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Thermal Simulation and Analysis of Cell Injury in Cryosurgery of Human Liver Cancer Ablation

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المحاكاة والتحليل الحراري للخلايا المصابة في جراحة التجميد خلال استئصال سرطان كبد الانسان

الملخص

تبحث الدراسة الحالية المحاكاة في تنفيذ جراحة التجميد عند استئصال سرطان الكبد للإنسان. يعد استئصال السرطان باستخدام تقنية التجميد ذات درجة الحرارة المنخفضة تقنية حديثة وصعبة وقد يوصى بها للمرضى مع الأنظمة الدوائية الأخرى، أو للمرضى الذين يعانون من مضاعفات صحية أثناء خضوعهم لأدوية السرطان التقليدية. على وجه التحديد، في هذه الدراسة، يتم محاكاة وتحليل نموذج يتكون من أنسجة سرطان كبد انسان بعد تعريضه للتبريد بدرجة حرارة منخفضة من خلال مسبار مبرد. تم افتراض أن جزء الأنسجة السرطانية في الكبد السليم يبلغ طوله 0.025 مترًا وعرضه 0.025 مترًا وعمقه 0.005 مترًا حيث ان افتراض ابعاد نموذج الانسجة السرطانية في الكبد اتى على أساس ان جزء من انسجة الكبد أصبحت سرطانية وليس جميع انسجة الكبد والتي تتوافق مع الحالات المرضية المسجلة والذي يمكن إيجاده في الدراسات السابقة. أيضا تم إجراء محاكاة النموذج عند درجة حرارة طرف المسبار المبرد – 100°م و– 200°م لمحاكاة الجراحة السريرية الحقيقي لهذه التقنية. وتم اجراء التحليل الحراري بناء على قياس درجات الحرارة عبر أنسجة الكبد السرطانية في 8 مواقع. وبناء على ذلك تم تحديد مراحل التبريد والتجميد والذوبان، وعليه توصي الدراسة الحالية الى تقدير تلك المراحل حيث يمكن استخدامهم لتحديد معلمات التاريخ الحراري المسؤولة عن إصابة الخلية. بالإضافة إلى ذلك، تمت دراسة آلية تكوين بلورات الجليد داخل الخلايا حيث يمكن ان يكون سبب تكونها

ناتج من تأثير الخصائص الديناميكية الحرارية في المجال المجهري إلى جانب المخطط الزمني الحراري للمعاملات مثل معدل التبريد ودرجة حرارة طرف المسبار المبرد فيما يتعلق بمراحل التبريد والتجميد والذوبان.

Thermal Simulation and Analysis of Cell Injury in Cryosurgery of Human Liver Cancer Ablation

Abstract

Our study investigates the implementation of cryosurgery for human liver cancer ablation. Cancer ablation using low-temperature refrigeration technique is a recent and challenging technique and may be recommended for patients parallel with other medication systems, or for patients who experience health complications undergoing other cancer medications. Specifically, in this study, a model consisting of human liver cancer tissues subjected to low-temperature refrigeration through a cryogenic probe is thermally simulated and analyzed using ANSYS software. The liver cancerous tissue was assumed to be 0.025 m in length, 0.025 m in width and 0.005 m in depth. The model simulations were performed at cryoprobe tip temperatures of – 100 ºC and – 200ºC to analog the real clinical implementation of the technique. The temperature distributions across the cancerous liver tissue are obtained in 8 locations. Based on the present thermal analysis the extension of cooling, freezing, and thawing stages are defined and suggested to be estimated prior the implementation of cryotherapy as they can be used to identify the thermal history parameters responsible for obtaining complete cell injury. In addition, intracellular ice crystals to the formation mechanism is studied and their formation is may be subjected to an influence of thermodynamic properties in the microscopic domain besides the thermal history parameters such as cooling rate, cryoprobe tip temperature in relation to the extent of cooling, freezing and thawing stages.

Keywords: Cryosurgery; Liver Cancer; Direct Cell Injury; Intracellular Ice Crystal; Cryotherapy; Cancer Treatment; ANSYS Software.

Introduction

Cancer research still for decades is been challenging area for scientists where they devote their efforts to exploring every possible means that can trigger its risks on human life as it constitute to the high death percentages among various diseases. Therefore, cancer treatments are still developing to adapt the variations of several factors such as cancer type, location, and condition as well as other factors like patient gender and age. A particular cancer therapy can be suitable for some patients whereas not for others having the same cancer type. These facts promoted oncologists to assess the interfering factors and select the best cancer therapy either chemotherapy, radiotherapy, surgery, or cryogenic therapy, for their patients before the

treatment program, to ensure cancer ablation and reduce any complications that can accompany the medication [1].
Radiotherapy is considered one of the major cancer management processes. In addition, radiotherapy can be considered an effective treatment as it decreases the pain and complications that are associated with advanced stages of cancers, however radiotherapy medications requires advanced management science since it requires knowledge of medical physics, radiobiology, radiation safety, radiotherapy planning, simulation and interaction of radiation therapy and other treatment processes (1, 2].

Besides radiotherapy, chemotherapy is considered a common medication for cancer. Chemotherapy drugs are used to destroy or inhibit cancer cells however experiments have shown undesired complications associated with the chemotherapy drugs, such as DNA damage or stopping chromosomal replication. Several chemotherapeutic drugs are naturally occurring compounds extracted from bacteria or plants whereas other chemicals are synthesized to be used for their cytotoxic effects [1, 3].
Surgery can be considered one of the most effective therapy for achieving cure and cancer management. Advances in surgical sciences have enabled surgeons to undertake successful re-sectioning of tumor tissues (1, 4].

It is well known that the number of deaths due to cancer diseases are the largest compared to other sever diseases [5]. This leads to implement much effort to develop all possible means for providing best treatment system and strategy to guarantee a cure and reduce complications associated for cancer patents including cancer type and patient condition. Although much development for classical cancer therapies is found, however efforts still are ongoing forward to provide best drugs and strategies to provide the optimum required results for these medication systems.
Cryotherapy one of promising therapies that is found to provide curative as some therapeutic benefits were found for some medical conditions that undergo cooling process. According to the literature Arnott in 1850 used a salt solution with some ice of temperature -18°C to -24°C to treat breast and uterine cancers [6]. Since then much efforts have been devoted to developing the implemented systems, the usage of commercial liquefied gases by Linde and Hampson enabling much progress in the field of cryotherapy and thus implemented for many diseases [6, 7].
As long as cancer medication is concerned, cryosurgery is undertaken to destroy the cancerous cells in various organs and it is usually recommended with other treatment systems like radiotherapy, or chemotherapy to guarantee complete destroying the cancerous cells. More specifically, cryosurgery allow the killing the cancerous cells present in tissue by exposing the targeting tissue to very low temperature through cryosurgery probe with the assistance of ultrasound, so undergoing controlled thermal treatment including cooling, freezing and thawing processes to achieve cell injury and thus damage tumor cells [5, 8].
Cryosurgery have been used to treat several diseases and disorders such as skin cancers and disorders, oral cancers, liver cancer, prostate cancer, lung cancer, cervical disorders, bone tumors [9]. Therefore, cryosurgery received much attention encouraging many researchers to explore various aspects of this technique through various experimental and clinical investigations to ensure its implementation successfully despite disorder type and location and the health condition of the patients.
As long as cancer treatment is concerned, cryosurgery is not considered as a primary treatment system at least for most cases however, it is recommended parallel with other treatment systems such as chemotherapy or surgery. In addition, cryosurgery can be recommended due to the risk factors of other implemented therapy, or in cases where the therapy develops clinical complications for some patients such as re-sectioning.

Therefore, cancer type, location, size and age as well as the patient's health condition should be determined carefully by the surgeons prior the initiation of the treatment system to obtain the expected optimum results that meet the patient's condition.
Cryosurgery is considered cheap in cost, less invasiveness and requires fewer hospitalization needs [10].
The scope of this study is dictated to the implementation of cryosurgery as treatment of primary and secondary tumors however much attention is more directed to the secondary tumors, which are a major concern for the surgeons who face complications including the mortality percentages with their patients. In the review study of Seifert and Junginger [11] revealed that 15-25% of liver metastasis are discovered in the time of diagnosis of primary colorectal cancer and 20 % will develop liver metastasis after re-sectioning of the primary tumor and the median survival of the liver metastasis patient undergoing no medication is less than a year. In addition, it was reported in the same study that liver re-sectioning is safe procedure with less than 5% perioperative mortality and acceptable levels of morbidity, moreover 20-50% of the patients will have 5-years survivor rate and cure can be achieved in some patient however, re-sectioning is possible for small proportion of patients. Thus, the remaining proportion of patients will have the cryosurgery treatment system as an option rather than re-sectioning.
Moreover, hepatocellular carcinoma is a complication resulted from hepatic cirrhosis besides chronic viral hepatitis B and C, which can develop cirrhosis which turn into heptococelluar carcinoma due to malignant transformation of a dysplastic regenerative nodule. Hepatocellular carcinoma is curatively treated through partial liver resection or liver transplantation where 15 % of hepatocellular carcinoma respond to the treatment system however, child with hepatitis C does not respond to liver partial resection and thus liver transplantation becomes the suggestive curative treatment system. In fact, hepatocellular carcinoma, heptocarcinoma, or heptoma lead to 90 % of primary liver cancer, which is the sixth most common type of cancer and showed a significant increase in its incidence ranking it the third cause of cancer mortality [12, 13]
Liver resection can be considered as a curative treatment system in some cases but in some other cases liver failure can be developed due to inadequate remaining functional liver tissue especially in Child B and C cirrhosis, and thus cryosurgery treatment system becomes a promising option that lead to focal destruction of cancerous tissues resulted from cirrhosis preserving the most remaining liver, where liver resection does not grantee [12, 14]. It should be noted that liver experience bleeding during the thermal treatment more likely to be during the thawing stage as found when examined the freezing stage of pigs liver as freezing stage is not hemostatic for major vessels however freezing can be useful in microcirculatory vessels [15, 16].
Liver has quick response of tumors to the treatment with cryosurgery. The liver lesion exposed (i.e., necrosis) to cryogenic temperature will experience coagulation as freezing will lead to occluding the blood supply to the liver however bleeding of the liver surface constitute a major problem during thermal treatment which can be controlled by cooling rate through means such as placing a thin felt layer between the liver and probe [15, 17, 18]. In addition, large rats liver volume was noted during freezing which was associated with cytokine release, which can be deleterious [15, 19].
The destruction of cancerous tissue is accomplished through the formation process of intracellular ice crystals that accompany the thermal treatment (cooling, freezing and thawing). This intracellular ice crystal is found to be responsible for direct cell injury and thus results destroy of the tumor tissues. Previous experimental and clinical studies have emphasized on the responsibility of attaining the desired destruction of the infected tissue but at the same time the presence and/or absence of intracellular ice crystals and its formation process is subjected to variations among the available studies in the literature and thus failure of reaching a known common and unique systematic mechanism of formation to exactly determine the destruction journey of the undesirable tissue. Understanding the formation mechanism of intracellular ice crystals is essential not only to ensure the required cell injury but also to determine overall picture of cryosurgery treatment success and

thus enable this technique to become universal treatment system despite the cancer and patient conditions. It should be agreed that the dominating cell injury mechanism is truly dictated to the thermal science field to understand various thermofluids processes associated this technique including the microscopic domain [20].
It became well known that intracellular ice formation is subjected to the thermal treatment process that established through sub-processes including cooling, freezing and thawing stages however they are intracellular ice crystals during the freezing stage when intracellular contents are subcooled to temperature beyond its freezing temperature at intracellular local pressure. And since the freezing stage is initiated just after the cooling stage, and thawing stage is after the freezing stage; thus, intracellular ice formation can be considered a function of the thermal treatment process in general, having in mind that the cooling stage contribute to the cell injury resulting in cell membrane disruption and spindle disruption. That is in fact the preparation stage of the next thermal treatment cycle i.e., next intracellular ice formation which is usually tumor tissue undergoes to ensure its curatively based on the tumor tissue type, location, condition and patient health which are usually determined by the surgeons assisted by ultrasound scan. Thus, the thermal treatment history can be established prior the treatment process is implemented.
The above discussion highlights the answers for two major question the first is why cryosurgery is recommended as an alternative treatment system for many medical condition? and second question is why cryotherapy is not considered as a universal treatment system for a particular health condition?
The answer of these questions can be discussed further in this study since there are still some major issues associated with cryosurgery implementation which are represented by some further unresolved question particularly the ones which prevent its implementation as a promising treatment system, among these questions are the followings:

- There is no particular definition in the literature that identify the extend of cooling, freezing and thawing stages in terms of temperature measure and tissue expose time duration, and their relation to the position or location of tissue exposed or subjected the treatment process.
- The presence of only partial explanation associated the thermal treatment of cryotherapy leaving several open issues unresolved clearly.
- The absence of exact definition of the formation of intracellular ice crystallization and its relationship with other affecting factors like tissue type, size and condition as well as the technique tool parameters.
- The absence of exact identification of the tissue type on the thermal treatment which can influence the heat transfer mechanisms and thus the progress of cooling, freezing and thawing processes and as sequences of that the exact cell injury effects associated with these stages. For example the formation of intracellular ice crystals in freezing stage.
- The absence of pre identification of the boundary conditions of the infected organ including organ physiological nature (composition) and organ thermal properties (pressure, density, temperature) that can significantly influence the thermodynamic processes of thermal treatment during cryotherapy because mild change in any related factor can cause larger effects on cell injury tools e.g., intracellular ice crystallization.

The present study aims to address the above needs, which is provided through discussing the simulation results of a liver cancer model that is exposed to cryogenic temperature by the cryoprobe of - 100 ºC and - 200ºC temperatures.

1. Methodology

2.1 Model description

The model in this study consists of mainly two parts; the first includes the cancer human tissues particularly human liver cancer, which is supposed to be subjected to cryosurgery whereas the second part includes the device, which is supposed to be implemented to produce the cryogenic effect particularly cryogenic probe. The following sections will address the general schematic diagram including all components, various descriptions and assumptions of models' components and simulation methodology with the mathematical formulations used.

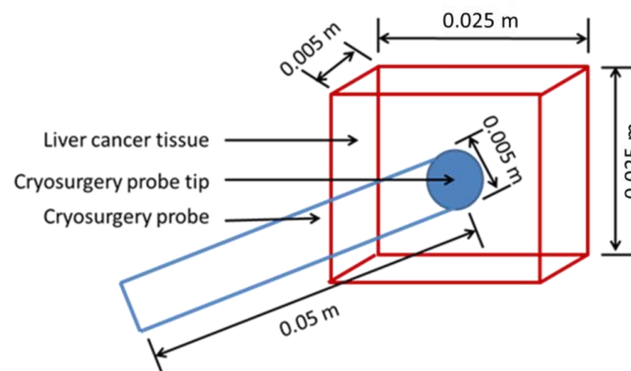


Figure 1: Schematic view of the investigated model

The human cancer tissue of the present model is supposed to be human liver cancer tissue. The physical properties of the liver cancer tissues are taken same as infected ones to analog reality of human liver cancer. The dimensions of the modeled human liver cancer tissue is supposed to be rectangular in shape with 0.025 m length, 0.025 m width, and 0.005 m depth, as shown in Figure 1. Therefore, the physical quantities of the modeled liver cancer with its dimensions were used for undertaking the model simulation; in addition, they are summarized in the following table.

Table1: Physical Properties and Dimensions of Modeled Human Liver Cancer

Physical quantity	Symbol	Unit	Value	Source
Length	L	m	0.025	Assumption
Width	W	M	0.025	Assumption
Depth	D	M	0.005	Assumption
Temperature	T	°C	37	Assumption
Specific heat	C	KJ/Kg. °C	3.62 (normal)	[21]
			3.76 (tumor)	
Thermal conductivity	K	W/(m.°C)	0.46 (normal)	[22]
Metabolic heat production	A^0	W/m^3	3181 (normal)	[22]

The cryosurgery probe used in the model is assumed to have a round-tip diameter of 0.005 m enabling direct contact with infected tissues. In addition, the model simulation was undertaken for two temperatures at the probe tip; the first case at T_p=-100°C and the second case at T_p=-200°C. The refrigeration effect is supposed to be produced by liquid nitrogen however other methods can be used as described in the introduction section. The two temperatures were chosen to give range of the implementation of the cryosurgery based on the type of the infected origins -and thus provide broader picture of cancer ablation mechanisms, and/or enable implementation of this technique as cryo-therapy for other medical conditions rather than cancer diseases such as skin and uterus lesion disorders [6].

2.2 Mathematical Calculation and Solution

The present results are calculated based on the following equations [23]. The first law of thermodynamics states that thermal energy is conserved specializing this to a differential control volume

$$\rho c\left\{\frac{\partial T}{\partial t} + (v)^T\ (L)T\right\} + (L)^T\ (q) = \dddot{q} \quad (1)$$

where,
ρ = Density of human cancer liver, m^3/kg. Input as DENS MP command
c = specific heat of human cancer liver, (kg / kJ °C), Input as C MP command
T = Temperature at lesion surface, (K), =T(x, y, z, t)
t = Time duration of the treatment, (s)

$$\{L\} = \begin{bmatrix} \frac{\partial}{\partial x} \\ \frac{\partial}{\partial y} \\ \frac{\partial}{\partial z} \end{bmatrix} = Vector\ Operator$$

$$\{L\} = \begin{bmatrix} vx \\ vy \\ vz \end{bmatrix} = Velocity\ vector$$

$(v)^T(L)T$ = Blood perfusion, (m^3/s.kg)

{q} = Heat flux vector (output as TFX, TFY, and TFZ)

$\dddot{q}$ = Metabolic heat generation per unit volume, input BF or BFE commands

It should be noted that the terms {L}T and $\{L\}^T$ {q} may indicate as ∇ T and ∇^*\{q\}, where ∇ represents the grad operator and ∇^* represents the divergence operator, respectively.

Next, Fourier's law is used to relate the heat flux vector to thermal gradient:

$$\{q\} = -[D]\{L\}T \qquad (2)$$

Where,

$$[D] = \begin{bmatrix} K_{xx} & 0 & 0 \\ 0 & K_{yy} & 0 \\ 0 & 0 & K_{zz} \end{bmatrix} = Conductivuty\ matrix$$

Where,

K_{xx}, K_{yy} and K_{zz} = Thermal conductivity in the element x, y, z directions respectively, input as K_{XX}, K_{YY}, and K_{ZZ} on MP command

Combining equation 1 and 2, we get

$$\rho c\left\{\frac{\partial T}{\partial t} + (v)^T\ (L)T\right\} = \ (L)^T\ ([D]\{L\}T) + \dddot{q} \qquad (3)$$

Expanding equation 3 to more familiar form, we get

$$\rho c\ \left\{\frac{\partial T}{\partial t} + vx\ \frac{\partial T}{\partial x} + vy\ \frac{\partial T}{\partial y} + vz\ \frac{\partial T}{\partial z}\ \right\} = \dddot{q} + \ \frac{\partial}{\partial x}\ \left[K_x\ \frac{\partial T}{\partial x}\right] + \frac{\partial}{\partial y}\ \left[K_y\ \frac{\partial T}{\partial y}\right] + \left[K_z\ \frac{\partial T}{\partial z}\right]$$

(4)

Assumptions:

It is assumed that all effects are in the global Cartesian system. Three types of boundary conditions are considered. It is presumed that these cover the entire element

1. Specified temperature acting over surface S_1

$$T = T^* \qquad (5)$$

Where T*is the specified temperature

2. Specified heat flows acting over surface S_2

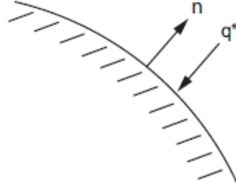
$$\{q\}T\ \{\eta\} = \ q^* \qquad (6)$$

Where,

$\{\eta\}$ = Heat transfer effectiveness through the tissue that is poured due to normal condition or tumor, which can influence the heat flow across the tissue as shown in Figure 2.

q^* = specific heat flow

Figure 2: Specific heat flow



3. Specified convection surfaces acting over surface S_3 (Newton's law of cooling):

$\{q\}^T \{\eta\} = h_f (T_S - T_B)$ (7)

Where,

h_f = Film coefficient evaluated at $(T_B + T_S)/2$ unless otherwise specified for the element

T_b = Bulk temperature of the adjacent fluid (input on SF or SFE commands)

T_s = temperature at the surface of the model

Note that positive specified heat flow is into the boundary (i.e., in the direction opposite of $\{\eta\}$, as shown in

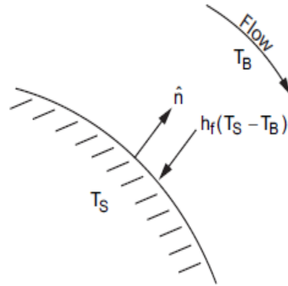


Figure 3, which accounts for the negative signs in equations 6 and 7

Figure 3: Specific convection surface

Now, combining equations 2, 6 and 7, we get

$\{\eta\}^T [D]\{L\} T = q^*$ (8)

$\{\eta\}^T [D]\{L\} T = h_f (T_B - T)$ (9)

Per multiplying equation 3 by virtual change in temperature and integrating over the volume of the element and combining with equations 8 and 9 with manipulation yields

$\int_{vol} [\rho c \, \delta T (\frac{\partial T}{\partial t} + \{v\}^T \{L\}T] + \{L\}^T (\delta T) ([D]\{L\}T) \, d(vol) =$

$\int_{S2} \delta T \, q^* \, d(S_2) + \int_{S3} \delta T \, h_F (T_B - T) \, d(S_3) +$

$\int_{vol} \delta T \, \dddot{q} \, d(vol)$ (10)

Where,

vol= volume of the element
δT = An allowable virtual temperature ($=\delta T(x, y, z, t)$)

2. Results and Discussion

Figure 4 shows the temperature distributions at surfaces of cryo probe and surface of the infected liver cancer for probe temperature equal to -100ºC. The simulation refrigeration treatment was carried for 600 seconds and as can see sharped decrease in the temperature is observed at the surface of the tissue in the first 10 seconds whereas after that a systematic increase in temperature through the remaining time. In addition, it can be seen that the surface temperature at the probe tip remain nearly constant during the first 10 seconds which is opposite trend to the distribution of infected surface tissue but start increasing till the end of simulation time i.e., t=600 seconds similarly same as the distribution of the tumor tissue for the same time however the shape of the two distributions are slightly different.

Figure 4 shows, the temperature distributions across the modeled liver cancer tissue for probe tip temperature – 100ºC, and as we can see in Figures 4a, the temperatures across the tumor tissue at distances of z_1=0.0001 m decrease rapidly due to may be the direct contact between the cryo probe and exposure of tumor tissues. These trends are observed in the same figure at locations z_2=0.0002 m and z_3=0.0003 m across the tissue and as the distances among z_1, z_2, and z_3 are small, the distributions are expected to be nearly identical. But if have a look in Figures 4a, b and c, we will find the trends of the temperatures distributions at z_4=0.001 m, z_5=0.002, z_6= 0.003 m, z_7=0.004 m, and z_8=0.005 m across the tumor tissue are similar to ones of z_1, z_3, and z_3 where the saddle up ends are smoothed in from the z_4 to z_8 ascending systematically with time.

The cooling process is known to disrupt the cell membrane and cell spindle [6]. Specifically, the cell membrane disruption due to cooling lead to uncontrolled mass transfer. Investigating the cooling region in Figure 4a, b, and c, we can see that the cooling regions begins from the first time exposure of the tissue of refrigeration process at first time tissue is exposed to cooling (t= zero) to a time where the temperature is less than zero (t<0), and if we examine this region in Figures 4a, b, and c we will find that cooling region is time and location dependent. In other words, as we deeper across the tissue from z_1 to z_8, the cooling region extends with time from t_1 to t_8 which is may be expected as tissue at deeper locations requires much time to be cooled in comparison to outer tissue locations. Figure 5 shows the temperature distributions across the modeled tumor tissue at probe temperature of -200ºC where similar trends can been seen as at probe temperature of -100ºC in Figure 4, However variation can be found in the extension of the various refrigeration treatments (i.e., cooling, freezing and thawing stages) which is attributed to the implementation of the probe temperature. The following tables focus on these variations for probe temperatures T_p=-100ºC and -200ºC.

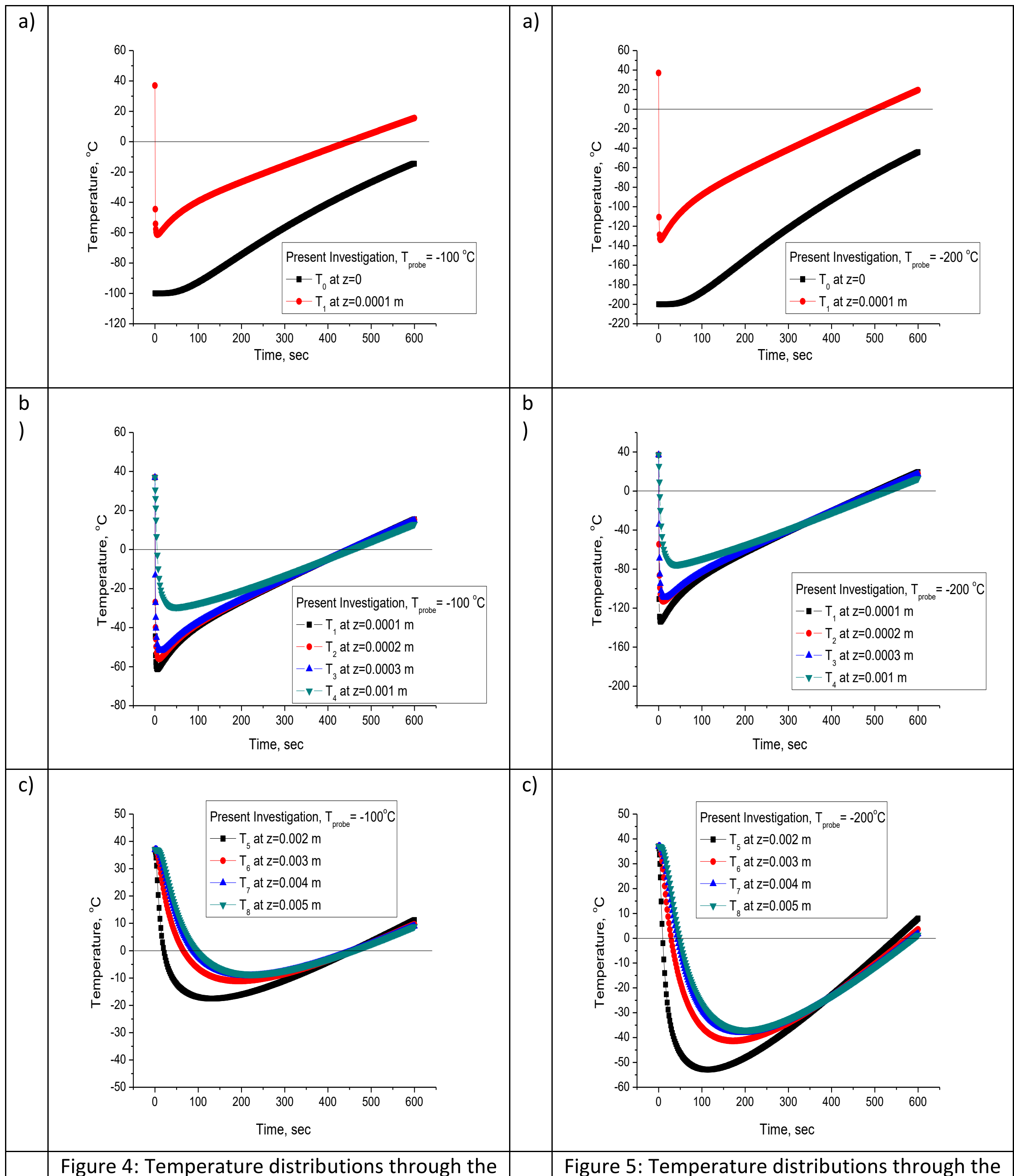


Figure 4: Temperature distributions through the

Figure 5: Temperature distributions through the

	cryogenic probe and cancer liver tissue for case 1, T_{probe}= -100ºC		cryogenic probe and cancer liver tissue for case 2, T_{probe}= -200ºC

Table 2 shows the details of starting and ending of cooling stages for probe temperature -100 ºC and -200ºC. As can be seen for probe temperature equal to -100ºC, the start of cooling zone begins for all locations across the tissue from initiation of cryosurgery treatment and thus the each duration is suspected to be closely from the treatment start e.g., 0.001 seconds and for all locations, and the initial temperatures corresponding these locations is expected to be the initial normal temperatures of the tissues' layers e.g. 37ºC, and this holds true for probe temperature equal to -200ºC as indicated in the same table. But when the cooling stage end is concerned as shown in Table 2 we will see that the cooling stage ends just before the freezing stage start however at further locations across the tissue it was found that the time interval of cooling stage i.e., cooling stage end is larger thus their cooling end duration is more and their corresponding temperatures at those locations is smaller, and this holds true for probe temperatures investigated (i.e., T_p=-100ºC and -200ºC).

Table 2: Cooling stage development at probe temperatures, T_p= -100ºC and -200 ºC

Probe Temperature =-100 ºC						Probe Temperature =-200 ºC					
cooling zone start			cooling zone end			cooling zone start			cooling zone end		
Location, z (m)	Duration, t (seconds)	Temperature, T (ºC)	Location, z (m)	Duration, t (seconds)	Temperature, T (ºC)	Location, z (m)	Duration, t (seconds)	Temperature, T (ºC)	Location, z (m)	Duration, t (seconds)	Temperature, T (ºC)
0.0001	0.001	37	0.0001	0.001	37	0.0001	0.001	37	0.0001	0.001	37
0.0002	0.001	37	0.0002	0.001	37	0.0002	0.001	37	0.0002	0.001	37
0.0003	0.001	37	0.0003	0.001	37	0.0003	0.001	37	0.0003	0.001	37
0.001	0.001	37	0.001	3.793	6.680	0.001	0.001	37	0.001	2	9.345
0.002	0.001	37	0.002	19.64	0.9590	0.002	0.001	37	0.002	8	5.769
0.003	0.001	37	0.003	63.64	0.5460	0.003	0.001	37	0.003	28	1.1389
0.00	0.001	37	0.00	87.64	0.156	0.00	0.001	37	0.004	42	1.2450

4			4		0	4					1
0.00 5	0.001	37	0.00 5	95.64	0.137 6	0.00 5	0.001	37	0.005	48	0.4491 59

In addition, it is may be of interest to note the dominating heat transfer mechanism across the tissue is conduction which is also influenced by medium type i.e., tissue type and thus we also should have in mind the nature of the treated tissue when investigating cooling region in cryosurgery as human organs are different and thus earlier determination before applying cryosurgery is important for cooling process besides tissue exposure time and location across the tissue as was mentioned early. This fact is derived from previous studies where there was identification of the differences between normal and cancerous tissue of living organs [24-27].

Cooling stage as was demonstrated early is a part of the thermal treatment during cryosurgery. It is considered as an essential stage since the required cell injury begins during cooling process. Previous investigations have demonstrated the impacts of cooling process on the cell injury.

It is well known that mammalian cells can resist the effects of low (non-freezing) temperatures but still it was found that exposing the cell to these low temperatures can impose complications on the cell function and thus may be on the cell life. The cell membrane is a bilayer consisting of protein which is impermeable except at locations where the protein is permitted to have mass transfer. Therefore, at low temperatures lipids is transformed into gel phase and as a result of the cooling process protein will be separated, leading to uncontrolled mass transfer. Moreover, as the cell membrane is more permeable, which leads to a change the ions of the cell and resulting in cell damage. In addition, it was found that cooling process influences the cytoskeleton, meiotic spindles, which is affected due to hypothermia leading to tubulin depolymerization. This is supported by the study of Zenzes and his team who found a shortened meiotic spindle when they cooled human oocytes to 0°C for 2 min to 3 min [6, 28].

In addition, cell injury is accomplished through other processes known as freezing process and thawing process which are respectively following the cooling process. Any refrigeration process begins normally from cooling until reaching the required freezing and if the freezing process ends, then thawing begins however thawing may occur even during the freezing in such complicating cryorefrigeration processes like the cryosurgery applications which is subjected to complex transient heat transfer and conduction heat transfer mechanisms as well as the interference of convection heat transfer that may take place in the tissues or even in inside the cell.

As we already know liquids freezes normally at zero centigrade at atmospheric pressure and thus as long as the origin of human tissues is water, we can expect the freezing process during cryosurgery starts at 0 °C (i.e., $T_f \geq 0°C$) and therefore Figure 1a, b, and c we will find that freezing process begins at the first few seconds at external tissue surfact (Figure 1a), and focusing on Figures 2a and b we see that freezing process is a function of time and distance across the tissue. For example, further tissues in location will have delayed starting of freezing process.

Table 3 demonstrates the trend of freezing zone at probe temperature T_p=-100°C and -200°C.

As we can see in Table 3, for cryoprobe temperature, T_p= -100ºC, starting of the freezing process was found to be delayed with location across the tissue and the end of freezing process was found to delay with the same location across the tissue. And for probe temperature, T_p= -200ºC the starting and ending trends of freezing process were be similar trends of probe temperature, T_p= -100ºC - that's delayed starting and ending of freezing process with the location across the tissue.

Table 3: Freezing stage development at probe temperatures, T_p= -100ºC and -200 ºC

Probe Temperature =-100 ºC						Probe Temperature =-200 ºC					
Freezing zone start			Freezing zone end			Freezing zone start			Freezing zone end		
Location, z (m)	Duration, t (seconds)	Temperature, T (ºC)	Location, z (m)	Duration, t (seconds)	Temperature, T (ºC)	Location, z (m)	Duration, t (seconds)	Temperature, T (ºC)	Location, z (m)	Duration, t (seconds)	Temperature, T (ºC)
0.0001	1	-44.49	0.0001	447.64	-0.046	0.0001	1	-110.8	0.0001	500	-0.3898
0.0002	1	-26.91	0.0002	447.64	-0.117	0.0002	1	-54.532	0.0002	506	-0.2570
0.0003	1	-13.12	0.0003	447.64	-0.183	0.0003	1	-34.245	0.0003	508	-0.2118
0.001	5.6	-2.82	0.001	453.64	-0.015	0.001	3	-5.763	0.001	526	-0.13761
0.002	21.64	-0.80	0.002	455.64	-0.074	0.002	10	-2.008	0.002	548	-0.30611

0.003	65.64	-0.003	0.003	455.64	-0.104	0.003	30	-1.078	0.003	574	-0.1213
0.004	89.64	-0.266	0.004	457.64	-0.018	0.004	44	-0.5119	0.004	586	-0.05054
0.005	97.64	-0.251	0.005	457.64	-0.012	0.005	50	-1.1927	0.005	590	-0.04953

However, key difference between starting and ending of the freezing process between the probe temperature investigated is at probe temperature of -100ºC starting of freezing process is delayed much in comparison to probe temperature -200ºC at the same locations across the tissue, and the ending of the freezing process at probe temperature is accomplished at a faster rate at probe temperature, T_p= -100ºC in comparison to probe temperature, T_p= -200ºC.
Moreover, freezing process during cryosurgery is considered an essential stage as was mentioned early besides other processes of thermal treatment that lead to the cell death through what is known as cell injury. A previous experimental and clinical review studies of Yiu and his team [6] have demonstrated the complications associated with the imposing of cells to very low temperatures i.e., freezing temperatures during cryosurgery. Among the hypothesis that was discussed in their informative review study is that the cell injury is a result of high solute concentration that leads to cell dehydration. Another hypothesis was also implicated that suggests the presence of intracellular ice formation that causes intracellular organelle and cell membrane disruption [6, 29]. Another theory that is associated to the cell injury due to freezing injury is related to the altering the immune system so that immune system becomes sensitized to destroy the frozen tissue and any tissue left behind is attacked by the host's immune system after the cryosurgery [30, 31]. The final theory that is believed to cause cell injury during freezing process is related to vascular injury where freezing leads to stasis of blood flow particularly in capillaries resulting in ischemia and thus finally leads to tissue necrosis [32].

The last stage of cryorefrigeration in cryosurgery is the thawing stage, which is believed to halt cell growth producing further cell injury besides cooling and freezing stages.
Careful examination of Figure 2a, b and c yields to put markers defining the thawing stage which can be identified at temperature above 0ºC (T≥ 0ºC) till the end of treatment, taking the temperature equal to zero centigrade attained as a reference point. Table 4 shows the limits of thawing zone for probe temperature -100ºC and -200ºC demonstrated for each location across the tissue and corresponding to the time and temperature. As shown in Table 4, for both probe temperature investigated, thawing zone begins at later time with location across the tissue. Another remarkable observation that for deep issue, which experience delayed thawing zone start, tissue layer temperature will be smaller in comparison to previous layer, which experiences faster thawing zone start.
Thawing process is considered one of the essential processes during cryoabltion of cancerous cells in targeted tissue. Previous investigations have found that the effectiveness of thawing process is related to the recrystallization and solute effect. For example, slow thawing permits the occurrence of maximum ice growth

during crystallization and solute effects. More specifically, it was found that solute effect and ice growth lead to the cell vanishing [33]. But also, rapid thawing after rapid cooling is also beneficial as ice crystal tends to be smaller with higher surface energies although larger ice crystal can be more destructive compared to smaller ones as larger ones impose larger forces during recrystallization. This fact finds support as red blood cells at faster cooling and warming rates have higher survival rates [34].

Table 4: Thawing stage development at probe temperatures, T_p= -100°C and -200 °C

Probe Temperature =-100 °C						Probe Temperature =-200 °C					
Thawing zone start			Thawing zone end			Thawing zone start			Thawing zone end		
Location, z (m)	Duration, t (seconds)	Temperature, T (°C)	Location, z (m)	Duration, t (seconds)	Temperature, T (°C)	Location, z (m)	Duration, t (seconds)	Temperature, T (°C)	Location, z (m)	Duration, t (seconds)	Temperature, T (°C)
0.0001	449.64	0.161	0.0001	600	15.52	0.0001	502	0.012	0.0001	600	19.47
0.0002	449.64	0.087	0.0002	600	15.25	0.0002	508	0.13	0.0002	600	17.81
0.0003	449.64	0.019	0.0003	600	14.99	0.0003	510	0.17	0.0003	600	17.44
0.001	455.64	0.162	0.001	600	13.02	0.001	528	0.21	0.001	600	12.90
0.002	457.64	0.076	0.002	600	11.19	0.002	550	0.008	0.002	600	7.92
0.003	457.64	0.022	0.003	600	9.64	0.003	576	0.161	0.003	600	3.56

0.004	459.64	0.098	0.004	600	9.03	0.004	588	0.220	0.004	600	1.85
0.005	459.64	0.010	0.005	600	8.83	0.005	592	0.2176	0.005	600	1.28

In common, cryosurgery applications usually rapid cooling, slow thawing, and repetition of the freezing/thawing cycle are used, as rapid cooling is more destructive than slow cooling and the response of cells to cooling rates are cell-dependent. In addition, it was found that tumor cells can retain more cellular water than normal one and thus tumor cells have lower degree of dehydration than normal cells. Also as long as dehydration is concerned it was found that there is a sensitivity among malignant cells for example higher dehydrations rates are found in normal human liver cells, metastatic colon cancrionma and primary heptomacellural carcinoma [35].

Moreover, it was found that lethality related freezing process is increasing with the decrease end temperature. Bischof and his associates [36] exposed AT-1 rat prostate tumor cells to similar cooling rates of normal cells, and they found that cell viability decreased which was a function of end temperature where it reached zero at about -60ºC. Following the previous study, present results suggest for the same freezing cycle, lowest end temperature can be obtained at external tissue surfaces than deeper tissue surfaces and more quickly in the external tissue compared to deeper tissue surfaces, across the hall tissue under treatment. This suggests that prior determination of the size of the treated tissue to determine the lowest end temperature and thus maintain it during cryosurgery to grantee maximum tumor cell lethality and thus permit higher cure rates.

Other factors can influence the cell viability rates beside the end temperature during freezing these factors include the time duration of the tissue as frozen condition. Gage and Baust [37] found that cooling duration i.e., holding time of human prostate cancer for 20 min at -10ºC has more destructive pattern compared holding it for 10 min time. In addition, Rubinsky [38] found that keeping the tissue frozen lead to progressive effects on the cells. These findings lead to carefully considering the time duration where the cells are kept frozen to reach the required complete cure of tumor cells during cryosurgery and thus pre-assessment of the tissue to be under treatment is important to estimate the time duration across layers of the tumor tissue that need to be in frozen condition as the described and shown in the present results for the freezing stage in both probe temperatures, T_p= -100ºC and -200ºC.

Figures 6 and 7 show the development of heat transferred through tumor tissue and cryoprobe for both temperature investigated (T_p=-100ºC and -200ºC) which both show the dramatic temperature color change during the treatment duration.

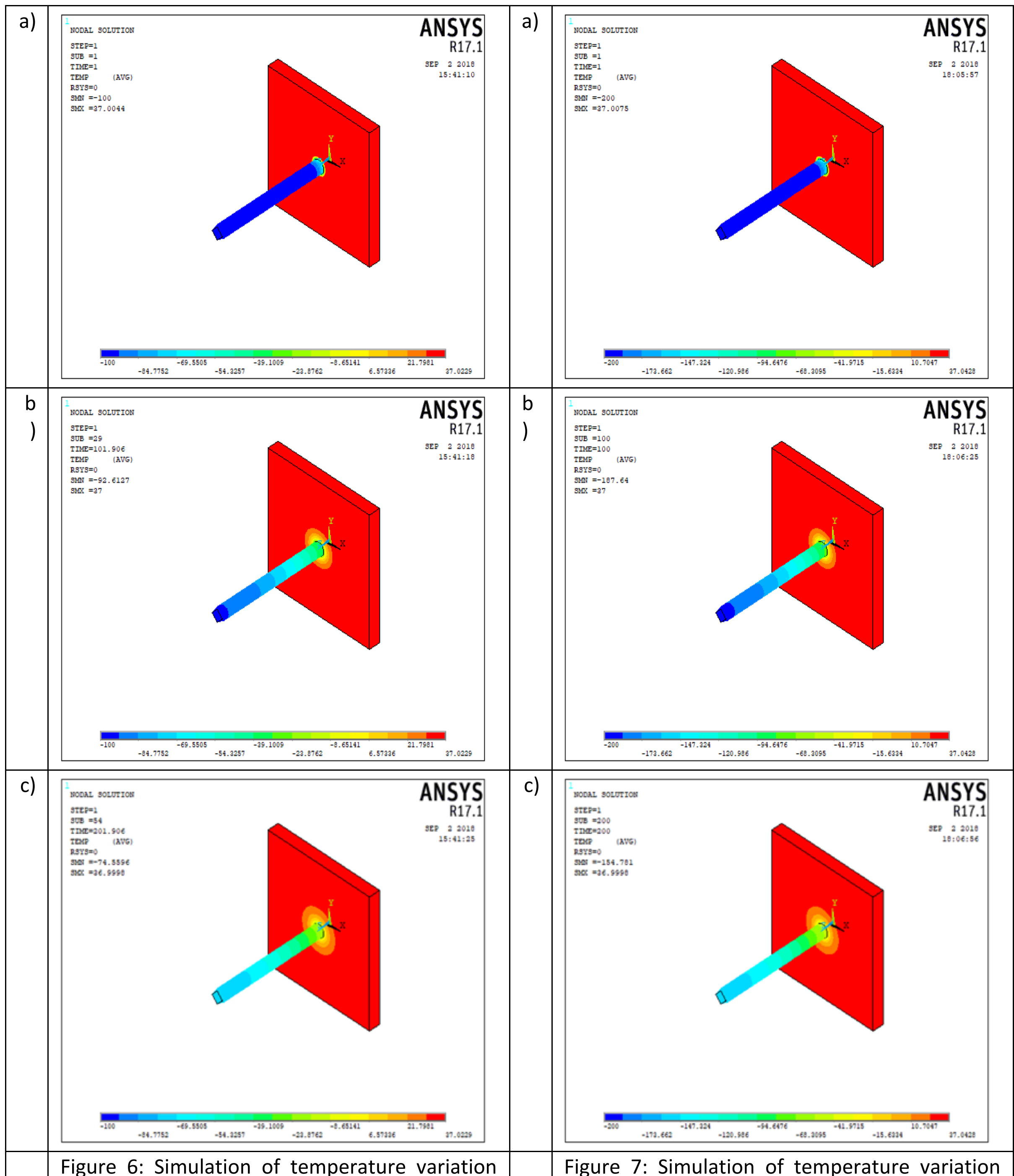


Figure 6: Simulation of temperature variation

Figure 7: Simulation of temperature variation

	development through the cryogenic probe and cancer liver tissue for case 1, T_{probe}= -100ºC		development through the cryogenic probe and cancer liver tissue for case 2, T_{probe}= -200ºC

2.1 Further insight into the cryogenic treatment during cryosurgery

Further investigations have related the direct cell injury to the two different dominating mechanisms one is the minimum volume hypothesis whereas the other mechanism of cell injury is related to cell membrane destabilization [6]. For the minimum volume hypothesis it is believed that during slow cooling rate freezing will take place in the extracellular space where the cells attempt to maintain equilibrium with an extracellular solution by osmosis [39]. This was explained as follows: the cells will be exposed to high extracellular solute concentration during slow cooling, the cell shrink. In addition, the solute concentration rises preventing the cell to remain in its volume then chemical potential gradients will be decreased by extracellular salt transfer to the cytoplasm leading to high intracellular solute concentration. Moreover, during thawing, the cell

contents are highly concentrated compared to extracellular solution, in addition, sudden exposure to hypotonic solution will lead to osmotic rupture of the cells [40].

However minimum volume hypothesis was used but two caveats regarding its application. The first is related to absence of actual minimum volume when precise measurement is performed. Whereas the second caveat is presence of similar hemolysis at different cell volumes using different glycerol concentrations. Thus, while cell shrinkage and re-expansion are essential causes of cell damage, reduction of cell volume is may not the dominating cause of cell injury [41].

Steponkus and Lynch [42] suggested another mechanism that is believed to cause direct cell injury which is related to the destabilization of cell membrane during freezing –thawing processes. More specifically, Steponkus and Lynch [42] found that almost of 80 % of the cell water was removed when they cooled their cells from 0ºC to -5 ºC where the nonacclimated protoplasts shrink to minimum volume during freezing induced dehydration. But when thawing process is applied, the cells re-expanded but lysed before reaching its original volume. Moreover, when cells were initially cooled to a lower temperature such as -10ºC, around 90% of cell water was removed osmotically and when cells were thawed, the cells were unresponsive osmotically and did not respond attributing this to cell membrane damage.

Regarding to the effect of intracellular ice formation on the cell injury, it was suggested that extreme cooling of cell water has higher chemical potential that force the cell water to leave the cell and thus freeze extracellularly, in addition when slow cooling is allowed the cell becomes dehydrated, however when the cooling rate is higher, then the intracellular ice will be formed [6]. Mazur [43] proposed the presence of intracellular formation is due to the propagation of extracellular ice through aqueous pore of cell membrane where this theory is called protein pore theory. Berger [44] and Acker et al., [45] experimented salivary gland and confluent cell monolayer, respectively, where both teams demonstrated the propagation of ice through gap junctions that strongly temperature dependence and thus support protein pore theory. Another theory that explained the presence of intracellular ice is related to the disruption of cell membrane at critical osmotic pressure gradient across the membrane during freezing process [46].

However, the intracellular ice formation is still interesting for research as a complete explanation of this mechanism is needed. Nevertheless, we may still discuss what other investigators reached and thus try to link their findings with the present work by applying thermal science theories.

The presence Intracellular ice formation is mainly related to freezing process and thus it is a function of cooling rate i.e., freezing temperature. In other words, the intracellular ice and its size should be related to the freezing temperature. Therefore, as long as cell injury is concerned, the presence and size of intracellular ice can determine the rate of cell injury and thus success of cryosurgery treatment. Easily, one can recognize the presence and size of intracellular ice is related to the thermal mechanisms that are associated with the freezing and thawing processes that result finally in cell injury. If we consider the theories that suggest the propagation of intracellular ice from outside the cell either through aqueous pores of the cell membrane or due to membrane disruption, we will find both theories may exist which are based on thermodynamics theories, however the application of a particular theory is subjected to various factors such as the slight effect of tissue nature and cooling rate. More specifically, if we consider the cooling rate effects, we will see that if slow cooling is allowed, the extracellular space will experience freezing temperatures thus content outside the cell is frozen. This will lead to have low pressure region due to low temperature outside the cell that will drive the cell water of higher pressure inside the cell to outer region of the cell. If such a case happened at slow freezing rate then the solute concentration inside the cell rises (because of water leaving the cell) until the freezing is penetrated to the cell inside. Therefore cell will experience shrinkage (i.e., minimum volume theory [6]). But if the cooling rate is faster, then cell outside will be exposed to faster freezing creating lower pressure region however the freezing will be penetrated more thus cell contents will be freezing rapidly preventing cell water of lower pressure to escape from cell inside (i.e., getting freezing outside and inside cell contents simultaneously or nearly simultaneously). In addition, formation of intracellular ice can be related to the entrance of extracellular ice to cell inside through cell membrane as proposed in previous studies [6, 15]. Considering thermodynamics principles we may add that formation of intracellular ice can be due to rapid cooling i.e., very low freezing temperatures, which penetrate freezing rapidly inside the cell thus preventing cell water from driven outside the cell resulting of forming intracellular ice. Therefore cell inside and outside can have different freezing point temperatures. Other factor that may affect the intracellular ice formation include the tissue type, which influences the dominating heat transfer mechanisms.
It is worth mentioning that the discussion of the present results is dictated to explore the direct cell injury, not immunological or vascular injuries, which are beyond the scope of the present study and thus left open for future research.

3. Conclusion

The present study investigated the direct cell injury in cryosurgery of a human liver cancer model at two cryoprobe temperature of -100ºC and -200ºC. The thermal analysis led to various findings that are worth to be considered in the field of cryosurgery treatment of cancer, among these findings:

- The extension of each stage of the thermal treatment process (i.e., cooling, freezing and thawing stages) have been defined in the present study which are essential to be estimated before the implementation of particular cryosurgery treatment for any patient. This pre-estimation is important to fix the boundary condition of the thermal history parameters such as tissue freezing lowest temperature, time duration of cooling, freezing and thawing stages following the tissue type, size, depth and cryoprobe tip temperature.
- The presence of intracellular ice crystals formation mechanism depends on:
  1) boundary conditions presented in above point
  2) thermodynamics properties (pressure and temperature) during any thermodynamic process and should be kept in mind that any phase change occur outside or inside the cell is sensitive to minor change of the correlated pressure-temperature and thus their minor change can be

translated to distinguished variations on the formation of intercellular ice crystals or even extracellular ice crystals formations.

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