Desiccation Tolerance of Adult Stem Cells in the Presence of Trehalose and Glycerol

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Abstract: Development of protocols for storing desiccated cells at ambient temperatures offers tremendous economic and practical advantages over traditional storage procedures like cryopreservation and freeze-drying. As a first step for developing such procedures for adult stem cells, we have measured the post-rehydration membrane integrity (PRMI) of two passages, Passage-0 (P0) and Passage-1 (P1), of human adipose-derived stem cells (ASCs). ASCs were dried using a convective stage at three different drying rates (slow, moderate and rapid) in D-PBS with trehalose (50 mM) and glycerol (384 mM). ASCs were incubated in the drying media for 30 mins prior to drying at the prescribed rate on the convective stage for 30 mins. After drying, the ASCs were stored for 48 hrs in three different conditions: i) at ambient temperature, ii) in plastic bags at ambient temperature and iii) in vacuum sealed plastic bags at ambient temperature. PRMI was assessed after incubating the rehydrated ASCs with stromal medium for a further 48 hrs. Our measurements show that the PRMI of ASCs was: i) higher when ASCs were dried slowly; ii) increased when they were stored in vacuum as opposed to at ambient or in plastic bags; and iii) decreased with increasing passage of ASCs, i.e. under similar drying and storage conditions P0 ASCs had higher PRMI than P1 ASCs. Our results suggest that the best PRMI (37% for P0 ASCs and ~14% for P1 ASCs) can be achieved when the ASCs were dried slowly and stored in vacuum.

INTRODUCTION

Human adipose tissue provides a uniquely abundant and accessible source of adult stem cells, ASCs [1]. In response to chemical, hormonal or structural stimuli, these adipose-derived ASCs can differentiate along multiple lineage pathways, including adipocytes, chondrocytes, myocytes, neurons and osteoblasts [1-6]. Successful storage techniques of ASCs could revolutionize the fields of tissue engineering and regenerative medicine industry. The two competing strategies for long term storage of ASCs are freezing (cryopreservation) and desiccation (drying). An optimized desiccation procedure gives significant advantages over a standard cryopreservation protocols because the process of desiccation is simpler, quicker and typically less toxic protectants are needed; additionally, storage conditions are less stringent and the logistics of transportation are greatly simplified [7].

The damage to biological systems during drying is primarily due to the changes in the physical state of membrane lipids and changes in the structure of sensitive proteins. Trehalose, a disaccharide sugar found in large concentrations in a wide variety of species, is particularly effective in stabilizing cells at drying. Studies on the drying preservation of both prokaryotes and eukaryotes have revealed that non-reducing sugars such as trehalose, sucrose, and maltose protect liposomes from the adverse effect of freezing and drying [8-10]. Desiccation studies on *E. coli* and *B. thuringiensis* have supported the postulation that an increase in tolerance to drying appears to result from an ability of the sugars to lower the phase transition temperature of the membrane [11,12]. Sugars like trehalose and sucrose have also been shown to protect not only the membranes but also intracellular proteins during bacterial drying [13-16]. Desiccation tolerance of different cell types ranging from microbial pathogens of humans [17] to several varieties of plant species such as rice (*Oryza sativa*), wild rice (*Zizania palustris*) [18] and soybean [19] have also been studied and shown to be related to the production of saccharides in response to an external environmental stress. Additionally, in nature, a variety of organisms including artic frog, salamanders, tardigrades and nematodes bacteria, yeast, fungi and rotifers have also been shown to survive extreme dehydration and dry conditions due to the presence of sugars [20-24]. More importantly, recent studies suggest that mammalian cells, including human primary foreskin fibroblasts, 3T3 murine fibroblasts, human mesenchymal stem cells (hMSCs), corneal epithelial cells and mouse spermatozoa, can tolerate a drying process in the presence of either intracellular or extracellular trehalose [25-31]. A listing of studies reporting the ability of trehalose to act as a protective agent during drying storage of mammalian cells is summarized in Table **1**. A more detailed listing of the use of sugars in cell storage, including cryopreservation, freeze-drying and drying, is presented elsewhere and is beyond the scope of the present study [32].

As stated earlier, successful drying and storage of ASCs using relatively simple methods would revolutionize the tissue engineering industry. However, to the best of our knowledge no work specifically related to drying storage of ASCs has been reported. In the present study, we report the post-rehydration membrane integrity (denoted as PRMI) of vari-

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Table 1. A Listing of Studies Reporting the Use of "Trehalose" as a Protective Agent for Drying Storage of Mammalian Cells

Cell Type	Sugars in Drying Medium	Post-Storage Assessment	Observations (Optimal Conditions)	Ref.
Human Primary Foreskin Fibroblasts	Biosynthesis of trehalose	Viability (membrane integrity)	Vacuum storage and 50 mM trehalose is optimal. >150 mM trehalose induces cytotoxicity.	[25,26]
3T3 Murine Fibroblasts*	0 to 400 mM trehalose	Viability (membrane integrity)	Membrane integrity decreases with moisture content. Greater integrity when stored at 4ºC and -20ºC.	[27]
Human Mesenchymal Stem Cells (hMSCs)	50 mM trehalose + 3% glycerol (incubated 24 hrs before drying)	Morphology; Adhesion; Viability; Proliferation.	Vacuum storage and incubating with trehalose before drying improved desiccation tolerance.	[28]
3T3 Fibroblasts*	200 mM intra- and extracellular trehalose	Viability (membrane integrity)	10% residual moisture; natural convection; initial osmolality of the sugar solutions adjusted to isotonic levels by reducing buffer concentration.	[29]
Human Corneal Epithelial Cells	2 to 200 mM of either trehalose or maltose.	Viability (membrane integrity);	< 20 mM trehalose not useful; maltose had no effect.	[30]
Mouse Spermatozoa*	500 mM trehalose	Blastocyst formation; Embryo Transfer	Desiccated in ambient temperature with 500mM of trehalose, stored at 4 ˚C.	[31]

*intracellular Trehalose (using α-hemolysin).

ous passages of ASCs dried in the presence of 50 mM trehalose and 384 mM glycerol. After drying for 30 minutes on a convective drying stage at three different drying rates (slow, moderate and rapid), the ASCs were stored for 48 hrs at three different storage conditions (ambient, plastic bags, and in vacuum). Upon rehydration the ASCs were incubated for 48 hrs in the stromal media, before the ability of the ASCs to exclude fluorescent dyes was assessed. The results are analyzed to further our understanding of the complex interactions between the drying rate, the storage conditions and the passage of cells on the post-rehydration membrane integrity of ASCs.

MATERIALS AND METHODS

Isolation, Collection and Culture of Adult Stem Cells

All human protocols were reviewed and approved by the Pennington Biomedical Research Center Institutional Review Board. All reagents were obtained from Sigma Chemicals (St. Louis, MO) unless otherwise stated. Subcutaneous adipose tissue liposuction aspirates were provided by plastic surgeons in Baton Rouge, LA. These, tissue samples (100 to 200 ml) were washed 3-4 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 g) at room temperature, resuspended, and the centrifugation step repeated. The supernatant was aspirated and the pellet re-suspended in Stromal Medium (DMEM high glucose, 10% fetal bovine serum, 100 units penicillin/ml, 100 µg streptomycin/ml, and 25 µg amphotericin/ml). The cell suspension was plated at a density equivalent to 0.125 ml of liposuction tissue per sq cm of surface area, using a 35 ml volume of Stromal Medium per T225 flask. Cells were cultured for 48 hrs in a 5% CO_2, humidified, 37 ˚C incubator. At which time, the adherent cells were rinsed once with prewarmed PBS and the cells fed with fresh Stromal Medium. The cells were fed with fresh Stromal Medium every 2-3 days until they reached approximately 75-80% confluence. The medium was then 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 to 5 min at 37 ˚C. The cells were suspended in Stromal Medium, centrifuged for 5 min at 1200 rpm (300 g), the pellet resuspended in a volume of 10 ml of Stromal Medium, and the cell count determined by trypan blue exclusion. These cells were identified as Passage 0 (P0). An aliquot of cells was reserved for drying studies. The remaining cells were seeded in T225 flasks at a density of 5 x 10^3 cells per sq cm. The cells were maintained in culture and passaged as described to obtain Passage 1 (P1) ASCs. Note that there is extensive data suggesting that these adherent cells exhibit multiple lineages when culture *in vitro* [1-6, 33,34], and are termed as human adipose derived adult stem cells (ASCs), in the present study.

Addition of Trehalose and Glycerol

For all drying experiments the ASC concentration was adjusted to be 1 x 10^6 cells/ml. Before drying, 1 ml stock solution of containing 100 mM trehalose and 768 mM glycerol was added to 1 ml of ASC suspension. Thus, the final concentration of trehalose and glycerol in the drying media was 50 mM and 384 mM, respectively. Prior to conducting the convective drying experiments described below, the ASCs were incubated at 37ºC, 5% CO_2 and 100% relative humidity (RH) for 30 minutes.

Convective Drying Stage

A convective drying stage similar to the one described earlier by Bhowmick *et al.* [35] was used in the present study to achieve controlled drying of P0 and P1 ASCs. Briefly, nitrogen gas (Doussan Inc. New Orleans, LA) from a pres-

surized cylinder was dried using an in line desiccator drierite (W.A. Hammond Drierite & Co., Xenia, OH) and then exhausted through a Plexiglass chamber. The chamber was designed to hold 3 glass microslides, with each grooved microslide capable of holding a 10 µl ASC droplet. To ensure repeatable conditions, the entire flow path and the flow chamber were tightly sealed to prevent leakage. To ensure 3 different drying rates, the flow of nitrogen gas was controlled by adjusting the exhaust pressure using a pressure regulator (Doussan Inc.). The 3 different drying rates are denoted as slow (with regulator pressure of 275 kPa), moderate (205 kPa) and rapid (140 kPa). After drying ASCs on the stage for 30 min, the ASCs were placed in a 6 well plate (Corning Plasticware Cell Culture, Sigma Aldrich, St Louis, MO) and stored at ambient temperature, or in plastic bags (Deni Magic Vac Bags, Keystone Manufacturing Company, Inc., Buffalo, NY) at ambient temperature and in a vacuum sealed bag (Deni Magic Vac™ Select, Keystone Manufacturing Company, Inc.,). The samples were then stored for 48 hours.

Rehydration Protocol

After storing the ASC samples for 48 hrs, they were rehydrated with 1 ml of D-PBS and 1 ml of stromal media. The mixture of the cell suspension and the media were allowed to equilibrate briefly ~ 5 mins, and then placed in an incubator (NAPCO-CO_2 incubator, Precision, VA) at 37ºC, 5% CO_2 and 100% RH. After 48 hrs, the cells were removed and their membrane integrity was assessed, as described below.

Cell Membrane Integrity Measurements

Cells were assayed for viability by fluorescent nucleic acid dyes (SYBR-14 & PI) as previously described [36-39]. ASC viability was determined using 2 fluorescent nucleic acid dyes obtained from Molecular Probes (Eugene, OR) in kit form according to the instructions supplied. SYBR-14 (live cell stain) and Propidium Iodide, PI (dead cell stain) were prepared fresh daily in HBS and used at final concentrations of 100 nM and 600 nM, respectively. Between 300-500 cells/sample were scored in each assay using a light microscope (Nikon Instruments Inc. Melville, NY, USA) at 200x magnification and FITC and Texas Red filter cubes [36-39]. The average cell survival, based on the ability of the rehydrated cell to exclude dyes, was defined as the ratio of the live cells to the total cells in the field of view.

Statistical Analysis

Experiments were repeated 6 times for each drying rate and storage condition. The data was analyzed by analysis of variance (ANOVA) using the SAS software 9.0. All data represented here represents a normal distribution and the data expressed is mean ± SEM. Unless otherwise stated, all statistically significant differences in the data are reported with a probability of $P < 0.05$.

RESULTS

Choice of Drying Media

Initially drying experiments were conducted to assess the relative impact of trehalose and glycerol on the post-rehydration membrane integrity (PRMI) of ASCs and to identify a suitable media for conducting the intensive drying experiments. To this end, equal aliquots of either P0 or P1 ASCs were incubated for 30 minutes in either D-PBS alone or in D-PBS with 384 mM glycerol or in D-PBS with 384 mM glycerol and 50 mM trehalose. The ASCs were then dried using a convective drying stage for 30 minutes at a moderate drying rate, as described above. The cells were stored in ambient temperature for a further 30 minutes, rehydrated and the % cell survival was estimated immediately (5 mins) after the rehydration process using membrane excluded dyes. These preliminary experiments showed that the % P0 ASC survival in the presence of trehalose was significantly higher ($P < 0.01$) than in its absence (72 ± 3% in the presence of trehalose and 56 ±5 % with glycerol and 26 ± 8% with D-PBS alone). Similar results were obtained for P1 ASCs. Thus, we proceeded to conduct exhaustive drying experiments with the drying media containing 50 mM trehalose with 384 mM glycerol. In summary, the experimental scheme in the present work comprises of drying two different passages of ASCs under three different convective drying conditions (slow, moderate or rapid) and three different storage conditions (ambient, plastic bags and in vacuum) in D-PBS with 50 mM trehalose and 384 mM glycerol.

Effect of the Drying Rate & Storage Condition on ASC Membrane Integrity

A comparison of the post-rehydration membrane integrity (PRMI) is shown in Table **2** for P0 and P1 ASCs dried in the presence of 50 mM trehalose and 384 mM glycerol. For ease of analysis, a graphical representation of the data is also shown in Figs. (**1** and **2**). In Fig. (**1**), a comparison of the ASC PRMI is made for the three drying rates at each storage condition while in Fig. (**2**), a comparison of ASC PRMI is made for the three storage conditions at each drying rate. An examination of the data shows that: i) for P0 ASCs the optimal drying and storage conditions are slow drying and vacuum storage, respectively and ~37% of rehydrated ASCs are able to exclude dyes when slow dried and stored in vacuum; ii) for P0 ASCs dried either at a moderate or rapid drying rate, the % of PRMI is independent of the storage condition; iii) for P1 ASCs at a given storage condition, the % of PRMI is independent of the drying rate, with the highest values (~12 to 14%) being obtained for vacuum stored samples; iv) for P1 ASCs, the % of PRMI for samples stored in either ambient environment or in plastic bags (~2 to 4%) are significantly smaller than vacuum stored samples (~12 to 14%); v) the maximum % of PRMI obtained is significantly smaller for P1 ASCs (~14%) when compared with P0 ASCs (~37%).

DISCUSSION

Anhydrobiosis & Choice of Drying Media

As described in the introduction, several organisms have developed a variety of novel and intriguing strategies for their survival when exposed to extreme cold, dryness, or heat, or the absence of oxygen [40]. This phenomena, named *anhydrobiosis*, involves a reversible suspension of the metabolism, an effective isolation from the environmental changes and the production of large amounts of saccharides [40-42]. The mechanisms whereby sugars may stabilize living systems during freeze-thaw, heat-cooling, or dehydra-

Table 2. Post-Rehydration Membrane Integrity of ASCs Dried in the Presence of 50 mM Trehalose and 384 mM Glycerol

ASC Cell Passage	Storage Conditions	Drying Rate		
		Slow	Moderate	Rapid
P0	Ambient	$13.5 \pm 2.5^{1,a}$	$12.9 \pm 2.2^{1,a}$	$17.5 \pm 2.9^{2,a}$
	Plastic Bags	$9.3 \pm 1.8^{1,a}$	$15.1 \pm 3.0^{1,a}$	$13.5 \pm 2.1^{1,a}$
	In Vacuum	$37.4 \pm 2.6^{1,b}$	$18.6 \pm 2.5^{2,a}$	$22.8 \pm 3.3^{3,b}$
P1	Ambient	$3.7 \pm 0.6^{1,c}$	$2.7 \pm 0.7^{1,b}$	$2.3 \pm 0.6^{1,c}$
	Plastic Bags	$3.0 \pm 1.0^{1,c}$	$4.0 \pm 1.0^{1,b}$	$2.1 \pm 0.3^{1,c}$
	In Vacuum	$13.5 \pm 2.9^{1,a}$	$13.1 \pm 2.4^{1,a}$	$12.1 \pm 2.2^{1,a}$

Each entry in the table has a superscript consisting of a "number" and an "alphabet". Different "numbers" within a given row denotes the differences in the measured post-rehydration membrane integrity are statistically significant ($P < 0.05$), while different "alphabets" within a given column denote statistically significant data ($P < 0.05$).

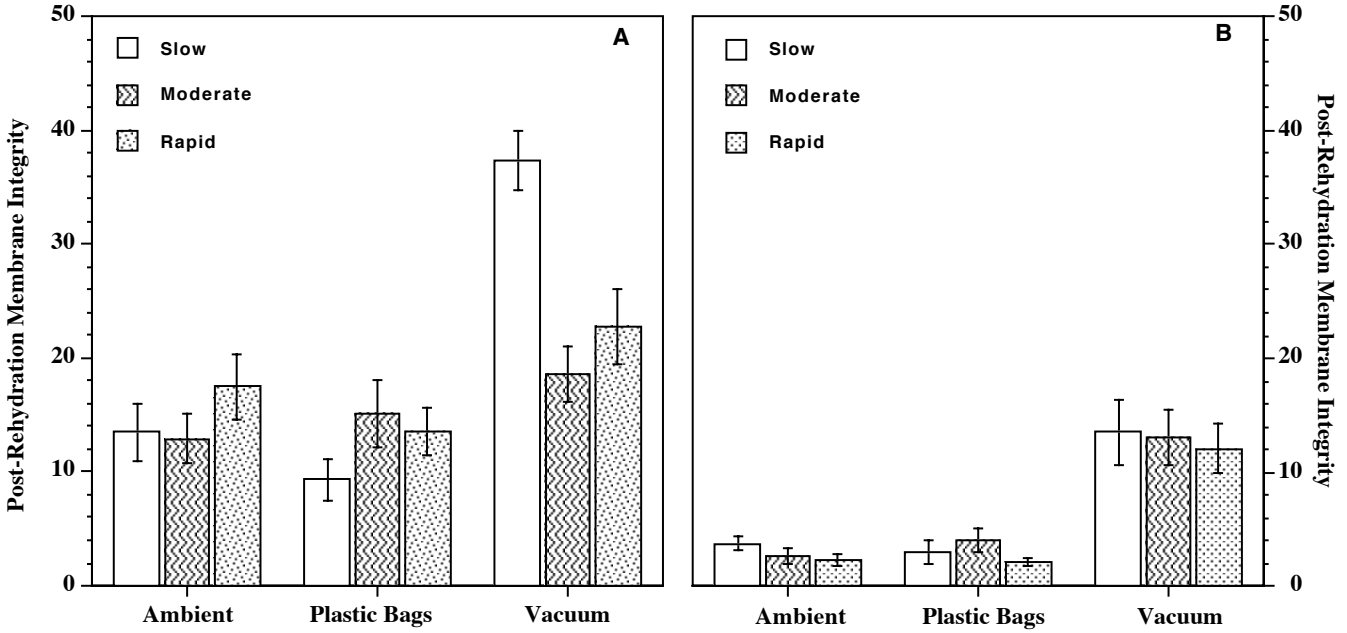

Fig. (1). A comparison of the post-rehydration membrane integrity for P0 (Fig. **1A**) and P1 (Fig. **1B**) ASCs. The three storage conditions: i) at ambient temperature, ii) in plastic bags at ambient temperature and iii) in vacuum sealed plastic bags at ambient temperature are represented on the y-axis while the post-rehydration membrane integrity is shown on the x-axis. For each storage condition, the post-rehydration membrane integrity is shown for the three drying rates studied: slow, moderate and rapid. The errors bars represent standard deviation in the data (n = 6).

tion-rehydration cycles remain a matter of debate. However three hypotheses, not necessarily mutually exclusive, have been developed to elucidate the protective effect of sugars. The "water-replacement" hypothesis suggests that during drying, sugars can substitute water molecules (in particular by forming hydrogen bonds) around the polar and charged groups present in phospholipid membranes and proteins, thereby stabilizing their native structure in the absence of water [43]. The "water-entrapment" hypothesis, in contrast, proposes that sugars concentrate residual water molecules close to the biostructure, thereby preserving to a large extent its solvation and native properties [44]. Finally, the "vitrification" hypothesis suggests that sugars found in anhydrobiotic systems, known to be good vitrifying agents, protect biostructures through the formation of amorphous glasses, thereby reducing structural fluctuations and preventing denaturation or mechanical disruption [45,46].

Among the sugars related with the mechanisms of anhydrobiosis, trehalose is one of the most effective protectants and has therefore been extensively studied [42,47,48]. The effect of trehalose during freezing preservation has also been extensively studied, including its effect on the cryopreservation of carrot and tobacco cells [49], mammalian sperm [50-52], pancreatic islets [53], yeast cells [54], oocytes [55], fetal skin [56] and recently for hematopoietic stem cells [57]. The effect of trehalose to stabilize membrane during freeze-drying has also been studied [58,59]. Consistent with prior experiments [25-31], our experiments also showed that a

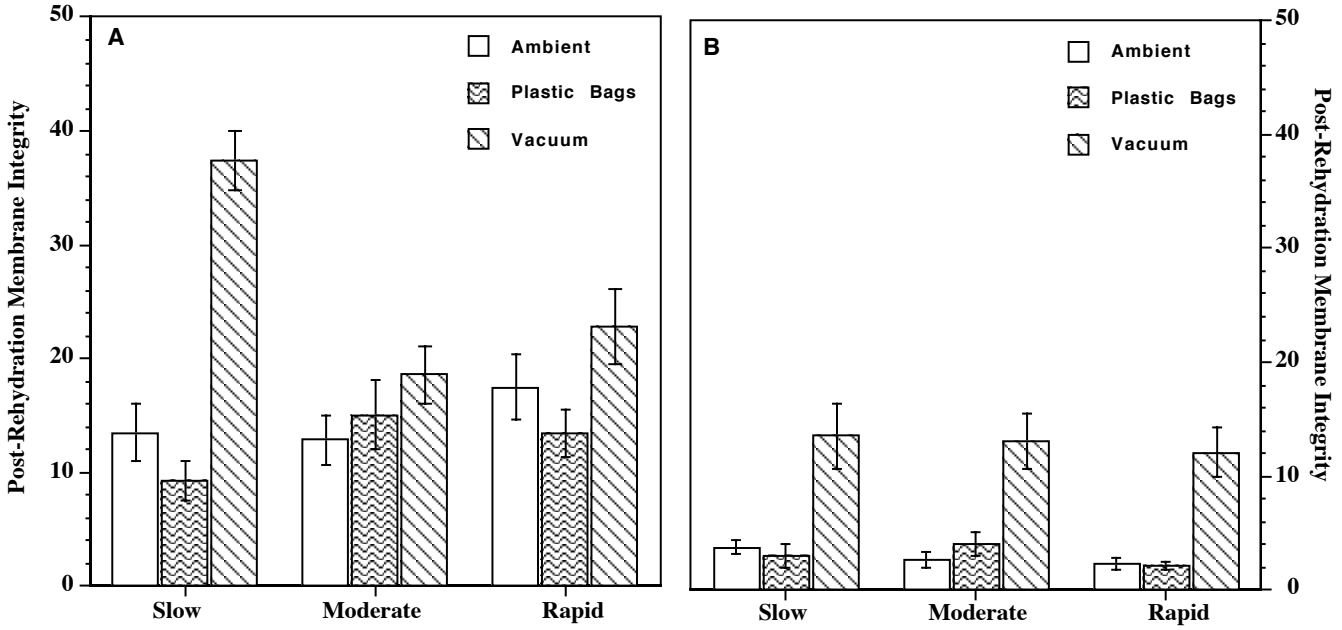

Fig. (2). A comparison of the post-rehydration membrane integrity for P0 (Fig. **2A**) and P1 (Fig. **2B**) ASCs. The three drying conditions: i) slow ii) moderate and iii) rapid are represented on the y-axis while the post-rehydration membrane integrity is shown on the x-axis. For each drying condition, the post-rehydration membrane integrity is shown for the three storage conditions studied: at ambient temperature, in plastic bags at ambient temperature and in vacuum sealed plastic bags at ambient temperature. The errors bars represent standard deviation in the data (n = 6).

significantly higher fraction of ASCs dried in the presence of trehalose retain their post-rehydration membrane integrity than its absence. Although, the exact reason for the protective action of trehalose is as yet unknown, it has been suggested that it reduces the mechanical stress imposed on the membrane during drying by maintaining the spacing between head groups and helps to keep the membrane in the fluid phase [60,61].

Intracellular vs. Extracellular Trehalose

Previous studies on the desiccation tolerance of human cells revealed that, trehalose could be expressed intracellularly with adenoviral vectors otsA and otsB genes [25]. Although, these genes are responsible for the conversion of uridine diphosphoglucose (UDP-Glucose) to trehalose, they exhibited significant cytotoxicity, particularly at multiplicities of infection [25]. Recently, Toner and colleagues reported the intracellular loading of trehalose by using a genetically engineered mutant of *Staphylococcus aureus* α-*hemolysin* to create pores in the cellular membrane [27,29, 31,62,63] or by microinjecting trehalose [55] into oocytes. de Castro and Tunnacliffe [64] report that 80 mM intracellular trehalose is able to confer increased resistance to the partial dehydration resulting from hypertonic stress in a genetically engineered mouse cell line, but does not enable survival of complete desiccation due to air drying. And finally, there is evidence to suggest that the presence of extracellular trehalose combined with permeating protectant (eg. glycerol, dimethylsulfoxide) is beneficial for freezing preservation of fetal skin [56]. As we made no attempt to either induce intracellular trehalose or load trehalose into ASCs using pore forming techniques, our results only relate the effect of extracellular trehalose and a permeating protectant (glycerol) on the post-rehydration membrane integrity of ASCs. However, it is possible that ASCs might have "taken up" the extracellular trehalose by fluid-phase endocytosis or other mechanisms. This assertion is based on experimental evidence that suggest, trehalose is rapidly taken up by human platelets at 37 ˚C, with loading efficiencies of 50% or greater *via* fluid-phase endocytosis or other mechanisms [58,59]. And finally, our method of adding glycerol and trehalose to ASC suspensions in a one step addition process might have caused osmotic shock to the ASCs and the consequent endogenous uptake or synthesis of osmolytes [26,65-67]. Future analysis by Fourier transform infrared spectroscopy are needed to demonstrate if the membrane and protein components of trehalose-loaded ASCs pre-dehydration, post-drying and post-rehydration are similar to fresh ASCs.

Concentration of Trehalose & Optimal Drying Conditions

As shown in Table **1**, a wide range of trehalose concentrations have been utilized during drying storage of mammalian cells. Puhlev *et al.* [26] report that a concentration of 50 mM trehalose is optimal for Basinger cells and results in the highest degreee of desiccation tolerance with minimum cytotoxicity. Concentrations higher than 150 mM trehalose were found to induce obvious cytotoxicity in Basinger cells, probably due to prolonged exposure to hyperosmotic solutions [26]. However, Matsuo [30] reports that trehalose concentrations of either 50 mM, or 100 mM or 200 mM provided desiccation tolerance to human corneal epithelial cells with no obvious cytotoxic effects at higher concentrations of trehalose. While, Ginnis *et al.* [31] report successful desiccation preservation of mouse spermatozoa in the presence of 500 mM trehalose. In the present study, we report the effect of 50 mM trehalose (with 384 mM glycerol) to provide desiccation tolerance to P0 and P1 ASCs. Clearly, further stud-

ies are needed with other concentrations of trehalose to assess the relative effect of concentration on the PRMI of ASCs.

To further assess the impact of drying ASCs in the presence of trehalose, we have also studied the effect of 3 different drying rates and 3 different storage conditions on the PRMI of P0 and P1 ASCs. For both P0 and P1 ASCs, slow drying and vacuum storage was found to be uniformally "optimal". Our observations are consistent with earlier studies on human foreskin fibroblasts [25,26] and human mesenchymal stem cells [28] which showed that slow drying coupled with vacuum storage has a dramatic positive effect on the retention of cellular viability of the desiccated cells. However, the actual mechanism involved in promoting the cellular viability in vacuum is as yet unknown. One possibility is that storage in vacuum reduces the amount of oxygen that is available to generate free radicals and minimizes the associated deleterious effects induced in the cells stored at ambient temperature or in plastic bags [68]. Another possibility is that the removal of air eliminates the meniscus effects that could damage the cells [25,26].

In general, previous drying studies on mammalian cells suggest that slow drying and/or natural convection drying is optimal with ~5 to 15% residual moisture content [25-31,35]. Our results are somewhat in agreement with this assertion, as slow drying resulted in a higher fraction of rehydrated ASCs retaining their membrane integrity, especially for cells stored in vacuum. However, our experiments with natural convection drying (i.e., no flow rate of dry nitrogen gas) resulted in <1% cell survival (data not shown). Additionally, rapid drying seems to be somewhat better than either moderate or slow drying for P0 ASCs stored in the ambient environment (Fig. **2A**). Although, the % final moisture content between the slowly, moderately and rapidly dried P0 ASCs was not significantly different (Fig. **1A**; 12.9% to 10.9% to 9%). This observation, i.e. rapid drying being more efficacious than slow drying, is in agreement with earlier desiccation studies on embryonic axes of recalcitrant jackfruit seeds [69]. Intriguingly, the PRMI of P1 ASCs dried was independent of the drying rate, i.e. for a given storage condition the rate of drying did not significantly effect the measured PRMI (Fig. **2B**). Clearly more detailed studies, possibly emulating a recent parametric study that described the effect of various freezing parameters on the post-freeze/thaw membrane integrity of ASCs [39], are needed to assess the relative impact of drying rate, the various components of the drying media, and the cell passage on ASC post-rehydration membrane integrity. Future experiments should (and will) be conducted to study the post-rehydration functionality and potentiality of ASCs as time and resources are available.

And finally, future experiments should be directed towards understanding the effect of rehydration kinetics on the membrane integrity of ASCs, a phenomena that has been long known to influence the post-rehydration integrity of micro-organisms [70]. Recent evidence in *E. coli* suggests that there exists an optimal rehydration rate that permits maximum survival of preserved bacteria [71,72]. This optimal rehydration is postulated to correspond to a rate that is slow enough to allow the cell membrane to keep its integrity but rapid enough to prevent harmful perturbations of cell metabolism [71,72]. Thus, it is quite possible that in future, determining the optimal magnitude and rate of rehydrating ASCs will lead to a further increase in their post-rehydration membrane integrity.

CONCLUSION

In conclusion, we report here the effect of desiccation tolerance of two different passages (P0 and P1) of adipose tissue derived adult stem cells (ASCs) dried in D-PBS with 50 mM trehalose and 384 mM glycerol at three different drying rates (rapid, moderate or slow) and at three different storage conditions (ambient, in plastic bags and in vacuum). An important finding of this study was that significantly higher post-rehydration ASC membrane integrity is achieved when they are slow dried and stored in vacuum. Additionally, for a given combination of drying rate and storage condition, P1 ASCs had a significantly lower post-rehydration membrane integrity than P0 ASCs. It is hoped that the data presented here will lead to a better understanding of desiccation tolerance, and in the development of optimal drying storage protocols for ASCs.

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