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Investigations on the heat transport capability of a cryogenic oscillating heat pipe and its application in achieving ultra-fast cooling rates for cell vitrification cryopreservation*

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Abstract

Theoretically, direct vitrification of cell suspensions with relatively low concentrations (~1 M) of permeating cryoprotective agents (CPA) is suitable for cryopreservation of almost all cell types and can be accomplished by ultra-fast cooling rates that are on the order of 10^{6-7} K/min. However, the methods and devices currently available for cell cryopreservation cannot achieve such high cooling rates. In this study, we constructed a novel cryogenic oscillating heat pipe (COHP) using liquid nitrogen as its working fluid and investigated its heat transport capability to assess its application for achieving ultra-fast cooling rates for cell cryopreservation. The experimental results showed that the apparent heat transfer coefficient of the COHP can reach 2×10^5 W/m²·K, which is two orders of the magnitude higher than traditional heat pipes. Theoretical analyzes showed that the average local heat transfer coefficient in the thin film evaporation region of the COHP can reach 1.2×10^6 W/m²·K, which is approximately 10^3 times higher than that achievable with standard pool-boiling approaches. Based on these results, a novel device design applying the COHP and microfabrication techniques is proposed and its efficiency for cell vitrification is demonstrated through numerical simulation. The estimated average cooling rates achieved through this approach is 10^{6-7} K/min, which is much faster than the currently available methods and sufficient for achieving vitrification with relatively low concentrations of CPA.

Keywords

Cryopreservation; Vitrification; Oscillating heat pipe; Microfabrication

Introduction

During cell cryopreservation, intracellular ice formation (IIF) is generally believed to be fatal to cells due to mechanical damage of the cellular ultrastructure either by the direct action or by the associated volumetric expansion [7,16,24,27]. Cryoprotectant agents (CPAs) are widely used to reduce the chance of IIF and achieve vitrification of intracellular solutions. Many CPAs are permeable to cells (permeating CPAs), with ethylene glycol (EG), glycerol and MeSO₄ (DMSO) as examples. For typical vitrification approaches, relatively high concentrations of permeating CPAs (usually 4–7 M) are used in combination with cooling rates higher than the critical cooling rates (the minimum cooling rate required to achieve vitrification) of the

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solutions, which are 10^3 – 4 K/min [3,21,22]. Currently available cooling methods, such as the open pulled straw (OPS), micro-droplet, cryo-loop, and cold surface solidification methods, can achieve these cooling rates [2,18–20,25]. However, the high concentrations of CPAs used in these procedures often have severe toxic and/or osmotic effects on cells, and the procedures to add/remove these high concentrations of CPAs into/from cells are complicated [7,17,26]. The sample volume used in these methods is very limited (~ 1 μ l) and that further constrains their applications [2,18–20,25].

Vitrification of cell suspensions with relatively low concentrations of permeating CPAs (~ 1 M) would be suitable for almost all cell types, and it is a potentially promising approach for universal cell cryopreservation. Theoretically, this approach can be achieved by employing an ultra-fast cooling rate. For example, based on the theoretical predictions made by the extrapolation of dynamic models established by Boutron and Ren [3,22], cooling rates as high as 10^6 K/min are required to vitrify a 1 M glycerol aqueous solution. Currently, the methods and devices applied in cell cryopreservation cannot achieve such cooling rates due to their technological limitations. This is especially true for the pool-boiling methods inherent in the typical OPS, cryo-loop, and micro-droplet methods [2,18–20,25]. The main disadvantage of pool-boiling methods is that direct plunging the sample into liquid nitrogen results in strong nitrogen vaporization around the sample surface, and this forms a “vapor coat” which acts as a heat-insulation layer. As a result, the heat transfer coefficient between the sample surface and liquid nitrogen is quite limited ($< 1 \times 10^3$ W/m²·K) due to poor thermal conduction of the vapor in the “vapor coat” [9,19]. This limitation prevents a further increase of the cooling rates. In addition, these pool-boiling methods, as well as the cold surface solidification method, produce an “open system” in which the samples are vulnerable to contamination when coming in to direct contact with cooling agents (liquid nitrogen, cold metal surface, *etc.*). Furthermore, the geometry of the samples used in these methods is generally semi-spherical and limits the ratio of the sample surface versus its volume ($R_{S/V}$). As a result, the heat conduction inside the samples significantly lowers the sample cooling rates and generates non-uniform cooling (the cooling rate on the surface of the sample is several orders of magnitude higher than the sample center). Therefore, a completely new approach is needed to achieve the required cooling rate to vitrify solutions with low CPA concentrations and overcome the shortcomings of current cooling methods.

A common approach to increasing the value of the heat transfer coefficient between the sample and coolant is the use of heat pipes, which are a package of pipes containing two-phase flows that continuously remove the heat released from the sample through phase change (evaporation and condensation) [1]. Among the different types of heat pipes, oscillation heat pipes (OHPs) [1,13,15,29] demonstrate several salient features that do not exist in traditional heat pipes. The most important of them is that it provides an ultra-high heat transfer coefficient ($> 10^6$ W/m²·K) between the sample and the working fluid mainly through thin film evaporation and oscillation motion of the working fluid [5,10–12]. If OHPs can be applied to increase the heat transfer coefficient between samples and liquid nitrogen during cell cooling procedures, the cooling rates are expected to be significantly increased. Our group recently proposed the idea of the application of a cryogenic OHP (COHP, an OHP using cryogenic working fluid, such as liquid nitrogen) and numerically investigated the vitrification tendency of the sample in an ultra-thin straw [9]. However, the values of the heat transfer coefficients used in the simulation were estimated from results of room temperature OHPs with water as their working fluid, rather than on the basis of the performance of a COHP, which significantly affects the accuracy of the simulation. In addition, the design using the so-called “ultra-thin straw” that we proposed in [9] is only a conceptual device, and considering the small dimension of walls and difficulties to perform the proposed operations, the design should be improved.

In this study to investigate the heat transport capabilities of an actual COHP, we constructed an OHP using liquid nitrogen as its working fluid and measured its apparent heat transfer coefficient. To the best of our knowledge, this is the first report of a working COHP. The local heat transfer coefficient due to thin film evaporation in the COHP was also obtained through theoretical investigation. To maximize the value of $R_{S/V}$, we designed a device to spread a 50–100 μl cell suspension into a thin layer of 50–100 μm in thickness in a sample holder made by microfabrication. Based on the theoretical investigation of the thin film evaporation in a COHP, we also numerically simulated the process of using COHP to cool the thin layer sample and demonstrated the efficiency of this device design.

Experimental method and theoretical analyzes

A copper tube (2 mm in outer diameter, 1 mm in inner diameter) with a total length of 2 m was meandered into 6 turns (15 cm length per turn) to form heat pipe turns, with an open liquid charging inlet. Two pairs of copper plates covered its two ends with several thermocouples inserted, and a flat electric heater controlled through variable transformers connected to a multimeter was sandwiched on the evaporator surface, as shown in Fig. 1. The evaporator section was sealed in a polyethylene cage to minimize the heat loss from the outer surface of the evaporator, so that the major heat flux from the electric heater is considered to be absorbed by the COHP. Then the whole device was plunged into a liquid nitrogen pool, with the open charging inlet 1–2 cm underneath the liquid nitrogen surface. Due to the lowering of the temperature of the COHP, the air inside is compressed and results in the partially filling of the COHP with liquid nitrogen. After the filling process was finished, the open inlet was then sealed by soldering. According to [13], the inner diameter (D) of an OHP should satisfy:

$$D \leq 1.84 \sqrt{\frac{\sigma}{g(\rho_l - \rho_v)}} = 1.9 \text{ (mm)}; \quad (1)$$

where σ is the surface tension, ρ_v and ρ_l are the vapor and liquid density, respectively, to hold nitrogen vapor bubbles and liquid slugs. Obviously, this condition is satisfied, for the value of D of this COHP is only 1.0 mm. Therefore, the liquid nitrogen and the remaining air inside the COHP will form liquid slugs and bubbles automatically (since over 80% of air is nitrogen, the bubbles are considered as nitrogen bubbles). The condenser temperature of the COHP remains at $\sim -196^\circ\text{C}$, the liquid nitrogen saturation temperature at ambient pressure. The temperature of its evaporator can be controlled by the power input of the electric heater. To test its performance, the apparent heat transfer coefficient h_a was calculated as:

$$h_a = \frac{\dot{q}}{S \Delta T}, \quad (\Delta T = T_e - T_c); \quad (2)$$

where $\dot{q}$ is the value of the power input measured through the multimeter, T_e is the average temperature of the evaporator and T_c is the average temperature of the condenser, and they are measured by the embedded thermocouples; S is the contact area between the electric heater and the COHP, and calculated as the product of the COHP inner diameter (1.0 mm) and the length of the COHP sections that contact with the electric heater (measured as 12.0 cm).

To apply the COHP to cell cooling processes, the local heat transfer coefficient (h_{local}) in its thin film evaporation region, is a critical parameter to calculate the cell cooling rate [9]. However, it is difficult to measure its value directly through experiments, due to the small dimension of the pipes. Therefore, we established a physical model for the thin film evaporation inside the pipes to obtain the value of h_{local} . First, we divide the contact region between the

nitrogen bubble and the liquid nitrogen slug into three sections near the wall of the pipe, as shown in Fig. 2: section I, the non-evaporating region, where the disjoining pressure is dominant, the thickness of the liquid nitrogen is so thin that vaporization in this region is negligible [5,8,11]; section II, the thin film evaporation region, where the temperature of the liquid film surface is controlled by both the disjoining pressure and surface tension, and strong evaporation occurs [5,8,11,14]; section III, the bulk liquid region, where a weak evaporation presents. Then, we apply Clausius–Clapeyron equation in the thin film evaporation region:

$$\left(\frac{dp}{dT}\right)_{\text{sat}} = \frac{h_{fg}}{T_{\text{sat}}[1/\rho_v - 1/\rho_l]}, \quad (3)$$

where p is the pressure, h_{fg} is the latent heat of vaporization, and the subscript sat denotes the interface between the liquid and vapor at the saturation state. Integrating Eq. (3) from the vapor temperature T_v to the interfacial temperature T_{lv} and neglecting the influence of the term $1/\rho_l$ (because $\rho_l/\rho_v \approx 650$, $1/\rho_v \gg 1/\rho_l$), Eq. (3) can be simplified as:

$$T_{lv} = T_v \left[1 + \frac{\Delta p}{\rho_v h_{fg}} \right]; \quad (4)$$

where Δp is the pressure difference between the liquid and vapor. Considering the disjoining pressure and surface tension,

$$\Delta p = \frac{\sigma}{r} + p_d, \quad (5)$$

where r is the radius of the meniscus, and p_d is the disjoining pressure [5] and can be calculated as:

$$p_d = \frac{A}{\delta^3}; \quad (6)$$

where A is the modified Hamaker constant [5] and δ is the thickness of the thin film. From the geometry of the meniscus as shown in Fig. 2, it is obvious that:

$$\delta = r - \sqrt{r^2 - x^2} + \delta_0; \quad (7)$$

where δ_0 is the thickness of the starting pointing of the meniscus, as well as the ending point of the non-evaporation region; x is the coordinate in the horizontal direction in the meniscus region, and when $x = 0$, $\delta = \delta_0$. Since the heat flux from the wall to liquid nitrogen is zero at $\delta = \delta_0$, the wall temperature T_w is equal to the interface temperature T_{lv} . Combining Eqs. (4)–(6), δ_0 can be calculated as:

$$\delta_0 = \left[\frac{A}{\left(\frac{T_w - T_v}{T_v} \right) \rho_v h_{fg} - \frac{\sigma}{r}} \right]^{1/3}. \quad (8)$$

Substituting the value of δ_0 into Eq. (7), the thickness of the meniscus region as a function of x is obtained. Meanwhile, the length of the meniscus is much larger than its thickness, so the heat transfer process from the COHP wall to the interface can be modeled one dimensionally:

$$q'' = k \frac{T_w - T_{lv}}{\delta}; \quad (9)$$

where q'' is the heat flux, and k is the thermal conductivity of liquid nitrogen. Then the local heat transfer coefficient in the thin film region is:

$$h_{\text{local}} = \frac{q''}{T_w - T_{lv}} = \frac{k}{\delta}, \text{ or} \\ h_{\text{local}} = k \cdot \left[r - \sqrt{r^2 - x^2} + \left(A / \left(\frac{T_w - T_v}{T_v} \right) \rho_v h_{fg} - \sigma / r \right)^{1/3} \right]^{-1}. \quad (10)$$

Integrating h_{local} along x (x ranges from 0 to 10 μm), the average value of the local heat transfer coefficient of the thin film region is obtained as:

$$\bar{h} = \int_0^x h_{\text{local}}(x) dx / x. \quad (11)$$

To apply the COHP for a cell cooling/cryopreservation process and maximize the value of $R_{S/V}$, we propose a sample holder design as shown in Fig. 3. The upper part of the sample holder is a flat container (100 or 50 μm in depth, 3.5×3.5 cm in width and length), into which a drop of cell suspension (~ 100 μl for a 100 μm deep container or 50 μl for a 50 μm deep container) will be spread by the cover and will fill the flat container to form a thin layer of the cell suspension. The lower part of the sample holder is the channels for liquid nitrogen flow from COHP. Fig. 4 is a cross-sectional view of the spatial relationship of the thin layer block of the cell suspension, container wall and liquid nitrogen passage channel (Z is the coordinate in the thickness direction of thin layer sample and the container wall; $Z = 0$ is the location where the container wall is in contact with the liquid nitrogen passage channel). Obviously, this design requires the container wall to be thin and made of a material with good thermal conducting properties in order to lower the thermal resistance between the sample and the liquid nitrogen flow. Since the thermal conductivity of silicon at cryogenic temperatures is very high (at -196 $^{\circ}\text{C}$, silicon thermal conductivity reaches ~ 1680 $\text{W/m}\cdot\text{K}$) and silicon is easy to process with chemical etching, the container will be made of silicon.

Fig. 5 demonstrates the proposed manufacturing process for the sample holder: (1) The flat cell container is carved on the top surface of a silicon wafer (the silicon wafer is 150 or 200 μm in thickness depending on the cell size: for cells with diameter less than 50 μm , the depth of the container is 50 and 150 μm wafer is used; for large cells, such as mammalian oocytes, the depth of the container is 100 and 200 μm wafer will be used) through microfabrication techniques (photolithography and chemical etching). (2) The container wall will form automatically after the flat container is produced (*e.g.* for a 200 μm silicon wafer, when the flat container is carved on the top surface as 100 μm in depth, then the container wall is 100 μm in thickness or equal to 200 μm minus 100 μm ; for a 150 μm silicon wafer, when the flat container is carved as 50 μm in depth, then the container wall is then also 100 μm in thickness); another Pyrex glass chip with grooves (30 grooves per chip; to simplify the figure, only 8 of them are shown), whose depth is 1 mm, is bound on the bottom surface of the container through anodic binding. Thus, the container wall will seal the hatches of these grooves to form the liquid nitrogen passage channels, and the wall of these channels will sustain the container wall to strengthen it.

The cooling process will be performed by injecting the liquid nitrogen flow into these passage channels with a “U”-shaped working section connected to a COHP, as shown in Fig. 6. The “U”-shaped working section has two layers of rectangular coolant passage channels: the upper and lower parts. The size of these channels is the same as the liquid nitrogen passage channels under the sample container. They also have common inlets and outlets that connect the COHP from its condenser and evaporator, as shown in Fig. 6, and each of the channel has 4 valves, named as V1,2, 3,4 (V1,2 are in the upper channels and V3,4 are in the lower channels). When there is no sample loaded, V1,2 are closed and V3,4 are open, so the upper channels are closed and the lower channels are connected with the COHP. After the sample container is loaded as shown in Fig. 7, the upper channels will be connected to the passage channels beneath the sample container. Then the V1,2 are open and V3,4 are closed, so the liquid nitrogen flow will be injected into the passage channels by the oscillating flow in the COHP and cool the thin layer block of the cell suspension through thin film evaporation of the liquid nitrogen flow. Then the V1,2 are closed and V3,4 are open, so the cell container can be removed and dropped into liquid nitrogen for long-term cryopreservation, and another cell container can be loaded and the above procedures can be repeated. To cool a large volume of cell suspension, the “U”-shaped working section can be connected to form a matrix as shown in Fig. 8.

To investigate efficiency of the proposed device, we performed numerical simulation on the cooling process using this device. Since the width of the thin layer block is much larger than its thickness, the model can be simplified as a one dimensional heat conduction process. The thermal conductivity and specific heat of the silicon is temperature dependant and the values can be obtained from [23]. The thermal conductivity and specific heat of the cell suspension is assumed the same as ice at 0 °C. For the boundary conditions, at $Z = 0$, because thin film evaporation of the liquid nitrogen flow inside the COHP is dominant, the Cauchy boundary condition will be written as:

$$-k \frac{\partial T}{\partial z} \Big|_{z=0} = \bar{h} [T_{\infty} - T|_{z=0}]; \quad (12)$$

where k is the thermal conductivity of silicon at $T|_{z=0}$, and T_{∞} is -196 °C. At the surface $Z = L$ (L is the summation of the container wall and thin film sample's thickness, as shown in Fig. 4), the sample is in contact with the cover. Because the cover can be made of materials of low thermal conductivity, such as polyethylene, the heat release from the sample to the cover is much less than that to the container wall and the adiabatic boundary is chosen as:

$$\frac{\partial T}{\partial z} \Big|_{z=L} = 0.$$

The algorithm is time implicit and the details can be found in [28]. A total of 100 nodes were sampled in the thin layer and container wall to achieve high precision (relative error less than 1%). The code for the simulation program was written using Microsoft Fortran Power-Station and the program was run on a PC.

Experimental and theoretical results

Figs. 9 and 10 show the temperature difference between the condenser and evaporator of the COHP shown in Fig. 1, when the input power from the flat electric heater is 40 and 80 W, respectively. The temperature of the liquid and the vapor inside the COHP is different, and the oscillating flow of these slugs and vapor bubbles results in the vibration of temperature as shown in Figs. 9 and 10, which is absent in traditional heat pipes. The relationship between

the value of $\dot{q}$ and ΔT is shown in Fig. 11 when the input power is between 30 and 120 W. Obviously, when $\dot{q}$ is in this range, $\dot{q}$ is a linear function of ΔT . Through linear regression and according to Eq. (2), the value of h_a of this COHP can be calculated as $2 \times 10^5 \text{ W/m}^2\cdot\text{K}$ ($R^2 = 0.97$).

According to Eq. (10), the value of h_{local} , as a function of x in the thin film evaporation region, is shown in Fig. 12. The value of $\bar{h}$ is calculated as $1.2 \times 10^6 \text{ W/m}^2\cdot\text{K}$ according to Eq. (11).

Substituting the value of $\bar{h}$ into Eq. (12), and solving the one-dimensional heat transfer discrete equations, the cooling process using the proposed device can be simulated. Defining $\bar{C}$ as the average cooling rate passing through the most probable nucleation region or the “dangerous temperature region” (from -20°C to -90°C) [22] at different locations (Z) of the thin layer, or:

$$\bar{C}(Z) = \int_{T_1=-20}^{T_2=-90} \frac{\partial T(Z)}{\partial t} dT / (T_1 - T_2);$$

the distribution of $\bar{C}$ was then calculated and the results are shown in Fig. 13A and B, for the $50 \mu\text{m}$ and $100 \mu\text{m}$ thick samples, respectively.

Discussion

For the COHP described here, the measured h_a is $2 \times 10^5 \text{ W/m}^2\cdot\text{K}$, which is within the same order of magnitude as other types of OHPs that use water or acetone as their working fluid, and is much higher than the h_a of traditional heat pipes (around 2 orders of magnitude higher) [1,29]. These results demonstrate the remarkable heat transfer capabilities of the COHP design and open numerous avenues of investigations regarding its application for ultra-fast cooling processes associated with cryopreservation. When $\dot{q}$ is less than 30 W, the experimental results show that the value of ΔT is too small to distinguish it from the background of the temperature vibration in the condenser or evaporator. When $\dot{q}$ is greater than 120 W, $\dot{q}$ approaches the dryout power input ($\sim 180 \text{ W}$) of the COHP, and the linear relationship between $\dot{q}$ and ΔT no longer exists [10,11,13] and the value of h_a is much less than $2 \times 10^5 \text{ W/m}^2\cdot\text{K}$. Therefore, the optimal working conditions for the power input of this COHP are between 30 and 120 W. The experimental error of this measurement is majorly generated by the heat release from the evaporator to the polyethylene cage and its value is technically difficult to measure. However, since the thermal conductivity of polyethylene is two orders of magnitude lower than that of copper, the material of the plates and COHP, it is reasonable to estimate that this part of thermal loss only generate the relative error less than 10% in the h_a measurement.

The theoretical investigation of the thin film evaporation process inside the COHP shows that the value of h_{local} is almost three orders higher of magnitude than that of a typical liquid nitrogen pool-boiling processes. In the physical model we established in Eqs. (3)–(10) to obtain the distribution of h_{local} , the energy dissipation due to friction is not considered. If an energy dissipation item is introduced into Eq. (9), considering friction between liquid nitrogen and the pipe wall, a difference of only 5% is observed for the results of h_{local} , due to the low viscosity of liquid nitrogen. Therefore, to simplify the analytical process, the frictional energy dissipation can be ignored for the COHP cooling process using liquid nitrogen as working fluid. Previous investigations have under-estimated the COHP heat transfer coefficient. For example, the maximum h_{local} used in [9] is only $2 \times 10^4 \text{ W/m}^2\cdot\text{K}$, which is much less than the value we have obtained. The ultra-thin straw proposed in [9] cannot be practically manufactured with current techniques, considering the shape and size of the straw and hardness of the materials. In contrast, the proposed device in this study can be easily produced through microfabrication.

This new design also spreads the sample into a thin layer, so the value of $R_{S/V}$ of the sample is independent of the sample volume, and always equal to the reciprocal of the depth of the container. In this way, the device overcomes the limitations of sample volumes used in OPS and micro-droplet methods, and even for the small sample volume such as 1 μL , $R_{S/V}$ of the sample in the proposed device is still 2–4 times higher than in an OPS or micro-droplet. This device is also a close system, where the sample layer is sealed between the cover and the container, and it is separated from liquid nitrogen by the silicon container wall, so this design will prevent cells from contamination and loss often encountered with traditional methods.

In the proposed device, after the liquid nitrogen flow from the COHP is injected into the passage channels under the sample container, because the dimension of the channels ($1 \times 1 \text{ mm}$) satisfies the requirement described by Eq. (1), the front edge of the flow will automatically form a thin film region contacting the saturated vapor. Obviously, this front-edge thin film evaporation region exists in every channel of the proposed device. Because the local heat transfer coefficient inside these front-edge thin film regions is several orders of magnitude higher than other non-evaporating regions of the flow in the COHP, we assume that, for any control point on the container wall surface that contacts with the oscillating flow (or at $Z = 0$), its cooling process starts when the front-edge thin film region contacts with it and ends when this region passes it. The process is shown in Fig. 14. Therefore, the duration of this cooling process is the same as the time it takes for the effective region of the front-edge thin film to pass the control point, and we use $\bar{h}$, the spatial average value of h_{local} in the thin film region, in Eq. (12) for the boundary condition at $Z = 0$. The loss of the liquid nitrogen in this front-edge thin film due to evaporation will be instantly compensated by the liquid nitrogen driven into the thin film by surface tension and disjoint pressure, so the cooling process is continuous and uniform for all the control points at $Z = 0$. The effective dimension of this front-edge region of thin film evaporation is on the order of 10 μm , as shown in Fig. 12. Since the speed of the oscillating flow of OHPs is generally on the order of 10 cm/s [13], the time it takes for this front-edge thin film region to pass for any control point of the sample is on the order of 10^{-1} millisecond or 10 μm divided by 10 cm/s. That time duration is also at the same order of the time duration for a cooling process from 0 $^{\circ}\text{C}$ to liquid nitrogen saturation temperature with the cooling rate on the order of 10^{7-8} K/min, which is the average cooling rate of control points on the silicon wall, as shown in Fig. 13. These results quantitatively agree with our assumption. From the simulation results, as shown in Fig. 13A and B, the minimum value of $\dot{C}$ locates at $Z = L$, and the value is 7.4×10^6 and 2.0×10^6 K/min, when the depth of the sample container is 50 and 100 μm , respectively. These cooling rates are high enough to achieve vitrification of 1 M EG or DMSO solutions [4,22].

During warming processes, devitrification occurs at a relatively low warming rate, and it is also a cell damaging process due to the ice crystals formed [6]. To prevent devitrification, the warming rate should be higher than the critical warming rate [4,6,22]. For the solutions of permeating CPA's, the critical warming rates are extremely high even for solutions with a high concentration of CPAs: for example, 30% L-2,3-Butanediol solution requires 3×10^7 K/min [22]. Therefore, the proposed device should also provide ultra-high warming rate during warming processes. In the proposed device, using water as working fluid in the OHP during the warming process can only achieve a warming rate of 10^7 K/min. However, we can significantly lower the critical warming rates of the solutions by adding low concentrations (5–10%) of non-permeating solutes [4,6,22], such as HES, PVP or PEG, which have no severe toxic effects on cells. Meanwhile, the molecules of large molecular weight inside the cells (such as proteins) should also have similar functions to enhance the survival of cells at the warming rates much lower than the calculated critical warming rates of extracellular solutions. In this way, the device may also be able to provide enough warming rates to diminish devitrification during warming.

Conclusion

Both experimental results and theoretical analyzes demonstrate the outstanding heat transfer ability of a COHP and lead to its novel application in cell cooling and cryopreservation processes. The proposed device applies COHP and microfabrication techniques to increase the heat transfer coefficient of the cooling process and maximize $R_{S/V}$ of the sample. The numerical simulation shows that the device can achieve 10^{6-7} K/min cooling rates and hence vitrification of >100 μ l cell suspensions with ~ 1 M CPA. The current investigations will help to design a highly efficient cooling device for cell cryopreservation.

Nomenclature

A	modified Hamaker constant (N/m^2)
C	cooling rate (K/min)
D	diameter (m)
h	heat transfer coefficient ($\text{W/m}^2\cdot\text{K}$)
h_{fg}	latent heat of vaporization (J/kg)
k	thermal conductivity ($\text{W/m}\cdot\text{K}$)
p	pressure (N/m^2)
$\dot{q}$	power (W)
q''	heat flux (W/m^2)
$R_{S/V}$	the ratio of the sample surface area versus its volume
r	meniscus radius (m)
S	area (m^2)
T	temperature (K or $^{\circ}\text{C}$)
t	time (second)
x	Z , coordinates (m)

Greek Symbols

σ	surface tension (N/m)
δ	thickness (m)
ρ	density (kg/m^3)

Subscripts

c	condenser
d	disjoining
e	evaporator
l	liquid
lv	liquid–vapor interface
sat	saturated
v	vapor

w wall

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Fig. 1.
A Schematic of the COHP construction and its performance test: (1) the copper pipe turns; (2) copper plate; (3) flat electric heaters; (4) a multimeter; (5) thermocouples.

Fig. 2.
A schematic of thin film evaporation model in a COHP.

Fig. 3.
The design of the sample holder to form the cell suspension into a thin layer block.

Fig. 4.

The cross section view of the sample holder shows the spatial relationship between the thin layer sample of cell suspension, the container's cover and wall, and the liquid nitrogen passage channel: Z is the thickness coordinate, and $Z = 0$ is the location where OHP working fluid is in contact with the container wall, and $Z = L$ is the location where the thin layer sample is in contact with the cover.

Fig. 5.

A schematic of the manufacturing process for the sample container through three steps: (1) carve the flat container on the top of the silicon wafer; (2) produce the grooves on the Pyrex glass chip; (3) the silicon wafer will seal the hatches of the grooves through anodic binding to form the complete coolant passage channels.

Fig. 6.

The design of the “U”-shaped working section. (A) The appearance of the whole device without the sample holder loaded: (1) the evaporator; (2) COHP; (3) the working section; (4) the condenser. (B) The appearance of the working section. (C) The cross section view of the working section: (5) the upper pipes; (6) the lower pipes; (7) coolant oscillating flow and the small red bars are the valves (V1–4).

Fig. 7.

The cooling procedures using the device. (A) and (B) The appearance when the working section is loaded with a sample container. (C) The cross section view: (1) the cover; (2) the thin layer sample (in green color); (3) the thin container wall under the sample; (4) the liquid nitrogen passage channel under the sample container. (D) To cool the thin film sample, V3,4 are closed and V1,2 are open, so the liquid nitrogen slugs and nitrogen bubbles will flow through the new passages under the thin container wall, and the thin film evaporation will absorb the major heat release.

Fig. 8. A schematic of integration of the whole device system with a common condenser and evaporator. This three-dimensional matrix of the sample holders and COHPs can process a total of 2 ~ 5 ml cell suspension.

Fig. 9.
Performance of COHP under 40W input.

Fig. 10.
Performance of COHP under 80W input.

Fig. 11.
The linear relationship between the power input and the temperature difference between the evaporator and condenser of the COHP when the input is between 30 and 120 W.

Fig. 12.
The theoretical result of the distribution of the local heat transfer coefficient in the thin film evaporation region of the COHP.

Fig. 13. Distribution of the average cooling rate of the sample in the proposed device to pass the nucleation temperature region.

Fig. 14.
The cooling process of the control point A at $Z = 0$ when the front-edge thin film region passes it.