International Journal of Heat and Mass Transfer 114 (2017) 1–7

Contents lists available at ScienceDirect

International Journal of Heat and Mass Transfer

journal homepage: www.elsevier.com/locate/ijhmt

Modeling and experimental studies of enhanced cooling by medical gauze for cell cryopreservation by vitrification

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ARTICLE INFO

Article history:
Received 1 November 2016
Received in revised form 21 April 2017
Accepted 10 June 2017

Keywords:
Vitrification
Traditional French-type straw
Ice crystallization
Film boiling
Enhanced heat transfer

ABSTRACT

Vitrification is considered as an important alternative approach to traditional slow freezing method for cryopreservation of cells. A typical cell vitrification procedure involves a non-equilibrium cooling process commonly accomplished in liquid nitrogen, while in which film boiling is believed to greatly hinder heat transfer surrounding the sample, resulting in incomplete vitrification or a much higher critical concentration. In this study, we developed a simple while effective approach, wrapping traditional French-type straw with medical gauze, to greatly enhance convective heat transfer during cooling by suppress film boiling. We further established a coupled heat transfer model for cooling and warming of cell suspensions to investigate the inherent thermodynamic mechanism in this approach. The model describes both the macroscale thermal distributions in extracellular solution and the microscale ice crystallization inside the cells. The simulation indicated that straws wrapped with medical gauze would increase cell survival subject to vitrification cryopreservation by significantly increasing the cooling rate to inhibit intracellular ice formation (IIF). Our experiments on human umbilical vein endothelial cells (HUVECs) further confirmed the predictions in that the cell survival rate was significantly increased by wrapping straws with medical gauze.

1. Introduction

Vitrification has been considered to be the most promising method for successful cryopreservation of cells and tissues [1]. In 1980s, Rall and Fahy introduced vitrification to the preservation of organs and mouse embryos and proposed some emerging principles on the vitrification protocol [2]. Vitrification has tremendous advantages over traditional slow freezing method [1,3–5]. On one hand, vitrification process is simple and easy to perform. It does not require controlled cooling rate, which is essential to traditional slow freezing method and should be implemented by an expensive controlled rate freezer. On the other hand, vitrification totally avoids the formation of intracellular ice formation (IIF) which is considered to be fundamental to all cryoinjuries. Vitrification experiments for cryopreservation are usually conducted by plunging vitrification devices with cell suspensions into liquid nitrogen (LN_2) [6]. Owing to the large temperature difference between sample and LN_2, this procedure is commonly accompanied by film boiling of LN_2 on device surface [7,8], which consequently blocks heat transfer surrounding the sample and leads to incomplete vitrification. Some researchers have worked on enhancing heat transfer during cooling in vitrification cryopreservation [8–10]. He et al. suggested that using a quartz micro-capillary would achieve ultra-fast cooling rates and enable vitrification of murine embryonic stem cells at a low concentration of cryoprotectants [9]. Zhou et al. utilized micro-channel array on the surface of vitrification device and reported that an ultra-high heat transfer coefficient can be obtained [8,10]. However, to our knowledge, little research has been done on eliminating film boiling regime. In order to investigate the inherent thermodynamic mechanism underlying vitrification procedure, a well-established heat transfer model is also needed [11–15]. In 1990s, Boutron carefully investigated the glass-forming tendency in cryoprotective systems and empirically introduced a non-equilibrium model for determination of ice formation in aqueous cryoprotective solutions [16]. This model has been widely used in the simulation of vitrification process. For example, Song et al. successfully optimized the droplet vitrification protocol by adopting this model [7]. Zhou et al. applied this model to demonstrate the efficiency of a novel microfluidic system for cell vitrification [8]. However, in all these studies, they used tempera-

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http://dx.doi.org/10.1016/j.ijheatmasstransfer.2017.06.036
0017-9310/

Nomenclature

t	time (s)
T	temperature (K)
k_a	crystallization characteristic constant (s^{-1} K^{-1})
T_m	final temperature of the freezing process (K)
T_∞	temperature of liquid nitrogen (K)
T_f	equilibrium freezing temperature of the cytoplasm (K)
Q	activation energy (J/mol)
R	gas constant (J/mol/K)
c	specific heat capacity (J/kg/K)
k	thermal conductivity (W/m/K)
r	distance from center of the straw along radial direction (m)
H	latent heat (J/kg)
h	convective heat transfer coefficient ($W/m^2/K$)
A_c	cell surface area (m^2)
N	number of water molecules in contract with the substrate (–)

Greek symbols

χ	degree of ice crystallization (–)
ρ	density (kg/m^3)
Ω	thermodynamic parameter for nucleation (–)
κ	kinetic parameter for nucleation (–)
η	viscosity of the cytoplasm (Pa s)

Subscripts

0	isotonic condition

ture dependent thermal properties which were measured isothermally. Thus the results might be inaccurate. Besides, Jiao et al. demonstrated that even in a narrow plastic straw, the temperature distribution during cooling could be quite non-uniform depending on the convective heat transfer coefficients [17]. Meanwhile, whether or not a cell is vitrified depends largely on cooling rates. Therefore, thermal non-uniformity of extracellular environments inside straws should be considered.

In this study, we developed a simple while effective approach, wrapping traditional French-type straw with medical gauze, to greatly enhance convective heat transfer during cooling by suppress film boiling. Furthermore, we established a coupled heat transfer model for cooling and warming of cell suspensions to investigate the inherent thermodynamic mechanism in this approach. The simulations indicated that wrapping straws with medical gauze would increase cell survival subject to vitrification preservation. To verify the prediction, we conducted experiments using human umbilical vein endothelial cells (HUVECs) and measured cell membrane integrity after cryopreservation. Finally, the simulative and experimental results were compared and analyzed.

2. Materials and methods

2.1. Modeling of the non-equilibrium crystallizing process

To predict the non-equilibrium crystallizing process, the following equation is used [16]:

$$\frac{d\chi}{dt} = k_a \chi^{2/3}(1-\chi)(T_m - T)e^{-Q/RT} \tag{1}$$

where χ is degree of ice crystallization ($0 < \chi < 1$), t is time, k_a is a characteristic constant, T_m is final temperature of the freezing process, Q is activation energy, and R is gas constant. Corresponding parameters for the model used in this study can be found in a previous research [18].

2.2. Modeling of the heat transfer process

Considering the primary mode of heat transfer in the straw is conduction rather than convection, we adopted the following energy equation to depict the temperature distribution inside the straws [19]:

$$\rho c \frac{\partial T}{\partial t} = k \frac{1}{r} \frac{\partial}{\partial r}\left(r \frac{\partial T}{\partial r}\right) + \rho L \frac{\partial \chi}{\partial t} \tag{2}$$

where T is temperature, r is radial coordinate starting from the center of the straw, ρ is density, c is specific heat capacity, k is thermal conductivity, and L is latent heat. To our knowledge, experimental data for thermal properties of supercooled and vitrified water are largely unknown at this time. Therefore, we adopted an approach depicted in [20] to calculate thermal properties.

The convective boundary condition is applied to the straw's wall:

$$k \frac{\partial T}{\partial r} = h(T_\infty - T) \tag{3}$$

where h refers to the convective heat transfer coefficient and T_∞ is the temperature of LN_2. The values of h remain largely unknown and are dependent on many factors including the constitutions of solutions in the straw, the material of the straw's wall as well as its roughness [21]. Thus in the current study the values of h were determined by fitting experimental temperature profiles.

Due to the fact that the heat transfer equation (Eq. (1)) is a nonlinear second order partial equation that is highly coupled with that of crystallization (Eq. (2)), it is difficult (if not impossible) to obtain analytical solutions of T. In this study, the values of T and χ were calculated simultaneously using numerical methods whereas temperature variations were computed using the method of lines (MOL) [22].

2.3. Modeling of probability of intracellular ice formation

As mentioned before, IIF is the main cause for all cryoinjuries. Thus it is highly correlated with cell viability after cryopreservation. To evaluate the efficiency of vitrification cryopreservation, we considered the probability of IIF (PIF) as an approximate evaluation of the final cell survival rate after cryopreservation. PIF can be estimated using the model as follows [23–25]:

$$P_{IIF} = P_{IIF}^{SCN} + \left(1 - P_{IIF}^{SCN}\right) P_{IIF}^{VCN} \tag{4}$$

$$P_{IIF}^{SCN} = 1 - \exp\left[-\int_0^t A_c I_{SCN} dt\right] \tag{5}$$

$$P_{IIF}^{VCN} = 1 - \exp\left[-\int_0^t V_c I_{VCN} dt\right] \tag{6}$$

where t is time, A_c and V_c are cell surface area and volume, respectively, and I is nucleation rate of that can be computed as follows: