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MODELING CELL OSMOTIC BEHAVIOR DURING COOLING AND WATER CRYSTALLIZATION

Selection of cryopreservation parameters, such as cooling rates, is crucial for the successful preservation of cell suspensions. The objective of this study was to assess the impact of cooling rates and nucleation on the survival of testicular interstitial cells (ICs) and to model cell osmotic behavior based on the Kedem-Katchalsky formalism during water crystallization. The ICs were cooled in the serum-free medium supplemented with 0.7 M DMSO and 100 mg/ml dextran 40 (0.7DMSO + D40). The modeling of IC osmotic behavior in the medium, based on experimentally determined water and DMSO membrane permeability coefficients and activation energies, revealed the potential to increase the cooling rate from the "standard" 1 °C/min. This possibility was tested experimentally using higher cooling rates of 2, 5, and 10 °C/min. A cooling rate of 2 °C/min, combined with controlled nucleation parameters, was shown to be beneficial for preserving ICs in 0.7DMSO + + D40. The approach including modeling, the use of defined serum-free compositions, controlled nucleation and optimization of cooling rates, can be applied to other cell types cryopreserved in multicomponent cryopreservation media.

Key word: *cooling rate, nucleation, Kedem-Katchalsky formalism, serum-free media, dextran, dimethyl sulfoxide, testicular interstitial cells, cryopreservation.*

Cryopreservation of cell suspensions consisting of cells that differ in size, volume, origin, and physical and chemical membrane properties (a heterogeneous cell suspension) is a very complex and challenging task. One of the key cryopreservation parameters is the cooling rate. According to Mazur's two-factor theory, excessively high cooling rates may lead to insufficient cell dehydration and, consequently, an increased probability of harmful intracellular ice formation, while excessively low rates can result in excessive cell volume loss and prolonged, unnecessary exposure to the hyperconcentrated solution formed by extracellular ice, leading to cell death by the "solution effect" [15]. Selecting optimal cooling rates is a typical task for a cryobiologist. However, an optimal cooling rate for one cell type may be far from optimal for another in a heterogeneous cell suspension. This task becomes even more complicated if some cells fall into the category of so-called poorly cryopreservable biological objects [25, 29].

One approach to address this challenge is to use the combined cryoprotective compositions, with components that create optimal conditions for most cell types during cryopreservation. In our previous studies [18], we identified several serum-free and serum-containing media that may be beneficial for cryopreserving testicular interstitial cells (ICs), a heterogeneous cell suspension that includes various cell types such as Leydig cells and immune

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system cells. In particular, the serum-free medium 0.7DMSO + D40, supplemented with 0.7 M DMSO and 100 mg/ml dextran 40 (D40), can promote moderate cell dehydration before cooling, influence the size and shape of ice crystals in a way that facilitates cell survival, inhibit eutectic crystallization/melting, provide an optimal glass transition temperature, and preserve the physical and chemical properties of cell membranes [19, 24]. This medium also presents a lower potential risk of infection transmission and DMSO toxicity compared to its serum-containing counterpart, 1.4DMSO + + FBS, which includes a higher DMSO concentration (1.4 M) and 10% FBS [21].

Another approach is to modify cryopreservation parameters, such as the duration of cell exposure to the cryoprotective solution before cooling and the cooling rates. The optimal exposure time and cooling rate for each individual cell depend on membrane permeability to water and cryoprotective agents (CPAs) such as DMSO. Based on the determined average osmotically inactive volume, permeability coefficients for water and DMSO, and corresponding activation energies, we modeled the IC osmotic behavior in the cryopreservation media, assessed the optimal exposure time, and confirmed it experimentally [20].

The objective of the present research was to model cell osmotic behavior based on the aforementioned averaged biophysical indices of cell membrane permeability and the Kedem-Katchalsky formalism during cooling at various rates, to correlate the modeling results with empirical data and explain the observed impact of cooling rates on the heterogeneous cell suspension of ICs in 0.7DMSO + + D40. Additionally, a technical issue related to stochastic nucleation was addressed, and the limitations of the Kedem-Katchalsky formalism for modeling cell osmotic behavior in certain cryoprotective media were discussed.

MATERIALS AND METHODS

Experimental animals. Male Wistar rats from the animal house of the Institute for Problems of Cryobiology and Cryomedicine of the NAS of Ukraine (Kharkiv) were used. The animals were provided with standard laboratory chow and water *ad libitum*. All manipulations with animals were approved by the Bioethics Committee of the Institute for Problems of Cryobiology and Cryomedicine of the NAS of Ukraine (Protocol No. 4, dated of June 16th, 2025) and comply with the main provisions of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (Strasbourg, 1986) and the Law of Ukraine 'On the Protection of Animals Against Cruelty'.

Isolation of testicular interstitial cells. Testis isolation was performed as described [5]. Cervical dislocation was used for the slaughtering of rats. The animals' bodies were immersed in 75% ethanol for 5 minutes. The testes were isolated, decapsulated, and the blood vessels were trimmed from the testes. They were then placed in 15-ml centrifuge tubes containing 4 mL DMEM F12 per testis, supplemented with enzymes: 0.2 mg/mL collagenase (type I) (Sigma-Aldrich, USA) and 0.1 mg/mL DNase I (Sigma-Aldrich, USA) for 10 minutes. The tubes were incubated in a thermostated shaking water bath at 90 cycles/min and 34 °C. Then, 10 mL of collagenase-free DMEM F12 (Biowest, France) were added to each tube. The seminiferous tubule mass was removed by filtration through doubled 100 μm nylon mesh. The filtrates were centrifuged at 325 g for 3 minutes at room temperature. Supernatants were discarded, and the residues were resuspended in 10 mL phosphate-buffered saline (PBS). The sedimentation procedure was repeated. Cell concentration was adjusted to 4×10^7 cells per mL with PBS. Then, the cell suspensions were diluted 1 : 1 with concentrated cryoprotective media to reach the final concentration of additives (see below). Thus, the final cell concentration in the sample came to 2×10^7 cells/mL.

For the measurement of cell survival parameters, ICs mixed with 0.4% Trypan Blue dye (1 : 1) were counted using a hemacytometer chamber. General cell survival was determined as the ratio of the total number of cells in the sample after incubation to the number of cells in PBS (300 mOsm) before incubation, multiplied by 100%. Cells unstained by Trypan Blue dye were considered to have intact membranes. The survival of cells with intact membranes was calculated as the ratio of the number of unstained cells in the sample after incubation to the number of unstained cells in PBS (300 mOsm) before incubation, multiplied by 100%.

To measure the IC metabolic activity after incubation, 50 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma-Aldrich, USA) solution in PBS (5 mg/mL) was added to 500 μL of the IC suspension following incuba-

tion at 4 °C and medium removal. The cells were incubated with MTT for 2 hours at 35 °C. Then, ICs were sedimented at 325 g for 3 minutes, and the supernatant was discarded. Next, 450 μL of DMSO was added to the stained pellet, and the samples were stirred. After 5 minutes, the samples were centrifuged, and the optical density (extinction) of the supernatant was measured at 530 nm. Samples containing no cells were used as a reference. Metabolic activity preservation was calculated as the ratio of the optical density of the sample after incubation to that of a sample in PBS (300 mOsm) before incubation, multiplied by 100%.

Modeling. The modeling was based on a nonlinear equation and the Kedem–Katchalsky model [13], as modified by Gordienko Ye.A. and Pushkar M.S. [8], and adapted for a single cell. It was used to determine the hydraulic conductivity, DMSO coefficient of penetration, the activation energies of water and DMSO transport across the membrane [20]. Prediction of the osmotic behavior of cells under various cooling rates was carried out using these previously determined values of activation energies as described by Tarusin D.N. *et al* [26]. The used activation energies are represented in Table 1. The kinetics of changes in the concentration of the solution surrounding the cells during crystallization were modeled by approximating the phase diagram of the melting process of aqueous DMSO solutions, as described by Todrin O.F. *et al.* [27].

The ICs were subdivided into two groups. One specific type was microscopically distinguishable from the other cells due to its noticeably larger volume. The diameters of these "large cells" came to about 18.3 to 19.8 μm. Thus, they were referred to as "large cells" (L-ICs), in contrast to the remaining cells within the ICs, which were designated as "small cells" (S-ICs).

Cryopreservation. A volume of 0.5 mL of ICs (4×10^7 cells/mL) was diluted 1 : 1 with concentrated cryoprotective media (4 °C) in 1.8 mL cryocontainers (Nunc, Denmark) to achieve the final cryoprotective media composition. The nucleation procedure, based on the capabilities of the freezer, was applied. However, the selection of parameters for this procedure was a technical task addressed in this study and described in detail in the "Results and Discussion" section. After nucleation initiation, samples were cooled at one of the programmable rates: 1, 2, 5, or 10 °C/min. A copper-constantan thermocouple was used to monitor temperature changes in control samples and within the cryochamber of the programmable freezer. Once the final temperature was reached, samples were held at –30 °C in the freezer chamber for 7 minutes to equalize the chamber and the sample temperature. After reaching the final temperature, cryocontainers were transferred to a 37 °C water bath and warmed. Samples remained in the water bath until the crystalline phase disappeared. Cryopreservation media were then replaced with isosmotic PBS by gradual dilution with isosmotic DMEM F12. Cell survival indices were subsequently measured.

Statistical analysis. Data were presented as median, 25^{th} and 75^{th} percentile, maximum, and minimum. The Mann-Whitney U test with Bonferroni correction was applied for paired comparisons; $p \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

The indices of membrane permeability can help model the degree of cell dehydration at different cooling rates [26]. The parameters of cell membrane permeability that determine cell osmotic behavior and the degree of dehydration—the osmotically inactive volume, hydraulic conductivity, coefficient of membrane penetration for DMSO, and corresponding activation energies for water and DMSO transport across IC membranes—were determined by applying the Kedem-Katchalsky formalism in our previous research [20]. In the present work, these parameters were first used to predict volume changes of ICs in cryopreservation media designed for IC suspension: 0.7DMSO + + D40 and 1.4DMSO + FBS. Additionally, we modeled osmotic changes for ICs during cooling in media supplemented only with DMSO for comparison, as this additive may exert a strong documented osmotic and toxic effect [7, 12, 21, 23, 28]. The ICs form a heterogeneous cell suspension in which cells can be conditionally subdivided into two populations: a more uniform population of large ICs (L-ICs) and a more heterogeneous population of small ICs (S-ICs).

According to the modeling in 0.7DMSO + D40, cooling at a rate of ≤0.75 °C/min can lead to a drop in L- and S-IC volume to the minimum (to the osmotically inactive — 0.3 and 0.2, respectively), which may result in cell damage due to the "solution effect" (Fig. 1*a*, *b*). Higher cooling rates of 1—2 °C/min may result in moderate volumetric chang-

es, where ICs lose about 15—50% of their volume depending on the rate and initial cell volume. A rate ≥5 °C/min may cause no change in cell volume, increasing the probability of intracellular ice formation.

However, the model does not take into account that membrane permeability may increase sharply at a critical temperature due to lipid membrane phase transitions and/or membrane damage under increased solute concentration [1]. So, cooling rates higher than 1—2 °C/min might also be beneficial. Interestingly, 0.1 °C/min in 0.7 M DMSO may cause significant dehydration of L-ICs to the minimum, while 0.5 and higher can result in water retention in the cells (Fig. 1*c*). In order to achieve moderate dehydration, higher concentrations of DMSO should be used for the cells (Fig. 1*e*), but this is fraught with the risk of toxic effects of DMSO described in our previous works [21, 23]. This strategy to increase cell survival after cryopreservation was implemented using the serum-containing medium 1.4DMSO + FBS, which has a similar profile of cell volumetric changes (Fig. 1*g*, *h*). Our previous research showed that excessive contact of ICs with this cryoprotective medium can decrease the survival of ICs after cryopreservation [20].

As for S-ICs, the range of cooling rates appears to be wider than for L-ICs (Fig. 1*b*, *d*, *f*, *h*). However, this group of cells is heterogeneous and includes many types of cells of different sizes and volumes. The S-ICs exhibit varying sensitivity to cooling rates. Therefore, for a heterogeneous group, partial dehydration before cooling, shown in [20, 21, 23] for ICs in 0.7DMSO + D40, can reduce sensitivity to cooling rates. The same applies to L-ICs. This feature of 0.7DMSO + D40 may be advantageous for the cryopreservation of heterogeneous cell suspensions such as ICs.

The modeling of cell osmotic changes upon water crystallization does not take into account the degree of supercooling. A 1-ml sample with ICs in 0.7DMSO + D40 tended to nucleate spontaneously at about −10.5 °C (Fig. 2) at a cooling rate of 1 °C/min in the interval 4…−80 °C (Table 2, Program 1). Nucleation caused a significant increase in sample temperature up to its melting point, followed by a "plateau" formed due to equilibrium between the exothermic latent heat release and heat removal through the cryocontainer walls. After the crystallization of bulk water, the cooling rate of the sample increased. This increase was driven by the significant temperature difference between the sample and the chamber. Subsequently, the temperature changes in the sample became similar to those in the chamber in the interval of −37…−80 °C.

The temperature of nucleation (the degree of supercooling) of a medium may considerably impact the degree of cell dehydration and the probability of intracellular ice formation. To compare the influence of cooling rates on cell survival, equal conditions must be guaranteed. Therefore, the second part of the work was devoted to developing parameters for controlled nucleation in samples with 0.7DMSO + D40. Sharp and potentially more dangerous intracellular dendritic ice crystals may form when the nucleation temperature drops well below the melting point of the solution [16, 24]. Controlled nucleation at temperatures near the melting point and subsequent crystallization under more equilibrium conditions has been shown to be beneficial for the cryopreservation of different types of cells [2, 4].

During the procedure of controlled ice nucleation with a programmable freezer, the chamber with samples is cooled rapidly, creating a "cold spot" somewhere on the wall of the cryocontainer,

Table 1. **Values of activation energies used for the modeling, kJ/mol**

Solutions	Large ICs		Small ICs	
	Water, kJ/mol	DMSO, kJ/mol	Water, kJ/mol	DMSO, kJ/mol
0.7 M DMSO	37.9 (37.4; 41.9)	64.6 (56.4; 66.2)	26.3 (25.4; 39.1)	63.9 (56.2; 64.2)
1.4 M DMSO	35.7 (32.6; 43.8)	61.0 (60.3; 61.3)	20.4 (17.6; 32.4)	66.4 (55.3; 73.5)
0.7DMSO + D40	29.8 (28.9; 37.0) *	42.6 (40.9; 62.6) *	34.5 (31.8; 36.6) *	30.1 (26.0; 35.6) *
1.4DMSO + FBS	31.9 (26.9; 41.6) *	65.5 (61.7; 67.9)	26.4 (18.8; 27.7)	63.8 (60.0; 67.3)

* Differences are statistically different from 0.7 M DMSO, $p \leq 0.05$.

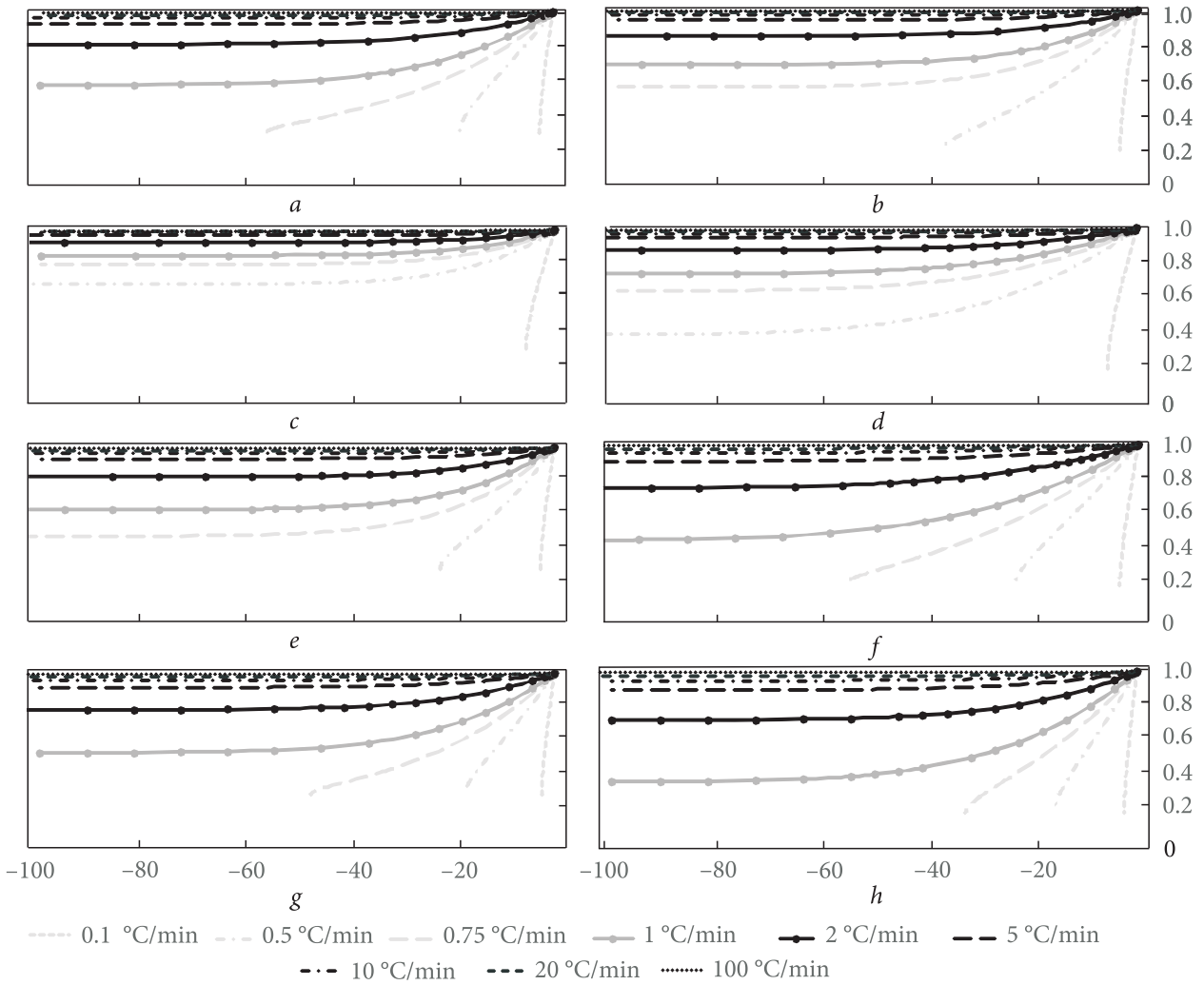

Fig. 1. Modeling volumetric changes of ICs depending on cooling rates: *a* — L-ICs in 0.7DMSO+D40; *b* — S-ICs in 0.7DMSO + D40; *c* — L-ICs in 0.7 M DMSO; *d* — S-ICs in 0.7 M DMSO; *e* — L-ICs in 1.4 M DMSO; *f* — S-ICs in 1.4 M DMSO; *g* — L-ICs in 1.4DMSO + FBS; *h* — S-ICs in 1.4DMSO + FBS

resulting in ice nucleation. This type of ice nucleation is called "shock cooling" or "seeding" [16]. The parameters of this procedure, such as the duration of the cooling ramp and its final temperature, were selected for 0.7DMSO + D40. Table 2 shows the parameters of the program steps without nucleation (Program 1) and with the selected parameters for controlled nucleation (Program 2). The use of the selected parameters prevented excessive supercooling of the samples (Fig. 3*a*). This also guaranteed more uniform cryopreservation conditions for all samples, eliminating the stochastic factor of spontaneous nucleation. It can be seen that, notwithstanding the rapid temperature drop in the cryochamber during nucleation initiation, the sample's temperature did not change significantly. However, this triggered nucleation near the melting temperature and water crystal growth, which can be observed as a "temperature plateau" on the thermogram.

Although running the two programs resulted in no difference in overall cell survival and plasma membrane preservation, Program 2 with the initiation of the crystallization step proved superior, as the index of IC metabolic activity was significantly higher (Table 2).

The final part of the work was to study the effect of cooling rates during real cryopreservation and to compare the empirical data with those obtained from modeling. Modeling showed that the cooling rates of the samples with ICs in 0.7DMSO + D40 can be increased relative to the rate of 1 °C/min used in the second part of the work. After the step of water crystallization initiation followed by a stabilization step, the samples were cooled at one of the selected rates: 1, 2, 5, or

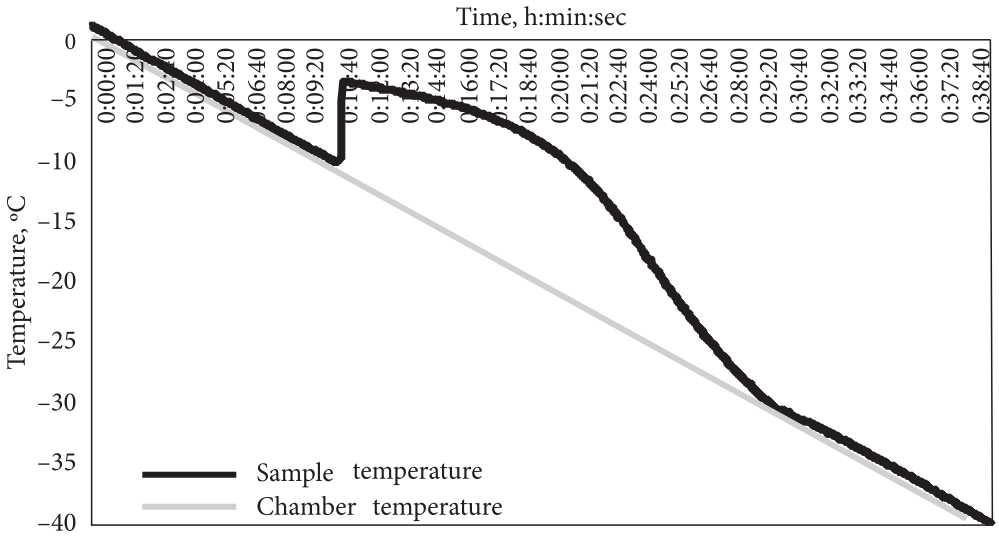

Fig. 2. Characteristic temperature changes in the chamber of the programmable freezer and in the sample with ICs in 0.7DMSO + D40 cooled using program 1

10 °C/min. Although the temperature change in the chamber of the programmable freezer was controlled, the temperature changes inside the samples lagged behind the chamber temperature (Fig. 3). Some average estimates of the cooling rates of the samples are given in Table 3.

Increasing the cooling rate to 2 °C/min led to a slight increase in general cell survival but no changes in preservation of the plasma membrane or metabolic activity. Higher cooling rates caused a significant decrease in the cell survival indices (Fig. 4). The cooling rates of the chamber and the samples were similar when the set cooling rate was within 1—2 °C/min. According to the modeling, L-ICs lost about 40 and 20 % of their volume when cryopreserved at cooling rates of 1 and 2 °C/min, respectively. As for S-ICs, they lost about 30 and 15% of their volume, respectively. Thus, the degree of

Table 2. **Steps of cooling programs without controlled nucleation (Program 1) and with the developed procedure of controlled nucleation (Program 2)**

Parameters	Program 1	Program 2
Program steps	CT, 3 min, 4 °C PK (loading samples) OH, 1 °C/min, −80 °C CT, 2 min, −80 °C PK (unloading samples)	CT, 3 min, 4 °C PK (loading samples) CT, 5 min, 4 °C KP, 90 sec, to −60 °C CT, 7 min, −4 °C OH, 1 °C/min, −80 °C CT, 2 min, −80 °C PK (unloading samples)
General cell survival, %	37 (36; 41)	37 (36; 41)
Plasma membrane preservation, %	24 (21; 34)	30 (28; 38)
Metabolic activity preservation, %	52 (46; 54)	67 (64; 68) *

CT — stabilization of the programmable freezer chamber at a given temperature and time; PK — loading/unloading of the samples to/from the chamber of the programmable freezer; OH — cooling of the chamber at a given rate and to the final temperature; KP — a procedure for initiating crystal formation provided by a programmable freezer, which involves rapidly lowering the chamber temperature over a certain period of time to a set minimum temperature. The parameter highlighted in bold and underlined could have changed when studying the effect of cooling rate. The indices of IC survival were represented as median (25^{th} and 75^{th} percentile). * The value was statistically different from the corresponding value of program 1, $p < 0.05$.

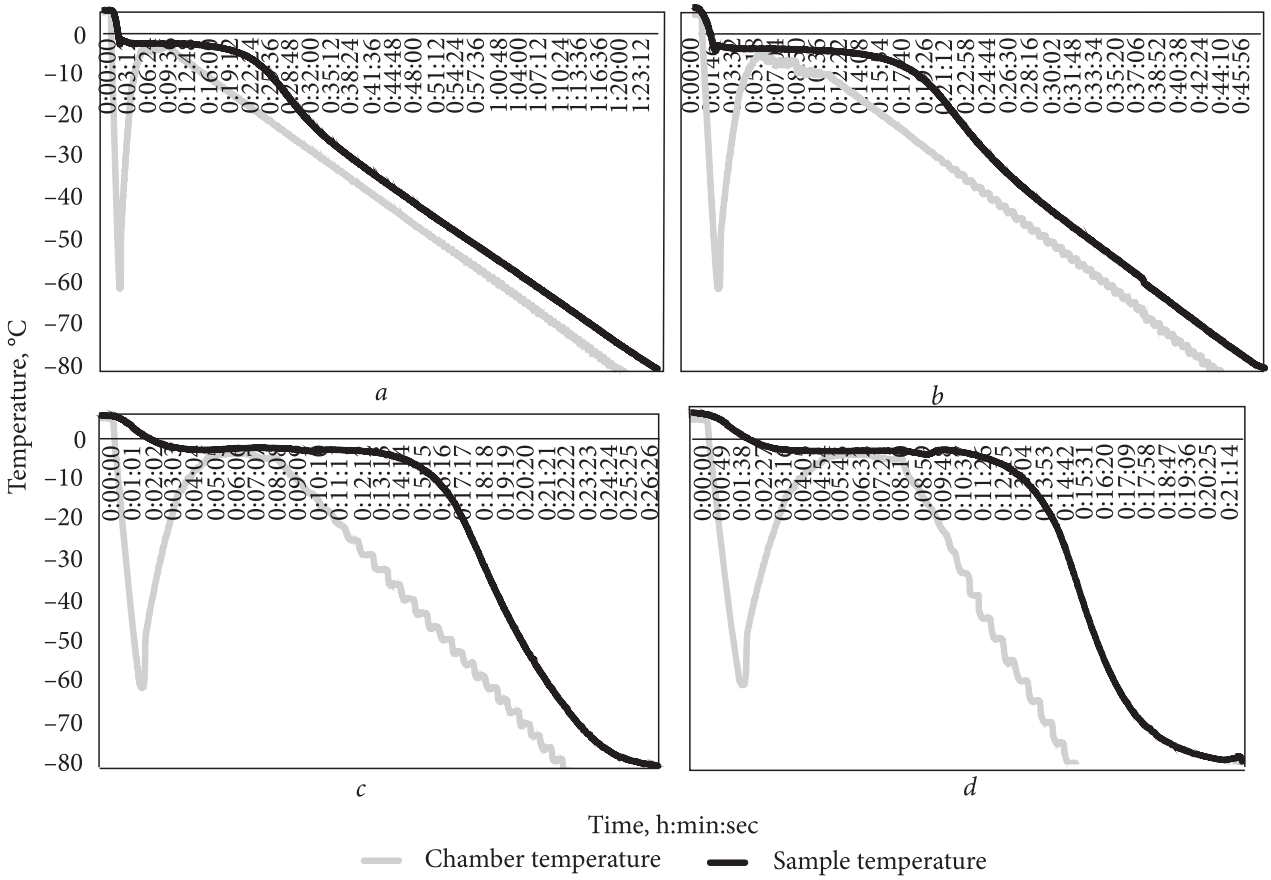

Fig. 3. The profile of temperature changes in the samples and in the chamber of the programmable freezer. The procedure of initiation of nucleation was applied. This nucleation step was followed by cooling at a rate: *a* — 1, *b* — 2, *c* — 5, *d* — 10 °C/min

dehydration during cryopreservation should be kept within the range of 20—30%. Higher rates of temperature changes in the samples (>2 °C/min) during cooling cause insufficient dehydration of ICs and, according to Mazur's two-factor theory, damaging intracellular ice formation. Furthermore, the modeling shows that the use of lower cooling rates (<1 °C/min) has no prospects, as it may increase the period of contact of the biological material with toxic DMSO at higher temperatures and expose cells to a hyperconcentrated solution formed upon extracellular ice formation during cooling. Lower indices of cell survival were also demonstrated experimentally at the same experimental conditions (not shown).

The modeling also demonstrates that the use of 0.7 M DMSO without D40 makes optimization of the cooling rate impossible: the cooling rates achievable with a programmable freezer either lead to a significant drop in volume or increase the prob-

Table 3. **Cooling rate assessments in the chamber of programmable freezer and in the samples with 0.7DMSO + D40, °C/min**

Conditions of cooling rate assessments	Cooling rate, °C/min			
Cooling rate of the chamber of the programmable freezer after initiation nucleation	1.0	2.0	5.0	10.0
Average cooling rate of the sample from 4 to −80 °C	1.0	1.8	3.1	3.8
Average cooling rate of the samples from the beginning of nucleation to −80 °C	1.1	1.8	3.5	5.9
Average cooling rate of the samples assessed in the interval after initiation of nucleation −4…−80 °C	1.1	2.2	5.8	6.9

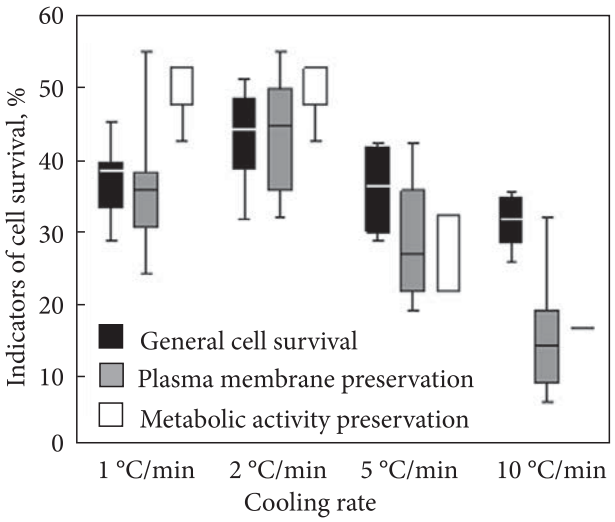

Fig. 4. Indices of IC survival under different cooling rates. The procedure of initiation of nucleation was applied. The general cell survival and plasma membrane preservation were assessed microscopically with Trypan Blue dye, metabolic activity preservation by MTT-test. *The indices are statistically different from 2 °C/min, ($p \leq 0.05$); # — indices are statistically different from 1 °C/min, ($p \leq 0.05$)

ability of intracellular crystallization. Considering that we are dealing with a heterogeneous cell suspension, such a solution will not provide moderate dehydration for most of the cells in this suspension. It was shown that D40 promotes partial dehydration of ICs before cryopreservation and prevents excessive cell volume excursion during cryopreservation [24]. D40 and its combination with 0.7 M DMSO, apart from other media, do not change the degree of membrane dehydration and ordering [22]. Additionally, dextran may cover some membrane defects [3]. In this work, we have shown that its use makes the cells in the heterogeneous suspension (ICs) less sensitive to deviations from the optimal cooling rate for each individual cell. Notably, the optimal cooling rate is difficult to determine and achieve (if it is even possible) when the cell suspension consists of different cell types, as conditions suitable for one type may be less suitable for another.

According to the modeling the concentration of DMSO can be increased instead of using D40, which, on the one hand, may help control cell dehydration over a wider range of cooling rates and decrease the probability of intracellular ice formation; on the other hand, this approach is linked with excessive DMSO concentrations potentiating its toxic effects [18, 24, 19, 21]. The use of a higher DMSO concentration (1.4 M) was demonstrated for the serum-containing medium 1.4DMSO + + FBS. However, despite the satisfactory cryopreservation outcome in terms of cell survival, the use of serum carries the risk of transmitting infections and batch-to-batch variability in medium composition [6, 11, 14]. Additionally, as mentioned above, a higher DMSO concentration increases the risk of toxicity. The optimization of cooling rates for this serum-containing medium was demonstrated in our previous works [9, 10, 17].

The presented model, based on the Kedem-Katchalsky formalism, is a simplification, failing to account for factors that develop during actual cryopreservation: changes in membrane permeability under the influence of CPAs and other substances, changes in membrane viscosity, fluidity, and order, especially at high concentrations, changes in membrane potential, solution properties such as deviations from ideality or significant stabilization of hydration shells around cryoprotectant molecules with decreasing temperature, phase transitions in membranes with temperature changes, membrane damage during crystal growth, cell behavior deviating from an "ideal osmometer", *etc*. Perhaps adjusting the model to account for these parameters could be fruitful in predicting cell behavior during cryopreservation. However, even in its original form, this model allows for understanding the main trends occurring in a cell suspension with changes in cryoprotectant concentration and cooling, and, accordingly, for adjusting cryopreservation parameters.

CONCLUSION

In this work, we demonstrated the possibility of optimizing cryopreservation parameters by manipulating nucleation and adjusting the cooling rate. Using modeling, we identified the optimal degree of cell dehydration and explained some mechanisms of cryoprotection when using a combined serum-free medium based on DMSO and dextran (0.7DMSO + D40). It was shown that this medium allows us to find cryopreservation parameters satisfying the demands of various cell types in heterogeneous suspensions. This, in turn, opens up prospects for the use of such media for cryopreservation of different biological samples in both research and clinical settings.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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МОДЕЛЮВАННЯ ОСМОТИЧНОЇ ПОВЕДІНКИ КЛІТИН ПІД ЧАС ОХОЛОДЖЕННЯ ТА КРИСТАЛІЗАЦІЇ ВОДИ

Для успішного збереження клітинних суспензій важливим є визначення такого параметра кріоконсервування, як швидкість охолодження. Метою цього дослідження було оцінити вплив швидкості охолодження та нуклеації на виживаність інтерстиціальних клітин (ІК) сім'яника, а також змоделювати осмотичну поведінку клітин на основі формалізму Кедема–Качальського під час кристалізації води. Клітини інтерстицію охолоджували в безсироватковому середовищі, який містив 0,7 М ДМСО та 100 мг/мл декстрану 40 (0,7ДМСО + Д40). Моделювання осмотичної поведінки КІ у середовищі, яке засноване на експериментально визначених показниках мембранної проникності для води та ДМСО і відповідних енергіях активації, показало потенційну можливість підвищення швидкості охолодження порівняно зі «стандартною» 1 °С/хв. Цю можливість було перевірено експериментально із застосуванням вищих швидкостей охолодження — 2, 5 та 10 °С/хв. Було показано, що швидкість охолодження 2 °С/хв у поєднанні з контрольованими параметрами нуклеації є сприятливою для збереження КІ у середовищі з декстраном і ДМСО. Підхід, який включає моделювання, використання хімічно визначених безсироваткових композицій, контрольовану нуклеацію та оптимізацію швидкостей охолодження, може бути застосований до кріоконсервування інших типів клітин у розчинах із хімічно визначеним станом.

***Ключові слова*:** швидкість охолодження, нуклеація, модель Кедема-Качальського, безсироваткові середовища, декстран, диметилсульфоксид, клітини інтерстицію сім'яника, кріоконсервування.