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Numerical Simulation of Cell Response in Freezing of Ternary Solution*

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Abstract: Using the continuum model for multi-component phase change system, the freezing process of ternary cell solution (H_2O-NaCl-CPA) was simulated numerically. In order to verify the results of numerical simulation, with some specific assumptions, an approximate analytical method was developed. Results of numerical simulation and analytical method were compared under the same conditions. Their coincidence shows the numerical simulation to be right basically.

Key words: phase change heat conduction; cell model; nucleation mechanism; cryoinjury

CLC number: Q274; Q273 **Document code**: A

0 Introduction

Cryopreservation of biological cells plays an important role in clinical application and scientific research. When cells and suspension are cooled below the freezing point of the solution, ice forms in the external medium preferentially. Presumably because the plasma membrane blocks the growth of ice crystals into the cytoplasm, cell contents remain unfrozen and supercooled[1]. The supercooled water in the cell has a higher chemical potential than that of water outside the cell, as a result the water inside the cell will diffuse out of the membrane to maintain the osmotic balance between the intra and extracellular media.

In 1963, Mazur[2] developed a model describing the kinetics of water loss from cells during freezing process. Using this model, many researchers have successfully predicted the damage of the cell at subzero temperatures[3~7]. Also some modifications of this model have been made, such as introducing of the activities in Toner's model[3], including of the CPA in the solution in Karlsson's study[4], and in Mansoori's study, he concerned the temperature difference between the cell and excellular media[8].

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However, these models are all based on a basic assumption that the temperature and solute concentration distributions are uniform in the cryopreserved cell suspensions. But when the preserved samples are large in size, the time dependent temperature and solute concentration distributions are not uniform during the cooling process. Viskanta[9] has calculated the temperature and salt concentration distributions during the solidification process of solution, and used these data to predict the kinetics of water loss from the cell. In his calculation, the cell suspension is a binary solution (H_2O-NaCl).

The purpose of this paper is use the continuum model developed by Bennon and Incropera [10] to simulate the freezing process of ternary solution (H_2O-NaCl-DMSO), then use the calculated temperature data, coupled with the model describing water loss from a cell to predict the cell response during the cooling process.

1 Freezing of ternary solution

For solidification of cell suspension, the following assumptions have been made, (1) neglecting convection of the solution, that is there is no fluid motion occurring in the melt; (2) neglecting the species diffusion process; (3) local equilibrium has been achieved. Under these assumptions, apply the continuum model describing multicomponent phase change system developed by Bennon *et al*[10], and the energy equation of the mixture can be given as:

$$\frac{\partial(\rho h)}{\partial t} = \nabla\left(\frac{k}{c_{ps}}\nabla(h)\right) + \nabla\left(\frac{k}{c_{ps}}\nabla(h_s - h)\right) \tag{1}$$

where h is enthalpy of the mixture, k is the thermal conductivity of the mixture, c_{ps} is the specific heat of solid phase, h_s is the enthalpy of the solid phase. h and k are all functions of f_l (mass fraction of liquid)

$$h = f_l h_l + (1 - f_l)h_s \tag{2}$$

$$k = f_l k_l + (1 - f_l)k_s \tag{3}$$

where h_l and h_s are the enthalpy of liquid and solid phase respectively , k_l and k_s are thermal conductivities of lquid and solid phase respectively. h_l and h_s are given by

$$h_l = c_{pl}T + L \tag{4}$$

$$h_s = c_{ps}T \tag{5}$$

where T is temperature, L is the heat of fusion, and c_{pl} is the specific heat of liquid phase.

Assuming that the temperature of the solution is always greater than the pseudo-binary eutectic temperature, which means that there is only one solid phase (ice) formed during freezing process, thus the ratio, R, of wt% DMSO to wt% NaCl will not change during the whole process. If the initial concentrations of NaCl and DMSO are C_s^0 and C_a^0 respectively, then

$$C_s^0 = f_l C_s(T) \tag{6}$$

$$C_a^0 = f_l C_a(T) \tag{7}$$

where $C_s(T)$ and $C_a(T)$ are concentrations of NaCl and DMSO in liquid phase at temperature T. By assuming local thermodynamic equilibrium, they can be determined from the ternary phase di-

agram of H_2O-NaCl-DMSO as long as R is given.

From eq. (6) and eq. (7), the mass fraction of liquid phase can be given by

$$f_l = \frac{C_s^0}{C_s(T)} = \frac{C_a^0}{C_a(T)} = \frac{C_{\mathrm{Tot}}^0}{C_{\mathrm{Tot}}(T)} \tag{8}$$

where C_{Tot}^0 is the initial concentration of total solutes (DMSO + NaCl). The mass fraction of liquid phase is the concentration of solute in liquid phase at temperature T.

The numerical procedure is as follows: f_l can be estimated from the previous time step, eq. (1) can be solved numerically, from the new h value obtained, f_l is updated, and the iterative process is continued until the solution converges. Once the solution has converged, we march to the next time step where the iterative cycle is followed once again.

2 Water loss from cell

The volume change rate of the cell is given by[2]:

$$\frac{\mathrm{d}V_{cv}}{\mathrm{d}t} = L_p A(\pi_i - \pi_e) \tag{9}$$

where V_{cv} is the intracellular solution volume, it excludes the inactive cell volume V_b. The total cell volume is thus $V_{\mathrm{cell}} = V_{cv} + V_b$. A is the surface area of the cell, L_p is the cell membrane permeability to water. π_i is the intracellular osmolality and π_e is the extracellular osmolality, all given by [11]

$$\pi_i = -\frac{R_u T}{v_w}\ln a_w^i \tag{10}$$

$$\pi_e = -\frac{R_u T}{v_w}\ln a_w^e \tag{11}$$

where v_w is the specific volume of the water, a_w^i and a_w^e are activities of intracellular and extracellular water, and R_u is universal gas constant. Substituting eq. (10) and eq. (11) to eq. (9), one obtain:

$$\frac{\mathrm{d}V_{cv}}{\mathrm{d}t} = \frac{L_p A R_u T}{v_w}\ln\left(\frac{a_w^e}{a_w^i}\right) \tag{12}$$

The intracellular solution is modeled as an ideal solution for which the water activity is equal to its mole fraction:

$$a_w^i = x_w^i = \frac{n_w}{n_w + n_s + n_a} = \frac{V_{cv} - (n_s v_s + n_a v_a)}{V_{cv} - (n_s v_s + n_a v_a) + v_w(v_s n_s + n_a)} \tag{13}$$

where n_w, n_s and n_a are the number of moles of water, NaCl and DMSO in the cell respectively, v_s is the dissociation constant of NaCl ($v_s = 2$), v_s and v_a are specific volume of NaCl and DMSO respetively.

The extracellular water activity can be written as[11]:

$$a_w^e = 1 + 0.00966(T - 273.15) + 4.1025 \times 10^{-5}(T - 273.15)^2 \tag{14}$$

Substitute eq. (13) and eq. (14) into eq. (12), the result is

$$\frac{\mathrm{d}V_{cv}}{\mathrm{d}t} = \frac{L_p A R_u T}{v_w} \ln\left[\frac{1 + 0.00966(T - 273.15) + 4.1025 \times 10^{-5}(T - 273.15)^2}{\dfrac{V_{cv} - (n_s v_s + n_a v_a)}{V_{cv} - (n_s v_s + n_a v_a) + v_w(v_s n_s + n_a)}}\right] \quad (15)$$

With the obtained temperature data from the previous numerical simulation of solidification process of ternary solution, and using the fourth-order Runge-Kutta scheme, eq. (15) can be solved numerically.

3 A sample study

We now consider the freezing of H_2O-NaCl-DMSO ternary solution to simulate the freezing of a cell suspension in a box. The equilibrium diagram for this ternary solution is given by Pegg[12]. By calculating the transient temperature, we can predict the cell response of different locations inside the box.

The model is given in Fig. 1. A box containing the cell suspension is considered a symmetric, one dimensional rectangular, the width of the box is $2L$, the temperature of the outside boundaries decreases at a constant cooling rate. In this study, we presume the temperature is always greater than the pseudo-binary eutectic temperature of the ternary solution. The initial temperature and concentration are uniform in the solution.

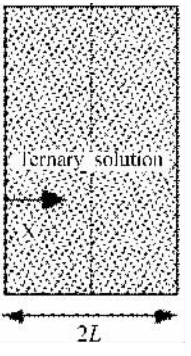

Fig. 1 Schematic diagram of the solution and coordinate system

We assume the thickness of the box is negligible. We neglect the volume change of the solution during freeing and thermophysical properties of the liquid and solid are independent of temperature and concentration. We also neglect convection caused by buoyancy forces. It is assumed that the growth of the mushy zone is controlled by heat conduction only. This assumption is based on the fact that the thermal diffusivity of the solution is much greater than the mass diffusivity.

The mathematical description of the above boundary and initial conditions are as follows:

$$t = 0 \qquad C_s(x,0) = C_s^0$$

$$C_a(x,0) = C_a^0$$

$$T(x,0) = T_i$$

$$x = 0 \qquad T(0,t) = T_0 - Bt$$

$$x = \gamma \qquad \frac{\partial T}{\partial x} = 0$$

where C_s^0 and C_a^0 are initial concentrations of NaCl and DMSO respectively, T_i is the initial temperature of the solution, T_0 is the temperature of the outside boundaries of the box at time 0 s, and B is the cooling rate.

With the assumption of local thermodynamic equilibrium in the mushy zone, the temperature and concentration of the solution follow the liquidus curve of the phase diagram. Since the bound-

ary temperature is always greater than pseudo-binary eutectic temperature, lever rule will apply, so eq. (8) can be used to determine the mass fraction of liquid phase.

Using the implicit scheme and control volume method, the enthalpy equation can be discretized, the solution procedure is summarized as follows: at each time step, f_l field is estimated from the previous time step, and eq. (1) can be solved. From the enthalpy obtained, the new temperature can be calculated, then the concentration can also be obtained from the phase diagram. With the temperature and concentration, f_l field is updated. The iterative process is continued until the solution converges, and the next time step with the same iterative process starts. Once we get the temperature and concentration of the solution, these data can be used to solve eq. (15) to predict the cell response in different locations of the box.

4 Parameters

The parameters in this numerical simulation are as follows:

Box thickness: $2L$ = 10 mm;

Initial NaCl concentration: 0.142 mol/L; Initial DMSO concentration: 1.5 mol/L

Initial temperature: 273.15 K; Cooling rate: −10 ℃/min

Cells: mouse oocytes; Cell membrane properties are from Agca *et al*[13].

5 Results and discussion

Fig. 2 shows the results of temperature distributions inside the box at different time. Because the left side boundary temperature is always greater than the pseudo-binary eutectic temperature, only liquid and mushy regions exit in the solidification system. Note that there are changes of slopes of these curves. They are due to the different thermophysical properties of the mushy zone and the liquid region. We can also see from the result at time t = 180 s that nearly all the region becomes mushy.

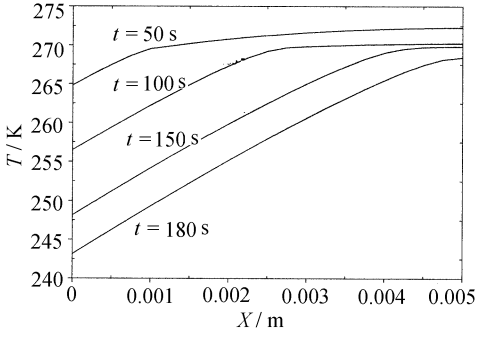

Fig. 2 Temperature distributions during freezing of H_2O + 0.142 mol/L NaCl + 1.5 mol/L DMSO ternary solution at a boundary cooling rate of −10 ℃/min

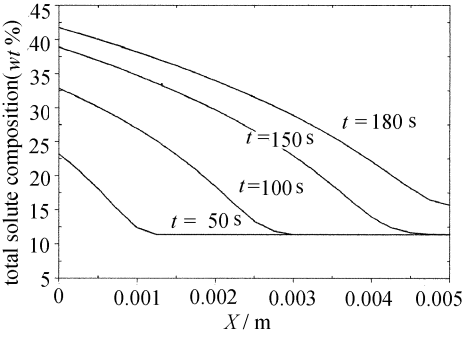

Fig. 3 Total solute concentration distributions during freezing of H_2O + 0.142 mol/L NaCl + 1.5 mol/L DMSO ternary solution at a boundary cooling rate of −10 ℃/min

The total solute (NaCl + DMSO) concentration distributions in the box at different times are illustrated in Fig. 3. As the solution freezes, ice forms in the solution, so the solute concentration increases sharply in the mushy region. Note that the total solute concentration is much higher near the boundary because that is where temperature is much lower and more ice crystals are formed.

Fig. 4 shows temperature variations with time at different locations in the box under the boundary cooling rate of -10℃/min. From the results we can see that the temperature profiles are quite different depending on the locations inside the box. At the region near the boundary of the box ($x/L = 0.25$), temperature remains at liquid temperature value for a relatively short time and the drops keep at a nearly constant rate. While at the region near the center of the box ($x/L = 1.0$), the temperature of the solution remains at liquid temperature value for a relatively long period of time before it starts decreasing suddenly. The temperature profiles at the two intermediate locations ($x/L = 0.5$, $x/L = 0.75$) have a similar behavior. From these results, we can see that the maximum of temperature gradient appears at the center of the sample.

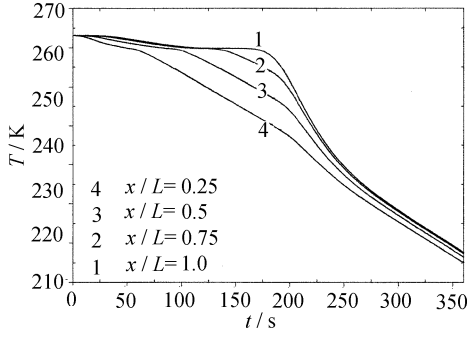

Fig. 4 Temperature variations with time at different locations during freezing of H_2O + 0. 142 mol/L NaCl + 1. 5mol/L DMSO ternary solution at a boundary cooling rate of -10 ℃/min

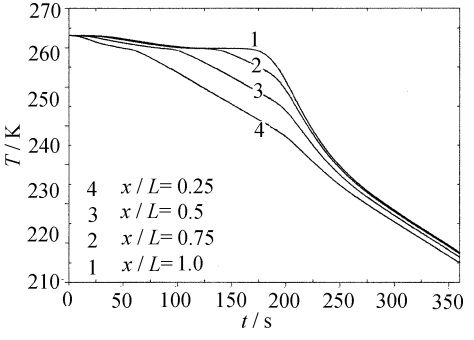

Fig. 5 Total solute concentration variations with time at different locations during freezing of H_2O + 0. 142mol/L NaCl + 1. 5 mol/L DMSO ternary solution at a boundary cooling rate of -10 ℃/min

Fig. 5 shows the total solute concentration profiles at the same locations as those in Fig. 4. The concentration profiles are similar for $x/L = 0.25$, $x/L = 0.5$ and $x/L = 0.75$. While the profile in the center of the box is quite different, it maintained constant for a relative long period, and through the concentration increased sharply.

From Fig. 2 ~ Fig. 5, we can see that for a given time the temperature and concentration are not uniform inside the box. Although the boundary temperature drops at a constant rate, the temperature at different locations inside the box undergoes quite different changes. So the cells in the solution will go through different cooling conditions which are determined by the locations inside the box.

6 Kinetics of water loss from cells at different locations inside the box

In this sample study, we used the calculated temperature data as the extracellular environ-

mental conditions to simulate the cell response in different locations inside the box. The parameters used in this calculations are as follows: $V_{cell0} = 2.62 \times 10^5\ \mu m^3$, $V_b = 5.58 \times 10^4\ \mu m^3$, $L_p = 5.29 \times 10^{-2}\ \mu m \cdot min^{-1} \cdot atm^{-1}$ (at temperature of 273.15 K), $E_{Lp} = 16.39$ Kcal $\cdot$ mol^{-1}. V_{cell0} is the cell volume at isotonic condition, V_b is osmotically inactive volume, L_p is the membrane permeability at reference temperature, E_{Lp} is the membrane permeability activation energy. These values are taken from the existing studies of mouse oocytes[4, 13].

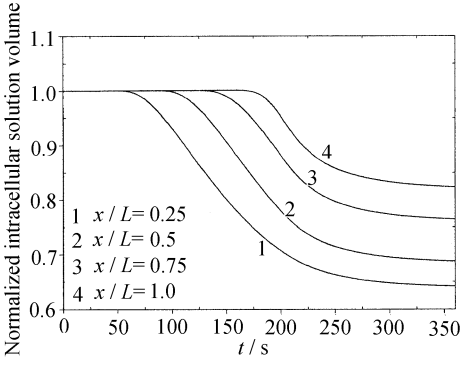

Fig. 6 Dependence of intracellular solution volume on time at different locations in the box with a boundary cooling rate of -10℃/min

The results of normalized intracellular solution volume variations with time at different locations during cooling process are illustrated in Fig. 6. From this figure, we can see that the cell volume change depends on the locations. At the location near the boundary ($x/L = 0.25$), cell dehydrates much earlier than that in other regions, and at the time $t = 360$ s, the volume shrinks to nearly 65% of its original volume. While at the region near the center of the box ($x/L = 1.0$), there is no water loss from the cell for a long time, and at time $t = 360$ s, the cell water volume reduces by only 20% of its original volume. This is because the local cooling rate in the center of the sample is greater than that near the boundary. Cells at $x/L = 0.5$ have a similar manner to those at $x/L = 0.75$, except that they maintain for a shorter time at their initial volumes. These results mean that even though the boundary cooling rate of the sample is constant, different locations inside the sample experience different temperature change histories. As a result, cells at different locations inside the sample undergo different volume changes.

7 Conclusion

A mathematical model describing heat transfer during the solidification process of ternary solution (H_2O-NaCl-DMSO) has been developed, and some results have been obtained from a sample study. With these calculated temperature data, coupled with cell model, the kinetics of water loss from cell during freezing was obtained. These results demonstrated that in relatively large dimensions, the temperature and concentration distributions are not uniform inside the solution during the solidification process. As a result, the cell response will be different depending on their locations inside the sample.

The exact knowledge of the temperature and concentration distribution in cell suspension is very important for cryobiology research and can be used to develop optimum cryopreservation protocols. Also, with these information, using the existing IIF model[3], the damage to cells during freezing at different locations of the suspension may be estimated.

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在三元溶液低温相变过程中细胞反应的数值模拟

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摘要: 应用多组分相变系统的连续体模型,对三元溶液(NaCl-H_2O-CPA)低温相变过程中的细胞反应进行数值模拟. 为了检验数值分析的结果,在特定条件下,提出一种解决多组分系统相变问题的近似分析方法,并在相同条件下,将此结果与数值分析的结果进行比较,两者基本一致.

关键词: 相变传热; 细胞模型; 成核机制; 低温损伤