FREEZING RESPONSE AND POST-FREEZE DIFFERENTIATION POTENTIAL OF HUMAN ADIPOSE DERIVED ADULT STEM CELL (huASCs)

Sreedhar Thirumala
Ph.D. Candidate

Faculty Advisor: Dr. Ram V Devireddy

ABSTRACT

This study presents volumetric shrinkage (water transport) during freezing of human adipose derived adult stem cells (huASCs) using a calorimetric technique in the presence and absence of cryoprotective agents (CPAs). By fitting a model of water transport to the experimentally obtained volumetric shrinkage data we determined the membrane permeability parameters (reference membrane permeability to water, Lpg or Lpg[CPA] and the activation energy, Elp or Elp[CPA]) [1-3]. The experimentally determined membrane permeability parameters were then used to calculate the optimal rates of freezing huASCs cells in the presence and absence of CPAs [1-3]. Additionally, we have investigated the ability of frozen-thawed huASCs to form a colony of osteoblasts using a colony-formation-unit (CFU) assay [4].

INTRODUCTION

Human adipose tissue provides a uniquely abundant and accessible source of adult stem cells. In response to chemical, hormonal or structural stimuli, these human adipose-derived adult stem cells (huASCs) can differentiate along multiple lineage pathways, including adipocytes, chondrocytes, myocytes, neurons and osteoblasts [4]. Successful cryopreservation of scientifically and commercially important huASCs would revolutionize the tissue engineering and regenerative medicine industry. All cell systems do share common cryobiological responses, which may be exploited to better understand and alleviate the specific problems of freezing in huASCs cells. The highest rates of cellular survival are typically found for cooling rates which are fast enough to minimize dehydration solute effects injury while still slow enough to preclude large amounts of intracellular ice. 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. We are unaware of any studies that report water transport or IIF parameters in any stem cells. This study aims to rectify this lack of cryobiological knowledge in stromal vascular fraction (SVF) and several passages (P0, P2, and P4) huASCs stem cells using a DSC technique. A more detailed explanation of the DSC technique is given elsewhere [5].

WATER TRANSPORT MODEL

Mazur [6] developed a mathematical model for the volumetric change in cells due to the water transport during freezing process in the presence of extracellular ice and CPAs. In this water transport model,

\[ \frac{dV}{dT} = f \left( L_{pg}, E_{lp}, SA, WV, Type, and Conc. of CPAs, B \right) \]

where \( dV/dT \) is the rate of volumetric shrinkage of a cell, \( L_{pg} \) or \( L_{pg}[CPA] \) is the reference membrane permeability, \( E_{lp} \) or \( E_{lp}[CPA] \) is the activation energy, \( SA \) is the surface area available for water transport, \( WV \) is the initial volume of intracellular water and \( B \) is the imposed cooling rate (˚C/min). A detailed description of the water transport model is presented elsewhere [5, 6]. And finally a nonlinear least squares curve fitting technique was implemented in a computer program to calculate the best fit membrane permeability parameters (\( L_{pg} \) and \( E_{lp} \)) or \( L_{pg}[CPA] \) and \( E_{lp}[CPA] \), as previously described [1,2,5]

RESULTS

Fig 1 shows the water transport data (filled circles) and simulation (------) for P2 cells using the ‘best fit’ parameters in the water transport model at cooling rate 20 ˚C/min the absence of CPAs (Fig. 1A), in the presence of 10% glycerol (Fig. 1B) and in the presence of 10% DMSO (Fig. 1C). The model simulated equilibrium cooling response is also shown for reference as (-----). \( L_{pg} \) and \( E_{lp} \) or \( L_{pg}[CPA] \) and \( E_{lp}[CPA] \) that best fit the DSC water transport data of SVF, P0, P2 and P4 cells were used to generate the optimal cooling rates (data not shown). The optimal cooling rate, \( B_{opt} \) was calculated using a recently developed Generic Optimal Cooling Rate Equation (GOCRE) [7] (data not shown). The GOCRE relates several cell level parameters (\( L_{pg} \) or \( L_{pg}[CPA] \), \( E_{lp} \) or \( E_{lp}[CPA] \); \( SA; WV \)) to \( B_{opt} \) as

\[ B_{opt} = 1009.5 \times e^{-0.0545 \times E_{lp} \times L_{pg} \times \left( SA / WV \right)} \]
have investigated the ability of Post-Freeze CFU-ALP to form a colony using a colony
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10% DMSO and 10% DMEM/F 12 Ham’s with 10% fetal bovine serum, 100U
penicillin, 100µg streptomycin and 0.25µg Fungizone and ii) DMEM/F-12 media with 10% (v/v) of DMSO. The cells
were frozen to an end temperature of -80 °C before they
were stored in LN2 for 30 days. The data is summarized in
Table 1.

Figure 1: Volumetric response of P1 of huASCs as a function of subzero temperatures – see text for further details

The permeability parameters obtained in this study predict an optimal rate of freezing for SVF cells ranging from 25 to 60 °C/min, for P0 huASCs from 25 to 100 °C/min, for P2 huASCs from 20 to 25 °C/min and for P4 huASCs from 19 to 22 °C/min.

Concomitantly, we have investigated the ability of frozen-thawed huASCs to form a colony using a colony-formation-unit (CFU) assay. For the initial series of experiments, the huASCs were placed in an isopropanol freezing container and put into the -80 freezer for 24 hours and then transferred to liquid nitrogen. The freezing media consisted of 80% FBS, 10% DMSO and 10% DMEM/F-12 media. The results are shown in Fig 2. In another study, we have investigated the ability of huASCs stored in liquid nitrogen for 30 days after a controlled freezing and then thawed to form a colony. The huASCs were frozen at three different cooling rates (1, 20 and 40 °C/min) in a controlled rate freezer in two different media: i) in stromal media or DMEM/F 12 Ham’s with 10% fetal bovine serum, 100U penicillin, 100µg streptomycin and 0.25µg Fungizone and ii) DMEM/F-12 media with 10% (v/v) of DMSO. The cells

Figure 2. Colony-Formation-Unit (CFU) analysis. CFU-Fibroblast (CFU-F), CFU-Alkaline Phosphatase (CFU-ALP), CFU-Adipocytes (CFU-AD) and CFU-Osteoblast (CFU-OB)

Table 1: CFU-OB Frequency of huASCs frozen at 3 cooling rates.

<table>
<thead>
<tr>
<th>Freezing Media Composition</th>
<th>Cooling Rate (°C/min)</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Stromal Media</td>
<td>49.6 ± 12.3</td>
</tr>
<tr>
<td>DMEM/F-12 + 10% (v/v) DMSO</td>
<td>2.9 ± 0.42</td>
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These initial studies suggest that the post-freeze osteogenic potential of human huASCs can be retained even when they are frozen in the absence of serum.

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REFERENCES