HeatSYNC™ Gel as a Thermal Accelerant and Drug Delivery System

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BACKGROUND Theromics' HeatSYNC™ gel is a protein-based formulation with the following properties: Firstly, the gel alters dielectric properties of target tissue to augment the applied energy; Secondly, the gel is a viscous solution so that it is stationary at a target site once deposited; Lastly, once reaches a specific temperature or higher (>70 °C), the gel becomes coagulated and becomes a part of the ablated tissue. We have previously demonstrated in several *in vivo* studies that the gel can improve performance of IGTA by producing the more spherical and larger ablation volume in a shorter time. [references] This effort was to address the local recurrence rate (ca. 30%) issue associated with the current IGTA technology by lowering the rate to the level of the surgical resection (ca. 10-15%).

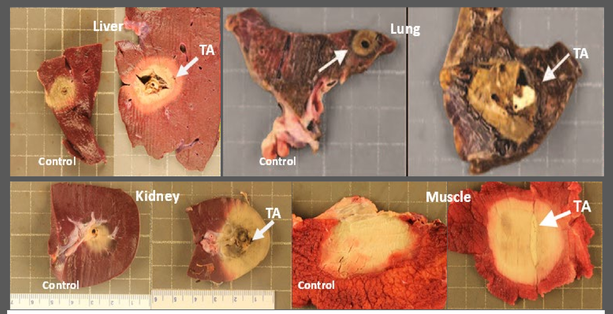

Figure 1. Images of the ablated tissue (*in vivo* porcine study with ablation conditions: 915 MHz, 60W, distance between antenna and HeatSYNC gel (2 mL) for 10 minutes). Post ablation, tissue were sectioned and treated with a triphenyltetrazolium chloride solution, a tissue viability stain: top left (A): the liver; top right (B): lung; bottom left (C): kidney; bottom right (D): gluteal muscle.

To advance our HeatSYNC technology even further, it was envisioned that the post-ablation coagulated gel could be used as a drug-depot and release it over time. Here, we designed a *POC* study that the HeatSYNC gel is miscible with the drugs of choice (i.e., Doxorubicin and Resiquimod) and performs as TA; the drugs are structurally stable during ablation within the coagulated protein meshwork; and the drugs are released from the protein meshwork over time.

OBJECTIVES of this study are to demonstrate that HeatSYNC gel can be used as a drug depot eluting anti-tumor agents after microwave ablation.

Aim 1 We demonstrate that Doxorubicin can be miscible with the HeatSYNC gel and is eluted out from the coagulated HeatSYNC gel after ablation.

Methods 1 In a 1 (w/v)% agarose gel as phantom, HeatSYNC gel (0.5 mL) with Doxorubicin HCl (2.0 mg) was placed 1 cm away from a MW antenna at ambient temperature. The ablation was performed at 60W for 10 minutes (MicroThermX/Varian 915 MHz). Post ablation, the cooled agarose gel was left at ambient temperature for 48 hours for diffusion of the Doxorubicin.

Results 1 Doxorubicin HCl (2.0 mg) is mixed with HeatSYNC gel (0.5 mL) to result in a transparent orange-colored liquid as shown in Figure 2. The mixture was placed in a pre-bored column using a 1 mL syringe followed by ablation. During ablation, coagulation started to appear at ca. 70 °C. Immediately after ablation, the orange–colored coagulated HeatSYNC gel showed no diffusion out of the protein meshwork. At 6 h diffusion of Doxorubicin to the surrounding area of the phantom was apparent, and the diffused area becomes steadily larger as the time increased as shown in Figure 2.

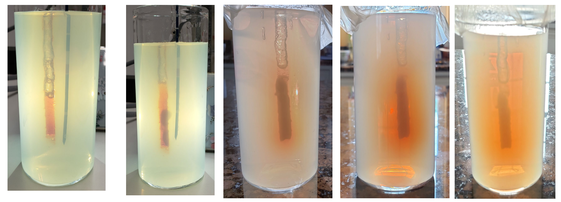

Figure 2. The drug elution of Doxorubicin+HeatSYNC Gel, before ablation (far left), during ablation (3 min), 6 h, 24h and 48h post ablation, respectively. 1 %(w/v) agarose phantom. The ablation conditions: 915 MHz, 60W for 10 minutes.

Aim 2 *In vitro* elution rate of various concentrations of Doxorubicin, a cytotoxic antitumor agent, is determined.

Methods 2 Absorbance values of three known concentrations of Doxorubicin samples (1.0 x 10^{-6}, 9.5 x 10^{-6} and 1.9 x 10^{-5} M) were obtained to established a linear relationship (Figure 3, Left) using an HPLC system: Agilent 1100; mobile phase = a gradient of ACN and H_2O with 1% ammonium acetate; wavelength = 500 nm; flow rate = 1 mL/min. Each data point was an average of three measurements. Under the HPLC conditions, the retention time of Doxorubicin was at 4.00 minutes. Separately, three different amounts of Doxorubicin (2.1, 1.1 and 0.6 mg) were mixed with HeatSYNC gel (0.5 mL each) and ablated using the conditions described in Aim 1. Once complete, the coagulated gel impregnated with Doxorubicin was collected and placed in a citrate buffer (5 mL, pH 7) in a water bath at 36.5 °C. At 1, 3, 24 and 72 h, a small quantity of solution (0.5 mL) was passed through a membrane filter (cutoff <7KDa) and injected into the HPLC system to quantify the eluted Doxorubicin. Each data point is triplicated.

Results 2 The elution behavior of Doxorubicin is shown in Figure 3, (Right). The drug was eluted out of the coagulated HeatSYNC gel in a dose-dependent manner (2.1 > 1.1 > 0.6 mg) and only a part of Doxorubicin was eluted out over time (57, 64 and 87%, respectively). In addition, the elution rate was the highest at 1 h and slowed down in the order of 1 > 3 > 24 > 72h.

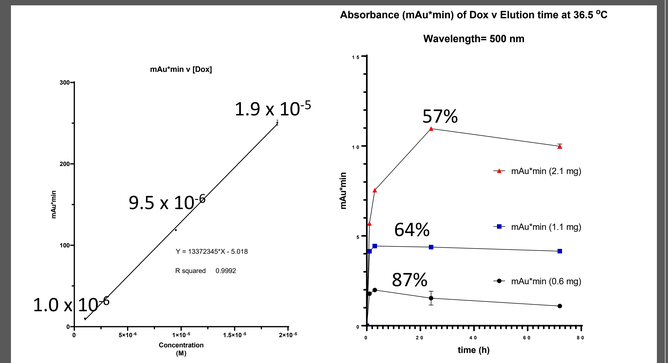

Figure 3. Left) A plot of absorbance v [Doxorubicin]; Right) A plot of absorbance of the eluted Doxorubicin from HeatSYNC gel over time.

Aim 3 *In vitro* elution rate of various concentrations of Resiquimod, a TLR 7/8 agonist, is determined.

Methods 3 Absorbance values of three known concentrations of Doxorubicin samples (4.3x 10^{-6}, 4.3 x 10^{-5} and 8.6 x 10^{-5} M) were obtained to established a linear relationship (Figure 4, Left) using the HPLC system as described in Methods 2 except wavelength = 328 nm and the retention time = 3.31 min.

Results 3 The elution behavior of Resiquimod is shown in Figure 4, (Right). The drug was eluted out of the coagulated HeatSYNC gel in a dose-dependent manner (1.25 > 0.63 > 0.063 mg) and only a part of Doxorubicin was eluted out over time (57, 64 and 87%, respectively). In addition, the elution rate was the highest at 1 h and slowed down in the order of 1 > 3 > 24 > 72h.

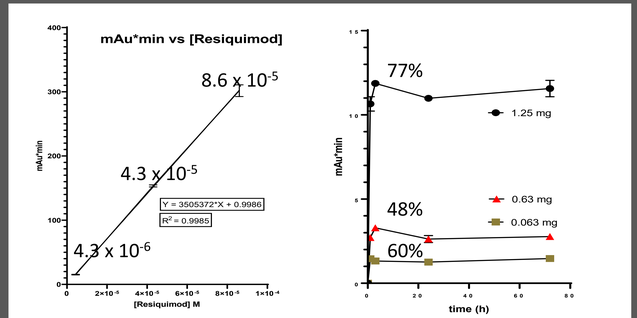

Figure 4. Left) A plot of absorbance v [Resiquimod]; Right) A plot of absorbance of the eluted Resiquimod from HeatSYNC gel over time.

DISCUSSION Here, we have demonstrated as a proof-of-concept trial that HeatSYNC gel impregnated with drugs can perform as thermal accelerant, and the selected drugs retain their structural integrity during ablation. Furthermore, the drugs entrapped in the coagulated HeatSYNC gel were eluted out of the protein meshwork over time and maintained the level of the elution over the observed time frame, i.e., 72 hours. Although the in vitro study design is limited to simulate the tumor environment of a living body, the present study results provide a distinct possibility of HeatSYNC gel to be useful for post-ablation anti-tumor treatment for further reduction of the local recurrence rate.

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