Numerical modeling of cryospray for treatment of skin tumor

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by

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ROURKELA

CERTIFICATE

This is to certify that the thesis entitled. "*Numerical modeling of cryospray for treatment of skin tumor"* submitted by Mr. Mujtabha Khursheed Magrey in partial fulfillment of the requirements for the award of Bachelor of Technology Degree in Biomedical Engineering at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by him under my guidance.

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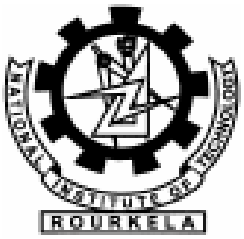

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ABSTRACT

Cryospray as a mode of cryosurgery has come up as a novel and revolutionary clinical technique to ablate undesired tissue, malignant and benign, ranging from skin lesions and boils to keratosis and skin tumors. Cryosurgery is effective and fast way to treat such skin abnormalities. A numerical model for the same is hence highly desirable that can prefigure the various thermal and physical characteristics changes in the targeted tissue. The numerical solutions are derived using a two-dimensional model of the skin having three layers namely epidermis, dermis and subcutaneous fat, these layers are all considered to be homogenous and the thermal and physical properties of these layers are incorporated into the model to generate results. The Pennes equation for bio heat transfer in polar coordinates is taken and different constraints for the blood perfusion and the metabolic heat generated in each layer are considered. The equation is solved using typical boundary conditions like Dirichlet and Robin's conditions for clinical use. We use implicit method for discretization of unsteady term and central difference scheme for discretizing diffusion term which give us second order accurate result in time and space. The model not only takes into account the freezing process but also puts into perspective the thawing process which is equally effective in the destruction and damage of the target tissue. The slow thawing increases the water content causing the low osmotic pressure on the outside and hence large uptake of water by cells leading to their rupture. The damage to the tissue is calibrated in terms of the propagation of the frozen area during the spray time and then during the thawing process.

We also study the temperature changes during the freeze-thaw cycle at various depths within the skin tissue for further understanding and establishment of the process. The results for the propagation of the frozen zone are expected to provide substantial numerical and graphical information about cryospray and hence give the basis for further improvements in the current cryosurgical procedures.

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CHAPTER 1

INTRODUCTION

1.1 Historical review

The therapeutic use of using low temperatures manipulations of the skin has been documented as far as 2500 B.C. Egyptian papyri [1, 2]. The use of ice for anti-inflammation and as a local anesthetic also dates back to the same time. Continuing into the 5th century Hippocrates described cold therapy as a treatment to relieve pain of trauma and diseases affecting the bones and joints [2]. This continues further as cold baths and wet sheets were used as means to reduce fever [3]. The anesthetic properties of the cold were used in operations particularly in case of amputations when the surgical means and techniques used gruesome red hot tools for operating, also blood loss could be prevented if the part under surgery was covered with ice [2, 4]. Benjamin Franklyn was making notes on this as soon as 1773.

However the cryosurgery in its original essence using the high sub zero temperatures was not developed until the 1850s, it was at this time that instrumentation and physics were at boom [5]. It was 1845 and 1851, that Dr. James Arnott [6], a British physician who applied this freezing ice formation therapy during a surgery of a patient with cancer. He used a solution of sodium chloride (NaCl) and crushed ice maintained at -12^{o}C to treat lesions of the breast and uterus. As far as the surface tumors were concerned, a water cushion with the solution was used that froze the lesion surface and developed a white hard appearance, without any discharge or hemorrhage. Even after thawing the tumor became less visible.

Then this was followed by a lag phase till stronger and more powerful cryoagents capable of producing extremely low temperatures were available. It was only in the late 19th century that developments came that put cryosurgery in all new stead [7]. Few of these developments have been enumerated below:

In 1877, Louis Paul Cailletet, a French mining engineer produced liquid oxygen by some adiabatic expansion system. In the same year, Raoul Pictet, a Swiss physicist successfully produced liquid oxygen by cascade process, and then in the early 1880s it was S. von Wroblewski and K. Olsenski who first liquefied nitrogen, which was shortly followed by Carl von Linde's realization of industrial liquefaction of air. For the coming few years all the other permanent gases were liquefied.

This also led to the development of the first vacuum-jacket vessel for the storage of cryogenic fluids in 1892 by James Dewar, a British professor, this vessel greatly prevented the evaporation of the cryogenic fluids and hence made progress possible in their preservation [3, 5]. The first documented medical use of liquefied air was reported by Dr.Campbell A. White [8] and Proffesor Charles Tripler in 1899 according to them it could treat warts, nevi, varicose leg ulcers, chancroids, boils, epitheliomas and herpes. Other cryogens viz CO_2, He, N_2, H_2, were also produced and in these processes liquid nitrogen (LN_2) was produced as a byproduct which latter on was reported to treat skin ailments by Allington [9].

The modern cryogenic probes came into being with the advent of the first cryoprobe by Dr. Irving Cooper [37] a simple closed system with continuous flow of liquid nitrogen (LN_2). This introduction of cryoprobe greatly boosted cryosurgery by adding new applications in the latter half of the 20^{th} century, examples of use of cryoprobes are; Gonder *et al.* [38] used cryoprobes in treatment of prostate diseases, Marcover and Miller used the same in orthopedics [39], Cahan and coworkers added a new structural element that was the heating element which could facilitate the easy removal of the probe from the frozen tissue surface [40].

Cryogenic spray: The first cryospray medical usage was done by Whitehouse in 1907 [10]. The design was a simple bottle for spraying liquid air onto the lesion surface. This liquid air was later on replaced by LN_2. The first commercial-grade spray was by Zacarian [11] in 1969 and the development since has brought newer and better spray devices into the market.

1.2 Literature review

The acceptance of cryosurgery has greatly increased with the better understanding and development of the procedure. Since long, cryosurgery has been used as novel means to treat skin abnormalities like boils, carbuncles, keratosis etc using cold temperatures [5]. These technologies or means used to reduce the temperature of the skin areas under consideration. These techniques can be broadly classified under three heads:

1. Cotton swab or copper cylindrical discs.
2. Cryoprobe.
3. Cryogenic spray.

The cotton swab technique applies LN_2 directly to the lesion surface using a cotton swab, but this technique has the drawback that deeper seated lesions cannot be destroyed using this technique. Cryoprobe on the other hand is a closed system with a continuous circulation of LN_2 and can be used to treat both surface and visceral tumors like kidney, liver etc. In cryospray a typical spray device comprises of a dewar bottle for storing the cryogenic fluid, a delivery pipe, trigger and changeable nozzles is used to directly spray the LN_2 and nitrogen mixture to the targeted tissue area.

The process of spraying is simple; the nozzle or the vent of the spray device is positioned at a distance of 1-1.5 cm over the lesion of abnormality and is triggered which results in the spray of fine droplets of LN_2 with nitrogen that get stuck to the skin surface and the fast rate of vaporization of this LN_2 causes quenching and hence the tissue freezes rapidly.

Cutaneous cryosurgery or cryospray can be used to treat actin keratosis, surfacial tumors, skin lesions, warts etc. Keratosis is the deposition of keratin and actin keratosis is a premalignant condition in which clusters of keratin are deposited on the skin in flat mounds or horn forms. It is more common in fair skinned people and more prone in areas of high sun exposure [33, 34]. This can proceed to squamous cell carcinoma if left untreated. We are considering this condition and treating it with liquid nitrogen cryospray. The cryospray technique is used in most cases as it is an effective, fast, cost efficient and aesthetically sound (since leads to less scarring) mode of skin abnormality treatment. As a technique being still nascent as compared to other surgical methods cryosurgery still faces an uphill task as there are still lots of doubts about the mechanism and

maturity of the process. A typical cryospray procedure requires the complete monitoring and right use of various parameters like freeze time, temperature achieved, cooling and thawing rate. All these have to be normalized in order to prevent healthy tissue loss and recurring of the abnormality, this can be modeled aptly using a stratified approach of the skin tissue and using finite volume analysis.

Cryotherapy has been globally accepted in the latter half of the 20^{th} century as the best technique for quick and effective treatment of skin abnormalities. The various advantages of cryosurgery are [35]:

1. No or minimal blood loss during the procedure which greatly reduces the risk factor, moreover lesions nearby major blood vessels areas can also be treated.
2. It is a highly directional technique and can be used to particularly ablate a specific area without adversely affecting the surrounding tissue; this is of great importance when it comes to lesions in the vicinity of visceral organs like liver, kidney.
3. Short duration of surgery and the quick recovery time.
4. Self anesthetic effect of the cold negates the use of anesthesia.
5. It can be used on both malignant and benign tumors.
6. Less offensive after the procedure, due to minimal scarring.
7. Lastly, it prevents the spread of tumor as there is no metastasis which is highly probable during operative procedures which involves incision and cutting of tumors.

Cryosurgery affects the tissues at the macro and micro scale leading to damage through various processes. The macro scale effect can be understood as the propagation of an ice-ball (frozen region) or ice front through the tissue which is definite and determines the boundary up till where the damage occurs. The living tissues do not freeze at a particular temperature they do so over a range during which a lot of phase changes occur. This range is known as the intermediate or the mushy zone which lies between 0^oC to -10^oC [36]; above 0^oC the tissue is normal and below -10^oC the tissue is completely frozen.

Various cryogens are used as agents to induce cold temperatures, among these are CO_2 , He, N_2, H_2, CH_4, O_2, F_2 and the properties of these gases are listed in table 1:

Table 1.Various cryogens are used as agents to induce cold temperatures among these are CO_2, He, N_2, H_2, CH_4, O_2, F_2

Gas	Helium - *He* -	Hydrogen - H_2 -	Methane - CH_4 -	Nitrogen - N_2 -	Oxygen - O_2 -	Fluorine - F_2 -
Density at NPT *(Kg/m^3)*	0.1649	0.0833	0.6647	1.1597	1.3247	1.5720
Boiling point at 1 atm *(^{o}C)*	-269	-253	-161	-196	-183	-188
Vapor density at boiling point *(Kg/m^3)*	16.9795	1.3455	1.7780	4.6133	4.7414	
Liquid density at boiling point *(Kg/m^3)*	122.0606	70	423.84	807.49	1141.63	1508.93
Heat of vaporization *(KJ/Kg)*	20.46	449	510	198	213.3	172
Critical Temperature*(^{o}C)*	-268	-239	-82	-147	-118	-129
Critical Pressure *(KPa)*	229	1294	4641	3394	5080	5573

Since our major focus is Liquid Nitrogen Cryospray so we will elucidate some more specific thermal and physical properties of LN_2 at 1 atm in table 2.

Table 2. Properties of liquid nitrogen LN_2

Property	Unit	value
Density	Kg/m^3	807.3
Normal Boiling Pt.	K	77.36
Latent Heat	KJ/Kg	199.3
Viscosity	Pa.s	$158x10^{-6}$
Speed of sound	m/s	856
Specific heat	KJ/Kg.K	2.05
Thermal Conductivity	W/m.K	0.1396

1.3 Mechanism of cryogenic ablation of tissue

There are three micro scale events that have been implied to cause the tissue injury during the freeze-thaw process, they are:

1. Extracellular Ice Formation (EIF)
2. Intracellular Ice Formation (IIF)
3. Cellular dehydration.

Extracellular ice formation is said to happen prior to IIF, as shown by Chambers and Hale [12] and simultaneous experiments by Koonz *et al*. [13] with poultry tissues. This was proposed to happen as the liquidation temperature of the extracellular fluid is higher than that of the intracellular fluid. This was observed by Hong *et al.* [41] who froze samples of normal and malignant tissues from the breast by immersing them in LN_2 and could observe large crystals of ice surrounding the fat cells.

Intracellular ice formation has been studied by Mazur [14] and is said to occur in cases when the cooling rate is high, this high cooling rate super cools the cytoplasm causing

formation of ice crystals by not allowing time for water to move out of the cell due to exosmosis..

Cell dehydration is said to take place when the cell freezes with a slow cooling rate. The process of cellular dehydration can be understood as follows: when the temperature drops to the liquidus temperature of the extracellular fluid around the cell the ice formation in the extracellular part increases the salt concentration there in and the cell membrane being semi-permeable which causes the osmotic pressure to rise and forces more and more water out of the cell due to exosmosis (since environmental solution is hypertonic). The phenomenon has been extensively studied by Mazur [14].

The mechanism by which the damage occurs has been subject to various proposed theories. This debate over the actual cause of tissue injury led to the doubt over the proposed usage of cryospray as a medical treatment and has recently led to extensive research in the said field. The two most widely accepted theories about cellular damage are [15, 16]:

1. Direct cellular damage
2. Vascular injury

Direct cellular injury theory proposes that maximum damage to the tissue occurs by means of intracellular ice formation and cellular dehydration. A model was also proposed for the same by Albin of freezing-stimulated immunological injury [19]. As discussed earlier, cellular dehydration also referred to as the solution effect, can damage the cells in many ways. Damage may be caused to the enzymatic machinery which may destabilize the cell membrane in presence of high solute concentration [20] or it may cause water removal from the cells leading to dehydration and hence necrosis. The IIF happens when the cooling rate is sufficiently high so that there is no time for the water movement out of the cell and leads to formation of ice crystals within the cell, disrupting the organelles and cellular mechanism.

Vascular injury theory proposes severe lack of oxygen (anoxia) or obstruction to normal fluid flow (stasis) as the means for necrosis of the tissue, same was proposed by Cohnheim [17] as he suggested that homeostasis caused frost-bite within the frozen tissue after the thawing process. The cooling causes the blood circulation to stop and may also cause direct injury to the capillary endothelial lining resulting in poor microcirculation, this in turn causes lack of oxygen and stagnation of blood in certain capillaries to complete blockage resulting in necrosis[18]. The extracellular ice formation also may cause vascular injury.

1.4 Factors and control measures for cryospray:

1. Cooling rate:

   It plays a vital role since it decides whether there is exosmosis when the cooling rate is slow or ice formation when it is sufficiently high. A specific temperature, which is very tissue dependant, called the lethal temperature is to be reached in order to damage the cells irreversibly [21-24]. Experiments done on various cancer cell lines have shown that these cells are more resistant to damage due to low temperatures than the normal body cells. It is considered to be apt that a temperature of -50 °C will cause cell destruction in all cancerous tissues [25].

2. Hold time:

   The time for which we have to freeze the target tissue so that the cells are completely destroyed [16]. It has been shown the longer the freeze time more is the tissue damage [22, 23, 24].

3. Thawing rate:

   This is also a pivotal factor and a slow thawing greatly increases the ice formation and also the solution effects causing more cell destruction which has been proved experimentally [15, 16].

CHAPTER 2

MATHEMATICAL MODELING

2.1 Model description

The problem at hand is a 4 mm radius tumor on the skin surface that has to be completely ablated. We take the domain size to be a square of side 10 mm because for our spray time $t_s = 10\,\text{s}$ the frozen zone grows to within this 10 mm radius thus giving us domain independence. Since we have to calculate the heat transfer from the cryospray to the skin tissue to calculate the damage extent, the problem is a complex one in the three dimensional domain. To simplify this we use an axisymmetric approach and define the skin as a tri-layered structure in the axial and the radial planes.

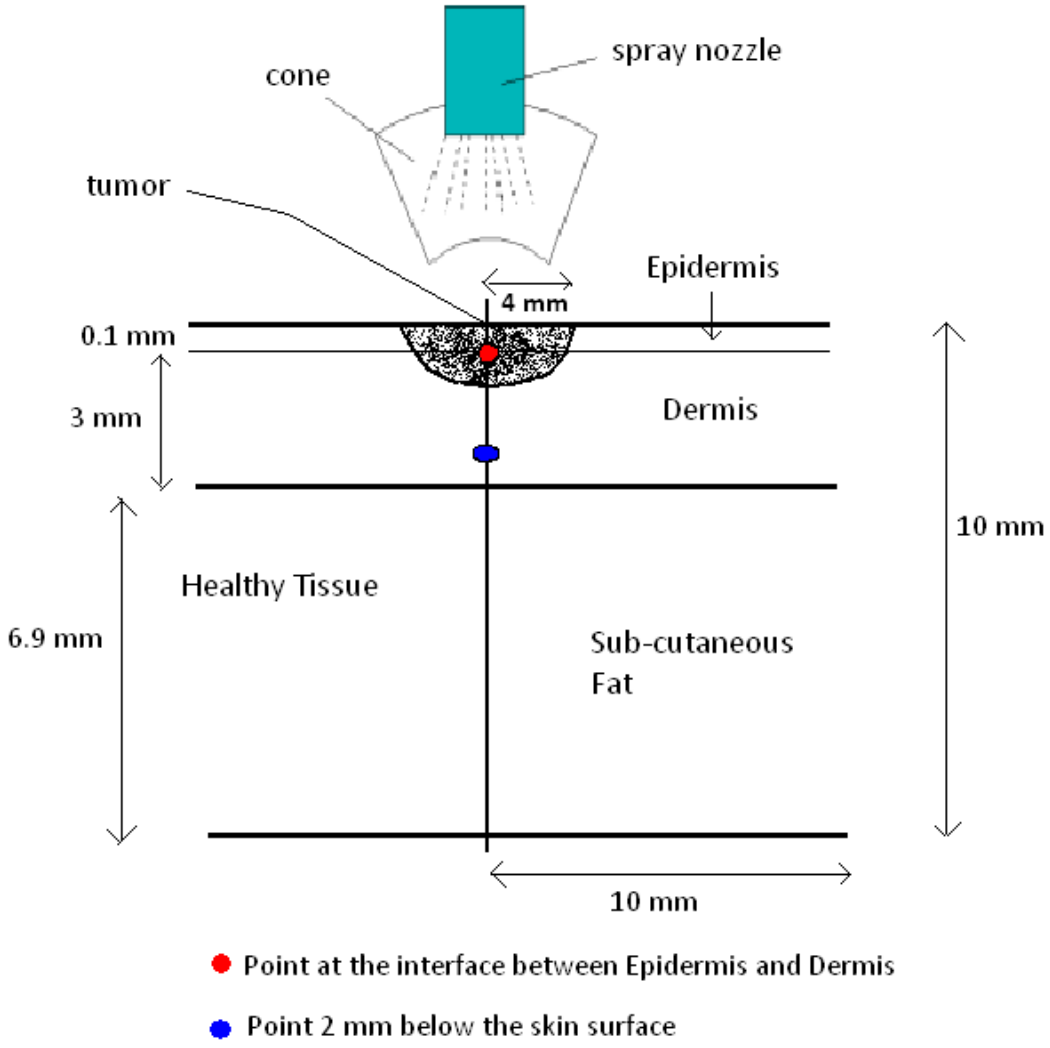

Figure 1.Model used for mathematical calculations

These three homogenous layers viz epidermis, dermis and subcutaneous fat are shown in Fig 1. The outermost layer which is very thin (taken as 0.1 mm for our model) is the epidermis, this in collaboration with the dermis forms what is known as cutis. The epidermis is formed of stratified squamous epithelium and the topmost layer of it is known as stratum corneum and made up of keratinised tissue. The second layer is the dermis (taken as 3 mm for our model); it mainly comprises of collagen, elastic fibers, and extra-fibrillar matrix and is richly supplied with blood and nervous tissue. The third and the innermost layer mainly comprises of fat cells (taken to be 6.9 mm for our model). For the model we propose the spray is directly impinged on the keratinized (tumor) site causing the deposition of nitrogen gas and fine droplets of LN_2 on the tumor surface and the rapid vaporization of the LN_2 causes the cooling of the tissue to a frozen state, this freezing propagates through the tissue, and when the spray is stopped the thawing process starts under the influence of the heat from the surrounding unfrozen tissue. This freeze-thaw cycle causes tissue destruction and leads to ablation.

The various assumptions considered for our mathematical model are:

1. The properties of the cancerous tissue and the normal tissue are taken as the same.
2. The processes at the micro scale level such as the IIF, EIF and cellular dehydration are not taken into account.
3. The metabolic heat generation for the epidermis and the subcutaneous fat are considered to be zero.
4. The properties of each layer of the skin are treated as homogenous and the temperature is taken to be a uniform 37^oC, and the thermal properties are estimated from the water content within the tissue.
5. The spray is considered to be uniform, i.e. the cooling at the surface is equal in all directions where the spray directly impinges.

2.2 Pennes bio-heat equation for the three skin layers

For the effective mathematical modeling of the skin thermodynamics we consider the Pennes equation of the bio-heat transfer [26]. We have considered the equation in polar coordinates and the problem as an axis-symmetric cylinder of radial coordinate r and axial coordinate z. The equation qualifies the thermal behavior of skin and adjoining tissues based on classical Fourier's law and has the following form:

$$\frac{1}{r}\frac{\partial}{\partial r}\left(kr\frac{\partial T}{\partial r}\right)+\frac{\partial}{\partial z}\left(k\frac{\partial T}{\partial z}\right)+\lambda\dot{q}+\psi\dot{w}_b\rho_b C_b(T_b-T)=\rho c\frac{\partial T}{\partial t}$$

where ρ, C , T denote the density, specific heat, and temperature of the tissue under consideration (epidermis, dermis or fat). The specific heat, density and the perfusion rate of blood is denoted by C_b, ρ_b, $\dot{w}_b$, respectively. The heat source is denoted by $\dot{q}$ and is taken as the sum of two components: metabolic heat generation and the heat generated by other heating methods (external). The coefficients λ and ψ are used to solve for the different values of blood perfusion rate and other metabolic constants for these three skin layers namely epidermis, dermis and subcutaneous fat and the values of these coefficients are listed in the table 3 for various physical states i.e. completely frozen (<-10°C) completely liquid/unfrozen (>-1°C) and the intermediate (-10<T<-1°C).

Table 3.Values assigned to λ and ψ during the freeze-thaw cycle:

Region	Epidermis	Dermis	Subcutaneous fat
Unfrozen	$\lambda=0;\psi=0$	$\lambda=1;\psi=1$	$\lambda=0;\psi=1$
Intermediate	$\lambda=0;\psi=0$	$\lambda=1;\psi=0$	$\lambda=0;\psi=0$
Frozen	$\lambda=0;\psi=0$	$\lambda=0;\psi=0$	$\lambda=0;\psi=0$

The values of $\rho_b$and C_b are taken to be 1060 Kg/m^3 and 3.84 KJ/Kg.K respectively [40]. The values of the various other thermal and physical properties of skin used for the solution of our model are given in table 4.

Table 4.Thermal and physical properties of the three skin layers used in computation [28, 29, 30]

Property	Denotation	Unit	Epidermis	Dermis	Subcutaneous Fat
Thermal conductivity (Unfrozen)	$\boldsymbol{k_u}$	W/m.K	0.2	0.498	0.268
Thermal Conductivity (Frozen)	$\boldsymbol{k_f}$	W/m.K	0.2	(273-T)$^{1.156}$x0.0039+1.463	0.268
Heat capacity (Unfrozen)	$\boldsymbol{C_u}$	J/Kg.K	3520	2721	2390
Heat capacity (Frozen)	$\boldsymbol{C_f}$	J/Kg.K	3520	89.7+5.04T	2390
Mass Density	$\boldsymbol{\rho}$	Kg/m^3	1500	1116	916
Latent heat	$\boldsymbol{L}$	J/Kg	0	217100	70808
Heat generation metabolic	$\boldsymbol{\dot{q}_m}$	W/m^3	0	1239	0
Blood Perfusion rate	$\boldsymbol{\dot{\omega}_b}$	1/s	0	0.002387	0.002387

2.3 Computational domain and boundary conditions

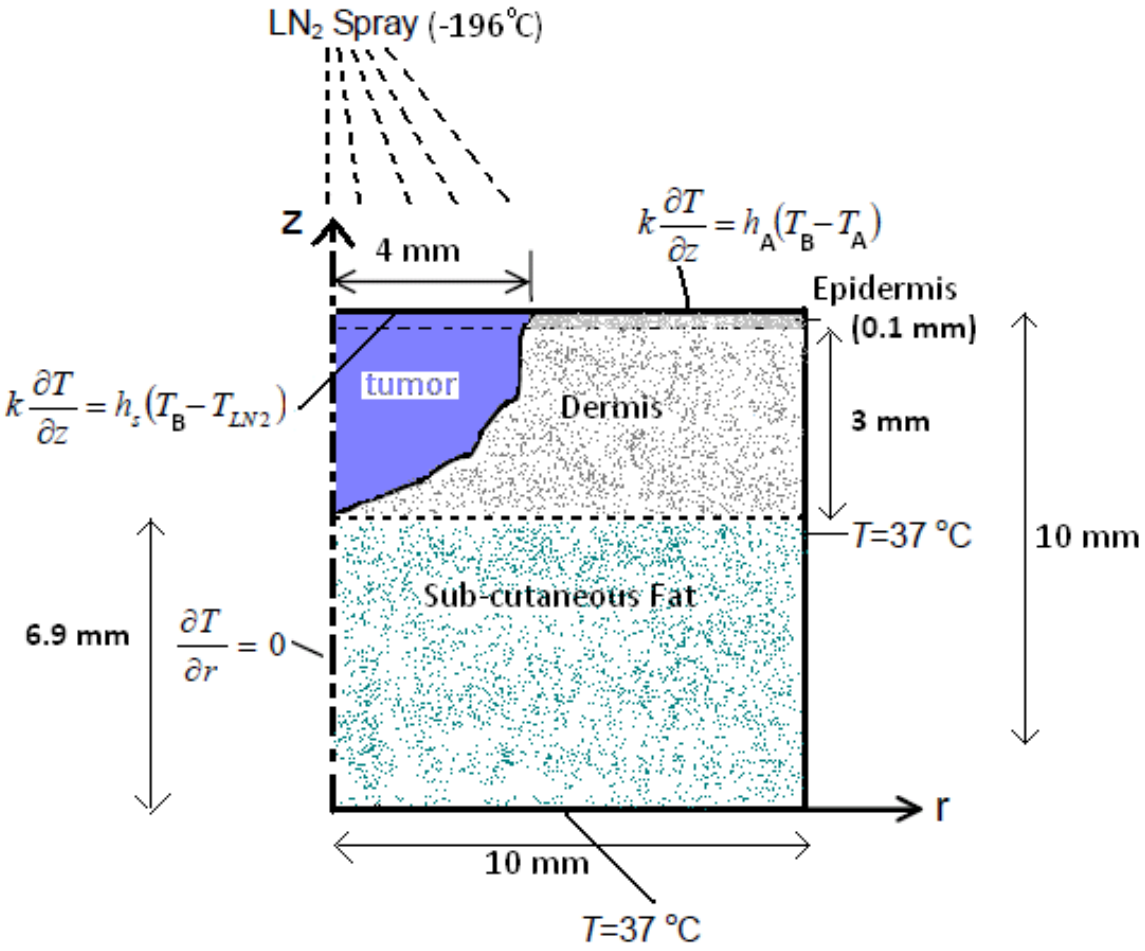

Figure 2.Representation of the computational domain with the various boundary conditions

The various boundary conditions used in the numerical solution are:

a) Convective heat transfer during the spray time (t_s), cooling process at the surface i.e. interface between air and skin and the spray and skin which is represented by $z = 10; 0 \leq r \leq 10$ is given by robin (or third type) boundary condition:

Case 1: Between the spray and skin for the direct spray region i.e. $t < t_s$

$$h_s\left(T_B - T_{LN_2}\right) = -k_B \frac{\partial T}{\partial z}$$

where T_B is the skin surface temperature, T_{LN_2} is the temperature of the spray, h_s is the convective heat transfer coefficient between spray and skin and k_B is the thermal conductivity of the epidermis.

Case 2: Between the air and skin for region where the spray does not fall directly, $t < t_s$

$$h_A(T_B - T_A) = -k_B \frac{\partial T}{\partial z}$$

where T_B is the skin surface temperature, T_A is the temperature of the air, h_A is the convective heat transfer coefficient between air and skin and k_B is the thermal conductivity of the epidermis.

For the thawing process the entire skin surface is in contact with air only since the spray has already been removed hence the condition is:

$$h_A(T_B - T_A) = -k_B \frac{\partial T}{\partial z}$$

for $t > t_s$.

The values of h_s and h_A have been taken to be 10^6 and 10 W/m^2.K respectively.

b) $r = 0; 0 \leq z \leq 10$, $\frac{\partial T}{\partial r} = 0$

c) $z = 0; 0 \leq r \leq 10$, $T = 37℃$

d) $r = 10; 0 \leq z \leq 10$, $T = 37℃$

2.4 Solution approach

The Pennes equation in a polar coordinate system is utilized to study the heat transfer during cryospray of liquid nitrogen on a skin tumor. The governing differential equation is solved on a square computational domain of size 10mm. As the explicit scheme demands use of very small time step for numerical stability, implicit three time level scheme is used for discretizing the unsteady term while central difference scheme is utilized for discretizing the diffusion term, thereby obtaining second order accurate solution in time as well as in space.

2.4.1 Grid independence test

Grid independence or grid convergence is used to gradually or stepwise improving the results by successively using smaller and smaller grid sizes (control volumes). The calculations should become more and more accurate as the mesh size becomes smaller hence denoted by the term grid convergence. The normal approach adopted in computational fluid dynamics is of starting with a coarse mesh and then gradually reducing the mesh size till the changes in the results are smaller than the predefined error magnitude. To check the grid independence we use three grid sizes: 50x50, 100x100 and 150x150. The first and the second term in the grid size are the control volumes along the radial and the axial directions.

Figure 3 indicates the variation of the liquidus temperature front (-1^0C in our model), i.e. the temperature above which the tissue is unfrozen and below which ice crystals form, at time 5 s.

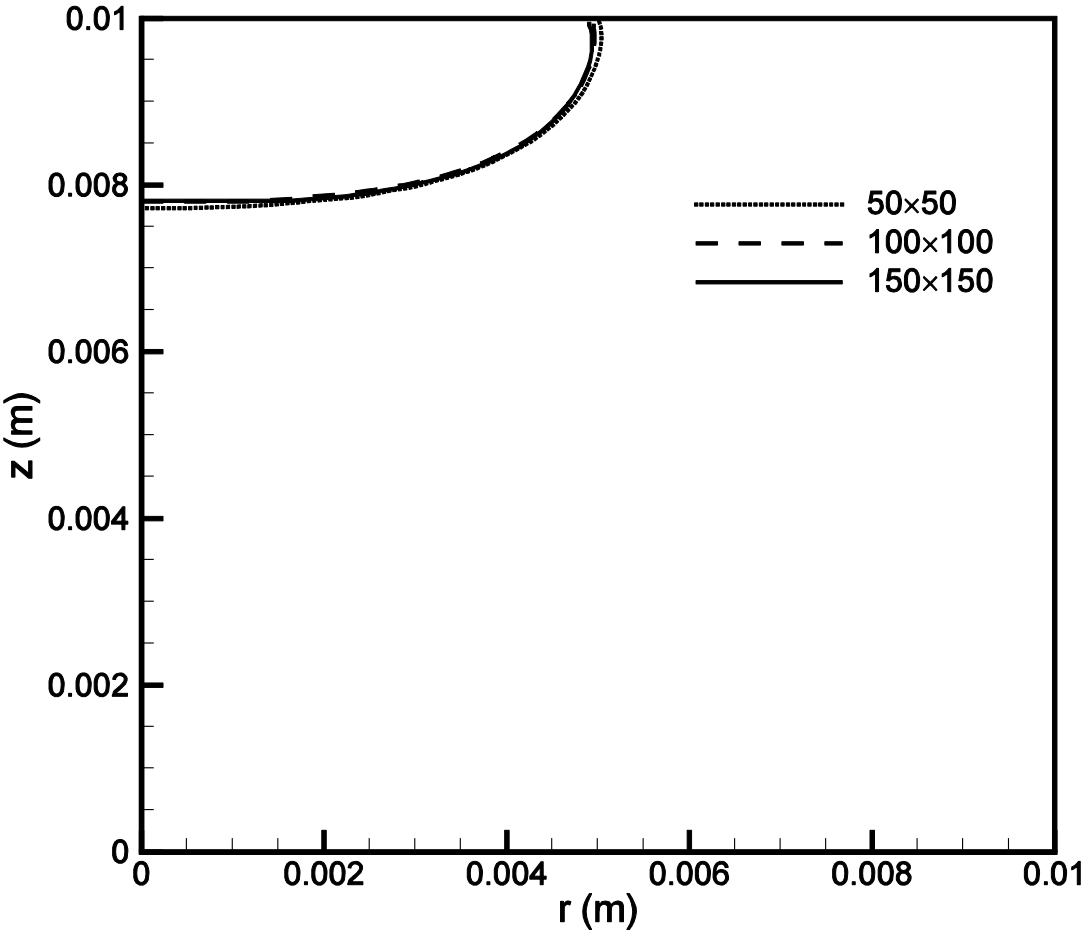

Figure 3. Liquidus temperature front at 5 s.

As can be deduced from the figure there is variation in the liquidus temperature front for 50x50, 100x100 and 150x150 grid sizes. This variation, when the grid size changes from 100x100 to 150x150 is small as compared to the variation when the grid size changes from 50x50 to 100x100, thereby showing that grid independence is reached after the grid size is greater than 100x100.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Time evolution of frozen zone

The spray impinges directly onto the skin surface where the tumor is located this abruptly cools the skin topmost layer and this cooling is related through the convective heat transfer coefficient h_s which is taken as a constant for the skin-spray interface. The value of h_s is equal to 10^6 W/m^2.K for skin-LN_2 interface. One of the most important parameters in cryospray is the duration of the spray. The effective cooling with time is represented through isotherms given in figures 4-9, representing the frozen zone evolution with time. The frozen zone propagates through the axial and radial directions but with varied rates. The rate of propagation is faster in the axial direction i.e. penetrating directly into the skin layers as compared to the radial direction, the one parallel to the skin surface. This results in the change of shape of the frozen zone with time from an initial perfect ellipse. Figure 4 shows the frozen zone at time 10 s after the start of the spraying process, the liquidus temperature front is the outline of the plot and the gray area shows the frozen region.

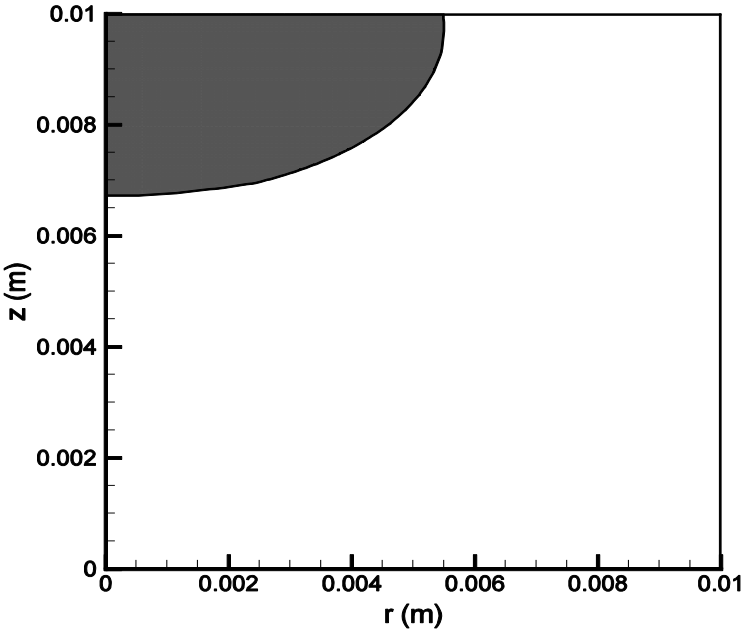

Figure 4. Frozen zone at 10 s after start of spray

Figure 5 shows the frozen zone at 20 s, this is about the time the frozen zone reaches the dermis and fat interface, up till this time the frozen front was smooth ellipse, and just at the interface (3.1 mm) there is a hump in the outline, this can be understood as a discontinuity in the graph showing a point of inflexion. This is because of the difference in the water content of the two layers, i.e. fat and dermis, fatty layer has 21% water while the dermis has a high 65 % water content which results in very different latent heats for these two tissues and causes difference in the heat needed to freeze the both of them.

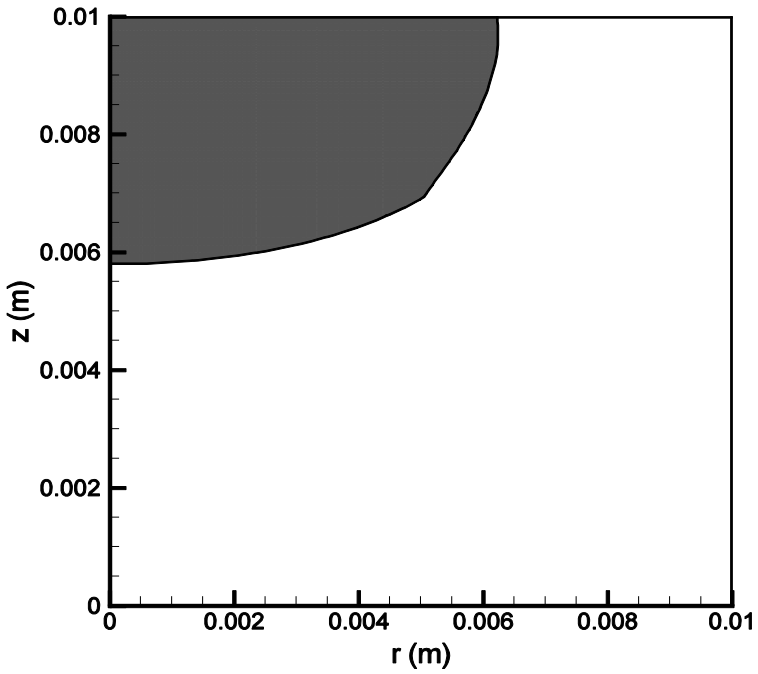

Figure 5. Frozen zone at 20 s after start of spray

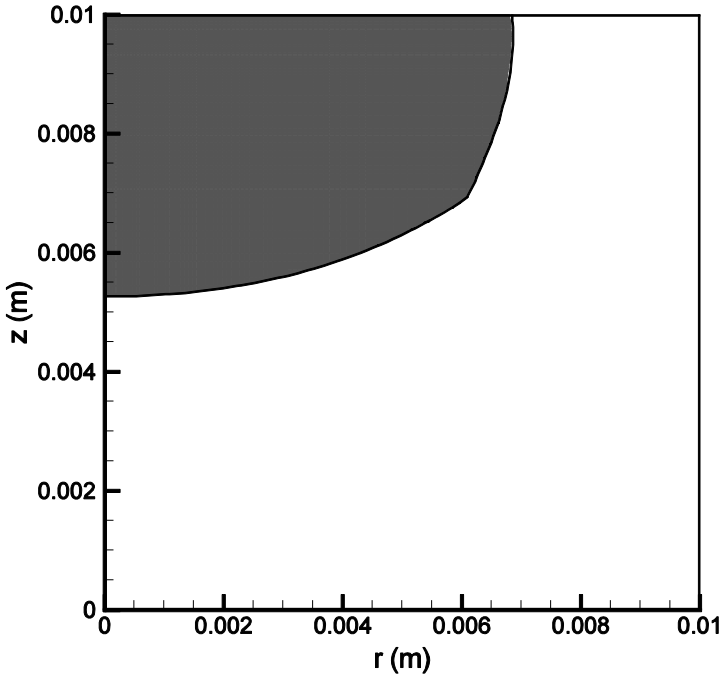

Figure 6. Frozen zone at 30 s after start of spray, spray is stopped now

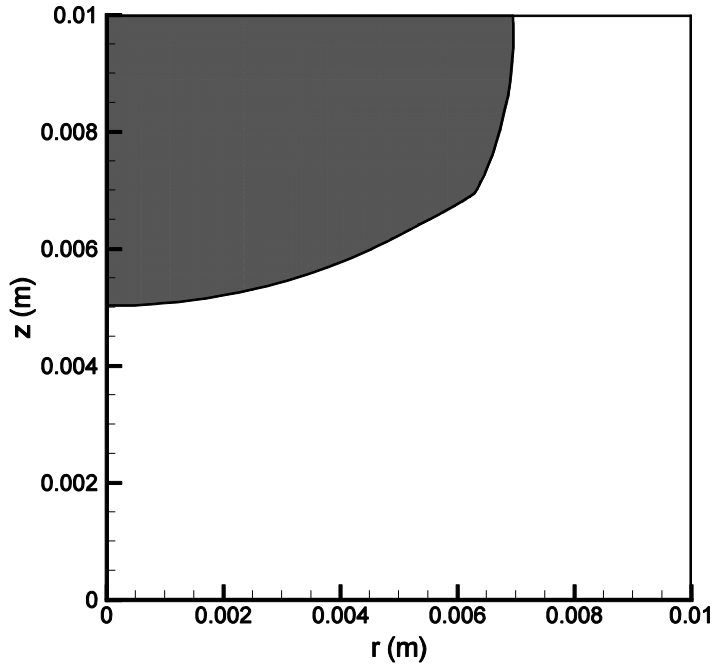

Figure 7. Frozen zone at 40 s after the start of spray, 10 s after start of thawing process

Figure 6 continues in the same way to show the frozen zone at 30 s post the start of the spraying process. It is at this time that we stop the LN_2 spray and let the thawing process begin under normal atmospheric conditions and at room temperature without any external agent.

Figure 7 shows the same frozen region 40 s after the start of spraying, i.e. 10 s post the spray has been stopped and the thawing process started. What stands out is that the frozen zone does not immediately melt but instead it holds on to its form even grows a bit, before melting.

Figure 8 shows the state of the frozen region at 20 s post thawing and 50 s from the time the spray was initialized, as can be inferred from the figure that the size of the frozen zone has started to decrease due to the melting of the previously frozen tissue.

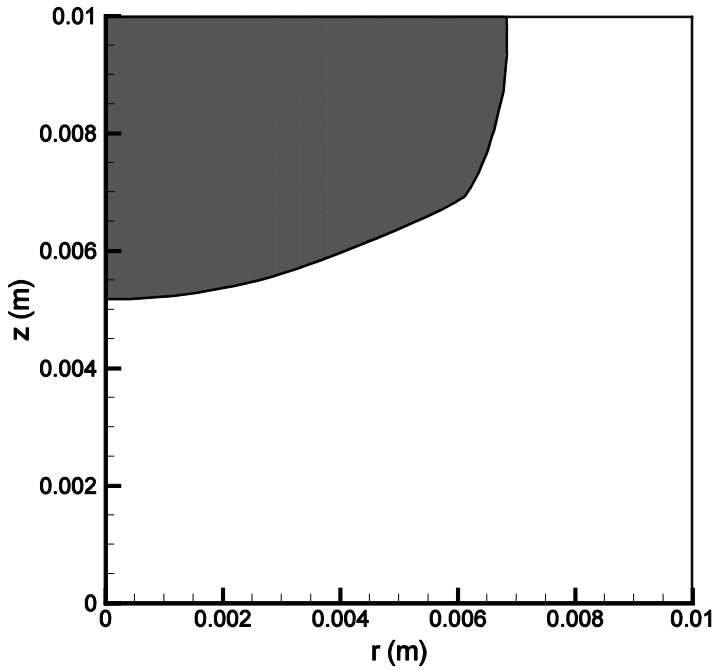

Figure 8. Frozen zone at 50 s after start of spray, 20 s into thawing

During the cryospray process one should know the lethal temperature for the tissue under consideration (taken as -50°C) and the frozen zone propagation with time in order to calculate whether the tumor tissue is completely ablated or not, and also it must be taken care of that no healthy tissue is damaged in the process.

3.2 Thermal histories inside the skin layers

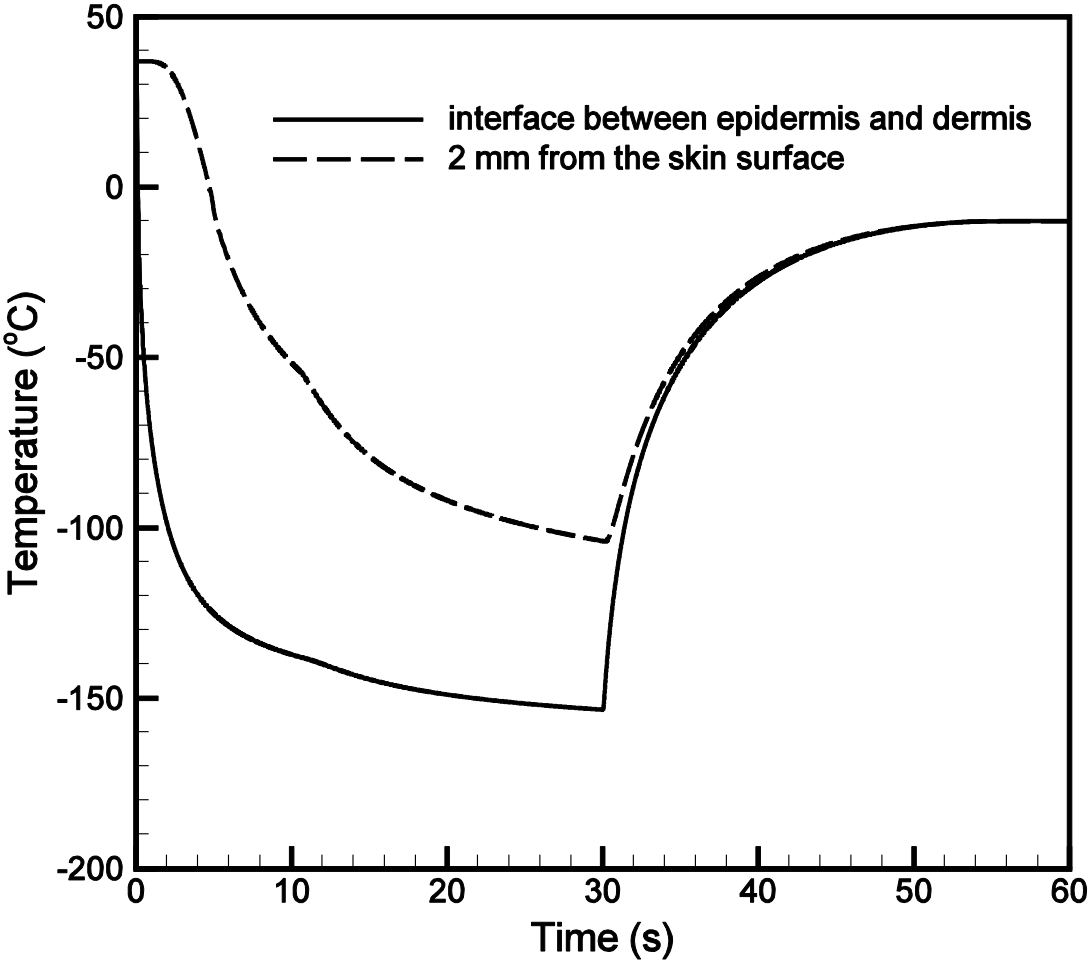

Figure 9. Temperature variation at two points during freeze-thaw process

Since it is not only the lethal temperature but also the hold time and the rate at which the tissue cools (thawing rate) that determine the extent of injury. In order to understand this, the temperature variation with time at two points along the axis, shown in figure 1, has been obtained. The two points are:

1. A point on the axis at the interface between the epidermis the dermis and
2. A point on the axis taken 2 mm below the skin surface.

The temperatures at these points during the freeze-thaw cycle are plotted in figure 9. Since the interface between the skin and the spray has a high convective heat transfer coefficient (h_s= 10^6), hence the surface cools very rapidly as compared to the skin layers below it, this is shown explicitly by the graph. The fast cooling of the epidermis results in IIF as the water quickly changes to ice with the abrupt temperature drop but in the inner regions the case is different as the cooling is slow so the major effect is the solution effect causing cellular dehydration as there is enough time for osmosis and balancing of the solutes, also the upper layers remain at a colder temperature for longer time causing the growth of the ice crystals hence formed and resulting in tissue membrane and organelle damage of the cells. Also the close monitoring of the curve brings to notice that there is a slight dip when the curve reaches 10 s because this is about the time when the ice front reaches the interface between the dermis and the fat. The water content in both these tissues is highly different, as discussed earlier, it is 21% in fat and 65% in case of dermis [32], due to this massive difference in the water content the latent heat for both the tissues is very different causing this hitch or drop in the graph. Due to the high convective coefficient of skin with respect to the nitrogen spray (10^6 W/m^2.K) as compared to the air (10 W/m^2.K) the thawing process is slower.

CONCLUSION

The frozen zone growth during the freeze-thaw cycle gives us an idea of the damage zone. This helps us understand the mechanism and extent of tissue injury, the areas covered by the liquidus temperature show regions where the tissue is affected by the cold temperatures and freezing occurs. The tissue within the frozen zone may or may not be at the lethal temperature (-50°C) which decides whether the tissue is destroyed irreversibly or not. During the spraying process IIF occurs particularly in the epidermis due to the abrupt temperature change, as for the dermis and fat the cooling is relatively slow and hence cellular dehydration also occurs. Moreover the thawing rate is slower than the freezing rate. The findings suggest that a cryospray of 30 s duration is capable enough to completely ablate the 4 mm tumor as it forms a frozen zone that encompasses a radius of around 5 mm in the axial direction and 7 mm in the radial direction, with temperatures reaching to as low as -100°C in the dermis and -150°C in the epidermis (both these temperatures being well below the lethal temperature).

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