A Thermoreversible Barrier for Hydrodissection during Ablation Procedures

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Abstract

Ablation is a relatively safe, effective, and minimally invasive procedure used to treat lesions in the liver, lungs, kidneys, and heart. To improve the safety of the procedure, hydrodissection is often used; an isotonic fluid (0.9% saline, 5% dextrose in water (D5W)) is injected between the ablation site and the surrounding healthy tissues to localize damage to the tumor. However, these fluids are prone to migration throughout the procedure, reducing their efficacy as a barrier to protect healthy tissue. Here, we investigate the potential of a thermoreversible poloxamer 407 solution to be used in place of these fluids. A 15.4 w/w% poloxamer solution would gel at 32°C. The kinematic viscosity of this solution was compared to D5W, and was found to be at least 11 times more viscous. The electrical impedance of the poloxamer solution was comparable to D5W. Both solutions were transparent when using ultrasound imaging, and could be differentiated from tissues during CT scanning. A 15.4 w/w% poloxamer solution preserves the favorable characteristics of current hydrodissection fluids. The formation of a gel between targeted and healthy tissues is expected to deter fluid migration and barrier degradation during ablation procedures. The use of this fluid stands to increase safety, reduce patient complications, and make ablation available to patients previously rejected because of tumor location.

Introduction

Ablation is a relatively non-invasive procedure frequently used to treat malignant tumors in the heart, kidney, lungs, and liver. In this procedure, a probe is inserted into the tumor using either ultrasound or CT scanning for guidance. To cause tissue necrosis, the probe is either heated with radio-frequency AC current, or cooled with liquid nitrogen (or argon) for RF and cyro-ablation, respectively [1][Callstrom, 2009]. This technique has yielded good patient results; successful resections are performed 85% of the time, and no tumor recurrence is seen in 52-67% of patients [2]. Due to the extreme temperatures, however, ablation probes have the possibility of damaging healthy tissues [3]. A procedure known as hydrodissection is used to prevent this [Brace, 2006].

During hydrodissection, fluid is injected between the target ablation site and the surrounding tissues to create a physical, thermal, and electrical barrier. Currently used liquids, like 5% dextrose in water (D5W) and saline have been relatively successful [4]. However, they lack the necessary viscosity and absorb quickly which results in unintended fluid migration and barrier degradation throughout the ablation procedure. Because of this, a large amount of liquid (> 1L) is typically required for adequate protection [4].

This study was conducted to develop a more viscous hydrodissection fluid while maintaining favorable characteristics of D5W and saline. Patient complications primarily arise when ablating tissue near extrahepatic organs, large vessels, or bile ducts [5-7]. A hydrodissection fluid capable of maintaining an adequate thermal and electrical barrier during ablation procedures stands to reduce the number of post-procedural patient complications. Additionally, this has the potential of making the ablation treatment available for hepatocellular carcinoma patients with previous contraindications due to tumor location. The primary goal was to increase untargeted tissue protection and hydrodissection efficacy by preventing fluid migration and barrier degradation.

This was accomplished with the development of a 15.4 w/w% poloxamer 407 (P407) solution. Poloxamers are polyethylene oxide- polypropylene oxide-polyethylene oxide (PEO-PPO-PEO) triblock copolymers and compose a group of nonionic surfactants [8]. This repeating triblock consists of two hydrophilic chains (PEO) flanking a hydrophobic (PEO) polymer core [8]. A unique characteristic of P407 is its ability to micellize in aqueous solution and form a thermoreversible gel. As the solution is heated, the hydrophobic PPO cores become dehydrated and clump together forming micelles. With continued temperature increase, more micelles form and the free hydrophilic PEO chains become entangled, forming an organized structure resulting in a transition from solution to gel. The temperature at which this transition occurs is termed the gelation temperature and is concentration dependent [8-10].

In this study the viscosity, impedance, cytotoxicity, and imaging ability of the poloxamer solution were evaluated and compared with D5W.

Methods

Gelation Testing

The gelation temperature was determined using a similar procedure to those previously reported by Gilbert et al. [11]. Poloxamer 407 was generously donated by BASF. Solutions were developed using the cold method: the poloxamer was slowly added to cold deionized water while mixing [12]. A water cycler 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 size 50 viscometer (Cannon-Fenske Cannon®) was used to measure D5W. Six milliliters of each solution was used for viscosity measurements (n = 3). The viscometer and fluid were placed in a water bath (Isotemp 1006S, Fisher Scientific) 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 kinetic viscosity constant multiplied by the elution time. Per manufacturer, the kinetic viscosity constant is 0.1 cSt/s and 0.004 cSt/s for the size 200 and size 50 viscometers, respectfully [13].

Impedance Testing

A Cool-Tip RF ablation machine (ValleyLab) was used to measure the impedance of the solutions tested. Two strips of aluminum tape were placed vertically on opposite sides of a 200 mL plastic beaker. To measure a blank, the cathode and the anode were placed in contact with each other. Approximately 40 mL of each solution (saline, D5W, P407) was placed within the container and the impedance was determined using the RF generator.

Imaging

Ultrasound imaging (SonixTOUCH) was used to qualitatively compare the transparency of poloxamer solution and D5W. Approximately 50 mL of each solution was placed into a flat bottomed container (solution height ~3 cm) and imaged with an ultrasound transducer.

Contrast measurements were taken using CT scan (750 HD, General Electric). Conical tubes (50 mL) were filled with either D5W or the poloxamer solution/gel. Iohexol (Omnipaque, General Electric) was added as a contrast agent to both D5W and the poloxamer solution. Image processing was performed using ImageJ. Images were taken of solutions with and without the addition of Iohexol.

Results

Gelation Testing

The gelation temperature was determined for several concentrations of poloxamer 407. Results are shown in Figure 1. These results indicate that a 15.4 w/w% poloxamer solution would gel at 32°C. This would allow the solution to be injected within the peritoneal cavity prior to an ablation procedure and form a gel between the target and adjacent tissues. With the formation of the gel, it's expected that the placement of the barrier will be maintained throughout the ablation procedure.

Viscosity

It was determined the poloxamer 407 had its lowest viscosity around 14°C. Initially the solution followed a typical fluid pattern with a decreasing viscosity as temperature increases; however, after 14°C the solution begins to micellize, causing an increase in viscosity. This property should allow the poloxamer to be injected as a liquid that becomes a viscous gel as it nears body temperature. To improve the ease of injection, the solution should be injected below 18°C; before micellization causes the viscosity increase.

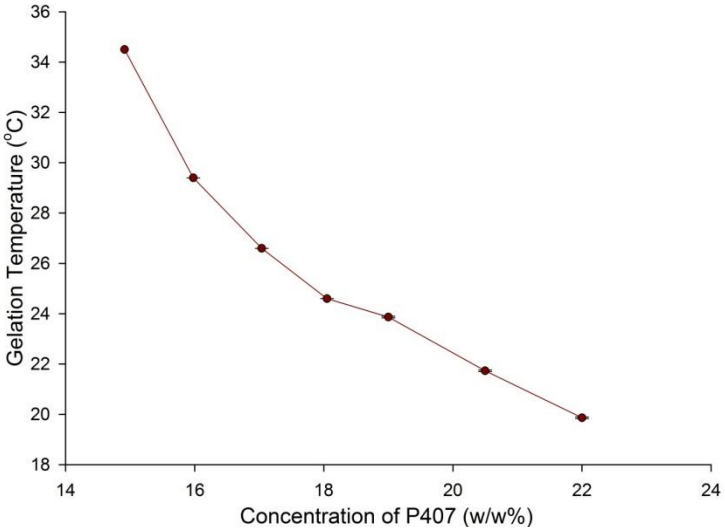

Figure 1 *Several concentrations of poloxamer 407 were formulated and 2 mL aliquots were prepared (n = 3) in 15 mL centrifuge tubes. The solution was considered gelled when the tube was rotated horizontally and the meniscus did not move. A correlation was developed between the concentration of poloxamer 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 the poloxamer solution to be injected as a liquid between then target and adjacent tissues where gelation would occur. The formation of a gel is expected to provide a barrier with greater integrity than D5W.*

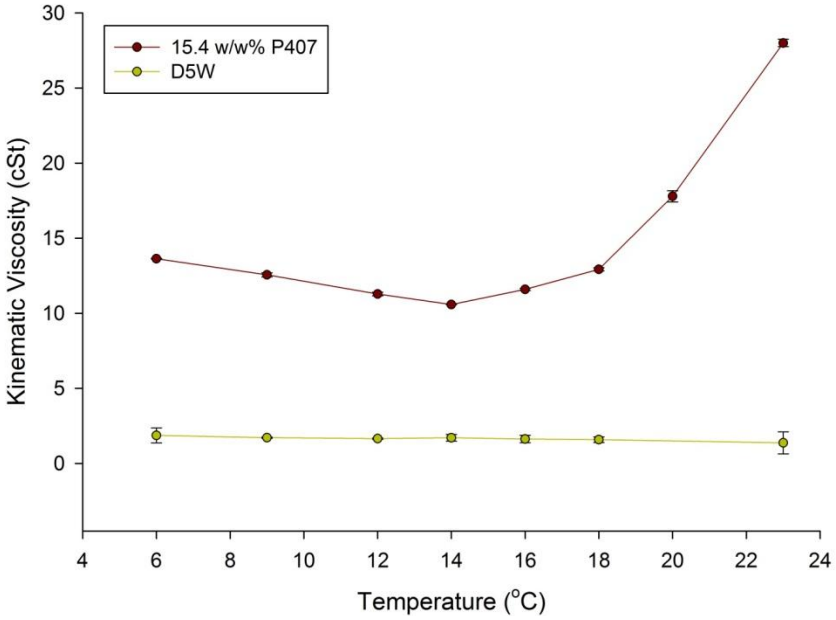

Figure 2 *Size 200 and size 50 viscometers were used to determine the elution times of poloxamer 407 and D5W, respectfully (n = 3). The viscometers were allowed to equilibrated for 15 minutes in a water bath (Isotemp 1006S, Fisher Scientific) at the specified temperatures. Elution times were measured using a stopwatch and kinematic viscosities were determined using the viscosity constant of each viscometer. The 15.4 w/w% P407 solution is more viscous than D5W.*

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

Table 1 *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 40mL of the solution was placed in the plastic beaker, and tested. Poloxamer had a very high impedance (over 1000 Ω), similar to D5W.*

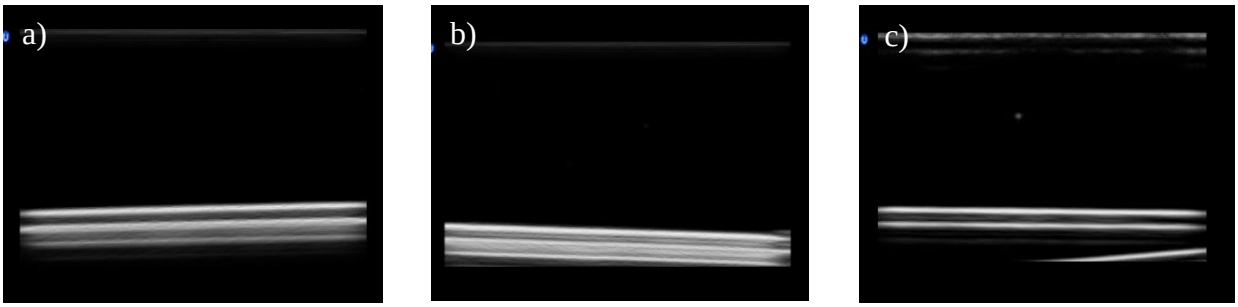

Figure 3 *Approximately 50mL of D5W (a), poloxamer solution (b), and poloxamer gel(c) was subjected to an Ultrasound System (SonixTOUCH). All of the images are quite clear, which will not inhibit ultrasound imaging during the ablation procedure.*

	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 2 *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

It was determined that the poloxamer 407 had similar impedance values to D5W as a solution and gel. The impedance registered on the RF ablation machine was larger than 1000 Ω for all three of these solutions. However, saline had an impedance of 88 Ω due to its ionic nature. These results suggest that poloxamer, both in a solution and gel, would act like an electrical insulator and protect surrounding tissue from damage during ablation procedures.

Imaging

Ultrasound imaging was examined first to compare the poloxamer solution, gel, and D5W. The poloxamer solution appeared comparable with D5W when the transparency of the solutions was qualitatively evaluated; however, once gelled, the poloxamer had an echo on the top of the gel, possibly resulting from air between the transducer and the gel surface. The images suggest that poloxamer solution will not prevent the use of ultrasound imaging during the ablation procedure.

Next, the contrast of the three solutions was evaluated using computed tomography (CT). Initially, all three of the samples had very low ROIs (region of interest), a measure of contrast in a specific area on a CT scan. After the addition of Iohexol, the ROIs increased; this indicates the use of the poloxamer solution will not inhibit the ability to differentiate between tissues and the solution during the ablation procedure.

Discussion

This study was conducted to develop a more viscous hydrodissection fluid to be used during ablation procedures. Currently used solutions tend to migrate throughout the peritoneal cavity resulting in barrier degradation and unwanted tissue necrosis. It was shown that a 15.4% poloxamer solution would gel at 32°C, just below body temperature (37°C). This would allow the hydrodissection fluid to be injected between the tumor and adjacent tissues with currently used hydrodissection techniques. Once placed between the tissues, transition would occur from solution to gel resulting in a stable, immobile barrier to act as an electrical and thermal insulator throughout the ablation procedure.

P407 was primarily chosen for this design because of its ability to form a thermoreversible gel. Additionally, with a molecular weight of less than 13 kDa, poloxamers are considered bioabsorbable [10]. Poloxamers are less attractive as biomaterials because of their low mechanical strength and rapid erosion rates [10]. These properties may expedite the removal process and minimize material residue. The low mechanical strength is not a concern since the patient is relatively immobile throughout the procedure. Poloxamers also have a gel melting temperature where the poloxamer begins to precipitate out of solution [10]. This is not of concern since the only edge closest to the ablation site will be affected, resulting in a fluid comparable to current hydrodissection fluids such as 5% dextrose in water (D5W).

It was shown that the viscosity of the poloxamer solution is significantly greater than that of the current standard, D5W. Additionally, as the temperature increases above 14°C, it was shown that the viscosity of the solution exponentially increases. These results are comparable to those reported by Cabana, A et al.,

Edsman, K. et al., and Ricci, E.J. et al. [14-16]. The high viscosity of the poloxamer solution necessitates that it be cooled prior to and throughout hydrodissection. The transient barrier provided by D5W is available immediately after hydrodissection, the ensuing degradation of the protective barrier happens over a period of time. With initial placement, it is expected the heat transfer to the poloxamer solution will occur fast enough to quickly gel when/where needed. The concentration of poloxamer 407 in solution could further be optimized for in vivo application to reduce gelation time and increase injectability during hydrodissection.

The impedance of the poloxamer solution is unaffected by gelation and is comparable to D5W, which has been shown to reduce unwanted tissue damage during RF ablations P. Laeseke, et al. [4]. The results reported here suggest the developed solution will provide an adequate barrier to reduce the effects of current-generated tissue death. Brace, et al. have previously shown that a 9 mm barrier of D5W is able to provide adequate thermal protection during ablation [17]. However, to achieve this barrier, large amounts of the hydrodissection fluid (> 1L) are often required [4]. The formation of the gel between the tissues, and the high specific heat of water, suggests the developed poloxamer solution should provide similar thermal insulation during ablation to D5W. It's expected that less than 100 mL of fluid would be required for proper hydrodissection.

A qualitative analysis of images from ultrasound and CT scans comparing D5W and poloxamer shows similar behavior. There is a slight echo in the ultrasound from the gelled poloxamer solution; however, it is expected this effect will not be present when used in vivo. Results suggest that, with the addition of a contrast agent, Iohexol, the poloxamer solution will be distinguishable from nearby tissues during a CT scan. The effects of Iohexol on solution/gel properties (i.e. change in gelation temperature, fluid viscosity) has not been examined in this study; however, the ability to gel remained unaffected by its addition.

To verify the effectiveness of the poloxamer solution for use in hydrodissection, evaluation in vivo is to be conducted using methods similar to those reported by Laekse et al. to compare protection provided by D5W and 15.4 w/w% P407 during ablation.

Conclusion

Here it has been shown that a 15.4 w/w% poloxamer solution incorporates the favorable characteristics of D5W for use as a hydrodissection fluid. The increase in solution viscosity compared to D5W would prevent fluid migration and barrier degradation during ablation procedures. This would reduce unwanted tissue damage and make ablation available to some of those previously rejected because of tumor location. With the high viscosity of the solution near room temperature (~22°C), the solution would have to be cooled (below 18°C) prior to and during hydrodissection. The formation of a gel as the solution warms to body temperature should prevent barrier degradation seen when using D5W, and thus improve the performance, efficacy, and safety of hydrodissection and ablation procedures

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