digestive system, and although MC is biocompatible, experiments indicate that MC may take over a month to fully degrade. Degradation products are shorter MC chains and CO_2, the latter of which is readily absorbed by the blood. It is unknown if the shorter MC chains have a mechanism for passing through the body. For these reasons, MC was given 15 points for this category.

Similar to poloxamer 407, poloxamer 188 is biocompatibile and non-ionic. It is FDA approved for intravenous injection for up to 0.60 w/w% [30]. Due to the relatively low value of this compared to PEG, poloxamer 188 was given 25 points out of 30.

Bioadhesion (25 pts)

Bioadhesion is the ability of a material to stick to surrounding biological tissues, typically mucus glands [49]. If it is more bioadhesive, the product is less likely to migrate within the peritoneal cavity, away from the ablation site. In addition, greater bioadhesion should also allow the gel to remain unaffected by small motion artifacts. Poloxamer 407 is already considered bioadhesive, so bioadhesion was deemed less important than reducing the solution viscosity [34]. Thus, this category was allotted 25 points.

Benzoic acid and PEG 400 have not been shown to affect the adhesion characteristics of poloxamer 407 solutions/gels; because of this; they were given zero points for this category.

The primary reason MC is mixed with poloxamer solutions is as a mucoadhesive surfactant stabilizer. A small concentration of MC (~1-2 w/w%) has been shown to greatly increase mucoadhesion without resulting in damage to surrounding mucosa. Because of this, MC was given the full points in this category.

Similar to MC, poloxamer 188 is a mucoadhesive. Although not as strong, poloxamer 188 will increase the adhesiveness of the gel to some degree; for this reason, poloxamer 188 was given 23 points in this category.

Cost (5 pts)

In order for this product to compete with existing ones, it must cost less than $200. It is been estimated that a product with favorable characteristics below this cost would receive

widespread use. Because of this, cost is less important than all other categories and was allotted 5 points.

It's expected that 0.5-2.0 w/w% benzoic acid would be used for the design, this results in a need for 1.25-5.00 grams. This would cost less than one dollar per 250 mL unit; hence, benzoic acid was given the max of five points [50].

A 5 w/w% PEG 400 addition is expected to reduce the viscosity of the solution enough for adequate injection. Buying PEG in bulk results in an estimated cost of three cents per gram [50]. The 5 w/w% addition is expected to cost 38 cents; hence, PEG 400 was given the max of five points. More PEG can be added to decrease viscosity even more which should not be a monetary concern since this will raise costs almost negligibly.

Methylcellulose costs about $0.30 per gram, which is relatively expensive compared to our other additives [50]. Since MC is so effective at such low concentrations, only a few grams would need to be added, however, this still results in a cost nearly double our other additives per 250 mL unit. For this reason, MC was given only four points in this category.

A 5 w/w% poloxamer 188 concentration is expected to be enough to increase bioadhesion of the solution. For this amount, a 250mL unit will cost an additional 90 cents [50]. Because of the small value added to the total cost of the unit, poloxamer 188 was given full points in this category.

MATERIALS AND METHODS

Solution Synthesis

The poloxamer solutions were prepared using the cold method [21]. To make the poloxamer solutions, the appropriate amount of poloxamer was measured using an analytical balance and slowly added to cold (4°C), filtered, deionized water while mixing. Filtered water

was used to make sure no contaminants were present; minor contamination could alter physical/chemical characteristics of the solution.

A slightly different procedure was used when making solutions of poloxamer and another element. PEG-400 is a liquid at room temperature, and was added after the poloxamer solution was synthesized, as previously described. Poloxamer 188 was added before poloxamer 407 to a beaker containing cold (4^{o}C) water and stirred at 300-1000 rpm. After dissolution, poloxamer 407 was added using the previous method. Methylcellulose was added to heated water (80^{o}C) and stirred between 300-1000 rpm as needed to break up the powder into separate particles. The solution was then cooled and continuously stirred until the methylcellulose dissolved. Poloxamer 407 was then added. Benzoic acid was stirred between 300-1000 rpm in hot (80-85^{o}C) water until it dissolved. Poloxamer was then immediately added and stirred in while the solution was cooled to 4^{o}C until complete dissolution was achieved. The protocol established for poloxamer fluid synthesis can be found in Appendix B.

Gelation Testing

Sol-gel transition temperature testing was conducted following procedures previously reported by Gilbert et al [28]. To begin the gelation testing, a water cycler (Fisher Scientific, Isotemp 1006S) was set to 10°C. Two milliliter aliquots of each solution were placed into 15 mL centrifuge tubes (n = 3). Tubes were placed in the water cycler and allowed to equilibrate for fifteen minutes; a control tube with 3 mL of deionized water was used to monitor temperature with a thermometer (as a secondary measurement to that of the water cycler). Temperature increments were changed with intervals as low as 0.1°C near gelation temperatures. The solutions were considered gelled when the centrifuge tube could be tilted horizontally with no movement of the meniscus. A correlation curve was generated, and the necessary concentration

to develop a solution with a 32°C gelation temperature determined. This solution was used for the remainder of the study.

Viscosity Testing

A Cannon-Fenske (Cannon®) size 200 viscometer was used to measure the kinematic viscosity of the 15.4 w/w% poloxamer 407 solution while a Cannon-Fenske (Cannon®) size 50 viscometer was used to measure D5W. A schematic of the viscometer can be seen in Figure 15. Six milliliters of each solution were used for viscosity measurements (n = 3). The viscometer and fluid were placed in a water bath (Fisher Scientific, Isotemp 1006S) which was used to cool or heat water to a specific temperature. After fifteen minutes of temperature equilibration, the elution time was determined using a stopwatch. The kinematic viscosity was calculated using the viscometer's viscosity constant and the elution time. Per manufacturer, viscosity constants used were 0.1 cSt/s and 0.004 cSt/s for the size 200 and size 50 viscometers, respectively [51].

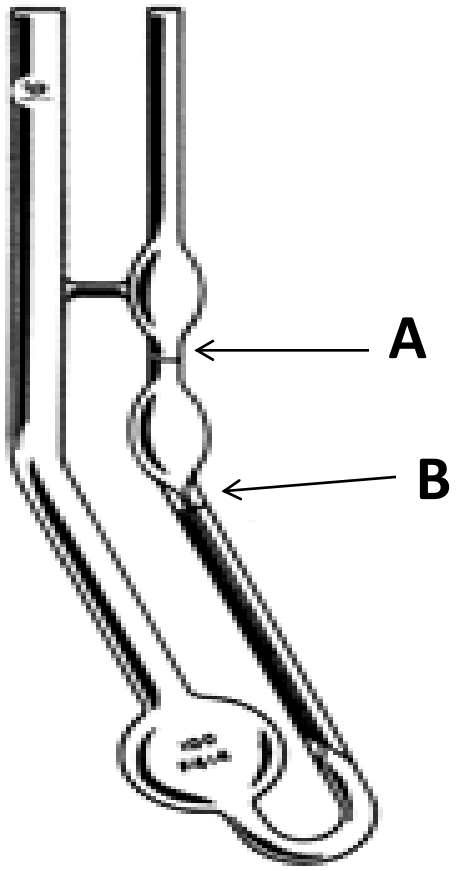

Figure 15 – *A Cannon-Fenske, size 200, viscometer was used to measure kinematic viscosity in previous tests. An analytical pipet is used to transfer 6 mL of solution into the viscometer. A bulb is used to force fluid ~1 cm past point A; when released the time taken for the fluid meniscus to travel from point A to B is directly proportional to the viscosity of the fluid. The viscosity of the poloxamer solution changes with temperature; because of this, the test must be conducted in a temperature controlled environment. Image from [52].*

Impedance Testing

A Cool-Tip RF ablation machine (ValleyLab) was used to measure the impedence of the solutions tested. Two strips of aluminum tape were placed vertically onto a ~200 mL plastic beaker on opposite sides to act as conductors. Two electrodes will be placed on opposite sides of the beaker attached to the aluminum tape. A test with both connections attached to the same piece of tape was done first as a blank. Approximately 40 mL of the solution was placed in the plastic beaker, enough to cover a portion of the tape. A current was passed between the electrodes and the machine displayed the impedance, as can be seen in Figure 16.

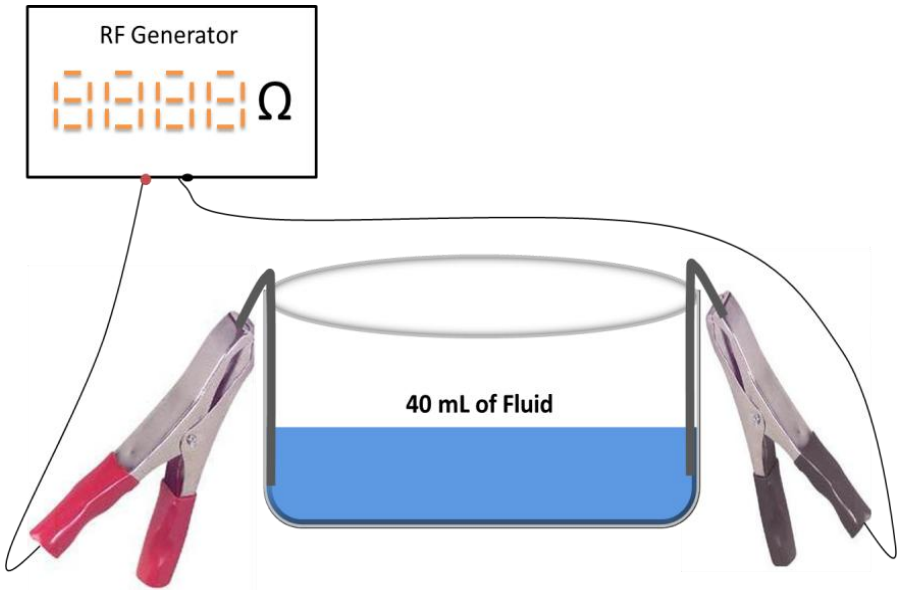

Figure 16 – *A schematic showing experimental set-up for impedance testing of the poloxamer solution and gel. An RF generator used for ablation procedures has the capability of measuring the impedance. Approximately 40 mL of solution will be placed in a 100 mL beaker; two electrodes will be placed on opposite sides of the beaker attached to aluminum tape. The impedance between electrodes, the impedance of the solution or gel, will be tested by the RF signal generator.*

Imaging

An Ultrasound System (SonixTOUCH) was used to compare the imaging of poloxamer to that of D5W. About 50 mL of each solution was placed into a flat container. An ultrasound was taken with three screenshots of each solution, and these images were compared.

One ~50 mL aliquot of D5W and two ~50 mL aliquots of 16.0% poloxamer were placed within 50 mL centrifuge tubes. One tube of poloxamer was allowed to gel in warm water. The tubes were then imaged in a CT scanner (750 HD, General Electric) and processed using ImageJ. After the first scan, another scan was conducted with Iohexol (Omnipaque, General Electric) added to both solutions (~1:50 dilution) to increase contrast.

CytotoxicityTesting

3T3 fibroblasts were cultured in T75 flasks with Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Cosmic Calf Serum and 1% Pen-strep at 37°C in 5% CO_2.

Cells were harvested at 80% confluency with Trypsin (5 min incubation) and plated in 24 well plates at 5.0 x 10^3 cells. Transwell inserts were used to expose the cells to the P407 gel. P407 solution (200 µL) was aliquoted into the transwells and placed at 37°C to allow for gelation. Transwells were placed within the wells; two conditions were evaluated, a control and P407 addition, at 6 and 24 hrs. Because of limited supplies, D5W was evaluated at the 24 hour mark only. A live/dead assay kit (Viability⁄Cytotoxicity Kit for mammalian cells, Invitrogen) was used per manufacturer protocol to assess cell death following exposure to the P407 gel compared to the control wells. The addition of 100 µL of 0.2% Triton X 100 (Promega) was used as a dead cell control. Fluorescence microscopy was used to determine the number of live and dead cells. Results, shown below in Figure 17, are reported as a percentage of live cells versus total cells at both 6 and 24 hrs (n = 3).

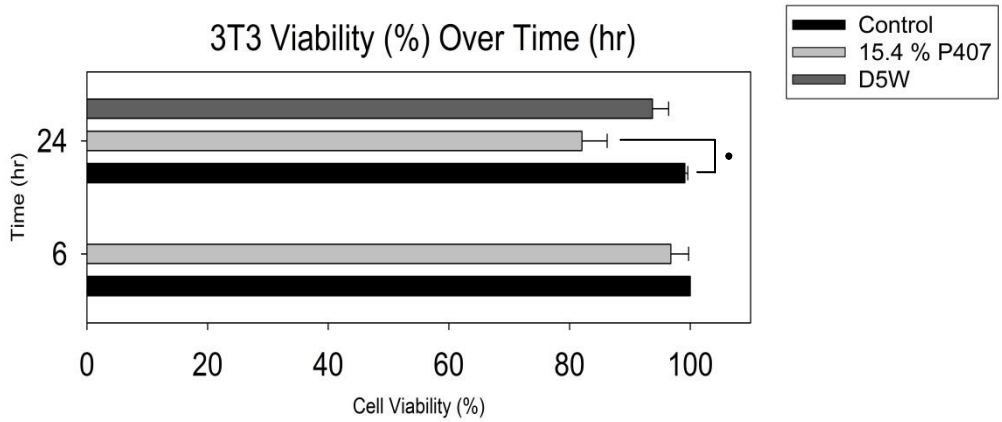

Figure 17 - *P407 cytotoxicity was measured over 24 hours and compared to a control consisting of DMEM, 10% Cosmic Calf Serum and 1% Pen-strep. There was a significant difference (p = 0.05) between P407 cell viability and the control at 24 hrs. However, the P407 was not significantly different (p = 0.05) than the D5W condition at 24 hrs.*

Density Testing

In order to find the density of the poloxamer 407 solution a 100 mL graduated cylinder and a 50 mL volumetric pipette (± 0.5 mL error) were zeroed on an analytical scale. Exactly 50 mL of poloxamer 407 solution was measured and immediately placed into the graduated

cylinder. The mass of the poloxamer 407 solution was recorded and the density was calculated at 25°C.

RESULTS

Imaging Testing

The first test completed on the poloxamer design was the imaging test. The poloxamer solution and gel were both subjected to two types of imaging commonly used during ablation procedures: a CT scan and an ultrasound scan. Results showed both, the solution and gel, were similar to D5W during imaging; however, the poloxamer had a slight echo when gelled under the ultrasound due to air between the transducer and the gel surface. The similarities to D5W suggest that imaging will not be inhibited by using the poloxamer solution as a hydrodissection fluid; results can be seen in Figure 18 and Table 3 below.

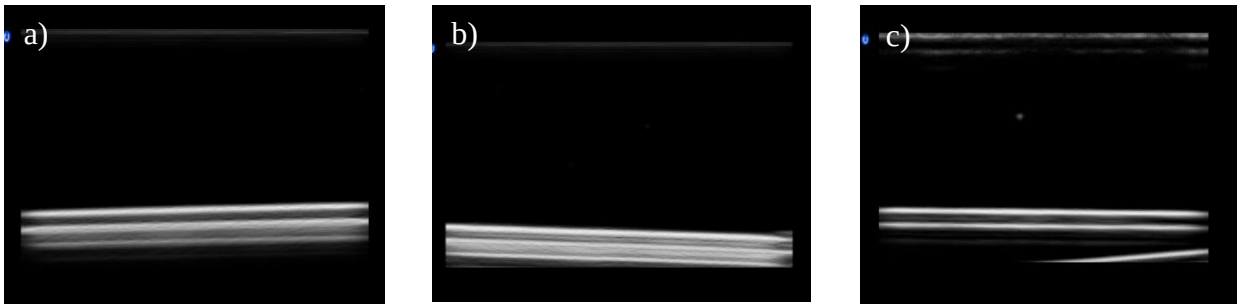

Figure 3 - *Approximately 50mL of D5W (a), poloxamer solution (b), and poloxamer gel(c) was subjected to an Ultrasound System (SonixTOUCH). All of the images are quite clear, which will not inhibit ultrasound imaging during the ablation procedure.*

	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 - *Approximately 50 mL of D5W and poloxamer was placed in 50 mL conical tubes in a CT scanner. After an initial test, Iohexol was added to increase contrast. The resulting ROIs were recorded. Both poloxamer and D5W have low contrast before the addition of Iohexol and high contrast afterwards.*

Impedance Testing

Another requirement for the poloxamer solution was to have high impedance. Both the poloxamer solution and gel had a high (>1000 Ω) impedance when tested with an RF generator, similar to D5W. These results, shown in Table 4, suggest the poloxamer solution will adequately act as an electrical insulator.

Sample	Impedance (Ω)
Blank	40
Saline	88
D5W	High (>1000)
15.4% P407 (solution)	High (>1000)
15.4% P407 (gel)	High (>1000)

Table 4 *A Cool-Tip RF ablation machine (ValleyLab) was used to measure the impedance of saline, D5W, and poloxamer 407. Two strips of aluminum tape were connected to the RF ablation machine and opposite sides of the beaker. Approximately 40 mL of the solution was placed in the plastic beaker, and tested. Poloxamer had a very high impedance (over 1000 Ω), similar to D5W.*

Density Testing

Density testing was done to calculate the force necessary to push poloxamer through a syringe. The 50 mL of poloxamer 407 solution weighed 51.20 g. This yields a density of 1.024 g/mL for the P407 solution.

Gelation Temperature Testing

The gelation temperature was tested for varying concentration of poloxamer 407 along with either the addition of methylcellulose or PEG-400. The results can be seen in Figure 19.

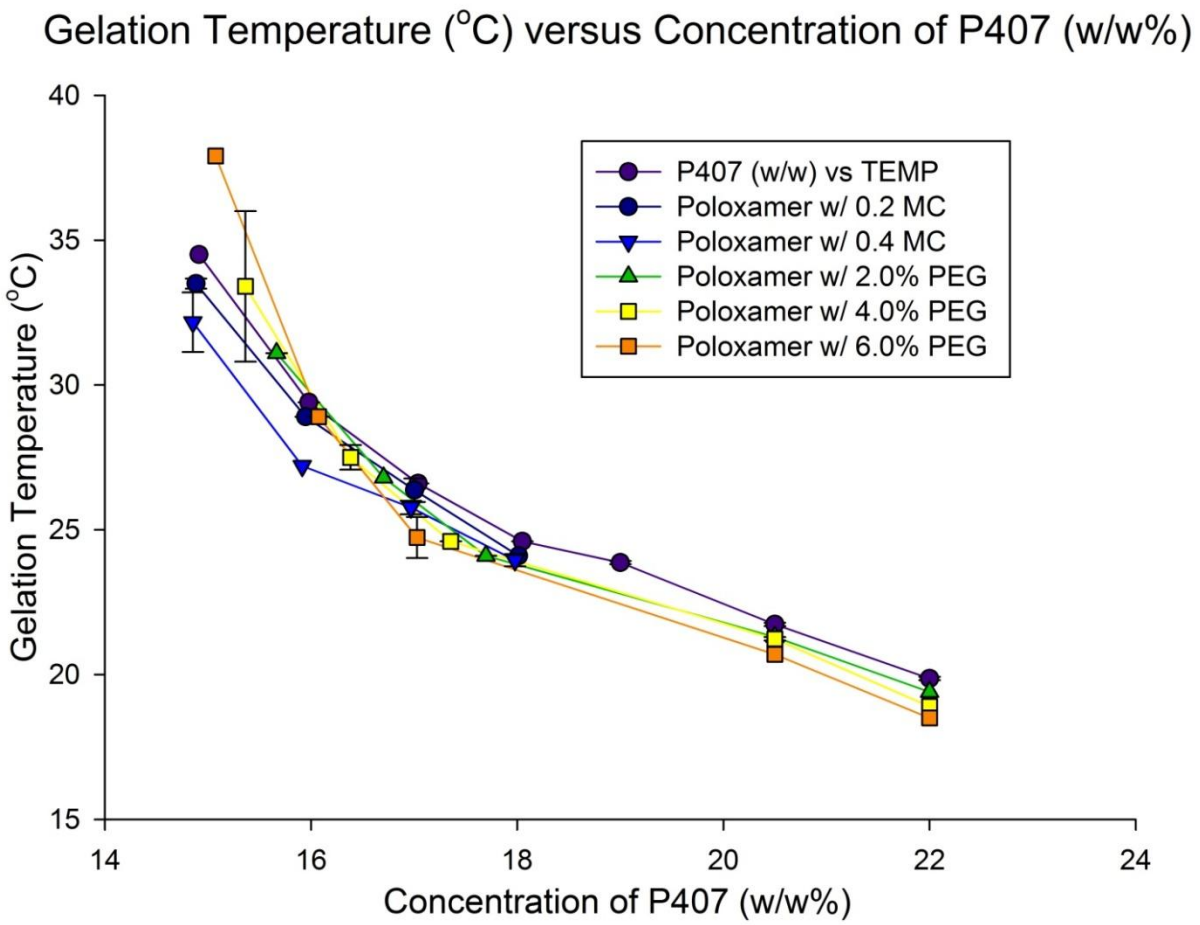

Figure 19 *- Several concentrations of different poloxamer 407 solutions (control solution, 0.2% MC, 0.4% MC, 2.0% PEG-400, 4.0% PEG-400, and 6.0% PEG-400) were formulated and 2 mL aliquots were prepared (n=3) in 15 mL centrifuge tubes. The solution was considered gelled when the tube was rotated into the horizontal position and the meniscus did not move. A correlation was developed between the concentration of poloxamer 407 (w/w%) and the gelation temperature (°C), this was used to determine that a 15.4 w/w% solution of P407 was needed to formulate a solution that would gel at 32°C. This would allow hydrodissection to take place as a fluid is injected between then target and adjacent tissues at which point the fluid temperature would raise and gelation would occur. The formation of a gel is expected to provide a barrier with greater integrity than that of D5W.*

For all solutions but 0.4% MC, solution of P407 varied in concentration ranging from 14.5% to 22% P407; difficulties in solution synthesis were experienced when making solutions greater than 18.0% P407 for 0.4 %MC.

Results show that the addition of MC reduced the gelation temperature of the poloxamer 407 solution; this coincides with results previously reported [38]. It was previously reported that PEG-400 reduced the gelation temperature of P407 solutions [46, 48]. Results of this study show

this is correct for solutions greater than 16.0% P407; however at concentration less than 16.0% P407 it was found that PEG-400 increased the gelation temperature. Additionally, this increase was found to be dependent on the concentration of PEG-400. It is expected this is due to a thermal transition temperature of PEG-400 at approximately 29°C; examination of the thermal properties of PEG-400 are required to confirm this.

The correlation found between the gelation temperature and the concentration of P407 was used to determine the needed concentration of P407 to formulate a solution which gelled at 32.0°C. Results can be found in Table 5.

Condition	Necessary Concentration (w/w%) of Poloxamer 407 for a 32°C Gelation Temperature
Poloxamer 407	15.4_1
Poloxamer 407 w/ 0.2% MC	15.1_4
Poloxamer 407 w/ 0.4% MC	14.8_5
Poloxamer 407 w/ 2.0% PEG-400	15.5_8
Poloxamer 407 w/ 4.0% PEG-400	15.6_6
Poloxamer 407 w/ 6.0% PEG-400	15.6_7

Table 5 - *It can be seen that PEG does not reduce the amount of poloxamer necessary to achieve a gelation temperature of 32°C; more poloxamer 407 is needed in solution to gel at 32°C compared to the solution with P407 alone. The addition of MC reduced the amount of poloxamer needed.*

Viscosity Testing

It was determined the poloxamer 407 had its lowest viscosity around 14ºC. Initially the solution followed a typical fluid pattern: a decreasing viscosity with an increasing temperature; however, after 14°C the micelles in solution begin to form causing a rapid increase in viscosity. This is adequate for this design since the P407 solution resists fluid migration by becoming viscous rapidly.

The addition of PEG-400 to the poloxamer solution made the solution much more viscous. The results show the PEG aided in the formation of micelles causing a rapid viscosity increase at a lower temperature than P407 alone.

The D5W solution had the lowest viscosity of the solutions tested (approximately 1.58 cSt at 18^{o}C). The D5W solution followed a typical fluid pattern of decreasing viscosity as temperature increased. At the optimal injection temperature of 14ºC, the poloxamer 407 solution is approximately eleven times more viscous than D5W. The results can be seen in Figure 20.

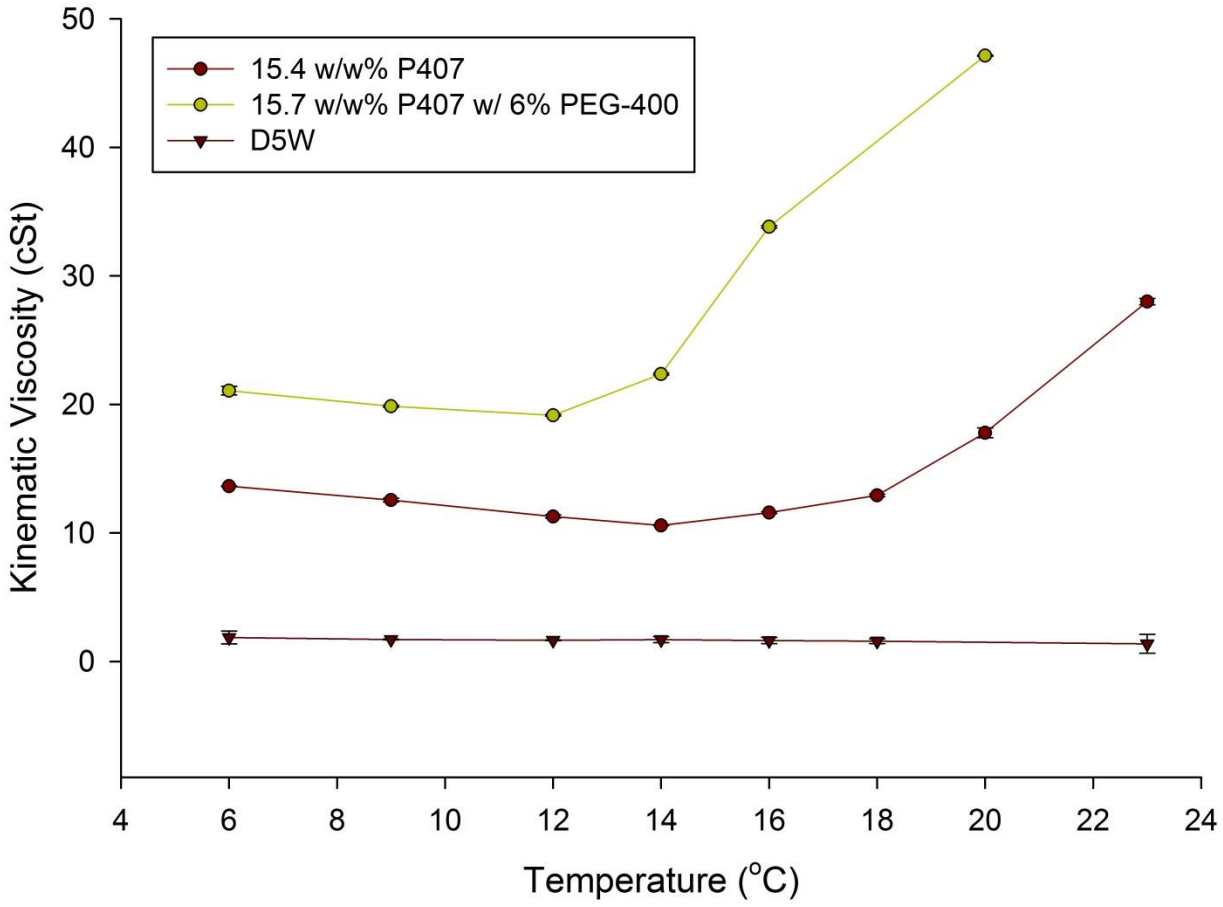

Figure 20 - *Viscometers were used to test the elution times of poloxamer 407, poloxamer 407 with PEG-400, and D5W (n=3). The 15.4 w/w% P407 solution is 11 times more viscous than D5W at 14 ºC. As the poloxamer solution increases in temperature, the viscosity increases greatly. Also, the addition of PEG causes the micelles to form at a lower temperature, causing the dramatic increase at a lower temperature.*

Cytotoxicity Testing

An initial cytotoxicity test showed that P407 induced significant cell death at 24 hrs compared to a control population. These results suggest there is a cellular response with the addition of P407. However, these are preliminary results for toxicity testing. When compared to D5W, there was no significant decrease in cell viability 24 hrs after exposure. Additional testing must be conducted in vivo to confirm in vitro results. In vivo testing will give vital information to whether immune responses are able to alleviate the toxicity seen in vitro. Additionally, poloxamer is expected to be broken down and cleared from the peritoneal cavity within 48-72 hrs [18].

FINAL DESIGN – 15.4 W/W% P407

The results suggest a 15.4 w/w% P407 solution will provide adequate protection while reducing fluid migration and barrier degradation during ablation procedures. Recent testing has shown that the poloxamer solution can be pushed through a needle; however, various syringe guns can be used to aid the user in injecting the viscous fluid [53-55].

FUTURE WORKS

Additional Design Testing

Testing was conducted on various poloxamer 407 solutions this semester to determine their sol-gel transition temperature, kinematic viscosity, impedance, imaging, and cytotoxicity. However, additional testing on these solutions could be done to better understand their properties, including: force of injection (syringability), dynamic viscosity, and bioadhesion strength.

With the help of the Rheology Research Center (RRC) at UW-Madison, the shear modulus, G', will be tested using a rheometer. The shear modulus has been shown to be proportional to

the bioadhesion of the material. Rheometer availability at RRC is limited; testing is to be conducted August 2011.

The adhesive properties can be tested by applying a tension force on the hydrodissection fluid in the gelled state. Because the solution will be used in vivo at 37^{o}C, the experimentation must be conducted in a temperature regulated environment. The design for the test is shown in Figure 21; this method was previously reported by Barakat et al. [24]. The search for other methods for adhesion testing is being conducted; however, thus far, this seems like the most viable option.

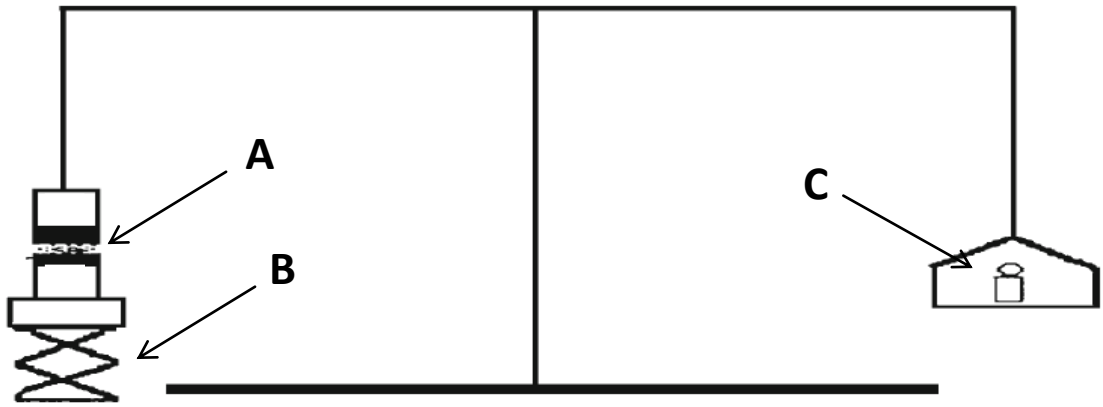

Figure 21 – *A schematic of the adhesion test reported by Barakat et al. Modifications may be made to this design. A piece of tissue is secured on top of a glass vial; two of these are formed, one is secured to an adjustable plate (B) and one to the balance. A gel (A) is placed between the two pieces of tissue. The diameter of the tissue/gel must be recorded for calculations. A mass (C) is place on the other side of the balance; additional weight is added until the gel and tissue separate. From this a stress can be determined as Force/Area = $4mg/\pi d^2$. Where m is the mass, g is the gravitational force, and d is the diameter. Image from [24].*

Previously a syringe pump was used to try to measure the force necessary to push the poloxamer solution through a 21 gauge (23 cm length, 0.514 mm diameter) needle. This test failed because the syringe pump was not able to generate the necessary force (max force = 40 N for most commercial syringe pumps). Upon further investigation of the 21 gauge needle, it was determined that the needle was partially clogged, effectively increasing drag and the force needed for proper delivery. This semester calculations were made to estimate the necessary force

needed for injection of the final design. The following assumptions were made for easier calculations: 1) a 60 mL syringe will be used for administration, 2) a 19 gauge needle will be used, 3) the kinematic viscosity of the P407 solution is 11 cSt, 4) the density of the P407 solution is 1.024 g/mL and 5) a flow rate of .833 mL/s (from injecting 1 L of fluid in 20 minutes). Calculations can be visualized in Appendix D. It was calculated that a pressure difference of 341.24 Pa is needed for a flow rate of .833 mL/s. So, an additional 341.24 Pa above the patient's intraperitoneal pressure (IPP) is necessary. Typical IPP values are 1265.2 ± 666.6 Pa for patients in the prone position [56]. For a maximal IPP value of 1931.8 Pa (average plus standard deviation), a 12.67 N force needs to be applied to the end of the 60 mL syringe for a flow rate of .833 mL/s. Of course, this value will increase if the flow rate is increased.

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 [11]. Protocol number M01814 will be followed for swine testing. A comparison of hydrodissection efficacy between the poloxamer solution and D5W will be made. 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. Poloxamer is expected to outperform the D5W in both maximizing protection of surrounding tissues and minimizing unwanted barrier degradation.

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 [57]. 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 [58]:
>
> **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 16.0 w/w% 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.

Cost Analysis

All chemical supplies for the design were provided by BASF as samples. The current project cost has totaled $93.00; a breakdown of these costs can be seen in Table 6. Also, the projected cost of materials for the final product, a single 250 mL unit, is $8.46; this is the expected cost based on the maximum quantity of each reagent necessary for design development. Breakdown of reagent costs can be seen in Table 7.

Material	**Cost**
Lab Supplies (i.e. pipets, gloves, etc)	$150.00
Fall Poster	$43.00
Centrifuge tubes, 500	$84.76
Methylcellulose, 100 grams	$10.79
Penicillin-Streptomycin, 100 mL	$7.18
Cosmic Calf Serum, 100 mL	$12.25
Benzoic Acid, 500 grams	$24.37
Size 200 Viscometer	$99.59
Spring Poster	$50.75
PROJECT TOTAL	**$482.69**

Table 6 – *The current project cost, thus far, has totaled $482.69.*

Material	Estimated Max Quantity	Cost
Poloxamer 407 ($120 for 1kg; Sigma Aldrich)	38.5 grams, 15.4 w/w%	$4.62
PROJECTED PRODUCT COST		**$4.62**

Table 7 – *A current estimation of materials cost for the final product design; maximum expected quantities/concentrations were used to determine cost. Projected cost is based on the cost of a single 250 mL unit.*

Intellectual Property

This product has been endorsed by WARF (Wisconsin Alumni Research Foundation). Necessary steps are being taken to process a patent application. Companies that may be interested in leasing the patent include, but are not limited to, ablation device companies and material research companies (i.e. Johnson & Johnson, Proctor & Gamble, etc.). A similar product, SpaceOAR, is available. However, it is not available for use in the United States and will not compete for the same market. More background on SpaceOAR is available in Appendix C.

REFERENCES

[1] S. A. Curley*, et al.*, "Early and late complications after radiofrequency ablation of malignant liver tumors in 608 patients," *Annals of surgery,* vol. 239, p. 450, 2004.

[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] D. C. Brace, "BME 517 Image Guided Interventional Oncology Presentation," ed, 2010.

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

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

[8] A. M. Paganini*, et al.*, "Cryosurgical ablation of hepatic colorectal metastases," *Surgical oncology,* vol. 16, pp. 137-140, 2007.

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

[10] 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.

[11] 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.

[12] 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.

[13] 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.

[14] 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.

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

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

[17] *Biology + MasteringBiology*: Benjamin-Cummings Pub Co, 2007.

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

[19] A. B. Dhanikula and R. Panchagnula, "Preparation and characterization of water-soluble prodrug, liposomes and micelles of paclitaxel," *Current Drug Delivery,* vol. 2, pp. 75-91, 2005.

[20] 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.

[21] 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.

[22] R. Rowe*, et al.*, *Pharmaceutical Handbook of Pharmaceutical Excipients*, 5th ed. Washington, USA: London UK and American Pharmaceutical Association, 2005.

[23] "Material Safety Data Sheet: Lutrol F 127 NF Prill," BASF, Ed., 2.2 ed, 2009.

[24] N. S. Barakat, "In Vitro and In Vivo Characteristics of a Thermogelling Rectal Delivery System of Etodolac," *AAPS PharmSciTech,* vol. 10, pp. 724-731, 2009.

[25] FDA. (2009, *Device Classification*. Available: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/ClassifyYourDevice/default.htm

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

[27] (2011, *Baxter ViaFlex IV Bags*. Available: http://www.buyemp.com/product/1121301.html

[28] J. C. Gilbert*, et al.*, "The effect of solutes and polymers on the gelation properties of Pluronic F-127 solutions for controlled drug delivery," *Journal of Controlled Release,* vol. 5, pp. 113-118, 1987.

[29] C. o. t. Day. 6 March). *Benzoic Acid*. Available: http://chemicaloftheday.squarespace.com/todays-chemical/2010/4/26/benzoic-acid.html

[30] FDA. 6 March). *Inactive Ingredient Search for Approved Drug Products "Benzoic Acid"*. Available: http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm

[31] EPA. 6 March). *Benzoic acid (CASRN 65-85-0)*. Available: http://www.epa.gov/iris/subst/0355.htm

[32] A. Organics. 6 March). *Benzoic acid*. Available: http://www.acros.be/DesktopModules/Acros_Search_Results/Acros_Search_Results.aspx?XX=_KFPNKUMENBQNKQNENMPOLWON_?tabID=21&alias=Rainbow&lang=en&search_type=2&search=&for

=acro2&sup=AcrosEU&pri=USF&rid=t&stock=t&server=&from=2876&show_rn=true&show_fw=true&bl=25&found=22605&border=no&bgcolor=%23DDEEFF&bgcolor_td=%23DDEEFF&bgcolor_th=%2399CCFF&bgcolor_th1=%236699CC&bgcolor_th2=%23DDEEFF&bgcolor_th3=%23DDEEFF&align_th1=center&align_th2=center&face_font=verdana,geneva,arial,helvetica,sans-serif&nlink=%23000000&color_font=%23000000&link=false&submitUrlSearch=&alink=&show_pict=&show_bp=&show_mp=&show_den=&show_hr=&tag=&query=

[33] BASF, "Lutrol L and Lutrol F-Grades."

[34] H. Qi*, et al.*, "Optimization and Physicochemical Characterization of Thermosensitive Poloxamer Gel Containing Puerarin for Ophthalmic Use," *Chemical & Pharmaceutical Bulletin,* vol. 54, pp. 1500-1507, 2006.

[35] (2011, *Methylcellulose*. Available: http://en.wikipedia.org/wiki/Methyl_cellulose

[36] N. Sarkar, "Thermal gelation properties of methyl and hydroxypropyl methylcellulose," *Journal of Applied Polymer Science,* vol. 24, pp. 1073-1087, 1979.

[37] FDA, 2011.

[38] A. Karmarkar*, et al.*, "Poloxamers and their applications," *Online international journal Pharmainfo. net,* 2008.

[39] L. Li, "Thermal gelation of methylcellulose in water: scaling and thermoreversibility," *Macromolecules,* vol. 35, pp. 5990-5998, 2002.

[40] F. Blanchard*, et al.*, "Biodegradability of [14C] methylcellulose by activated sludge," *Applied and Environmental Microbiology,* vol. 32, p. 557, 1976.

[41] T. Miyamoto*, et al.*, "Tissue biocompatibility of cellulose and its derivatives," *Journal of Biomedical Materials Research,* vol. 23, pp. 125-133, 1989.

[42] S. Smith*, et al.*, "Safety and efficacy of 2% methylcellulose in cat and monkey cataract-implant surgery," *Journal-American Intra-Ocular Implant Society,* vol. 10, p. 160, 1984.

[43] M. C. Tate*, et al.*, "Biocompatibility of methylcellulose-based constructs designed for intracerebral gelation following experimental traumatic brain injury," *Biomaterials,* vol. 22, pp. 1113-1123, 2001.

[44] *Material Safety Data Sheet, Methylcellulose, 15 cps MSDS*, 2011.

[45] T. Ma*, et al.*, "PEG 400, a hydrophilic molecular probe for measuring intestinal permeability," *Gastroenterology,* vol. 98, p. 39, 1990.

[46] E. Ricci*, et al.*, "Rheological characterization of Poloxamer 407 lidocaine hydrochloride gels," *European Journal of Pharmaceutical Sciences,* vol. 17, pp. 161-167, 2002.

[47] E. Ricci*, et al.*, "Sustained release of lidocaine from Poloxamer 407 gels," *International journal of pharmaceutics,* vol. 288, pp. 235-244, 2005.

[48] N. K. Pandit and H. J. McIntyre, "Cosolvent Effects on the Gel Formation and Gel Melting Transitions of Pluronic® F127 Gels," *Pharmaceutical Development and Technology,* vol. 2, pp. 181-184, 1997.

[49] S. B. Ruddy*, et al.*, "Formulations of oral gastrointestinal therapeutic agents in combination with pharmaceutically acceptable clays," ed: Google Patents, 1996.

[50] S. Aldrich. (2011. Available: http://www.sigmaaldrich.com/

[51] (2011, *Cole-Palmer*. Available: http://www.coleparmer.com/

[52] *Capillary Viscometer*. Available: http://www.google.com/imgres?imgurl=http://www.tamson.com/product/41_img_cat31_num0.jpg&imgrefurl=http://www.tamson.com/en/content/accessories/130/a/mid130&usg=__G4hBwNg3jal48ertD1Hlr3srp1A=&h=400&w=600&sz=10&hl=en&start=74&zoom=1&tbnid=qdJ-w_qUN60bKM:&tbnh=127&tbnw=172&ei=ssx2TZm4MMSx0QGk4MnfBg&prev=/images%3Fq%3Dcapillary%2Bviscometer%26um%3D1%26hl%3Den%26sa%3DN%26biw%3D1920%26bih%3D989%26tbs%3Disch:10,700&um=1&itbs=1&iact=rc&dur=495&oei=h8x2Tb_1JpKJrQH6ofm7CQ&page=2&ndsp=75&ved=1t:429,r:28,s:74&tx=95&ty=75&biw=1920&bih=989

[53] (2010, 12/03/2010). *Syrine Gun*. Available: http://www.dispensinglink.com/syringe_gun.htm

[54] (2010, 12/03/2010). *Aspiration Biopsy Syringe Gun*. Available: http://www.sthelens.com.cn/english/inrad-2.htm

[55] (2010, 12/03/2010). *Manual Syringe Gun*. Available: http://www.findtheneedle.co.uk/products/2388675-manual-syringe-gun.asp?tab=jobs

[56] A. K. Al-Hwiesh*, et al.*, "INTRAPERITONEAL PRESSURE AND INTRA-ABDOMINAL PRESSURE: ARE THEY THE SAME?," *Peritoneal Dialysis International,* p. pdi. 2010.00057 v1, 2011.

[57] 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

[58] J. Sieber, "Ethical considerations in planning and conducting research on human subjects," *Academic Medicine,* vol. 68, p. S9, 1993.

APPENDIX A

PRODUCT DESIGN SPECIFICATIONS May 1, 2011

Team Members: *Patrick Cassidy (Leader)*
Sean Heyrman (Communicator)
Alex Johnson (BWIG)
A.J. Sprangers (BSAC)
Advisor: *Dr. John Puccinelli*

A common technique to facilitate a percutaneous thermal ablation is hydrodissection. During hydrodissection, fluid is injected between the target ablation site and any surrounding tissues which require thermal protection. The hydrodissection fluid creates a physical, thermal and, in the case of non-ionic fluids, electrical barrier to protect such vulnerable tissues. Current fluids are relatively non-viscous, prone to migration in the abdominal cavity, and readily absorbed by the body. As a result, large fluid volumes are often required (~1 L) to create an effective barrier. Even with large volumes, the fluid barrier can degrade substantially during a procedure.

Previously, a 16.0% poloxamer 407 solution in DI water was formulated and tested to prevent fluid migration and barrier degradation while retaining the useful characteristics of currently used hydrodissection fluids. Poloxamer 407 could be injected as a fluid, then form a thermoreversible gel in vivo at body temperature. This does solve the main problem with other hydrodissection fluids; however, it also presents new problems to solve. The main issue with the current design is that it is too viscous of a fluid to readily inject through a 21 gauge needle. In addition, initial animal testing has shown that excessive motion between the ablation site and surrounding tissue can inhibit and even prevent gelation in vivo.

Client Requirements:

- The fluid must prevent migration of solution within the body cavity during hydrodissection and ablation.
- The fluid will be used in minimally invasive procedures and must be able to inject easily through a 21 gauge needle
- The designed fluid must retain the favorable characteristics of the current product:
    - 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 the 16.0% poloxamer solution is minimal, approximately ten dollars per unit.

Design Requirements:

1. Physical and Operational Characteristics

a. *Performance requirements*: The product must retain all favorable characteristics of current hydrodissection methods: biocompatibility, thermal and electrical insulation, fluid migration prevention, and reasonable cost. In addition, it must be easier to inject and form a stronger gel not prone to breakdown upon excessive motion between tissues.
b. *Safety*: Since the fluid is to be introduced into the body cavity, the final design must be non-toxic, biocompatible, bioabsorbable, and hypo-allergenic.
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 to three hours.
d. *Life in Service*: The product is to be used for hydrodissection during radiofrequency ablation lasting approximately one to three hours. Prior to treatment, the fluid will be stored in 250 mL IV bags at room temperature, though refrigeration would be ideal.
e. *Shelf Life*: The fluid will be packaged in 250 mL IV bags and should 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. It should also be isotonic to the peritoneal fluid.
g. *Ergonomics:* The final design must have a low enough viscosity to inject through a 21 gauge needle. The ability of the fluid to be introduced through a 21 gauge needle is imperative for successful, minimally invasive operations.
h. *Size:* A single effective treatment should require one 250 mL unit or less.
i. *Weight:* Weight requirements are not applicable to this product.
j. *Materials:* All the materials used in this design must meet the standards of the Food and Drug Administration (FDA) for class III medical devices, as it is designed for use on human subjects.
k. *Aesthetics, Appearance, and Finish:* Requirements for the design necessitate distinction between the fluid and the malignant tissue during procedural imaging.

2. Production Characteristics
    a. *Quantity:* A volume of 250 mL or less should be sufficient for treatment.
    b. *Target Product Cost:* Less than $200 per unit. Minimizing cost is essential to market success of the product.
3. Miscellaneous
    a. *Standards and Specifications:* The final product will require the approval of the FDA for class III medical devices for use in the human body.
    b. *Customer:* Prospective customers of this product would require it to produce effective hydrodissection, be ergonomically efficient, have a reasonable cost, and be biocompatible. The primary customers are medical personnel performing radiofrequency or cryoablation procedures. This product will be an alternative to current hydrodissection techniques during patient consults.
    c. *Patient-related concerns:* Patient safety is the primary concern; the prevention of non-targeted tissue damage is essential. Additionally, patient comfort should be maximized during and after treatment.
    d. *Competition:* Five percent dextrose in water (D5W) is the most commonly used hydrodissection fluid, and fulfills many requirements for an ideal hydrodissection fluid. Though it is only $2.50 per 250 mL unit, large volumes (> 1L) are often required to prevent ablation damage due to migration within the peritoneal cavity. Saline solutions have also been used in similar quantities; however, they conduct electricity and do not see as much use.

APPENDIX B

POLOXAMER FLUID SYNTHESIS - PROTOCOL

Purpose:

To develop a poloxamer solution that will effectively form a gel at body temperature, approximately 37°C. Because the gel becomes more viscous as temperature increases, a solution that gels between 32-34°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 filtered, deionized water and cool to 4°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. Determine the amount of additive, Ex. 5 w/w% poloxamer 188
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. Weigh out required amount of poloxamer. NOTE: 1 ml of water = 1 gram of water.

$$Mass\ of\ reageant = Concentration\ (\%\, w/w) * Volume\ of\ Liquid$$

6. Slowly pour the additive, then poloxamer into the deionized water.
7. Mix until all poloxamer is in solution; this could take up to 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.
8. Poloxamer solution can be stored at room temperature or 4°C (recommended).

APPENDIX C

OTHER PRODUCTS-SPACEOAR™

Only one other product with similar purpose to poloxamer 407 has been found. This product deemed SpaceOAR™ was created by Augmenix. It works in similar fashion to P407 in that you inject it in to the body cavity and it forms a gel. However, this gel is a PEG gel and requires crosslink agents to bind the PEG molecules together. This causes the injection to need two syringes, both being pushed through the same needle together. Another difference is the degradation time. While P407 is absorbed in about 2-3 days, SpaceOAR™ takes over 6 months to fully degrade. These qualities are both undesirable in a hydrodissection fluid as they are harder to inject properly and are in the body for a longer period of time. Also, this product currently is not accepted for use in the United States. For all of these reasons, this product should not harm P407's marketability in the United States.