

Cellular Response of Adipose Derived Passage-4 Adult Stem Cells to Freezing Stress

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A differential scanning calorimeter technique was used to generate experimental data for volumetric shrinkage during cooling at 20°C/min in adipose derived adult stem cells (ASCs) in the presence and absence of cryoprotective agents (CPAs). By fitting a model of water transport to the experimentally determined volumetric shrinkage data, the membrane permeability parameters of ASCs were obtained. For passage-4 (P4) ASCs, the reference hydraulic conductivity L_{pg} and the value of the apparent activation energy E_{Lp} were determined to be $1.2 \times 10^{-13} \text{ m}^3/\text{Ns}$ and 177.8 kJ/mole, respectively. We found that the addition of either glycerol or dimethylsulfoxide (DMSO) significantly decreased the value of the reference hydraulic conductivity $L_{pg}(cpa)$ and the value of the apparent activation energy $E_{Lp}(cpa)$ in P4 ASCs. The values of $L_{pg}(cpa)$ in the presence of glycerol and DMSO were determined as 0.39×10^{-13} and $0.50 \times 10^{-13} \text{ m}^3/\text{Ns}$, respectively, while the corresponding values of $E_{Lp}(cpa)$ were 51.0 and 61.5 kJ/mole. Numerical simulations of water transport were then performed under a variety of cooling rates (5–100°C/min) using the experimentally determined membrane permeability parameters. And finally, the simulation results were analyzed to predict the optimal rates of freezing P4 adipose derived cells in the presence and absence of CPAs.

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Introduction

Preservation of adult stem cells in the frozen state for later clinical and biotechnological use is an area of ongoing interest. Cryopreserved adipose-derived adult stem cells (or progenitor cells isolated from fat tissue exhibiting multilineage potential and differentiation in vitro toward fat, cartilage, muscle, bone, and nerve cells) could provide a steady supply of these cells to develop tissue engineering strategies and cell-based therapies [1–4]. To rationally develop optimal freezing storage protocols, it is instructive to perform quantitative freezing studies of isolated adipose derived adult stem cells (ASCs) in the presence and absence of cryoprotective agents.

During freezing ice forms first in the extracellular space surrounding the cells, then depending upon the cooling rate, water transport and/or intracellular ice formation will occur. At sufficiently slow cooling rates, as the amount of ice increases, the effective osmolarity of the extracellular unfrozen fraction increases over that of the unfrozen intracellular solution. This concentration gradient drives water out of the cell in an attempt to maintain equilibrium with the extracellular solution. During this dehydration process, cellular injury can result from long term exposure to the high concentrations of electrolytes which is termed “solute effects” injury. Alternately, if cooling occurs too rapidly for any water transport to occur (rapid cooling), the water inside the cell supercools, nucleates, and grows into large stable intracellular ice crystals which have been directly correlated to cell death after a freeze-thaw protocol. A plot of survival versus cooling rate is usually shaped like an inverted “U,” with maximum survival being attained at intermediate rates where intracellular ice formation is avoided and yet the cooling process occurs quickly enough to reduce “solute effects” injury [5]. Thus, to optimize and generate a firm biophysical understanding of the freezing process

in any biological system, both water transport (dehydration) and intracellular ice formation (IIF) need to be experimentally determined. However, biophysical knowledge of either one is typically sufficient to construct an optimal cryopreservation protocol for that cell type (since water transport parameters can be used to predict potential supercooling leading to IIF and cellular dehydration as shown in the present study; and conversely, ice nucleation parameters can be used to predict cooling rates that potentially cause excessive cellular dehydration and IIF).

This study reports the experimental measurements of the changes in the volumetric shrinkage and biophysical parameters of passage-4 adipose derived adult stem cells (ASCs) during freezing in the presence of commonly used cryoprotective agents, glycerol and DMSO. The biophysical water transport parameters L_{pg} and E_{Lp} were determined for additions of 0% and 10% (v/v) concentrations of dimethylsulfoxide (DMSO) and glycerol. The experimentally determined ASC membrane permeability parameters were used to numerically predict the loss of intracellular water under a variety of cooling rates (5–100°C/min, i.e., a range of cooling rates that encompasses both excessive cellular dehydration and the entrapment of a significant portion of the intracellular water). And finally, the numerical predictions were analyzed to predict the optimal rate of freezing adipose derived adult stem cells.

Theoretical Background

During subzero transport (as occurs in the presence of extracellular ice during freezing) the available data suggests that water transport occurs while cryoprotective agent (CPA) and solute (electrolyte) transport is negligible [6]. We further assume that the major resistance to the passage of water is the plasma membrane and not the cytoplasm or the extracellular space [7]. Assuming that water is essentially the only species transported on the basis of the above stated evidence, and that the membrane represents the major resistance to its flow out of the cell, a thermodynamic model for the prediction of water transport in the presence of CPAs was selected as [7–10]

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$$\frac{dV_{\text{cell}}}{dT} = -\frac{L_p A R T}{B \nu_w} \times \left[\ln \left(\frac{(V_{\text{cell}} - V_b) - (n_s \nu_s + n_{\text{cpa}} \nu_{\text{cpa}})}{(V_{\text{cell}} - V_b) - (n_s \nu_s + n_{\text{cpa}} \nu_{\text{cpa}}) + \nu_w (\psi_s n_s + n_{\text{cpa}})} \right) - \frac{\Delta H_f}{R} \left(\frac{1}{T_R} - \frac{1}{T} \right) \right], \quad (1)$$

where R is the universal gas constant; V_{cell} is the cell volume; V_b is the osmotically inactive cell volume; A is the constant cell surface area; T is the absolute temperature; T_R is the reference temperature (273.15 K); B is the cooling rate ($^{\circ}\text{C}/\text{min}$); ν_w is the molar specific volume of water; ν_{cpa} is the specific molar volume of DMSO; n_{cpa} is the number of moles of CPA; ψ_s is the dissociation constant for salt in water ($=2$); n_s is the number of moles of salt initially inside the cell; ΔH_f is the latent heat of fusion of water; L_p is the permeability of the plasma membrane to water. The permeability of the plasma membrane to water, L_p , is assumed to be a function of temperature and concentration of solute, CPA and water, and is given as

$$L_p = L_{pg}(\text{cpa}) \exp \left[-\frac{E_{Lp}(\text{cpa})}{R} \left(\frac{1}{T} - \frac{1}{T_R} \right) \right], \quad (2)$$

where R is the universal gas constant; $L_{pg}(\text{cpa})$ is the permeability of the cell membrane to water at the reference temperature (273.15 K); $E_{Lp}(\text{cpa})$ is the apparent activation energy for the process and is the absolute temperature. The modification simply allows for the biophysical parameters $L_{pg}(\text{cpa})$ and $E_{Lp}(\text{cpa})$ to depend on the concentration of a chemical additive such as DMSO or glycerol. Model assumptions include membrane limited transport, with negligible temperature and pressure differentials across the plasma membrane, the external solution in equilibrium with extracellular ice, and an approximately ideal internal solution [7–10].

Materials and Methods

Isolation, Collection, and Culture of ASCs. All the human protocols were reviewed and approved by the Pennington Biomedical Research Center Institutional Review Board. Subcutaneous adipose tissue liposuction aspirates were provided by plastic surgeons in Baton Rouge, LA. All procedures were conducted under aseptic conditions according to a modification of methods outlined elsewhere [2,3]. Tissue samples (100–200 ml) were washed three to four times in phosphate buffered saline (PBS) prewarmed to 37°C , suspended in PBS supplemented with 1% bovine serum albumin and 0.1% collagenase (Type I, Worthington Biochemicals, Lakewood, NJ), and digested with gentle rocking for 45–60 min at 37°C . The digests were centrifuged for 5 min at 1200 rpm ($300 \times g$) at room temperature, resuspended, and the centrifugation step repeated. The supernatant was aspirated and the pellet resuspended in culture medium (DMEM high glucose, 10% fetal bovine serum, 100 units penicillin/ml, 100 mcg streptomycin/ml, and 25 mcg amphotericin/ml). The cell suspension was plated at a density equivalent to 0.156 ml of liposuction tissue per sq cm of surface area, using a 35 ml volume of culture medium per T225 flask. Cells were cultured for 48 h in a 5% CO_2 , humidified, 37°C incubator. At that time, the adherent cells were rinsed once with prewarmed PBS and the cells fed with fresh culture medium. The cells were fed with fresh culture medium every 2–3 days until they reached approximately 75–80% confluence. At this time, the medium was aspirated, the cells were rinsed with prewarmed PBS, and harvested by digestion with 0.05% trypsin solution (5–8 ml per T225 flask) for 3–5 min at 37°C . The cells were suspended in culture medium, centrifuged for 5 min at 1200 rpm ($300 \times g$), the pellet resuspended in a volume of 10 ml of culture medium, and the cell count determined by trypan blue

exclusion. These cells were identified as passage 0 (P0). The cells were reseeded in T225 flasks at a density of 5×10^3 cells per sq cm. The cells were maintained in culture and passaged as described through successive passages 0–4 (P0–P4). In this study P4 ASCs were used to evaluate the membrane permeability parameters and consequently optimal cooling rates for freezing adipose derived stem cells.

Differential Scanning Calorimeter (DSC) Experiments. DSC experiments were carried out both in the absence and presence of CPAs on adipose derived cells. Six separate DSC experiments were conducted in the absence of any CPAs and in the presence of two permeating CPAs, glycerol (10%, v/v), and dimethylsulfoxide (DMSO; 10%, v/v). The DSC dynamic cooling protocol used to measure the water transport out of the adipose derived cells was the same as that reported in earlier studies on other cell systems [11–16] and will only be briefly stated here. In the DSC technique, heat releases from the same cell suspension are measured: (i) during freezing of osmotically active (live) cells in media and; (ii) during freezing of osmotically inactive (dead) cells in media. The difference in heat release measured between the two cooling runs was correlated to water transport and was denoted as Δq_{dsc} . This has been demonstrated in a variety of cellular systems [11–16] and independently verified [17,18]. Should the cells be osmotically inactive or lysed prior to the start of the experiment, the DSC cooling protocol will measure no difference in heat release, as described in earlier studies [11–16].

Translation of DSC Heat Release Data to Water Transport Data. The heat release measurements of interest were Δq_{dsc} and $\Delta q(T)_{\text{dsc}}$ which are the total and fractional difference between the heat releases measured by integration of the heat flows during freezing of osmotically active (live) cells in media, q_{initial} , and during freezing of osmotically inactive (dead) cells in media, q_{final} (i.e., $\Delta q_{\text{dsc}} = q_{\text{initial}} - q_{\text{final}}$; $\Delta q(T)_{\text{dsc}} = q(T)_{\text{initial}} - q(T)_{\text{final}}$). As defined in a series of earlier studies by Devireddy and colleagues, this difference in heat release has been shown to be related to cell volume changes in several biological systems [11–16] as

$$V(T) = V_i - \frac{\Delta q(T)_{\text{dsc}}}{\Delta q_{\text{dsc}}} \cdot (V_i - V_e). \quad (3)$$

The unknowns needed in Eq. (3) apart from the DSC heat release readings are V_i (the initial cell volume) and V_e (the end or the final cell volume). The initial volumes (V_i) of the adipose derived (P4) cells in the medium with no CPAs was assumed to be the initial isotonic cell volume, V_o (the P4 ASC is found to be approximately spherical with a radius of 25 μm). Similarly, the end volume in the medium with no CPAs was assumed to be the osmotically inactive cell volume, $V_b (=0.6V_i)$. To ensure the accuracy and repeatability of the experimental data, a set of calibration and control experiments were performed as detailed previously for a DSC-7 (Perkin Elmer Corporation, Norwalk, CT) machine [11].

Biophysical Parameter Estimation. The biophysical parameters for water transport were then estimated using a nonlinear regression algorithm as previously reported [10–16,19]. The experimentally measured water transport data at $20^{\circ}\text{C}/\text{min}$ and molarity of CPA (either of glycerol or DMSO) were compared to theoretically predicted curves at $20^{\circ}\text{C}/\text{min}$ and molarity of CPA (Eqs. (1) and (2)). The biophysical parameters of water transport, $L_{pg}(\text{cpa})$ and $E_{Lp}(\text{cpa})$, were iteratively adjusted until the chi-squared variance, χ^2 , was minimized (or a goodness of fit parameter R^2 was maximized: a value of 1 representing a perfect fit while a value of 0 represents a flawed fit) using a gradient search method as described in Bevington and Robinson [20]. All reported parameters were obtained for curve fits with a variance $\chi^2 < 10^{-3}$ or an $R^2 > 0.99$. To simulate the biophysical response of an ASC under a variety of cooling rates, the best-fit parameters were substituted in the water transport equation which was then nu-

merically solved using a fourth-order Runge-Kutta method using a custom written FORTRAN code on a Mac Powerbook G4 (Apple Computer Inc, Cupertino, CA).

Results and Discussion

Dynamic Cooling Response and Membrane Permeability Parameters. Figure 1 shows the water transport data and simulation for P4 ASCs using the best-fit parameters in Eq. (1) at a cooling rate of 20°C/min in culture medium with no CPAs (Fig. 1(A)), in culture medium with 10% glycerol (Fig. 1(B)), and in culture medium with 10% DMSO (Fig. 1(C)). The dynamic portion of the cooling curve (region where the initial and final heat release thermograms are distinct and separate) is found to be between -0.6 and -14 °C in medium with no CPAs, between -3.1 and -24 °C with either 10% glycerol or 10% DMSO. The P4 ASC membrane permeability parameter values that best fit the water transport data in the absence and presence of CPAs are shown in Table 1. The model simulated equilibrium cooling response (equilibrium is achieved at each temperature when the internal and external osmotic pressures are equal) is also shown as reference as dotted line (- -). The equilibrium cooling response represents the volumetric shrinkage response of an ASC cooled infinitely slowly and is significantly different (>99% confidence level using the student's *t* test) from the dynamic water transport data obtained at 20°C/min.

Water Transport Simulations and Optimal Rates of Freezing. Water transport simulations obtained using the best fit parameters (shown in Table 1) in Eq. (1) are shown for a variety of cooling rates (5–100°C/min) in Fig. 2. From these water transport simulations, the amount of trapped water (or a lower bound on the intracellular ice volume or IIV) was computed as a ratio of the volume of the water trapped inside the ASCs at a temperature, *T* (~-30°C) relative to the initial ASC water volume, a strategy previously applied in several cell systems [10–16,19]. Hence IIV is represented as $(V - V_e)/(V_i - V_e)$ where *V* is the end volume after water transport ceases (at ~-30°C), and *V_i* and *V_e* are the initial (isotonic) and final (osmotically inactive) ASC volumes, respectively. A listing of the various values for IIV and the corresponding end volumes for P4 ASCs at various cooling rates is provided in Table 2.

As described earlier, the cooling rate which optimizes the freeze/thaw response of any cellular system can be defined as the fastest cooling rate in a given media without forming damaging intracellular ice formation, IIF [5]. Mazur [21] defines IIF as damaging and lethal if >10–15 % of the initial intracellular water is involved. We defined, for the adipose derived cells studied, the “optimal cooling rate” as the cooling rate at which 5% of the initial osmotically active water volume (or 5% IIV) is trapped inside the cells at temperature, *T* ~ -30°C. For P4 cells, the simulations (shown in Figs. 2(A)–2(C)) suggest the following cooling rates as optimal in the three media investigated: 21°C/min with no CPAs, 29°C/min with 10% glycerol as the CPA, and 22°C/min with 10% DMSO as the CPA (as shown in Table 1). Note that if intracellular ice formation (IIF) occurs by a heterogeneous or a surface catalyzed nucleation mechanism [22] (generally between -5 and -20°C for a variety of single cells), which our model does not predict, then potentially even more water will be trapped in the ASCs than predicted by water transport alone (i.e., the lower bound of intracellular ice discussed above). Thus, the “optimal cooling rates” (stated above) based on the lower bound of intracellular ice are probably overestimated.

Membrane Permeability Parametric Space. To assess the variability of the parametric space that best fit the measured water transport data the goodness of fit parameter *R*² was generated for a wide range of *L_{pg}* and *E_{Lp}* (or *L_{pg}*[cpa] and *E_{Lp}*[cpa]) values. To summarize the results of this analysis a series of contour plots are shown in Fig. 3. Any combination of *L_{pg}* and *E_{Lp}* (or *L_{pg}*[cpa] and

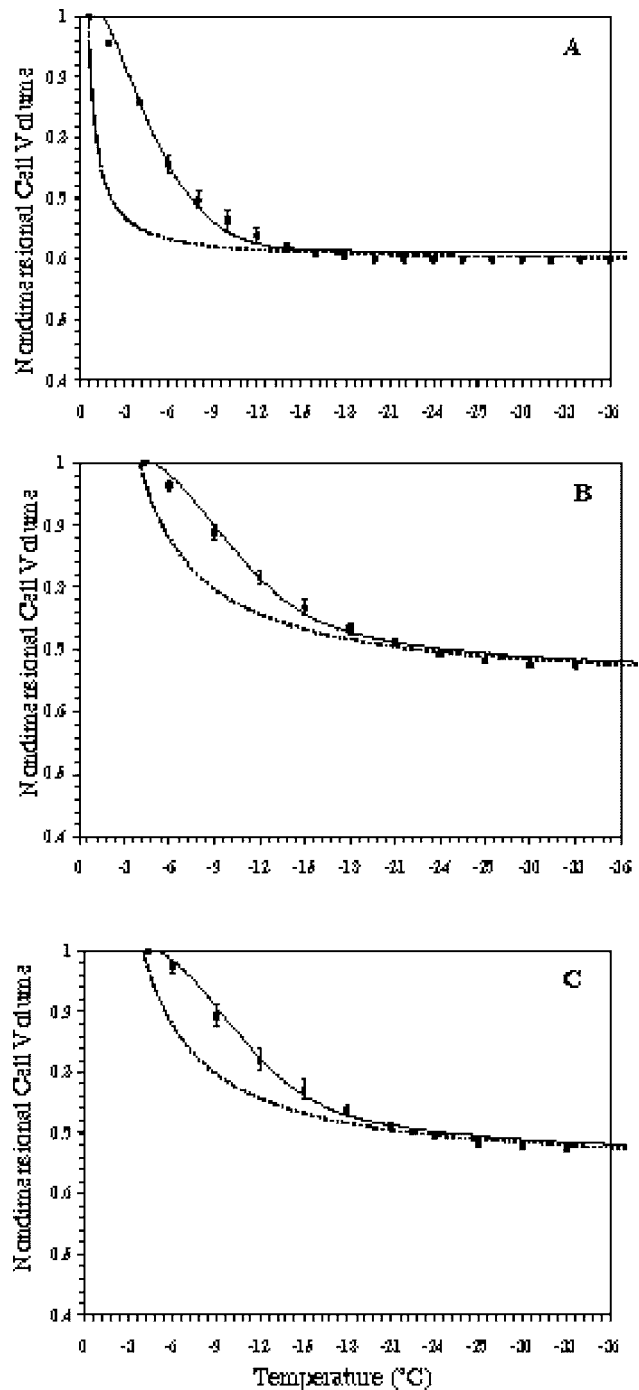


Fig. 1 Volumetric response of P4 ASCs as a function of subzero temperatures obtained using the DSC technique in the presence of extracellular ice (A), in the presence of extracellular ice and glycerol (B), and in the presence of extracellular ice and DMSO (C). The filled circles represent the experimentally obtained water transport (volumetric shrinkage) at a cooling rate of 20°C/min. The model-simulated dynamic cooling response at 20°C/min is shown as a solid line and was obtained by using the best-fit membrane permeability parameters (*L_{pg}* and *E_{Lp}* or *L_{pg}*[cpa] and *E_{Lp}*[cpa]) (Table 1) in the water transport equation (Eqs. (1) and (2)). The model-simulated equilibrium cooling response obtained is shown as a dotted line in all the figures. The nondimensional volume is plotted along the y axis and the subzero temperatures are shown along the x axis. The error bars represent the standard deviation for the mean values of six separate DSC experiments (*n*=6).

Table 1 Predicted subzero membrane permeability parameters (the reference membrane permeability in the absence and presence of CPAs, L_{pg} or L_{pg} [cpa], and activation energy in the absence and presence of CPAs, E_{Lp} or E_{Lp} [cpa]) and optimal rates of freezing for P4 ASCs. Note that the goodness of fit R^2 value was always greater than >0.99 . The values in parentheses in columns 3 and 4 represent the magnitude of L_{pg} or L_{pg} [cpa] in $\mu\text{m}/\text{min-atm}$ and the magnitudes of E_{Lp} or E_{Lp} [cpa] in Kcal/mole, respectively.

Assumed Inactive Cell Volume (V_b)	Concentration of CPA (v/v)	L_{pg} or L_{pg} [cpa] $\times 10^{13} \text{m}^3/\text{Ns}$ ($\mu\text{m}/\text{min-atm}$)	E_{Lp} or E_{Lp} [cpa] kJ/mol (kcal/mol)	Predicted Optimal Cooling Rate $^\circ\text{C}/\text{min}$
0.4 V_o	No CPA	1.86 (1.09)	180.4 (43.2)	21
	10% Glycerol	0.74 (0.43)	61.0 (14.6)	25
	10% DMSO	0.68 (0.40)	57.7 (13.8)	24
0.6 V_o	No CPA	1.20 (0.7)	177.8 (42.5)	21
	10% Glycerol	0.39 (0.23)	61.0 (12.2)	29
	10% DMSO	0.50 (0.29)	61.5 (14.7)	22
0.8 V_o	No CPA	0.56 (0.33)	171.4 (41.0)	22
	10% Glycerol	0.27 (0.16)	62.7 (15.0)	24
	10% DMSO	0.26 (0.15)	62.8 (15.0)	19

E_{Lp} [cpa]) shown to be within the contour in the Fig. 3 will “fit” the water transport data in that media with an R^2 value >0.98 . The three contours within Fig. 3 represent the three different media investigated, namely culture medium with no CPAs, culture media with 10% glycerol, and culture medium with 10% DMSO. Note that the contour for the media containing 10% glycerol and 10% DMSO are almost identical. This suggests that the membrane permeability parameters obtained in the presence of 10% DMSO could predict the volumetric response of the adipose derived (P4) cells in the presence of 10% glycerol quite accurately and vice versa. The reasons for the similarity in the water transport response (and consequently the membrane permeability parameters) in the presence of DMSO and glycerol for P4 ASCs are beyond the scope of this study and cannot be fully answered unless a mechanistic model describing the interactions and modifications caused by different chemicals on different cell membranes can be developed (such models are currently unavailable). Also note that the parameters obtained in the absence of CPAs are significantly different than those obtained in their presence (the contour plot corresponding to 0% of CPAs has no overlap with the contours obtained with 10% glycerol or 10% DMSO).

Effect of Varying V_b . There are no prior experimental studies that report the osmotically inactive cell volume fraction (V_b) of adipose derived adult stem cells (ASCs). Thus, we assigned a value of $0.6V_o$ as the inactive cell volume, a value that is commonly used for most mammalian cells [10–16,20,23]. To further study the effect of varying the V_b on measured membrane permeability parameters (L_{pg} and E_{Lp}) and consequently on the predicted optimal cooling rates, the value of V_b was increased to $0.8V_o$ and decreased to $0.4V_o$. The DSC data was correspondingly modified (using Eq. (3)) and the modified DSC water transport data was curve fitted to the water transport model (Eqs. (1) and (2)) using the nonlinear least-squares curve fitting technique as previously described. The predicted values of the membrane permeability parameters (L_{pg} and E_{Lp}) for adipose derived cells using a value of $0.4V_o$ and $0.8V_o$ as the osmotically inactive cell volume are also shown in Table 1. Note that as the assumed value of V_b increases the model predicted values of L_{pg} decreases in all the media. However, the behavior of E_{Lp} is more complex, without CPAs, it decreases as V_b increases. In the presence of 10% DMSO, E_{Lp} increases as V_b increases. While in the presence of

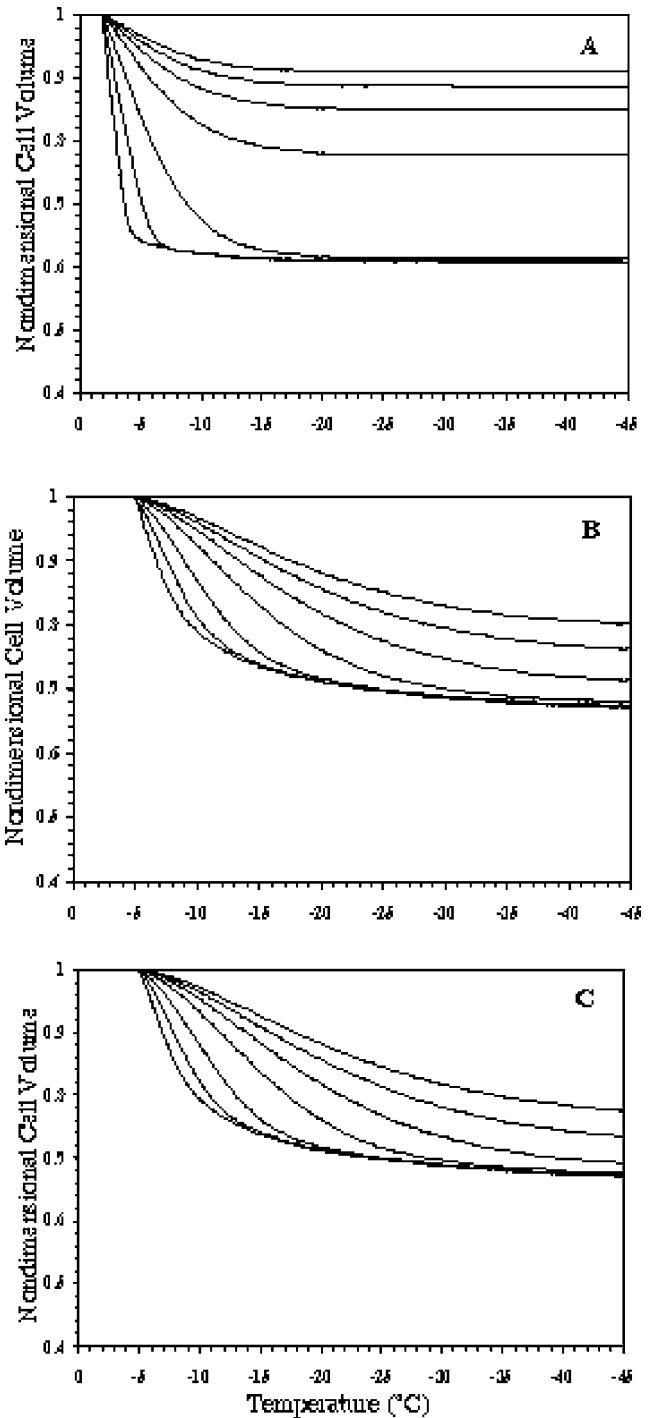


Fig. 2 Volumetric response of adipose derived (P4) cells at various cooling rates as a function of subzero temperatures using the best-fit membrane permeability parameters (shown in Table 1). The changes in the normalized cell volume (V/V_o) are shown as a function of temperature for different cooling rates in P4 ASC suspensions without CPA (A), with 10% glycerol (B), and with 10% DMSO (C). In all the figures, the water transport curves (solid lines) represent the model-simulated response for different cooling rates (from left to right: 5, 10, 20, 40, 60, 80, and $100^\circ\text{C}/\text{min}$). The subzero temperatures are shown along the x axis while the nondimensional volume is plotted along the y axis.

10% glycerol, E_{Lp} has a minimum at an assumed V_b value of $0.6V_o$ and increases as the value of V_b is varied, irrespective of

Table 2 Numerically predicted end volumes ($T \sim -30^\circ\text{C}$) and the amount of trapped water (or a lower bound on the intracellular ice volume or IIV) at various cooling rates, assuming an osmotically inactive cell volume of $0.6V_o$. IIV was computed as a ratio of the volume of the water trapped inside the ASCs at a temperature, $T(\sim -30^\circ\text{C})$ relative to the initial ASC water volume (see text for further details)

Concentration of CPA (v/v)		Cooling Rate ($^\circ\text{C}/\text{min}$)				
		<20	40	60	80	100
No CPA	End Volume at -30°C	$0.614V_i$	$0.778V_i$	$0.850V_i$	$0.879V_i$	$0.909V_i$
	% IIV	<3.5	44.5	62.5	69.8	77.3
10% Glycerol	End Volume at -30°C	$\leq 0.689V_i$	$0.700V_i$	$0.747V_i$	$0.795V_i$	$0.830V_i$
	% IIV	<4.8	8.2	28.1	37.1	47.9
10% DMSO	End Volume at -30°C	$\leq 0.689V_i$	$0.696V_i$	$0.734V_i$	$0.781V_i$	$0.818V_i$
	% IIV	<4.6	7.1	18.2	32.8	44.0

whether it is lowered to $0.4V_o$ or increased to $0.8V_o$. The exact reason for this anomalous behavior of P4 ASCs in the presence of glycerol is unclear and warrants further study.

Despite the differences in the predicted permeability (L_{pg} and $L_{pg}[\text{cpa}]$) values of P4 ASCs for different V_b values, a similar value ($\pm 20\%$) is obtained for the predicted optimal cooling rate (Table 1). This relative insensitivity of the predicted optimal cooling rate to the assumed value of V_b has been reported earlier for a variety of biological systems [10–16,23,24]. A possible explanation for the independence of the predicted optimal cooling rate from V_b could be due the following concatenation of events: (i) assuming V_b decreases (or the initial water volume increases), the ratio of surface area available for water transport (A) to the initial water volume ($V_o - V_b$) decreases; (ii) a simple analysis of Eq. (1), shows that as the ratio of A and ($V_o - V_b$) decreases, L_{pg} has to

increase (this trend of increasing L_{pg} with decreasing V_b is clear from the data shown Table 1). The effect of varying V_b on E_{LP} is not quite as straightforward due to the exponential (arrhenius) relationship between L_p and E_{LP} , as described earlier in Eq. (2); (iii) and if we realize, as revealed in a recent study by Thirumala and Devireddy [10], that the optimal cooling rate is a direct and linear function of L_{pg} and the ratio $A/(V_o - V_b)$ we conclude that changes in V_b will effect the value of L_{pg} but should not dramatically alter the optimal cooling rate (as is clear from the data shown in Table 1). And finally, it is important to note that the effect of E_{LP} on the predicted optimal cooling rate is not linear and in fact decreases exponentially as it increases [10]. In essence, the most significant measurements that determines the optimal cooling rate are the water transport (volumetric shrinkage) response shown in Fig. 1 and the ratio of the cell surface area available for water transport to the initial amount of water present in the cell.

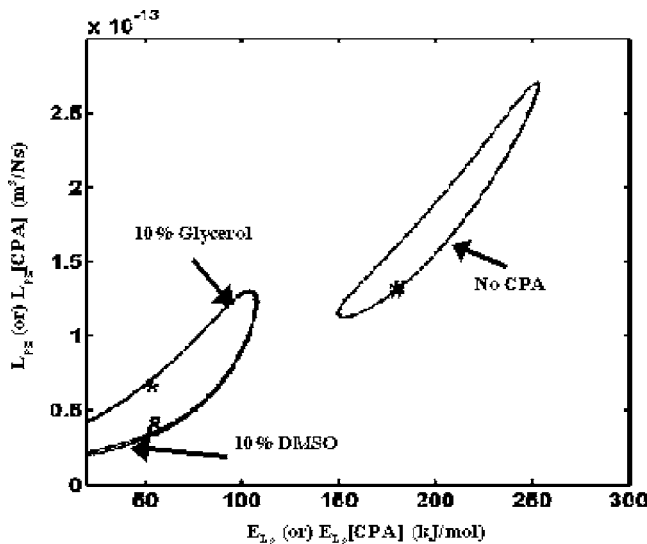


Fig. 3 Contour plots of the goodness of fit parameter $R^2(=0.98)$ for water transport response in P4 ASCs in medium with no CPAs, in medium with 10% glycerol, and in medium with 10% DMSO. The enclosed region corresponds to the range of parameters that best fit the water transport data at a cooling rate of $20^\circ\text{C}/\text{min}$ with $R^2=0.98$. Note that the best-fit parameters (Table 1; $V_b=0.6V_o$) are represented within the contours by a “#” (absence of CPA), “&” (10% glycerol), and “*” (with 10% DMSO). The membrane permeability at 0°C , L_{pg} (or $L_{pg}[\text{cpa}]$) (m^3/Ns), is plotted on the y axis while the apparent activation energy of the membrane, E_{LP} ($E_{LP}[\text{cpa}]$) (kJ/mol) is shown on the x axis.

Conclusion

Volumetric shrinkage during freezing of adipose derived adult stem cells was obtained in the presence of extracellular ice and CPAs (10% DMSO and 10% glycerol). The measured water transport data in the presence and absence of CPAs was curve fitted to a model of water transport, to predict the membrane permeability parameters L_{pg} or $L_{pg}[\text{cpa}]$ and E_{LP} or $E_{LP}[\text{cpa}]$ of adipose derived cells. The permeability parameters obtained in this study predict an optimal rate of freezing for P4 ASCs ranging from 17 to $30^\circ\text{C}/\text{min}$. The water permeability parameters presented in this study will help to establish cryopreservation of adipose derived adult stem cells on a firm biophysical basis. Future studies should make direct comparisons of the optimal cooling rates predicted using the water transport models developed here with experimentally determined optimal cooling rate values for ASCs.

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