Numerical Analysis to Determine the Performance of Different Oocyte Vitrification Devices for Cryopreservation

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Abstract

BACKGROUND: Vitrification is widely used for cryopreservation of oocytes. **OBJECTIVE:** The present study is to compare the cooling rate in various oocyte vitrification systems (Micro-droplet, Open Pulled Straws, Quartz Micro-Capillary, Cryotop) when immersed in liquid nitrogen, and to explore the effects of different process parameters on the cooling rate in Cryotop via numerical analysis. **METHODS:** The present study has built the theoretical models of four vitrification systems, and performed numerical analysis to predict the cooling rates. **RESULT:** The numerical analysis shows that the average cooling rate of the cryotop system was higher than those of other three systems between 298K and 100K. In addition, the effects of other process parameters on the cooling rate with the cryotop system were also investigated, including the thickness of the carrier, the volume of cryoprotectant agent, the temperature of cold source as well as the heat transfer coefficient, when plunging into liquid nitrogen.

Keywords: Numerical analysis, vitrification, oocyte, cooling rate.

INTRODUCTION

Cryopreservation of oocytes plays an important role in assisted human reproduction and animal breeding programs. Oocytes are often damaged directly or indirectly by ice formation upon freezing. Vitrification is the process that solidification occurs without the formation of ice crystals, and is currently used for cryopreservation of oocytes (1, 2). Vitrification could be achieved by either rapid cooling rates or high concentrated CPA. However, the cooling rates that can be obtained in practice are limited and the high concentrations of CPA are toxic for cells. Therefore, vitrification protocol is a deliberate balance between the highest possible cooling rates and the highest CPA concentrations that are not toxic to cells. In order to reduce CPA concentration and avoid the related toxicity problem, it is important to obtain high cooling rates (3, 4).

Various approaches have been developed to achieve high cooling rate, including the open-pulled straws (OPS), the electron microscopy copper grid, and the Cryoloop and Cryotop, which are reviewed recently (5). Since the probe used to measure temperature may well contribute to a distortion of small biologic samples, the measurement of accurate cooling rates are difficult for all of approaches. Thus, the comparison in the efficacy among available vitrification systems can only be based on oocyte survival and embryo developmental rates after warming, which are time-consuming and often lack of consistency. This problem motivates us to perform a numerical analysis, and to evaluate the cooling rates in different vitrification systems.

It is reported that Cryotop has made inspiring progress in embryology. About 50 babies were born worldwide after oocyte vitrification with Cryotop (6), more than any other cryopreservation technologies for this purpose. However, from the perspective of heat transfer performance, the design of Cryotop system could be technically improved further. Several process parameters, such as carrier

thickness, CPA volume, temperature of cold source and heat transfer coefficient, could affect the cooling rate in Cryotop. Numerical analysis will provide some useful insight into designing experimental devices that enable cooling biologic material at high rates.

For these reasons, the objectives of the present study were (1) to compare the cooling rate in various oocyte vitrification systems (Micro-droplet, Open Pulled Straws, Quartz Micro-Capillary, Cryotop) when immersed in liquid nitrogen; (2) to explore the effects of different process parameters on the cooling rate in Cryotop via numerical analysis.

MATERIALS AND METHODS

Physical Models

(a) The Micro-droplet method refers to dropping the sample droplets without any container directly into liquid nitrogen. It is easy to operate, but difficult to control the size of droplet. This model can be described as two concentric spheres: the oocyte and its surrounding CPA solution as shown in Figure 1a. The surface exposed to liquid nitrogen is the outer surface of CPA solution.

(b) The OPS consists of a traditional French type plastic straw. Straws were warmed and pulled by hand, then cut at the thinnest point with a razor blade. The oocytes were loaded by capillary effect and plugged into liquid nitrogen. The OPS model was composed of two concentric cylinders: the internal cylinder represents CPA solution and the oocyte inside, the external cylindrical tube represents OPS (i.e., the PVC material), the oocyte is positioned in the center of straw tip as shown in Figure 2b. The area exposed to liquid nitrogen includes the outer surface of the OPS and the opening bottom of cylinder.

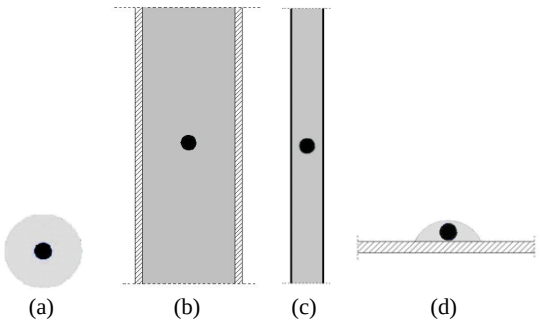

Figure 1. Geometry of four physical models used for numerical simulation: (a) the Micro-droplet model, (b) the OPS model, (c) the QMC model, and (d) the Cryotop model. The black dots represent the oocyte, whereas the gray domain stands for CPA solution. The domains with diagonal stripes in (b) and (d) stand for PVC and black line in (c) stands for quartz.

(c) QMC is an improved method of OPS. Compared with OPS, the radius of quartz capillary tube decreased, and the heat conductivity of straw increased. The QMC model is similar to OPS model, which is shown in Figure 1c. Since the length-diameter ratio of quartz capillary is sufficiently large, the axial heat flow contribution is negligible and the exposing part to liquid nitrogen is only the outer wall of QMC.

(d) Cryotop, first proposed by Kuwayama in 2005, is made of a narrow strip of thin plastic film attached to a hard plastic handle. The oocytes were loaded onto the plastic film by the glass capillary with the diameter of approximately 140 μm (equal to the diameter of the oocyte) under stereomicroscope, then nearly all solution was removed, leaving oocytes covered by a thin layer of CPA solution (<0.1 μl). Finally the Cryotop with oocytes is plunged into liquid nitrogen (3). The Cryotop model can be simplified as a ball on a panel and the ball was covered by a spherical cap–shaped CPA environment as shown in Figure 1d. The exposing parts to liquid nitrogen include the surface of plastic film and spherical cap–shaped CPA environment.

The dimensional parameters of four models are listed in Table 1. To obtain a numerical solution, the diameter of oocyte was set at 0.1mm (100 μm) and the height of spherical cap-shaped CPA environment on the carrier of Cryotop was set at 0.12mm (120 μm).

Thermophysical Parameters

It was assumed that vitrification avoided ice crystal formation during cooling. The oocyte was considered homogeneous and its thermal physical parameters were approximated with properties of water. Thermal properties of CPA solution were assumed equivalent to properties of 40% ethylene glycol since high concentration of ethylene glycol was often used as CPA. All the thermophysical properties were considered constant, based on the thermal property at room temperature (3, 4, 7, 8, 9, 10).

The material of OPS and Cryotop are PVC, and the material of QMC is quartz. The thermal properties of materials used in the numerical simulation are shown in Table 2.

Numerical analysis

There was no internal heat source in computational models. The boundary condition of the third kind was used between different surfaces. The external heat transfer coefficient in four models were assumed to be the same, where h =2000 W/m^2·K. The thermal contact between each component was considered ideal, without thermal contact resistance. The heat conduction partial differential equation was represented as follows (11):

$$\frac{\partial T}{\partial t} = a\nabla \cdot \nabla T$$

The boundary condition of the third kind：

$$\lambda \frac{\partial T}{\partial n} = h(T - T_e)$$

where $a = \lambda / \rho \cdot c_p$ refers to the thermal diffusion coefficient, ρ is density, c_p is specific heat capacity of the material, T is transient temperature and t is time, T_e is cold source temperature, n is normal direction of heat transfer surface, h is external heat transfer coefficient, and λ is thermal conductivity.

Table 1. Dimensions of Main items in simulation

Vitrification Method	Outer Diameter (mm)	Inner Diameter (mm)	Length (mm)	Width (mm)	Thickness (mm)
Microdroplet	0.70	0.10			
OPS	0.95	0.80	3		
QMC	0.20	0.18	3		
Cryotop			3	0.4	0.1

Table 2. Thermophysical Parameters used in simulation

	Density kg/m^3	Specific heat $J/kg \cdot K$	Heat conductivity $W/m \cdot K$
ethylene glycol	1082.2	4430	0.3
Cell	998.2	4182	0.6
PVC	1200	1500	0.2
Quartz	2430	891.38	1.38

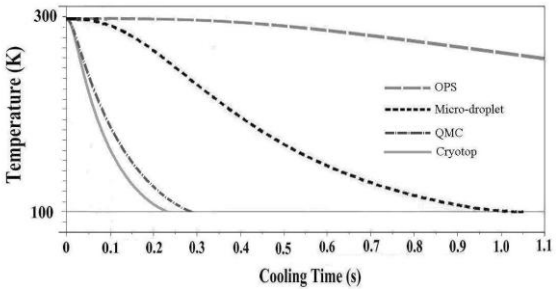

Figure 2. The predicted temperature at the warmest point of the oocyte in four different vitrification systems immersed in liquid nitrogen for an external heat transfer coefficient h = 2000 W/m^2·K.

Initial temperature condition: T_0 =298K.

The grid was developed by the grid generator program Gambit. All grid cells generated for the computational domain defined were hexahedron, except that the carrier of Cryotop was tetrahedron. The interval size of grid was 0.01. The commercial code Fluent 6.2 was used for Numerical Analysis. A segregated implicit unsteady solver was employed to solve the discretized differential equations. The first-order upwind scheme was adopted to solve energy equation. The unsteady time step size was 0.01 seconds and the max iteration per time step was 20 times in calculation.

RESULTS

Comparing cooling rates in four vitrification systems

The temperature and the cooling rate are space dependent inside the cell. For the purpose of simplification, the cooling rate was evaluated at the warmest point of the cell. The vitrification process was assumed to have completed when the temperature of the warmest point in the cell reaches 100K (9, 10). The average cooling rate was defined as follows:

$$\bar{\upsilon}_c = (T_0 - T_z)/t = \Delta T/t$$

where $\bar{\upsilon}_c$ is the average cooling rate of cells, T_0 is initial temperature(298K), T_z is end temperature(100K), t is the time needed to reduce the temperature of the warmest point in cells from 298K to 100K.

Predicted temperature changes (between 298K and 100K) at the warmest point of the cell in the different vitrification systems immersed in liquid nitrogen are compared in Figure 2. The time t needed in microdroplet, OPS, QMC, Cryotop was 1.05 s, 2.25 s, 0.275 s, 0.221 s, respectively. Correspondingly the average cooling rate in four methods was 7,143, 3,334, 27,272 and 53,756 K/min. Obviously, the average cooling rate in Cryotop was the fastest, while in OPS was the lowest one.

Effect of thicknesses of carrier on cooling rate

When initial temperature T_0 was at 298 K, the temperature of the cold source T_e was at 77 K, h was 2000 W/m^2·K, and the basal radius of spherical cap-shaped CPA was 0.12mm, the effect of thickness of PVC carrier on cooling rate in Cryotop was analyzed. The thickness of PVC carrier was set at 0.06mm, 0.08mm, 0.10mm and 0.12mm. The results showed the cooling time of each PVC carrier was 0.195, 0.212, 0.221 and 0.235 s, respectively, which corresponded to 60,923, 56,038, 53,756 and 50,553K/min (Figure 3a). when the thickness of PVC decreased from 0.12mm to 0.06mm, the cooling rate increased by 20.5%.

Effect of volume of CPA on cooling rate in Cryotop

When the thickness of carrier was set at 0.06mm, initial temperature T_o was at 298K, the cold source temperature Te was at 77K, h was 2000 W/m^2·K , the effect of basal radius of spherical cap-shaped CPA on cooling rate in Cryotop was analyzed. Suppose the basal radius of the spherical cap was 0.12mm, 0.14mm, 0.16mm, 0.18mm, 0.20mm, the results showed the cooling time was 0.195s, 0.235s, 0.275s, 0.32s, 0.361s, respectively. The cooling rate was correspondingly 60,923 K/min, 50,553K/min, 43,200K/min, 37,125K/min and 32,909K/min (Figure 3b). When the basal radius of the CPA decreased from 0.20mm to 0.12mm, the cooling rate increased by 85.1%.

Effect of the temperature of cold source on cooling rate in Cryotop

When the thickness of carrier was 0.06mm, the basal radius of spherical cap was 0.12mm, initial temperature T_0 was at 298K, heat transfer coefficient h was 2000 W/m^2·K, the effect of

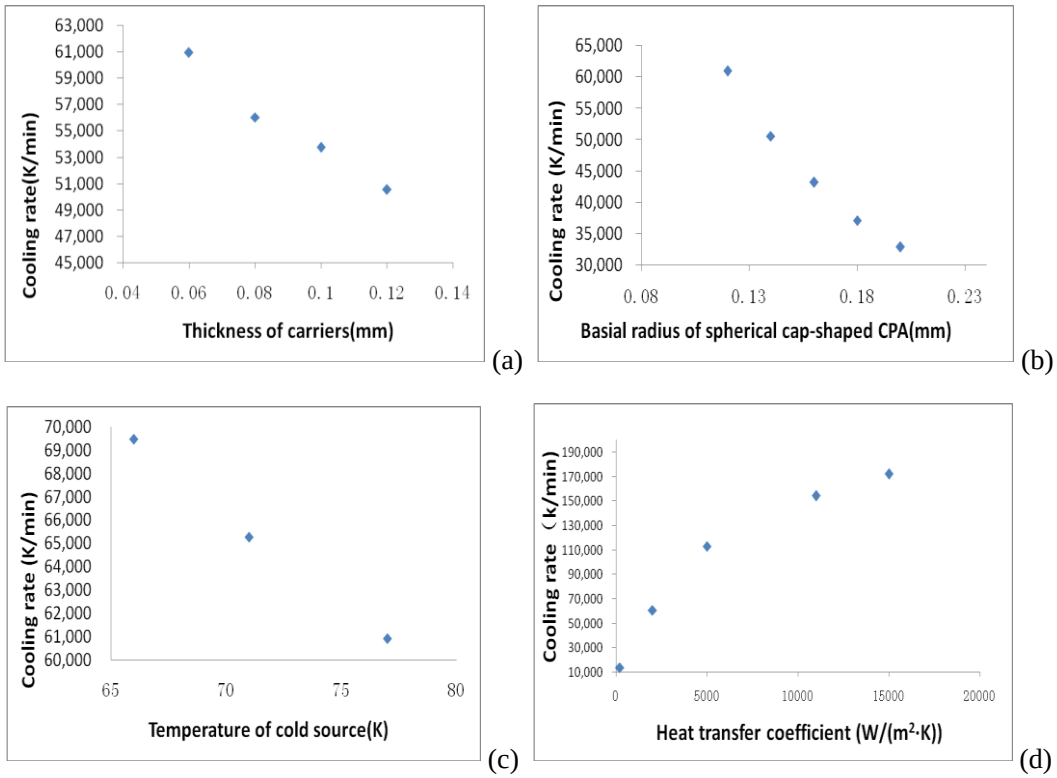

Figure 3. Effect of thicknesses of carrier (a), basal radius of spherical cap-shaped CPA (b), temperature of cold source (c), and heat transfer coefficient (d) on the cooling rate in Cryotop.

different cold source temperatures on cooling rate in Cryotop was studied. When the cold source temperature was at 77K, 71K, and 66K, the cooling time was 0.195s, 0.182s, and 0.171s, thus the corresponding cooling rate was 60,923 K/min, 65,275 K/min and 69,474 K/min. Effect of different cold source temperatures on the cooling rate in the Cryotop system is shown in Figure 3c. When the temperature of cold source decreased from 77K to 66K, the cooling rate increased by 14.0%.

Effect of heat transfer coefficient on cooling rate

When the thickness of carrier was 0.06mm, the basal radius of spherical cap was 0.12mm, initial temperature T_0 was at 298K, and the cold source temperature T_e was at 66K, the effect of different heat transfer coefficient on cooling rate in Cryotop was studied. When h was 200 $W/m^2 \cdot K$, 2000$W/m^2 \cdot K$, 5000$W/m^2 \cdot K$, 11000$W/m^2 \cdot K$, 15000$W/m^2 \cdot K$, the results showed the cooling time for each heat transfer coefficient was 0.85s, 0.195s, 0.105s, 0.077s, 0.069s. The corresponding cooling rate was 13,976 K/min, 60,923K/min, 113,143K/min, 154,286K/min and 172,174K/min, respectively. The results were shown in Figure 3d. As the heat transfer coefficient increased from 200 $W/m^2 \cdot K$ to 15000 $W/m^2 \cdot K$, the cooling rate increased by 11.3 times.

DISCUSSION

Sansinena et al.(9) have evaluated different oocyte vitrification systems (Cryoloop , Cryotop, Miniflex, and Open Pulled Straw) immersed in liquid nitrogen using numerical simulation to predict their cooling rates, the highest cooling rate was achieved in Cryoloop system. In their study, Cryoloop system was simplified as the solution with a minimum thickness of 20μm, the calculated cooling rate reached 180,000K/min. However, the diameter of oocyte is about 100μm, the cooling rate calculated in their study can not reflect the actual cooling rate of oocyte during vitrification. For their simulation in Cryotop system, the volume of CPA is much larger than in actual process, so the cooling rate (37,500 K/ min) is lower than the real value. In fact, the volume of CPA in Cryotop system is tiny, when the basal radius of spherical cap-shaped CPA was set at 0.12mm (0.12mm in height and 0.004μl in volume), the cooling rate reached 53,756 K/min in this study.

The complex influence factors in the vitrification process include fluid dynamics, surface chemistry and heat transfer etc. This study evaluated the achievable cooling rate in Cryotop by using the numerical analysis. As the thickness of PVC carrier decreases, the heat resistance dramatically decreases, but the thickness of carrier could not be reduced infinitely due to intension of material. If the thickness of carrier in Cryotop is less than 0.06 mm, the carrier is too soft to support the oocytes. The volume of CPA is another key factor which influences the cooling rate. As too much CPA can increase the thermal capacity, the volume of CPA should be as small as possible in actual operation process. Obviously, reducing temperature of cold source can increase the cooling rate. 77K is the temperature of liquid nitrogen under standard atmospheric pressure and the lower temperature can be attained by reducing the pressure. Actually, when the temperature of liquid nitrogen is decreased, the slush nitrogen formed, the nitrogen gas from liquid nitrogen can be reduced or even avoided, the heat transfer coefficient between CPA and cold source will hereby increase. The cooling rate increases with the increase of heat transfer coefficient (h), but when h surpasses some value (about 10000 $W/m^2 \cdot K$), the cooling rate will not increase strikingly. The main factors affecting h include the cold source temperature, the physical state of cold source and the relative speed of vitrification device and cold source. In actual process, h can be increased by accelerating the speed of insertion and changing the state of liquid nitrogen into slush nitrogen.

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