ENHANCED UPTAKE OF CRYOPROTECTANT AND ADVANCED THERAPY WITH MAMMALIAN CELLS

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Cryopreservation at very low temperatures has been used extensively to store cells, tissues, embryos and microorganisms for long term use. Challenges with cryopreservation include post-thaw cell viability, growth, maintenance of function and differentiation ability issues, as well as alteration in gene expression. 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. The main functions of trehalose include the provision of energy and the regulation of certain metabolic pathways that affect growth. The aim of the current study was to develop and optimise an applicable method for biological research based on the use of trehalose. To improve the loading of trehalose into mammalian cells, the trehalose concentration and the osmotic pressure between the inside and outside of the cells were manipulated using phosphate buffered saline (PBS). The study also aimed to examine the efficacy of a cell-penetrating peptide (CPP) as a trehalose delivery system. The utilised CPP, termed PLR (P21LK158R), has previously been shown to enhance delivery. Trehalose delivery was measured using the anthrone assay, and metabolic activity was evaluated using the presto-blue assay. The data indicate that manipulating osmolarity and the concentration gradient increases membrane permeability; however, they also affect cell stability, possibly due to osmotic shock.