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Design and Validation of a Thermoreversible Material for Percutaneous Tissue Hydrodissection

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Abstract

Interventional oncology procedures such as thermal ablation are becoming routine for many cancers. Hydrodissection— separating tissues with fluids—protects tissues near the treatment zone to improve ablation's safety and facilitate more aggressive treatments. However, currently used fluids such as normal saline and 5% dextrose in water (D5W) migrate in the peritoneum, reducing their protective efficacy. As a hydrodissection alternative, we investigated a thermoreversible poloxamer 407 (P407) solution. Such a material can be injected as a liquid which then forms a semi-solid gel at body temperature without syneresis. The desired gelation temperature of 32°C was achieved with 15.4 wt/wt % P407. Viscosity analysis revealed the lowest viscosity and ideal injection point was at 14°C. Solution viscosity increased during gelation, to a peak of 65 kPa*s at 40°C. The electrical impedance of P407 was significantly greater than isotonic saline, but lower than D5W, indicating its potential for electrical protection. The P407 gel was similar to other hydrodissection fluids at ultrasound and CT imaging. *Ex vivo* liver ablations showed that P407 protects neighboring tissues, but may require a thicker barrier for comparable protection to D5W. Overall, we found that the P407 solution is a feasible alternative to traditional hydrodissection fluids and warrants additional study.

Keywords

ablation; poloxamer 407; hydrodissection; D5W; thermoreversible

INTRODUCTION

Thermal ablation is a minimally invasive procedure frequently used to treat tumors in the liver, kidneys, lungs, and bone.^{1–7} During a thermal ablation procedure, one or more energy applicators are inserted into the tumor, typically using medical imaging, such as ultrasound or computed tomography (CT), for guidance. The applicator alters the surrounding tissue temperature either by internal cooling (cryoablation) or by heating with energy such as radiofrequency (RF) electrical current, microwaves (MWs), laser light, or ultrasound

waves.^{4,8–10} Non-thermal techniques such as irreversible electroporation are also under investigation.¹¹ The choice of energy depends on the institution, tumor type and location, and available equipment. To date, the greatest clinical experiences worldwide have utilized radiofrequency, MW, and cryoablation.^{4,8,9,12,13}

Several studies have demonstrated that thermal ablation can provide nearly equivalent survival rates when compared with surgical resection, but with reduced procedural mortality, morbidity and complications, and faster recovery.^{3,5,12,14} These results are promising; however, local recurrence rates have been somewhat higher for thermal ablation.^{5,15} Inadequate coverage of the entire tumor with an ablative margin of at least 5 mm has been identified as a significant predictor of local recurrence. Therefore, newer systems and approaches aim to increase the size of the ablation zone achievable in one treatment session, and many tumors are treated aggressively to ensure an adequate treatment margin.

One possible side effect of more aggressive treatment is thermal damage to healthy tissues peripheral to the treatment zone such as muscles of the body wall, diaphragm, stomach, pancreas, bowel, colon, nerves, ureter, and bladder.^{6,7,16,17} Damage to these tissues can cause minor complications such as pain and breathing difficulties to major complications such as bowel perforation and death.^{6,7,17}

Hydrodissection is the most widely utilized adjunctive technique to lower the risk of such complications.⁶ During hydrodissection, fluid is injected between the treatment zone and susceptible tissues to provide a physical, thermal, and potential electrical barrier to protect the susceptible tissues. Solutions such as 5% dextrose in water (D5W) and normal saline have been effective at reducing complications from peripheral thermal damage.^{6,7,16–18} However, these liquids are highly mobile, can migrate into the peritoneal space and can be absorbed quickly, potentially degrading the barrier during the ablation procedure. As a result, a relatively large amount of liquid (1–3 L) or continuous infusion is often necessary to ensure adequate protection which can result in patient discomfort following the procedure.^{6,17–23}

We evaluated the feasibility of an alternative to conventional liquids: an aqueous poloxamer 407 (P407) hydrodissection material. Poloxamers are polyethylene oxidepolypropylene oxide-polyethylene oxide (PEO-PPO-PEO) triblock copolymers that traditionally serve as nonionic surfactants.^{24,25} A unique characteristic of P407 is its ability to form a thermoreversible gel without syneresis.²⁶ As the liquid solution is heated, the hydrophobic PPO cores become dehydrated and clump together, forming micelles. With continued temperature elevation, more micelles form and the free hydrophilic PEO chains become entangled, leading to an organized, semi-solid gel structure. The temperature at which this transition occurs is termed the gelation (or sol–gel) temperature and is concentration dependent.^{24,27–29} Therefore, a P407 solution may be injected *in vivo* at or below room temperature and gel at body temperature. The objective of our study was to use an engineering design approach to create an optimized aqueous P407 solution for tissue hydrodissection, and compare its use and efficacy in a controlled *ex vivo* environment to normal saline and D5W during thermal ablation. This proof-of-concept study will be used to guide later preclinical and clinical evaluation studies.

MATERIALS AND METHODS

Concentration optimization

The target sol–gel temperature was determined to be 32°C to allow rapid gelation at a core body temperature of 34–37°C but a low-viscosity liquid at room temperature (~20°C). The sol–gel temperature was evaluated using the procedures reported by Gilbert et al.³⁰ Serial dilutions of 10–25 wt/wt % P407 (molecular weight of 12,500 Da, Sigma Aldrich, St. Louis, MO) in water were prepared using the “cold method” at 10°C described by Schmolka.³¹ This approach was necessary for higher P407 concentration solutions (greater than 20 wt/wt %) as their gelation temperatures were lower than room temperature; however, lower P407 concentration solutions (less than 20 wt/wt %) can be synthesized at room temperature. Two milliliter aliquots of each dilution were placed into 15 mL centrifuge tubes ($n = 3$ for each dilution). Tubes were placed into a controlled water cycler along with a 3 mL control sample of deionized water. Water temperature was increased incrementally at intervals of 0.1–2.0°C. Material phase was evaluated by tilting the sample container; gelled samples were characterized by a lack of meniscus movement.³⁰ Sol–gel transition temperatures were plotted against P407 concentration and the optimal concentration for a 32°C sol–gel transition temperature was interpolated.

Viscosity testing

Viscosities of D5W and 15.4 wt/wt % P407 were measured from 5 to 65°C in 5°C increments using a rheometer at shear rates from 0.001 rad/s to 1.0 rad/s (TA Instruments, ARES-LS2, New Castle, DE). Samples were evaluated using a 25 mm parallel-plate configuration with 1–2 mm of fluid between the plates. Temperature in the environmental chamber was allowed to equilibrate prior to starting each trial and never deviated more than 0.5°C during each trial. For tests under room temperature, the environmental chamber of the rheometer was cooled using liquid nitrogen.

Viscosities at temperatures less than 25°C were below the dynamic range of the rheometer, so kinematic viscosity was also evaluated from 6 to 24°C using glass capillary viscometers (Cannon-Fenske, State College, PA). Six milliliter aliquots of each solution ($n = 3$ each) were equilibrated for 15 min using a controlled water bath (Isotemp 1006S, Fisher Scientific) and then injected into the viscometer. A size 50 viscometer was used for the D5W while a size 200 viscometer was used for the 15.4 wt/wt % P407. Kinematic viscosity was calculated as the product of the efflux time and kinetic viscosity constant (0.1 cSt/s for size 200, 0.004 cSt/s for size 50). Dynamic viscosity was then calculated by multiplying the kinematic viscosity by the solution's density, where density was determined using a graduated cylinder and an analytic scale. The P407 solution was also injected through 19-gauge needles to determine the effect of viscosity on injectability.

Electrical impedance testing

To evaluate the ability of the gel material to provide electrical protection during RF ablations, we measured the relative impedances of normal saline, D5W, and 15.4 wt/wt % P407 barriers. Approximately, 1.5 mL of each solution (saline, D5W, 15.4 wt/wt % P407) was placed within 1.5 mL sample tubes containing two diametrically opposed electrodes

(5mm × 20 mm). To create a lumped impedance, a 1000-Ω resistor was added in parallel with the fluid such that the total lumped impedance, Z_{Total} , was:

$$1/Z_{\text{Total}} = 1/1000\ \Omega + 1/Z_{\text{Fluid}}$$

and the impedance of the fluid, Z_{Fluid} , could be calculated as:

$$Z_{\text{Fluid}} = (1000\ \Omega \times Z_{\text{Total}}) / (1000\ \Omega - Z_{\text{Total}}).$$

A thermocouple was centered in the tube to monitor temperature. The tubes were then heated in a controlled water bath (VWR International, 89032-216, Radnor, PA) to achieve temperatures of 25–78°C. The total circuit impedance (0–1000 Ω) was measured using a 500 kHz Radiofrequency (RF) ablation generator (CC-1, Radionics, Mansfield, MA) with a dynamic range of 0–1000 Ω and a precision of 1 Ω.

Imaging

Ultrasound imaging was used to compare the 15.4 wt/wt % P407 liquid, P407 gel, normal saline, and D5W. Approximately 50 mL of each solution was placed into a flat container (solution height ~3 cm) and imaged with a 14.5 MHz ultrasound transducer (Siemens S2000, Malvern, PA). Images were qualitatively inspected to identify any significant scattering, attenuation, or other artifacts.

Similarly, 100 mL of each solution was evaluated using CT (750 HD, General Electric, Waukesha, WI) at 120 kVp and 200 mA. In addition, one sample each of D5W, saline and two samples of P407 were each infused with 2 vol/vol % iohexol (Omnipaque 300 mg/mL, General Electric, Waukesha, WI) prior to CT imaging. One of the P407 samples with iohexol was left as a liquid at room temperature while the other P407 sample with iohexol was gelled in a 40°C water bath prior to imaging. Following CT acquisition, mean pixel intensities and standard deviations from a square region of interest (ROI) in the center of the sample containers were compared between materials.

Ex vivo ablation testing

The ability to protect neighboring tissues during RF and MW ablation was evaluated using the setup depicted in Figure 1(a). Briefly, two bovine liver pieces (equilibrated to 37°C using a waterbath) were separated by a known hydrodissection fluid thickness, d , which was maintained by two plastic gates with fiberglass mesh-covered openings [Figure 1(b)]. Hydrodissection barriers of 5, 8, and 11 mm were evaluated (approximately 30 mL, 50 mL, and 70 mL of fluid, respectively) commensurate with previous laboratory and clinical studies.^{6,7,17,18} The ablation applicator was placed vertically 4.5 cm into the liver tissue, 1 cm from the fluid barrier. For RF ablations, a 3 cm water-cooled electrode (Covidien, Mansfield, MA) delivered a maximum power of 200 W from the RF ablation generator using internal impedance control for 10 min. For MW ablations, a gas-cooled triaxial antenna delivered 90 W from a 2.45 GHz generator (NeuWave Medical, Madison, WI) for 5 min. These settings were used to produce similar ablation zone sizes between RF and MW

ablations. Temperatures were monitored 3.5 cm deep at four locations [see Figure 1(a)] every 1 s using fiberoptic temperature probes (Neoptix T1, Toronto, Canada). Tissue protection was determined by comparing the total temperature elevation at probe 3, and by comparing the differential temperature elevation between probes 2 and 3. Temperature data were plotted over time and compared between groups using unpaired, two-tailed Student's *t*-tests.

RESULTS

Gelation testing

The relationship between gelation temperature and P407 concentration was approximately exponential; as concentration increased, gelation temperature decreased (Figure 2). This relationship agrees almost identically with previously published data.²⁴ By linear interpolation, the necessary P407 concentration for a gelation temperature of 32°C was 15.4 wt/wt % (Figure 3). Hereafter, P407 refers to this specific concentration (15.4 wt/wt %) unless otherwise noted.

Viscosity

Dynamic viscosity was determined directly from low shear rate rheometer measurements [Figure 4(a)]. As micelles begin to form upon heating, viscosity increased steeply to a maximum of 65 kPa*s at 40°C where micelle entanglement is maximal and gel formation is most complete. As temperature continues to increase, gel viscosity decreases until the gel became a viscous liquid at 55°C. Upon further heating the minimum viscosity of 36 kPa*s was seen at 60°C.

Dynamic viscosities of D5W and P407 using the glass viscometers are shown in Figure 4(b). The viscosity of D5W was relatively constant in this temperature range, with a dynamic value of 2 mPa*s at 6°C. By contrast, the P407 solution viscosity was nonlinear with temperature. From 6 to 14°C the solution behaved linearly with a peak dynamic viscosity of 14 mPa*s at 6°C. However, above 14°C the viscosity increased exponentially as the solution began to micellize. The viscosity exceeded the viscometer's dynamic range above 24°C.

Impedance

Overall, the electrical impedance of the P407 solution at 500 kHz was less than D5W but greater than saline (Figure 5). At 26°C, the impedances of D5W, P407, and normal saline were 5369 Ω , 1740 Ω , and 54 Ω , with linear temperature coefficients of $-32.55 \text{ } \Omega/^{\circ}\text{C}$, $-18.05 \text{ } \Omega/^{\circ}\text{C}$, and $-0.22 \text{ } \Omega/^{\circ}\text{C}$, respectively. No inflection in the impedance was noted during the sol-gel transition, suggesting that P407's electrical properties are unaffected by the phase change.

Imaging

On ultrasound imaging, all solutions appeared qualitatively similar with no notable backscatter or attenuation [Figure 6]. On CT, all solutions were relatively isodense at 17.6 ± 4.47 Hounsfield unit (HU), 15.76 ± 5.38 HU, 16.47 ± 3.92 and 6.3 ± 5.01 HU for D5W, saline, P407 liquid, and P407 gel, respectively. The approximate 10 HU difference in ROI

values for the P407 gel (at 40°C) and the P407 liquid (at 20°C) was due primarily to thermal expansion in the warmer gel material; however, this slight difference is practically insignificant and is nearly indiscernible in a clinical CT acquisition due to background noise. The addition of 2 vol/vol % iohexol into the P407 liquid solution did not affect gelation, but did increase its relative intensity on CT to 189.32 ± 4.87 HU. The enhanced CT intensity due to iohexol addition is desirable to visualize the hydrodissection barrier continuously throughout the ablation procedure. As iohexol is water soluble, it diffuses and absorbs along with the hydrodissection material—whether D5W or P407. The iohexol addition also resulted in more similar ROI values between the P407 liquid and gel states (Table I).

Ex vivo RF ablation testing

Analysis of temperatures measured during RF ablations in the *ex vivo* phantom setup demonstrated complex interactions between thermal conduction, free convection, and heat generation in each hydrodissection material. With 5 mm barriers, mean temperature elevations at probe 1 were not statistically different at $(52.5 \pm 7.1)^\circ\text{C}$ and $(46.5 \pm 16.7)^\circ\text{C}$ for D5W and P407, respectively ($p > 0.58$). Mean temperature elevations at probe 3 were statistically significantly different at $(5.7 \pm 1.7)^\circ\text{C}$ and $(19.7 \pm 4.8)^\circ\text{C}$ for D5W and P407, respectively ($p < 0.001$) (Figure 7, and Table II). Thus a 5 mm thickness of P407 does thermally protect the neighboring liver tissue (temperature elevation at probe 3 was less than at probe 1), but not as well as the same thickness of D5W. The temperature differentials between probes 2 and 3 were $(3.2 \pm 2.9)^\circ\text{C}$ and $(3.3 \pm 2.2)^\circ\text{C}$, respectively, and were not statistically different. A D5W barrier of 5 mm was shown to be thermally protective since the temperature elevation at probe 3 did not increase greater than 10°C . An elevation of more than 10°C is associated with some amount of tissue damage and cellular necrosis when maintained for over several of minutes.^{32–35} As a result, additional D5W tests with greater thicknesses were inessential and were not performed. However, increasing the P407 barrier thickness was performed to evaluate what thickness would be comparable to 5 mm of D5W. P407 thicknesses of 8 mm and 11 mm yielded more comparable results to the 5 mm D5W thickness (Figure 8). In particular, the average temperature increase at probe 3 for 11 mm of P407 was $(8.3 \pm 1.1)^\circ\text{C}$. Also, the temperature differential between probes 2 and 3 increased with increasing P407 thickness: $(3.3 \pm 2.2)^\circ\text{C}$, $(11.7 \pm 3.7)^\circ\text{C}$, and $(22.4 \pm 7.3)^\circ\text{C}$ for 5 mm, 8 mm, and 11 mm of P407, respectively. A summary of results can be seen in Table II. From these tests it was determined that an 11 mm P407 barrier would adequately protect neighboring tissues during RF ablation since the temperature elevation at probe 3 was under 10 degrees. It should be noted that the temperature elevation at probe 1 for all P407 conditions was not statistically significant from the temperature elevation at probe 1 for 5 mm of D5W ($p > 0.1$).

Ex vivo microwave ablation testing

Analysis of temperatures measured during MW ablations in the *ex vivo* set up demonstrated similar relationships of heat dissipation to those seen in the RF ablation tests. Plots showing increase in temperature versus time can be seen in Figure 9. With a 5 mm barrier, temperature elevations at probe 3 were $(5.0 \pm 3.0)^\circ\text{C}$ and $(14.5 \pm 5.3)^\circ\text{C}$ for D5W and P407, respectively, and were statistically significantly different ($p < 0.01$). The temperature differentials between probes 2 and 3 were not statistically significant and were $(5.4 \pm 1.9)^\circ\text{C}$

and $(10.0 \pm 9.2)^\circ\text{C}$, respectively. Increasing the P407 distance to 8 mm protected much better than 5 mm of P407 and was statistically comparable to a 5 mm barrier of D5W. Here, probe 3 experienced an increase of only $(5.0 \pm 3.1)^\circ\text{C}$ and the temperature differential between probes 2 and 3 was $(13.3 \pm 6.0)^\circ\text{C}$. A summary of results can be seen in Table II. From these tests, it was determined that an 8 mm P407 barrier would provide adequate protection during MW ablations. It should be noted that the temperature elevation at probe 1 for all P407 conditions was not statistically significant from the temperature elevation at probe 1 for 5 mm of D5W ($p > 0.9$).

DISCUSSION

P407 was chosen for this application primarily because of its ability to be injected as a liquid and transition into a more mechanically stable gel inside the body. Currently used solutions tend to migrate throughout the peritoneal cavity resulting in barrier degradation and potential damage to adjacent vulnerable structures. With a molecular weight of less than 13 kDa, poloxamers are considered bioabsorbable and are excreted within days after injection.^{27,36} In the work by Pec et al., P407 excretion from rats was examined following intraperitoneum injection of a 33 wt/wt % P407 solution (1.7 g P407/kg body weight).³⁶ The P407 gel was found to have a half-life of approximately 21 h and was fully excreted around 96 h post-injection.³⁶ Since our P407 concentration (15.4 wt/wt %) is approximately half that described in Pec et al., it is hypothesized that excretion of our material would be more rapid; however, the dissolution and excretion rates likely depend on physiological properties such as heart rate, glomerular filtration rate, and so forth. Additionally, we chose P407 over other poloxamers because it is arguably the most studied poloxamer formulation, and a lower concentration of P407 is needed for a given gelation temperature than other poloxamer formulations.^{24,37,38} P407 is already currently used in many pharmaceutical and cosmetic applications due to its low toxicity and weak immunogenic properties.^{25,28} Also, P407 lacks any inherent myotoxicity following single or multiple intramuscular injections; in previous works toxicity was comparable to that of saline or peanut oil.³⁹ Furthermore, P407 is also well tolerated when administered via daily IP injections (for four days) to rats at a concentration of 0.33 g/kg.^{39,40} Lastly, P407 is classified as non-hazardous by the Occupational Safety and Health Administration (OSHA).⁴¹

The target gelation temperature for this application was set to be 32°C as this would allow for gelation to occur under body temperature (37°C) while also minimizing the amount of poloxamer in solution and the temperature differential between body and gelation temperatures. Lowering the amount of P407 in solution will decrease the fluid's viscosity, making it easier to inject; minimizing the temperature differential between body and gelation temperatures will minimize the time necessary for gelation. A 15.4 wt/wt % P407 solution was found to gel at the target temperature (32°C), allowing the solution to be injected before gelling *in situ*. The gel was found to be compatible with ultrasound and CT imaging techniques used for treatment guidance and monitoring. The P407 solution was also noted to be a better electrical insulator than normal saline, but slightly less insulating than D5W. Thermal protection afforded by the P407 gel was slightly less effective than D5W for thin barriers while increasing the P407 barrier thickness resulted in comparable protection. Additionally, the gel was also more immobile than a D5W barrier.

Viscosity testing revealed that the ideal temperature for P407 injection (that with the lowest viscosity) is approximately 14°C, or just below room temperature. Below 14°C the fluid behaves like a normal Newtonian fluid, linearly decreasing viscosity as temperature increases. Above 14°C, the P407 viscosity increased exponentially; these observations agree well with data in the literature.⁴² This drastic viscosity increase when approaching the sol–gel temperature is due to the poor solubility of the hydrophobic PPO polymer cores as solution temperature increases; the resulting micelle formation minimizes the amount of free energy in solution.⁴² As a liquid, the P407 solution had a greater viscosity than D5W, owing primarily to the fact that the P407 solution contains polymers while D5W only contains glucose monomers in solution. Additionally, the P407 has a greater concentration of solute (15.4 wt/wt % for P407 vs 5 wt/wt % for D5W). Despite the high viscosity of the P407 solution, it was still injectable through a 19-gauge needle (at fridge and room temperatures) and created a gel almost immediately after injection between *ex vivo* tissues at body temperature; similar results would be anticipated *in vivo*. Previous studies have also shown 17–19 wt/wt % P407 solutions to be injectable through smaller 23–27 gauge needles.⁴³

While the impedance of D5W was the most affected by temperature changes (highest $\Omega/^\circ\text{C}$ slope), its initial high impedance value at 26°C easily offsets these effects; this can be seen by an impedance drop of 25% over the studied temperature range (48°C). The P407's impedance was also greatly dependent on temperature change and experienced a 52% decrease over the same temperature range. Thus, P407 was shown to be more electrically protective than normal saline, but less than D5W. As electrical impedance decreases with temperature, it is expected that greater RF current could flow through the P407 barrier than a similar D5W barrier. This effect may explain the reduced protection in P407 barriers compared to D5W barriers of equivalent thickness. Therefore, a thicker (11 mm) P407 barrier may need to be employed during RF ablations. Even though the temperature elevation at probe 3 for 11 mm of P407 was statistically different than that of 5 mm of D5W, 11 mm of P407 will be adequate since an 8.3°C increase in temperature would be well tolerated by neighboring tissues *in vivo*.^{32,35} Additionally, since the P407 barrier is mechanically more stable than a liquid, it is anticipated that such a barrier could be created and maintained with less material than necessary when using D5W or normal saline.

During MW ablation, the P407 gel did not protect as well as D5W for a barrier thickness of 5 mm, but an 8 mm thickness of P407 was statistically comparable. Additionally, increasing the P407 distance did increase the temperature differential between probes 2 and 3 as expected; however, this was just above being statistically significant ($p = 0.07$). We note that, while electrical current flowing through the hydrodissection barrier can cause heating for RF ablation, the mechanism for MW heating is similar between P407 and D5W since the MWs mainly interact with water molecules. This suggests that thermal property differences between the liquid D5W and gelled P407 may be more important contributors to the differences in performance for equivalent hydrodissection distances.

Free convection of heated liquid (D5W or normal saline) appears to play a significant role in thermal dissipation. In D5W, heat was freely convected away from the location where it entered the hydrodissection barrier (probe 2) and is redistributed throughout the entire volume of the hydrodissection fluid, whereas little to no convective heat dissipation was

present in the P407 gel. This would help explain why D5W's probe 2 temperature was lower than all P407 probe 2 temperatures even when there was a larger P407 barrier. The difference in effect can be directly seen by comparing 5 mm of P407 to 5 mm of D5W during MW ablation. In this experiment, the 5 mm of P407 and D5W exhibited an increase in temperature of $(14.5 \pm 5.3)^{\circ}\text{C}$ and $(5.0 \pm 3.0)^{\circ}\text{C}$ at probe 3, respectively, and a temperature differential between probes 2 and 3 of $(10.0 \pm 9.2)^{\circ}\text{C}$ and $(5.4 \pm 1.9)^{\circ}\text{C}$, respectively. This results in more of the energy being used to heat up the D5W volume rather than the linear transfer of heat through the fluid, resulting in a smaller temperature differential between probes 2 and 3. In contradistinction, as the P407 is in the gel state during these tests, there is no convective heat dissipation, only radial, conductive heat dissipation from the ablation probe through the gel. As the shortest radius exists from the ablation probe to probe 2 and then to probe 3, both probes 2 and 3 experience a greater temperature increase. So even though more heat gets to probe 3 in the P407 barrier, the P407 barrier is most effective at insulating (due to the higher probe 2 and 3 temperature differential). This may also be due to the possibility that the P407 gel may have a lower thermal diffusivity than water. This would result in an insulating effect as the heat dissipation would occur slower in the gel. The varying modes of heat transfer by free convection or conduction, in addition to P407 having a lower thermal diffusivity would help explain the differences between D5W and P407 in both RF and MW experiments, especially the differences seen at probe 2 and the temperature differential between probes 2 and 3. More detailed experimentation and analysis of thermal protection in hydrodissection fluids is beyond the scope of this study, but is ongoing. Additionally, the possibility of combining separate P407 and D5W barriers is being examined and might be able to capture advantages of both materials for hydrodissection.

Additional limitations of this study include that the setup of the *ex vivo* ablation experiments likely exacerbated the differences between fluids, particularly during RF ablation experiments. The ablation applicator was placed only 1 cm away from the edge of the liver tissue, which is relatively close compared to a typical clinical procedure. Therefore, while the overall trends may hold, the absolute temperature elevations in the neighboring tissues were likely exaggerated in this study. Additionally, in the RF ablation experiments there are only two electrical current paths, essentially forcing current to pass through the hydrodissection barrier. In a clinical setting, the current paths are more numerous and diffuse, and would dissipate more of the electrical current outside of the high-impedance hydrodissection barrier. Lastly, the overall setup was not within a closed system. However, the effect of free convection from the entire set up is likely small compared to the internal heat transfer within the hydrodissection barrier.

Previous studies suggested that a 5–9 mm barrier of D5W was able to provide adequate protection during ablation.^{6,7,18} However, to achieve such a barrier, potentially large amounts of D5W (1–3 L) may be required clinically.^{6,17,20–22} Additionally, due to the low viscosity of D5W these barriers displace rather immediately, particularly in renal ablations and may require continuous D5W infusion to maintain the barrier's integrity.²³ During initial clinical trials of D5W hydrodissection, Hinshaw et al. report that an average of 963 mL of D5W (range: 350–1500 mL) was necessary to create an average 8.5 mm

hydrodissection barrier (range: 3–17 mm).⁶ In another clinical study, Kondo et al. used an average D5W volume of 681 mL (range 250–3000 mL) to achieve separation of the liver tumors from the gastrointestinal tract (here a separation distance was not specified).²² Lastly, it has been shown that D5W hydrodissection can displace the tumor away from one critical structure but may move it toward other critical structures.²³ Maintaining D5W barriers in multiple locations, if feasible, would likely prolong procedure time and increase postoperative discomfort for the patient.

Based on our studies, we estimate that a single injection of less than 100 mL of P407 would be required to achieve a 5–10 mm barrier thickness. As such a small amount is being injection, and there would be no need for continual infusion, we believe that P407 hydrodissection may help expedite the hydrodissection and ablation procedures. A basic estimate of material costs also suggests that 100 mL of P407 would be ~\$8 (Sigma Aldrich), which compares favorably to ~\$3 for 1000 mL of D5W (Hospira, Lake Forest, IL).

CONCLUSION

A 15.4 wt/wt % P407 solution was evaluated as a hydrodissection barrier for thermal ablation procedures. The liquid solution was injectable through a 19-gauge needle and formed a mechanically stable gel above 32°C. *Ex vivo* testing revealed that while the P407 gel thermally protected neighboring tissues, thicker barriers were required compared to D5W. Free convection in fluids, such as D5W, appears to play a significant role in thermal dissipation and, likewise, the protection of neighboring tissues. However, as the P407 solution is more viscous and forms a gel above 32°C it may prevent barrier degradation associated with fluids like D5W. Additional study in an *in vivo* model appears warranted.

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FIGURE 1.

(a) Setup for *ex vivo* ablation experiments in a plastic container. Two bovine liver pieces are held at a constant distance, d , by two plastic gates. This separation enables the establishment of a hydrodissection fluid barrier. The ablation probe and temperature probes are separated by a distance, l , which is always 1 cm. (b) Plastic gate construction. Screen mesh was drilled over an opening on a piece of plastic using plastic screws. The mesh covered opening allows for direct heat transfer from the neighboring liver pieces to the hydrodissection fluid while maintaining a constant hydrodissection distance. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 2.

Several concentrations of P407 were formulated and 2 mL aliquots were prepared (AVE $\pm$ SD, $n = 3$) in 15 mL centrifuge tubes. The solution was considered gelled when the tube was rotated horizontally and the meniscus did not move. A relationship was developed between the concentration of P407 (wt/wt %) and the gelation temperature ($^{\circ}$ C). Linear interpolation was used to determine that a 15.4 wt/wt % solution of P407 would gel at 32° C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 3.

(a) About 5 mL of 15.4 wt/wt % P407 in liquid form at room temperature ($\sim 20^{\circ}\text{C}$). Gravitational force is from the top to the bottom of the pictures. (b) This same solution was then incubated in a 40°C water bath for 5 min to allow for gelation of the P407. The tube was then inverted and the resulting gel does not flow or move. Arrow designates a small bubble that would normally float against gravity; however, the bubble is unable to move due to the gel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 4.

(a) A rheometer (TA instruments, ARES-LS2) equipped with an environmental chamber and 25 mm parallel plates was used to determine the viscosity of 15.4 wt/wt % P407 solutions and gels from 5°C to 65°C at a shear rate under 0.005 rad/s (AVE±SD, $n = 3$). Viscosity measurements lower than 25°C were below the dynamic range of the rheometer. A maximum viscosity of 65 kPa*s was seen at 40°C followed by a minimum of 36 kPa*s at 60°C following gel melting. (b) Size 200 and size 50 glass capillary viscometers were used to determine the elution times of P407 and D5W, respectively (AVE±SD, $n = 3$). The viscometers were allowed to equilibrate for 15 min 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 dynamic viscosities were then calculated using the densities of the fluids. The 15.4 wt/wt % P407 solution is more viscous than D5W and exhibits a minimum viscosity at 14°C followed by an exponential viscosity increase as micelles form due to increasing temperatures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 5.

A Cool-Tip RF ablation machine (Radionics) was used to measure the impedance of approximately 1.5 mL of hydrodissection fluids at different temperatures. P407 has a greater impedance than isotonic saline but a lower impedance than D5W at all temperature points. P407's impedance is greatly dependent on temperature changes and this relationship does not change during the different phases of P407. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 6.

Approximately 50 mL of D5W (a), poloxamer solution (b), and poloxamer gel (c) was evaluated with ultrasound imaging (Sonix-TOUCH). The poloxamer solution was sonolucent with no inhibition of sound waves during the ultrasound imaging. Thus, the poloxamer will not inhibit ultrasound imaging during the ablation procedure. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 7.

Graph a is for 5 mm of D5W while graph b is for 5 mm of P407. A Cool-Tip RF ablation machine (Radionics) was used to perform RF ablations on liver pieces depicted in the experimental setup, Figure 1. The ablation was performed for 10 min and temperatures at the corresponding locations were obtained every second. Here, temperature readings at every 30 s are plotted. P407 does protect the neighboring tissue but not as well as D5W. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 8.

Graph a is for 8 mm of P407 while graph b is for 11 mm of P407. The effectiveness of increasing the barrier distance is easily seen by the increase in temperature difference between probes 2 and 3 and the decrease in temperature experienced by probe 3. From these graphs, it was determined that an 11 mm P407 barrier would be sufficient during RF ablation procedures as an 8.5°C increase in temperature at the neighboring tissue would be well tolerated. Increasing the P407 barrier distance is one way to circumvent its issue of increased electrical conductivity. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 9.

Graph a is 5 mm of D5W, graph b is 5 mm of P407, and graph c is 8 mm of P407. A MW ablation machine (Neuwave) was used to perform MW ablations on liver pieces depicted in the experimental setup (Figure 1) for 5 min at 90 W. P407 performs better during these tests since there is no impedance associated heating during MW ablation. A 5 mm of D5W protects better than 5 mm of P407; however, 8 mm of P407 is comparable to 5 mm of D5W since these two conditions experience about a five degree increase at probe 3. This slight increase in temperature would be well tolerated by neighboring tissues during an ablation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE I

Approximately 100 mL of D5W, Saline and Poloxamer Was Placed in 250 mL Conical Tubes in a CT Scanner and Run at 120 kVp and 200 mA

Fluid	Iohexol	ROI (HU)
D5W	No	17.6±4.47
	Yes	202.05±5.25
Saline	No	15.76±5.38
	Yes	187.1±5.7
P407 (liquid)	No	16.47±3.92
	Yes	195.34±3.98
P407 (gel)	No	6.3±5.01
	Yes	189.32±4.87

Some solutions were infused with 2 vol/vol % Iohexol to increase contrast. The resulting Hounsfield units (HU) of region-of-interests (ROIs) were recorded. Poloxamer, saline, and D5W all have low contrast before the addition of Iohexol and high contrast afterwards.

TABLE II

Ex Vivo Ablation Results for Different Hydrodissection Fluids and Distances

Hydrodissection distance and fluid	RF Ablation		MW Ablation	
	Temperature (°C) at probe 3	Temperature (°C) differential between probes 2 and 3	Temperature (°C) at probe 3	Temperature (°C) differential between probes 2 and 3
5 mm D5W	5.7±1.7	3.2±2.9	5.0±3.0	5.4±1.9
5 mm P407	19.7±4.8**	3.3±2.2	14.5±5.3*	10.0±9.2
8 mm P407	21.0±3.4**	11.7±3.7*	5.0±3.1	13.3±6.0
11 mm P407	8.3±1.1*	22.4±7.3**	X	X

Ability to protect neighboring tissues was evaluated by the increase in temperature at probe 3 and the temperature differential between probes 2 and 3. For RF ablation, 11 mm of P407 was comparable to 5 mm of D5W while for MW ablation 8 mm of P407 was comparable to 5mm of D5W. A larger P407 barrier is needed during RF ablation since P407 has a lower electrical impedance than D5W. P407 results were statistically compared to the results from 5 mm of D5W using a two-tailed, unpaired student's *t*-test.

* and ** designate an alpha value lower than 0.05 and 0.01, respectively.