ENHANCED UPTAKE OF CRYOPROTECTANT AND ADVANCED THERAPY WITH MAMMALIAN CELLS

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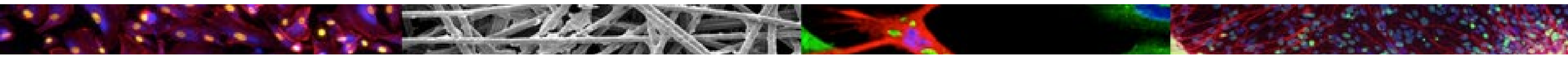

Introduction

Cell-based medicines are undergoing continual development and their applications are expanding, so there is an urgent need for safe and effective approaches for long-term storage and rapid use of viable live cells (Pancrazio et al., 2007). Cryopreservation has been used extensively to store cells as cell banking, tissues and microorganisms for long term use. Cryopreservation requires the use of cryoprotective agents (CPAs) that protect cells from damage caused by cooling and warming processes. Commonly used CPAs, such as dimethyl sulfoxide (DMSO), can be toxic and affect cell viability; they must be removed before the clinical administration of the cells. Trehalose, a disaccharide glucose synthesised by several organisms, is a potential nontoxic CPA that could replace DMSO in the preservation of different cell types. Cell-penetrating peptides (CPPs), also known as protein transduction domains (PTDs), are effective tools that have the ability to cross the cell membrane and enter cells. The CPP P21LK158R (PLR) has previously been shown to enhance cargo delivery (Dixon et al., 2016). Here, the ability of PLR to deliver trehalose into mammalian cells was examined. The results show that manipulating osmolarity increases membrane permeability; however, cell stability is also affected, possibly due to osmotic shock. The use of PLR resulted in an increased trehalose concentration in cells compared to the use of no PLR and a positive control but did not protect cells stored at very low temperatures for long-term application.

Aims

- Develop and optimise an applicable method for long-term cell storage based on the use of trehalose.
- Improve the loading of trehalose into mammalian cells by manipulating the osmolarity using phosphate-buffered saline (PBS).
- Examine the efficacy of the CPP termed PLR as a trehalose delivery system.

Methods

Fluid face endocytosis

Cells were incubated in the presence of a high extracellular concentration of trehalose. Trehalose was internalised via vesicles that penetrated the plasma membrane.

Phospholipid phase transition

The concentration gradient between the inside and the outside of the cells was manipulated to increase membrane permeability. Cells were incubated in a solution that contained a trehalose concentration that was higher than the isotonic condition.

Peptide-assisted delivery

Cells were incubated in solutions containing different concentrations of PLR (1, 10 and 100 µM) with trehalose.

Results

Different trehalose concentration effect NIH3T3 mouse fibroblast viability

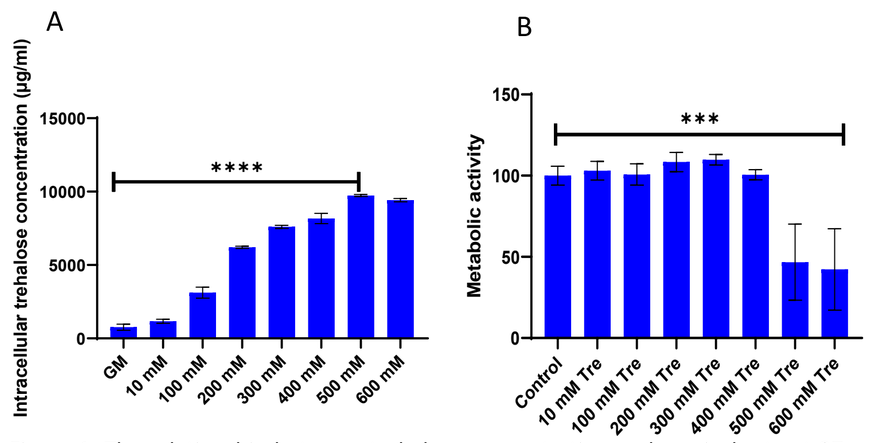

Figure 1: The relationship between trehalose concentration and survival rate at 37 ℃. Different concentrations of trehalose were tested (10, 100, 200, 300, 400, 500 and 600 mM). (A) Intracellular trehalose concentration assessed using an anthrone assay. (B) Cell metabolic activity assessed using a presto-blue assay.

Intracellular trehalose uptake is time and temperature dependent

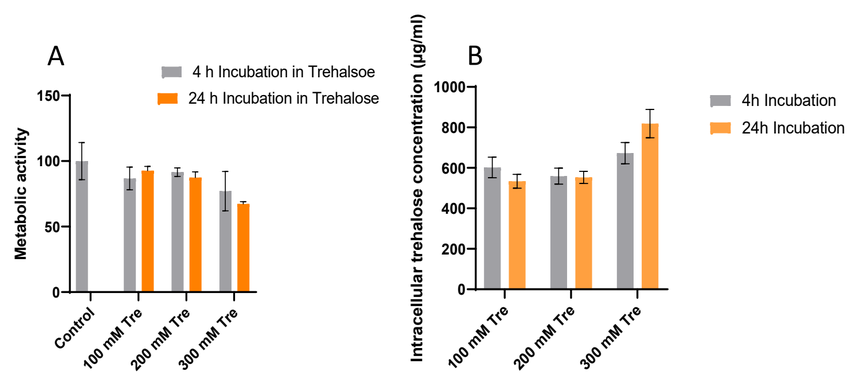

Figure 2: Effect of extracellular trehalose concentration and incubation time on survival rate and intracellular trehalose concentration. (A) Metabolic activity. (B) Intracellular trehalose concentration (µg/mL).

NIH3T3 mouse fibroblast incubated with different PBS concentration to manipulate osmolarity

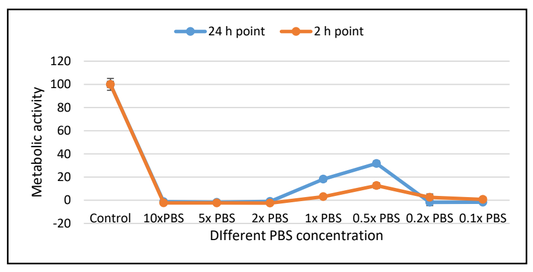

Figure 3: (A) Cell viability after incubation with different PBS concentrations for 2 h at 37 ℃. Viability was assessed at 2 h (red line) and 24 h (blue line) after incubation with PBS.

Effect of combined cryopreservation trehalose and PBS at 37 ℃

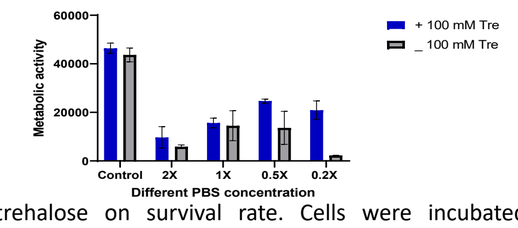

Figure 4: Effect of trehalose on survival rate. Cells were incubated in PBS supplemented with 100 mM trehalose (blue columns) or in PBS without trehalose (grey columns).

Effect of PBS on cryopreservation after 2 h at – 80 ℃

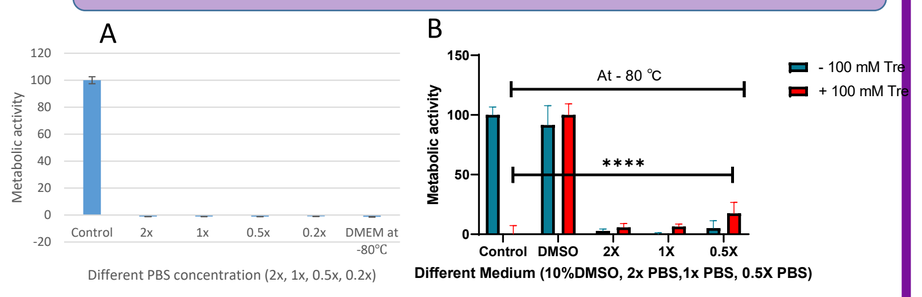

Figure 5: Viability of 3T3 cells after incubation for 30 min in PBS supplemented with 100 mM trehalose before storage at –80 ℃. (B) Cell viability after resuspension of the pellet in 200 µL of DMEM containing 100 mM trehalose (red columns) and without trehalose (blue columns).

Improve trehalose delivery using PLR in the presence of PBS and DMSO

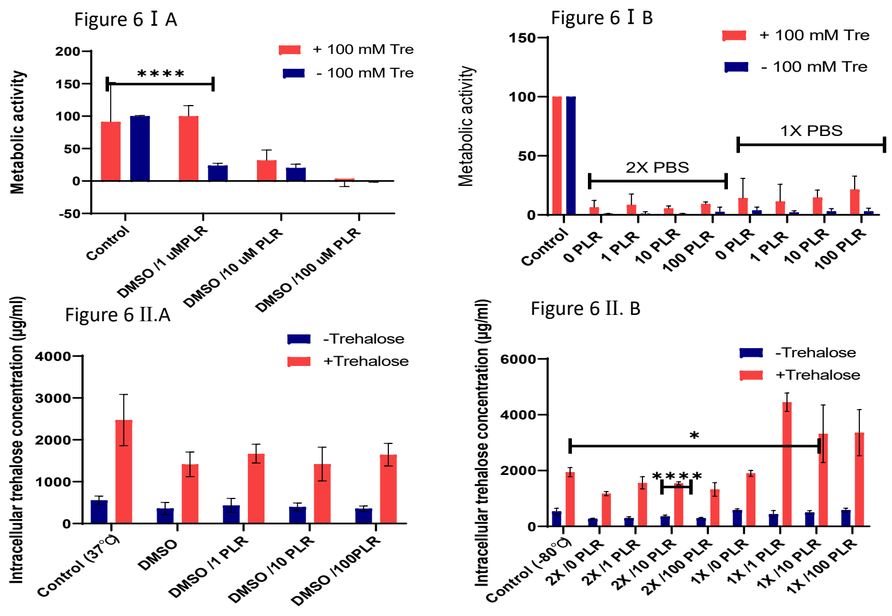

Figure 6: Effect of PLR on trehalose entry into cells. PLR (1, 10 and 100 µM) was added in PBS/ DMSO supplemented with 100 mM trehalose (blue columns) or PBS/ DMSO without trehalose (red columns). Figure (6 Ⅰ A and 6 Ⅰ B) represent Viability. Figure (6 Ⅱ A and 6 Ⅱ B) for Intracellular trehalose concentration (µg/mL).

Discussion and Conclusions

Cryopreservation is a challenging procedure and ideally would allow cells to be used immediately after thawing, without the need to remove agents or further processing steps. The loading of trehalose is time- and temperature-dependent, and successful cryopreservation requires the trehalose concentration to be balanced on both sides of the cell membrane. However, we have demonstrated here that methods used to deliver trehalose, such as fluid face endocytosis, phospholipid-phase transition via osmotic stress and CPPs, can improve trehalose cell uptake. However, undesired small cytotoxic particles can also enter the cells, since the methods are not selective for trehalose, which could ultimately result in cell death. Further optimisation may lead to a rapid and simplistic method to preserve cells as data showed that resuspending pellet after freezing at -80 ℃ in DMEM with trehalose improve the survival rate to 26 %. Techniques that allow the selective entry of trehalose are thus more desirable. Nanoparticle encapsulations seem to be a more promising approach for the specific delivery of trehalose, and they might form the basis of a revolutionary trehalose delivery system for live cells that is free of DMSO and allows long-term storage and use.

References

Dixon, J. E., Osman, G., Morris, G. E., Markides, H., Rotherham, M., Bayoussef, Z., El haj, A. J., Denning, C. & Shakesheff, K. M. 2016. Highly efficient delivery of functional cargoes by the synergistic effect of GAG binding motifs and cell-penetrating peptides. Proc Natl Acad Sci U S A, 113, E291-9.

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