Modeling a Freeze-Thaw Cycle to Treat Lung Cancer

BEE 4530: Computer-Aided Engineering:
Applications to Biomedical Processes

Group 11:
Lauren Dugard
Scott Hayes
Tara Holter
Julie Leviter

May 1, 2009

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Executive Summary

This study examines the effects of pre-freezing on the RF ablation of lung cancer, a widespread disease in the United States. While current treatments utilize cryosurgery or RF ablation to destroy lung tumors, neither method ensures the tumor destruction. Sun *et al*. (2008) describes an alternative treatment combining both cryosurgery and RF heating techniques, consisting of 10 minutes of pre-freezing with a -150°C probe followed by 30 minutes of RF heating (1). Pre-freezing acts to lower the inactivation energy of the tissue, resulting in an increased radius of tumor death for the same duration of resistive heating. The study aims to examine the effects of pre-freezing on RF ablation surgery of a lung tumor, verify the findings of Sun et al. using COMSOL, and examine the sensitivity of the freeze-thaw procedure to tumor and tissue material properties.

COMSOL Multiphysics was used to model the freeze-thaw procedure for a lung tumor with a 16.7 mm radius, in comparison with simple RF heating. Pre-freezing was simulated as heat transfer by conduction with a constant -150°C temperature probe with a 2.5 mm probe radius, and accounted for latent heat in tabulated data for apparent specific heat of the tissue. RF heating was simulated by implementing the voltage equation to account for resistive heat generation in the tissue. Cell radius of tumor death was calculated using an equation for cell death due to heating formulated by Sun *et al.* (2008).

The COMSOL model was verified by comparing the cell death radius to values reported by Sun (2008). The applied voltage was first set to 17.6 V to destroy a tumor radius of 8.7 mm with simple RF heating as observed by Sun *et al.* (2008). The freeze-thaw procedure was implemented for a range of inactivation energy values from 136 to150 kcal/mol. The energy of inactivation energy required for a tumor death radius of 12.7 mm was143200 cal/mol, a 0.0485% difference from the literature reported of 143,898 cal/mol. For the tumor we modeled in COMSOL, the voltage was adjusted to 20 V to destroy the entire area of tumor and minimize damage to normal tissue. A sensitivity analysis was conducted for thermal conductivity, density, and specific heats of the tissue and tumor, and inactivation energy.

The model demonstrated that ten minutes of pre-freezing can increase the effectiveness of RF ablation. This resulted in a larger area of tumor destruction and allows for a lower voltage or reduced duration of probe contact. Furthermore, the material properties of the tumor and surrounding tissue had a minimal effect on the radius of tumor death, suggesting variation

between patients and tumor composition would have little effect on the effectiveness of the freeze-thaw treatment.

Before the procedure could be used for animal trials or human use, the required voltage for the freeze-thaw treatment of various tumor sizes and geometries must be calculated, and the model should be run using all biologically probable parameters. Nonetheless, the freeze-thaw procedure combines cryosurgical and RF ablation surgical techniques that have already been proven safe and effective for human use. Therefore, the freeze-thaw procedure may improve the outcome of lung cancer cases with minimal cost to develop and comparable patient risk to current treatment procedures.

Introduction

Lung cancer is a very prevalent disease in the United States and can be treated using a variety of methods including: invasive surgery, chemotherapy, radiation, and localized thermal treatment (2). Thermal treatment methods include both cryosurgery and radiofrequency (RF) heating. Cryosurgery is a localized and minimally invasive method of treatment, but it can cause direct cellular injury, vascular injury, and possible immune reactions. RF heating penetrates deep into tissue but can initiate metastasis. Unfortunately, neither method of treatment guarantees complete destruction of the tumor (1). A novel system developed to treat lung cancer has combined both of these treatment methods, using a system designed to freeze and then warm the tissue in a series of cycles. The combination of these two stages causes more damage than either alone because the deep tissue heat generation in frozen tissue causes heterogeneity and damaging thermal stress.

Much medical research has been aimed at treating tumor tissue with minimal destruction of surrounding normal tissue. To improve tumor shape confirmation, several techniques have been tried, including cool tip RF probes and multi-probe arrays (1). In 2004, Cabrera *et al.* submitted a student project that investigated the use of cryosurgery to treat a cancerous cylindrical mass in lung tissue (3). From this project an optimal freezing time was determined that would successfully freeze the tumor while minimizing damage to the surrounding normal tissue. In 1982, Gage *et al*. proposed the method of combining freezing and heating for the first time (4). Liu *et al.* then designed a cryo-probe system with vapor heating, and concluded that cooling immediately followed by vapor heating would improve treatment effect due to thermal stress. Sun *et al*. (1) recently used a nude mouse dorsal skin flab chamber tumor model to study this type of system. To provide Nitrogen cooling and RF heating alternatively, they used a metal probe with circulating fluid for a freezing effect, and a current running through a wire to generate an RF effect. For analysis they used a bio heat transfer model to show temperature changes and to predict the therapeutic effect at the cellular and tissue levels. The study demonstrated that tumor cells and vessels were completely destroyed after alternate treatment, whereas neither cooling nor heating alone achieved same effects.

The alternative treatment process entails ten minutes of freezing followed by thirty minutes of RF heating treatment. In the cryosurgical freezing process, the probe is quickly cooled by the flow of liquid nitrogen. During this ten minute cooling stage a ball of ice is formed in the affected tumor tissue and blood perfusion is assumed to be inhibited in all regions that reach a temperature below 0°C. The probe then switches function and enters a thirty minute RF heating stage. In this heating stage deep tissue heat generation causes thermal stress and heterogeneity in the affected tissue such that it becomes more damaged that normal homogeneous material.

Design Schematic:

The schematic used to model the destruction of a tumor in healthy lung tissue shows boundary conditions for both freezing and heating stages (Figure 1). The left boundary has been set as an axis of symmetry to create a 3D model in COMSOL. The probe, which does not appear in this schematic, is placed along the axis of symmetry on top of the tumor. The temporal plot of the alternative treatment shows the timescale of the procedure (Figure 2).

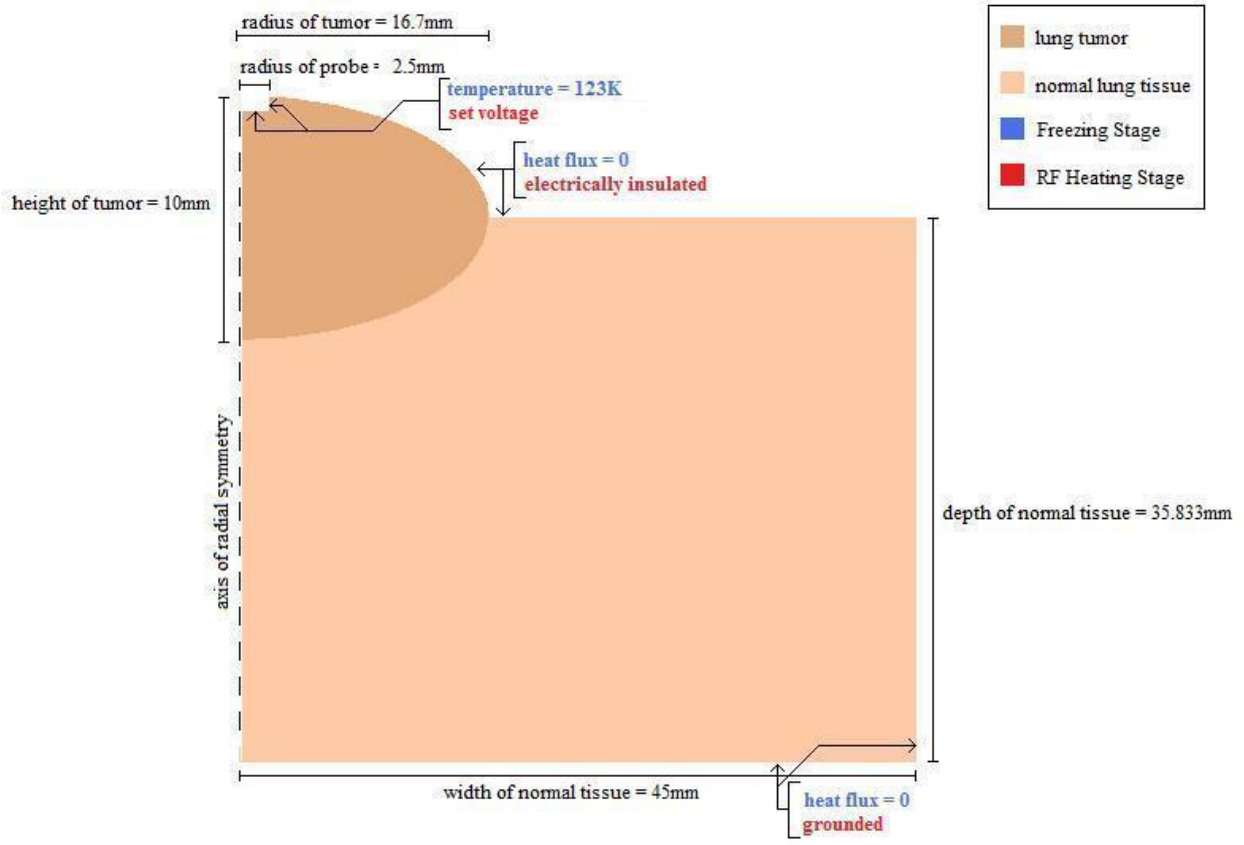

Figure 1: A schematic of the lung tumor and tissue system to be modeled in COMSOL.

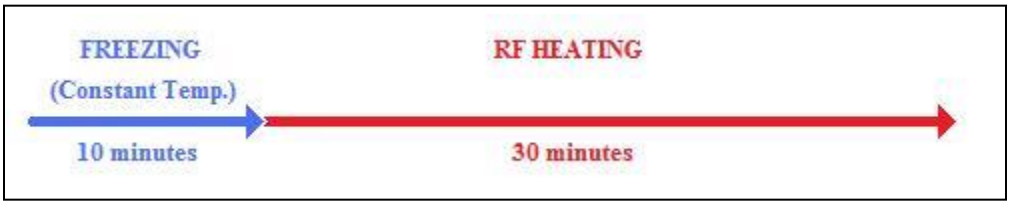

Figure 2: Temporal plot of the alternative treatment to visually represent the timescale and relationship between different parts of the thermal cycling treatment.

Establishing the Model in COMSOL

We selected the two dimensional axial symmetry to most accurately model the structures we desired and then initiated two different multiphysics systems: transient heat transfer (for heating and cooling processes) and conductive media DC (for RF heating). The value for the apparent specific heat (c_{pa}) was included in the governing equation (Appendix A, Equation 1A) for cooling to account for the freezing of the material. The expression for c_{pa} describing the changes in c_{pa} during the freezing process is given in Equation 1b. For this step, the heat flux along the edges of our model was considered to be negligible.

The governing equation for heat transfer during RF heating (Appendix A, Equation 2A) used the same equation as for freezing, but included a heat generation term. This generation term comes from the heat generated by resistive heating of the radiofrequency heating (RF) probe, which is modeled by an equation relating electric field to voltage and position (Appendix A, Equation 3A). For the voltage equation, boundary conditions were set such that the tissue in contact with air was insulated, the boundary along the axis of symmetry had axial symmetry, and the tissue in contact with the rest of the body was grounded. For the heating stage, each edge was considered to have a no flux boundary condition (Appendix A, Equation 4A).

Within COMSOL, we input the parameter values in the appropriate locations under subdomain settings (Appendix A, Table 1A). For the heat capacity, we defined a function called heat_cap that was based on interpolation between points we entered (Appendix A, Table 2A). Under global expressions, we included equations for k_s and cell_survival_heating in order to determine the extent of damage caused by variations in procedure based on the equations provided in Sun *et al*. (2008).

The equation used by Sun *et al*. to find cell survival rate is given by (Appendix B, Equation 1B). In this equation, n = 100 and F(t) is a function quantifying the amount of damaged protein. A calculation of 1 indicates cell survival, 0 indicates cell death. The variable k_s is the rate of protein denaturization, and is modeled by Equation 2B (Appendix B). The normal value for ΔH, given by Sun *et al*., is 145149 cal/mole, and that after pre-freezing is 143898 cal/mole. Δs is an extensive state function that accounts for the effect of irreversibility in a thermodynamic system. The smallest functional unit in a living system, a cell, can be considered as such a system, hence our use of it in this model. The value used here was -374.5 cal K^{-1} $mole^{-1}$.

Design Objectives

The first design objective was to accurately model a thermal cycling treatment in COMSOL. This included implementing the governing equations for energy transfer and connecting the separate thermal stages in an overall model. COMSOL was used to create a model of the lung tumor and to analyze the effects of applying a thermal cycling probe placed in the middle of the lung tumor. The second design objective was to develop a model that would minimize cell survival rate in cancerous tissue and maximize the survival rate in the surrounding healthy tissue. The probe's optimal heating and cooling cycles were determined by using the cell death equations provided by Sun *et. al*. (Appendix B, Equation 1B).

Methods and Results

Mesh Convergence

Initial examination of our model required a mesh optimization in order to determine the minimum mesh size that would achieve convergence. This optimization was important because convergence is necessary in order to yield accurate results but the smaller the mesh, the longer the computation time. Therefore, a mesh convergence was performed for both of the subdomains (tumor area and tissue area) independently in order to obtain optimal results.

From the mesh convergence, it was determined that a free mesh with a total of 2928 elements would suffice. The tumor subdomain has a total of 589 elements and a maximum element size 0.05 and the normal tissue subdomain has a total of 2339 elements with a maximum element size of 0.1. These optimizations were determined based on when the average temperature reached a temperature that was stable with increasing elements in the mesh. (Figure 3, Appendix B, Table 1B).

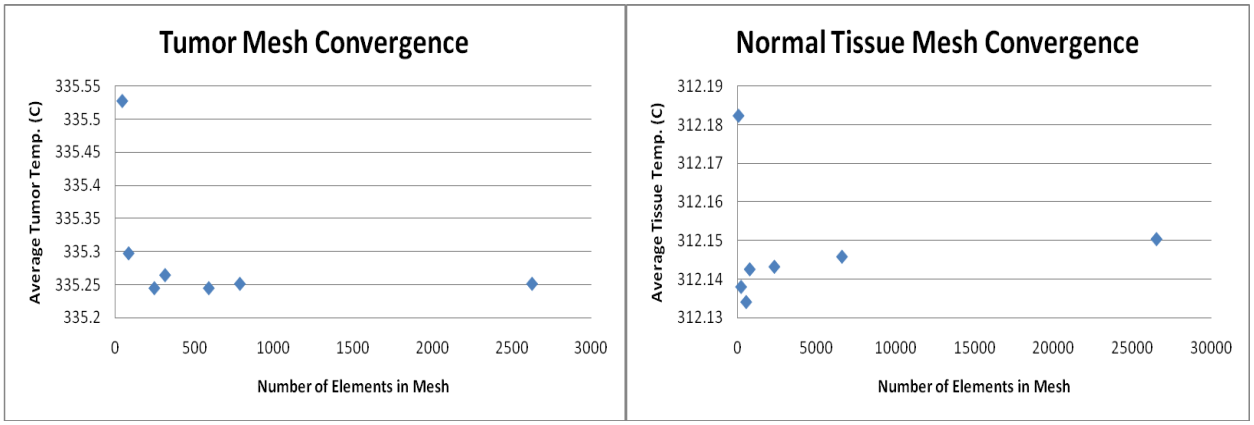

Figure 3: Mesh convergence data shown graphically. This representation makes it clear that the values chosen for tissue and tumor mesh are within the region of convergence but are also small enough that there is not excessive computation time.

Modeling Steps

In order to accurately model the alternate treatment proposed, we had to first model the freezing of the tumor with a probe cooled by liquid nitrogen and then apply those conditions as initial conditions to the RF heating treatment of the probe. This was done by first establishing a 123K temperature boundary at the probe boundary and running the heat transfer model for ten minutes of freezing. This solution was stored in the COMSOL solver manager and then could be applied as the initial condition once we changed the boundary to a constant voltage and asked the program to solve for both temperature and voltage for the thirty minute RF heating stage.

Creating an accurate model was accomplished in three steps. The first was to establish the voltage used during the RF heating stage. Sun *et al*. (2008) reported that a treatment of just heating the tumor (without pre-freezing) killed the cells of the tumor in a radius of 8.5mm. Therefore, the voltage was experimentally varied in order to yield this radius of cell death according to the output of the equation cell_survival_heating. The next step was to apply the

same voltage to the model for alternate treatment and vary the energy of inactivation to yield a radius of tumor cell death of 9.5mm (1). The final step was to use the determined inactivation energy and again vary the voltage of the RF heating stage in order to kill the radius of the tumor cell modeled in COMSOL.

Freezing Model

The first model established in COMSOL is that of the freezing stage. The surface plot is for the final temperature after ten minutes of contact with the probe and temperature graphs given are for the entire process (Appendix C Figures 2C, 3C).

Heating Model: Voltage Determination

Using the COMSOL model for heating only, we determined that the optimal voltage for the RF heating stage was 17.6V as that yielded a radius of cell death that agrees with the results of Sun et al. (2008) (Appendix C Table 2C). The surface plot is for the final temperature after thirty minutes of contact with the probe and temperature graphs given are for the entire process (Figure 4, Appendix C Figures 4C, 5C).

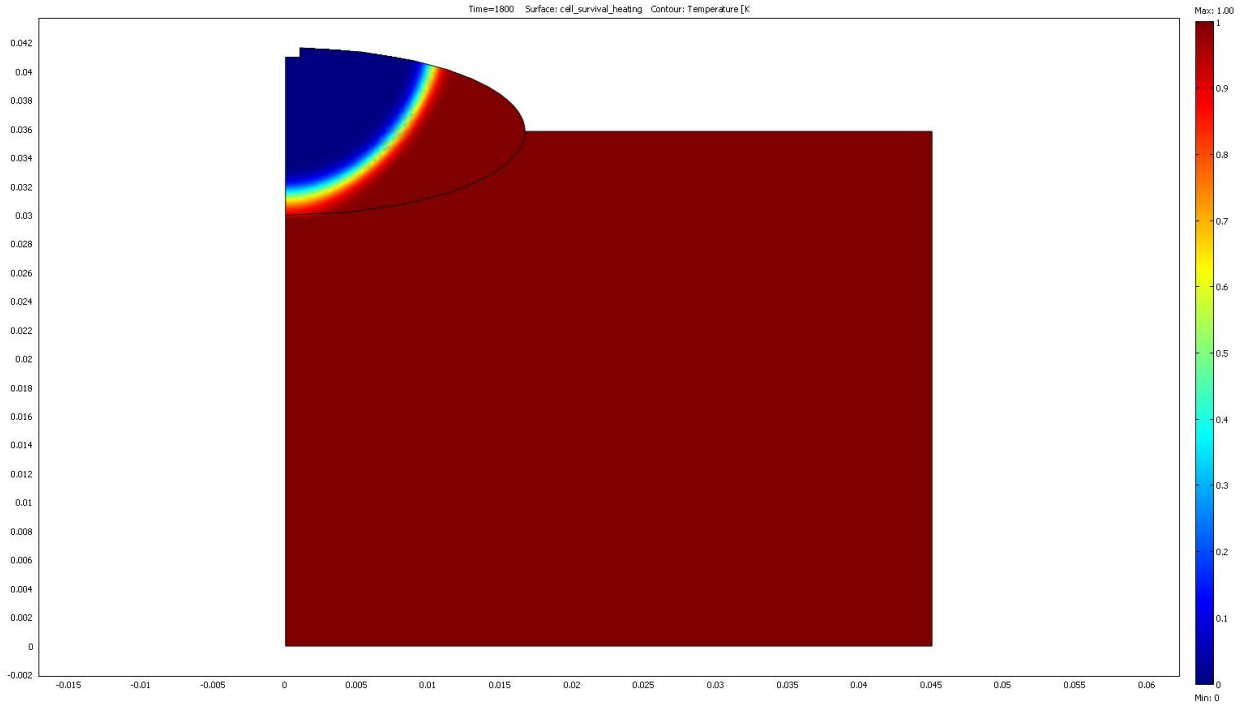

Figure 4: The surface plot after heating for thirty minutes of the equation given by Sun *et al.* (2008) for the cellular survival rate defined as cell_survival_heating within the COMSOL program. The blue areas indicate areas of cell death and the red indicates cell survival.

Alternative Treatment: Inactivation Energy Determination

The energy of inactivation was determined using the model for the alternate treatment (freezing the tumor for ten minutes and then RF heating for thirty minutes). By fitting the radius

of cell death to the results of the paper (9.5mm), the energy of inactivation was determined to be 143,200cal/mol (Appendix C Table 3C). The surface surface plots show the final temperature and cell survival after the full forty minutes and the temperature graphs given are for the RF heating process following the ten minute freezing (Figure 5, Appendix C Figures 6C, 7C).

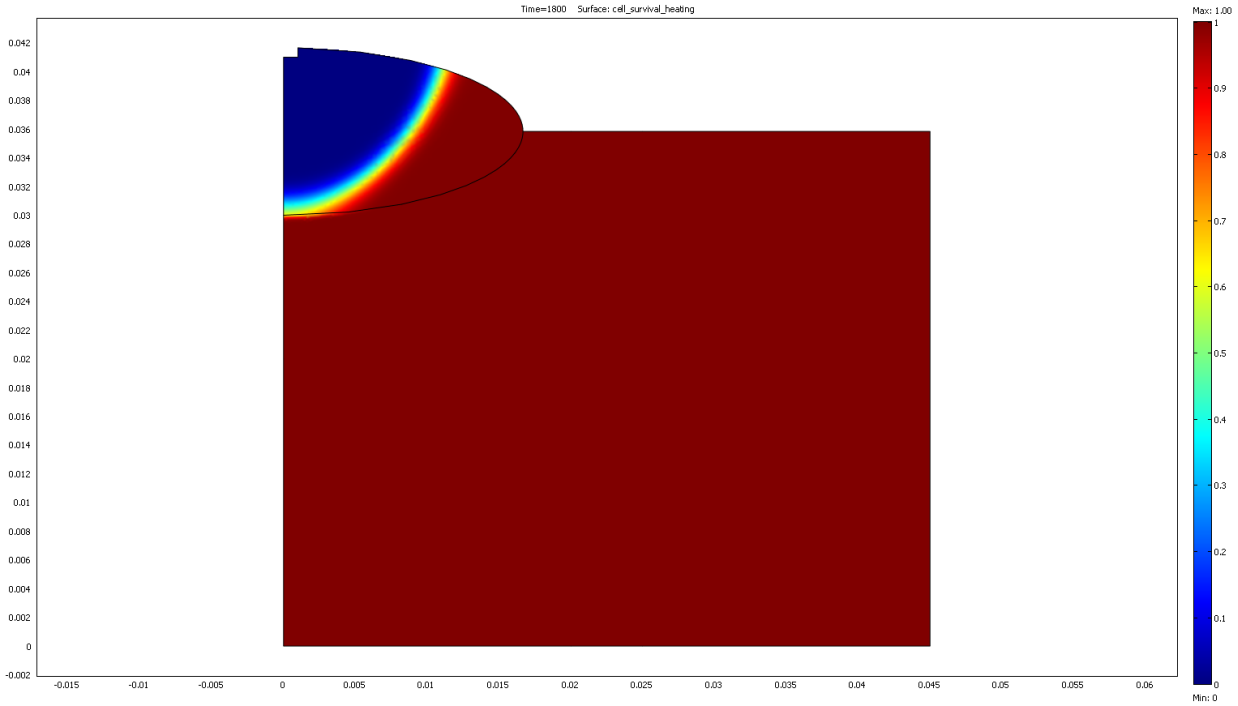

Figure 5: The surface plot after the alternate treatment using the equation for cellular survival rate. This was fit to the results given by Sun- the radius of cell death is 9.5mm.

Alternative Treatment: Adjustment to Optimize Tumor Death

The voltage required to destroy the entire tumor modeled was determined using the energy of inactivation (143,200cal/mol) and varying the voltage to fit the radius of the physical tumor. This voltage was determined to be 20V (Appendix C, Table 4C). The surface plots show the final temperature and cell survival after the full forty minutes of treatment and the termperature graphs given are for the RF heating process following the ten minutes of pre-freezing (Figure 6, Appendix C Figures 8C, 9C).

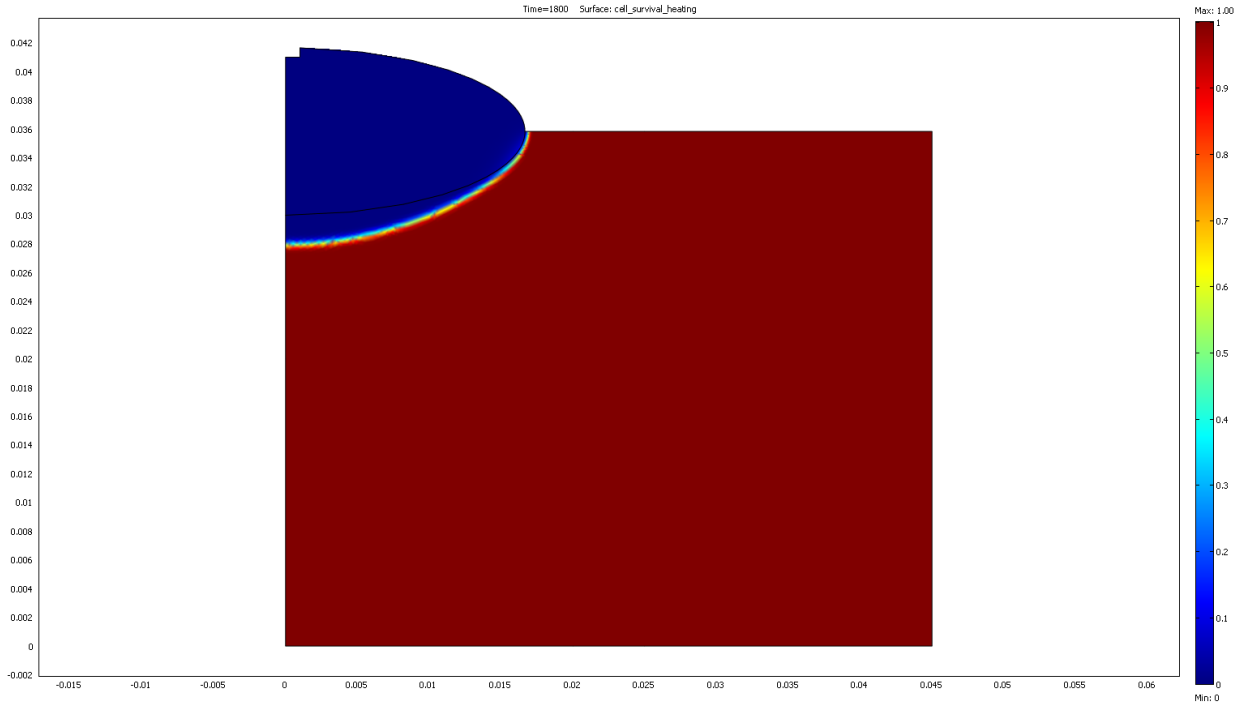

Figure 6: The surface plot after the alternate treatment with RF heating voltage = 20V using the equation for cellular survival rate. It is clear that the entire tumor area was destroyed and minimal normal tissue was compromised.

Sensitivity Analysis

We tested the sensitivity of our model by varying tissue density and thermal conductivity, tumor density and thermal conductivity, and heat capacity. We did this by altering values by ±20%, and observing the difference in results (Figure 7).

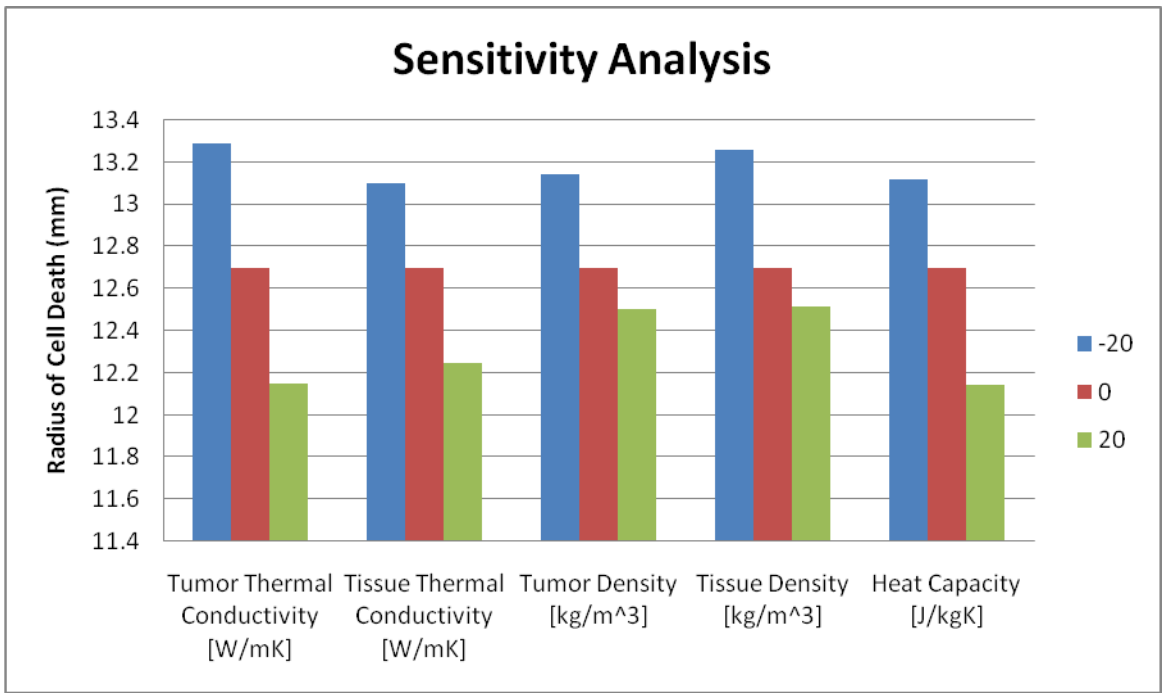

Figure 7: Sensitivity analysis of thermal parameters. The 0% figure is the input parameter for the corresponding property (Appendix A, Table 1A). It is clear that even a 20% variance of these parameters leads to minimal changes to the outcome of the COMSOL model.

We also performed a sensitivity analysis of the energy of inactivation, but used smaller percent differences (±5%, ±2.5%, ±1%) given that it is such a large value even these small changes can have a significant impact on the results (Figure 8, Appendix C Table 5C).

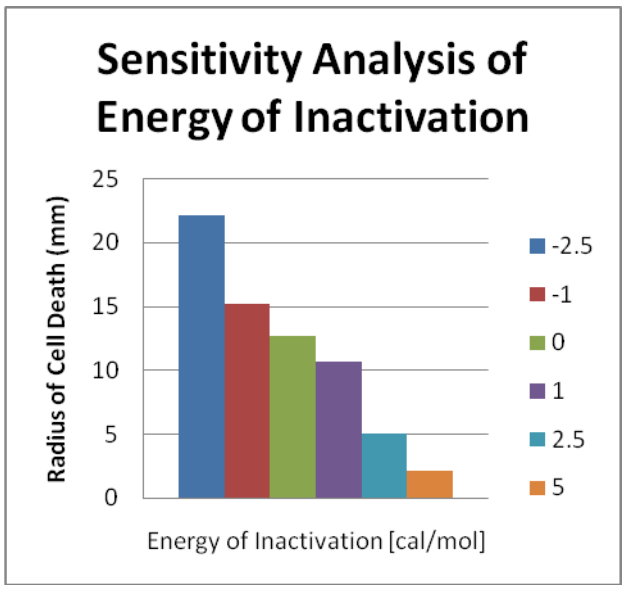

Figure 8: Sensitivity analysis of the energy of inactivation is shown graphically. The value for a percent difference of -5% is excluded as the COMSOL model predicted the destruction of the entire tissue.

Validation

First, it was verified that 17.6 V and 20.0 V are resonable voltage values for RF ablation. Nikolic *et al.* (2009) report that RF probes for tissue ablation typically have a current of 140 +/- 35 mA, and a power of 2-5 W (7). Using Ohm's Law (Power=IV) for the typical current range, 17.6 V yields a power between 1.85 W and 3.08 W, and 20 V yields a power between 2.10 W and 3.50 W (Appendix B Table 2B, Equation 3B). Therefore the voltage of 20 V is reasonable for the entire range of possible currents, while the voltage of 17.6 V is reasonable as long as the current is set above 115 mA.

The model we developed using the COMSOL software was validated primarily by comparing the energy of inactivation we found experimentally (143200cal/mol) to the energy of inactivation provided by the paper (143,898cal/mol). The calculated percent error was 0.485%, which is well within acceptable range for percent error. The calculation is shown below (Equation 1).

$$Percent\ Error = 100 \times \left(\frac{actual\ value - calculated\ value}{actual\ value}\right) = 100 \times \left(\frac{143{,}898 - 143{,}200}{143{,}898}\right) = 0.0485\% \quad (1)$$

Conclusion and Design Recommendations

Conclusions

The tumor ablation model implemented in COMSOL demonstrated that freeze-thaw cycling increased the radius of tumor death by 9.6% compared to RF heating. Furthermore, we found that the increased effectiveness of freeze-thaw cycling is relatively independent from the material properties of the tumor or the surrounding normal tissue such as the specific heat, density, and thermal conductivity, but is highly dependent on the inactivation energy of the tissue.

In our model, freeze-thaw cycling increased the radius of tumor death from 8.684 mm to 9.514 mm, compared with simple RF heating at 17.6 V. The model was verified by implementing the RF heating and freeze-thaw cycle treatment implemented by Sun (2008), and comparing the tumor death diameter to the literature reported value. We determined that the inactivation energy required to destroy a 12.7 mm tumor radius was 143200cal/mol, a 0.0485% difference from the inactivation energy value of 143,898 cal/mol proposed by Sun et al.

The use of a computer model is crucial to demonstrating the effect of pre-freezing on tumor death radius. If an experiment was conducted in vivo, each replicate of an experiment would require a new tumor. Therefore, if an *in vivo* experiment were used, it would be very difficult to confirm that the increased cell death is due to the freeze-thaw cycling and not variation between individual tumors.

Our group determined that the voltage of the probe should be increased to 20 V to successfully destroy the tumor as defined in the model with a radius of 12.5 mm. The sensitivity analysis shows that varying the material properties by 20% resulted in cell death radius to vary by less than 0.5 mm. More strikingly, the COMSOL model uses specific heat, density, and thermal conductivities from sources outside of Sun et al, but the results are very similar regardless of the differing material properties.

Design Recommendations

The COMSOL model could be used to examine the effects of many procedural variations in tumor treatment. Many different freeze-thaw procedures can be implemented by changing the heating and cooling time, probe diameter, number of freeze-thaw cycles, and number of probes in the model. While it was confirmed that freeze-thaw cycling is more effective than RF heating, the model must be tested for all biologically relevant parameter ranges before it is tested on animals or humans. To examine the feasibility of freeze-thaw treatment in human cases, a Monte Carlo analysis could be conducted to test many different tumor parameters. MRI images of real tumor geometries could also be used to determine the effects of freeze-thaw cycling using more realistic tumor geometry. While our model demonstrates that freeze-thaw cycling can be beneficial in tumor treatment, future research must determine which geometries and tumor types would benefit from the freeze-thaw cycling tumor treatment.

Modeling in COMSOL is both faster and more humane than using animal models for testing purposes, avoiding the ethical concerns of animal research. Through the model, one could iteratively optimize all aspects of the problem and then test a finalized procedure experimentally. The model also provides a direct comparison of freeze-thaw and RF heating treatment procedures on the same tumor, which would not possible to achieve through animal testing.

Given that lung tumors vary greatly in size and geometry, the data from the COMSOL model could be used by doctors to set an appropriate voltage for a freeze-thaw cryosurgery procedure. The geometry could be scaled to different sizes to create a chart indicating the appropriate applied voltage for a given tumor size. The relationship between tumor size and appropriate probe voltage would be crucial in minimizing damage to healthy tissue, and must be determined before the probe could be used in human trials.

The freeze-thaw procedure combines both cryosurgical and RF ablation, two commonly used surgical techniques. By incorporating two approaches that are already in human use, clinical trials for freeze-thaw cycling would be safer than testing a completely new approach, such as experimental medications. Additionally, the freeze-thaw probe combines a common RF probe, with a liquid nitrogen cooled tip, a small variation on an existing device that could be easily manufactured. Combining two common approaches would be fairly inexpensive to develop and produce, may result in faster approval from the FDA than a completely new approach, and provide more effective lung tumor treatment with comparable patient risk to current treatment procedures.

Appendix A: Mathematical statement of the problem

Governing Equations

Freezing

$$\rho c_{pa}\left(\frac{\partial T}{\partial t}\right)=k\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial T}{\partial r}\right)+\frac{\partial^2 T}{\partial z^2}\right] \quad (1A)$$

Table 2A: Values for the apparent specific heat

Temperature, T [K]	Apparent specific heat, c_{pa} [J/KgK]
18	4180
248	4180
261	5000
265	10000
268	20000
269	80000
270	44000
270.5	20000
271	4180
333	4180

Heating

$$\rho c_{pa}\left(\frac{\partial T}{\partial t}\right)=k\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial T}{\partial r}\right)+\frac{\partial^2 T}{\partial z^2}\right]+Q \quad (2A)$$

$Q = \sigma E^2$, where E is defined by the voltage equation

$$\boldsymbol{E}=\frac{\delta \boldsymbol{V}}{\partial \boldsymbol{x}} \quad (3A)$$

$$-k\frac{\partial T}{\partial r}\Big|_{all\,boundaries}=0\ J/m^2 \quad (4A)$$

Input Parameters

Table 1A: Parameters used for the model in COMSOL.

Parameter Name	**Units**	**Value**	**Source**	**Notes**
Thermal Conductivity of Healthy Tissue (k)	W/mK	0.245	4, 5	Original values taken from (5). Values for frozen and unfrozen materials averaged in (4)
Thermal Conductivity of Tumor (k)	W/mK	1.401	4,5	" "
Density of Healthy Tissue (p)	kg/m^3	960	4, 5	Original values taken from (5). Values for frozen and unfrozen materials averaged in (4)
Density of Tumor (p)	kg/m^3	200	4,5	" "
Specific Heat of both Tumor and Tissue (Cp)	$J*(kg\ K)^{-1}$	Tabulated	3	Tablulated Values refer to the specific heat of water (refer to chart in project text)
Intitial Temperature (T_int)	K	310	4	Body temperature
Probe Temperature	K	123	4	
Water Content	kg/kg	0.8	4	
Latent Heat	kJ/kg	333	4	Assumed to be latent heat of water based on high water content

Appendix B: Solution Strategy

Cell Death Equation

$$S_{heat} = 1 - [F(t)]^n = 1 - (1 - e^{-kst})^n \quad (1B)$$

$$k_s = 2.05 \times (10)^{10} \times T \times e^{\Delta s/2} \times e^{-\Delta H/2T} \quad (2B)$$

Mesh Convergence

TUMOR				NORMAL TISSUE		
No. Elements	Sub Int	Average Temp.		No. Elements	Sub Int	Average Temp.
44	0.051018	335.527523		65	0.479512	312.1822917
84	0.050983	335.2973402		226	0.479444	312.1380208
246	0.050975	335.244727		551	0.479438	312.1341146
314	0.050978	335.264457		775	0.479451	312.1425781
589	0.050975	335.244727		2339	0.479452	312.1432292
785	0.050976	335.2513037		6621	0.479456	312.1458333
2626	0.050976	335.2513037		26549	0.479463	312.1503906

Table 1B: Mesh convergence analysis for both the tumor and normal tissue subdomains in the model. For the tumor, the mesh with 589 subunits was chosen and for the normal tissue, the mesh with 2339 subunits was chosen.

RF Voltage Validation

P=VI (3B)

Voltage = 20V	
Current (mA)	*Power (W)*
Low = 105mA	2.10
Average = 140mA	2.80
High = 175mA	3.50
Voltage = 17.6V	
Current (mA)	*Power (W)*
Low = 105mA	1.85
Average = 140mA	2.46
High = 175mA	3.08

Table 2B: Shows the calculation for the power of both of the voltages applied to the RF probe using different current values. The normal range of power is 2-5W, thus it is clear that all of the values are within that range except using a low current for the applied voltage of 17.6V. This can easily be avoided by using a higher current for the treatment.

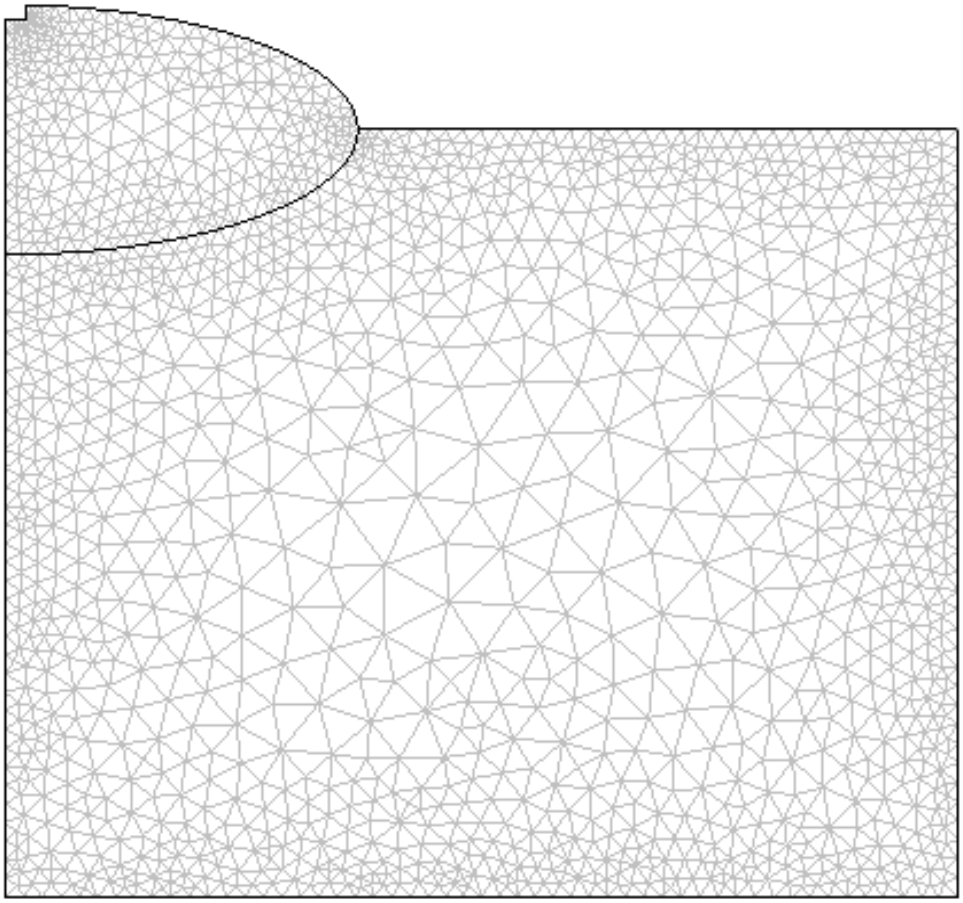

Figure 1B: Mesh plot with 589 subunits for the normal tissue and 2339 subunits for the tumor.

Appendix C: Additional Visuals

For each of the different models reported, both surface plots and point plots over time are given to express relevant data. For the point plots, three are given (points A, B, and C) for each model run in COMSOL. These points are consistent throughout the report to allow direct comparison. All of the points are within the tumor: A is close to the probe, B is in the center of the tumor, and C is near the tumor-normal tissue interface (Table 2, Figure 3).

Location (all within tumor)	r	z
(A) near probe	0.002128	0.039082
(B) center	0.007877	0.034992
(C) boundary	0.011019	0.031673

Table 1C: The coordinates of the points plotted in each of the COMSOL simulations.

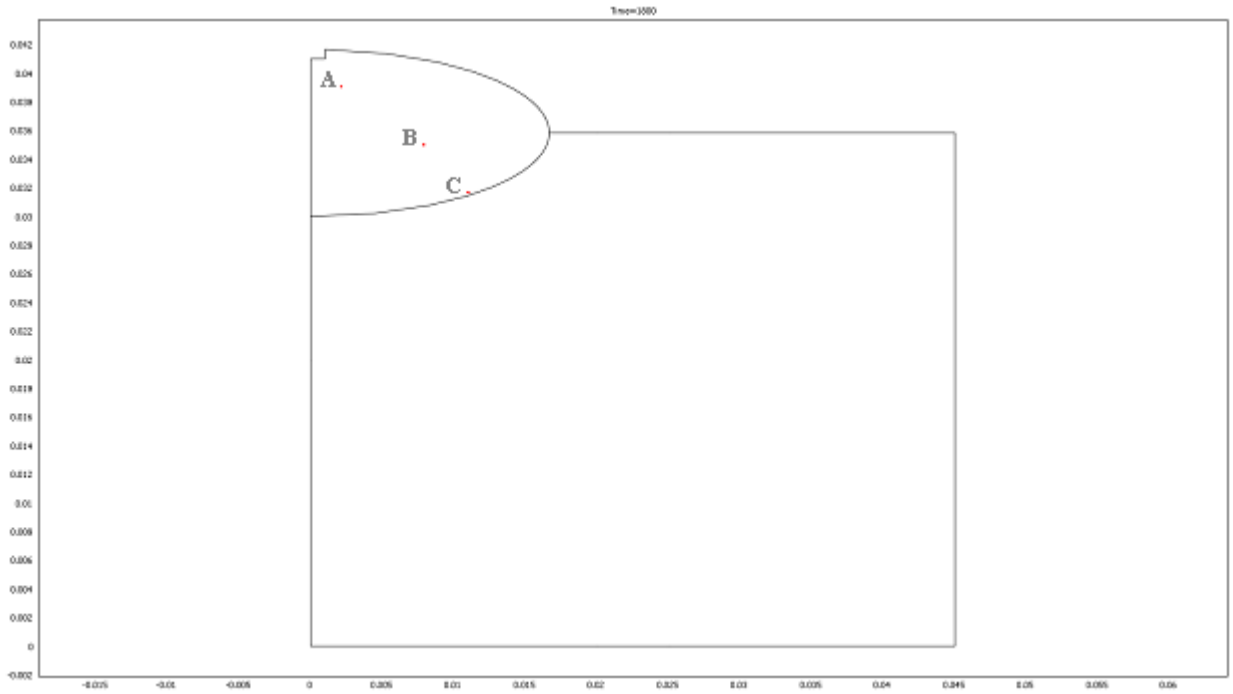

Figure 1C: The physical location of each of the points (A, B, and C) plotted for the different COMSOL simulations.

Freezing Model

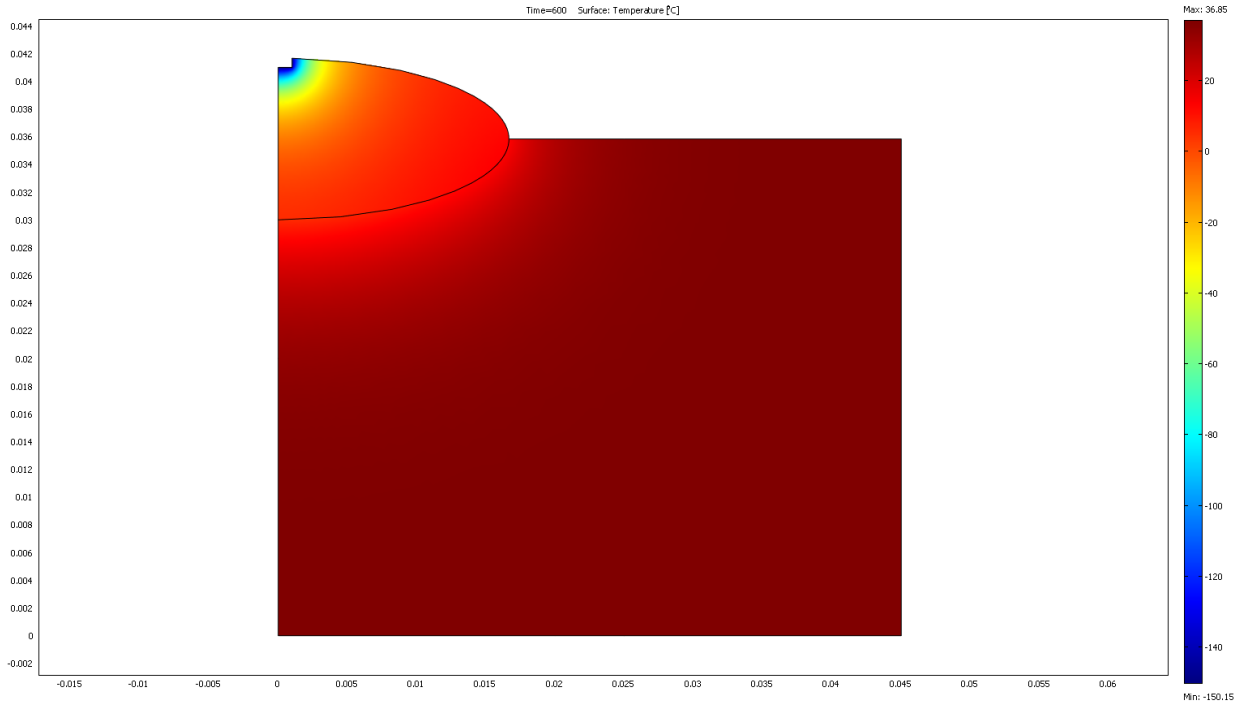

Figure 2C: The temperature surface plot after freezing shows that the tumor tissue immediately surrounding the probe has reached a temperature of -150°C (123K) after ten minutes of contact with the probe. The data from this simulation was saved in COMSOL and then used as the initial condition for the RF heating phase.

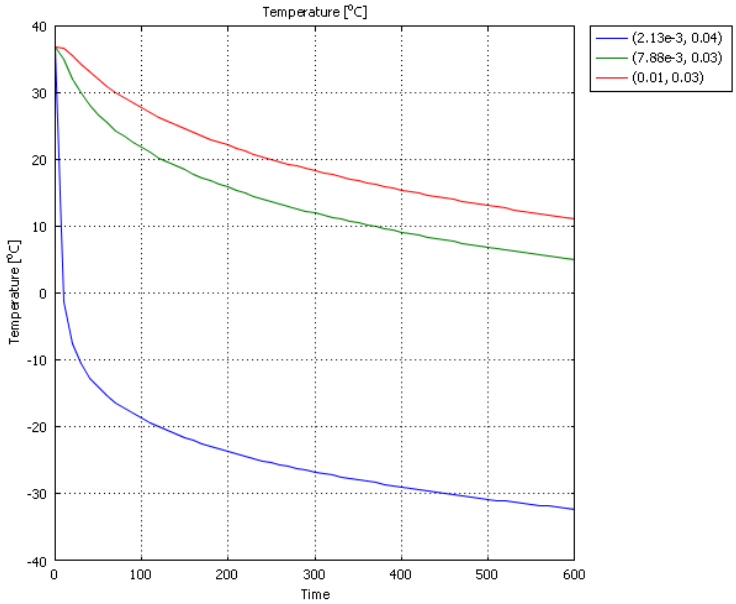

Figure 3C: Temperature profiles after freezing for points A, B, and C defined previously. As expected, the steepest temperature drop is observed closest to the probe in the tumor within the first minute of cooling. The other two points demonstrate a more gradual cooling over the duration of probe contact.

Heating Model: Voltage Determination

Voltage (V)	Radius of Cell Death (mm)
15	3.837
17	6.826
17.5	8.194
17.6	8.684
17.7	8.996
18	9.824
19	11.587
20	12.316

Table 2C: Demonstrates the experimental variation of the voltage of the RF heating probe and the resulting tumor cell death. The optimal voltage was determined to be 17.6V (highlighted).

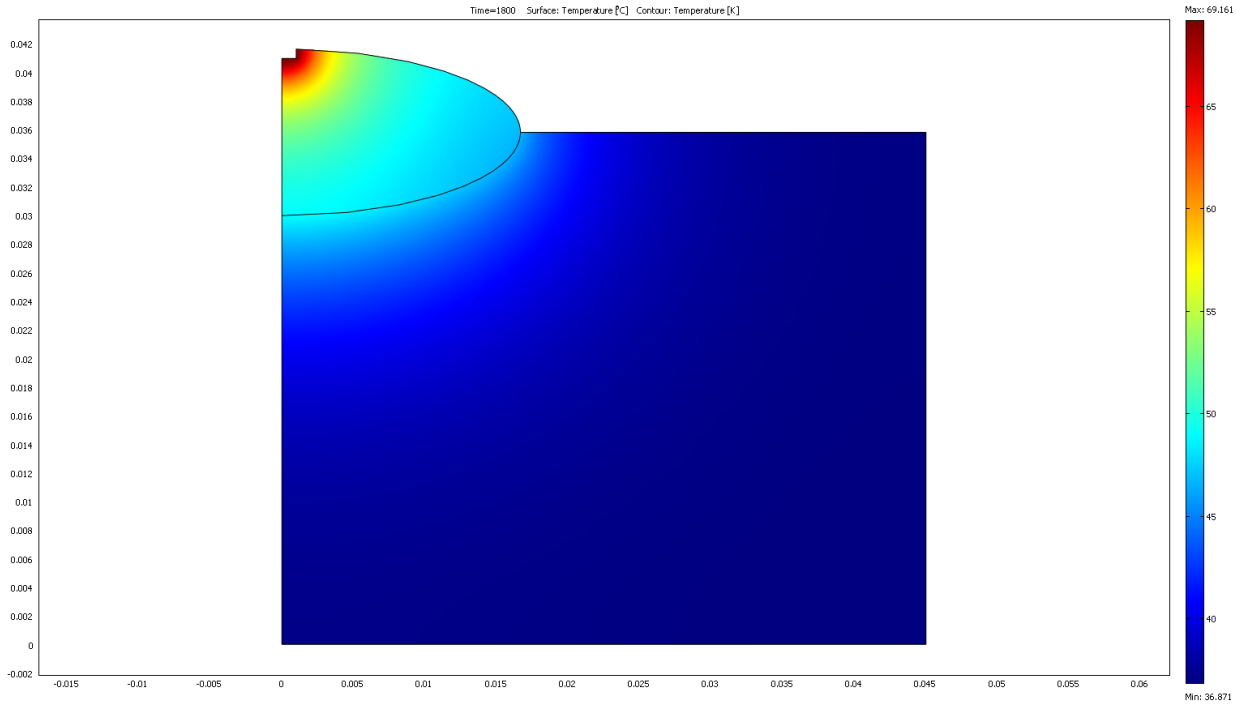

Figure 4C: The temperature surface plot after heating for thirty minutes shows that the tumor tissue immediately surrounding the probe has reached a high temperature of 69.2°C (342.2K) and that the majority of the normal tissue remains at body temperature.

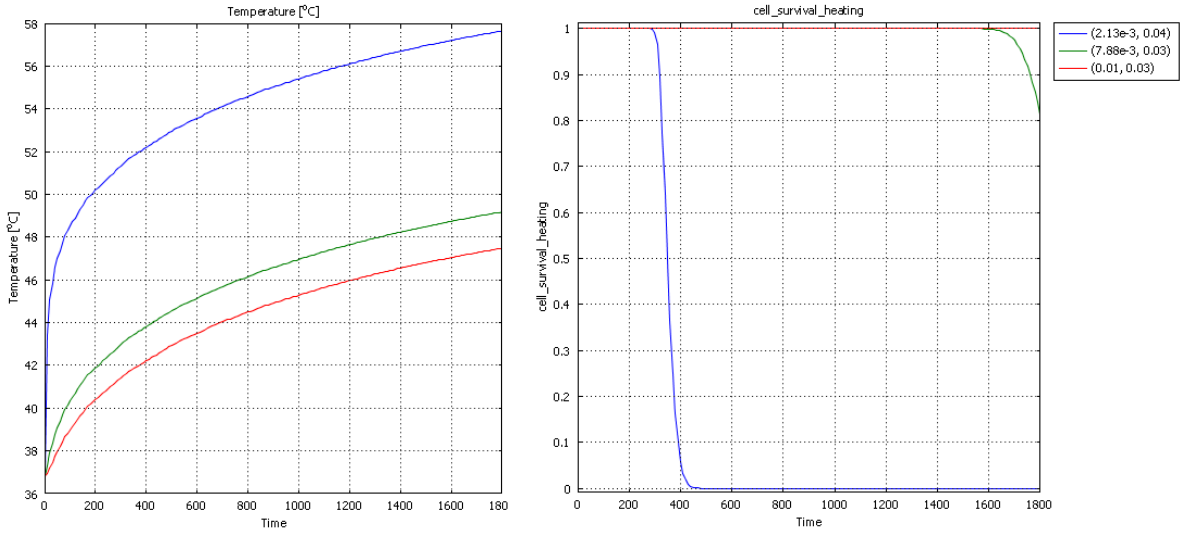

Figure 5C: Temperature and cell survival rate plots for points A, B, and C. For the cell survival plot, it is clear that the equation calculated is designed to either solve for living or death as the interval over which a cell dies is approximately 150s.

Alternative Treatment: Inactivation Energy Determination

Energy of Inactivation (cal/mol)	Radius of Cell Death (mm)
140000	17.686
141000	14.655
142000	12.519
143000	10.676
143100	9.954
143200	9.514
143300	8.909
143500	8.096
143700	7.366
143898	6.772
144000	6.468
145000	4.521
150000	1.7

Table 3C: Demonstrates the experimental variation of the energy of inactivation (ΔH) caused by the pre-freezing and the resulting tumor cell death. The optimal ΔH was determined to be 143,200cal/mol (highlighted).

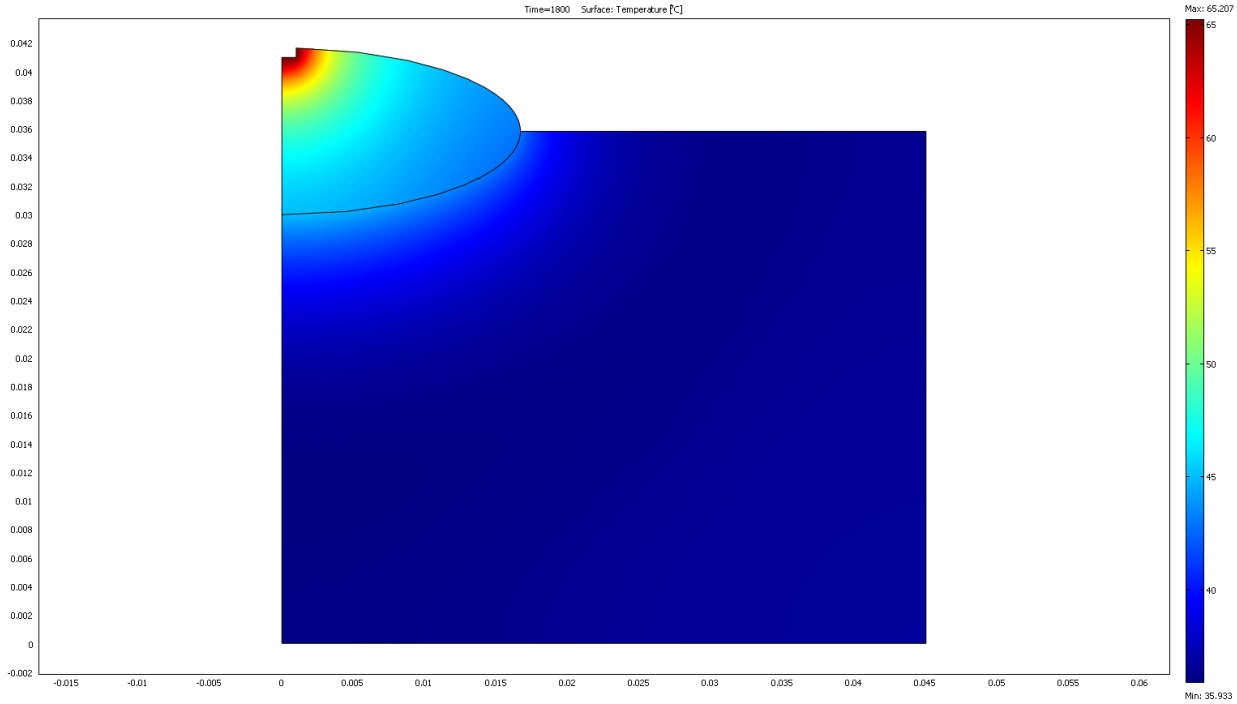

Figure 6C: The temperature surface plot after alternate treatment. This result is very similar to the heating only surface plot, but the maximum temperature is 65.2°C (338.2K), which is less than the maximum temperature of the heating model because the initial temperature was significantly lower due to the ten minute pre-freezing.

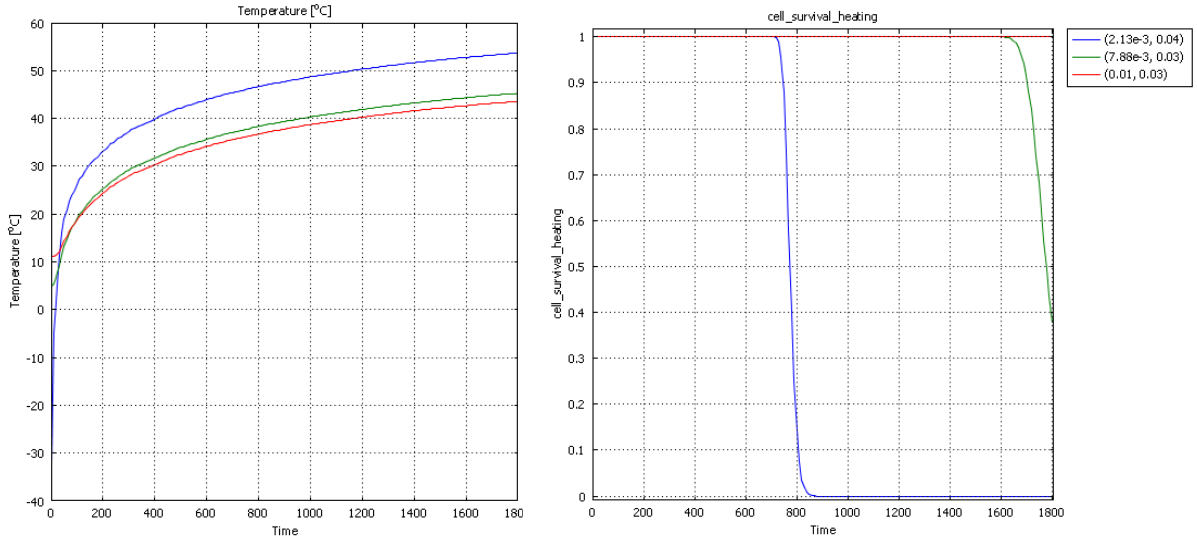

Figure 7C: Temperature and cell survival rate plots for points A, B, and C for the RF heating stage of the alternate treatment. From the initial temperatures of each of the points, it is clear that the initial conditions for the heating stage were yielded from the end result of ten minutes of freezing.

Alternative Treatment: Adjustment to Optimize Tumor Death

Voltage (V)	Radius of Cell Death (mm)
17.6	9.514
18	10.981
19	11.806
20	12.698
21	13.504
22	14.334

Table 4C: Demonstrates the experimental variation of the voltage and the resulting tumor cell death. The optimal voltage for the COMSOL model was determined to be 20V (highlighted).

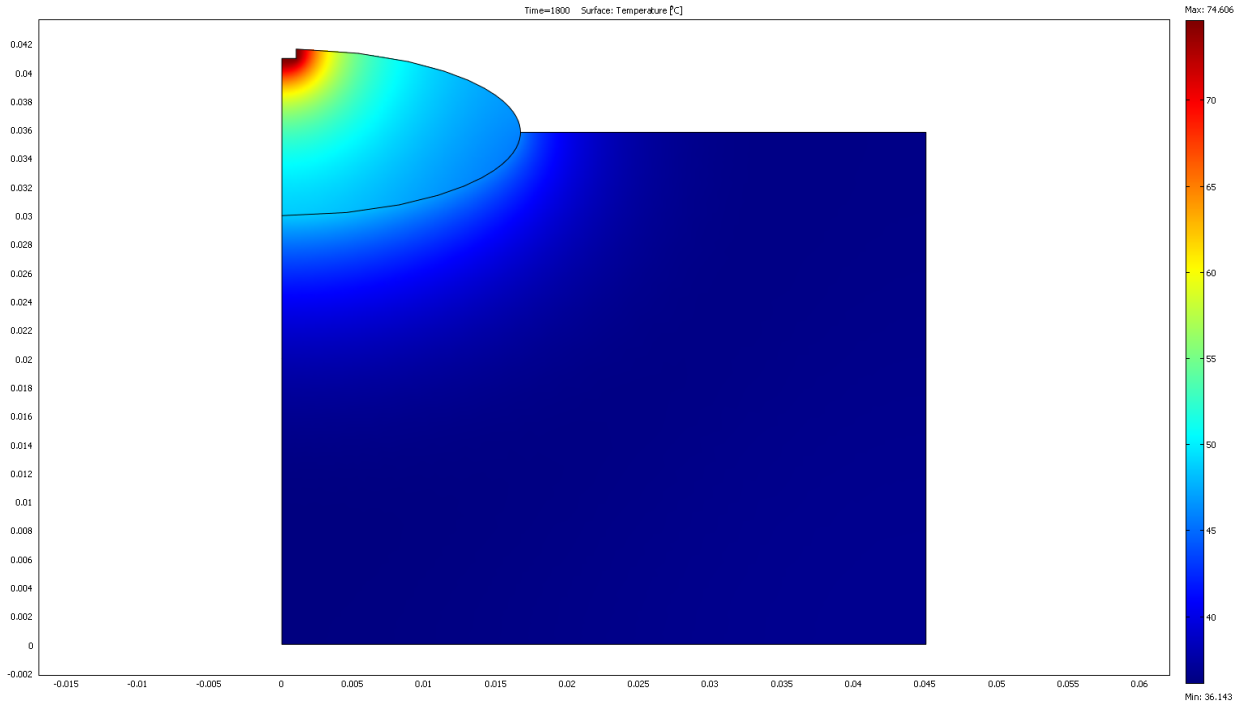

Figure 8C: The temperature surface plot after alternate treatment fit to the COMSOL model. The result is very similar to the alternate treatment modeled with RF heating voltage = 17.6V except the maximum temperature is higher given the higher voltage of RF heating.

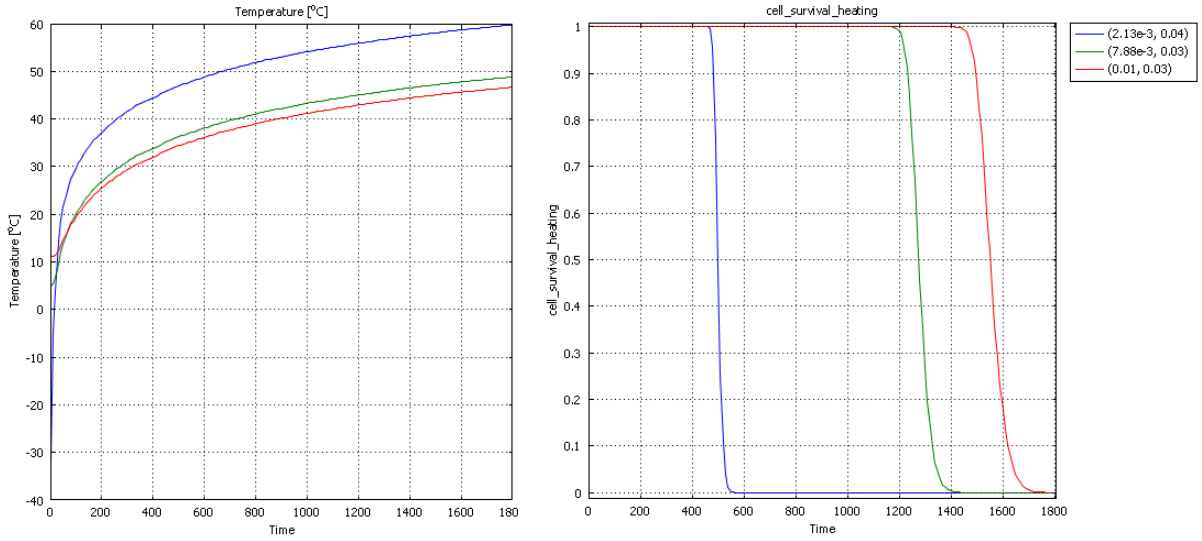

Figure 9C: Temperature and cell survival rate plots for points A, B, and C for the RF heating stage of the alternate treatment. It is clear that all of the points demonstrate tumor cell death at the end of the forty minute treatment.

Sensitivity Analysis

Percent Difference	Energy of Inactivation [cal/mol]	Radius of Cell Death [mm]
-5	136040	Error
-4.5	136756	Error
-4	137472	Error
-3.5	138188	Error
-3	138904	Error
-2.7	139262	Error
-2.5	139620	22.111
-1	141768	15.189
0	143200	12.698
1	144632	10.611
2.5	146780	5.038
5	150360	2.136

Table 5C: Sensitivity analysis of the energy of inactivation was determined and is shown here in table form. The "error" entries indicate values of ΔH for which the program predicted that all of the tissue (tumor and normal) would be killed by the procedure.

Appendix D: References

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