SMARTFREEZ
PRECISION CRYOSYSTEMS

CELL

A new cryopreservation method to improve cell viability

CELL

CELL bottom-up freezing

by SMARTFREEZ

CELL is a controlled-rate freezer with a bottom-up heat transfer geometry, reducing cryo-concentration and consequently decreasing mechanical stresses, resulting in higher cell viability after freezing-thawing.

- Enables precise control of ice-nucleation, reducing the supercooling effect.
- Controlled crystal growth (velocity and direction).
- Less harmful to the integrity of cells, allowing a significantly higher rate of cell survival with smaller concentrations of the cryoprotectant Dimethyl sulfoxide (DMSO).

The CELL bottom-up freezing geometry

uniform ice growth velocity, attenuating mechanical stresses during freezing.

CELL

Improving cell viability and reproducibility

The conventional methods for freezing cells in vials or bags have a general tendency to cause supercooling inside the container. This leads to extreme high ice growth velocities which can cause several stresses to cells. In the CELL the stress to cells is minimized by preventing the supercooling. Only a "sacrificial layer" of the liquid (5% volume) supercools to induce controlled ice-nucleation (Figure 1). Then, ice grows from the bottom (sacrificial layer of ice) to the top in a controlled rate preventing cryoconcentration and mechanical stresses caused by pressure.

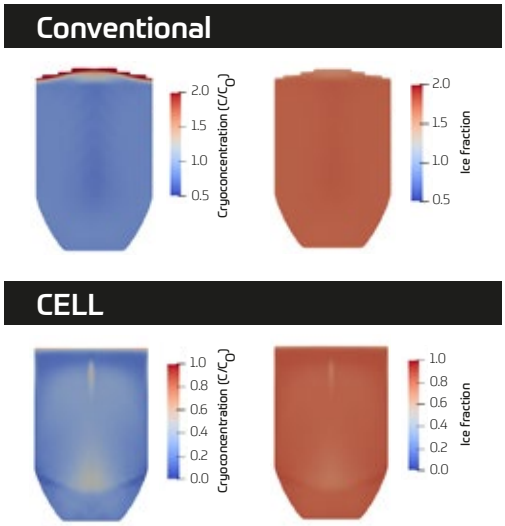
Fig. 1 *- Sacrificial layer of ice (in dark blue) formed after controlled nucleation in 2 mL vials.*

Quality by Design optimization
of cryopreservation protocols

CELL equipment has a digital twin, that allows to design your own methods for optimal cryopreservation methods, saving time (more rational design and less trial-error) and saving of biological material.

The SMARTFREEZSIM® simulation platform will provide data to understand the impact of your parameters on key stresses for cells` viability (Figure 2).

Fig. 2 *- Cryoconcentration and Ice fraction in 2 mL vials simulated at the SMARTFREEZSIM®.*



Case study 1

Impact of freezing geometry

on the cryopreservation of Hematopoietic stem cells

Umbilical cord blood mononuclear cells` were frozen using the CELL and a conventional radial freezing method.

Flow cytometry analysis for the CD34+ cells (hematopoietic stem cells and hematopoietic progenitor cells) revealed that when using 10% DMSO v/v, the level of expression of the CD34 marker was approximately three times higher for the CELL freezing compared to the conventional method. The bottom-up freezing geometry enables to reduce DMSO concentration to 2.5% with minor impact on expression levels (Figure 3).

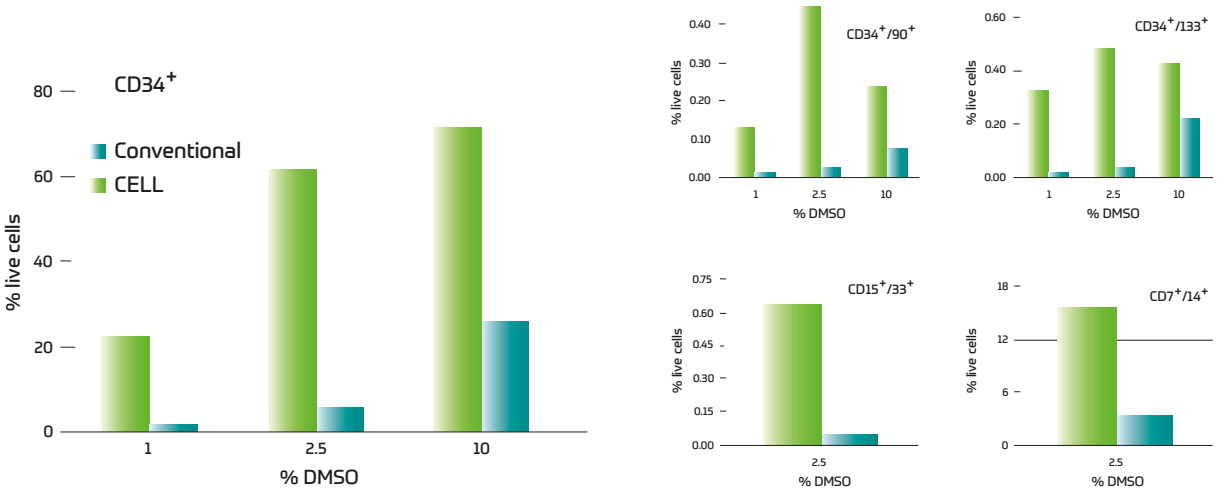


Fig. 3 *- Expression of CD34+ and the subpopulations CD34+/CD90+, CD34+/CD133+, CD15+/CD33+, CD7+/CD14+ after freezing, using the CELL and conventional radial freezing method.*

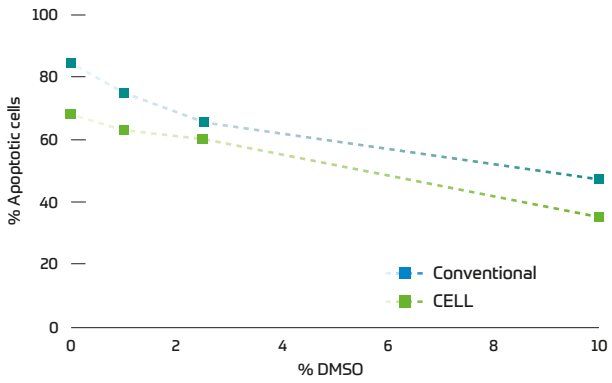


Fig. 4 *- Apoptotic cells (%) after freezing using the CELL and conventional radial freezing method.*

When comparing both methods, the conventional radial freezing always shows approximately more 10% to 20% of cells in an apoptotic state than the controlled freezing method using CELL (Figure 4).

Case study 2

Reducing DMSO concentration

during freezing of hIPSc

Human induced pluripotent stem cells (hIPSc) were frozen using the CELL and a conventional freezing method, with different DMSO concentrations and cooling-rates.

CELL allows to decrease the DMSO content below 5 % (v/v) and still obtain a cell survival of 80 % (Figure 5).

- A cooling rate of 1 °C/min enables to decrease the DMSO content to 4% v/v while maintaining the cells` viability above 90%.

- A cooling rate of 5 °C/min enables to decrease the DMSO content to 2% v/v with approximately 80% of cells` viability.

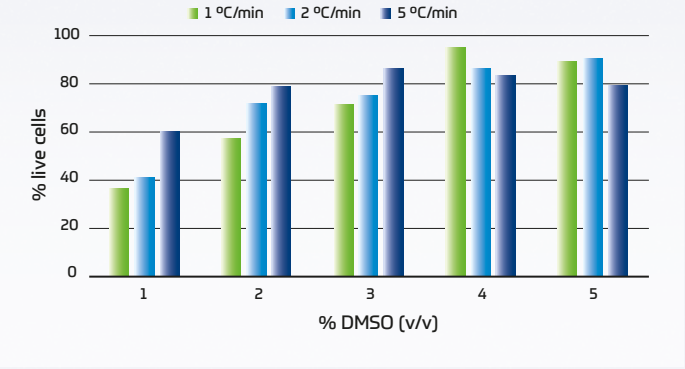


Fig. 5 *- Percentage of live cells after freezing using the CELL (bottom-up freezing) for DMSO concentrations below 5 % v/v.*

CELL bottom-up freezing results in higher cells` survival for both DMSO concentrations (1% and 5% v/v). For 1% of DMSO and a cooling rate of 5 °C/min, the bottom-up freezing resulted in 6 X more live cells than the conventional radial freezing (Figure 6).

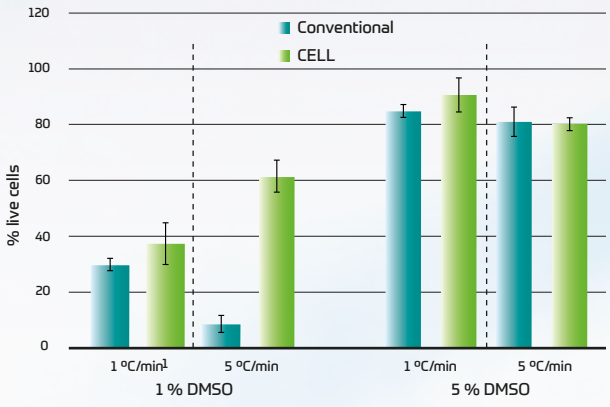


Fig. 6 *- Percentage of live cells after freezing using the CELL (bottom-up freezing) and conventional freezing method (radial freezing) for DMSO concentrations of 1 and 5 % v/v.*

Key
Specifications

CAPACITY	• *1 holder for 24 x 2 mL vials* • *1 holder for 3 x 30 mL bags*
DIMENSIONS (mm)	*460 (d) x 360 (w) x 600 (h)*
TEMPERATURE	*-85 °C to 25 °C*
WEIGHT	*40 Kg*
PATENT STATUS	*Pending*

Customizable to your needs

the CELL can be configured for vials and bags of different sizes and shapes.

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