HeatSYNC™ Gel: Mechanism of Action as Thermal Accelerant and Development of Drug-Eluting System for Anti-tumor Agents Post-Ablation

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INTRODUCTION

We have previously demonstrated that HeatSYNC gel effectively absorbs microwave energy during ablation because protein molecules, a major component of HeatSYNC gel, have much higher dipole moment (700 D, D=Debye) than that of a water molecule (1.85 D). When exposed to microwave energy, these molecules oscillate along the energy wave during which intermolecular collision occurs. These collisions produce thermal energy (heat). Since the protein molecules have larger polarity and size (ca. x3,700) than water molecules, more effective microwave heating will be observed using HeatSYNC gel than water **(Figure 1a).** To demonstrate the HeatSYNC gel's effectiveness, It was devised that the gel was placed at 1-1.5 cm from the feed point of the antenna in agarose phantom **(Figure 1b)**. When temperature was measured under the MWA conditions: 915 MHz, 60W, 10 minutes, a rapid increase was observed (> 60 °C within 15 s; 70-90 °C within 3 min; >100 °C while the carrier or water was not effective as thermal accelerant **(Figure 1c).** As the main component of HeatSYNC gel is a human protein, the gel coagulates as the temperature increases to 70-90 °C . At this temperature range, the protein loses its structural (i.e., conformational) integrity along with the dipole moment. Thus, the temperature must drop beyond 3 minutes of ablation. However temperature is maintained at around 100 °C even after all protein molecules were coagulated as shown in **Figure 1c.**

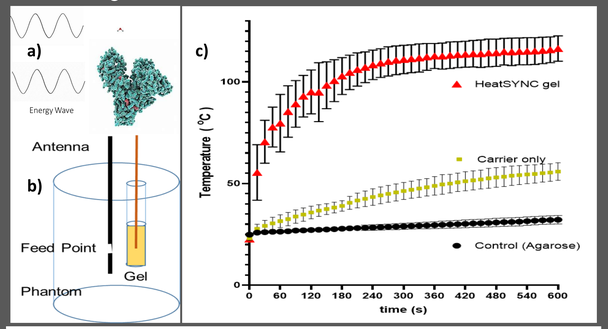


Figure 1 a) water vs. HeatSYNC gel upon exposure to energy wave; b) in vitro thermal acceleration design; c) Thermal profile of HeatSYNC gel (red), a carrier only (yellow) and control (agarose).

MECHANISM OF ACTION

To elucidate the observed mechanism of thermal behavior during ablation, we investigated its electrical conductivity, a key measure of the power absorbed per unit volume of the gel, and its change during ablation in a wide range of temperatures (i.e., 20-90 °C), and heat transfer to its surroundings. The measurements provided information on the energy absorption property of HeatSYNC gel from ambient temperature to the elevated temperatures during ablation.

The electrical conductivity σ of a material is the key parameter to understand its properties. The s value directly scales with the power absorbed per unit volume according to the following expression, ARD = $\sigma|E|^2$, where ARD is watt m^{-3}, and |E| is the magnitude of the electrical field produced by the MW antenna at the point of interest in the tissue. We measured σ of HeatSYNC gel (N=4) over the temperature range of 20-90° C at both 915 and 2450 MHz (**Figure 2**).

Electrical conductivity increased linearly as temperature increased at both frequencies with a similar magnitude: σ_{950} (S/m) = 0.05113 T (°C) + 0.9393 (R^2 =0.9822); σ_{2450} (S/m) = 0.05248 T (°C) + 1.830 (R^2 =0.9754) **(Figure 2).**

It is noteworthy that the electrical conductivity of HeatSYNC gel kept its positive linearity in the coagulation temperature range (70-90 °C) suggesting that the structural transformation of HSA is independent with the electrical conductivity.

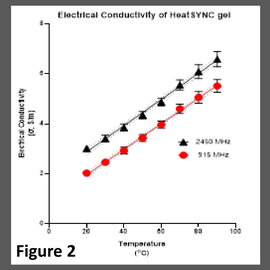
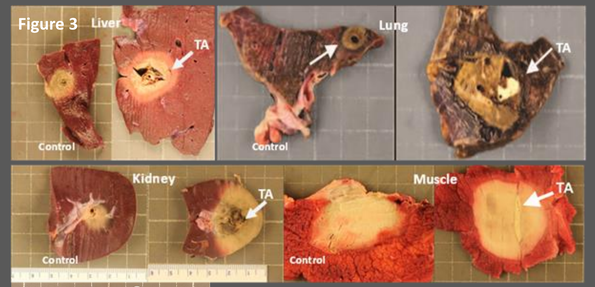


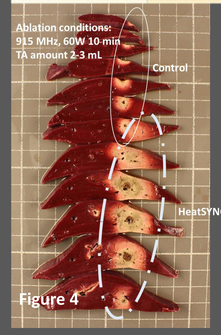
Figure 2

Therefore, the initial temperature acceleration of HeatSYNC gel is the combined results of the heat caused by the HSA's kinetic movement and the electrical conductivity of the gel. After coagulation of the gel (i.e., >3 minute), the plateaued thermal profile is attributed mainly to the electrical conductivity of the HeatSYNC gel.

IN VIVO ABLATION WITH HEATSYNC GEL

In order to test the effectiveness of HeatSYNC gel, ablations in various tissue were performed using porcine model with the ablation conditions: 915 MHz, 60W, 10 minutes for liver, lung, muscle and kidney **(Figure 3).**





In perfused tissue such as liver or lung, the ablation with HeatSYNC gel volume is as twice large as the control**.** The impact of the gel is less prominent in muscle as the tissue is less perfused. It is believed that the large ablation with HeatSYNC gel in kidney is attributed to its compartmentalized anatomy whereby the gel is less diffused. Simultaneous ablations (**Figure 4**) were performed in the porcine liver using two antennas, one without the gel and the other with the gel: 915 MHz, 60W each, 10 minutes, the gel (2-3 mL) injected 1 cm laterally. As shown in **Figure 4**, ablation with the gel shows much larger ablation volume and improved eccentricity.

HEATSYNC GEL AS DRUG-ELUTING SYSTEM (Doxorubicin + HeatSYNC gel)

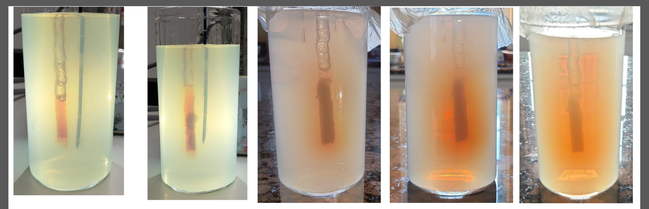


Figure 5 The drug elution of Doxorubicin+HeatSYNC Gel, before ablation (far left), during ablation (3 min), 6 h, 24h and 48h post ablation, respectively. 1 %(w/v) agarose phantom. The ablation conditions: 915 MHz, 60W for 10 minutes.

Aim *In vitro* elution rate of various concentrations of Doxorubicin, a cytotoxic antitumor agent, is determined after in vitro dox elution was observed (**Figure 5**).

Methods 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 6**, Left) using an HPLC system: Agilant 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 the Aim. 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 The elution behavior of Doxorubicin is shown in **Figure 6**, (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.

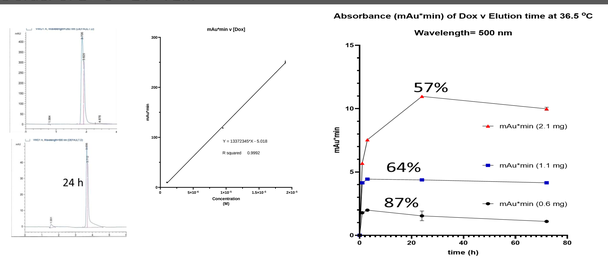


Figure 6. Left) HPLC traces of free Dox and Dox 24 h post ablation; Middle) A plot of absorbance v [Resiquimod]; Right) A plot of absorbance of the eluted Dox from HeatSYNC gel over time.

HEATSYNC GEL AS DRUG-ELUTING SYSTEM (Resiquimod + HeatSYNC gel)

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

Methods 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 7**, Left) using the HPLC system as described in Methods 2 except wavelength = 328 nm and the retention time = 3.31 min.

Results The elution behavior of Resiquimod is shown in **Figure 7**, (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.

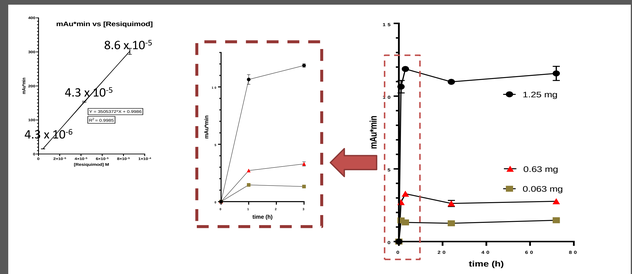


Figure 7. Left) A plot of absorbance v [Resiquimod]; Right) A plot of absorbance of the eluted Resiquimod from HeatSYNC gel over time.

DISCUSSION AND FUTURE WORK

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. 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. So far, the selected drugs include, small molecule kinase inhibitors (e.g., doxorubicin, paclitaxel, sorafenib), DNA (cyclic dinucleotides such as cGAMP STING agonists, TLR9 agonists such as Oligo deoxynucleotide). The study results show a distinct potential that HeatSYNC gel can be useful for post-ablation anti-tumor treatment and reduction of the drug-associated adverse effect.

Theromics are currently investigating that the HeatSYNC gel containing a STING agonist (CDN) can induce an abscopal effect: CT26 adenocarcinoma cell injected in both flanks of BALB/c mice. RFA is performed in one side of tumor. Over 3 weeks, reduction of tumor size in the other side will be measured. In the study histopathological examination will be made on the distant tumors. Tumor infiltrating lymphocytes (TILs) identified with CD3, CD4, CD8, FoxP3 will be analyzed by flow cytometry. In order to study the function of peripheral T lymphocytes, the ability of T cells from the spleen to produce IFN-γ, IL-6 and TNF-α will be analyzed. Immune checkpoint Inhibitors (ICI) such as anti-CTLA-4 (e.g., ipilimumab), anti-PD-1, anti-PD-L1 Abs can be used to augment the abscopal effect.

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