PhD Open Days

Impact of Freezing geometry on the cryopreservation of stem cells:
reducing DMSO concentration

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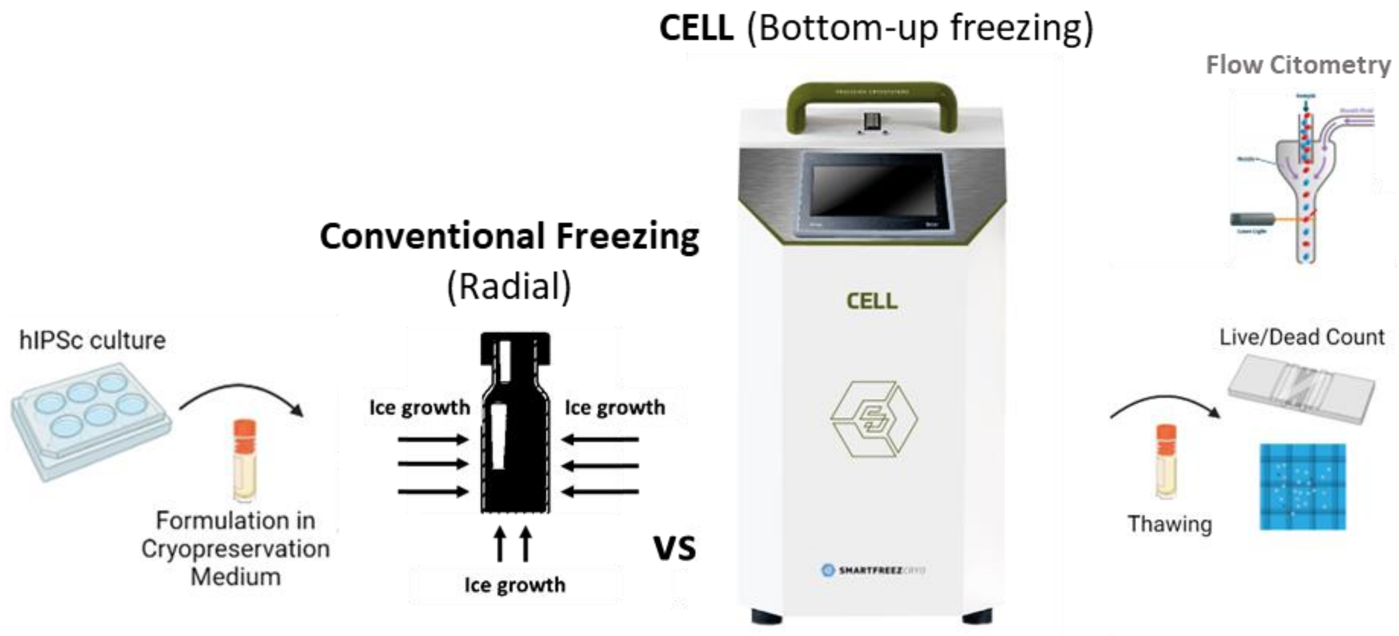
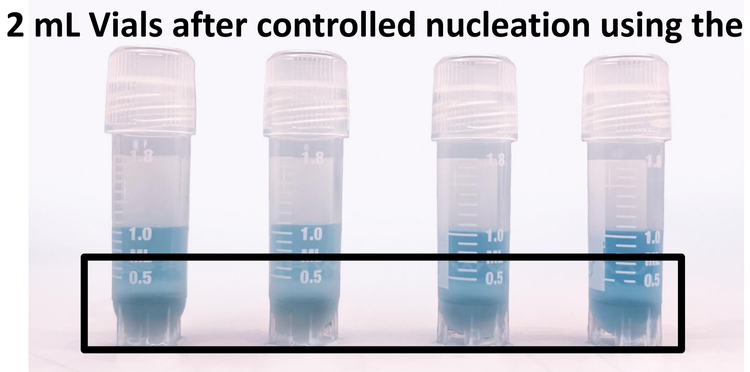
Background

Developing efficient cryopreservation systems for freezing cells has become essential for many applications such as cell therapies, reproductive medicine and organ preservation. Most cryopreservation methods rely on high concentrations of DMSO (10% v/v) to avoid critical freezing damage. However, it is desirable to reduce DMSO concentration, because it is toxic to cells and can also cause side effects on patients once DMSO-cryopreserved cells are infused.



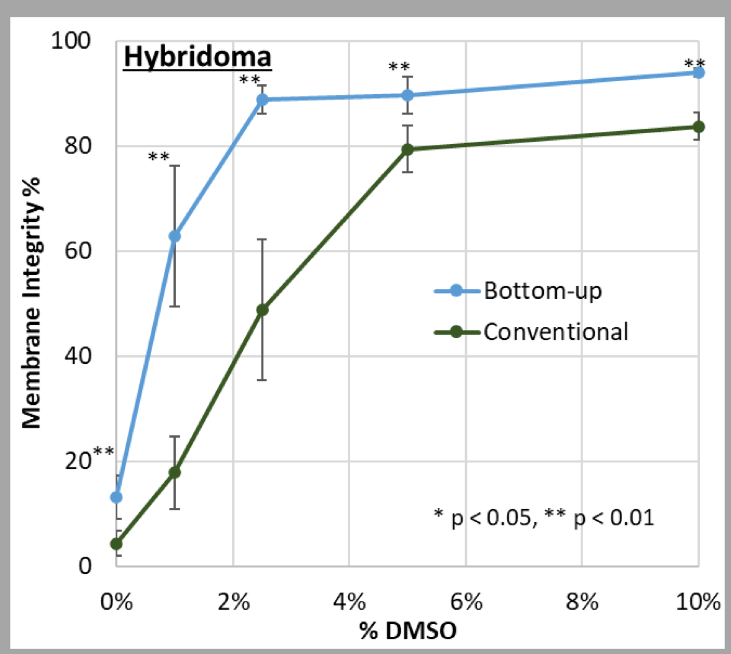
2 mL Vials after controlled nucleation using the CELL

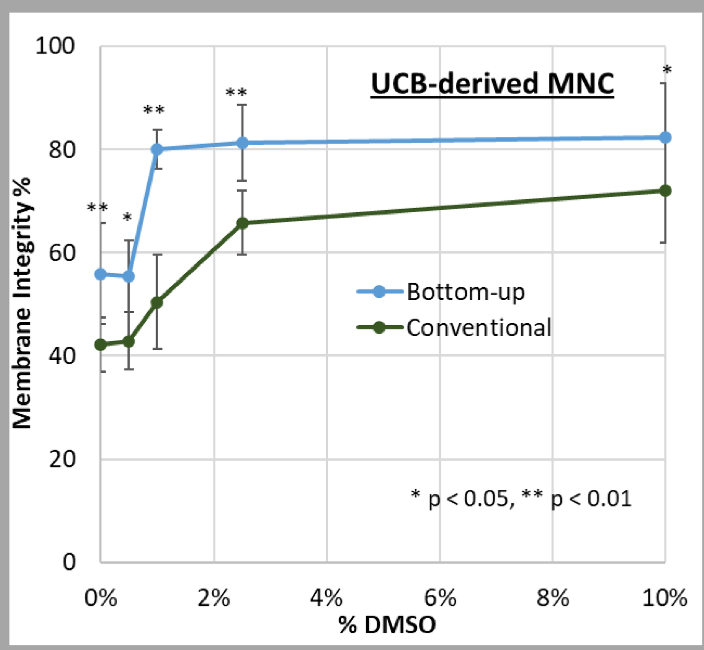


CELL

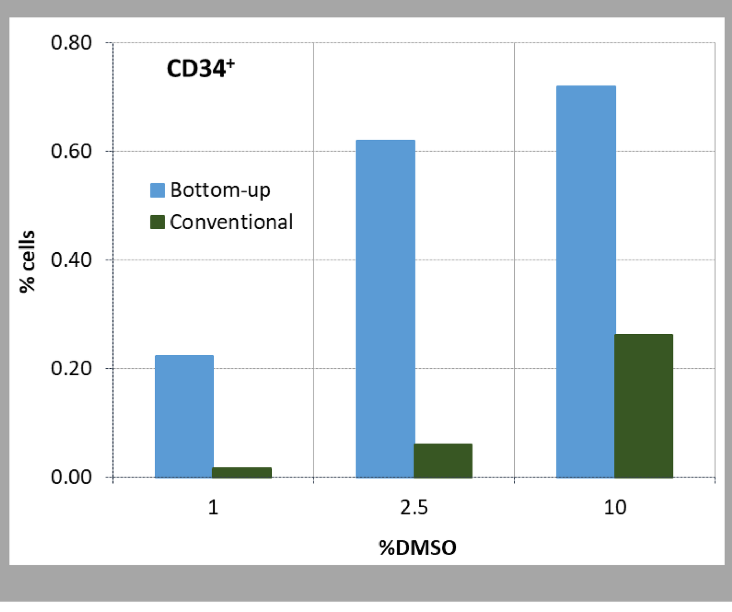
- A controlled rate freezer with a bottom-up heat-transfer geometry that attenuates freezing mechanical stresses.
- Enables precise control of ice-nucleation and crystal growth (velocity and direction).

CELL vs Conventional Freezing on Hybridoma and UCB-derived MNCS



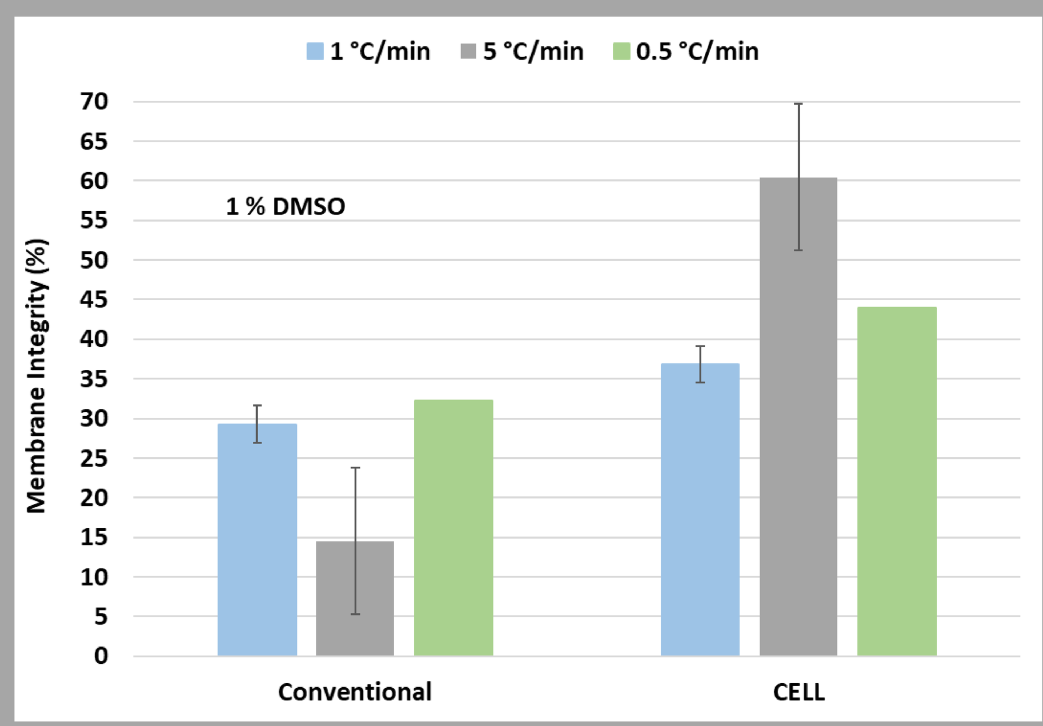


Cell (Bottom-up Freezing) results in higher membrane integrity



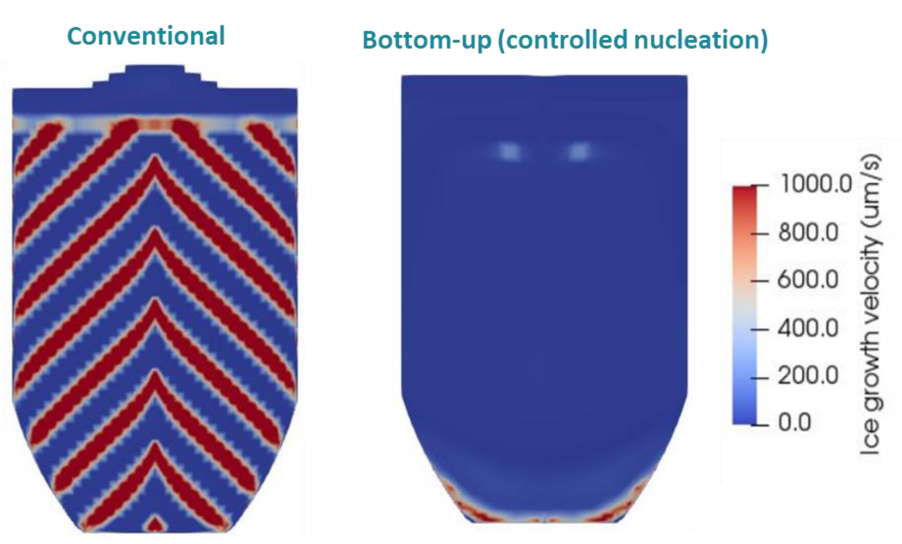
For 10% DMSO, the level of expression of the CD34 marker was 3x higher when bottom-up freezing was used

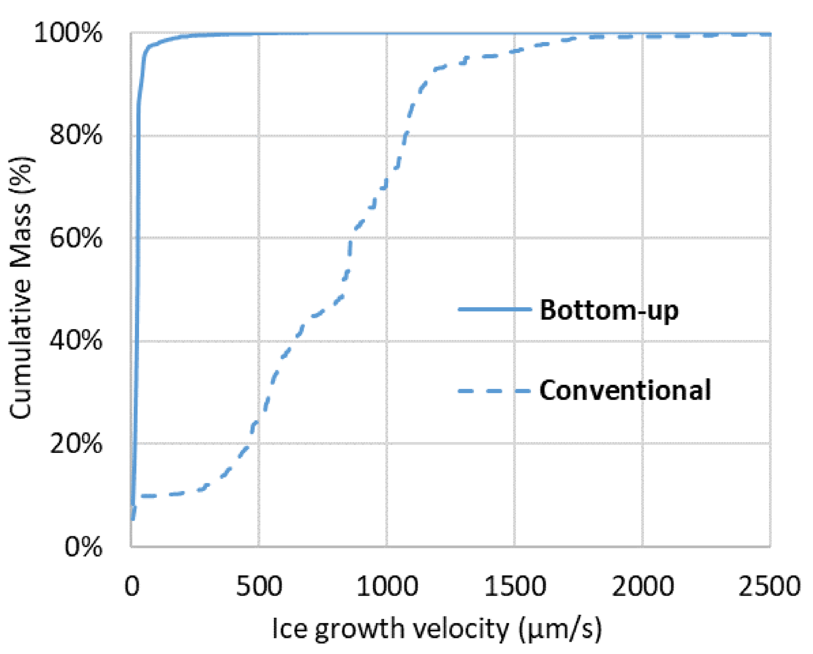
CELL vs Conventional Freezing on hIPS cells



- Using the CELL (Bottom-up), membrane integrity is always higher even for 1 % DMSO.
- A cooling rate of 5 ºC/min shows 3x higher cell survival when using the CELL.

Understanding cell survival during freezing by means of CFD

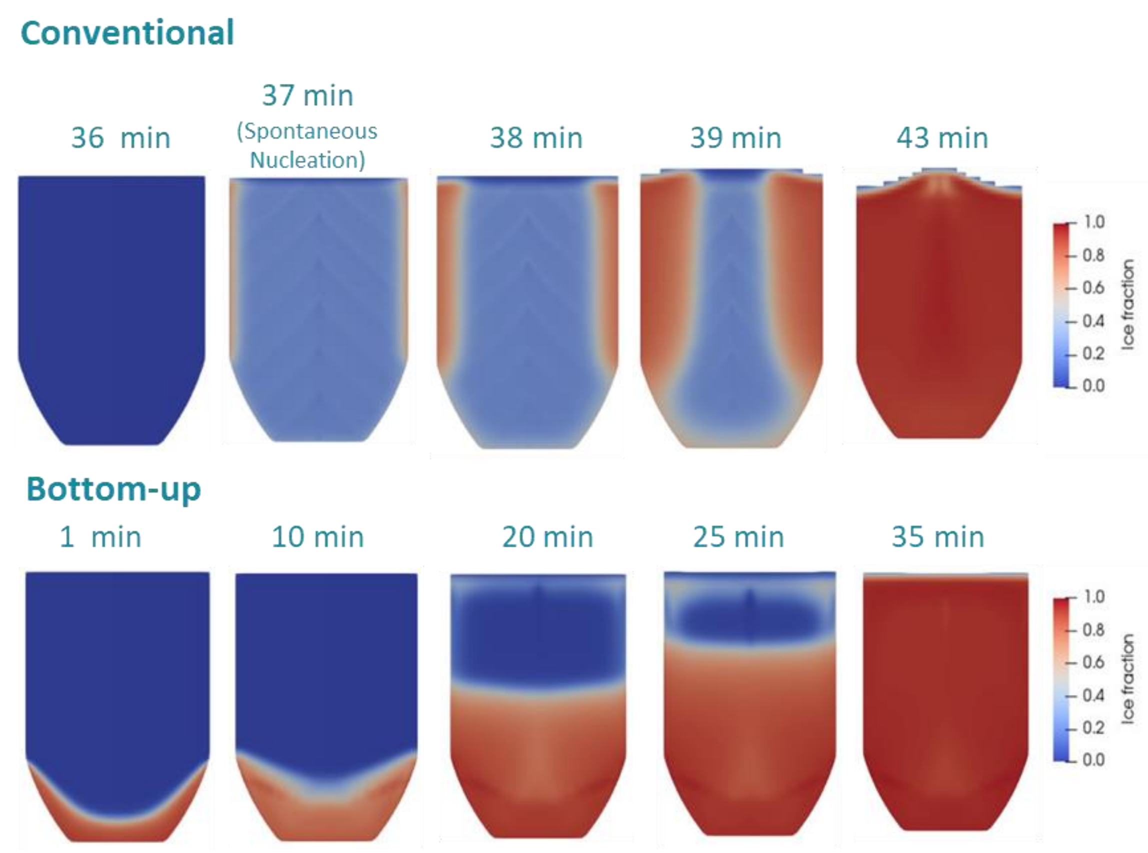


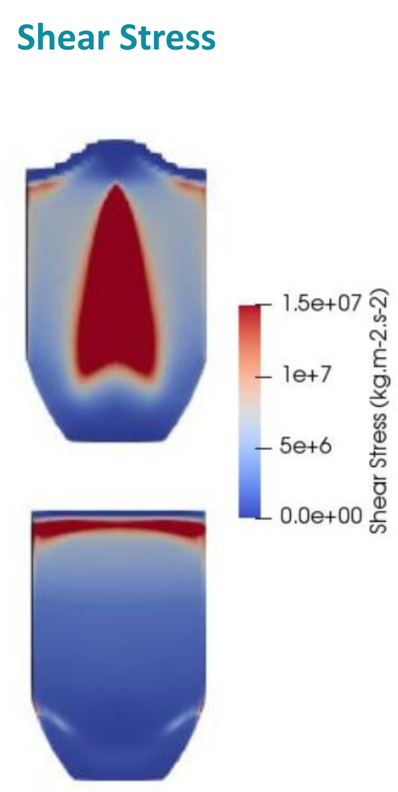


Bottom-up freezing shows a uniform ice growth velocity (between 10 to 20 μm/s).

Conventional freezing causes **high supercooling**, leading to burst freezing with extreme ice growth velocities.

Conventional radial freezing also leads **to higher mechanical stress (shear stress)** which can originate percolation of the liquid phase (cells) inside the ice matrix.





Conclusions

The study concludes that the CELL bottom-up freezing geometry allows to preserve cells (> 80% cell survival) with less than 4% v/v DMSO concentration. The CELL bottom-up geometry results in an uniform ice growth velocity attenuating mechanical stresses during freezing.

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