Title
Numerical prediction of the intracellular ice formation zone during cryosurgery on a nodular basal cell carcinoma using liquid nitrogen spray

Permalink
https://escholarship.org/uc/item/0469n0jd

Journal
INTERNATIONAL JOURNAL OF SPRAY AND COMBUSTION DYNAMICS, 4(4)

ISSN
1756-8277

Authors
Sun, F
Martinez-Suastegui, L
Wang, G-X
et al.

Publication Date
2012-12-01

DOI
10.1260/1756-8277.4.4.341

License
https://creativecommons.org/licenses/by/4.0/ 4.0

Peer reviewed
Numerical prediction of the intracellular ice formation zone during cryosurgery on a nodular basal cell carcinoma using liquid nitrogen spray

F. Sun1, L. Martínez-Suástegui2, G.-X. Wang3, K.M. Kelly4, and G. Aguilar1,*

1Department of Mechanical Engineering, University of California, Riverside, CA 92521, USA
e-mail: gaguilar@ucr.engr.ucr.edu
2ESIME Azcapotzalco, Instituto Politécnico Nacional, Avenida de las Granjas No. 682, Colonia Santa Catarina, Delegación Azcapotzalco, México Distrito Federal 02250, Mexico
3Department of Mechanical Engineering, University of Akron, OH 44325, USA
4Beckman Laser Institute, University of California, Irvine, CA 92612, USA

Submission May 10, 2012; Revised Submission July 20, 2012; Acceptance August 1, 2012

ABSTRACT
Cryosurgery is a surgical technique that employs freezing to destroy target tumor tissue. While the main objective during a cryosurgical procedure is to ensure tissue destruction within the cryolesion, the greatest challenge is how to spare the surrounding healthy tissues from cryoablation. In this work, a historical review of the field of cryosurgery is presented followed by a theoretical study where a mathematical model is developed for cryosurgery on a basal cell carcinoma (BCC) using liquid nitrogen ($\text{LN}_2$) spray. The model takes into consideration the anatomic structure of skin tissue and the irregular geometry of BCC. In particular, a methodology for quantitatively determining the extent of the intracellular ice formation ($\text{IIF}$) zone based on the tissue temperature and cooling rate (CR) is proposed, which can directly relate to the necrosis of the cancer cells after cryosurgery. The model is then used to analyze formation of the $\text{IIF}$ zone during cryosurgery on a BCC and quantification is provided for the volume of the final $\text{IIF}$ zone. A parametric study is also carried out to investigate the effect of various protocol parameters on the final $\text{IIF}$ zone. The results should be useful for dermatologists for pre-treatment surgery planning.

NOMENCLATURE
$a$ dimensionless constant from regression
$b$ dimensionless constant from regression

International journal of spray and combustion dynamics · Volume 4 · Number 4 · 2012 – pages 341 – 380
Numerical prediction of the intracellular ice formation zone during cryosurgery on a nodular basal cell carcinoma using liquid nitrogen spray

\[ C \quad \text{specific heat (J Kg}^{-1} \text{K}^{-1}) \]
\[ h \quad \text{convective heat transfer coefficient (W m}^{-2} \text{K}^{-1}) \]
\[ H \quad \text{height of BCC (m)} \]
\[ k \quad \text{thermal conductivity (W m}^{-1} \text{K}^{-1}) \]
\[ L \quad \text{latent heat of tissue freezing (J Kg}^{-1}) \]
\[ n \quad \text{normal direction of skin surface} \]
\[ Nu \quad \text{Nusselt number} \]
\[ \dot{q}_m \quad \text{metabolic heat generation rate (W m}^{-3}) \]
\[ r \quad \text{coordinate in radial direction (m)} \]
\[ r^* \quad \text{dimensionless coordinate in radial direction} \]
\[ r_s \quad \text{radius of LN}_2 \text{ spray cooling site (m)} \]
\[ R_0 \quad \text{radius of BCC (m)} \]
\[ t \quad \text{time (s)} \]
\[ T \quad \text{temperature (°C)} \]
\[ V \quad \text{volume (m}^3) \]
\[ V_0^* \quad \text{dimensionless volume from regression} \]
\[ V_{IIF} \quad \text{volume of tissue in IIF zone (m}^3) \]
\[ V_{ref} \quad \text{reference volume (m}^3) \]
\[ V_{IIF}^* \quad \text{dimensionless volume of tissue in IIF zone} \]
\[ z \quad \text{coordinate in axial direction (m)} \]
\[ z^* \quad \text{dimensionless coordinate in axial direction} \]

\textbf{Greek}

\[ \rho \quad \text{mass density (Kg m}^{-3}) \]
\[ \varepsilon \quad \text{mass fraction of tissue component} \]
\[ \eta \quad \text{coefficient controlling metabolic heat generation} \]
\[ \xi \quad \text{coefficient controlling thermal effect of blood perfusion} \]
\[ \dot{\omega}_b \quad \text{volumetric flow rate of blood perfusion} \]

\textbf{Subscript}

\[ a \quad \text{air} \]
\[ b \quad \text{blood} \]
\[ BCC \quad \text{basal cell carcinoma} \]
\[ epi \quad \text{epidermis} \]
\[ f \quad \text{frozen} \]
\[ IIF \quad \text{intracellular ice formation} \]
\[ in \quad \text{initial} \]
\[ LN2 \quad \text{liquid nitrogen} \]
\[ m \quad \text{metabolism} \]
\[ ref \quad \text{reference} \]
\[ s \quad LN_2 \text{ spray} \]
\[ surf \quad \text{surface} \]
\[ u \quad \text{unfrozen} \]
1. INTRODUCTION

Basal cell carcinoma (BCC), the most common skin malignancy, affects more than 500,000 Americans each year [1]. It’s estimated that 2.9 million BCCs are diagnosed annually in the United States, and the number of cases continues to increase. The increased incidence has been proposed to be due to increased sun exposure associated with the thinning ozone layer and extensive outdoor leisure activities [2]. To date, there are several modalities available for the treatment of BCC, including Mohs surgery, photodynamic therapy, radiation therapy and cryosurgery [3]. Merits of cryosurgery for treating BCC include minimal or no bleeding, low cost, high cure rate, relative ease of implementation and good cosmetic outcome.

Cryosurgery is a surgical technique using low temperature and tissue freezing to achieve tissue damage. In dermatological practice, the most commonly used cryogen is liquid nitrogen (\(LN_2\)) due to its low cost, wide availability, high efficiency and safety [4]. \(LN_2\) can be sprayed directly onto the surface of the BCC, i.e. cryospray technique, as schematically shown in Figure 1. At the cooling site, the sprayed \(LN_2\) boils and evaporates, cooling the underlying tissue to below the phase change temperature of cancerous tissue, resulting in freezing.

Two primary mechanisms are thought to result in tissue damage: direct cell injury and vascular injury [5]. Direct cell injury includes solution-effect associated with slow freezing and intracellular ice formation (IIF) due to a high cooling rate (CR). During tissue freezing, ice formation occurs first in the extracellular space. The resulting segregation of solutes leads to increased concentration of the extracellular solution, which forms osmotic pressure, which drives the transport of intracellular water through

---

**Figure 1:** Schematic of cryosurgery on a nodular basal cell carcinoma using \(LN_2\) spray.
the cell membrane. For a sufficiently high CR, there is inadequate time for water transport across the membrane. Therefore, ice crystals will nucleate and propagate intracellularly, i.e. \textit{IIF} occurs. \textit{IIF} is lethal to the survival of living cells because intracellular ice crystals disrupt cell membranes and intracellular organelles [5]. \textit{IIF} zone therefore represents a region where cell damage is of high probability. On the other hand, if the tissue freezes with a low CR allowing enough time for water transport, cell dehydration and concentration of intracellular electrolytes will result, i.e. the solution-effect (SE). The solution-effect can injure the cell in several hypothesized ways, e.g. damage to the enzymatic machinery and cell membrane destabilization [6]. However, significant cell injury requires a prolonged time to develop, and therefore, the solution effect may not always be lethal to cells [5]. It can be seen that under sub-zero temperature, the dominance of the above two events (SE and \textit{IIF}) depends greatly on the CR. Quantification of \textit{IIF} zone requires a threshold value of the CR, i.e. the lowest CR that can trigger \textit{IIF}, under which solution-effect dominates and precludes \textit{IIF}. This threshold CR can be referred as critical cooling rate (CCR).

Vascular injury is the eventual outcome of a series of events during and after tissue freezing. Damage to the vessel wall due to direct endothelium cell injury causes increased permeability of fluid and plasma proteins through the capillary wall. Edema, platelet aggregation and microthrombus formation take place when blood perfusion resumes during the thawing process. These changes result in the stagnation of microcirculation and eventual necrosis of tissue due to ischemia and anoxia [5, 6].

It is well accepted that the extent of injury in biological tissues by freezing depends on the thermal history, which in turn depends on five parameters: cooling rate, end temperature, time spent at the end temperature, thawing rate, and number of freeze/thaw cycles. Extensive research has been performed to understand the vascular effect of changing each thermal parameter with the ultimate goal of defining the threshold between viability and predictable tissue necrosis. Examples where qualitative and quantitative data that correlates thermal damage with the aforementioned parameters has been studied follow. Farrant and Walter [7] discussed the relationship between cell death and thermal history in cryosurgery. Gage and Baust [5] addressed the mechanisms of tissue injury in cryosurgery and established the basic features of cryosurgical technique: rapid freezing, slow thawing, and repetition of the freeze-thaw cycle. Hoffman and Bischof [6, 8] studied the cryobiology of cryosurgical injury and its correlation with the time-temperature history experienced by tissue during a thermal insult. The effects of freezing on cell viability and mechanisms of cell death in a human prostate cell line were addressed by Hollister et al. [9]. Ismail et al. [10] presented a novel approach to increase the sensitivity to cryotherapy using combined treatment of tumor necrosis factor related apoptosis-inducing ligand and cryotherapy in an in vitro cryotherapy model. Rabin [11] outlined the key issues that must be addressed in bioheat transfer simulations for the application of cryosurgery planning. Liu et al. [12] performed experiments where freezing was immediately followed by a rapid and strong heating of the target tissues in order to significantly improve the treatment effect. The same authors [13] developed a new cryoprobe system with a heating feature aimed at performing both cryosurgery and hyperthermia on target tumors and discussed the implementations of this new system in
clinical cryosurgery or hyperthermia. Mazur [14] addressed the effects of intracellular freezing on cell survival. Yang et al. [15] studied the changes in ice growth speed and its influence on the distance between the ice front and cell death boundary. Cai et al. [16] summarized the current state-of-the-art of gold nanoparticles in biomedical applications targeting cancer. Gage et al. [17] provided a review with a global overview of experimentation in vivo and its relationship to the optimal methods of technique of freezing to achieve efficacious therapy. Langenhuijsen et al. [18] provided an update on clinical results of modern cryotechnology and concluded that modern cryosurgery is reliable and results are promising with minimal morbidity. A summary of the avenues for research and development in cryosurgery treatment planning was provided by Sandison [19].

In order to provide solutions to generalized heat transfer models with phase change, extensive numerical works have been published on the thermal history of target tissue during cryosurgery [20–24]. Heat transfer problems involving change of phase in which the boundary conditions are specified at the external stationary surface are usually referred to as “Stefan problems”. Problems where the boundary conditions are specified at the moving phase-change front are termed “inverse Stefan problems [25–28]. Budman et al. [29] presented an integral solution for a one-dimensional inverse Stefan problem and discussed the problems associated with the control of the warming rate during the melting stage. Rubinsky and Shitzer [30] presented an analysis of a Stefan-like problem in the in vivo freezing of a biological tissue that includes blood perfusion, metabolic heat and tissue heat capacity effects. Baish et al. [31] used analytical and numerical methods to predict the temperature distribution in tissue by taking into account three models of thermally significant blood vessels, namely an array of unidirectional vessels, an array of countercurrent vessels, and a set of large vessels feeding small vessels which then drain into large vessels. Their results show how the vascular geometry and blood flow rate affect the thermal response of these models and provide a clearer physical insight into the mechanisms by which heat is transported in vascular tissue. Klemick et al. [32] implemented the adaptive grid generation scheme of body-fitted coordinates to study numerically the effect of blood perfusion on the heat transport of a cylindrical tissue heated by microwave radiation and assessed the effects of varying heat transfer coefficients and equilibration lengths. Bischof et al. [33] predicted the thermal history around a 3-mm diameter cylindrical cryosurgical probe by solving the bioheat equation in a one-dimensional cylindrical geometry with the aim to determine the influence of the time held at an end temperature, thawing rate, and vascular response to enhance the destructive effect of cryosurgery. Rewcastle et al. [34, 35] predicted the three-dimensional thermal histories for several probe configurations in order to compute their ablative ratio. Deng and Liu [36] analyzed the enhancement of freezing by injecting solutions with appropriate thermal properties into the target tissues during cryosurgery. The same authors [37] used the dual reciprocity boundary element method to model the multidimensional freezing problem during cryosurgery. Since it was first proposed by Bonacina et al. [38], the heat capacity method has been used by many investigators to solve phase-change problems [39–51]. In this method, the latent heat effect is approximated by a large effective specific heat
over a small temperature range. Although the effective capacity is a highly non-linear function of temperature within the phase change temperature range, it can be substituted by a linear relationship. The advantage of this method is that numerical computation is based on a fixed grid [52] and the formulation avoids complex front tracking algorithms. However, the formulation is only valid when there is a continuous variation of the properties across phase boundaries. Also, the limitation of this method is that it is very sensitive to the choice of phase change temperature interval [53] and to the chosen time step for the transient process, since computation is divergent when implemented with implicit time integration schemes [54]. Gong and Majumdar [55] provided the cause and cure of non-convergence in effective heat capacity methods for the numerical solution of phase change problems. Later, an average heat capacity algorithm based on a finite difference method where the time step limitation was eliminated was introduced by Hsiao [56], and Lee and Chiou [57] modified the latter for the finite element technique. Examples where the average heat capacity method for the analysis of conjugate heat transfer during a two-phase solidification process has been used and successfully implemented and can be found in Amin [58] and Amin and Greif [59]. Comini et al. [60] provided an algorithm for multidimensional phase change conduction problems. A brief review of current source for modeling solidification phase change systems was provided by Voller and Swaminathan [61]. Deng and Liu [62] implemented the Monte Carlo method to solve the direct bioheat transfer problems that are often encountered in the treatment planning of cancer hyperthermia. The same authors [63], developed a numerical algorithm based on the dual reciprocity boundary element method to solve the multidimensional phase change problem of biological tissues subjected to cryosurgery. Jankun et al. [64] developed a software package to assist in cryoablation therapy through computer modeling, simulation, and visualization in order to predict the outcome of cryosurgery. One of the most common approaches is the enthalpy method, where latent heat content is a function of temperature and the energy equation is solved either in the enthalpy form or with an effective specific heat to incorporate latent heat [65–68]. Rabin and Korin [69] developed a simple and efficient numerical technique based on the enthalpy method for solving transient multidimensional heat transfer problems with melting/solidification processes. Rabin [70] performed heat transfer simulations and obtained a closed-form solution of the one-dimensional temperature distribution in frozen water and blood by taking into account the effect of temperature-dependent thermal conductivity.

Devireddy et al. [71] attempted to incorporate microscopic models of cellular water transport and probability of IIF (PIF) into macroscopic tissue freezing calculations, where cellular-level repetitive unit is described by a Krogh cylinder. The results indicate that the thermal history predicted by the coupled model presents little significant difference compared to that by an uncoupled model. Zhang et al. [72] studied IIF and cell dehydration during cryosurgery of breast cancer, combining the macroscopic bioheat transfer model with the microscopic models of PIF, intercellular ice propagation and water transport in clusters of closely packed cells. Different cooling boundary conditions were employed. They reported that the constant heat flux induces greater cellular dehydration and higher probability of IIF. However, discrepancy exists for the
intercellular ice propagation model, since a different model has been proposed for the identical process [73]. Zhang et al. [72] applied classic bioheat equation to model tissue heat transfer and freezing processes during cryosurgery on prostate cancer. Three-dimensional geometry of prostate and cryosurgical techniques, e.g. multiple probe and urethral warmer, were considered, and thermal history was studied in terms of the evolution of critical isotherms. Gonder et al. [74–76] assessed the chemical and morphologic changes in the prostate following extreme cooling. The temperature-time relationship for tissue destruction in human benign prostatic hyperplasia was experimentally studied by Bhowmick et al. [77]. Dow and Waterhouse [78] studied experimentally the effect of lethal freezing temperatures of the prostate gland. Smith et al. [79] experimentally studied the connection between thermal history and cell injury in AT-1 rat prostate tumor cells using four parameters: cooling rate, end temperature, hold time, and thawing rate. Jiang et al. [80] demonstrated that tumor necrosis factor alpha (TNF-α) can yield complete destruction of prostate cancer within a cryosurgical iceball when given 4h prior to cryosurgery. Robilotto et al. [81] developed a three-dimensional, tissue engineered human prostate cancer model to simulate and assess the effects of cryotherapy. Klossner et al. [82] performed experiments using an in vitro prostate cancer model to study the effects of end temperature, cooling rate, duration of the freezing episode, and repetition of the freezing cycle in terms of providing maximum cell destruction. Larson et al. [83] determined the critical temperatures below which human prostatic tissue can be cryoablated in situ and assessed the advantages of the cryoablative efficacy of single versus double-freeze cryosurgery. Tatsutani et al. [84] provided quantitative values for the relationship between thermal variables during freezing and the destruction of human primary prostatic adenocarcinoma cells. Turk et al. [85] determined that freezing prostate cancer to temperatures below −40°C reliably kills prostate cancer regardless of the number of freeze/thaw cycles. Han et al. [86] developed a cryoinjury model using tissue engineering technology to determine thermal thresholds of cryoinjury with two different prostate cancer cell lines. Cohen [87] reviewed the techniques and indications for cryosurgery of the prostate. Babaian et al. [88] provided a best practice statement on cryosurgery for the treatment of localized prostate cancer cells. The efficacy and safety with targeted cryoablation of prostate cancer was retrospectively reviewed by Bahn et al. [89] and by Katz and Rewcastle [90]. Technological advances for minimally invasive cryosurgery were provided by Baust et al. [91] and by Baust and Gage [92]. Langenhuijsen et al. [18] provided an update on clinical results of modern cryotechnology and concluded that modern cryosurgery is reliable and results are promising with minimal morbidity. A summary of the avenues for research and development in cryosurgery treatment planning was provided by Sandison [19]. Recently, a new methodology for preventing freezing damage beyond pre-specified boundaries during prostate cryosurgery was proposed [93, 94], where laser heating counteracts tissue freezing to better confine damage to the targeted cancerous tissue within a lethal low-temperature isothermal boundary, an approach referred to as laser-assisted cryosurgery (LAC). The advantage of this new methodology is that there is no need to stop the freezing process prior to achieving the target lethal temperature within preset boundaries and therefore only one freeze/thaw
cycle is required. Martínez-Suástegui et al. [95] performed LAC procedures in ex vivo mice hepatic tissue. Their results show that since the effective cryosurgical tissue destruction zone is enlarged by producing large thermal gradients, the frozen region can be shaped to selectively destroy malignant tissue. This approach is particularly advantageous for the treatment of large or irregularly shaped tumors.

The problem of cryosurgery optimization was first considered by Keanini and Rubinsky [96]. The authors described a general technique for optimizing cryosurgical procedures based on three parameters: the number of cryoprobes used, the freezing length per cryoprobe, and the cryoprobe diameter. Baissalov et al. [97, 98] presented a model for treatment planning of multiprobe cryosurgery by simultaneously optimizing multiple cryoprobe placements and their thermal protocol for one freeze-thaw cycle. Chua et al. [99] performed simulations based on solving the transient bioheat equation using the finite volume scheme for a single or multiple-probe geometry and provided the basis for designing an optimized cryosurgical protocol which incorporates thermal effects and the extent of cell destruction within tumors. Ivarsson et al. [100] used a microprocessor controlled power regulation and thermometry system to maintain a stable target temperature and produce lesion volumes adequate for treating relatively large tumors in a single session. Lung et al. [101] developed a computerized planning tool to determine the best locations to insert the cryoprobes based on the bioheat transfer simulations using the force-field analogy. In order to optimize cryo-needle placement and operation protocols, Magalov et al. [102] performed three-dimensional numerical simulations of multi-cryo-needle surgery. Rossi et al. [103, 104] presented a computerized planning scheme for prostate cryosurgery using a variable insertion depth strategy. Tanaka et al. [105] proposed a method to generate computationally a preferred cryoprobe layout for the pullback operation using the bubble-packing method. In Rossi et al. [106] developed a numerical scheme for bioheat transfer simulations with application to phase change problems of cryosurgery using variable grid size and time intervals. Tanaka et al. [107–109] developed an optimization method based on the bubble-packing and force-field analogy methods to obtain the optimal arrangement of the cryoprobes during cryosurgical procedures. Maruyama et al. [110, 111] assessed a novel and rapid cooling system which employs a heat transfer control device based on the application of a thermoelectric principle (Peltier effect).

The majority of the published works concentrate on the cryoprobe technique [112–133], and few studies have evaluated tissue thermal history during cutaneous cryosurgery using LN\textsubscript{2} spray. Such a numerical tool could be very useful in dermatological practice for protocol optimization.

In an attempt to develop such a numerical model, the authors started with a homogeneous model, in which heat transfer within tissue is described by classic bioheat equation and target skin tissue is treated as a homogeneous media [134]. Anatomic structure of human skin tissue, i.e. multi-layer structure, was then considered and both freezing and thawing processes were included in the calculations [135]. The model was later applied to a specific case, i.e. BCC, where the target tissue has irregular geometry [136]. In these works, parameters of tissue thermal history were studied systematically,
including the freezing interfaces, CR, lethal isotherm, etc. Microscopic models are not considered by the authors thus far, since the biophysical parameters in microscopic models are highly cell-type dependent [71, 137] and most are unknown for skin cells, e.g. permeability of water through cell membrane and activation energy for water transport, etc.

In this paper, the model is used to study cryosurgery on a nodular BCC using \( \text{LN}_2 \) spray. In particular, a mechanistic methodology quantifying the extent of IIF zone is presented based on transient profiles of tissue temperature and cooling rate. The effects of protocol parameters on the extent and the volume of final IIF zone are investigated, which include the heat transfer coefficient of spray cooling \( (h_s) \), the critical cooling rate (CCR), and the cooling site radius \( (r_s) \). Results of the enclosed work can be used to estimate cryosurgical injury outcome and optimize cryospray protocol parameters.

2. MATHEMATICAL MODEL AND NUMERICAL METHOD

2.1. Anatomic and physiological basis

It is customary to utilize the classic bioheat equation [138] to describe heat transfer processes in biological tissue, where a dense capillary network supplies slow blood perfusion [139–143]. In clinical practice, target tissue has complex anatomy, which must be understood and considered for successful modeling.

Human skin is composed of three primary layers: epidermis, dermis and subcutaneous fat. The epidermis is the outermost avascular portion and varies in thickness with site, ranging from 0.1 mm on the eyelids to about 1 mm in acral sites [144]. The dermis lies underneath the epidermis and hosts abundant capillaries, nerves and lymph vessels. It is believed that blood perfusion produces profound thermal effects in the dermis layer and as such, can be treated uniformly therein [145, 146]. Below the dermis is the subcutaneous fat layer. This layer is not highly vascular, but is transversed by vessels branching and proceeding from deeper tissue to the capillary vasculature in the dermis [147]. These transverse vessels are believed to be thermally insignificant [148] and the thermal effect of blood perfusion is therefore usually neglected in the subcutaneous fat [147].

2.2. Physical model and mathematical formulation

Based on the above considerations, the following assumptions were utilized for our model of the thermal history of a nodular BCC during \( \text{LN}_2 \) spray cooling:

1. The BCC is presumed to have the geometry of a spherical cap and \( \text{LN}_2 \) spray is controlled to have a concentric circular cooling site on the surface of the BCC with a radius of \( r_s \), as shown in Figure 2.

2. The cooling site is subjected to convective heat transfer during spray cooling, and an average heat transfer coefficient, \( h_s \), over the entire sprayed area is used and assumed to be constant. However, it is well known that the heat transfer coefficient at the sprayed surface is non-uniform and varies between central and peripheral locations [149–153]. To overcome this issue, our preliminary case study considered the spatial distribution of the heat transfer coefficient over the target skin
In this case study, a comparison between a Gaussian distributed heat transfer coefficient and a constant heat transfer coefficient over the target area revealed that the spatial distribution does not affect the tissue temperature variation and growth of the IIF zone, whereas the magnitude of the heat transfer coefficient is the dominant factor. In addition, since the degree and spatial/temporal distribution of tissue cooling can be achieved in a controlled manner by adjusting the cryogen spurt duration [154] and models that enable accurate prediction of the heat transfer coefficient that account for the asymmetric deposition and spreading of the liquid cryogen are available in the literature [155–158], the assumption of a constant heat transfer coefficient over the entire sprayed area seems valid. The heat extraction induced by cryogenic sprays (R134a) across the surface of skin phantom was measured by Franco et al. [159]. The sprays were produced by typical straight tube nozzles used for dermatological lasers aimed perpendicularly or at a small angle (< 15°) to the skin surface. These nozzles were known to yield high pressure jets or finely atomized cones depending on the nozzle diameter.
(varying between 0.5–1.5 mm in diameter) [160] and they produced consistent narrow size and velocity distributions [161], where the aiming angle had a small effect on the heat extraction [162]. The skin phantom was instrumented with multiple miniature temperature sensors that measured simultaneously (every 1 mm across a 16 mm diameter cone) the temperature decrease as a function of time. Franco et al. [159] observed that while there were appreciable differences between the heat flux extracted around the sprayed zone center and the periphery, there was a subregion close to the center where the variations were minimal. This was later confirmed also by Franco et al. [163] in terms of the uniformity in the protection these sprays could provide when used in conjunction with laser irradiation. Nevertheless, the reader must not forget that non-uniformities in the heat transfer coefficient over the entire spray area exist and depend on the following parameters: geometric parameters such as nozzle diameter-length [164], nozzle-to-skin distance [165] and nozzle angle [162], cryogen spurt duration [166–173], ambient pressure [174–176], humidity and frost formation effects [177–180], skin indentation effects [181, 182], and the dynamics of cryogen spray deposition, such as cryogenic spray shape, the droplet velocity and diameter [183].

3. Epidermis, dermis and subcutaneous fat are assumed to be homogeneous in terms of thermal, physical and physiological properties.

4. Cancer tissue is generally assumed to have the same thermal and physical properties with surrounding tissue [71, 72, 137]. The assumption is also applied for BCC herein, since we are not aware of any reliable data evaluating thermal and physical properties of BCC over the cryosurgical temperature range.

5. The epidermis and subcutaneous fat are assumed to be free of blood perfusion thermal effect and metabolic heat generation [145, 147] while both are taken into account in the dermis before freezing occurs.

6. The freezing process of skin tissue initiates and completes at −0.5 and −10°C, respectively [8, 184, 185]. Once the tissue starts to freeze, blood perfusion and metabolism are assumed to cease immediately.

According to the above assumptions, heat transfer within the target tissue can be described as

\[
\rho_{\text{i}} C_{\text{i}} \frac{\partial T}{\partial t} = \nabla \cdot (k_{\text{i}} \nabla T_{\text{i}}) + \xi \dot{\omega}_{b} \rho_{b} C_{b} (T_{b} - T) + \eta \dot{q}_{m}
\]

where the index, i, identifies epidermis, BCC, dermis and subcutaneous fat, respectively; subscripts b and m represent blood and metabolism; \(\dot{\omega}_{b}\) stands for the volumetric flow rate of blood perfusion, 2.387 \times 10^{-3} (Kg blood) s^{-1} (Kg tissue)^{-1} [186]; \(\dot{q}_{m}\), 1240 W m^{-3} [186] is the metabolic heat generation rate; \(\rho_{b}\) (1060 Kg m^{-3}) and \(C_{b}\) (3840 J Kg^{-1} K^{-1}) are density and specific heat of blood [187], respectively; and \(T_{b}\) (37°C) the blood temperature. Coefficients \(\xi\) and \(\eta\) serve to implement assumption No. 5 and are defined according to anatomic structure and temperature ranges as shown in Table 1.
2.3. Computational domain and boundary conditions

Considering a nodular BCC with a radius \( R_0 \) of 5 mm, the axially symmetric two-dimensional computational domain is established as schematically shown in Figure 2. The thickness of epidermis, dermis and subcutaneous fat are 0.075 mm, 1.5 mm and 3.425 mm, respectively [145]; and the radial extension of the domain is much greater than its axial thickness. The BCC is elevated \( H = 2 \) mm from the surrounding skin surface. Before LN\(_2\) spray, the whole domain presents a uniform initial temperature \( T_{\text{in}} \) of 37°C, the normal human body temperature. Once the spray starts, the sprayed LN\(_2\) provides a convective thermal boundary on the cooling site, represented by a heat transfer coefficient, \( h_s \). Skin surface uncovered by the cooling site experiences a convection heat interaction with room air, which can be expressed as:

\[
k_{\text{epi}} \left\{ \begin{array}{ll}
h_s \left[ T(r, z, t) - T_{\text{LN}_2} \right] & 0 \leq r \leq r_s; \ t > 0 \\
h_a \left[ T(r, z, t) - T_a \right] & r > r_s; \ t > 0
\end{array} \right.
\]

where \( n \) is the normal direction of the skin surface; subscripts \( s \) and \( a \) represent spray cooling and air, while \( \text{epi} \) and \( \text{surf} \) stand for epidermis and skin surface, respectively; \( T_{\text{LN}_2} \) is –196°C, the boiling point temperature of LN\(_2\) at 1 atm [188]; \( h_s \) is the convection heat transfer coefficient of room air, 10 W m\(^{-2}\) K\(^{-1}\) [189]; and \( T_a \) is the room temperature, 25°C. The central axis is subject to an axially symmetric thermal boundary, and the constant temperature, 37°C, is applied to the other two boundaries as indicated in Figure 2, making the assumption that little thermal perturbation is produced in these boundaries during the spray cooling. A Dirichlet boundary condition of 37°C was justified by a preliminary case study where the thermal boundary condition at the bottom of the domain was adiabatic, while the other thermal and initial boundary conditions remained unchanged [135, 136]. Results indicate that after 30 s spray, the temperature at the bottom of the domain showed little change. The primary reason is that in our model, the thermal effect from the blood perfusion term is proportional to the tissue temperature difference with human body temperature (37°C). Before fully frozen, tissue with a lower temperature will generate more heat per unit volume. On the other hand, as spray cooling proceeds, more tissue starts to provide this thermal effect to prevent the tissue freezing front from moving towards deep tissue. As a result, deep tissue temperature is not affected. Considering the space limit, this preliminary case study was not reported in this work.

### Table 1: Values of \( \xi \) and \( \eta \) in equation (1)

| Tissue             | \( T \leq -0.5^\circ \text{C} \) | \( T > -0.5^\circ \text{C} \) |
|--------------------|----------------------------------|--------------------------------|
| Epidermis          | \( \xi = 0; \eta = 0; \)        | \( \xi = 0; \eta = 0; \)        |
| BCC                | \( \xi = 0; \eta = 0; \)        | \( \xi = 1; \eta = 1; \)        |
| Dermis             | \( \xi = 0; \eta = 0; \)        | \( \xi = 1; \eta = 1; \)        |
| Subcutaneous Fat   | \( \xi = 0; \eta = 0; \)        | \( \xi = 0; \eta = 0; \)        |
2.4. Tissue properties
In the present study, the thermal and physical properties of unfrozen tissues, including blood, are obtained from [190] and [187]. Volumetric flow rate of skin blood perfusion and metabolic heat generation are calculated from [186]. Thermal conductivity and specific heat of frozen dermal tissue are estimated based on the principles proposed by Duck [187]:

\[ k = \rho \sum_{n=1}^{3} \frac{k_n \varepsilon_n}{\rho_n} \]  

\[ C = \sum_{n=1}^{3} \varepsilon_n C_n \]  

where the index \( n \) represents water, protein and lipid/fat, since these three components account for approximately 99% of human skin tissue mass [187]. \( \varepsilon \) is the mass fraction of the component. The estimation also assumes that only thermal conductivity and specific heat of water are temperature dependent according to Alexiades et al. [65] while those of protein and lipid/fat are constant. The phase change latent heat is estimated based on water percentage and the latent heat of pure water. The specific heat of frozen subcutaneous fat (a time-dependent piecewise linear function) comes from the data of porcine fat at low temperatures [187]. Table 2 provides tissue properties used in this study. As can be seen, density for the epidermis, dermis/BCC and subcutaneous fat are assumed to be constant and no density average is performed in the calculation. Data are from Duck [187] unless otherwise indicated.

2.5. Numerical models
The above mathematical problem was solved by a finite-volume-method-based commercial software package, FLUENT 6.2 (Fluent Inc., New Hampshire), and the governing equation (1) was discretized by a second-order upwind scheme. It is important to mention that the energy equation in FLUENT addresses the energy transfer due to conduction, convection, species diffusion, viscous dissipation, chemical reaction

### Table 2: Thermal physical properties used in the study

| Properties | Epidermis | Dermis/BCC | Sub fat |
|------------|-----------|------------|---------|
| \( k_u \) (W m\(^{-1}\) K\(^{-1}\)) | 0.209 \(^{(a)}\) | 0.498 | 0.268 |
| \( k_f \) (W m\(^{-1}\) K\(^{-1}\)) | 0.209 \(^{(a)}\) | 1.553 + 0.0039(273-\(T\))\(^{1.156}\) \(^{(b)}\) | 0.268 |
| \( C_u \) (J Kg\(^{-1}\) K\(^{-1}\)) | 3530 | 3150 | 2400 |
| \( C_f \) (J Kg\(^{-1}\) K\(^{-1}\)) | 3530 | 521.4 + 4.65\(T\) \(^{(b)}\) | piecewise linear function |
| \( \rho \) (Kg m\(^{-3}\)) | 1150 | 1116 | 916 |
| \( L \) (J Kg\(^{-1}\)) | 0 | 217100 | 70808 |

\(^{(a)}\) from Bowman et al. [190]; \(^{(b)}\) estimated from Alexiades et al. [65].
and other volumetric heat sources, so that the solver is fairly adaptive for various applications. Hence, although our governing equation (1) does not include a convection term, the FLUENT solver still solves the comprehensive energy equation although the velocity is zero. Also, FLUENT provides several spatial discretization schemes for the energy equation, such as first and second-order upwind, Power Law, QUICK, etc. In this work, a second-order upwind scheme was used because of its improved spatial accuracy and easy convergence. The thermal effects of blood perfusion and metabolic heat generation are treated by source terms and implemented by user-defined-function. Latent heat released during freezing is addressed by the apparent heat capacity method, since this method is robust [191] and easy to implement. The benchmark of the method was conducted by calculating a one-dimensional ice formation process, and results were in good agreement with the analytic solution [135].

To evaluate detailed information from the areas of prime concern, a structured grid was constructed with small grid cells within the BCC and immediate surrounding areas of the epidermis and dermis, and larger grid cells for areas farther away from the cooling site. Such a non-uniform grid significantly reduces memory consumption and grid-independent solutions can be achieved with quick convergence. All results reported in the paper are obtained from this kind of grid.

2.6. Data processing

The present paper uses a series of dimensionless parameters in the analysis of results. To study the extent of the final IIF zone, the dimensionless coordinates, $r^*$ and $z^*$, are defined according to the characteristic length, $R_0$ (5 mm), the radius of the assumed BCC:

$$r^* = \frac{r}{R_0}$$

$$z^* = \frac{z}{R_0}$$

The heat transfer coefficient of spray cooling, $h_s$, can be represented by Nusselt number $(Nu)$:

$$Nu = \frac{h R_0}{k_{LN_2}}$$

where $k_{LN_2}$ is the thermal conductivity of $LN_2$, 0.1396 W m$^{-1}$ K$^{-1}$ [188]. In addition, the axisymmetric model allows rotation of the boundary of the IIF zone around the central axis, $z$, providing the volume of the IIF zone, $V_{IIF}$, which can be represented by the dimensionless form:

$$V_{IIF}^* = \frac{V_{IIF}}{V_{ref}}$$

where $V_{ref}$ is the reference volume, i.e. the volume of the spherical cap in Figure 2.
3. METHODOLOGY FOR DETERMINING IIF ZONE

As aforementioned, IIF requires simultaneous low temperature for phase change and a sufficiently high CR to trigger IIF. The extent of the IIF zone can therefore be delineated by determining target tissue regions where both of the prerequisites occur simultaneously. The methodology determining the extent of the IIF zone is illustrated in Figures 3 to 5.

Figure 3 shows the propagation of the isotherms of –5°C and –10°C, the upper and lower limits of tissue freezing temperature range, respectively, when \( r_s/R_0 = 0.6 \) and the heat transfer coefficient of spray cooling \( (h_s) \) is \( 10^6 \text{ W m}^{-2} \text{ K}^{-1} \). As noted in assumption No. 6, isotherm of –10°C outlines the boundary between fully frozen tissue and tissue where freezing is still in process, while –0.5°C represents the interface of freezing and non-frozen tissue. Between the isotherms is the mushy zone, the region where tissue freezing takes place. Relying merely on Figure 3, one cannot determine whether tissue freezing will result in IIF, as one must also know the distribution of CR, as shown in Figure 4.

Figure 4 presents the contours of the calculated local CR in the target tissue when \( t = 3 \text{ s} \), while other parameters are identical with those in Figure 3: \( r_s/R_0 = 0.6 \) and \( h_s = 10^6 \text{ W m}^{-2} \text{ K}^{-1} \). The transient local CR is calculated using a second order central difference scheme:

\[
CR' = \frac{T^{i-1} - T^{i+1}}{2\Delta t} \tag{9}
\]

![Figure 3: Propagation of isotherms of –5°C and –10°C (or mushy zone) in the target tissue \((h_s = 10^6 \text{ W m}^{-2} \text{ K}^{-1}, r_s/R_0 = 0.6)\).](image-url)
Numerical prediction of the intracellular ice formation zone during cryosurgery on a nodular basal cell carcinoma using liquid nitrogen spray

Figure 4: Contours of local cooling rate (CR) in the target tissue at $t = 3$ s, ($r_s/R_0 = 0.6$, $h_s = 10^6$ W m$^{-2}$ K$^{-1}$). Note that the local cooling rate (CR) varies with time.

Figure 5: Illustration of how to determine the IIF zone (shaded area) at a given time $t = 3$ s. The IIF zone is the shaded area that is commonly covered by both the mushy zone and the contour of 17°C s$^{-1}$ ($r_s/R_0 = 0.6$, $h_s = 10^6$ W m$^{-2}$ K$^{-1}$).
where superscripts, $i$, $i-1$ and $i+1$ are time index; $\Delta t$ is the time step which is constant. Each point on the contour presents the identical CR as indicated in the figure. During the cryosurgery, the variation of CR is highly dynamic [136]: as LN$_2$ spray proceeds, the high CR zone moves from epidermis (under LN$_2$ spray) to deeper tissue with the maximum CR dropping significantly.

CR is a critical parameter determining which mechanism of direct cell injury will dominate, IIIF or solution-effect. The present study defines the lowest CR that can trigger IIIF as the critical cooling rate (CCR). For the time being, it is reasonable to assume a CCR of 17°C s$^{-1}$ [192] for BCC and dermal cells. Using information on the temperature distribution and the local CR at $t=3$ s, one can determine where IIIF occurs. This is shown in Figure 5, where the isotherms of −0.5 and −10°C ($t=3$ s) are presented together with the simultaneous CR contour of 17°C s$^{-1}$. From Figure 5, one can see that there exists an area (shaded in the figure) commonly enclosed by the contour of 17°C s$^{-1}$ and the two isotherms (−0.5 and −10°C). In this area, the two prerequisites of IIIF are satisfied and one can therefore presume that IIIF occurs in the shaded area when $t=3$ s. Using our method, areas where IIIF takes place can be determined for consecutive time steps starting from $t=0$ through the end of the LN$_2$ spray, and the envelope of these areas will eventually outline the final IIIF zone.

Applying the above-described methodology, Figure 6 shows the propagation of the IIIF zone in a snapshot manner for $r_s/R_0 = 0.6$ and $h_s = 10^6$ W m$^{-2}$ K$^{-1}$. As one can see, at an early stage of the cryosurgery ($t \leq 3$ s), IIIF zone forms underneath the LN$_2$ spray. Using information on the temperature distribution and the local CR at $t=3$ s, one can therefore presume that IIIF occurs at $t=3$ s. This is shown in Figure 5, where the isotherms of −0.5 and −10°C ($t=3$ s) are presented together with the simultaneous CR contour of 17°C s$^{-1}$. From Figure 5, one can see that there exists an area (shaded in the figure) commonly enclosed by the contour of 17°C s$^{-1}$ and the two isotherms (−0.5 and −10°C). In this area, the two prerequisites of IIIF are satisfied and one can therefore presume that IIIF occurs in the shaded area when $t=3$ s. Using our method, areas where IIIF takes place can be determined for consecutive time steps starting from $t=0$ through the end of the LN$_2$ spray, and the envelope of these areas will eventually outline the final IIIF zone.

Applying the above-described methodology, Figure 6 shows the propagation of the IIIF zone in a snapshot manner for $r_s/R_0 = 0.6$ and $h_s = 10^6$ W m$^{-2}$ K$^{-1}$. As one can see, at an early stage of the cryosurgery ($t \leq 3$ s), IIIF zone forms underneath the LN$_2$ spray. Using information on the temperature distribution and the local CR at $t=3$ s, one can therefore presume that IIIF occurs in the shaded area when $t=3$ s. Using our method, areas where IIIF takes place can be determined for consecutive time steps starting from $t=0$ through the end of the LN$_2$ spray, and the envelope of these areas will eventually outline the final IIIF zone.

Figure 6: Propagation of IIIF zone ($r_s/R_0 = 0.6$, $h_s = 10^6$ W m$^{-2}$ K$^{-1}$, CCR = 17°C s$^{-1}$). Note that the propagation of the IIIF zone stops after $t = 6$ s.
and propagates in both radial and axial directions. As the spray cooling proceeds (3 s < t < 6 s), the IIF zone keeps growing in the axial direction toward the dermal layer, however propagation in the radial direction stops. This can be understood by re-investigating Figure 5, where one can see that the shaded area does not extend to skin surface. Further, propagation of the IIF zone along the axial direction is fastest initially and then slows down as cryosurgery proceeds. This is reflected in the distance that the IIF zone marches in each second along the central axis: longest in the 1st second (0.82 mm from skin surface), and shortest (0.19 mm) during seconds 5 – 6. When t = 6 s, the growth of the IIF zone stops, since the highest CR in the domain has dropped to 16.3 °C s⁻¹, less than the CCR (17°C s⁻¹) required for IIF. The red curve in Figure 5 is the envelope of the IIF zones for t = 0 – 6 s, which outlines the extent of the final IIF zone under the conditions of $r_s/R_0 = 0.6$, $h_s = 10^6$ W m⁻² K⁻¹ and CCR = 17°C s⁻¹. The methodology is implemented by an algorithm, which is incorporated into the calculation by user-defined-function in Fluent [193]. It is worth noting that this methodology can also be applied in other situations to estimate the IIF zone in the treated tissue, e.g. the cryosurgery using cryoprobe technique and the cryopreservation of human tissues.

4. RESULTS AND DISCUSSIONS

Calculations are carried out for series combinations of protocol parameters, including $h_s$ ($10^3$ ~ $10^6$ W m⁻² K⁻¹), CCR ($5$ ~ $17°C$ s⁻¹) and $r_s/R_0$ (0.6 ~ 1.0). Investigation of the results reveals that the modeling parameters have significant impact on the extent and volume of the final IIF zone, which is studied in detail herein.

4.1 Numerical models

4.1.1. Heat transfer coefficient of LN$_2$ spray cooling ($h_s$)

The present model describes the heat interaction between LN$_2$ and subjacent tissue using a convective thermal boundary with constant heat transfer coefficient, $h_s$. Different values of $h_s$, ranging from $10^3$ to $10^6$ W m⁻² K⁻¹, have been applied at the cooling site, and the resultant final IIF zones are shown in Figure 7. From Figure 7 one can see that a higher heat transfer coefficient, $h_s$, produces a larger final IIF zone. Increasing $h_s$ from $10^3$ to $5 \times 10^3$ W m⁻² K⁻¹ results in a dramatic growth of the final IIF zone, while the effect becomes relatively insignificant when $h_s$ is further increased from $5 \times 10^3$ to $5 \times 10^4$ W m⁻² K⁻¹. Calculation results also indicate that further increase of $h_s$ produces little effect on the extent of the final IIF zone. One cannot even differentiate the boundaries of IIF zones produced by $h_s = 5 \times 10^4$ and $10^6$ W m⁻² K⁻¹ since the boundaries almost overlap each other.

4.1.2. Critical cooling rate (CCR)

Previous experimental studies have reported a broad range of CR under which IIF is observed in vitro in different tissues. For example, Bischof et al. [194] reported that IIF can be observed in normal human liver tissue when tissue freezes with a CR ≥ 360°C min⁻¹; in metastatic colon carcinoma of human liver when CR ≥ 60°C min⁻¹; and in primary hepatocellular carcinoma of human liver when CR ≥ 22°C min⁻¹. In
cryosurgery, the thermal history is defined by parameters such as CR, end temperature, hold time, and thawing rate. A summary of experimental data where the time-temperature history experienced by multiple tissue systems during a thermal insult and its relation to vascular damage were assessed can be found in Hoffmann and Bischof [6]. However, little information is available about the CCR for BCC and skin tissues. The present work first assumes CCR = 17°C s⁻¹ to illustrate the methodology of determining the $IIF$ zone. In Figure 8, different values of CCR are used to study the effect of varying this value on the final $IIF$ zone ($r_j/R_0 = 0.6; h_s = 10^3$ W m⁻² K⁻¹). As one can see, with a lower CCR, e.g. 5°C s⁻¹, the final $IIF$ zone penetrates deeper and wider than results with higher CCRs. The boundaries of the final $IIF$ zones determined using different CCRs, have approximately the same curvature.

Further, Figure 8 provides important information on the CR when tissue is freezing. In the figure, the boundaries of the final $IIF$ zones by three CCRs split the target tissue into four areas: A, B, C and D. Since area A is enclosed by the boundary of CCR = 17°C s⁻¹ (blue curve in the figure), when the tissue within area A freezes, the CR is higher than 17°C s⁻¹. Similarly, one can estimate that the tissue in area B freezes with the CR between 10 and 17°C s⁻¹, area C with the CR of 5 – 10°C s⁻¹, and area D with the CR lower than 5°C s⁻¹.

In vitro experimental observations [33, 194, 195] have indicated that frozen tissue can have three morphologies depending on the CR during tissue freezing: 1) $IIF$ and

\[ r_j/R_0 = 0.6 \]

\[ h_s = 10^3 \text{ W/m}^2 \text{- K} \]

\[ 2 \times 10^3 \]

\[ 5 \times 10^3 \]

\[ 10^4 \]

\[ 5 \times 10^4 \]

\[ CCR = 17^\circ C/s \]
extracellular ice formation (EIF) with little cell dehydration, associated with sufficiently higher CRs; 2) IIF, EIF and cell dehydration, resulting from moderate CRs; 3) EIF together with dehydrated cells without IIF, caused by low CRs. Accordingly, Figure 8 can be applied to estimate the resultant tissue morphology, as discussed below.

Assuming that the CCR is 5°C s⁻¹ for BCC and dermal cells and 17°C s⁻¹ is the CR high enough to preclude any cell dehydration, IIF and cell dehydration then coexist within the CR range of 5~17°C s⁻¹, as schematically illustrated in Figure 9.

**Figure 8:** Effect of CCR on the final IIF zone: CCR = 5, 10 and 17°C s⁻¹ ($r_s/R_0 = 0.6$, $h_s = 10^6$ W m⁻² K⁻¹).

**Figure 9:** Schematic of how the cooling rates and CCR affects the IIF and cell dehydration.
Combining Figures 8 and 9 allows one to estimate the morphology in frozen tissue: area A, directly underneath the LN\textsubscript{2} cooling site, is frozen with a high CR (>17°C s\textsuperscript{-1}) and should be dominated by IIF accompanied with EIF, with little cell dehydration; areas B and C freeze with moderate CR (5 ~ 17°C s\textsuperscript{-1}) and are predicted to experience IIF, EIF and cell dehydration simultaneously; area D, furthest away from the cooling site, should experience a low CR (<5°C s\textsuperscript{-1}), resulting in EIF and possibly cell dehydration without IIF.

4.1.3. Radius of the LN\textsubscript{2} spray (r\textsubscript{s})

Figure 10 shows the final IIF zones when r\textsubscript{s}/R\textsubscript{0} = 0.6, 0.8, and 1.0, respectively, while other parameters are h\textsubscript{s} = 5 \times 10\textsuperscript{4} W m\textsuperscript{-2} K\textsuperscript{-1} and CCR = 17°C s\textsuperscript{-1}. As one can see, increasing r\textsubscript{s}/R\textsubscript{0} produces a greater final IIF zone, which penetrates deeper into skin tissue. This information reminds the clinician that the LN\textsubscript{2} spray should be controlled to avoid a larger than necessary cooling area on the skin surface in order to minimize the final scar. A large cooling area will also result in a deeper final IIF zone, which may injure important tissue underlying the treated skin.

4.2. Effects of modeling parameters on the volume of final IIF zone, V\textsubscript{IIF}.

Given the boundary of the final IIF zone, one can obtain its volume (V\textsubscript{IIF}) by rotating the boundary around the central axis. From figure 7 one can see a trend that V\textsubscript{IIF} grows as h\textsubscript{s} increases, which is studied quantitatively for different CCRs in figure 11, where V\textsubscript{IIF} takes the dimensionless form, V\textsubscript{IIF}*\textsuperscript{h}, and h\textsubscript{s} is represented by the Nusselt number, Nu.

![Figure 10](image_url) **Figure 10:** Effect of r\textsubscript{s} on the final IIF zone (h\textsubscript{s} = 5 \times 10\textsuperscript{4} W m\textsuperscript{-2} K\textsuperscript{-1}, CCR = 17°C s\textsuperscript{-1}).
Regression of the results reveals that the variation of \( V_{IIF}^{*} \) can be described by an exponential decay function of \( Nu \):

\[
V_{IIF}^{*} = V_{0}^{*} - a \exp(-b \cdot Nu) \tag{10}
\]

where \( V_{0}^{*} \), \( a \) and \( b \) are constants obtained from the regression, as shown in Table 3.

As one can see, when \( Nu \) exceeds 600, \( V_{IIF}^{*} \) shows little variation and approaches \( V_{0}^{*} \). \( V_{0}^{*} \) represents the dimensionless volume of the \( IIIF \) zone when the cooling site is subject to a Dirichlet thermal boundary: \( T(r, z, t) = -196^\circ C \). For spray cooling with \( Nu \geq 600 \) therefore, one can use \( V_{0}^{*} \) to estimate the tissue volume of the final \( IIIF \) zone without significant error. Figure 11 also indicates that the variation of \( V_{0}^{*} \) is the function of two parameters: CCR and \( r_s \), which is studied in Figure 12.

![Figure 11: Variation of the volume of the final IIIF zone (\( V_{IIF}^{*} \)) with the Nusselt number (\( Nu \)) of spray cooling for three CCRs: 5, 10, 17°C s\(^{-1}\) (\( r_s/R_0 = 0.6 \)). Note that \( V_{IIF}^{*} \) reaches a constant at about \( Nu = 600 \) for all CCRs.](image)

**Table 3: Regression results for \( V_{0}^{*} \), \( a \) and \( b \) in equation (10)**

| CCR (°C s\(^{-1}\)) | \( V_{0}^{*} \) | \( a \)      | \( b \)        |
|----------------------|----------------|-------------|----------------|
| 5                    | 1.678          | 1.23        | \( 7.944 \times 10^{-3} \) |
| 10                   | 1.678          | 0.823       | \( 7.31 \times 10^{-3} \)  |
| 17                   | 0.7506         | 0.6144      | \( 6.694 \times 10^{-3} \) |
In Figure 12, a dimensional CR is used for the data presentation, since there is no apparent reference cooling rate that can be used to non-dimensionalize the CR. From Figure 12 it can be seen that $V^*_0$ decreases as CCR increases. The three curves are all relatively steep at the left end and flat at the right end, implying that at lower levels ($< 7^\circ$C s$^{-1}$), variation of the CCR produces significant change on $V_{IIIF}$, while the effect becomes insignificant when CCR takes higher values ($>15^\circ$C s$^{-1}$), especially for the case of $r_s/R_0 = 0.6$. On the other hand, increasing $r_s$ results in dramatic growth of $V^*_0$, which implies that the greater cooling site area can significantly enhance the cooling efficiency.

4.3. Clinic implication

In clinical practice, an optimal protocol is desired for cryosurgery of BCC to achieve the best outcome: destruction of all cancerous tissue with minimal injury to surrounding healthy tissue. To ensure the former, the cryosurgery protocol needs to be controlled to generate a final $IIF$ zone enclosing the entire BCC. Assuming the geometry of BCC can be detected as shown by the dashed line in Figure 13, one can determine the final $IIF$ zone applying our methodology for a spray of 30 s based on CCR = $17^\circ$C s$^{-1}$ and $5^\circ$C s$^{-1}$ ($r_s/R_0 = 0.6$, $h_i = 10^6$ W m$^{-2}$ K$^{-1}$). During the cryosurgery, $IIF$ zone propagation stops once the local CR drops below CCR. The mushy zone, however, continues to propagate until the spray ends ($t = 30$ s in our demonstration). Utilizing the analysis described in Section 4.1.2, one can determine the injury mechanisms in different portions of target tissue: tissue enclosed within the final $IIF$ zone by CCR = $17^\circ$C s$^{-1}$ (dark red line) is injured by $IIF$ and
EIF; between the dark red line and the green line, tissue experiences IIF, EIF and SE, simultaneously; EIF and SE dominate between the green line and the dotted blue line; beyond the red dotted line, the tissue are safe from any freezing injury. From Figure 13 one can see that the peripheral portion of the BCC tissue is not enclosed in the final IIF zone \( \text{CCR} = 5^\circ \text{C s}^{-1} \), and the tissue therein experiences EIF and SE when spray cooling ends at \( t = 30 \text{ s} \). Since SE requires prolonged time to achieve significant injury effect, the survival of the cancerous tissue therein is of relatively high probability after the treatment. Therefore, we would recommend that \( r_s/R_0 = 0.6 \) should be replaced by an alternative higher value, i.e. a larger spray cooling site, by which the final IIF zone can enclose the entire BCC tissue. It is, however, worth noting again that a large cooling site will result in a greater final scar, and possible injury to the underlying tissue, all of which must be considered in determination of the optimal cooling site area.

5. CONCLUSIONS
A two dimensional mathematic model has been established to address the heat transfer and tissue freezing processes during a cutaneous cryosurgery on a nodular BCC. A multi-layer skin tissue model is implemented based on the anatomic structure and physiological characteristics of human skin tissue. Pennes equation is applied to describe the heat transfer within target tissue and apparent heat capacity method is used...
to address the liberation of latent heat during tissue freezing. The cooling site is subject to a convective thermal boundary.

A methodology to determine the extent of the IIIF zone, using transient temperature and CR results, is presented. Using this methodology, the extents and volumes of final IIIF zones under various working conditions are studied. Results indicate that the IIIF zone forms directly underneath the cooling site and propagates in both axial and radial directions during the early stage of the cryosurgery. As spray cooling proceeds, propagation in the radial direction stops first, while that along the axial direction continues until the CR cannot support IIIF. The morphology of frozen tissue is discussed: directly underneath the cooling site, tissue is dominated by IIIF and EIF and this area is surrounded by a tissue layer where IIIF, EIF and cell dehydration coexist. In the tissue further away from the cooling site, EIF and possibly cell dehydration occur without IIIF.

The volume of the final IIIF zone, $V_{IIIF}$, depends on spray cooling $Nu$ number by an exponential decay function and the sensitivity concentrates in the range of $Nu < 600$. $V_{IIIF}$ increases as CCR decreases and this variation is most pronounced for lower CCR values. Increasing the cooling site area can greatly enhance the cooling efficiency in terms of $V_{IIIF}$.

This work provides the numerical means to study IIIF quantitatively, therefore contributing to the thorough understanding of thermal history and tissue injury during cutaneous cryosurgery using $LN_2$ spray. Results can be applied for optimization of protocol parameters. The methodology determining the IIIF zone can also be used in other applications, e.g. cryosurgery utilizing the cryoprobe technique and cryopreservation of human tissues.

REFERENCES

[1] C. A. Turkington and J. S. Dover, Skin Deep: an A-Z of Skin Disorders, Treatments and Health, Facts on File, 1996.

[2] I. M. Freedberg, Fitzpatrick's Dermatology in General Medicine, McGraw-Hill, 1999.

[3] D. I. M. Kuipers, M. R. T. M. Thissen and M. H. A. Neumann, Basal cell carcinoma, American Journal of Clinical Dermatology, 2002, 3, 247–259.

[4] B. Rubinsky, Cryosurgery, Annual Review of Biomedical Engineering, 2000, 2, 157–187.

[5] A. A. Gage and J. G. Baust, Mechanisms of tissue injury in cryosurgery, Cryobiology, 1998, 37, 171–186.

[6] N. E. Hoffmann and J. C. Bischof, The cryobiology of cryosurgical injury, Urology, 2002, Suppl 2A, 40–49.

[7] J. Farrant and C. A. Walter, The cryobiological basis for cryosurgery, Journal of Dermatologic Surgery & Oncology, 1997, 3, 403–407.

[8] N. E. Hoffman and J. C. Bischof, Cryosurgery of normal and tumor tissue in the dorsal skin flap chamber: Part I-thermal response, ASME Journal of Biomechanical Engineering, 2001, 123, 301–309.
[9] W. R. Hollister, A. J. Mathew, J. G. Baust and R. G. V. Buskirk, Effects of freezing on cell viability and mechanisms of cell death in a human prostate cell line, *Molecular Urology*, 1998, 2, 3–18.

[10] M. Ismail, R. Morgan, K. Harrington, J. Davies and H. Pandha, Enhancing prostate cancer cryotherapy using tumour necrosis factor related apoptosis-inducing ligand (TRAIL) sensitisation in an *in vitro* cryotherapy model, *Cryobiology*, 2009, 59(2), 207–213.

[11] Y. Rabin, Key issues in bioheat transfer simulations for the application of cryosurgery planning, *Cryobiology*, 2008, 56, 248–250.

[12] J. Liu, Y. X. Zhou, T. H. Yu, L. Gui, Z. S. Deng and Y. G. Lv, New cryoprobe system with powerful heating features and its performance tests on biomaterials, *ASME International Mechanical Engineering Congress and RD&D Expo, Washington, DC, November, 2003*, 14–23.

[13] J. Liu, Y. X. Zhou, T. H. Yu, L. Gui, Z. S. Deng and Y. G. Lv, Minimally Invasive system capable of performing both cryosurgery and hyperthermia, *Minimally Invasive Therapy & Allied Technologies, 2004*, 13, 47–57.

[14] P. Mazur, Freezing of living cells: mechanisms and implications, *American Journal of Physics*, 1984, 143, C125–C142.

[15] W. H. Yang, S. T. Liao, S. Y. Shen and H. C. Chang, The speed of ice growth as an important indicator in cryosurgery, *The Journal of Urology*, 2004, 172(1), 345–348.

[16] W. Cai, T. Gao, H. Hong and J. Sun, Applications of gold nanoparticles in cancer nanotechnology, *Nanotechnology, Science and Applications*, 2008, 1, 17–32.

[17] A. A. Gage, J. M. Baust and J. G. Baust, Experimental cryosurgery investigations *in vivo*, *Cryobiology*, 2009, 59(3), 229–243.

[18] J. F. Langenhuijsen, E. M. P. Eveline and H. Vergunst, Cryosurgery for prostate cancer: an update on clinical results of modern cryotechnology, *European Urology*, 2009, 55(1), 76–86.

[19] G. A. Sandison, Future directions for cryosurgery computer treatment planning, *Urology*, 2002, 60(2a), 50–55.

[20] L. J. Hayes, K. R. Diller, H. J. Chang and H. S. Lee, Prediction of local cooling rates and cell survival during the freezing of a cylindrical specimen, *Cryobiology*, 1998, 25, 67–82.

[21] G. J. Müller and A. Roggan, *Laser-Induced Interstitial Thermotherapy*, SPIE The International Society for Optical Engineering, Berlin, 1995.

[22] A. Weill, A. Shitzer and P. Bar-Yoseph, Finite element analysis of the temperature field around two adjacent cryo-probes, *ASME Journal of Biomechanical Engineering*, 1993, 115, 374–379.

[23] B. Yang, R. G. Wan, K. B. Muldrew and B. J. Donnelly, A finite element model for cryosurgery with coupled phase change and thermal stress aspects, *Finite Elements in Analysis and Design*, 2008, 44(5), 288–297.
[24] Y. T. Zhang and J. Liu, Numerical study on three-region thawing problem during cryosurgical re-warming, *Medical Engineering & Physics*, 2002, 24, 265–277.

[25] Y. Rabin and A. Shitzer, Exact solution to the one-dimensional inverse-Stefan problem in nonideal biological tissues, *ASME Journal of Heat Transfer*, 1995, 117, 425–431.

[26] Y. Rabin and A. Shitzer, Combined solution of the inverse Stefan problem for successive freezing/thawing in nonideal biological tissues, *Transactions of the ASME*, 1997, 119, 146–152.

[27] Y. Rabin and A. Shitzer, Numerical solution of the multidimensional freezing problem during cryosurgery, *ASME Journal of Biomechanical Engineering*, 1998, 120(1), 32–37.

[28] J. Zhang, J. T. C. Hua and E. T. Chen, Experimental measurement and theoretical analyses of the freezing-thawing processes around a probe, *CryoLetters*, 2000, 21, 245–254.

[29] H. Budman, A. Shitzer and J. Dayan, Analysis of the inverse problem of freezing and thawing of a binary solution during cryosurgical processes, *ASME Journal of Biomechanical Engineering*, 1995, 117, 193–202.

[30] B. Rubinsky and A. Shitzer, Analysis of a Stefan-like problem in a biological tissue around a cryosurgical probe, *ASME Journal of Heat Transfer*, 1976, 98, 514–519.

[31] J. W. Baish, P. S. Ayyaswamy and K. R. Foster, Heat transport mechanisms in vascular tissues: a model comparison, *Journal of Biochemical Engineering*, 1986, 108(4), 324–331.

[32] S. G. Klemick, M. A. Jog and P. S. Ayyaswamy, Numerical evaluation of heat clearance properties of a radiatively heated biological tissue by adaptive grid scheme, *Numerical Heat Transfer, Part A*, 1997, 31, 451–467.

[33] J. C. Bischof, D. Smith, P. V. Pazhayannur, C. Manivel, J. Hulbert and K. P. Roberts, Cryosurgery of dunning AT-1 rat prostate tumor: Thermal biophysical, and viability response at the cellular and tissue level, *Cryobiology*, 1997, 34, 42–69.

[34] J. C. Rewcastle and G. A. Sandison, A model for the time dependent three-dimensional thermal distribution within iceballs surrounding multiple cryoprobes, *Medical Physics*, 2011, 28(6), 1125–1137.

[35] J. C. Rewcastle, G. A. Sandison, L. J. Hahn, J. C. Saliken, J. G. McKinnon and B. J. Donnelly, A model for the time-dependent thermal distribution within an iceball surrounding a cryprobe, *Physics in Medicine and Biology*, 1998, 43, 3519–3534.

[36] D. Zhong-Shan and J. Liu, Numerical simulation of selective freezing of target biological tissues following injection of solutions with specific thermal properties, *Cryobiology*, 2005, 50, 183–192.

[37] Z.-H. Deng and J. Liu, Modeling of multidimensional freezing problem during
cryosurgery by the dual reciprocity boundary element method, *Engineering Analysis with Boundary Elements*, 2004, 28, 97–108.

[38] C. Bonacina, G. Comini, A. Fasano and M. Primicero, Numerical solution of phase-change problems, *International Journal of Heat and Mass Transfer*, 1973, 16, 1825–1832.

[39] T. Basak and K. G. Ayappa, Role of length scales on microwave thawing dynamics in 2D cylinders, *International Journal of Heat and Mass Transfer*, 2002, 45, 4543–4559.

[40] C. Chen, H. Guo, Y. Liu, H. Yue and C. Wang, A new kind of phase change material (PCM) for energy-storing wallboard, *Energy and Buildings*, 2008, 40, 882–890.

[41] A. J. Dalhuijsen and A. Segal, Comparison of finite element techniques for solidification problems, *International Journal for Numerical Methods in Engineering*, 1986, 23, 1807–1829.

[42] J. A. Dantzig, Modelling liquid-solid phase changes with melt convection, *International Journal for Numerical Methods in Engineering*, 1989, 28, 1769–1785.

[43] P. Eslami-nejad and M. Bernier, Freezing of geothermal borehole surroundings: A numerical and experimental assessment with applications, *Applied Energy*, 2012, 98, 333–345.

[44] C. W. Foong, O. J. Nydal and J. Løvseth, Investigation of a small scale double-reflector solar concentrating system with high temperature heat storage, *Applied Thermal Engineering*, 2011, 31, 1807–1815.

[45] D. K. Gartling, Finite element analysis of convective heat transfer problems with change of phase, *Computer Methods in Fluids*, K. Morgan, C. Taylor and C. Brebbia, eds., Pentech Press, 1980.

[46] S. D. Guidice, G. Comini and R. W. Lewis, Finite element simulation of freezing processes in soils, *International Journal for Numerical and Analytical Methods in Geomechanics*, 1978, 2(3), 223–235.

[47] H. T. Hashemi and C. M. Sliepcevich, Effect of seepage stream on artificial soil freezing, *ASCE Journal of the Soil Mechanics and Foundations Division*, 1973, 99(SM3), 267–289.

[48] D. Heim, Isothermal storage of solar energy in building construction, *Renewable Energy*, 2010, 35, 788–796.

[49] D. Heim and J. A. Clarke, Numerical modelling and thermal simulation of PCM-gypsum composites with ESP-r, *Energy and Buildings*, 2004, 36, 795–805.

[50] Q. T. Pham, The use of lumped capacitance in the finite-element solution of heat conduction problems with phase change, *International Journal of Heat and Mass Transfer*, 1986, 29, 285–291.

[51] M. Zukowski, Mathematical modeling and numerical simulation of a short term thermal energy storage system using phase change material for heating applications, *Energy Conversion and Management*, 2007, 48, 155–165.
[52] V. R. Voller, C. R. Swaminathan and B. G. Thomas, Fixed grid techniques for phase change problems: A review, *International Journal for Numerical Methods in Engineering*, 1990, 30, 875–898.

[53] F. Civan and C. M. Sliepcevich, Limitation in the apparent heat capacity formulation for heat transfer with phase change, *Proceedings of the Oklahoma Academy of Science*, 1987.

[54] Z. X. Gong and A. S. Mujumdar, Non-convergence versus non-conservation in effective heat capacity methods for phase change problems, *International Journal of Numerical Methods for Heat and Fluid Flow*, 1997, 7(6), 565–579.

[55] Z. X. Gong and A. S. Mujumdar, Non-conservation in effective heat capacity methods for phase change problems, *International Journal of Numerical Methods for Heat and Fluid Flow*, 1997, 17, 565–579.

[56] J. S. Hsiao, An efficient algorithm for finite-difference analyses of heat transfer with melting and solidification, *Numerical Heat Transfer, Part A*, 1985, 8, 653–666.

[57] R. T. Lee and W. Y. Chiou, Finite-element analysis of phase change problems using multilevel techniques, *Numerical Heat Transfer, Part B*, 1995, 27, 277–290.

[58] M. R. Amin, Thermal analysis during continuous casting process using effective heat capacity method, *Journal of Thermophysics and Heat Transfer*, 2000, 14, 170–176.

[59] M. R. Amin and D. Greif, Conjugate heat transfer during two-phase solidification process in a continuously moving metal using average heat capacity method, *International Journal of Heat and Mass Transfer*, 1999, 42, 2883–2895.

[60] G. Comini, S. D. Guidice and O. Saro, A conservative algorithm for multidimensional conduction phase change, *International Journal for Numerical Methods in Engineering*, 1990, 30, 697–709.

[61] V. R. Voller and C. R. Swaminathan, Generalized source-based method for solidification phase change, *Numerical Heat Transfer, Part B*, 1991, 19(2), 175–189.

[62] Z. S. Deng and J. Liu, Monte Carlo method to solve multidimensional bioheat transfer problem, *Numerical Heat Transfer, Part B*, 2002, 42, 543–567.

[63] Z. S. Deng and J. Liu, Modeling of multidimensional freezing problem during cryosurgery by the dual reciprocity boundary element method, *Engineering Analysis with Boundary Elements*, 2004, 28, 97–108.

[64] M. Jankun, T. J. Kelly, A. Zaim, K. Young, R. W. Keck, S. H. Selman and J. Jankun, Computer model for cryosurgery of the prostate, *Computer Aided Surgery*, 1999, 4(4), 193–199.

[65] V. Alexiades and A. D. Solomon, *Mathematical modeling of melting and freezing processes*, Hemisphere, Washington DC, Chap. 4, 1993.
[66] R. I. Andrushkiw, Mathematical modeling of freezing front propagation in biological tissue, *Mathematical and Computer Modelling*, 1990, 13, 1–9.

[67] H. Budman, A. Shitzer and S. D. Guidice, Investigation of temperature fields around embedded cryoprobes, *ASME Journal of Biomechanical Engineering*, 1986, 108, 42–48.

[68] S. S. Shastri and R. M. Allen, Method of lines and enthalpy method for solving moving boundary problems, *International Communications in Heat and Mass Transfer*, 1998, 25(4), 531–540.

[69] Y. Rabin and E. Korin, An efficient numerical solution for the multidimensional solidification (or melting) problem using a microcomputer, *International Journal of Heat and Mass Transfer*, 1993, 36(3), 673–683.

[70] Y. Rabin, The effect of temperature-dependent thermal conductivity in heat transfer simulations of frozen biomaterials, *CryoLetters*, 2000, 21, 163–170.

[71] R. V. Devireddy, D. J. Smith and J. C. Bischof, Effect of microscale mass transport and phase change on numerical prediction of freezing in biological tissues, *ASME Journal of Heat Transfer*, 2002, 124, 365–374.

[72] J. Zhang, G. A. Sandison, J. Y. Murthy and L. X. Xu, Numerical simulation for heat transfer in prostate cancer cryosurgery, *ASME Journal of Biomechanical Engineering*, 2005, 127, 279–294.

[73] J. O. M. Karlsson, Theoretical analysis of unidirectional intercellular ice propagation in stratified cell clusters, *Cryobiology*, 2004, 48, 357–361.

[74] M. J. Gonder, W. A. Soanes and S. Shulman, Cryosurgical treatment of the prostate, *Investigative Urology*, 1966, 4, 372–378.

[75] M. J. Gonder, W. A. Soanes and V. Smith, Experimental prostate cryosurgery, *Investigative Urology*, 1964, 1(6), 10–19.

[76] M. J. Gonder, W. A. Soanes and V. Smith, Chemical and morphologic changes in the prostate following extreme cooling, *Annals of the New York Academy of Sciences*, 1965, 125, 716–729.

[77] P. Bhowmick, J. E. Coad, S. Bhowmick, J. L. Pryor, T. Larson, J. D. I. Rosette and J. C. Bischof, *In vitro* assessment of the efficacy of thermal therapy in human benign prostatic hyperplasia, *International Journal of Hyperthermia*, 2004, 20(4), 421–439.

[78] J. A. Dow and K. Waterhouse, An experimental study in lethal freezing temperatures of the prostate gland, *Journal of Urology*, 1970, 103, 454–457.

[79] D. J. Smith, W. M. Fahssi, D. J. Swanlund and J. C. Bischof, A parametric study of freezing injury in AT-1 rat prostate tumor cells, *Cryobiology*, 1999, 39, 13–28.

[80] J. Jiang, R. Goel, S. Schmechel, G. Vercellotti, C. Forster and J. Bischof, Preconditioning cryosurgery: Cellular and molecular mechanisms and dynamics of TNF-α enhanced cryotherapy in an in vivo prostate cancer model system, *Cryobiology*, 2010, 61(3), 280–288.
[81] A. T. Robilotto, D. M. Clarke, J. M. Baust, R. G. V. Buskirk, A. A. Gage and J. G. Baust, Development of a tissue engineered human prostate tumor equivalent for use in evaluation of cryoablative techniques, *Technology in Cancer Research & Treatment*, 2007, 6(2), 81–90.

[82] D. P. Klossner, A. T. Robilotto, D. M. Clarke, R. G. VanBuskirk, J. M. Baust, A. A. Gage and J. G. Baust, Cryosurgical technique: Assessment of fundamental variables using human prostate cancer model systems, *Cryobiology*, 2007, 55, 189–199.

[83] T. R. Larson, D. W. Robertson, A. Corica and D. G. Bostwick, In vivo interstitial temperature mapping of the human prostate during cryosurgery with correlation to histopathologic outcomes, *Urology*, 2000, 55(4), 547–552.

[84] K. Tatsutani, B. Rubinsky, G. Onik and R. Dahiya, Effect of thermal variables on frozen human primary prostatic adenocarcinoma cells, *Urology*, 1996, 48, 441–447.

[85] T. M. Turk, M. A. Rees, C. E. Myers, S. E. Mills and J. Y. Gillenwater, Determination of optimal freezing parameters of human prostate cancer in a nude mouse model, *Prostate*, 1999, 38, 137–143.

[86] B. Han, E. D. Grassl, V. H. Barocas, J. E. Coad and J. C. Bischof, A cryoinjury model using engineered tissue equivalents for cryosurgical applications, *Annals of Biomedical Engineering*, 2005, 33(7), 972–982.

[87] J. K. Cohen, Cryosurgery of the prostate: techniques and indications, *Reviews in Urology*, 2004, 6(4), S20–S26.

[88] R. J. Babaian, B. Donnelly, D. Bahn, J. G. Baust, M. Dineen, D. Ellis, A. Kats, L. Pisters, D. Rukstalis, K. Shinohara and J. B. Thrasher, Best practice statement on cryosurgery for the treatment of localized prostate cancer cells, *The Journal of Urology*, 2008, 180(5), 1993–2004.

[89] D. K. Bahn, F. Lee, R. Badalament, A. Kumar, J. Grescki and M. Chernick, Targeted cryoablation of the prostate: 7-year outcomes in the primary treatment of prostate cancer, *Urology*, 2002, 60(Supplement 2A), 3–11.

[90] A. E. Katz and J. C. Rewcastle, The current and potential role of cryoablation as a primary therapy for localized prostate cancer, *Current Oncology Reports*, 2003, 5(3), 231–238.

[91] J. Baust, A. A. Gage, H. Ma and C. Zhang, Minimally invasive cryosurgery—technological advances, *Cryobiology*, 1997, 34, 373–384.

[92] J. G. Baust and A. A. Gage, Progress toward optimization of cryosurgery, *Technology in Cancer Research & Treatment*, 2004, 3, 95–101.

[93] R. Romero-Méndez, K. Chu, H. Vu, W. Franco and G. Aguilar, Confinement of freezing front by laser irradiation during cryosurgery, *Proceedings of 2004 ASME-Summer Heat Transfer Conference*, San Francisco, CA, 2005.

[94] R. Romero-Méndez, W. Franco and G. Aguilar, Laser-assisted cryosurgery of prostate: numerical study, *Physics in Medicine and Biology*, 2007, 52(2), 463–478.
L. Martínez-Suástegui, B. Duperray, F. Godínez, G. Guillén, A. Slade and G. Aguilar, Laser-assisted cryosurgery in ex vivo mice hepatic tissue: viability assays using green fluorescent protein, *Annals of Biomedical Engineering*, 2011, 39(2), 636–648.

F. G. Keanini and B. Rubinsky, Optimization of multiprobe cryosurgery, *Transactions of the ASME*, 1992, 114, 796–802.

R. Baissalov, G. A. Sandison, B. J. Donnelly, J. C. Saliken, J. G. McKinnon, K. Muldrew and J. C. Rewcastle, A semi-empirical treatment planning model for optimization of multiprobe cryosurgery, *Physics in Medicine and Biology*, 2000, 45, 1085–1098.

R. Baissalov, G. A. Sandison, D. Reynolds and D. Muldrew, Simultaneous optimization of cryoprobe placement and thermal protocol for cryosurgery, *Physics in Medicine and Biology*, 2001, 46, 1799–1814.

K. J. Chua, S. J. Chou and J. C. Ho, An analytical study on the thermal effects of cryosurgery on selective cell destruction, *Journal of Biomechanics*, 2007, 40(1), 100–116.

K. Ivarsson, J. Olsrud, C. Sturesson, P. H. Möller, B. R. Persson and K.-G. Tranberg, Feedback interstitial diode laser (805 nm) thermotherapy system: ex vivo evaluation and mathematical modeling with one and four-fibers, *Lasers in Surgery and Medicine*, 1998, 22, 86–96.

D. C. Lung, T. F. Stahovich and Y. Rabin, Computerized planning for multiprobe cryosurgery using a force-field analogy, *Computer Methods in Biomechanics and Biomedical Engineering*, 2004, 7(2), 101–110.

Z. Magalov, A. Shitzer and D. Degani, Isothermal volume contours generated in a freezing gel by embedded cryo-needles with applications to cryo-surgery, *Cryobiology*, 2007, 55(2), 127–137.

M. R. Rossi, D. Tanaka, K. Shimada and Y. Rabin, Computerized planning of cryosurgery using bubble packing: An experimental validation on a phantom material, *International Journal of Heat and Mass Transfer*, 2008, 51, 5671–5678.

M. R. Rossi, D. Tanaka, K. Shimada and Y. Rabin, Computerized planning of prostate cryosurgery using variable cryoprobe insertion depth, *Cryobiology*, 2010, 60(1), 71–79.

D. Tanaka, K. Shimada, M. R. Rossi and Y. Rabin, Computerized planning of prostate cryosurgery with pullback operation, *Computer Aided Surgery*, 2008, 13(1), 1–13.

M. R. Rossi, D. Tanaka, K. Shimada and Y. Rabin, An efficient numerical technique for bioheat simulations and its application to computerized cryosurgery planning, *Journal of Endourology*, 2007, 85(1), 41–50.

D. Tanaka, K. Shimada and Y. Rabin, Two-phase computerized planning of cryosurgery using bubble-packing and force-field analogy, *Journal of Biomechanical Engineering*, 2005, 128(1), 49–59.
[108] D. Tanaka, K. Shimada, M. R. Rossi and Y. Rabin, Towards intra-operative computerized planning of prostate cryosurgery, *International Journal of Medical Robotics and Computer Assisted Surgery*, 2007, 3(1), 10–19.

[109] D. Tanaka, K. Shimada, M. R. Rossi and Y. Rabin, Cryosurgery planning using bubble packing in 3D, *Computer Methods in Biomechanics and Biomedical Engineering*, 2007, 11(2), 113–121.

[110] S. Maruyama, E. Nino and G. Ruocco, Analysis of a thermoelectrical device for active heat transfer control, *International Journal of Thermal Sciences*, 2001, 40, 911–916.

[111] S. Maruyama, K. Nakagawa, H. Takeda, S. Aiba and A. Komiya, The flexible cryoprobe using Peltier effect for heat transfer control, *Journal of Biochemical Science and Engineering*, 2008, 138–150.

[112] J. C. Bischof, N. Merry and J. Hulbert, Rectal protection during prostate cryosurgery: design and characterization of an insulating probe, *Cryobiology*, 1997, 34, 80–92.

[113] J. C. Bischof, N. Merry and J. Hulbert, Design and characterization of an insulating probe, *Cryobiology*, 1997, 34, 80–92.

[114] H. M. Budman, J. Dayan and A. Shitzer, Controlled freezing of nonideal solutions with application to cryosurgical processes, *Journal of Biochemical Engineering*, 1991, 113, 430–437.

[115] Z. Chang, J. J. Finkelstein, H. Ma and J. G. Baust, Development of high-performance multiprobe cryosurgical device, *Biomedical Instrumentation Technology*, 1994, 28, 383–390.

[116] I. Cooper and A. Lee, Cryostatic congelation. A system for producing a limited controlled region of cooling or freezing of biological tissue, *The Journal of Nervous and Mental Disease*, 1961, 133, 259–266.

[117] W. E. Fahmy and N. K. Bissada, Cryosurgery for prostate cancer, *Archives of Andrology*, 2003, 49, 397–407.

[118] A. A. Gage, History of cryosurgery, *Seminars in Surgical Oncology*, 1998, 14, 99.

[119] M. A. Ghafar, C. W. Johnson, A. D. L. Taille, M. C. Benson, E. Bagiella, M. Fatal, C. A. Olsson and A. E. Katz, Salvage cryotherapy using an argon based system for locally recurrent prostate cancer after radiation therapy: the Columbia experience, *Journal of Urology*, 2001, 166(4), 1333–1337.

[120] B. Gowardhan and D. Greene, Cryotherapy for the prostate: an *in vitro* and clinical study of the two new developments; advanced cryoneedles and a temperature monitoring system, *BJU International*, 2007, 100(2), 295–302.

[121] B. Gowardhan, B. Thomas, S. Asterling, N. Sheikh and D. R. Greene, Cryosurgery for prostate cancer-Experience with third-generation cryosurgery and novel developments in the field, *European Association of Urology*, 2007, 6, 516–520.
K. R. Han, J. K. Cohen, R. J. Miller, A. J. Pantuck, D. G. Freitas, C. A. Cuevas, H. L. Kim, J. Lugg, S. J. Childs, B. Shuman, M. A. Jayson, N. D. Shore, Y. Moore, A. Zisman, J. Y. Lee, R. Ugarte, L. A. Mynderse, T. M. Wilson, S. D. Sweat, H. Zincke and A. S. Beldegrun, Treatment of organ confined prostate cancer with third generation cryosurgery: preliminary multicenter experience, *Journal of Urology*, 2003, 170, 1123–1130.

K.-R. Han and A. S. Beldegrun, Third generation cryosurgery for primary and recurrent prostate cancer, *BJU International*, 2004, 93, 14–18.

S. G. Hubosky, M. D. Fabrizio, P. F. Schellhammer, B. b. Barone, C. M. Tepera and R. W. Given, Single center experience with third-generation cryosurgery for management of organ-confined prostate cancer: Critical evaluation of short-term outcomes, complications, and patient quality of life, *Journal of Endourology*, 2007, 21, 1521–1531.

S. Kasuto, Prostate cancer: cryotherapy, *Urologic Clinics of North America*, 2003, 30, 725–736.

F. Lee, D. K. Bahn and R. A. Badalament, Cryosurgery for prostate cancer: Improved glandular ablation by use of 6 to 8 cryoprobes, *Urology*, 1999, 54, 135–140.

P. J. Littrup, B. Jallad, V. Vorugu, G. Littrup, B. Currier, M. George and D. Herring, Lethal isotherms of cryoablation in a phantom study: effects of heat load, probe size and number, *Journal of Vascular and Interventional Radiology*, 2009, 20(10), 1329–1341.

B. Z. Maytal, Fast Joule-Thomson cryocycling device for cryosurgical applications, *Advances in Cryogenic Engineering*, 1998, 43, 911–917.

T. J. Polascik, I. Nosnik, J. M. Mayes and V. Mouraviev, Short-term cancer control after primary cryosurgical ablation for clinically localized prostate cancer using third-generation cryotechnology, *Urology*, 2007, 70, 117–121.

S. L. Qi, P. Zhang, R. Z. Wang, A. L. Zhang and L. X. Xu, Development and performance test of a cryoprobe with heat transfer enhancement configuration, *Cryogenics*, 2006, 46, 881–887.

H. Takeda, S. Muruyuma, J. Okajima, S. Aiba and A. Komiya, Development and estimation of a novel cryoprobe utilizing the Peltier effect for precise and safe cryosurgery, *Cryobiology*, 2009, 59, 275–284.

A. Zisman, D. Leibovici, Y. I. Siegel and A. Lindner, Prostate cryoablation without an insertion kit using direct transperineal placement of ultrathin freezing probes, *Techniques in Urology*, 2000, 6, 34–36.

A. Zisman, A. J. Pantuck, J. K. Cohen and A. S. Beldegrun, Prostate cryoablation using direct transperineal placement of ultrathin probes through a 17-gauge brachytherapy template-technique and preliminary results, *Urology*, 2001, 58, 988–993.

J. Sun, A. Zhang and L. X. Xu, Evaluation of alternate cooling and heating for tumor treatment, *International Journal of Heat and Mass Transfer*, 2008, 51, 5478–5485.
[135] F. Sun, G. X. Wang, K. M. Kelly and G. Aguilar, Numerical modeling of the tissue freeze-thaw cycle during cutaneous cryosurgery using liquid nitrogen spray, Proceedings of International Mechanical Engineering Congress and Exposition (IMECE), Orlando, Florida, 2005.

[136] F. Sun, G. Aguilar, K. M. Kelly and X. W. G., Thermal analysis of cryosurgery of nodular basal cell carcinoma, In Proceedings of International Mechanical Engineering Congress and Exposition (IMECE), Chicago, Illinois, 2006.

[137] A. Zhang, L. X. Xu, G. A. Sandison and J. Zhang, A microscale model for prediction of breast cancer cell damage during cryosurgery, Cryobiology, 2003, 47, 143–154.

[138] H. H. Pennes, Analysis of tissue and arterial blood temperatures in the resting human forearm, Journal of Applied Physiology, 1948, 1, 93–122.

[139] L. M. Jiji and P. Ganatos, Freezing of blood perfused tissue: An improved quasi-steady solution, International Journal of Heat and Mass Transfer, 2008, 51, 5679–5686.

[140] J. S. Jones, Effect of aberrant vasculature during cryosurgery for prostate cancer, Urology, 2009, 73(4), 754–755.

[141] L. Massalha and A. Shitzer, Freezing by a flat, circular surface cryoprobe of a tissue phantom with an embedded cylindrical heat source simulating a blood vessel, Transactions of the ASME, 2004, 126(6), 736–744.

[142] J. Shi, Z. Chen and M. Shi, Simulation of heat transfer of biological tissue during cryosurgery based on vascular trees, Applied Thermal Engineering, 2009, 29(8-9), 1792–1798.

[143] G. Zhao, H.-F. Zhang, X.-J. Guo, D.-W. Luo and D.-Y. Gao, Effect of blood flow and metabolism on multidimensional heat transfer during cryosurgery, Medical Engineering & Physics, 2007, 29, 205–215.

[144] B. R. Smoller and K. M. Hiatt, Dermatopathology: The Basics, Springer, 2009.

[145] K. R. Diller and L. J. Hayes, A finite element model of burn injury of blood-perfused skin, Journal of Biochemical Engineering, 1983, 105, 300–307.

[146] D. A. Hodson, J. C. Baebebel and G. Eason, Modeling transient heat transfer through the skin and a contact material, Physics in Medicine and Biology, 1989, 34, 490–507.

[147] S. B. Wilson and V. A. Spence, A tissue heat transfer model for relating dynamic skin temperature changes to physiological parameters, Physics in Medicine and Biology, 1988, 33, 895–912.

[148] L. M. Jiji, S. Weinbaum and D. E. Lemons, Theory and experiment for the effect of vascular microstructure on surface tissue heat transfer-part II: model formulation and solution, ASME Journal of Heat Transfer, 1984, 106, 331–341.

[149] W.-L. Cheng, F.-Y. Han, Q.-N. Liu, R. Zhao and H.-L. Fan, Experimental and theoretical investigation of surface temperature non-uniformity of spray cooling, Energy, 2011, 36, 249–257.
K. A. Estes and I. Mudawar, Correlation of Sauter mean diameter and critical heat flux for spraying cooling of small surfaces, *International Journal of Heat and Mass Transfer*, 1995, 38, 2985–2996.

W. Franco, G. X. Wang, E. Karapetian, J. S. Nelson and G. Aguilar, Radial heat transfer dynamics during cryogen spray cooling, in *Proc. ILASS Americas 17th Annual Conference on Liquid Atomization and Spray Systems*, San Jose, CA, 2004.

W. Franco, G. X. Wang, E. Karapetian, J. S. Nelson and G. Aguilar, Effect of surface thermal variations during cryogen spray cooling in dermatologic laser therapy, in *Proc. ILASS Americas 17th Annual conference on Liquid Atomization and Spray Systems*, San Jose, CA, 2004.

B. M. Pikkula, J. W. Tunnell and B. Anvari, Methodology for characterizing heat removal mechanism in human skin during cryogen spray cooling, *Annals of Biomedical Engineering*, 2003, 31, 493–504.

B. Anvari, T. E. Milner, B. S. Tanenbaum, S. Kimel, L. O. Svaasand and J. S. Nelson, Selective cooling of biological tissues: application for thermally mediated therapeutic procedures, *Physics in Medicine and Biology*, 1995, 40, 241–252.

M. M. Elkobt, Fuel atomization for spray modeling, *Progress in Energy and Combustion Science*, 1982, 8, 61–91.

D. B. Harmon, Drop sizes from low speed jets, *Journal of the Franklin Institute*, 1955, 259, 519–522.

L. O. Svaasand, L. L. Randeberg, G. Aguilar, B. Majaron, S. Kimel, E. J. Lavernia and J. S. Nelson, Cooling efficiency of cryogen spray during laser therapy skin, *Lasers in Surgery and Medicine*, 2003, 32, 137–142.

Y. Tanasawa and S. Toyoda, On the atomization of a liquid jet issuing from a cylindrical nozzle, *Technical Report of Tohoku University*, 1955.

W. Franco, J. Liu, G.-X. Wang, J. S. Nelson and G. Aguilar, Radial and temporal variations in surface heat transfer during cryogen spray cooling, *Physics in Medicine and Biology*, 2005, 50, 387–397.

G. Aguilar, W. Verkruysse, B. Majaron, L. O. Svaasand, E. J. Lavernia and J. S. Nelson, Measurement of heat flux and heat transfer coefficient during continuous cryogen spray cooling for laser dermatologic surgery, *IEEE Journal on Selected Topics in Quantum Electronics*, 2001, 7(6), 1013–1021.

G. Aguilar, B. Majaron, E. Karapetian, E. J. Lavernia and J. S. Nelson, Experimental study of cryogen spray properties for application in dermatologic laser surgery, *IEEE Transactions on Biomedical Engineering*, 2003, 50(7), 863–869.

G. Aguilar, H. Vu and J. S. Nelson, Influence of angle between the nozzle and skin surface on the heat flux and overall heat extraction during cryogen spray cooling, *Physics in Medicine and Biology*, 2004, 49, N147–N153.
[163] W. Franco, J. Liu, R. Romero-Méndez, W. Jia, J. S. Nelson and G. Aguilar, Extent of lateral epidermal protection afforded by a cryogen spray against laser irradiation, *Lasers in Surgery and Medicine*, 2007, 39, 414–421.

[164] E. Karapetian, G. Aguilar, S. Kimel, E. J. Lavernia and J. S. Nelson, Effects of mass flow rate and droplet velocity on surface heat flux during cryogen spray cooling, *Physics in Medicine and Biology*, 2003, 48(1), N1–N6.

[165] S. S. Hsieh and H. H. Tsai, Thermal and flow measurements of continuous cryogen spray cooling, *Archives of Dermatological Research*, 2006, 298(2), 82–95.

[166] G. Aguilar, G.-X. Wang and J. S. Nelson, Effect of spurt duration on the heat transfer dynamics during cryogen spray cooling, *Physics in Medicine and Biology*, 2003, 48, 2169–2188.

[167] N. Datrice, J. Ramirez-San-Juan, R. Azhang, A. Meshkinpour, G. Aguilar, J. S. Nelson and K. M. Kelly, Cutaneous effects of cryogen spray cooling on in vivo human skin, *Dermatologic Surgery*, 2006, 32, 1009–1014.

[168] B. Kao, K. M. Kelly, G. Aguilar, Y. Hosaka, R. J. Barr and J. S. Nelson, Evaluation of cryogen spray cooling exposure on in vitro model human skin, *Lasers in Surgery and Medicine*, 2004, 34, 146–154.

[169] A. L. N. Moreira, J. Carvalho and R. O. Panão, An experimental methodology to quantify the spray cooling event at intermittent spray impact, *International Journal of Heat and Fluid Flow*, 2007, 28, 191–202.

[170] A. L. N. Moreira and M. R. O. Panão, Heat transfer at multiple-intermittent impacts of a hollow cone spray, *International Journal of Heat and Mass Transfer*, 2006, 49, 4132–4151.

[171] J. C. Ramirez-San-Juan, G. Aguilar, A. T. Tuqan, K. M. Kelly and J. S. Nelson, Skin model surface temperatures during single and multiple cryogen spurts used in laser dermatologic surgery, *Lasers in Surgery and Medicine*, 2005, 36, 141–146.

[172] J. W. Tunnell, J. S. Nelson, J. H. Torres and B. Anvari, Epidermal protection with cryogen spray cooling during high fluence pulsed dye laser irradiation. An ex vivo study, *Lasers in Surgery and Medicine*, 2000, 27, 373–383.

[173] A. T. Tuqan, K. M. Kelly, G. Aguilar, J. C. Ramirez-San-Juan, C.-H. Sun, D. Cassarino, D. Derienzo, R. J. Barr and J. S. Nelson, Evaluation of single versus multiple cryogen spray cooling spurts on in vitro model human skin, *Lasers in Medical Science*, 2005, 20, 80–86.

[174] G. Aguilar, W. Franco, J. Liu, L. O. Svaasand and J. S. Nelson, Effects of hypobaric pressure on human skin: implications for cryogen spray cooling (Part II), *Lasers in Surgery and Medicine*, 2005, 36, 130–135.

[175] G. Aguilar, L. O. Svaasand and J. S. Nelson, Effects of hypobaric pressure on human skin: feasibility study for port wine stain laser therapy (Part I), *Lasers in Surgery and Medicine*, 2004, 34, 124–129.
L. O. Svaasand, E. J. Fiskerstrand, G. Kopstad, L. T. Norvang, E. K. Svaasand, J. S. Nelson and M. W. Berns, Therapeutic response during pulsed laser treatment of port-wine stains; dependence on vessel diameter and depth in dermis, *Lasers in Medical Science*, 1995, 10, 235–243.

B. Choi, G. Aguilar, G. Vargas, A. J. Welch and J. S. Nelson, Dynamic measurement of laser light attenuation by cryogen film and frost formation, *Proceedings of SPIE*, San Jose, CA, 2002.

B. Majaron, Sequential cryogen spraying for heat flux control at the skin surface, *Proceedings of SPIE*, New York, 2001, 4244, 74–81.

B. Majaron, S. Kimel, W. Verkruyse, G. Aguilar, K. Pope, L. O. Svaasand, E. J. Lavernia and J. S. Nelson, Cryogen spray cooling in laser dermatology: effects of ambient humidity and frost formation, *Lasers in Surgery and Medicine*, 2001, 28, 469–476.

J. C. Ramirez-San-Juan, B. Choi, W. Franco, J. S. Nelson and G. Aguilar, Effect of ambient humidity on light transmittance through skin phantoms during cryogen spray cooling, *Physics in Medicine and Biology*, 2006, 51, 113–120.

B. Basinger, G. Aguilar and J. S. Nelson, Effect of skin indentation on heat transfer during cryogen spray cooling, *Lasers in Surgery and Medicine*, 2004, 34, 155–163.

J. Liu, W. Franco and G. Aguilar, Effect of surface roughness on single cryogen droplet spreading, *Journal of Fluids Engineering*, 2008, 130(4), 041402.

Z. Zhou, B. Chen, Y. Wang, L. Guo and G. Wang, An experimental study on pulsed spray cooling with refrigerant R-404a in laser surgery, *Applied Thermal Engineering*, 2012, 39, 29–36.

G. Comini and S. D. Guidice, Thermal aspects of cryosurgery, *ASME Journal of Heat Transfer*, 1976, 98, 543–549.

N. E. Hoffmann and J. C. Bischof, Cryosurgery of normal and tumor tissue in the dorsal skin flap chamber: Part II-Injury response, *Journal of Biochemical Engineering*, 2001, 123(4), 310–317.

A. Shitzer and R. C. Eberhart, *Heat Transfer in Medicine and Biology: Analysis and Application (1)*, Springer, 1985.

F. A. Duck, *Physical Properties of Tissue*, Academic Press, 1990.

R. F. Barron, *Cryogenic Systems*, 1985.

Y. A. Cengel, *Heat Transfer: A Practical Approach*, McGraw-Hill, 1998.

J. F. Bowman, E. G. Gravalho and M. Woods, Theory, measurement and application of thermal properties of biomaterials, *Annual Review of Biomedical Engineering*, 1975, 4, 43–80.

X. He and J. Bischof, Quantifications of temperature and injury response in thermal therapy and cryosurgery, *Critical Reviews in Biomedical Engineering*, 2003, 35, 355–421.
[192] P. Mazur and C. Koshimoto, Is intracellular ice formation the cause of death of mouse sperm frozen at high cooling rate?, *Biology of Reproduction*, 2002, 66, 1485–1490.

[193] Fluent Inc., 2005.

[194] J. Bischof, K. Christov and B. Rubinsky, A morphological study of cooling rate response in normal and neoplastic human liver tissue: cryosurgical implications, *Cryobiology*, 1993, 30, 482–492.

[195] M. A. Zieger, E. E. Tredget and L. E. McGann, Mechanisms of cryoinjury and cryoprotection in split-thickness skin, *Cryobiology*, 1996, 33, 376–389.