# Investigating ice nucleation temperature effects on mesenchymal stem cell recovery from cryostorage

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## Introduction

During the cryopreservation process, cells are frozen gradually before transfer to cryogenic temperatures for long term storage. During this process, ice crystals form at nucleation sites within the storage solution. As a stochastic process, the structure of crystallisation is governed by chaos, so each time the freezing process takes place, the temperature of crystal formation and the structure changes. During crystal formulation, temperature spikes occur, linked to a change in phase, which can result in supercooling events that damage cells due to osmotic gradients and intracellular ice formation.

By controlling the ice nucleation temperature, the randomised formation effects could be curtailed potentially increasing the recovery of the cell product from storage.

## Methods

Mesenchymal stem cells (MSCs) were seeded at 4500 cells/well into a 96-well plate and cultured in DMEM+10%FBS overnight. The culture medium was then replaced with growth medium+10% DMSO. A commercially available ice nucleation array (IceStart, Asymptote) was then placed into half of the 96-well plate and then the whole plate placed into a controlled rate freezing device (ViaFreeze, Asymptote). Once at -80oC the plate was removed to liquid nitrogen storage (vapour phase). Each condition was analysed using MTT, one and four days after the implementation of slow (30 minute) and fast (5 minute) thawing conditions and removal of the IceStart.

## Results

The addition of IceStart moves the nucleation temperature closer to the melting point of the cryomedium. Furthermore, MTT analysis shows that IceStart Arrays enhance the viability of the MSCs significantly ($p \leq 0.05$) after just one day of culture in comparison to no-IceStart controls.

## Conclusions

The addition of a known nucleation site reduces the damaging variation observed during a standard freezing process without optimisation of cryomedium and in a high throughput setting.