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Convective heat transfer coefficients of open and closed Cryotop® systems under different warming conditions

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ABSTRACT

The warming of cryopreserved samples supported by small volume devices is governed by heat transfer phenomena which are mathematically described by the solution of the transient heat conduction partial differential equations; the convective heat transfer coefficient (h) is an important parameter involved in the boundary condition which is related to the fluid dynamic behavior at the interface device-warming fluid (water, sucrose solution or air). Unfortunately, h values for small volume devices (i.e. Cryotop®) have not been experimentally determined. Moreover, heat transfer coefficients during warming of Cryotop® cannot be obtained through classical dimensionless correlations expressed in terms of Nusselt vs. Reynolds and Prandtl numbers that are available for regular geometries and single materials.

It is the purpose of present work to determine the convective heat transfer coefficients (h) by numerically solving the heat transfer equation applying the finite element method. Numerical simulations allowed to predict time-temperature histories and warming rates under different protocols in Cryotop® system which were compared with literature warming rates reported for this device. The h values were calculated considering the heterogeneous structure of the domain (microdrop, plastic-support) and the irregular three-dimensional geometry. The warming conditions analyzed were: a) open system in contact with air and sucrose solution at 23 °C) and b) closed system in contact with air and water at 23 °C. The h values of the Cryotop® open system immersed in sucrose solution (23 °C), that according to literature achieved a survival in the order of 80%, are in the range of 1800–2200 W/m^2K. The h values obtained in this work for warming conditions are critical parameters for cryobiologists when studying heat transfer rate in this small volume device.

1. Introduction

Thermal histories during cooling, storage, and warming are fundamental aspects that critically influence the cryosurvival of reproductive cells. Vitrification has become the method of choice for low temperature preservation of large-volume cells such as oocytes and embryos and has replaced equilibrium freezing in most clinical settings [5,6,14]. This phenomenon is a non-equilibrium process in cryoprotective solutions (CPS) which suppresses ice crystal formation while achieving an amorphous state. Because these solutions usually contain permeating cryoprotectants with varying degrees of cytotoxicity [25], multiple exposure steps and high cooling rates (> 10,000 °C/min) are necessary in order to avoid osmotic effects while reducing exposure time to minimize toxicity; cells are typically loaded with minimal volume onto vitrification supports and plunged in liquid nitrogen. Minimal volume systems such as the Cryotop® have been shown to achieve these high cooling rates [12].

Studies of different vitrification carrier systems have mostly focused on the cooling process and the quantification of the cooling rates necessary to achieve vitrification. However, several works proposed that the warming rate of vitrified samples might be the most important factor that determines cell cryosurvival [13,20,21]. The work by Seki

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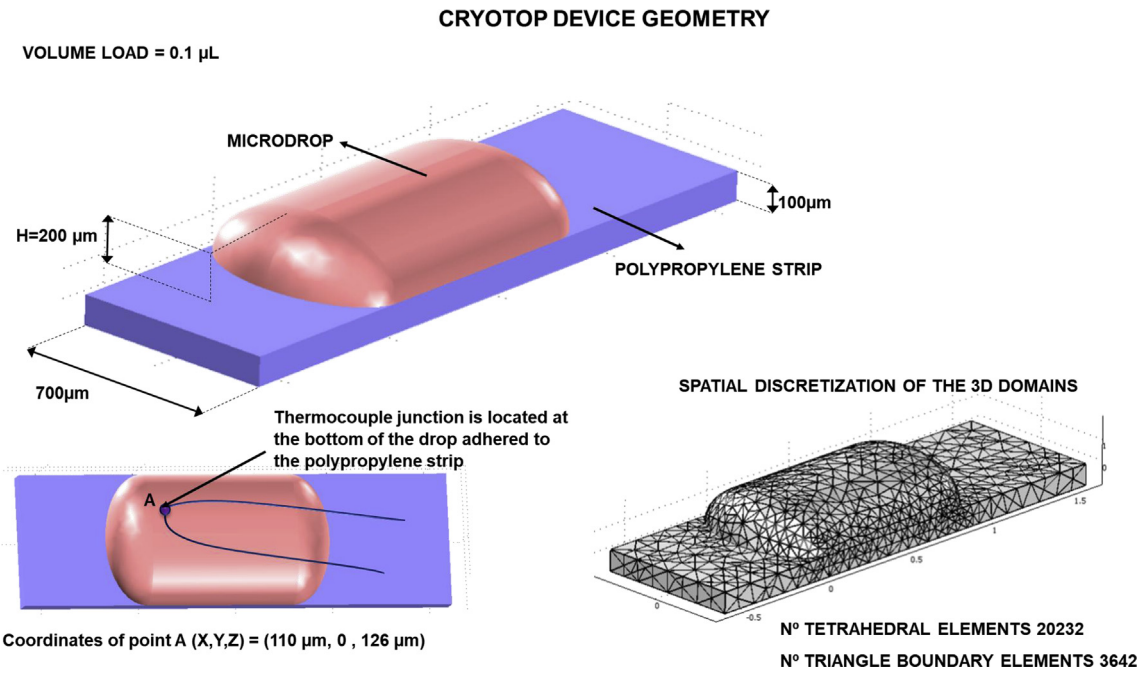

Fig. 1. a) Cryotop® system (microdrop on top of the fine polypropylene strip) with a volume of 0.1 µL. Spatial representation of the irregularly shaped body using tetrahedral and triangular elements. Location of the thermocouple defined using the photograph published by Kleinhans et al. [11] whose thermal history was simulated in the present work.

and Mazur [22] was the first report which showed the dominant effect of warming rate over cooling rate on the survival of mouse oocytes, and was later corroborated specifically for Cryotop® in 2012 [23]. Their results indicated that, irrespective of cooling rate, murine oocyte survival was 70–85% when warming was performed at the highest rate (96,000–117,000 °C/min) [23]. In this study, the authors measured the time-temperature histories during cooling and warming of a sample mounted on Cryotop® using a 50 µm copper-constantan thermocouple, and recorded data with a computer-oscilloscope. This simple experimental procedure allowed for the quantification of the warming rates achieved in a Cryotop® under several operating conditions.

The Cryotop® is a heterogeneous system consisting of a fine polypropylene strip supporting the micro-drop of the biological sample. The whole system is a complex irregular three-dimensional domain with materials of different thermophysical properties that cannot be assimilated to a simple regular geometry of a homogeneous material.

The warming process of cryopreserved samples is governed by heat transfer phenomena that can be mathematically described by the solution of the transient heat conduction partial differential equations. The time-temperature histories and warming rates in cryo-devices under different protocols can be predicted by numerical simulations of these partial differential equations that must be experimentally validated. The finite element method (FEM) is a powerful technique originally developed for the numerical solution of complex problems in structural mechanics, it has been extensively applied in many engineering problems that involve mass and energy transfer. In order to simulate heat transfer in Cryotop® and predict time temperature curves, FEM is considered the method of choice since it can deal with the high level of complexity encountered in this type of systems: irregular geometry and heterogeneous domain of the device.

The application of mathematical models requires the knowledge of the thermophysical properties of the biological fluid and the plastic support material. In the past, authors have used equilibrium thermophysical properties that considered the presence of ice for cell suspensions; however, vitrification is a non-equilibrium process which requires specific properties.

The surface heat transfer coefficient (h) is an important parameter involved in the boundary condition which is related to the fluid dynamic behavior at the interface device-warming fluid (water and/or air). Numerical calculations of warming rates require the knowledge of accurate h values that will predict the performance of a specific cryobiological procedure. Heat transfer coefficients during warming of Cryotop® system cannot be obtained using classical dimensionless correlations expressed in terms of Nusselt vs. Reynolds and Prandtl numbers that are available for regular geometries and single materials. In order to determine the h values that represent the warming rates of each protocol heat transfer numerical solutions must be compared with experimental time-temperature measurements. Santos et al. [18,19] have reported surface heat transfer coefficients in several cryopreservation systems (plastic French straws, Cryoloop®, Cryotop®, OPS among others) in order to estimate the performance of different cooling protocols and procedures (direct plunging in liquid nitrogen or freezing in nitrogen vapor).

Information about convective heat transfer coefficients during the warming process of Cryotop® have not yet been reported in literature; however, these coefficients are needed for the optimization of warming protocols [26].

The main objective of the present study was to determine heat transfer coefficients during warming using Cryotop® systems under different conditions, while considering the effects of the thermophysical properties and the loading volume. The warming conditions included in the analysis are: a) Cryotop® (open system in contact with air and sucrose solution at 23 °C), b) Cryotop® (closed system in contact with air and water at 23 °C).

2. Materials and methods

2.1. Vitrification system

The Cryotop® vitrification carrier, consists of a fine strip of polypropylene transparent film of 0.7 mm wide, 20 mm long and 0.1 mm thick [10,23], attached to a plastic handle resistant to liquid nitrogen. It is interesting to note that in different publications [11–13] a strip width of 0.4 mm was reported, however the actual value is 0.7 mm (Fig. 1).

The polypropylene tip has a flat film area where a minimal volume can be loaded (0.1–0.2 µL containing 4–8 oocytes or embryos) and subsequently plunged into liquid nitrogen. The Cryotop® allows for a sample to be cooled at a very high rate in order to achieve vitrification. Samples can be vitrified either in direct contact with liquid nitrogen (open system) or contained within a protective cap that isolates the loaded sample from the cryogenic fluid (closed system, Cryotop® SC Kitazato Supply, Inc, JP). Once vitrified, the warming protocols have been shown by Mazur and Seki (2011) to be a key aspect of cell survival.

3. Numerical modeling

3.1. Cryotop® dimensions and geometry. Support material

The geometry of the Cryotop® system used for the heat transfer numerical simulation was based on the information published by Jin et al. [10] and Seki and Mazur [23]. The two domains (microdrop and polypropylene strip) are shown in Fig. 1. Besides the position of the thermocouple junction used by Kleinhans et al. [11] whose experimental measurements were simulated in the present work are also shown Fig. 1. The selected point corresponding to the thermocouple position has the following spatial coordinates: x = 110 µm, y = 0, z = 126 µm (Fig. 1).

The spatial discretization of the 3D domains was implemented using tetrahedral and triangular elements for the inner and boundary domains, respectively (Fig. 1). The Cryotop® protocol requires the minimal volume droplet to be carefully spread into a thin film over the plastic polypropylene strip. Two different drop volumes (0.1 and 0.2 µL) were simulated in order to study the effect of the loaded microdrop on the warming rate. In Fig. 1 the height of the droplet (H) corresponds to 0.1 µL droplet volume.

The warming modeling conditions selected for the present study (Fig. 2) were based on earlier reports by Mazur and Seki (2011) [13], in which warming rates were experimentally measured.

3.2. Mathematical modeling of heat transfer

The partial differential equations that represent conductive heat transfer in the Cryotop® system (negligible convective contribution) during warming can be described as a 3D problem using Cartesian coordinates:

$$\rho_s Cp_s \frac{\partial T}{\partial t} = -\nabla(-k_s \nabla T) \text{ at } \Omega 1 \quad (1)$$

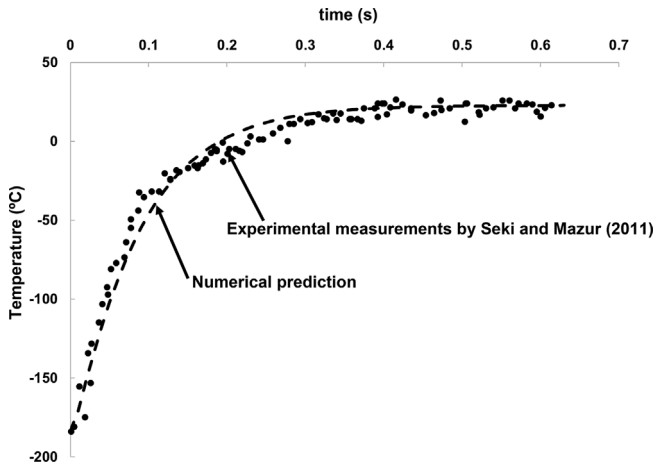

Fig. 2. Warming protocols modeled in the present study using open and closed Cryotop®.

$$\rho_p Cp_p \frac{\partial T}{\partial t} = -\nabla(-k_p \nabla T) \text{ at } \Omega 2 \quad (2)$$

where T is temperature, ρ is the density, Cp specific heat, k thermal conductivity. The effect of temperature on the thermo-physical properties of the biological solution was considered.

The subscript s corresponds to the domain Ω1 (droplet of biological solution) and p to the plastic material (Ω2).

The initial temperature condition was considered uniform in both material domains.

$$T0 = -196\ ^\circ C \text{ at } t = 0 \text{ for } \Omega 1 \text{ and } \Omega 2 \quad (3)$$

According to the simulated warming protocols (Fig. 2) the convective boundary conditions for the plastic support and for the microdroplet in contact with the external media (air or liquid warming medium) are expressed as follows:

$$-k_p \nabla T n = h\,(T - T_{ext}) \text{ at } \partial\Omega 1 \quad (4)$$

$$-k_s \nabla T n = h\,(T - T_{ext}) \text{ at } \partial\Omega 2 \quad (5)$$

where, h is the average surface heat transfer coefficient at ∂Ω1 (interface of the plastic strip) and ∂Ω2 (interface of the droplet); T is the variable surface temperature of the microdroplet or the plastic strip exposed to the external medium; T_{ext} is the external temperature and its value depends on the protocol used as warming process, n is the normal outward vector.

The heat transfer resistance of the closed Cryotop® (with a cap) is given by the sum of several serial heat transfer resistances: air insulation, the thickness of the plastic cap and the external fluid which can be air or water.

It is interesting to note that Kleinhans et al. [11] measured the response of a Cryotop system with incudes a thermocouple as part of the mass to be warmed. These authors estimated that the heat capacity of the thermocouple represented only 5% of the total thermal mass. Therefore in the present work the influence of the thermocouple was considered negligible.

The differential equations (1)–(5) that represent the warming process were numerically solved using the finite element method in COMSOL 3.5 AB Multiphysics (lic. 1048485).

3.3. Thermophysical properties

The thermophysical properties used in the model (specific heat, thermal conductivity and density) of the polypropylene strip, ice and vitrified water are summarized in Table 1 for the temperature range −196 to 0 °C.

There is a wide range of biological formulations used for cryopreservation purposes that vary in terms of the type of cryoprotective agents incorporated.

The thermal properties therefore are important parameters that must be carefully selected to simulate heat transfer phenomena and these properties depend on both the protocol (warming rate) which is related to the volume load of the sample and the proximate composition of the biological solution.

Ehlich et al. [7] experimentally determined the thermal conductivity of water-DMSO solutions using the hot wire technique; results showed that in a DMSO solution ranging between 2 and 6 M crystallization occurs and the thermal conductivity increases as the temperature decreases. In contrast, above approximately 7.05 M DSMO vitrification occurs and the thermal conductivity is independent of the concentration of solutes and of temperature. The presence or absence of ice was observed in the experiments using cryomicroscope images. Choi and Bischof [3] have also reported thermophysical properties (k, ρ, Cp) of biologically relevant solutions, liquids, and tissues that are important in the cryobiology field.

If the warming rate is sufficiently high to avoid recrystallization or

Table 1
Effect of temperature on thermophysical properties used in the simulations.

Materials	Thermophysical properties			
	k (W/m^2 K)	ρ (Kg/m^3)	Cp (J/kg K)	References
Polypropylene	0.22 (−196 °C, 23 °C)	920 (−196 °C, 23 °C)	1900 (−196 °C, 23 °C)	[11]
Glassy Water	1.1 (−196 °C, 23 °C)	940 (−196 °C, 23 °C)	1078.88 (−154.18 °C) 1120.55 (−150.77 °C) 1173.33 (−146.83 °C) 1216.11 (−142.99 °C)	[1,2,4,24]
Ice	2.22 (0 °C) 2.25 (−5 °C) 2.3 (−10 °C) 2.34 (−15 °C) 2.39 (−20 °C) 2.45 (−25 °C) 2.5 (−30 °C) 2.57 (−35 °C) 2.63 (−40 °C) 2.76 (−50 °C) 2.9 (−60 °C) 3.05 (−70 °C) 3.19 (−80 °C) 3.34 (−90 °C) 3.7 (−100 °C) 4.1 (−110 °C) 4.3 (−120 °C) 4.7 (−130 °C) 5.2 (−140 °C) 5.6 (−150 °C) 6 (−180 °C)	917.2 (0 °C) 924.13 (−50 °C) 929.3 (−100 °C) 931.0 (−150 °C)	2100 (0 °C) 1967 (−20 °C) 1833 (−40 °C) 1700 (−60 °C) 1566 (−80 °C) 1433 (−100 °C)	[3,7,9,15]

devitrification, then the thermophysical properties of a vitreous biological solution should be applied. On the contrary, when the warming rates are low and there is a partial or total crystallization of ice, then thermophysical properties under equilibrium conditions should be applied. In the last case, the use of Differential Scanning Calorimetry can be helpful for the estimation of thermal properties such as specific heat (Cp) which is strongly temperature dependent.

Due to the scarce information concerning the thermophysical properties of the specific biological fluid used by Mazur and Seki [13] a simplification was implemented using thermophysical properties of ice (for low warming rates) or vitrified water (for high warming rates) instead of the actual values of the biological complex systems.

The implementation of these properties has been previously applied for numerical simulations of the performance of several vitrificaction devices [16].

For an open Cryotop® system directly immersed in a 23 °C solution, the warming rate corresponds to the highest value (96,000–117,000 °C/min) that allowed to achieve the highest oocyte survival independent of the cooling rate applied [13].

Therefore, in this scenario, the numerical model applied in the present work was that of vitreous water; the thermophysical properties as a function of temperature are shown in Table 1.

In the case of other simulated protocols with lower warming rates, partial formation of ice due to recrystallization or devitrification phenomena could be responsible for the observed decrease in survival rates [13,17]. Therefore, in the present work, one set of simulations were carried out considering the thermophysical properties of ice and another set assuming vitreous water, in order to find the range of surface heat transfer coefficients that describe the warming process. In addition, the effect of varying these properties on the h values calculated were assessed. The thermophysical properties of ice which were considered dependent on temperature in the numerical model are also shown in Table 1.

Table 2
Warming protocols simulated using FEM for different droplet volumes (0.1 and 0.2 μL).

Warming Protocol	Cryotop® System	Description
P1	Open	Sucrose solution (23 °C)[a]
P2	Open	Holding in Air (23 °C)
P3	Closed	Immersion in water (23 °C)
P4	Closed	Holding in Air (23 °C)

[a] 0.5 M sucrose solution.

3.4. Warming simulations under different conditions

Table 2 shows the simulated warming conditions for which the surface heat transfer coefficients were calculated.

Mazur and Seki [13] measured the time-temperature curves during warming protocols consisting in the exposure of a closed Cryotop® system (CS) to an external temperature of 23 °C in air and in a liquid warming media (water or sucrose solution). In the case of the open Cryotop® (OS) system, the experiments were carried out with immediate immersion into a liquid warming media or in air both at 23 °C.

In the case of the open Cryotop® with direct immersion in warming solution at 23 °C Mazur and Seki [13] reported the entire time-temperature data. The numerical FEM was applied by varying the h value and then comparing the predicted time-temperature curve with the experimental curve reported by the authors. The heat transfer coefficient that minimized the absolute error of the temperature history was selected.

For the other protocols (P2, P3, and P4) time-temperature data are not available; only warming rates were reported, therefore the numerical FEM was applied to estimate the surface heat transfer coefficients (h) by comparing the experimental warming rates with the predicted ones obtained through the numerical thermal histories calculated by the model. The heat transfer coefficient that minimized the absolute error of the warming rates was selected. Mazur and Seki [13] defined the warming rate as the initial straight slope of their experimental temperature - time curves before the warming rate starts to slow down (from −170 °C to −30 °C).

4. Results and discussion

4.1. Surface heat transfer coefficients during warming under different operating conditions

4.1.1. Protocol 1. Open Cryotop® immersed in sucrose solution

Fig. 3 shows the numerical simulations considering a volume load of 0.1 μL and vitreous water. The h value that best fitted experimental data was 1850 W/m^2 K; as can be observed there is an excellent agreement between predicted and experimental curves which allows the determination of the h value that represents the warming process under vitreous conditions. The same numerical procedure was applied with a volume load of 0.2 μL and the h calculated value was 2200 W/m^{2}K. These values are representative of the range of h that generate a warming rate of 96,000 °C/min. As was mentioned before the h values cannot be estimated by the dimensionless Nusselt correlations. The numerical finite element program allowed to obtain the time-temperature distribution at any point inside the domains as time elapses.

A tetrahedral mesh using Lagrange elements of order 2 was applied to discretize the domains. The number of elements that constituted the mesh for the microdroplet with different volumes and plastic support are shown in Table 3. The time discretization scheme used was a Backward Euler Differentiation (minimum order 1 and maximum order 5) with a tuning step having a maximum of 0.1 s and a minimum initial

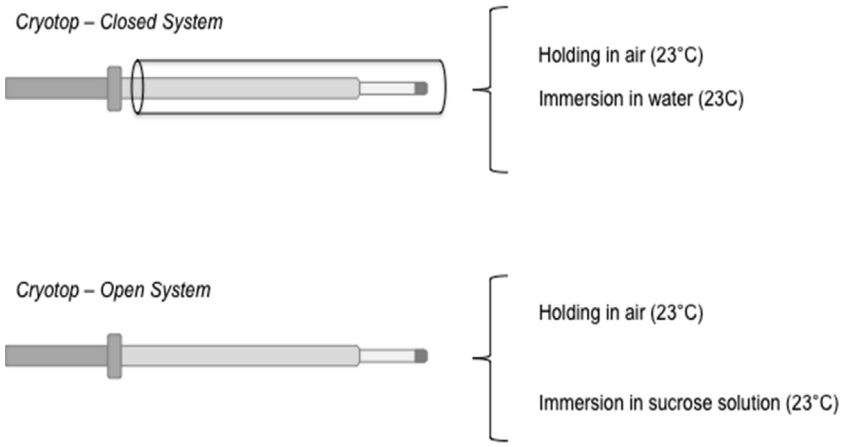

Fig. 3. Time-temperature measurements adapted from Kleinhans et al. [11] and numerical prediction for the warming process in an Open Cryotop® system by immersion in a sucrose solution at 23 °C. Experimental warming rate of 96000 °C/min.

Table 3
Dimensions of the droplets and mesh parameters.

	Mesh parameters		
	N° Tetrahedral elements (domain)	N° Triangular elements (boundary)	Total node points
Cryotop with 0.1 μL H = 200 μm[a]	20232	3642	4426
Cryotop with 0.2 μL H = 280 μm[a]	12162	2516	2735

[a] H = height of the droplet according to the volume loaded (See Fig. 1).

starting value of 0.001s. The absolute and relative tolerances for each integration step were 0.001 and 0.01, respectively.

All the numerical runs were tested for their computational speed, the maximum CPU time was less than 5 min for the 3D model runs using a PC Intel(R) Core(TM) i3 6300 with a processor speed of 3.80 GHz and a RAM of 4 GB.

Fig. 4 a, b shows the numerical simulations and time-temperature distribution in the whole system with the inner views at different positions.

The values of h determined in the present work for the Protocol 1, that achieved a survival in the order of 80% according to Mazur and Seki (2011) are in the range 1800–2200 W/m^{2}K which are higher than expected for different solids such as thin plates or small cylinders immersed in stagnant fluids (represented by sucrose solution). These high values may indicate nucleate boiling of the liquid nitrogen that is moistening or in intimate contact adhered to the surface of the open Cryotop® system (PP strip and droplet). This liquid film generates nitrogen vapor and bubbles when it comes into contact with the warm solution that rapidly escape from the warming media (it must be taken into account that LN2 boils at −196 °C at atmospheric pressure). This phenomenon is commonly observed when a cryobiological device coming from a liquid nitrogen container is rapidly immersed in a warming solution. The nitrogen bubbles that escape from the device

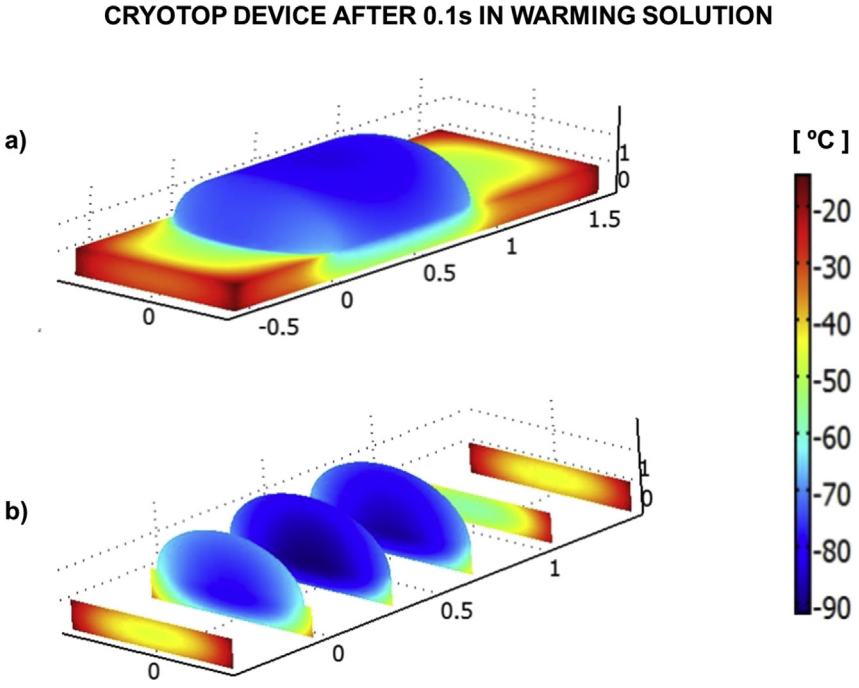

Fig. 4. Temperature distribution after 0.1 s at:a) the external surface of the droplet and PP strip, b) inner points of the microdrop at different consecutive slices in the axial direction, considering a volume load of 0.1 μL, initial temperature of −196 °C, h = 1100 W/m^{2}K, and a warming solution (sucrose) at a temperature of 23 °C.

Table 4
Convective heat transfer coefficients (h), experimental warming rates and predicted values using numerical simulations of the Cryotop® system under different warming protocols, droplet volumes and thermophysical properties of the simulated fluid (ice or glassy water).

Warming Protocol Cryotop®	Simulated fluid	Droplet volume loaded (μL)	Heat transfer coefficients h (W/m^2 K)	Predicted warming rate using FEM (°C/min)	Experimental warming rate (Mazur and Seki, 2011) (°C/min)
P2 OPEN in air	Ice	0.1	90	7758	7850 ± 415
		0.2	110	7828	
	Glassy Water	0.1	120	7845	
		0.2	140	7868	
P3 CLOSED with cap in water	Ice	0.1	40	3985	4050 ± 328
		0.2	50	4033	
	Glassy Water	0.1	53	4157	
		0.2	60	4034	
P4 CLOSED with cap in air	Ice	0.1	5.5	642	612 ± 40
		0.2	6	618	
	Glassy Water	0.1	5.5	618	
		0.2	6.5	630	

generate a fluid dynamic pattern that is far from the stagnant conditions, leading to higher h values. Another possible source of convective contribution can be originated by the laboratory operator through the swirling of the Cryotop® to homogenize temperature profiles.

4.1.2. Warming protocols of open and closed Cryotop® immersed in water and air

Table 4 shows the heat transfer coefficients and warming rates predicted with the numerical simulations of the Cryotop® under different conditions and using thermophysical properties for both glassy water and ice.

As can be observed the h values at the lowest possible warming rates (Protocol 4, Cryotop® closed system warmed in air) correspond to an external and internal stagnant fluid (air inside and outside the cap of the Cryotop®). The volume loaded on the Cryotop® for this protocol did not influence the low h value (h = 5.5–6.5 W/m^2K). As was mentioned previously, the h value for the Cryotop® with cap is in fact a global heat transfer coefficient, because it takes into account the sum of in series individual resistances given by: air insulation, the thickness of the plastic cap, and the external fluid. In this protocol (P4) in which the presence of air led to low heat transfer rates, there is a high possibility of ice formation due to devitrification or recrystallization; therefore, simulations were carried out introducing the thermal properties of ice.

In order to analyze the effect of the thermophysical properties on h values during warming, simulations of P4 using glassy water properties were also conducted. Obtained results showed that there was not an appreciable difference between the h values using both set of properties: h = 5.5.-6.5 W/m^2K for glassy water and h = 5.5–6 W/m^2K for ice.

The radiation heat transfer was calculated for this Protocol according to the method proposed by Geankoplis [8] and the obtained value of h for radiation was 1.98 W/m^2 K. This value implies that there is a significant contribution of radiation to the total heat transfer during the warming process.

Table 4 shows that thermophysical properties and the volume loaded did not affect in a significant manner the warming rate. In terms of finding the bottleneck of the warming process it can be concluded that in the case of Protocol 4 there is an external heat control of the system, therefore the process is governed by the thermal resistance of the external fluid (air).

When the Protocol 3 (Cryotop® closed system warmed in water) is used, the external control decreased compared to Protocol 4 since water as immersion warming fluid allows a higher heat transfer rate. Additionally, if the closed Cryotop® is swirled, the movement of the water solution generates convective conditions. For Protocol 3 h values ranged between 40 and 50 W/m^2K when ice properties were used in the simulations and higher values of h (53–60 W/m^2K) were obtained when glassy water properties were introduced in the model.

In the case of Protocol 2, an Open Cryotop® in contact with air was simulated obtaining higher h values (> 90 W/m^2 K) when compared to typical values of h in stagnant air and to Protocol 3 (closed Cryotop® immersed in water). This result can be attributed to the fact that the liquid nitrogen film adhered to the Cryotop® device evaporates when it is exposed to the warm air. Nitrogen vapor released from the sample produced a higher convective flow that led to higher h values. The individual contribution of radiation to the total rate of heat transfer was calculated resulting in less than 3%.

5. Conclusions

Surface heat transfer coefficients (h) under different warming protocols for Cryotop® systems were estimated using numerical finite element simulations considering the irregular 3D shape and the heterogeneous structure. Four warming protocols were simulated: a) Cryotop® open system immersed in air and sucrose solution at 23 °C; b) Cryotop® closed system in direct contact with air and water at 23 °C.

Time-temperature curves and warming rates were predicted and compared with published experimental data. Numerical simulations using different volume loads and thermophysical properties associated to non-equilibrium warming (glassy water) or equilibrium conditions (that generates ice crystals formation or devitrification) allowed to analyze the mechanisms governing the heat transfer rate for each Protocol.

The h values of the Cryotop® open system immersed in sucrose solution at 23 °C (Protocol 1), that achieved a survival in the order of 80% according to Mazur and Seki (2011) are in the range of 1800–2200 W/m^2K. Lower h values were observed for the other simulated warming protocols with a lower dependence on the loaded volume and thermophysical properties of the simulated fluid (ice or glassy water).

The h values obtained in this work for warming conditions are critical parameters for cryobiologists when studying technologies associated with vitrification systems, and limited information about these values are found in literature.

The present work contributes to the calculation of h values that represent the heat transfer rate during warming of vitrified samples which might be one of the limiting steps in cell survival.

Conflicts of interest

Authors declare no conflict of interest in the present study.

Role of the funding source

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