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Numerical analysis of an enhanced cooling rate cryopreservation process in a biological tissue

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ABSTRACT

Cryopreservation is the method of preservation of biological tissues for future references without causing significant damages to their physical and functional properties. This can be done by exposing them to very low cryogenic temperature that involves a greater heat removal rate. A two dimensional numerical model is developed to study the temperature distribution, cooling rate attained and movement of the freezing front during the cryopreservation process. The Pennes Bio-heat model is used for current study. The Finite Volume Method is employed for discretization of the governing differential equations while the Tri-Diagonal Matrix Algorithm is used to solve the discretized algebraic equations to find temperature distribution inside the domain. The Enthalpy-Porosity method is used to track the solid-liquid interfaces during the freezing process. The current model is first validated with the result of the existing literature. In the present work, freezing of tissue is done from one and two sides in two separate cases and the resulting temperature distribution inside the tissue and cooling rate in the two cases are compared. It is found that the freezing rate of tissue is enhanced about two times when it is freezed from two sides as compared to freezing from single side. Further, it is observed that a lower value of blood perfusion rate causes a lower value of the final temperature of the tissue after freezing. Thus, it can be concluded that the tissue with high blood perfusion rate is to be freezed in a lower cooling medium temperature. In the present condition, metabolic heat generation plays no significant role in the temperature distribution inside the healthy tissue.

1. Introduction

Freezing and frozen storage has been an old practice for long term preservation of fruits and vegetables. Freezing increases the stored life of these products by reducing activities of water content and enzymes inside them and prohibiting growth of micro-organisms. The scientific developments in this area have revealed the utilisation of freezing technique for preservation of biological tissues for future references. Organ preservation for implantation in future has further increased its utility. As per many researchers, a very quick freezing rate results high quality frozen goods and increases the preservation time. The development of intracellular and extracellular ice crystals in the frozen goods during the slow freezing processes, increases the tendency of enzymatic oxidation that causes destruction of tissues and reduction in preservation time. Therefore, the advanced freezing process of cryopreservation by vitrification has been developed. In cryopreservation, the object to be preserved is exposed to liquid nitrogen. As the boiling point of liquid nitrogen is − 196 °C, the very low temperature of liquid nitrogen brings the temperature of the object to be stored to a very low temperature. Vitrification is a very rapid cooling process that brings the temperature of the liquid inside the object to be preserved to such a low value that it gets converted into amorphous solid instead of solid crystals, thus arresting the enzymatic oxidation and enhancing the time of preservation. Cryopreservation by vitrification preserves the fruits, vegetables, meat or biological tissues for longer durations without altering its physical and morphological properties. It has wide range of applications in the fields of cryotherapy, stem cells and tissue engineering, organ preservation and assisted reproduction. In cryotherapy or cryosurgery, the very low temperature developed is used to freeze the tumour developed in the human body thus arresting biological activities inside it.

In the recent years, so many research work is focussed on the development of cryogenic preservation techniques with the aim of obtaining greater cooling rates, with longer preservation time without significant changes to the properties and arresting biological activities within the preserved article. Afrin et al. (2012) studied about the extent of thermal damage occurring to the living biological tissues subjected to laser irradiation considering the Dual Phase Lag model. Ahmadikia and Moradi (2012) developed an enthalpy model to study Fourier and non-

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Nomenclature

T	temperature
C_p	specific heat
k	thermal conductivity
H	total enthalpy
h_{sen}	sensible heat
L_f	latent heat of fusion
S	average source term
S_h	enthalpy source term
a_p, a_p^o	coefficient in discretized algebraic equation
g_L	liquid volume fraction
t	time
Q_m	volumetric metabolic heat generation rate

Greek Symbols

ρ	density
α	thermal diffusivity
λ	under-relaxation parameter
Δt	size of time step
ΔV_P	volume of control volume
ω_b	blood perfusion rate
Φ_1, Φ_2	Constants

Subscript

s	solid
l	liquid
P	control volume
f	freezing point
b	blood
nb	neighbouring nodes
init	initial

Superscripts

n	iteration number
0	previous time step

Fourier heat conduction inside a biological tissue during a non-isothermal freezing process. They also investigated the effect of thermal relaxation time on heat transfer during the non-Fourier process. Ahmadikia et al. (2012) investigated parabolic and hyperbolic heat transfer in a one-dimensional skin tissue considering constant and transient heat fluxes being applied at the boundary. They also studied the effect of blood perfusion rate on the temperature distribution inside the specimen. Alhamdan et al. (2015) studied about textural changes occurred to the fruits during cryopreservation. They also studied the changes in concentration of different sugars inside the fruit during the preservation period due to enzymatic actions. Antaki (2005) conducted studies on non-Fourier heat conduction inside a specimen of processed meat using the dual phase lag model and derived possibilities of using this model to analyse the extent of damage caused during thermal burning of human tissues. Bullen et al. (2014) conducted experimental studies and employed a rapid freezing technique for efficient preservation of inner ear of human. Chua (2013) studied on the effect of blood perfusion rate on the effectiveness of cryosurgery and efficiencies of different nanoparticles in enhancing heat transfer rates in order to achieve the lowest temperature inside the tumour during the cryosurgery process. Da Silva et al. (2017) conducted experimental study to cryopreserve pollen of wild pineapple for a longer duration. Deng and Liu (2004a) investigated about the freezing of tumour tissues and effect of freezing on normal tissue during cryosurgery and put forward their results regarding precise application of cryoprobes during the process in order to avoid irreversible damage caused to the surrounding normal tissues during the process. Deng and Liu (2004b) developed a novel treatment method combining cryosurgery and hyperthermia treatment for the cure of large and irregular shaped tumors. Deng and Liu (2012) conducted numerical studies in order to develop a novel combined method of nano-cryosurgery and RF hyperthermia for the treatment of tumour and found flexibility of the method in controlling temperature inside the tumour. Dessai (2018) effectively used 15% glycerol for cryopreservation of cultured mantle cells of Paphia malabarica. It also acted as one of the best cryoprotectant for cryopreservation of freshly dissociated cells that reduced morphological changes to the tissue by intercellular ice formation. Ehrlich et al. (2018) conducted thermal analysis of rabbit and human kidney during vitrification and found non-uniform temperature distribution with increase in kidney size because of non-uniform cooling inside it. Kumar and Katiyar (2010) studied freezing and thawing processes in a biological tissue considering it as a porous medium taking the effect of metabolism under consideration. They found that the rate of heat transfer during thawing is less as compared to freezing because of increased porosity due to formation of ice during freezing that presided thawing. Li et al. (2009) developed a 3-dimensional model to study heat transfer in a biological tissue during cryopreservation and cryosurgery considering some complex domains. Loken and Demetrick (2005) studied about the cryopreservation of tissues for future studies by preserving them inside cryosection molds containing cryogenic medium like liquid nitrogen. O'Brien et al. (2016) studied about requirements of proper dehydration of avocado germplasm before cryopreservation and about possible occurrence of toxicity to the cryopreserved specimen upon exposure to cryopreservation solution for an extended time period. The stress resulting in a biological tissue during its freezing has been studied by Olien and Livingston (2006). Rabin and Shitzer (2008) studied about freezing of a biological tissue during cryosurgery and obtained poorer thermal efficiencies of the cryosurgical devices and greater coolant consumption during the process. An increase in the rate of metabolic heat generation value inside the tissue results in greater final temperature of the concerned tissue following cryosurgery (Zhao et al., 2007; Singh and Kumar, 2013). Singh and Kumar (2014) numerically studied about the freezing of a three layered skin tissue considering the Dual Phase Lag model. Su et al. (2017) used dimethyl sulfoxide as cryopreservant and cryoprotectant to improve the cooling rate to about 150000 °C/min and increase the preservation time. Torvi and Dale (2008) developed a finite element model to predict the thermal damage occurring in human skin tissue subjected to a flash fire. Wang et al. (2007) developed a one-dimensional finite difference model to study temperature distribution inside the food stuffs like cucumber, meat etc. during their preservation. They also predicted successfully the probable freezing time for each specimen. Zhang et al. (2017) concluded vitrification as the most efficient method of cryopreservation involving non-equilibrium cooling accomplished in liquid nitrogen. They developed an innovative method by wrapping French-type straws with medical gauze around the biological tissue to be vitrified in order to avoid film boiling of liquid nitrogen during the cryopreservation process. Zheng et al. (2018) developed a novel technique for repeated and gradual loading and unloading of cryoprotectant in order to improve the post-preservation viability of the biological tissue.

From the above study, it is understood that many experimental works have been already performed to understand the freezing phenomenon during the cryopreservation process by lowering of temperature of the food articles or the biological tissues by dipping them inside the liquid nitrogen. The researchers have obtained greater cooling rates and lower preservation temperature during the process

which are essential for extended storage life. This is a field of study which demands to develop numerical models to know the influence of various parameters such as cryopreservation solution temperature, blood perfusion rate and metabolic heat generation rate to control the freezing rate. In this work, a numerical model is developed to study the cryopreservation of a living biological tissue by exposing it to cryogenic temperature. A comparative study is carried out by cooling the tissue from one side and the same from two sides. It is observed that cooling from both sides of tissue enhances the cooling rate and thus freezing time is reduced which is desirable for cryopreservation process. The effect of blood perfusion rate and metabolic heat generation in controlling the freezing rate is also discussed.

2. Physical model

A two dimensional biological tissue of length 1 cm and width 1 cm is considered for freezing The tissue is initially kept at uniform temperature, $T_{init} = 37\,^{\circ}C$ and then it is subjected to cryogenic temperature of $-196\,^{\circ}C$ as shown in the Fig. 1. Fig. 1(a) shows freezing from one side where as Fig. 1(b) shows freezing from two sides by exposing the concerned sides of the tissue to $-196\,^{\circ}C$ temperature. The physical properties of the biological tissue are mentioned in the Table 1. Three points A, B and C at 1 mm, 5 mm and 9 mm from the left wall of the tissue are considered inside the specimens where the temperature variation and cooling rate are studied. These points are considered on the line joining the mid-points of the left and right sides of the specimen.

2.1. Governing equations

The governing equations for the current study are given below.
Pennes Bio-heat equation:

$$\rho c_p \frac{\partial T}{\partial t} = k\nabla^2 T + \omega_b \rho_b c_b \left(T_b - T\right) + Q_m \tag{1}$$

$$\text{When } \rho_b = 0,\ Q_m = 0,\ \text{it reduces to } \rho c_p \frac{\partial T}{\partial t} k\nabla^2 T \tag{2}$$

The governing equation for freezing of a biological tissue in terms of enthalpy is considered as follows.

$$\frac{\partial(\rho H)}{\partial t} = k\nabla^2 T + \omega_b \rho_b c_b \left(T_b - T\right) + Q_m \tag{3}$$

$$H = h_{sen} + \Delta H \tag{4}$$

$$h_{sen} = C_p \Delta T \tag{5}$$

Then Eq. (3) becomes

$$\frac{\partial(\rho C_p T)}{\partial t} = k\nabla^2 T + \omega_b \rho_b c_b \left(T_b - T\right) + Q_m - \frac{\partial(\rho \Delta H)}{\partial t} \tag{6}$$

Table 1
Physical Properties of biological tissue.

Properties	Value	Unit
Density in Unfrozen state	1200.0	Kg/ m^3
Density in Frozen state	921.0	Kg/ m^3
Specific heat in Unfrozen state	3400.0	J/kg °C
Specific heat in Frozen state	1800.0	J/kg °C
Thermal Conductivity in Unfrozen state	0.52	W/m °C
Thermal Conductivity in Frozen state	2.0	W/m °C
Latent heat of fusion	250000.0	J/kg
Solidus Temperature	− 8.0	°C
Liquidus Temperature	− 1.0	°C
Initial temperature	37.0	°C
Metabolic Heat Generation	368.1	W/m^3
Blood Perfusion Rate	0.00125	s^{-1}
Blood Temperature	37.0	°C
Blood Specific Heat	3770.0	J/kg °C
Density of Blood	1060.0	Kg/ m^3

By introducing the liquid volume fraction term, g_L in Eq. (6), the equation becomes

$$\frac{\partial(\rho C_p T)}{\partial t} = k\nabla^2 T + \omega_b \rho_b c_b \left(T_b - T\right) + Q_m - \frac{\partial(\rho L_f g_L)}{\partial t} \tag{7}$$

$$\text{The Enthalpy source term, } S_h = -\frac{\partial}{\partial t}(\rho L_f g_L) = -\rho L_f \left(\frac{g_L - g_L^0}{\Delta t}\right) \tag{8}$$

Initial Condition:

$$T(x, y, t = 0) = T_{init} \tag{9}$$

Boundary Conditions:
For single side freezing,

$$-k\frac{\partial T}{\partial y} = 0;\ y = 0,\ 0 \le x \le L \tag{10 a}$$

$$-k\frac{\partial T}{\partial y} = 0;\ y = L,\ 0 \le x \le L \tag{10 b}$$

$$T = -196°C;\ x = 0,\ 0 \le y \le L \tag{10c}$$

$$-k\frac{\partial T}{\partial x} = 0;\ x = L,\ 0 \le y \le L \tag{10 d}$$

For double side freezing,

$$-k\frac{\partial T}{\partial y} = 0;\ y = 0,\ 0 \le x \le L \tag{11 a}$$

$$-k\frac{\partial T}{\partial y} = 0;\ y = L,\ 0 \le x \le L \tag{11 b}$$

$$T = -196°C;\ x = 0,\ 0 \le y \le L \tag{11c}$$

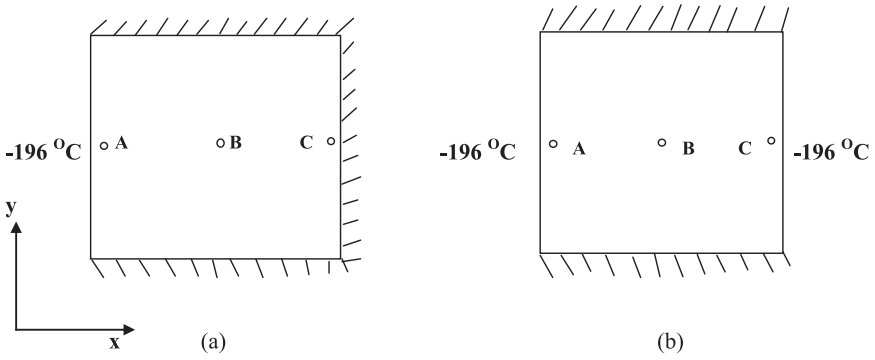

Fig. 1. Schematic diagram of the tissue (a) Freezing from one side (b) freezing from two sides.

$$T = -196°C;\ x = L,\ 0 \le y \le L \tag{11 d}$$

3. Formulation of numerical model

Finite Volume Method (Patankar, 1980) is used to discretize the governing differential equations while the resulting algebraic equations are solved using the Tri-Diagonal Matrix Algorithm to obtain temperature distribution inside the computational domain. The discretized enthalpy source term is given as

$$\text{The Enthalpy source term, } S_h = -\frac{\partial}{\partial t}(\rho L_f g_L) = -\rho L_f \left(\frac{g_L - g_L^0}{\Delta t}\right) \tag{12}$$

Here, g_L^0 is the volume fraction of liquid inside a control volume from previous time step and g_L is the volume fraction of liquid inside the control volume at the current time step. An iterative method is developed to update the value of g_L for freezing in each control volume at each time step depending upon the latent heat content inside it.

Eq. (6) is integrated over the time step, Δt and control volume, ΔV_P to obtain the discretized algebraic equation for the n^{th} iteration.

$$a_P T_P^n = a_P^0 T_P^0 + \sum a_{nb} T_{nb}^n + \omega_b \rho_b c_b T_b \Delta V_P - \omega_b \rho_b c_b T \Delta V_P + Q_m \Delta V_P - \rho L_f \Delta V_P \left[\frac{g_L^n - g_L^0}{\Delta t}\right] \tag{13}$$

Let's assume that in the $(n+1)^{th}$ iteration, the control volume attains freezing temperature, T_f. So, for the $(n+1)^{th}$ iteration,

$$a_P T_P^{n+1} = a_P^0 T_P^0 + \sum a_{nb} T_{nb}^{n+1} + \omega_b \rho_b c_b T_b \Delta V_P - \omega_b \rho_b c_b T \Delta V_P + Q_m \Delta V_P - \rho L_f \Delta V_P \left[\frac{g_L^{n+1} - g_L^0}{\Delta t}\right] \tag{14}$$

As $T_P^{n+1} = T_f$,

$$a_P T_f = a_P^0 T_P^0 + \sum a_{nb} T_{nb}^{n+1} + \omega_b \rho_b c_b T_b \Delta V_P - \omega_b \rho_b c_b T \Delta V_P + Q_m \Delta V_P - \rho L_f \Delta V_P \left[\frac{g_L^{n+1} - g_L^0}{\Delta t}\right] \tag{15}$$

Subtracting Eq. (15) from Eq. (14) and neglecting the changes in T_{nb}

$$a_P \left(T_P^n - T_f\right) = \rho L_f \Delta V_P \left[\frac{g_L^{n+1} - g_L^n}{\Delta t}\right] \tag{16}$$

$$\text{Then, } g_L^{n+1} = g_L^n + \frac{a_P \Delta t}{\rho L_f \Delta V_P}(T_P^n - T_f) \tag{17}$$

It can be noted that difference in the T_{nb} value between two consecutive iterations is not zero, but in order to simplify the liquid volume fraction update equation, this change has been neglected. When convergence of solution occurs, change in T_{nb} value between two successive iterations is zero, so there is no effect of neglecting the difference in T_{nb} on the ultimate solution achieved. By introducing an under-relaxation parameter, λ to avoid divergence in the iterative solution, Eq. (17) becomes

$$g_L^{n+1} = g_L^n + \frac{\lambda a_P \Delta t}{\rho L_f \Delta V_P}(T_P^n - T_f) \tag{18}$$

Eq. (18) is used when freezing is an isothermal process, but in the current work, freezing of tissue is considered over a range of temperature between liquidus temperature, T_L and solidus temperature, T_S. For this, a linear relation between liquid volume fraction and temperature is assumed.

$$g_L = \phi_1 T + \phi_2 \tag{19}$$

When $g_L = 0, T = T_S$

$$\Rightarrow \phi_1 T_S + \phi_2 = 0$$

$$\Rightarrow \phi_2 = -\phi_1 T_S \tag{20}$$

When $g_L = 1, T = T_L$

$$\Rightarrow 1 = \phi_1 T_L + \phi_2 = \phi_1 T_L - \phi_1 T_S$$

$$\Rightarrow \phi_1 = \frac{1}{T_L - T_S} \tag{21}$$

$$\Rightarrow \phi_2 = -\frac{T_S}{T_L - T_S} \tag{22}$$

$$\Rightarrow g_L = \frac{1}{T_L - T_S} T - \frac{T_S}{T_L - T_S} \tag{23}$$

When $T = T_f$,

$$g_L = \frac{1}{T_L - T_S} T_f - \frac{T_S}{T_L - T_S} \Rightarrow T_f = g_L \left(T_L - T_S\right) + T_S \tag{24}$$

Substituting the value of T_f in Eq. (24) in Eq. (18), the iterative equation to update liquid volume fraction inside a control volume in each time step for a non-isothermal freezing is given by

$$g_L^{n+1} = g_L^n + \frac{\lambda a_P \Delta t}{\rho L_f \Delta v_p}[T_P^n - \{g_L^n (T_L - T_S) + T_S\}] \tag{25}$$

T_P^n is the temperature of the control volume at the n^{th} iteration and volume of a single control volume under consideration is ΔV_P. When the value of g_L becomes less than zero, it is made zero and when the g_L value goes beyond one, it is made one in order to avoid overshooting and undershooting problems during the numerical computation process respectively. When $g_L = 0$, the control volume is considered to be completely solid and when $g_L = 1$, the control volume is considered to be completely liquid. The Enthalpy-porosity technique (Brent et al., 1988) is used to track the solid-liquid interface implicitly inside the domain at any instant of time during the freezing process. The convergence criteria of the iterative solution process has been set to 10^{-6} in the current simulation.

4. Results and discussion

4.1. Validation

To validate the present numerical model, at first a grid independence test is carried out as shown in the Fig. 2. The temperature distribution along the center line of tissue is plotted at time, t = 40 s. It is found that beyond the grid size of 41 × 41, there is no changes in the temperature field. So, this grid size is chosen to produce further results. A time step independent test is shown in Fig. 3 taking different values of time step sizes. It is observed that beyond time step size of 0.01 s, there is no significant variation in the temperature at the point C in the tissue.

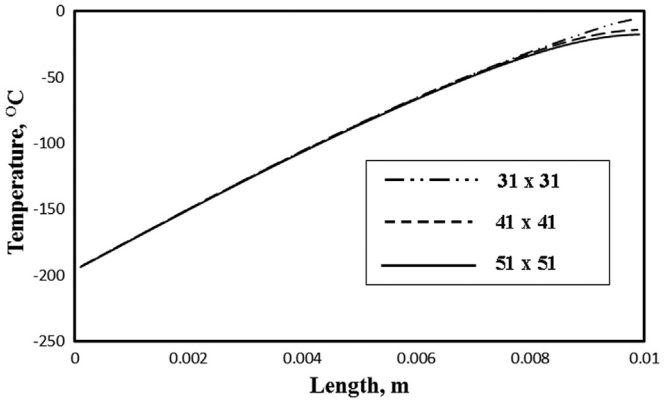

Fig. 2. Temperature distribution along the center line of the tissue at time, t = 40 s with different grid sizes.

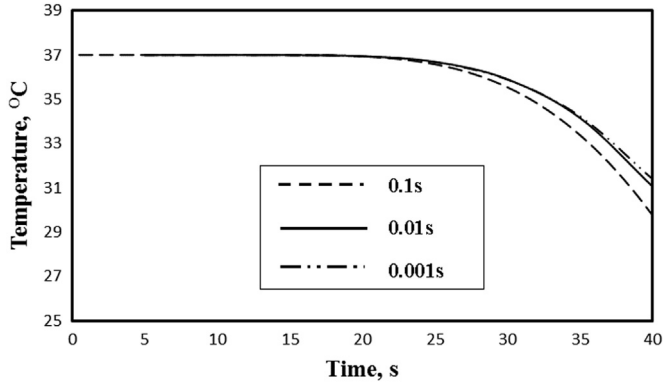

Fig. 3. Temperature variation at the location 9 mm (point C) from the left wall of the tissue at different freezing times with different time step sizes.

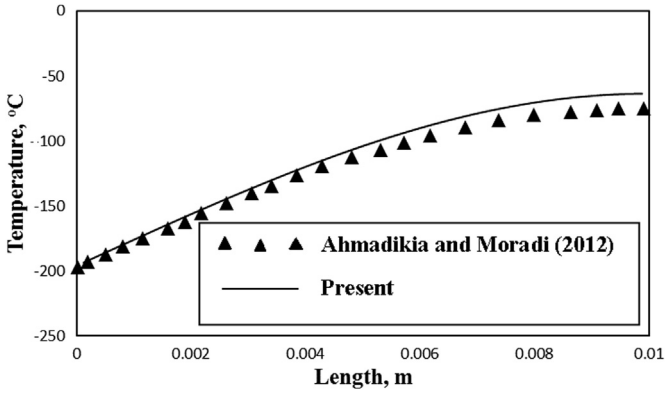

Fig. 4. Temperature variation along the length of the tissue at time, t = 90 s validated with the existing literature (Ahmadikia and Moradi, 2012).

Fig. 4 shows the validation of the model with the existing literature (Ahmadikia and Moradi, 2012). It is found that the result obtained using the current model agrees quite well.

The model is then extended to develop a two dimensional freezing model to study the cryopreservation process of a biological tissue considering the effects of blood perfusion rate and metabolic heat generation. A comparison is made to show the enhancement of cooling rate while freezing is done from two sides. The physical properties of the tissue are mentioned in Table 1. Freezing of the biological tissue considered here is a non-isothermal process as phase change occurs over a range of temperature between liquidus temperature −1 °C and solidus temperature −8 °C. The convergence criteria for liquid volume fraction (g_L) is set to be 10^{-6}.

4.2. Comparison of one sided and two sided freezing process of a biological tissue

Fig. 5(a) shows the variation of temperature at point A situated at 1 mm right to the left wall with respect to time. When the tissue is cooled either from one side or from both sides to a temperature of −196 °C, then there occurs a rapid release of heat at this point that causes a sharp decrease in temperature. Till about 20 s of freezing, the fall in temperature both for the single and double side freezing is nearly same. After 20 s, a remarkable difference in dropping of temperature is noted. Release of heat from both sides now makes the freezing more rapid as compared to the freezing from one side. It is very interesting to see that at the end of 60 s of freezing, the temperature at point A reaches about −163 °C for freezing only from one side where as it reaches −196 °C if freezing is done from both sides. The cooling rate of the tissue is shown in the Fig. 5(b). Initially the cooling rate is high for both the cases of freezing. The cooling rate is very high when the point A is in unfrozen state and the rate of heat release from this point is very high due to a greater temperature difference at point A and the cooling wall. As this point freezes, the cooling rate decreases due to decrease in temperature difference between the wall and the point. The release of heat from both sides in case of double side freezing increases the cooling rate at this point in the frozen state that results in attainment of further lower temperature at point A as compared to single side freezing.

The variation in temperature and cooling rate at the Point B situated 5 mm right to the left wall (at the middle of the tissue) with time is shown in the Fig. 6. At the end of 60 s of freezing, it is found that the temperature at the middle point of the tissue reaches −70 °C if freezing is done from one side while it reaches −196 °C if cooling is done from both the sides. So, in a fixed time of freezing, freezing from both the sides simultaneously results in a lower temperature at this point which is essential for proper cryopreservation and increasing the duration of preservation. Fig. 6(b) shows that the maximum cooling rate in case of both side freezing is nearly 250 °C/min while in case of single side freezing, it is only 90 °C/min. So, both side freezing is resulting a cooling rate nearly about three times faster as compared to the single side freezing. It is evident that in case of both side freezing, the cooling rate at point B is initially low and then it increases till attaining the maximum value. Then it decreases gradually. This is because initially it is in unfrozen state and then it freezes and the frozen thermal conductivity and thermal diffusivity are greater than those for the unfrozen state. This increases the rate of heat loss and leads to rise in cooling rate when complete freezing is attained at the point B. With further cooling, the difference between temperature at the point B and the cold walls decreases and it leads to decrease in cooling rate. In case of single side freezing, the maximum cooling rate at point B is also obtained after

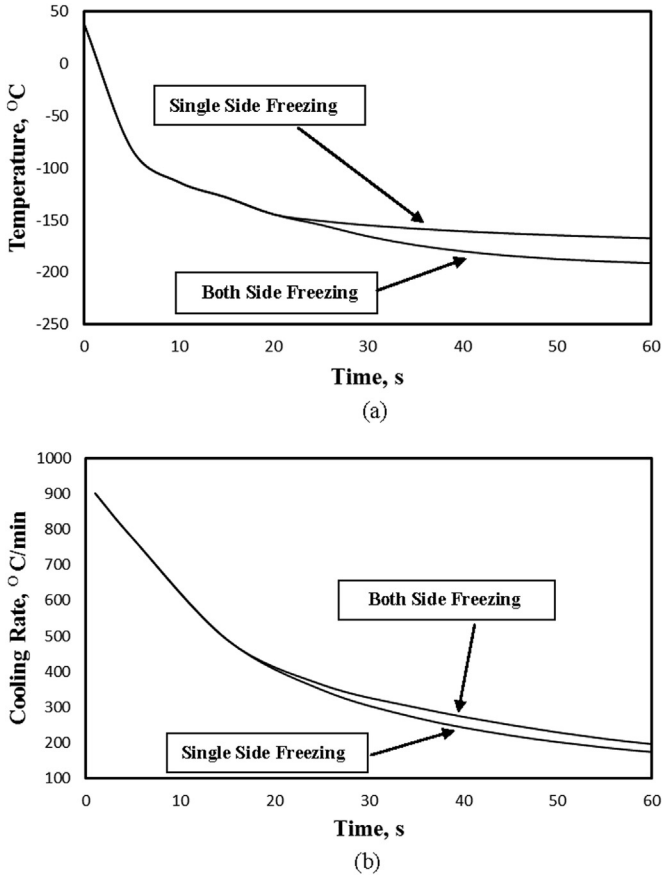

Fig. 5. Variation of (a) temperature and (b) cooling rate at the point A situated at 1 mm right to the left wall of the biological tissue specimen at different times of freezing (Q_m = 368.1 W/m^3, ω_b = 0.00125 s^{-1}).

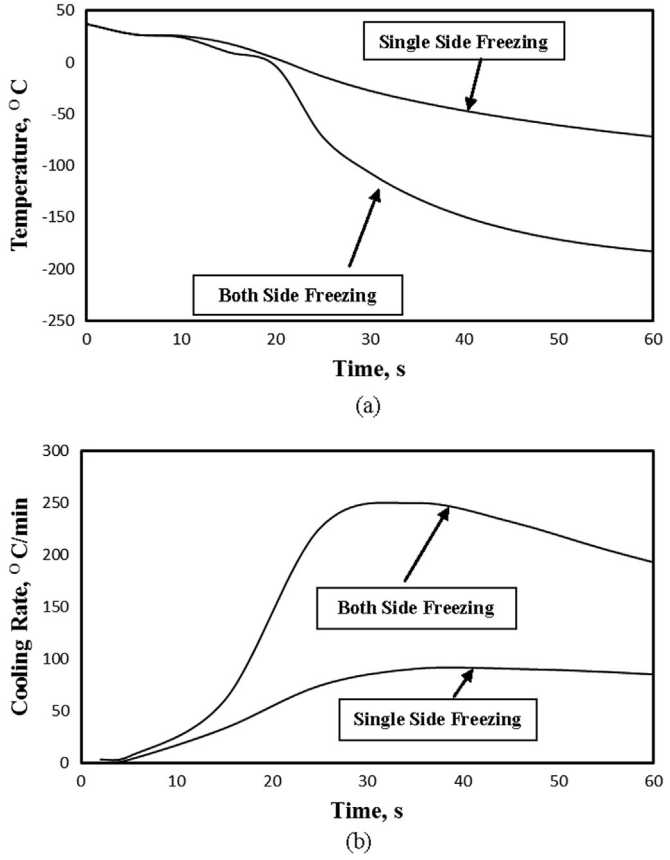

Fig. 6. Variation of (a) temperature and (b) cooling rate at the point B situated at 5 mm right to the left wall of the biological tissue specimen at different times of freezing (Q_m = 368.1 W/m^3, ω_b = 0.00125 s^{-1}).

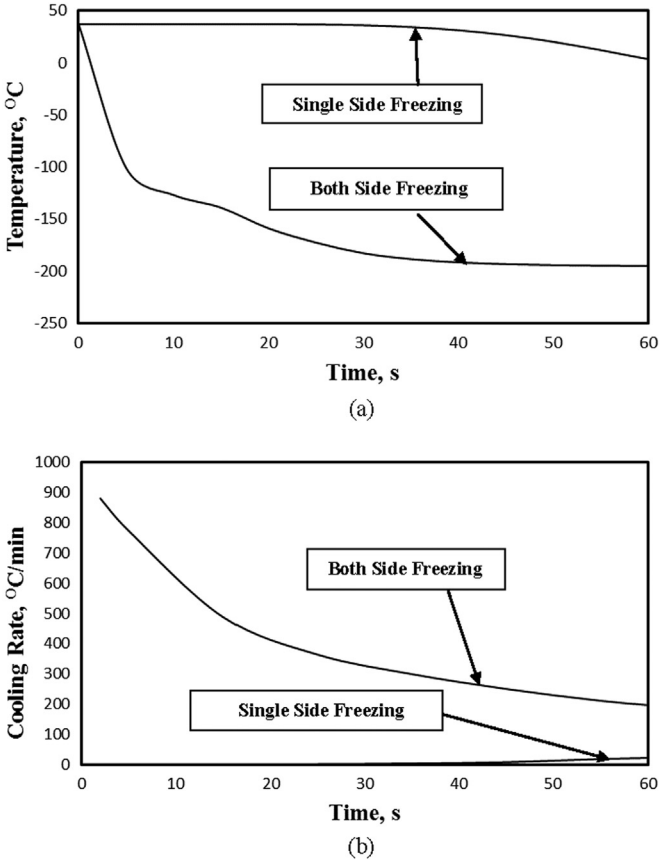

Fig. 7. Variation of (a) temperature and (b) cooling rate at the point C situated at 9 mm right to the left wall of the biological tissue specimen at different times of freezing (Q_m = 368.1 W/m^3, ω_b = 0.00125 s^{-1}).

occurrence of complete freezing at this point, but it does not decrease like double side cooling and remain almost constant as the temperature difference between point B and the cooling wall is still higher.

The variation in temperature and cooling rate with time at the Point C situated closer to the right wall during the freezing of biological tissue is shown in the Fig. 7. Fig. 7(a) shows that temperature at point C reaches −196 °C temperature when it is cooled from both sides. But for single side freezing, even after 60 s of cooling, freezing is not initiated at the point C. The significant difference in cooling rates for both side and single side freezing is observed in the Fig. 7(b). When freezing is done from one side only, the slow cooling process leads to formation of intracellular ice crystals inside the tissue specimen which damages the stored tissue during the preservation process. When the tissue is cooled from two sides, cooling at the Point C occurs at a very high rate. The high cooling rate reduces the time of freezing at that point and freezing also occurs at the point C. Initial cooling rate achieved at this point is nearly 900 °C/min. The cooling rate gradually decreases as the temperature difference between the point C and the right wall decreases and the temperature at the Point C approaches the cold wall temperature. During the preservation of biological tissue, in the unfrozen state, blood perfusion and metabolic heat generation add to the amount of heat to be removed. When the tissue is completely frozen, the metabolic heat generation and blood perfusion inside the tissue are completely ceased to happen. This reduces the amount of heat to be removed during the cooling process. The frozen thermal conductivity and diffusivity values are higher than the unfrozen values. Further no release of metabolic heat generation and no blood perfusion help the tissue approaching the cold wall temperature as quickly as possible.

Fig. 8 shows the variation of temperature along the length of the tissue at time 60 s. It is observed that when freezing is done from both

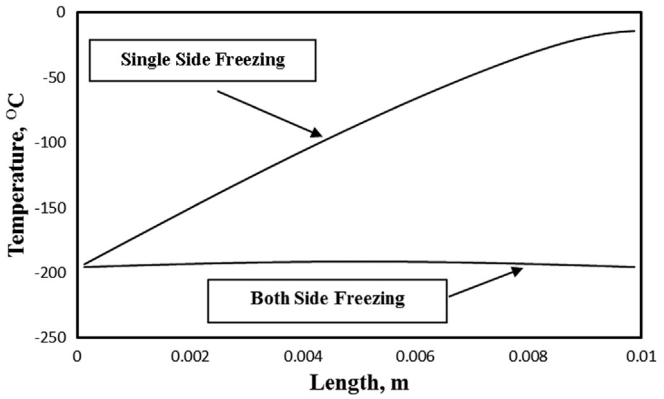

Fig. 8. Variation of temperature along the length of the biological tissue at time, t = 60 s (Q_m = 368.1 W/m^3, ω_b = 0.00125 s^{-1}).

sides, the whole slice is freezed completely within this time period and nearly attains the cooling wall temperature of −196 °C. But if freezing is done from one side, only a part of the tissue is freezed and it leaves a portion of the tissue close to the right wall still unfrozen. Presence of unfrozen portion leads to damage of the tissue during preservation. Then, to completely freeze the tissue specimen, the cooling time is required to be increased. The main motive of cryopreservation is to completely dehydrate the tissue within a very short time period to prevent growth of lethal intracellular ice crystals responsible for severe damage to the preserved tissue. It is also required to bring the whole tissue to be preserved to a very low and nearly uniform temperature to arrest the enzymatic oxidation inside the specimen so that it can be

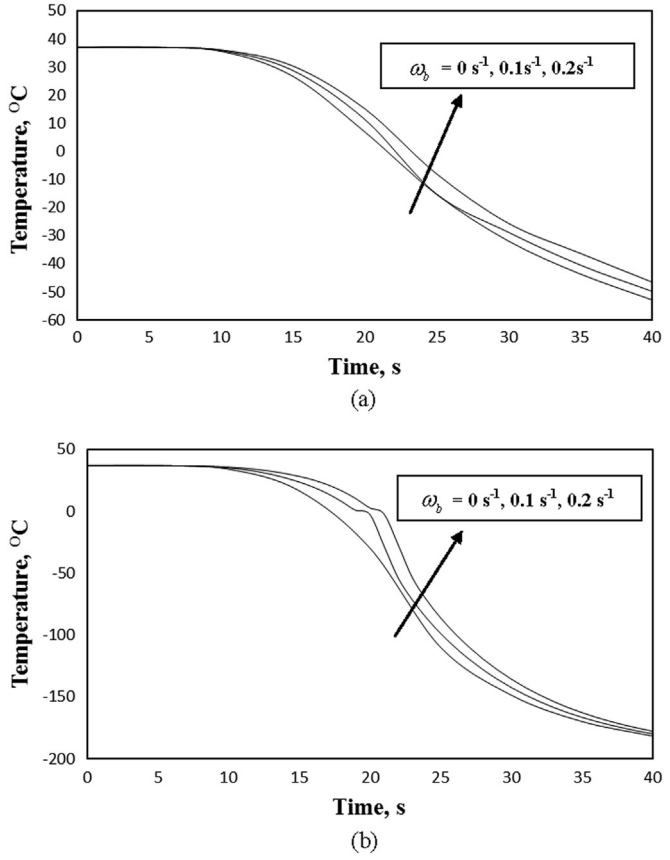

Fig. 9. Effect of blood perfusion rate on the variation of temperature at the center of the tissue at different freezing times for (a) single side freezing (b) both side freezing (Q_m = 368.1 W/m^3).

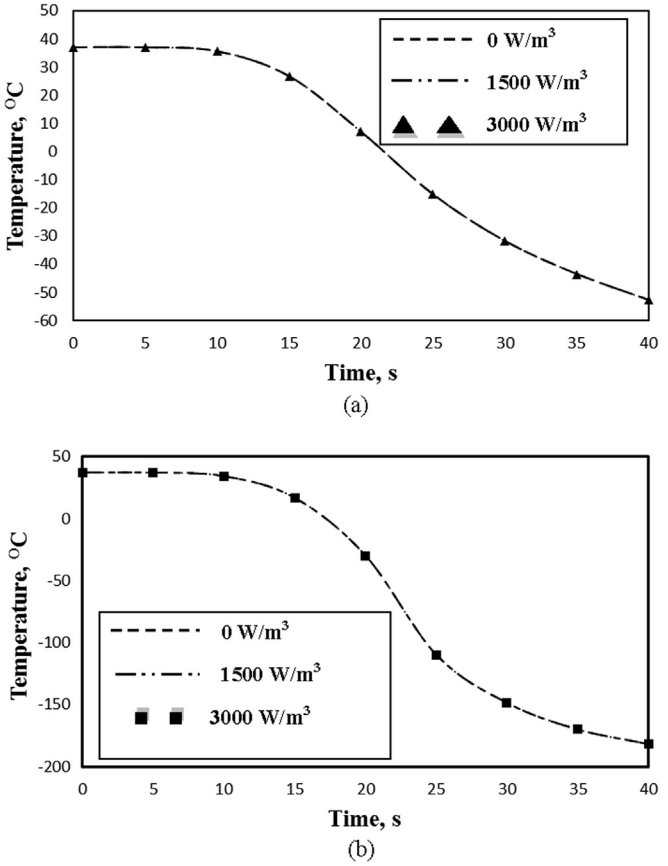

Fig. 10. Variation of temperature at the center of the tissue (point B) with different values of metabolic heat generation for (a) single side freezing (b) both side freezing (ω_b = 0.00125 s^{-1}).

preserved for a longer duration. This is achieveable in case of freezing from both sides which is evident from the Fig. 8.

4.3. Effect of Blood perfusion rate on freezing of biological tissue

The Fig. 9 shows the dependency of blood perfusion rate on freezing of the biological tissue. With the increase in blood perfusion rate, the rate of decrease in temperature is reduced. The flowing blood in the tissue carries heat from the unfrozen region towards the frozen region continuously till the whole tissue is completely frozen. When the blood perfusion rate is zero, heat carried by blood becomes zero and with the increase in blood perfusion rate, the heat carrying capacity of blood also increases which reduces the rate of decrease in temperature and increases the time required to completely freeze the tissue. So, in order to freeze the tissues with higher blood perfusion rates, the cryopreservation temperature should be further reduced in order to achieve desired cooling rate and final cryopreservation temperature.

4.4. Effect of Metabolic Heat generation on freezing of biological tissue

The dependency of freezing of biological tissue on metabolic heat generation is shown in the Fig. 10. Metabolic heat generation rate decreases exponentially with decrease in temperature in the unfrozen region of the tissue during the cooling process. Further, when a portion of the tissue is completely frozen, the metabolic heat generated in that portion becomes zero. Both these effects combined negate the effect of metabolic heat generation on the cooling rate and the time required for complete freezing of the tissue during cryopreservation.

5. Conclusion

In the current work, a two dimensional numerical model is developed to study the effect of cryopreservation in a square shaped biological tissue. In one case, the tissue specimen is freezed from one side while in another case, it is freezed from two sides keeping the other two sides insulated. Both side freezing resulted in achieving greater cooling rates and shorter freezing time during the freezing process as compared to the single side freezing. The time required for complete freezing of the tissue specimen is nearly halved for both side freezing. The entire tissue specimen achieved nearly a uniform temperature at the end of freezing which is equal to the cryogenic temperature or the temperature of the cold walls in case of double side freezing. Specimens are required to be cooled at a very high cooling rate to bring the entire specimen to a very low uniform temperature in shorter period for effective cryopreservation for a longer duration, which is achieved through both side freezing. It is witnessed that in a biological tissue, with increase in blood perfusion rate, the cooling rate reduces which necessitates the application of lower cryopreservation temperature for freezing of biological tissues with higher blood perfusion rates. It is also concluded that metabolic heat generation inside the biological tissue has a negligible role to play in the freezing of biological tissue.

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