Cryobiology 70 (2015) 32–37

Contents lists available at ScienceDirect

Cryobiology

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

Numerical investigation into thermal effects of pre-cooling zone in vitrification-based cryopreservation process ☆

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ARTICLE INFO

Article history:
Received 9 July 2014
Accepted 26 November 2014
Available online 4 December 2014

Keywords:
Ultra-fast cryopreservation
Vitrification
Ice crystal formation
Pre-cooling
Convective–conductive heat transfer

ABSTRACT

Most studies on ultra-fast cryopreservation assume an immediate placement of the cryopreservation tube in the liquid nitrogen tank. However, in practice, before the tube is placed into the liquid nitrogen, it passes through a space containing gaseous nitrogen (pre-cooling zone) formed via the evaporation of the bulk liquid nitrogen. Comparing with ultra-fast cryopreservation, the cooling rate is insufficiently high during the falling transition to vitrify the liquid. As the tube passes through this region, its temperature may fall to the temperature required for the formation of ice crystals, and thus cell damage may occur. Consequently, in optimizing the cryopreservation process, the effects of this transition region should be properly understood. Accordingly, the present study utilizes a thermal model to investigate the temperature variation in the tube as it falls through the pre-cooling region. The simulation results show that the cooling rate within the tube increases with an increasing tube velocity. Furthermore, the results reveal that the cooling rate at the front end of the tube is higher than that at any other position of the tube. Thus, to prevent the formation of ice crystals, the material used to seal the front end of the tube should have a low thermal conductivity. In addition, a streamlined design of the front end of the tube is advised. Finally, the cooling rate within the tube depends on the tube material as well as the falling speed. The height of the pre-cooling zone needs to be carefully designed based on the tube material and falling speed, thus the ice crystal formation can be prevented.

Introduction

The preservation of biological cells and tissues is essential in such fields as artificial insemination, visceral organ transplantation, and virus research. Such biological samples are generally preserved by means of vitrification. However, during the cooling process, the liquid contents within the sample may form ice crystals, which then lead to cellular damage. Two main methods have been developed to avoid the formation of ice crystals, namely slow programmable freezing and vitrification [8,6,10,11,15,17,12,21]. Slow programmable freezing comprises a set of well-established techniques which freeze biological samples by means of programmable sequences. However, these sequences are usually lengthy and require the use of expensive instrumentation [1]. Accordingly, vitrification, an ultra-fast cryopreservation process in which the sample is cryopreserved within a glass-like matrix through an ultra-fast cooling process has attracted increasing interest in recent years.

The success of the vitrification process relies principally on achieving an ultra-fast cooling rate and determining an appropriate concentration of the cryoprotective agent used to prevent ice formation during the cooling process [2–4,7]. Although highly concentrated vitrification solution lowers the ice formation temperature, it is toxic and might cause damage to the cell. Consequently, reducing the cryoprotectants (CPAs) concentration by enhancing the cooling rate is an important research focus in the field of vitrification. Many researches such as replacing the tube materials or cryogens [18,14] have been conducted to increase the cooling rate. However, the melting point of the sample increased as the concentration of CPAs decreased. This may increase the risk of ice formation during the tube placement transition phase of the vitrification process.

In addition, previous studies on the vitrification process have assumed that the cryopreservation tube is placed directly into

☆ *Statement of funding:* This research was funded by the National Chung Cheng University and National Pingtung University.

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http://dx.doi.org/10.1016/j.cryobiol.2014.11.003
0011-2240/

the liquid nitrogen tank [3,4,9,19,13,20]. However, in practice, as the cryopreservation tube approaches the liquid nitrogen, it passes through a region of gaseous nitrogen formed via the evaporation of the bulk nitrogen liquid in the tank. As it passes through this region, a significant reduction in the temperature occurs. In extreme cases, this temperature reduction may be sufficient to prompt the formation of ice crystals, which then damage the cells and tissues of the biological sample. Thus, in optimizing the vitrification process, the effects of this pre-cooling zone must be taken into account.

Accordingly, the present study assumes the tube is filled with DI water (i.e., no CPA was added), and performs a series of numerical simulations to investigate the flow and temperature fields around the tube as it falls through the pre-cooling region. The simulations are based on a thermal model constructed on the assumptions of thermal convection heat transfer in the region surrounding the tube and thermal conduction heat transfer within the tube. The simulations focus specifically on the effects of the tube velocity and tube material in determining the cooling rate of the liquid within the tube.

The remainder of this paper is organized as follows. Section 'Modeling and analysis' introduces the thermal model used in the simulations and describes the associated assumptions. Section 'Results and discussion' presents and discusses the simulation results. Finally, Section 'Conclusion' provides some brief concluding remarks.

Modeling and analysis

Fig. 1 presents a schematic illustration of ultra-cooling system. As shown, the cryopreservation tube, with a length a and outer diameter b, is held by means of a mechanical clip. The tank has a diameter c, and is filled with liquid nitrogen to a height d below the mouth of the tank. The cryopreservation tube was assumed to have a length (a) of 20 mm, an outer diameter (b) of 0.2 mm, and a wall thickness of 0.01 mm. Moreover, the diameter (c) of the tank was specified as 478 mm, while the height of gaseous nitrogen (d) was set as 480 mm.

In conducting the analysis, the cryopreservation tube was assumed to be filled with de-ionized (DI) water (i.e., no CPAs was added) at room temperature (300 K). Since the size of the cryopreservation tube is much smaller than the tank (i.e., $b/c \ll 1$, $a/d \ll 1$), the tank diameter (c) and the height of gaseous nitrogen (d) was assumed to be infinite. Five different drop speeds of the tube were considered, namely 1, 2, 3, 4 and 5 m/s. The drop speed of the tube was assumed to be constant. To simplify the calculations, the temperature within the pre-cooling zone was also assumed to be constant (125 K). Moreover, some studies [16,5] indicate that water has a tendency to freeze at a temperature below the melting point (generally −2 to −5 °C [5]. Thus, the melting point of water was assumed to be −4 °C (269 K). The process of phase change was not considered. The simulation was terminated when the temperature of any point inside the tube reaches 269 K.

From the thermal transfer point of view, the system can be further simplified as a fixed tube with gaseous nitrogen flowing over its surface at a certain relative speed. Based on this system model, two different heat transfer mechanisms apply, namely thermal convection on the outer surface of the tube, and thermal conduction within the tube. Thus, the temperature fields within the two regions of the problem domain, i.e., outside of the tube (M) and within the tube (N), were computed separately, as shown in Fig. 2. As described above, the operating space (c and d) was assumed to be infinite, and the relative velocity between the tube and the gaseous nitrogen was set as $v = 1$–5 m/s. The convective heat transfer within area M of the problem domain was modeled as follows:

Continuity equation:

$$\frac{1}{r}\frac{\partial\left(\rho_f r v_r\right)}{\partial r}+\frac{\partial\left(\rho_f v_z\right)}{\partial z}=0. \tag{1}$$

Momentum equation in r-direction:

$$\rho_f\left[v_r\frac{\partial(v_r)}{\partial r}+v_z\frac{\partial(v_r)}{\partial z}\right]=-\frac{\partial p}{\partial r}+\mu_f\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial v_r}{\partial r}\right)+\frac{\partial^2 v_r}{\partial z^2}-\frac{v_r}{r^2}\right]. \tag{2}$$

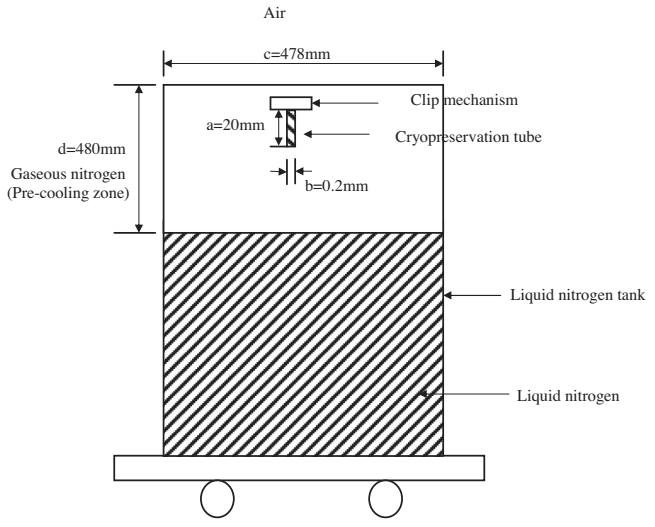

Fig. 1. Schematic illustration of ultra-fast cooling system.

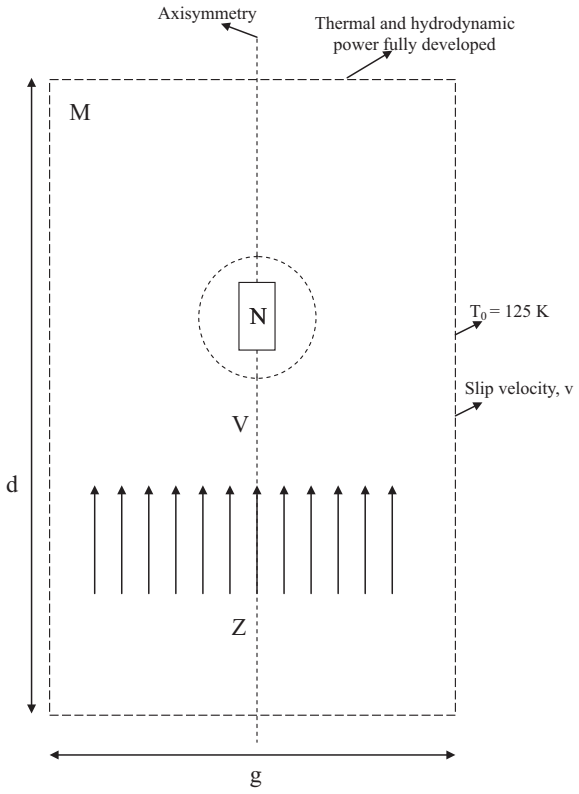

Fig. 2. Simulation model.