

# Entropy generation theory for characterizing the freezing and thawing injury of biological materials

Li-Na Yu · Jing Liu

Received: 20 December 2006  
© Springer-Verlag 2007

**Abstract** As an important approach for long-term preservation of biological material, cryopreservation has been widely studied and applied in clinics. However, one of the most critical issues involved—the injury of biology material induced during freezing and thawing process still remains to be incompletely answered due to complexity of the problem itself. In this paper, we proposed for the first time to interpret the freezing or thermal injury by using the irreversible thermodynamics theory which is generalized in concept and has wide applicability. Comprehensive entropy generation analysis was performed on several typical freezing/thawing processes of biological tissues subject to cryopreservation. Particularly, variation of entropy generation rate due to injury induced change of thermal properties of the biological materials was investigated. Several useful indexes were suggested to quantify the freezing injury or viability of the biological material, through a combination with certain specific measurements. This study may possibly open a new theoretical strategy for evaluating the final output of either cryobiology or hyperthermia practices.

## List of symbols

$\vec{A}_j$	Chemical affinities of reaction $j$
$c_k$	Mass percent for the component $k$
$C_f$	Heat capacities of frozen tissue ( $\text{MJ}/\text{m}^3 \cdot ^\circ\text{C}$ )

This work was partially supported by the National Natural Science Foundation of China under Grant #50325622 and #50436030.

L.N. Yu · J. Liu (✉)  
Cryogenics Laboratory,  
Technical Institute of Physics and Chemistry,  
Chinese Academy of Sciences,  
P.O. Box 2711, 100080 Beijing, P.R. China  
e-mail: jliu@cl.cryo.ac.cn

$C_p$	Heat capacity of cryopreserved sample ( $\text{MJ}/\text{m}^3 \cdot ^\circ\text{C}$ )
$C_u$	Heat capacities of unfrozen tissue ( $\text{MJ}/\text{m}^3 \cdot ^\circ\text{C}$ )
$dS$	Total entropy of the system (J/K)
$d_e S$	Flow of entropy due to interactions with the exterior environment (J/K)
$d_i S$	Flow of entropy due to changes inside the system (J/K)
$\vec{F}_k$	External force
$g_{x_0}$	Local entropy generation change rate
$\vec{J}_j$	Chemical reaction rate
$\vec{J}_k$	Diffusion flux
$\vec{J}_q$	Heat flux ( $\text{W}/\text{m}^2$ )
$\vec{J}_S$	Entropy flux ( $\text{J}/\text{s} \cdot \text{K} \cdot \text{m}^2$ )
$\vec{J}_{s,\text{tot}}$	Total entropy flow per unit area and unit time ( $\text{J}/\text{s} \cdot \text{K} \cdot \text{m}^2$ )
$k_f$	Thermal conductivity of frozen tissue ( $\text{W}/\text{m} \cdot ^\circ\text{C}$ )
$k_u$	Thermal conductivity of unfrozen tissue ( $\text{W}/\text{m} \cdot ^\circ\text{C}$ )
$L$	Width of the calculation domain (m)
$n$	Unit outward normal
$p$	Equilibrium pressure (Pa)
$Q_l$	Latent heat of tissue ( $\text{MJ}/\text{m}^3$ )
$s$	Entropy per unit mass ( $\text{J}/\text{K} \cdot \text{m}^3$ )
$T$	Absolute temperature (K)
$T_0$	Initial constant temperature (K)
$T_a$	Surface cooling temperature (K)
$T_f(x, t)$	Temperatures of frozen tissue
$T_m$	Freezing point of tissue
$T_u(x, t)$	Temperatures of unfrozen tissue ( $^\circ\text{C}$ )
$u$	Internal energy per unit mass ( $\text{J}/\text{m}^3$ )
$v$	Specific volume ( $\text{m}^3$ )
$\mathbf{v}$	Velocity of the medium (m/s)
$V_n$	Normal velocity of moving interface (m/s)
$w(t)$	Moving interface resulted by freezing or thawing (m)
$x$	Coordinate in one dimension (m)

## Greek symbols

$\rho$	Density of the medium ( $\text{kg}/\text{m}^3$ )
$\sigma$	Entropy generation rate ( $\text{J}/\text{s} \cdot \text{K} \cdot \text{m}^3$ )
$\mu_k$	Thermodynamic or chemical potential of component $k$
$\vec{\Pi}$	Momentum flux or viscous pressure tensor
$\tau_1, \tau_2$	Initial and the ending time (s)
$\Gamma$	Total entropy generation for the whole sample ( $\text{J}/\text{K} \cdot \text{m}^2$ )
$\Gamma_x$	Local entropy generation at a specific position ( $\text{J}/\text{K} \cdot \text{m}^3$ )
$\Gamma'_{x_0}$	Local entropy generation for the injured material ( $\text{J}/\text{K} \cdot \text{m}^3$ )

## 1 Introduction

Cryopreservation has been widely proved to be an ideal way for long-term preservation of biological materials. This process generally includes three steps: the freezing stage, the preserving stage, and the rewarming stage, respectively. Unfortunately, the material is prone to be injured over the whole process of freezing and rewarming [1]. One of the most critical issues in cryopreservation is therefore to precisely comprehend the fundamental mechanisms of the freezing or thermal injury process, and to evaluate the degree of the viability after completion of cryopreservation.

When the material is subjected to freezing and thawing, the internal microstructures of the biological material may generally be changed permanently, and the cell membrane of the biological tissues is often the part most easily destroyed. The content of the cells will flow out and lead to the exchange of solutes and water content over the intracellular domain. As a result, these changes will influence the thermal properties of the tissue, and thus affect the heat transfer process of the tissue.

Up to now there have been a few techniques ever developed to evaluate the freezing and thawing injury after the cryopreservation. The most typical ways generally include [2, 3]: morphological and physiological observation, cell culture followed by biochemical testing, fluorescence detection, long-term histological evaluation, cell accounting by flow cytometric analysis, liquid chromatography, composition analysis, isotope marker method, electron spin resonance spectroscopy, nuclear magnetic resonance spectroscopy, oxygen or glucose consumption analysis, dye exclusion and dopamine release, dielectric measurement, differential scanning calorimetry, effective thermal conductivity measurement, and minimum cell-to-volume ratio, etc. These methods provided the new sight of experimental detection of the viability and analysis of freezing or thermal injury of the biological material because of their practicability and maneuverability.

However, from the theoretical viewpoint, most of the currently available works are still limited to the framework of the classical “two factors hypothesis” proposed by Mazur et al in 1972 [4]. Investigations of using Mazur type equations for cell dehydration to quantify the local cell injury and physicochemical environment have been extensively performed during the past few decades [5–8]. Although this theory has been tackled in many different aspects, the basic concept and the research method still remain to be in the same category. Extensions of the Mazur approach have also been made by several groups [9–11] and many interesting freezing injury pictures occurred in the cellular scale were revealed. However, a major feature of these theories lies in that it deals with the freezing and thawing behavior of a single cell through a microcosmic approach. The overall quantification of the macroscopical phenomenon is not easy to handle, which may limit its application in developing a practical algorithm to comprehensively evaluate the biological samples subject to a specific cryopreservation procedure. Therefore, there is currently a strong desire to find out a new theoretical strategy, which is mathematically tractable and generalized in physical and chemical mechanisms, to evaluate the cryopreservation injury of biomaterials, which occurred and accumulated over a time and space dependent process.

To partially fulfill such object in developing a method to interpret and quantify the freezing and thawing injury through a generalized way, we turn to the basic entropy generation concept of the thermodynamics, which has been widely applied before to nearly all kinds of fields and proven to be a unified method for depicting the complex process related to irreversible thermal process. The irreversible thermodynamics, especially, is helpful to solve such thermal injury problems, in which, a typical irreversible course induced by heat transfer is strongly involved. In fact, irreversible thermodynamics has been applied in many fields and the entropy generation analysis method has become a very useful tool for evaluating the intrinsic irreversibility associated with a given process or device [12–16]. In addition, many efforts have been paid to optimize processes by minimizing entropy generation [13], especially in the field of performance evaluation and optimization of heat transfer process [17] or characterization of chemical / biochemical reaction [18]. But as far as one knows, theoretical analysis on the freezing and thawing injury based on irreversible thermodynamics has not been tried up to now. In this paper, we proposed for the first time to interpret the freezing or thawing injury of biological materials subject to cryopreservation using irreversible thermodynamics. The critical issue of the thermal injury is pointed out and discussed, and some important indexes were established as the quantitative parameter for reflecting the freezing or thermal injury. Although the entropy analysis as given in this paper is still

preliminary, its basic concept may open some new possibilities in developing a generalized theory for injury evaluation and would be instructive for proposing further experimental techniques for such practices in the near future.

## 2 Irreversible thermodynamic principle

Before illustrating the irreversible thermodynamics approach in quantifying the freeze-thawing injury of biological materials, we will first briefly review the basic concept of the irreversible thermodynamics. Although the equations can be separately found in various textbooks, it is still very useful to provide a detailed derivation and then discuss its specific applications in the present study. The innovated component of the paper is the motivation to use entropy generation to describe the cryopreservation process.

As is well known, the second principle of thermodynamics postulates the existence of a function of state, called entropy. The change of entropy of a system can be splitted into two parts. Denoting by  $d_e S$  the flow of entropy, due to interactions with the exterior, and by  $d_i S$  the contribution due to changes inside the system, we can then have the total entropy of the system as

$$dS = d_e S + d_i S. \tag{1}$$

According to the second principle of thermodynamics, the entropy increase  $d_i S$  will never be negative. It is zero when the system undergoes a reversible change only, while it is positive if the system is subjected to an irreversible process. Then one has

$$d_i S \geq 0. \tag{2}$$

Referring to reference [19], one can rewrite Eqs. 1 and 2 as follows

$$S = \int^V \rho s dV \tag{3}$$

$$\frac{d_e S}{dt} = - \int^{\Omega} \vec{J}_{s,tot} \cdot d\Omega \tag{4}$$

$$\frac{d_i S}{dt} = \int^V \sigma dV \tag{5}$$

where,  $s$  is the entropy per unit mass,  $\rho$  the density of the medium,  $\vec{J}_{s,tot}$  the total entropy flow per unit area and unit time, and  $\sigma$  the entropy source strength or entropy generation per unit volume and unit time.

Through a combination among Eqs. 3, 4 and 5 and by following the Gauss theorem, Eq. 1 can be rewritten as

$$\int^V \left( \frac{\partial \rho s}{\partial t} + \text{div} \cdot \vec{J}_{s,tot} - \sigma \right) dV = 0. \tag{6}$$

Since Eqs. 1 and 2 hold true for an arbitrary volume, one gets

$$\frac{\partial \rho s}{\partial t} = - \text{div} \cdot \vec{J}_{s,tot} + \sigma \tag{7}$$

and,

$$\sigma \geq 0. \tag{8}$$

Using the following equation

$$\vec{J}_S = \vec{J}_{S,tot} - \rho s \mathbf{v} \tag{9}$$

Eq. 7 can be expressed in another different form

$$\rho \frac{Ds}{Dt} = - \text{div} \cdot \vec{J}_S + \sigma \tag{10}$$

where  $\vec{J}_S$  is entropy flux, which is the difference between the total entropy flux and a convective term  $\rho s \mathbf{v}$  ( $\mathbf{v}$  is the velocity of the medium). The following calculation gives the explicit form of the entropy flux  $\vec{J}_S$  and the entropy source strength  $\sigma$ .

According to the theorem of thermodynamics, in equilibrium, the total differential of  $s$  is given by the Gibbs relation

$$T ds = du + p dv - \sum_{k=1}^n \mu_k dc_k \tag{11}$$

where,  $p$  is the equilibrium pressure, and  $\mu_k$  the thermodynamic or chemical potential of component  $k$ ,  $u$  is the internal energy per unit mass,  $v$  is the specific volume,  $c_k$  is the mass percent for the component  $k$  which is defined by  $c_k = \frac{\rho_k}{\rho}$ , and  $T$  is absolute temperature.

Now it was proposed that although the whole system is not actually in equilibrium, for a small enough mass unit, the hypothesis of “local” equilibrium can generally be acceptable. Especially, we assume that Eq. 11 remains valid for a mass element followed along its center of gravity motion, then one has

$$T \frac{ds}{dt} = \frac{du}{dt} + p \frac{dv}{dt} - \sum_{k=1}^n \mu_k \frac{dc_k}{dt}. \tag{12}$$

Rewriting the two items  $\frac{du}{dt}$  and  $\frac{dc_k}{dt}$  in more concrete forms, one can get

$$\begin{aligned} \rho \frac{Ds}{Dt} = & - \frac{\text{div} \cdot \vec{J}_q}{T} - \frac{1}{T} \vec{\Pi} : \text{Grad} \mathbf{v} + \frac{1}{T} \sum_{k=1}^n \vec{J}_k \cdot \vec{F}_k \\ & + \frac{1}{T} \sum_{k=1}^n \mu_k \text{div} \cdot \vec{J}_k - \frac{1}{T} \sum_{j=1}^r \vec{J}_j \cdot \vec{A}_j \end{aligned} \tag{13}$$

where, the differential expression  $d/dt$  has been replaced by  $D/Dt$  as usually written;  $\vec{J}_q$  donates heat flux,  $\vec{J}_k$  the diffusion flux,  $\vec{\Pi}$  the momentum flux or viscous pressure tensor, and  $\vec{J}_j$  the chemical reaction rate.  $\vec{F}_k$  is the external force, and  $\vec{A}_j$  the chemical affinities of reaction  $j$  ( $j = 1, 2, \dots, \gamma$ ).

Transforming Eq. 13 to the same form as Eq. 10 one gets

$$\begin{aligned} \rho \frac{Ds}{Dt} = & -\text{div} \cdot \left( \frac{\vec{J}_q - \sum_{k=1}^n \vec{\mu}_k}{T} \right) - \frac{1}{T^2} \vec{J}_q \cdot \text{grad } T \\ & - \frac{1}{T} \sum_{k=1}^n \vec{J}_k \cdot \left( T \text{grad} \frac{\vec{\mu}_k}{T} - \vec{F}_k \right) \\ & - \frac{1}{T} \vec{\Pi} : \text{grad } \mathbf{v} - \frac{1}{T} \sum_{j=1}^r \vec{J}_j \cdot \vec{A}_j. \end{aligned} \quad (14)$$

Though a comparison between Eqs. 10 and 14 we obtain the expressions of entropy flux and the entropy generation rate as follows

$$\vec{J}_s = \frac{1}{T} \left( \vec{J}_q - \sum_{k=1}^n \mu_k \vec{J}_k \right) \quad (15)$$

$$\begin{aligned} \sigma = & -\frac{1}{T^2} \vec{J}_q \cdot \text{grad } T - \frac{1}{T} \sum_{k=1}^n \vec{J}_k \cdot \left( T \text{grad} \frac{\vec{\mu}_k}{T} - \vec{F}_k \right) \\ & - \frac{1}{T} \vec{\Pi} : \text{grad } \mathbf{v} - \frac{1}{T} \sum_{j=1}^r \vec{J}_j \cdot \vec{A}_j. \end{aligned} \quad (16)$$

Considering in more detail the expression for entropy generation rate, it can be concluded that the entropy generation rate comes from four different contributions: heat conduction, mass diffusion, gradients of velocity field and chemical reactions, respectively. When facing an irreversible process, the analysis of entropy generation field is also rather significant besides evaluating the fields of temperature, the density field and the velocity. Because for a non-equilibrium thermodynamic process, entropy is produced at any time and anywhere over the whole area of the system, and the strength of the entropy source at different positions will be different. It is such strength of the entropy source and the entropy generation that demonstrate the quantity of the irreversibility [19]. Therefore, analysis of entropy generation will lead to an important understanding on an irreversible process. In fact, such efforts had ever been tried for many years in the field of performance evaluation of heat radiator or characterization of chemical reaction. And entropy generation rate (entropy source strength) has been proved to be an important parameter to quantify the irreversibility of the process.

During the freezing and thawing of the biological material, which is a typical irreversible process, the entropy generation is always inevitable. Clearly, heat transfer process contributes significantly to the entropy generation. Besides, the mass diffusion occurring in cellular micro scale, the micro scale however maybe rapid deformation due to thermal stress and chemical reaction will also affect the entropy generation in the cryopreservation process. In this paper, as the first effort to illustrate the basic concept of

the new evaluation approach, only the most basic part, the entropy generation caused by heat conduction and its related effect will be considered. Meanwhile, all other factors such as mass diffusion and chemical reaction were not specifically treated for simplicity, which is in the sense that their contributions were attributed to the apparent effects of the conduction term alone and the varied thermal properties thus caused by the freezing and thawing. Such issue will also be explained a little more in later section through additional parametric study. Further, only one dimensional problem will be considered for brief, although the same analysis can be performed on multi-dimensional process.

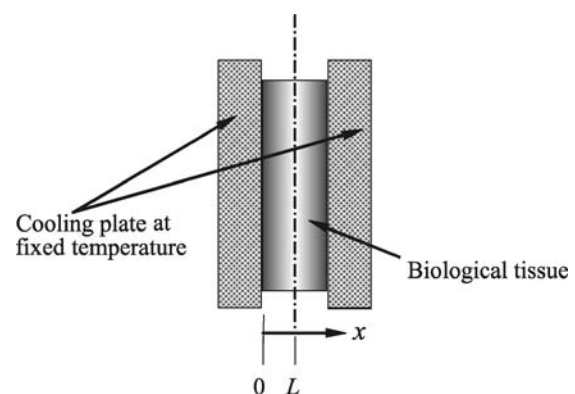
### 3 Heat transfer model

In the following calculation, typical cryopreservation process will be considered: a flat biological sample sandwiched and cooled by two ferreous plates (see Fig. 1). This resembles the clinical situation where a sheet of thin skin was frozen for a long term preservation. Due to symmetrical development of the thermal history in the sample, only one half of the domain will be considered. In this case, the heat balance for the unfrozen region and frozen region can be expressed respectively as follows

$$C_u \frac{\partial T_u(x, t)}{\partial t} = \nabla \cdot k_u \nabla [T_u(x, t)], \quad 0 < x < w(t), t > 0 \quad (17)$$

$$C_f \frac{\partial T_f(x, t)}{\partial t} = \nabla \cdot k_f \nabla [T_f(x, t)], \quad w(t) < x < L, t > 0 \quad (18)$$

where  $C_u, C_f$  are the heat capacities of unfrozen tissue and frozen tissue, respectively;  $T_u(x, t), T_f(x, t)$  are the temperatures of unfrozen tissue and frozen tissue, respectively.  $w(t)$  is the moving interface resulted by freezing or thawing; and  $k_u, k_f$  the thermal conductivity of unfrozen tissue and frozen



**Fig. 1** Schematic illustration of the condition where the biological tissue is sandwiched by two pre-cooled ferreous plates

tissue, and were treated as constant here for brief, respectively. The initial and boundary conditions for the case of Fig. 1 can be written as

$$T_u(x, t) = T_0 \quad 0 \leq x \leq 2L, t = 0 \quad (19)$$

$$T_f(x, t) = T_a \quad x = 0, t > 0 \quad (20)$$

$$\frac{\partial T_f(x, t)}{\partial x} = 0 \quad x = L, t > 0 \quad (21)$$

where,  $T_0$  is the initial constant temperature,  $T_a$  is the surface cooling temperature.

The temperature continuum and energy balance equations at the moving interface are

$$T_f(x, t) = T_u(x, t) = T_m, \quad x = w(t) \quad (22)$$

$$k_f \frac{\partial T_f(x, t)}{\partial x} - k_u \frac{\partial T_u(x, t)}{\partial x} = Q_l V_n, \quad x = w(t) \quad (23)$$

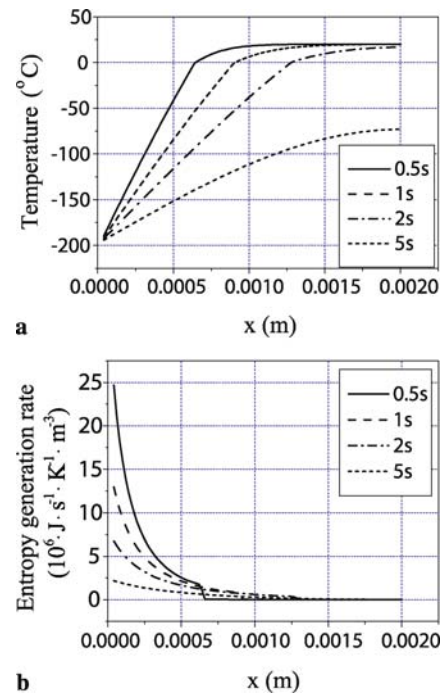
where,  $n$  denotes the unit outward normal;  $Q_l$ ,  $T_m$  are the latent heat and freezing point of tissue, respectively; and  $V_n$  is the normal velocity of moving interface. In order to avoid the time-consuming iteration at the moving boundary, the effective heat capacity method is used in this study. The essence of effective heat capacity method lies in that the latent heat is approximated by a generalized effective heat capacity over a small temperature range near the freezing point. Since its first proposition, the effective heat capacity method has been used by many investigators to solve phase change problems [20–22]. In fact, it has been a routine technique to simulate the heat transfer process with phase change. The effective heat capacity equation is equivalent in physical meaning with that by solving the heat transfer equations separately in the solid phase and liquid phase of tissues, respectively. For brief, the details about the method will not be repeated here. The readers can refer to [21] for more information. Based on the above one dimensional phase change heat transfer model, entropy generation analysis was performed on several typical heat transfer process. The typical intact tissue properties used in calculation are given in Table 1.

It should be pointed out that, different phase change heat transfer modeling may lead to a some what different temperature prediction. However, the basic concept as proposed in the present paper, i.e., using thermal dynamics theory to characterize the freezing and thawing injury of biological material, still holds true. In the near future, perhaps more

different heat transfer models can be tested on their effects to modeling the temperature and entropy generation. A lot of works along this direction are worth of pursuing.

#### 4 Entropy generation analysis on typical freezing and thawing process

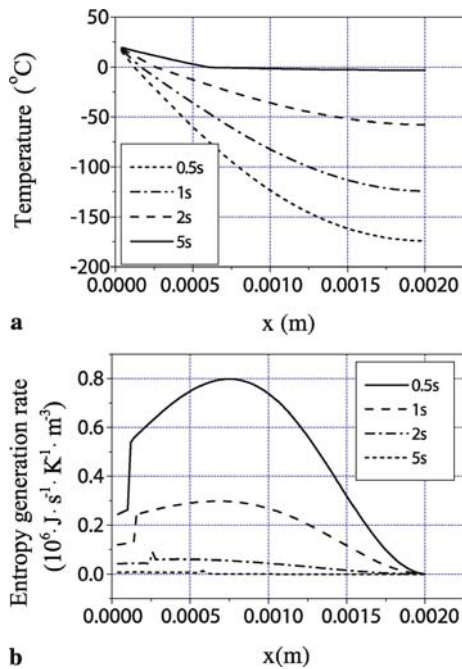
What presented in Fig. 2 are the distributions of temperature and entropy generation rate across the sample during the cooling process. It can be seen that the surface temperature was quickly reduced due to contact with the cooling plate, and the phase change interface moves to the center of the sample within only 3 s. Then the tissue was completely frozen. Figure 2b gives the entropy generation rate distribution at several times corresponding to Fig. 2a, respectively. Clearly, entropy generation rate of the sample near the cooling surface is tremendous at the initial stage of freezing due to the produced high temperature gradient there. However, it gets much less in the interior of the tissue, where the minimum value occurs at the center of the sample. Further, one can notice that, at the phase change interface, there appears a sudden drop in the entropy generation curve, whose reason can partially be attributed to the heat released when liquid phase tissue becomes solidified due to continuous freezing. The entropy generation rate levels off with the advancement of time, and approaches zero within only a few seconds



**Fig. 2** Distributions of the temperature (a) and entropy generation rate (b) in the biological tissue at several times during the freezing process

**Table 1** Typical thermophysical properties of biological tissues

	Unit	Value
Heat capacity of the frozen tissue	MJ/m <sup>3</sup> ·°C	1.8
Heat capacity of the unfrozen tissue	MJ/m <sup>3</sup> ·°C	3.6
Thermal conductivity of the frozen tissue	W/m·°C	0.5
Thermal conductivity of the unfrozen tissue	W/m·°C	2
Latent heat	MJ/m <sup>3</sup>	250

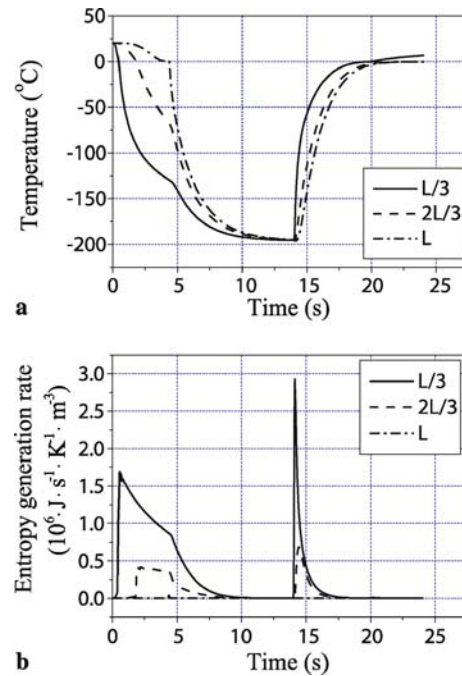


**Fig. 3** Distributions of the temperature (a) and entropy generation rate (b) of the biological tissue at several times during the thawing process

when the temperature becomes almost uniform throughout the tissue.

Figure 3 depicts the distribution of temperature and entropy generation rate during the thawing process. Clearly, the distribution of entropy generation appears much different compared with that in the process of freezing. It turns out to be degressive from the surface to the center of the sample. The peak of the entropy generation occurs at certain position inside the sample at different times during the thawing process. The sudden jump in the entropy generation curve in Fig. 3b indicated clearly the beginning of phase change stage. However, the temperature curve in Fig. 3a, corresponding to these times, appears as a gradually decreasing curve, and the phase change stage is not evident. Similar to the cooling process, with the progress of the thawing, temperature inside the tissue will level off and the entropy generation will approach zero finally.

Figure 4 indicates the transient temperature and entropy generation rate during the whole freezing and thawing process. Before initiating the freezing, the temperature throughout the whole tissue is uniform and the entropy generation rate is zero. As soon as the cooling process starts, the entropy generation rate at the surface of the sample increases rapidly, and reaches its maximum value when the phase change begins. By examining the corresponding curves at different positions of  $x = L/3, 2L/3$  and  $L$ , one can observe that the phase change stage starts at 0.45 s, 1.8 s and 3 s, respectively. In addition, comparing the two figures of Fig. 4a and b, it can be found out that there is also a sudden drop for



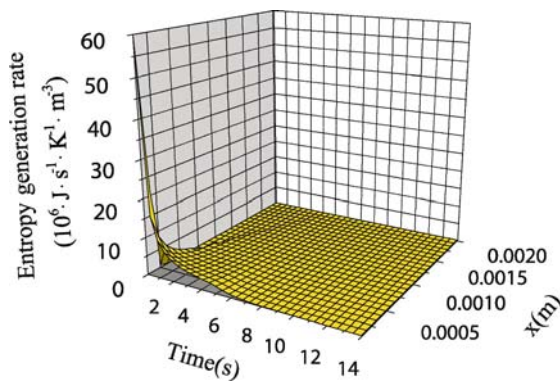
**Fig. 4** The transient temperature (a) and entropy generation rate (b) at several specific positions during the freezing and thawing process

the entropy generation rate when the phase change finishes. Then the rapid increase of entropy generation rate indicates the beginning of heating, and decreases gradually when the temperature inside the sample becomes uniform.

From the above analysis, in which the variation of the entropy generation rate curves caused by phase change heat transfer is clearly illustrated, it can be concluded that the value of entropy generation rate is the function of not only the temperature of the sample, but also the temperature gradient over the sample. So it is possible to reveal more information about the whole biological system during the process of cooling and rewarming by testing the spatial and transient distributions of entropy generation rate over the whole sample.

To be more intuitionistic, Fig. 5 gives the transient entropy generation rate distribution. It is clear that entropy generation rate evolution for a given position is very different according to the distance to the surface of the sample. For the purpose of better understanding the effect of freezing and thawing injury on the biological tissue, herein we defined two types of entropy generation: the local entropy generation at a specific position  $\Gamma_x$  and the total entropy generation for the whole sample  $\Gamma$ . At a specific spatial position, if integrating the entropy generation rate from time  $\tau_1$  to  $\tau_2$ , one can get the local entropy generation at the specified position through out the time period of  $\tau_1$  and  $\tau_2$

$$\Gamma_x = \int_{\tau_1}^{\tau_2} \sigma_{\tau,x} dt \tag{24}$$



**Fig. 5** Entropy generation rate vs. time and position

where,  $\sigma_{\tau,x}$  is the entropy generation rate for the position of  $x$  at time  $\tau$ , which can be obtained from Eq. 16; and  $\tau_1, \tau_2$  are the initial and the ending times of the integrating domain respectively. For example, in Fig. 4b, for the position of  $L/3$ , calculating the entropy generation of the whole cooling and rewarming process leads to  $\Gamma_{L/3} = 7.62966E6$ .

For the whole sample, one can also get its total entropy generation of  $\Gamma$  over the whole freezing and heating process as

$$\Gamma = \int_0^{2L} \int_{\tau_1}^{\tau_2} \sigma_{\tau,x} d\tau dx \tag{25}$$

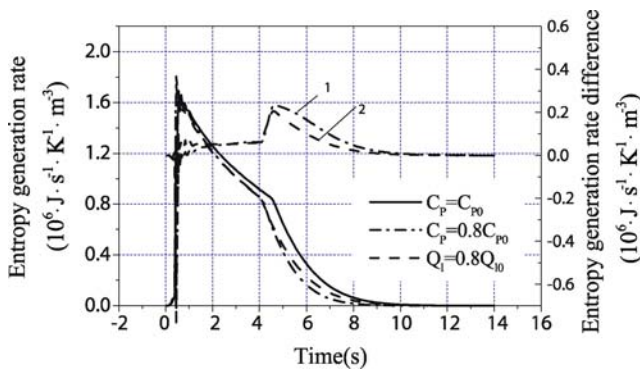
Here, the local information for entropy generation has been given for characterizing the state of a specific site inside the sample. In reality, the sample can be either very large such as in tissue scale or extremely small such as in cell level. Considering that sometimes part of the damage may not be able to well reflect the sample status on the whole, an integration term  $\Gamma$  as above is thus particularly introduced to quantify the overall property of the studied biological sample. This is rather useful for characterizing various sized samples, such as a thin layer of skin or even a single cell or part of its interior domain. What presented in the paper may have a generalized purpose.

For a practical freezing process, there would have different physical and chemical process to occur. Here, only the thermal process will be evaluated for simplicity. In fact, contribution of the heat induced side effects can be intuitively treated through an apparent heat transfer equation as proposed before. Therefore, the present model is still capable of providing a clear picture for understanding such process, although effects of the non-thermal processes have been attributed to that of the thermal one. If only taking the effect of heat transfer into consideration, the result of  $\Gamma_x$  and  $\Gamma$  which is initiated by an irreversible thermal process and its related event, can be regarded as the entropy generation at the position  $x$  and the total entropy generation of the whole

sample respectively. And they would serve well as the parameter to measure the irreversibility of the process from  $\tau_1$  to  $\tau_2$ . Therefore, the  $\sigma_{\tau,x}$  in Eqs. 24 and 25 can be obtained from Eq. 16 by just detecting the temperature transient and the other physical and chemical parameters. Based on these data, the  $\sigma_{\tau,x}$  can be calculated through a combined consideration of these factors. Therefore there in fact exists a crytic relation between the entropy generation and the temperature evolution of the sample. This is also the reason that the experimental techniques based on the temperature detection to evaluate the viability of the biological sample [2, 3] can give out the information of the total entropy generation and irreversible injury during the freezing and/or thawing process. The entropy analysis in this paper provides a theoretical explanation to some experimental techniques developed so far.

### 5 Entropy generation variation due to freezing or thawing injury

For a practical process, more complex situations can often be encountered. When the biological material is subjected to the freezing or thermal injury, the thermal parameters for the materials will most possibly be permanently changed implying the injury, which will result in a variation of entropy generation, compared with that of the intact material without modifications in thermal parameters. It is a common fact in cryobiology that the interior structure of the biomaterials is generally destroyed by the thermally induced behavior with the ice crystal formation in the freezing process and recrystallization during rewarming process. In addition, the synthesis and decomposition of the substance due to biochemical reaction will also irreversibly change the microstructure of the sample. These changes consequentially lead to the variation between the thermal properties of the cryopreserved biomaterial and that of the intact ones, such as that the heat capacity and the latent heat per unit mass of the sample generally reduces to some extent according to the strength of freezing injury. The heat transfer process will thus be varied by the change of the sample thermal properties. As a result, the temperature transient as well as distribution is influenced and so does the entropy generation rate calculated by Eq. 16 which directly corresponds to the sample temperature history. This in fact consists of the foundation of using the freezing curve based monitoring to evaluate the viability of the biological materials subject to freezing injury [2]. Now we will make a comparison on the entropy generation due to varied thermal property. Taking the freezing process for instance, at the position of  $L/3$ , if the heat capacity and the latent heat per unit changes 20% after freezing compared to that of the intact one, the transient entropy generation rate under the same boundary conditions as in the above-mentioned case in Fig. 5 was cal-



**Fig. 6** The local entropy generation rate at position  $L/3$  due to varied  $C_p$  and  $Q_l$

culated and displayed in Fig. 6. It can be seen that cryopreserved sample with smaller heat capacity  $C_p$  and latent heat per unit mass  $Q_l$  finishes its phase change stage at an earlier time (about 4.3 s) compared to that in the intact one (about 5.0 s), and its transient entropy generation rate is smaller than that of the intact one over the whole freezing process. Furthermore, the effect of  $C_p$  to the temperature and entropy generation is greater than that of  $Q_l$  if they vary by the same extent such as 20 percent. Especially when the phase change over the whole sample is accomplished, the effect of  $C_p$  reduction on the difference between the entropy generation rate of the cryopreserved sample and the intact one is more evident (as shown in the curves of 1 ( $C_p = 0.8C_{p0}$ ) and 2 ( $Q_l = 0.8Q_{l0}$ ) in Fig. 6). In fact, when the biomaterial is subject to freezing and thermal injury, the reduction of  $C_p$  and  $Q_l$  generally occurs synchronously. So the actual consequence is a combination of the effects of the both parameters.

For a specific position, if calculating the entropy generation of a freezing and thawing process using varied thermal parameters, the irreversibility of the freezing and thawing injury of the biomaterial can be seen more obviously. Taking the freezing process for example and calculating the entropy generation at  $L/3$  by using Eq. 17, we get

$$\begin{aligned} \Gamma_{L/3} &= 6.11416E6 & (C_p = C_{p0}) \\ \Gamma_{L/3} &= 5.41358E6 & (C_p = 0.8C_{p0}) \\ \Gamma_{L/3} &= 5.56497E6 & (Q_l = 0.8Q_{l0}) \\ \Gamma_{L/3} &= 4.89433E6 & (C_p = 0.8C_{p0}, Q_l = 0.8Q_{l0}) \end{aligned}$$

From these results, it can be seen that at the position of  $L/3$  when lowering  $C_p$  or  $Q_l$ , the entropy generation of the freezing process is reduced. To quantify such relative change, we define the local entropy generation change rate (LEPCR)  $g_{x_0}$  at a given position  $x_0$  as

$$g_{x_0} = \frac{|\Gamma_{x_0} - \Gamma'_{x_0}|}{\Gamma_{x_0}} \quad (26)$$

where,  $\Gamma_{x_0}$  is the local entropy generation at  $x_0$  for an intact material in an idealistic irreversible process, and  $\Gamma'_{x_0}$  for the injured material whose thermal properties was changed due to injury. Since the strength of the entropy source (entropy generation rate) and the total entropy generation represent the quantity of irreversibility, the variation of the entropy generation for a given position and the total sample can represent the information of the extent of irreversible freezing and thawing injury during the cryopreservation process. For the cryopreserved biological sample, which may be subjected to the intracellular freezing or excessive dehydration, the heat capacity and the latent heat are changed irreversibly. This irreversible change represents certain part of the irreversibility of the whole process. So when the freezing/thawing injury occurs, the local entropy generation changes, and the extent of variation will indicate the extent of the freezing injury. Referring to the above definition, we can depict the extent of freezing injury by using the index  $g_{x_0}$ -the local entropy generation change rate. If we calculate the corresponding value of entropy generation change rate for the freezing process after the change of  $C_p$  and  $Q_l$  at the position  $L/3$ , we get  $g_{L/3} = 11.5\%$  ( $C_p = 0.8C_{p0}$ ),  $g_{L/3} = 9.0\%$  ( $Q_l = 0.8Q_{l0}$ ) and  $g_{L/3} = 19.95\%$  ( $C_p = 0.8C_{p0}, Q_l = 0.8Q_{l0}$ ). The magnitude of the  $g_{x_0}$  can serve as an index for quantifying the degree of freezing and thawing injury for a specific position, and similarly, we can also define the total entropy generation change rate for the whole tissue to indicate the freezing /thawing injury on a whole:

$$g = \frac{|\Gamma - \Gamma'|}{\Gamma} \quad (27)$$

For a practical measurement, the varied thermal properties and temperature history can be recorded through experiment. Therefore, if submitting them to the above equation, the injury of biomaterials due to freezing or thermal injury can be quantified. Previously, the temperature was suggested to represent the thermal injury. However, it is still hard to know how to incorporate the thermal history into the quantification of the injury. Efforts made in this paper provide a very promising way for such purpose. It will possibly find significant applications in the viability evaluation of biomaterial through combination with a necessary experimental measurement.

## 6 Discussion and conclusion

As mentioned above, the recently proposed experimental techniques such as freezing curve-based monitoring method, biological electrical impedance detection method, and thermal conductivity evaluation method are proved efficient to evaluate the freezing and thawing injury of biological material. In these techniques, the variation of

thermophysical parameters and heat transfer process caused by freezing and thawing injury are observed and recorded, and a close correlation is found between the variation of thermophysical parameters and the viability of cells and tissue. Although the experimental data are abundant and persuasive, and different parameters are proposed to determine the extent of thermal and thawing injury, none of them can be regarded as generalized due to the lack of quantitative theoretical background. On the contrary, in this paper we suggested for the first time to use the entropy generation analysis method for such purpose. Some parameters have been proposed to interpret the freezing and thawing injury of biological material during cryopreservation, which has the concrete thermodynamical foundation—the quantity of irreversibility. So it is directly connected with the freezing and thawing injury, demonstrating the irreversible change of biological material in terms of heat transfer, mass transfer and chemical reaction when the biological material is subject to freezing.

It should be pointed out that, the irreversible thermodynamics is presently mainly useful in characterizing freezing and thawing process in tissue level. Analyzing the cellular injury needs additional work. Nevertheless, direct evaluation on tissues' viability is more commonly encountered in the practice of cryopreservation, compared with the evaluation of a single cell. The entropy generation represents certain story of the irreversibility of the whole process. Its change between the intact sample and the injured one serves as perhaps the most appropriate fundamental link between the sample injury and entropy generation. As is widely accepted, entropy generation is an ideal physical quantity to characterize the irreversible process. No other quantities can be found better than this. However, it should be emphasized that, the entropy generation itself does not necessarily reflect the injury strength. It is the difference between the intact and injuring process that serves as the quantity to characterize the viability variation. This was also one of the key points clarified by the present study. Most important of all, integration of entropy generation over the whole process completely reflects the whole injury (whether weak or serious) caused by the freezing and thawing process. Any of the other properties such as temperature curve, change of thermal properties etc. can only partially reflect the viability change.

We should also mention that the entropy generation analysis presented in this paper is still preliminary, in which only the most important term, the influence of heat transfer for entropy generation is taken into consideration, and other factors are ignored for simplifying the analysis. In future study, more complex and complete entropy generation calculation should be carried out by including multiple effects such as the mass diffusion, gradients of velocity field and chemical reactions inside the biomaterial during freezing

and thawing process. Characterization on the injury process of a single cell by using the present approach is also worth of perusing. In this way the entropy generation analysis can provide more comprehensive information and reveal more detailed physical picture.

Furthermore, the microstructure transformation of biomaterial is related to all the factors which induced the irreversibility of the process, and has been extensively studied in the past few decades. It may be beneficial to combine these knowledge and technology in microstructure transformation of biomaterial with the current theory of irreversible thermodynamics, especially entropy generation analysis method. Then, the problem of freezing or thawing injury of biological material can be interpreted from a new viewpoint. Besides, as is gradually noticed, the concept of entropy generation and minimization is becoming a highly modern and interesting concept in physics and engineering. In the field of cryopreservation, the minimization on the entropy generation is of particular importance in the sense of decreasing freezing-thawing injury and optimizing a practical freezing protocol. In this paper, the entropy generation difference of the injured sample and the intact one indicated the extent of freezing injury. Therefore, minimization on such entropy generation difference would lead to an optimum protocol for a successful cryopreservation. Previously, many efforts had ever been made for the operation, and the typical experimental methods include adding appropriate cryoprotective agents, optimizing the freezing and thawing rate, etc. The practical output of these experimental studies can possibly be evaluated by the principle of entropy generation difference analysis proposed in this study. Efforts made in this paper will stimulate more investigations along this direction in the near future.

## References

1. Karlsson JOM, Toner M (1996) Long-term storage of tissues by cryopreservation: critical issues. *Biomaterials* 17:243–256
2. Liu J, Zhou YX (2003) Freezing curve-based monitoring to quickly evaluate the viability of biological materials subject to freezing or thermal injury. *Anal Bioanal Chem* 277:173–181
3. Yu TH, Liu J, Zhou YX (2004) Electrical impedance detection to evaluate the viability of biomaterials subject to freezing or thermal injury. *Anal Bioanal Chem* 378, 1793–1800
4. Mazur P, Leibo SP, Chu EHY (1972) A two factor hypothesis of freezing injury—evidence from chinese hamster tissue culture cells. *Exp Cell Res* 71:345–355
5. Hayashi Y, Momose N, Tada Y, Jiang R (1995) Microbehavior of biological cell during freezing and thawing. *Proc ASME-JSME Therm Eng Joint Conf* 4:589–594
6. He XM, Bischof JC (2003) Quantification of temperature and injury response in thermal therapy and cryosurgery. *Crit Rev Biomed Eng* 31:355–422
7. Leibo SP, McGrath JJ, Cravalho EG (1978) Microscopic observation of intracellular ice formation in unfertilized mouse ova as a function of cooling rate. *Cryobiology* 15:257–271

8. Ujihira M, Yamaguchi R, Tanishita NK (1995) Injury of biological tissue by extracellular freezing. *Heat Transf Japan Res* 24:457–475
9. Devireddy RV, Barratt PR, Storey KB and Bischof JC (1999) Liver freezing response of the freeze tolerant wood frog, *Rana sylvatica*, in the presence and absence of glucose. II. Mathematical modeling. *Cryobiology* 38:327–338
10. Karlsson JOM (2001) A theoretical model of intracellular devitrification. *Cryobiology* 42:154–169
11. Kleinhans FW (1998) Membrane permeability modeling: Kedem-Katchalsky vs a two parameter formalism. *Cryobiology* 37:271–289
12. Bejan A (1982) *Entropy Generation Through Heat and Fluid Flow*. Wiley, New York
13. Bejan A (1996) *Entropy Generation Minimization*. CRC Press, Boca Raton
14. Baytas AC (1997) Optimization in an inclined enclosure for minimum entropy generation in natural convection. *J Non-Equil Thermodyn* 22:145–155
15. Demirel Y, Kahraman R (1999) Entropy generation in a rectangular packed duct with wall heat flux. *Int J Heat Mass Transf* 42:2337–2344
16. Hijleh BA, Qudais MA, Nada EA (1998) Entropy generation due to laminar natural convection from a horizontal isothermal cylinder. *ASME J Heat Transf* 120:1089–1090
17. Kolenda Z, Donizak J, Hubert J (2004) On the minimum entropy production in steady state heat conduction processes. *Energy* 29:2441–2460
18. Lems S, Kooi HJ, Arons JS (2003) Thermodynamic optimization of energy transfer in (bio)chemical reaction systems. *Chem Eng Sci* 58:2001–2009
19. de Groot SR, Mazur P (1962) *Non-equilibrium thermodynamics*. North-Holland Pub. Co., Amsterdam
20. Amin MR (2000) Thermal analysis during continuous casting process using effective heat capacity method. *AIAA J. Thermophys. Heat Transf* 14:170–176
21. Deng ZS, Liu J (2004) Numerical simulation on 3-d freezing and heating problems for the combined cryosurgery and hyperthermia therapy. *Num Heat Transf A* 46:587–611
22. Pham QT (1986) The use of lumped capacitance in the finite-element solution of heat conduction problems with phase change. *Int J Heat Mass Transf* 29:285–291