



SMARTFREEZ

PRECISION CRYOSYSTEMS

Rational systems *for safe and reliable cryopreservation*

Engineering Ice-Growth Geometry for Improved Cryopreservation of Protein and Cell Therapies

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Outline

1. *A habitat of convoluted stresses (Introduction to freezing complexity)*
2. *How to compare freeze-thaw in different containers (Introduction to engineering challenges)*
3. *Freezing with equations – (Multi-physics CFD modelling)*
4. *Un**bottle** freeze-thaw stresses – (Engineering processes with radial containers)*
5. ***Baggy** issues - (Engineering processes with slab geometry)*
6. *Ice nucleation challenges in small volumes (engineering with gravity)*
7. *A geometry case study on Stem Cell cryopreservation (insight from modelling)*
8. *Final remarks (please remember us...)*



Introduction to freezing complexity – a habitat of convoluted stresses

How is the inside of a frozen matrix?

Ionic force Dehydration
Cold denaturation Cryoconcentration
Osmotic pressure Oxidation
pH drop Precipitation Aggregation
Adsorption to ice interface

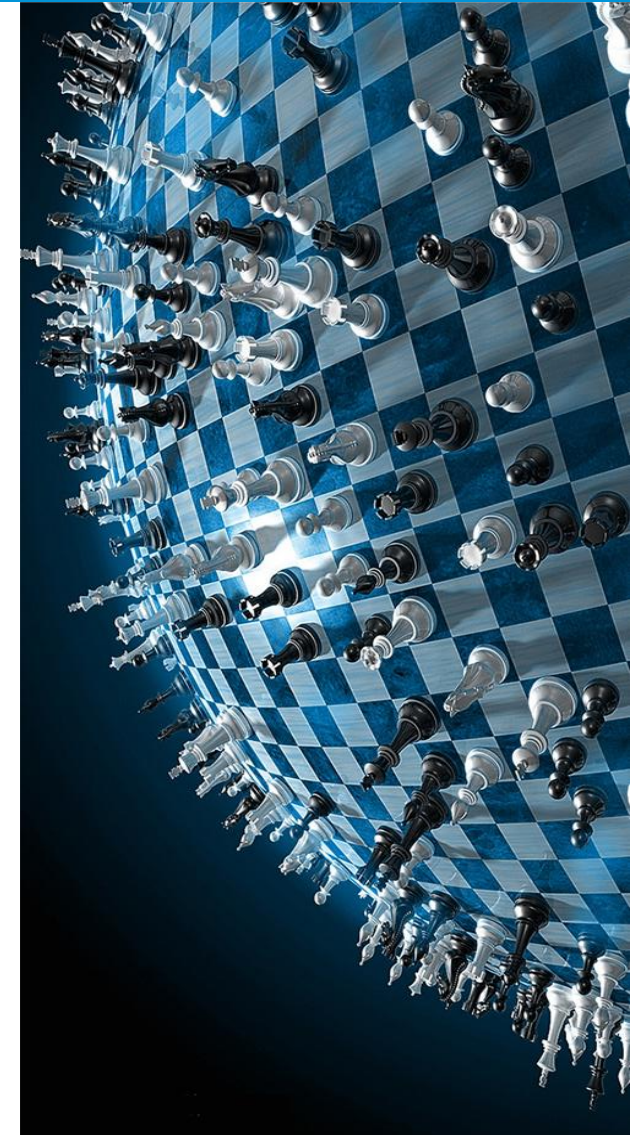
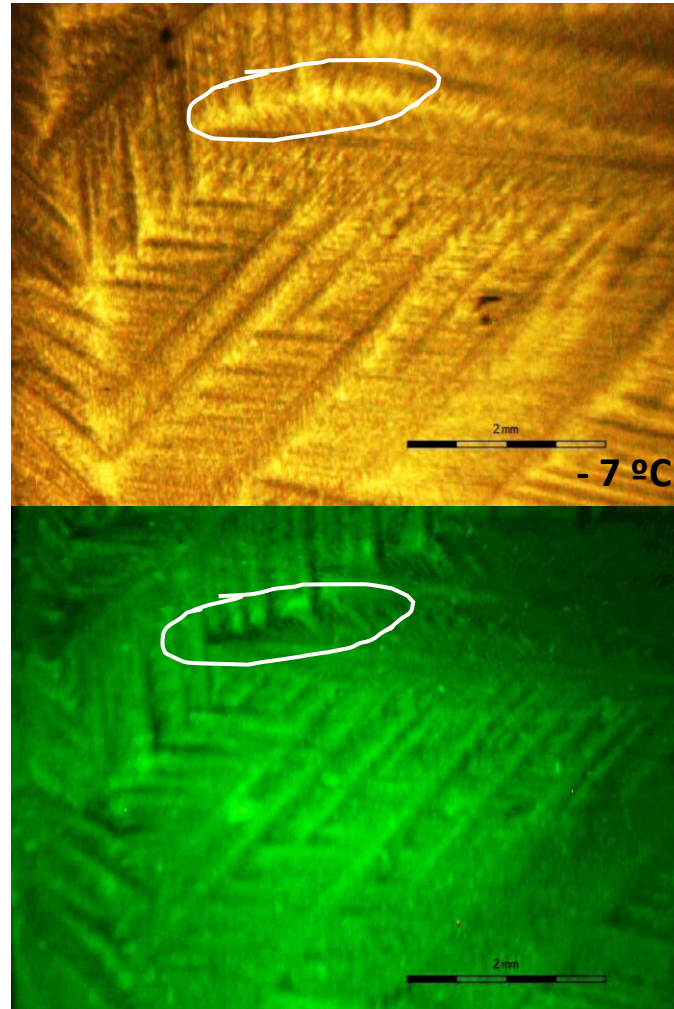
Where to start from?

The Excipients, The Concentrations, The Rates...



the possibilities are too many

and freezing encloses many uncontrolled mechanisms



How to compare freeze-thaw processes in different containers?



10 L



16 L



10 mL



2 mL



100 mL



6 L



10 L



20 L

Slab vs Radial



100 L

How to compare freeze-thaw processes in different containers?

Match external conditions?

Specific surface area is neglectable

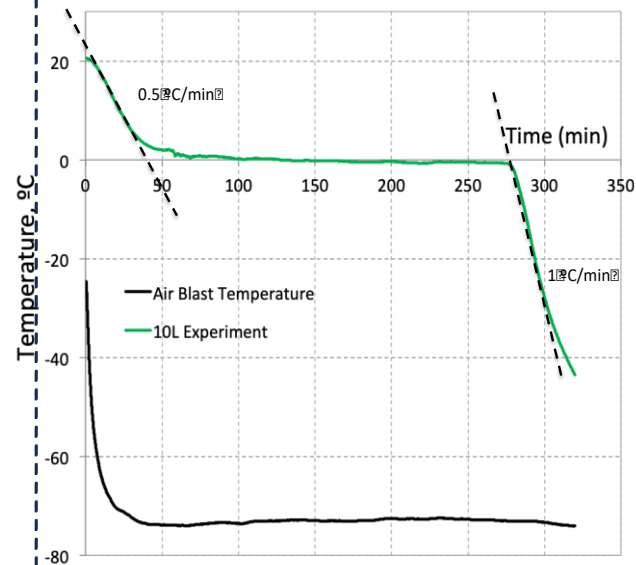


same boundary conditions



Match total process time?

Ice-growth rate is neglectable



Vial must freeze in 5h?!

Select $T(t)$... nucleate...

Planar ice growth...

1 D – Classical approaches

Match Ice growth-rate?

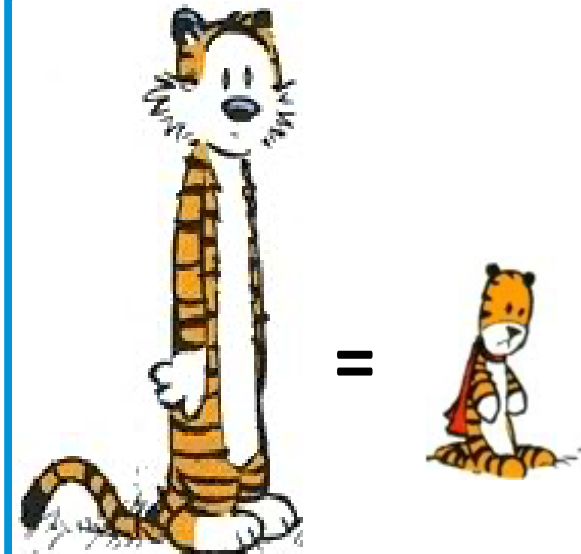
Holding time under stress is neglectable



1 h
to reach -40 °C



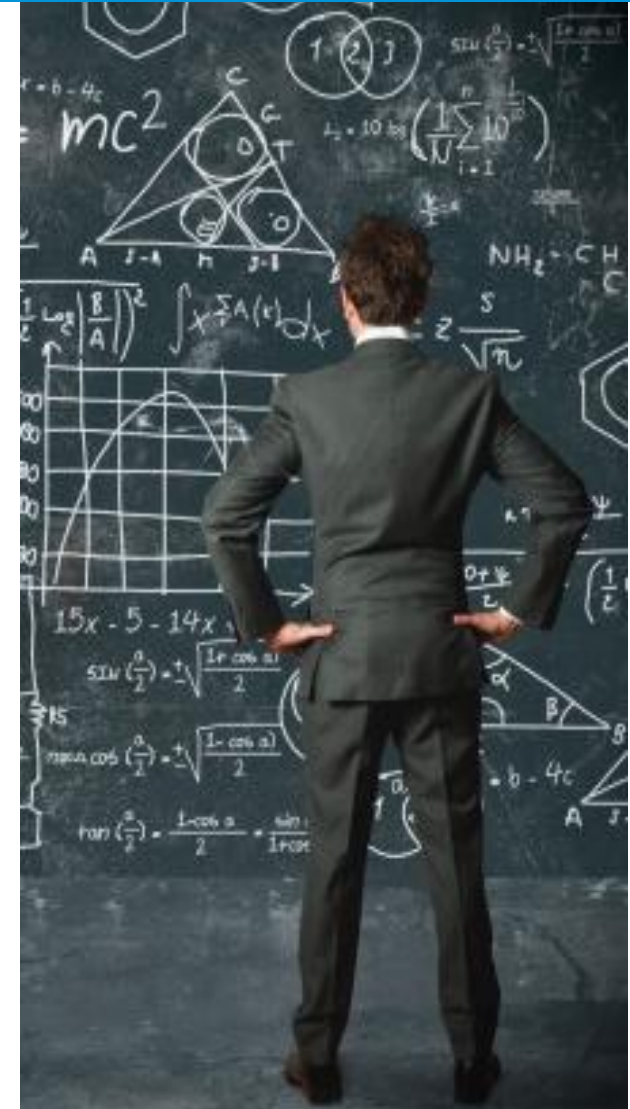
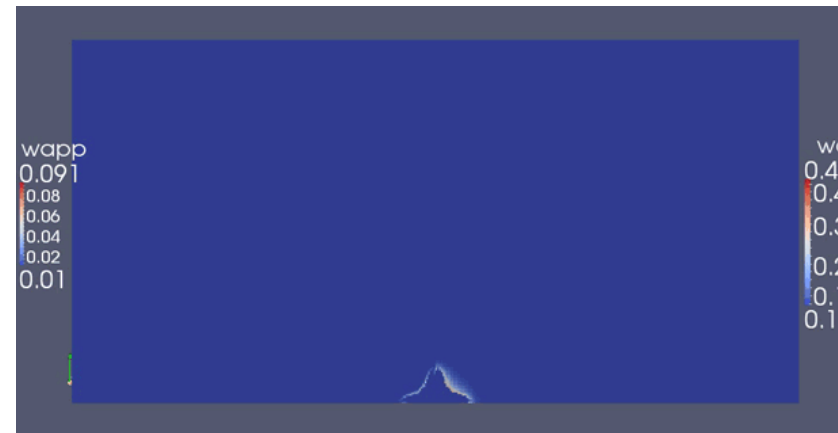
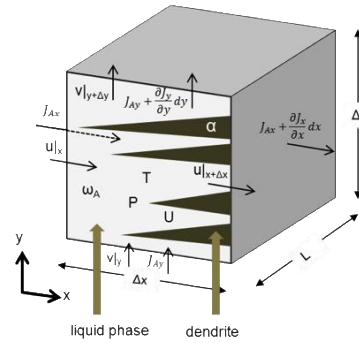
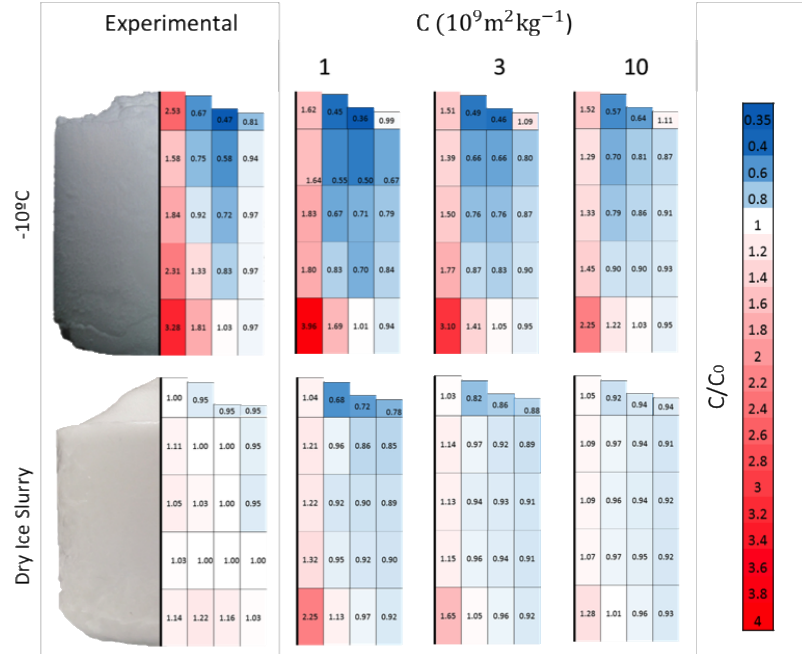
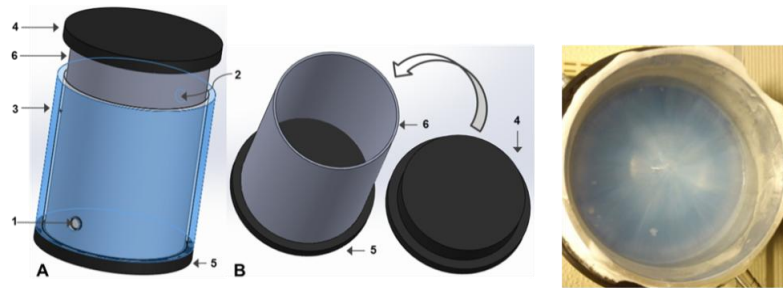
10 h
to reach -40 °C



Freezing with equations, the multi-physics of CFD SmartfreezSIM™

Computational Fluid Dynamics

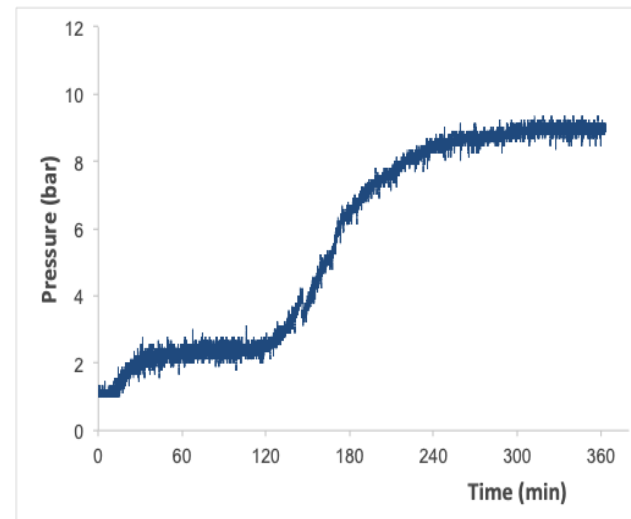
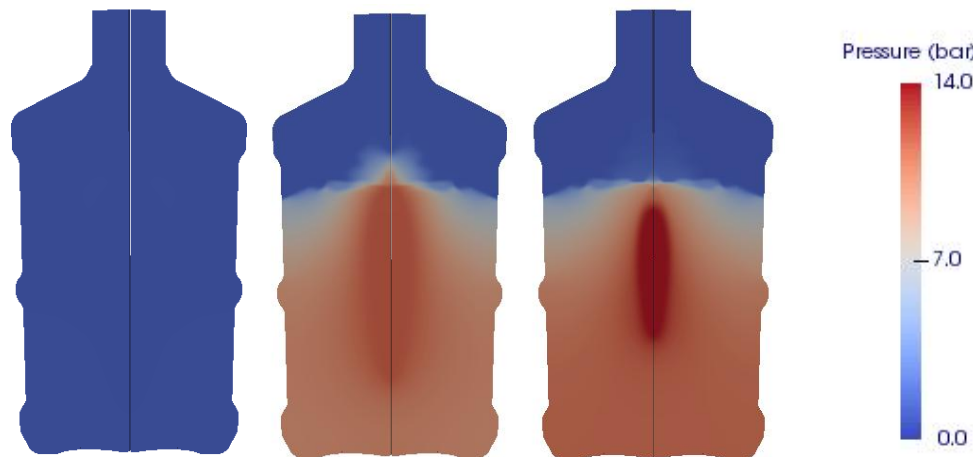
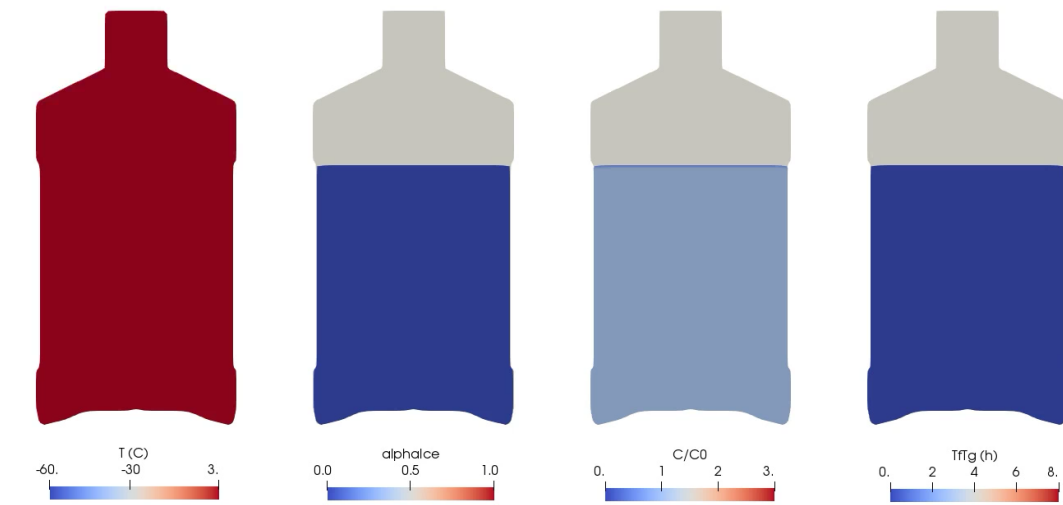
Geraldes et al.2020 J. Pham. Sci. 109:1078–1989



Unbottling the stresses - Engineering Radial Freezing

Time: 0.0 (h)

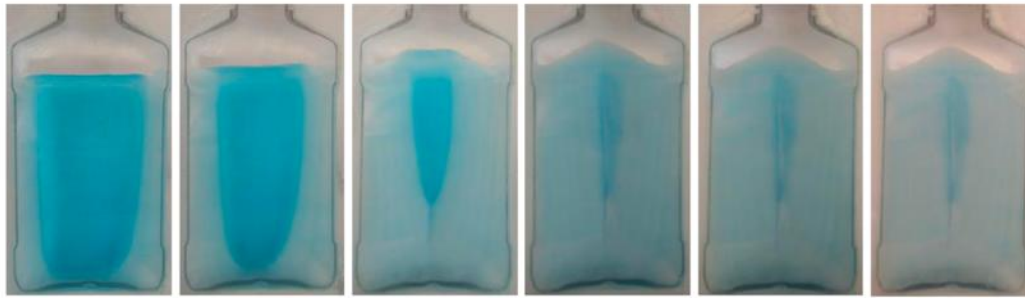
Duarte et al. 2020 AAPS PharmSciTech 2020 DOI: 10.1208/s12249-020-01794-x



Attenuating Mechanical Stresses - Engineering Radial Freezing



2L BOTTLE



Freeze-concentration
(C/C_0)

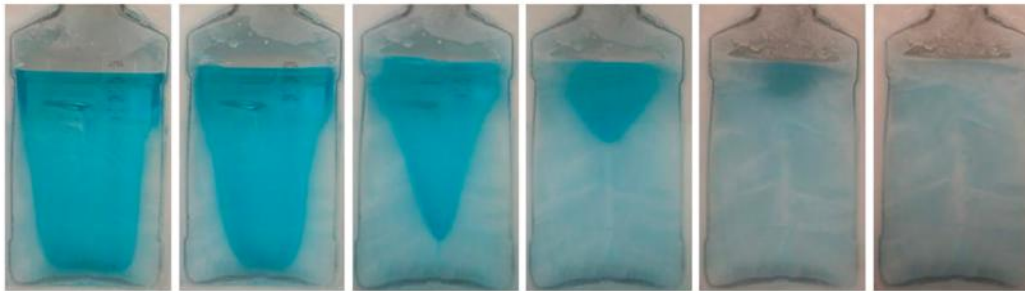
1.0	1.3	1.9	2.1	2.3	2.4
0.7	0.8	0.6	0.6	1.1	1.4
0.7	0.9	0.8	1.2	0.9	1.2
0.9	1.1	1.3	1.6	1.3	1.1
1.4	1.2	1.2	2.2	1.2	1.4
1.0	1.5	1.4	2.1	1.4	1.5
0.9	0.9	1.5	2.8	1.5	0.9
1.0	0.9	1.5	3.1	1.5	0.9
1.2	1.2	2.1	3.1	2.1	1.2

Aggregates increase
%

1.0	2.6	1.8	1.8	1.5	2.8
4.2	3.2	3.8	3.8	3.4	2.8
3.1	3.5	2.5	3.1	2.7	7.8
3.2	3.5	2.1	2.1	2.1	3.5
2.9	3.4	1.9	1.8	1.9	3.4
6.4	1.4	1.9	1.7	1.9	6.4
3.4	3.5	1.7	1.5	1.7	3.5
3.3	4.6	2.9	0.7	2.9	4.6
3.6	2.6	1.3	0.5	1.3	2.6



2L BOTTLE
INSIDE NORMABLAST



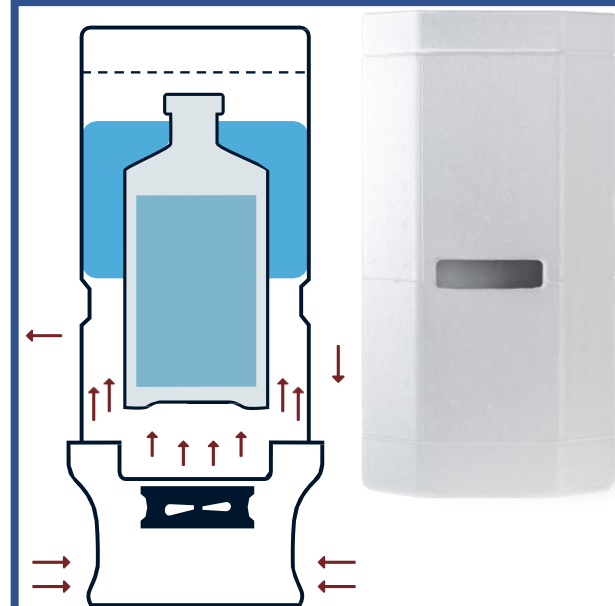
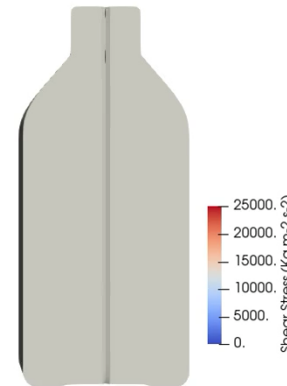
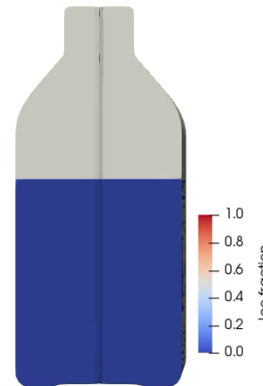
Freeze-concentration
(C/C_0)

1.2					
0.8	0.5	0.7	1.1	0.8	0.6
0.6	0.6	1.0	1.7	1.4	0.8
0.6	0.6	1.2	1.7	1.4	0.9
0.7	0.7	1.0	1.7	1.0	0.7
0.8	0.8	1.1	1.7	1.1	0.8
0.8	0.9	1.4	2.2	1.4	0.9
0.8	1.0	1.1	2.2	1.1	0.8
0.9	1.0	1.7	2.7	1.7	0.9
1.0	1.3	1.6	2.5	1.6	1.3

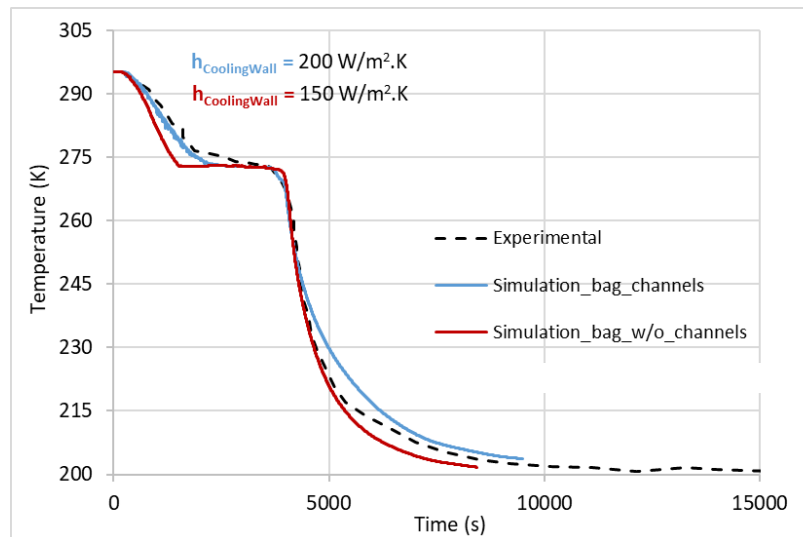
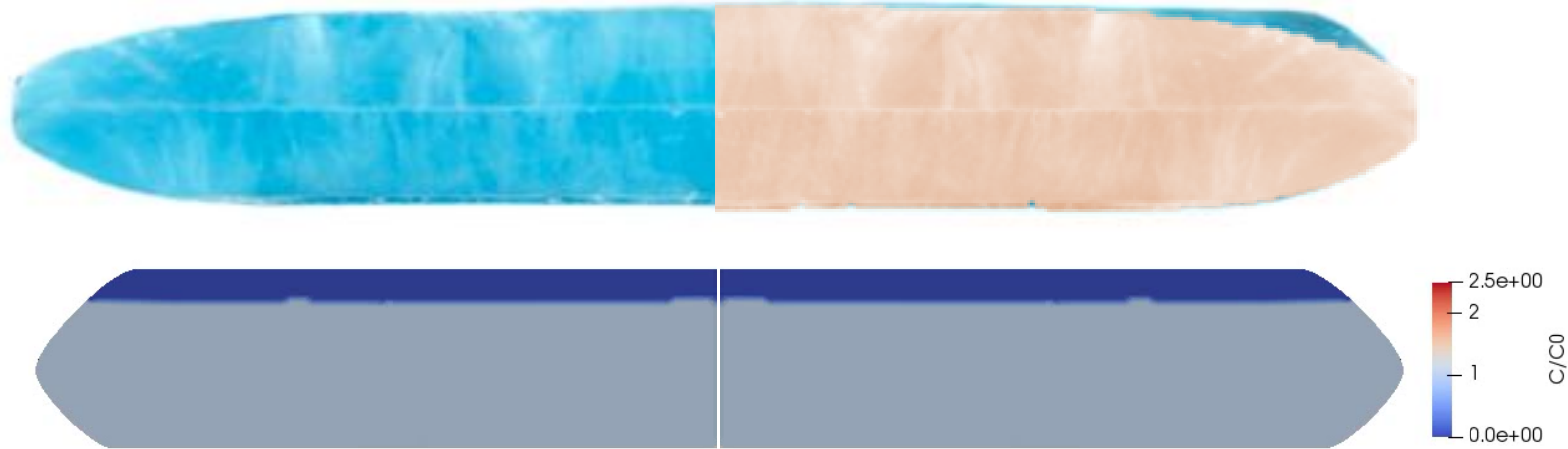
Aggregates increase
%

1.0					
2.7	2.0	2.1	0.2	1.0	1.3
0.2	2.3	1.3	0.0	0.4	1.7
1.5	3.4	1.0	0.2	0.3	1.9
1.8	1.7	1.4	0.2	1.4	1.7
1.7	1.3	1.4	0.0	1.4	1.7
0.2	1.3	0.4	0.1	0.4	1.3
1.7	1.6	1.2	0.5	1.2	1.6
1.8	1.6	0.6	0.9	0.6	1.6
1.6	1.0	0.3	0.5	0.3	1.0

Time: 0 min



Baggy issues - Engineering Slab freezing



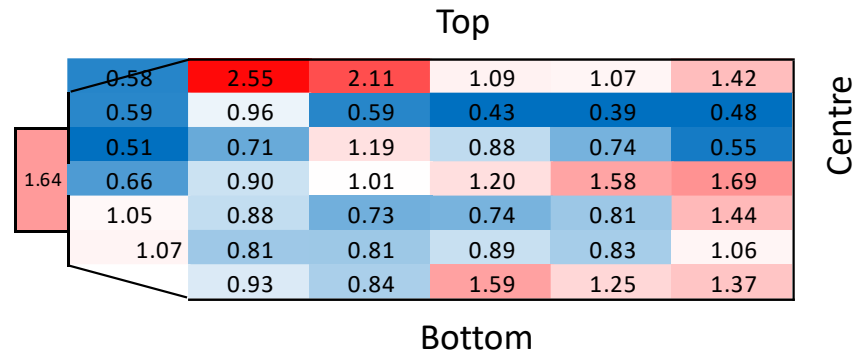
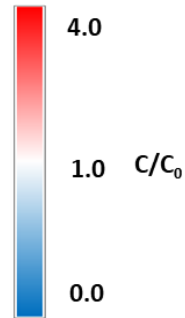
Attenuating mechanical stresses and anisotropy – Engineering Slab Freezing



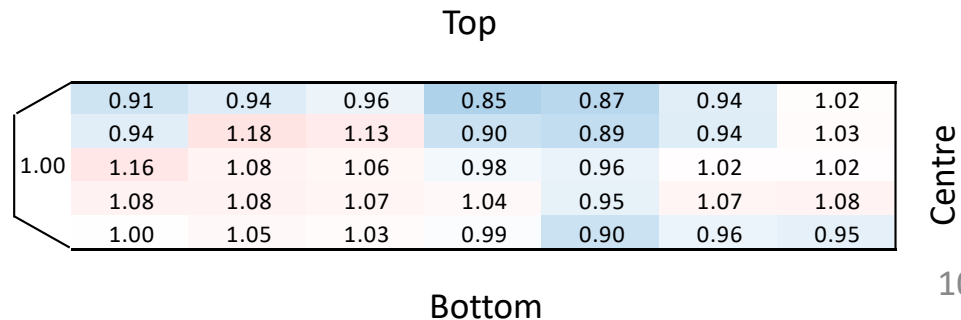
Bi-directional



Bottom-up - unidirectional



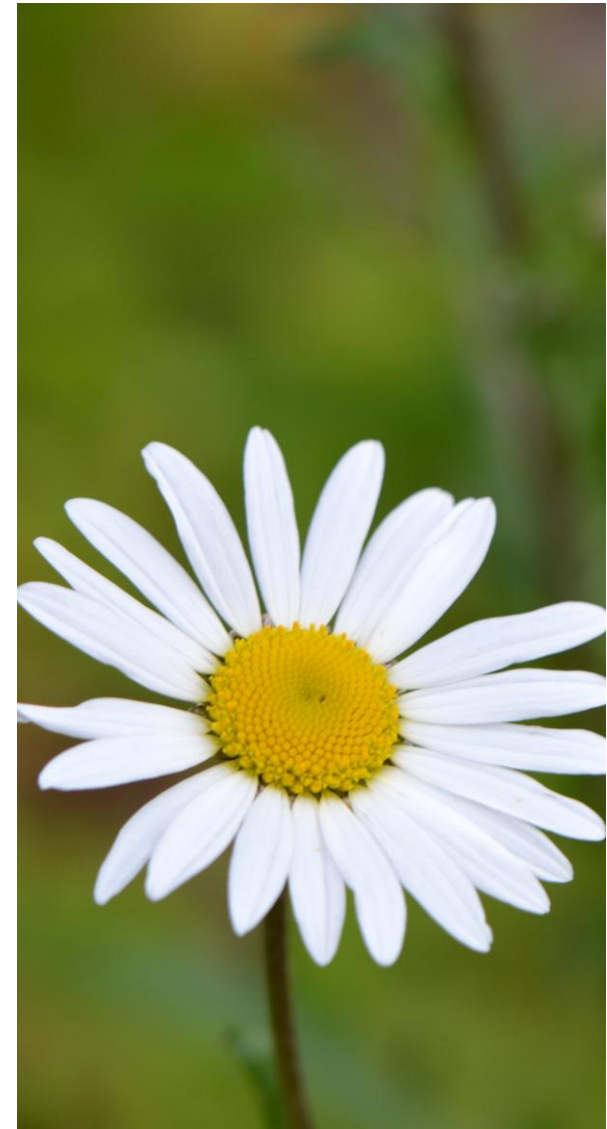
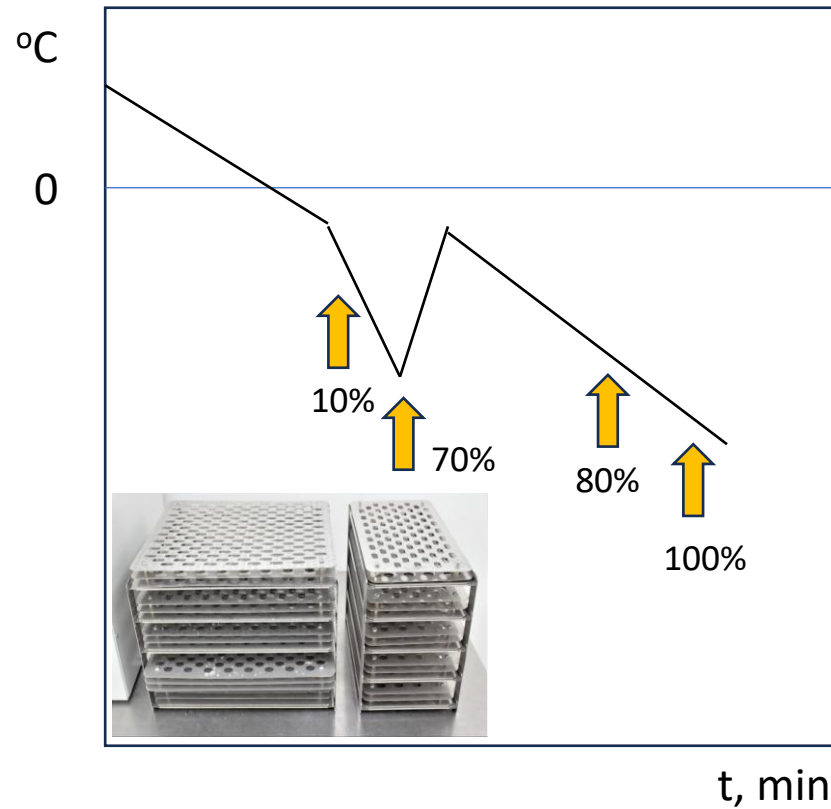
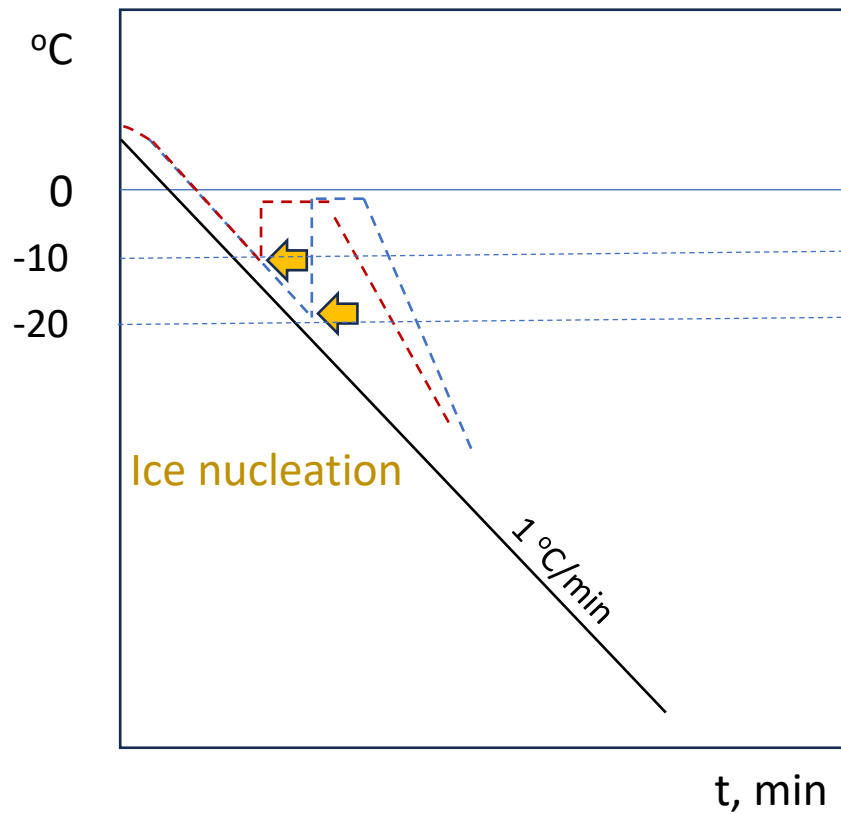
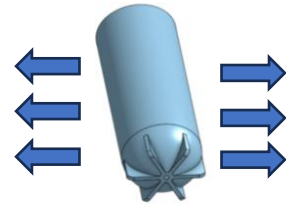
Local protein concentration divided by initial protein concentration
(determined by SEC-HPLC after thawing ice samples)



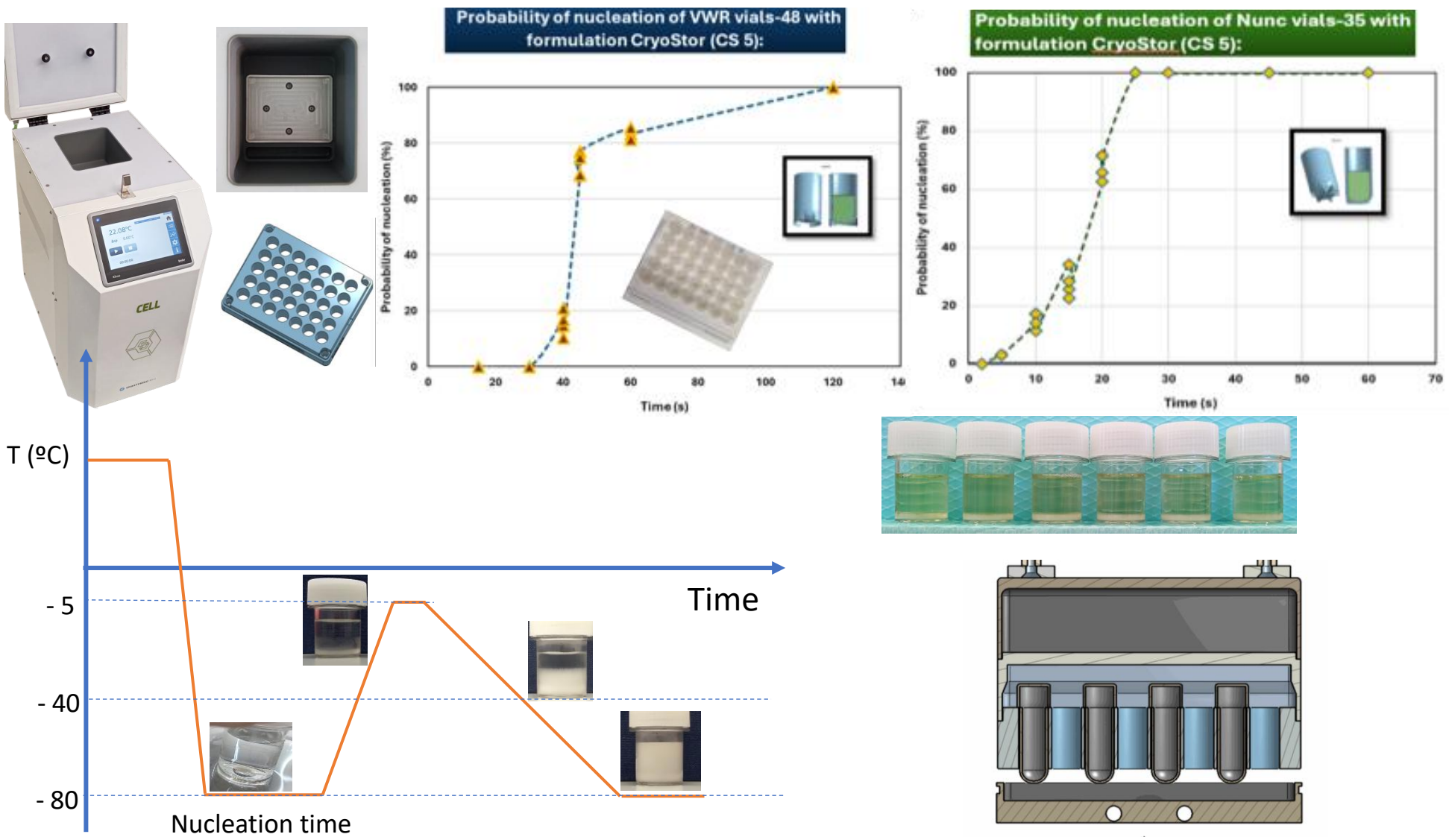


Ice loves me, or loves me not... - Engineering Ice Nucleation

Small volumes
Radial heat exchange
Enhances supercooling



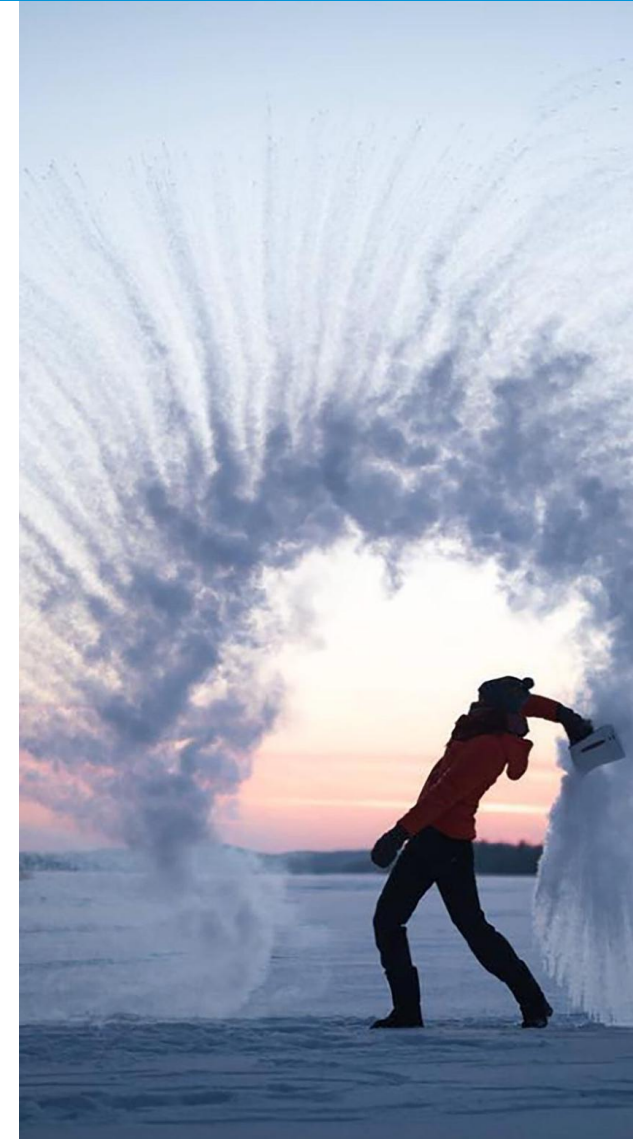
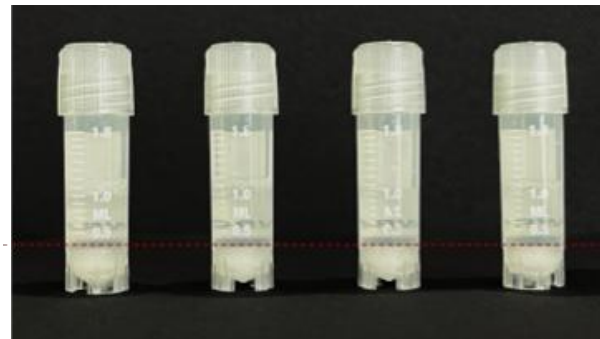
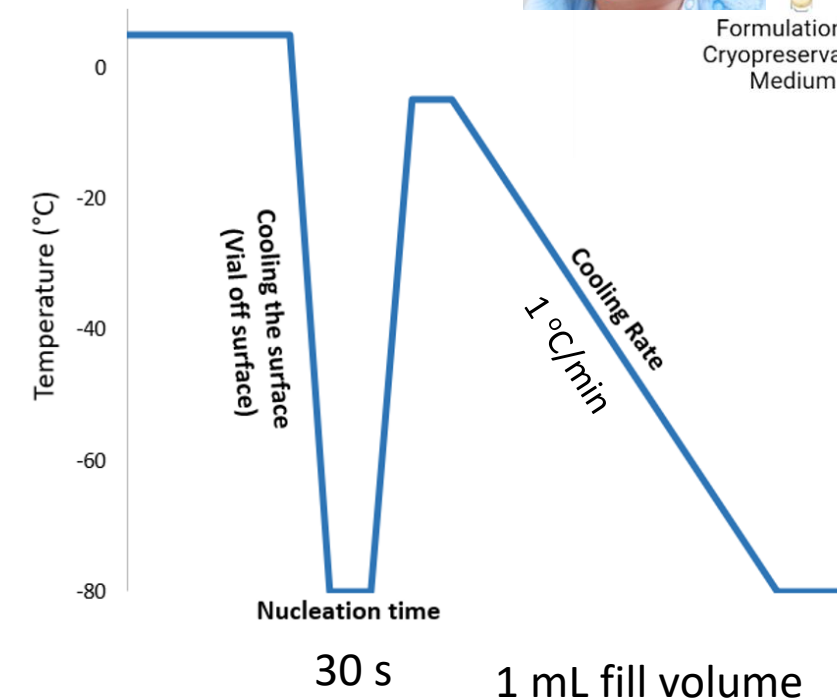
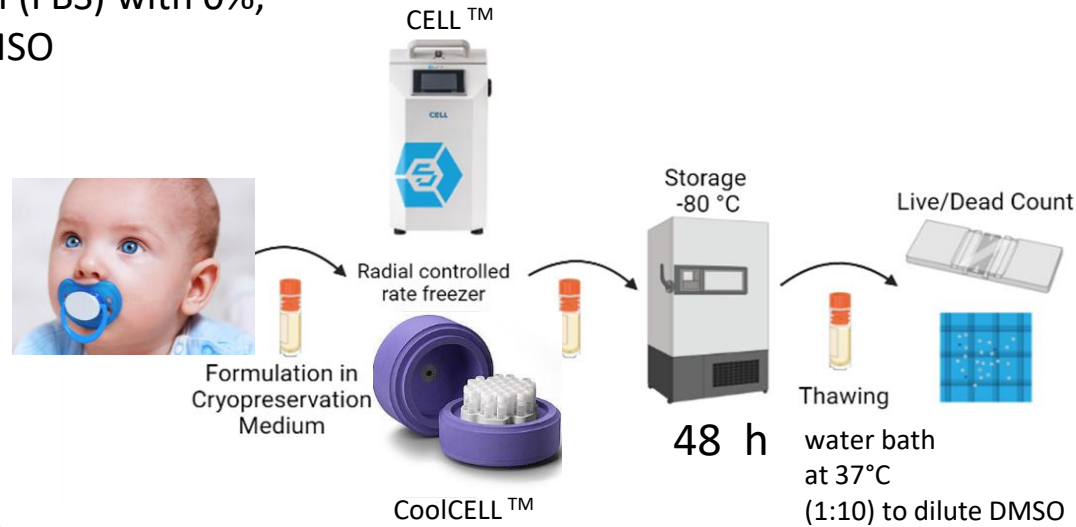
Attenuating supercooling - Engineering Ice Nucleation



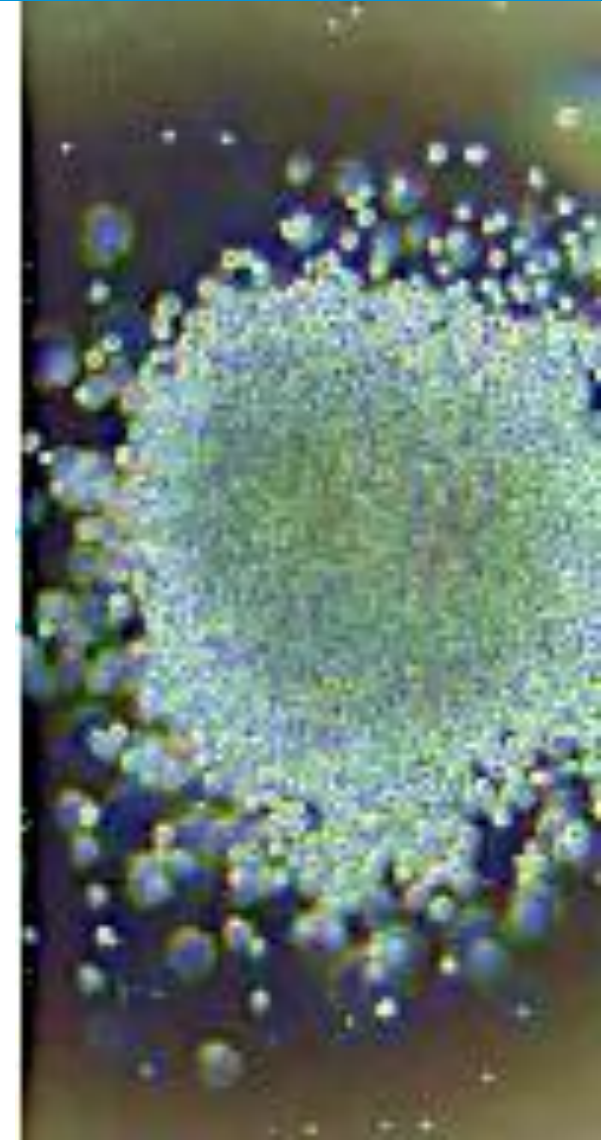
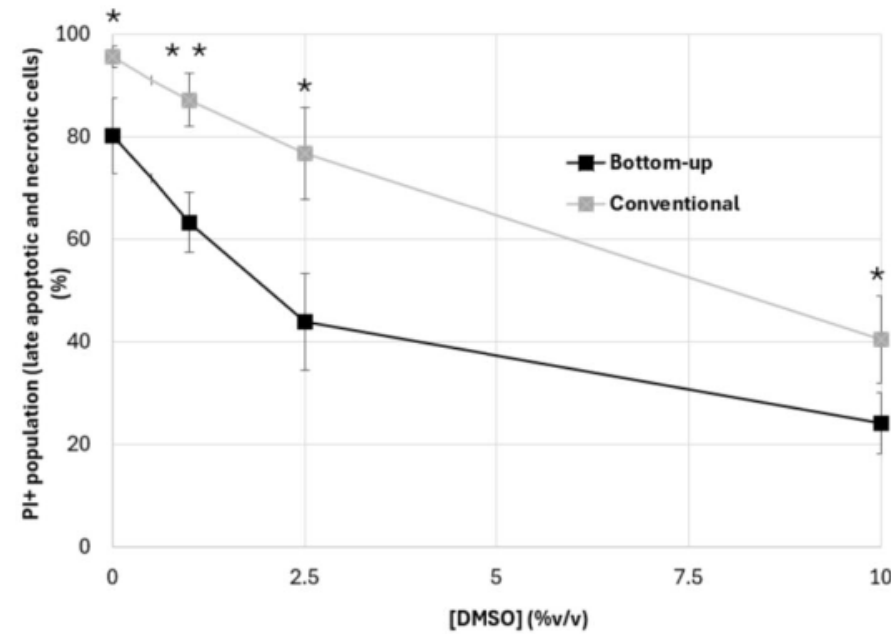
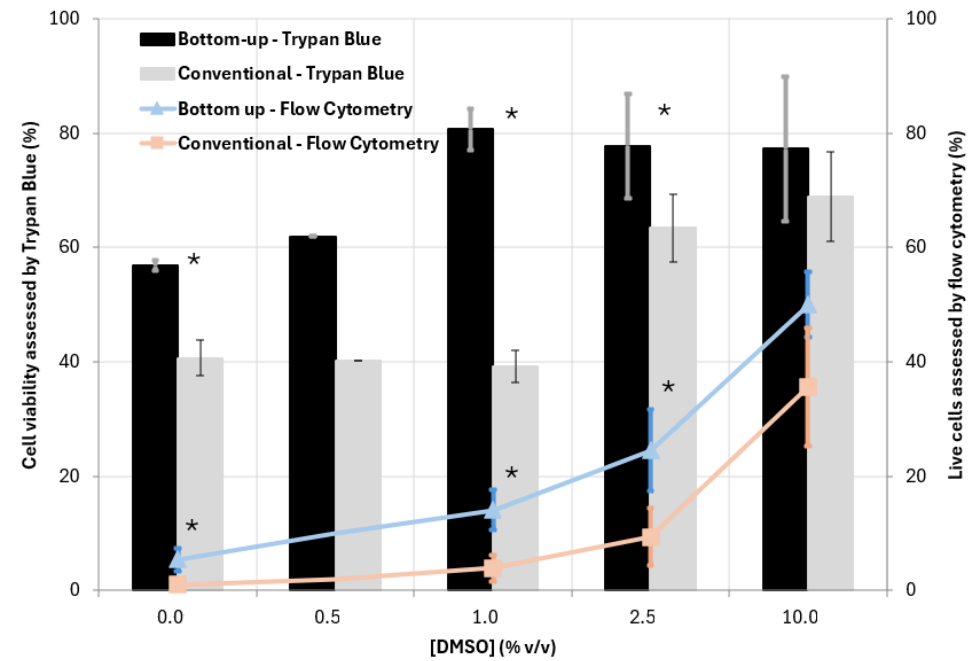
Bottom-up vs Radial - Geometry Case-Study on the Cryopreservation of Stem Cells

Media: Fetal Bovine Serum (FBS) with 0%, 0.5%, 1%, 2.5%, or 10% DMSO

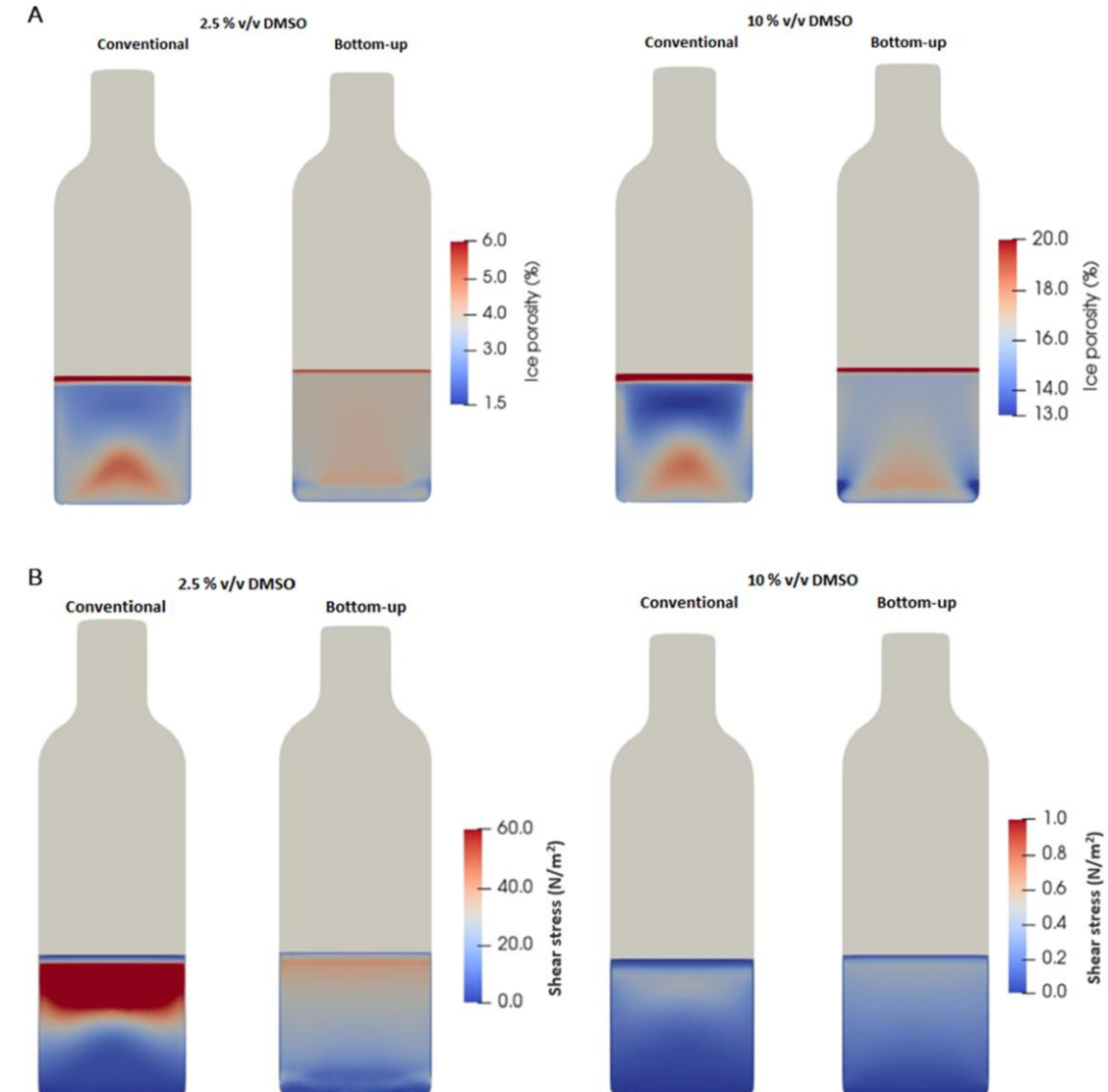
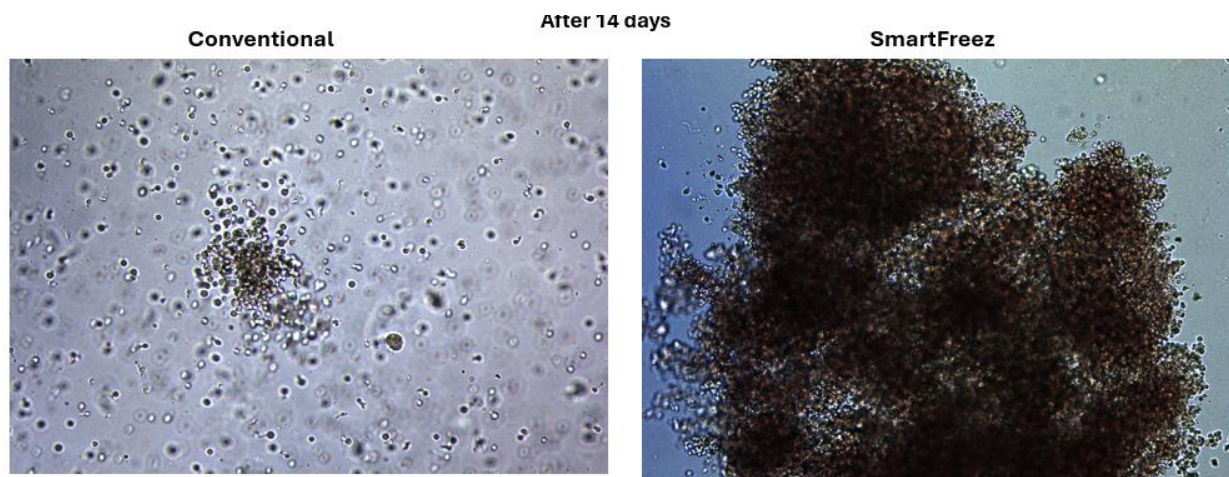
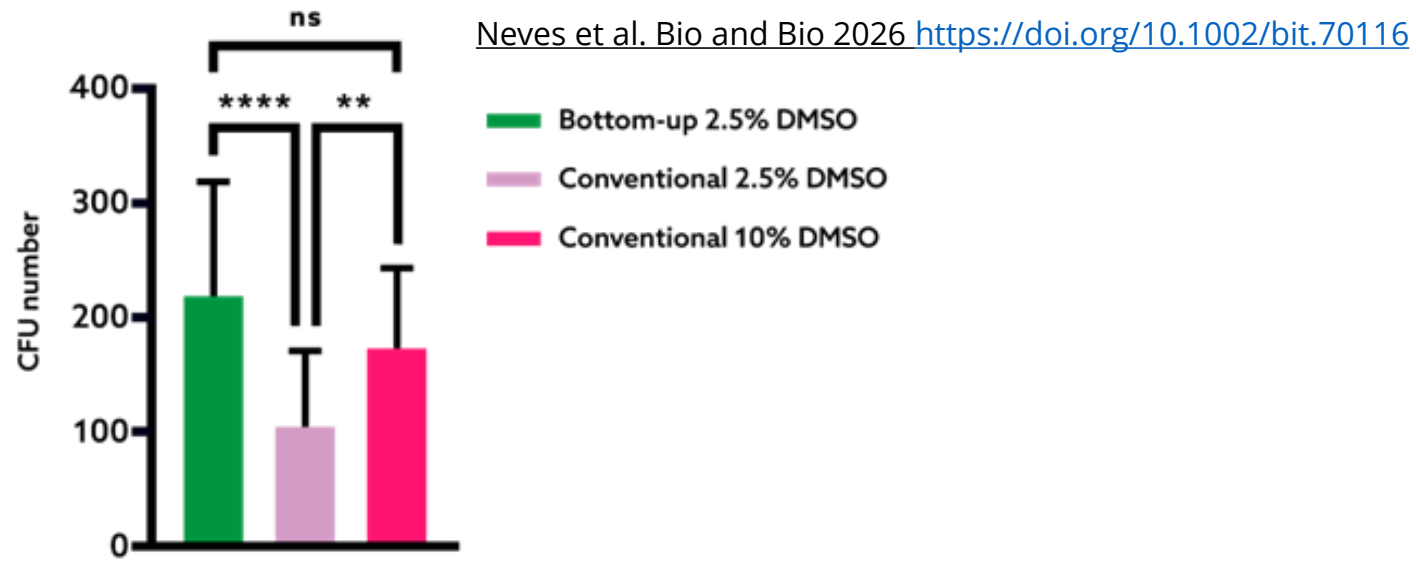
Neves et al. Bio and Bio 2026 <https://doi.org/10.1002/bit.70116>



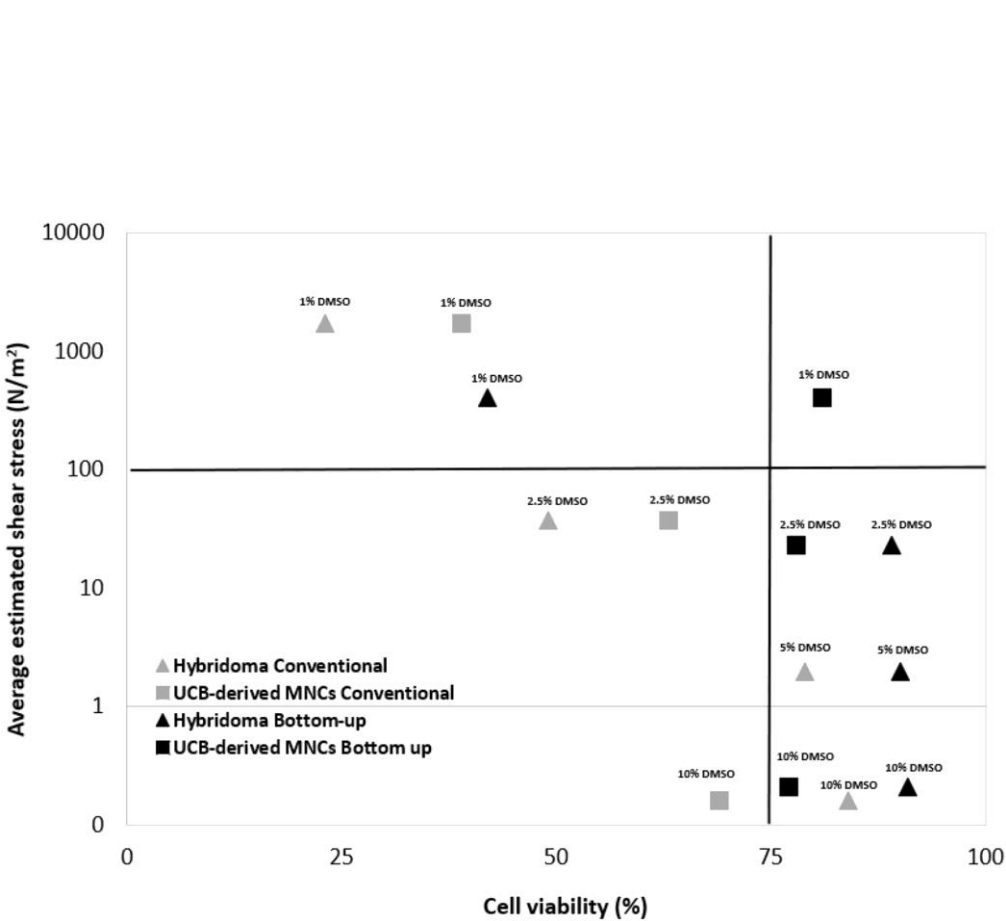
Stem Cells UCB-derived MNCs - Bottom-up vs radial (conventional) Case study



Stem Cells UCB-derived MNCs - Bottom-up vs radial (conventional) case study



Variable found... - Multi-physics insight for selecting geometries that reduce mechanical stresses



Time: 0 min

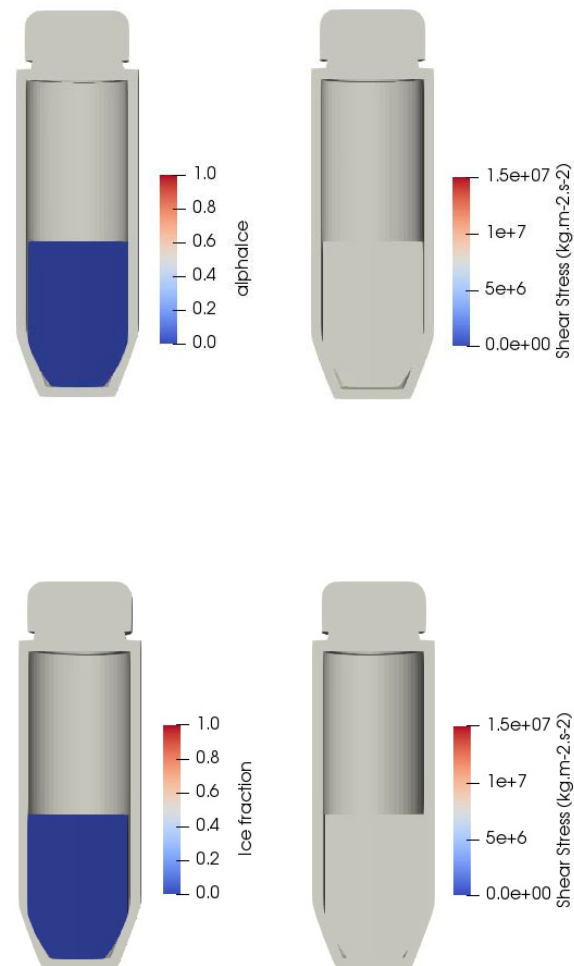
1% DMSO

CELL
1% DMSO
Cooling rate: 1C/min

Time: 0 min

1% DMSO

Conventional freezing
1% DMSO
Cooling rate: 1 C/min



Final Remarks

Processes are more rationally optimized (and scaled) using computational fluid dynamics

*Different sizes freeze differently, **don't use 1D downscale models***

Stresses can be quantified between very different sizes, geometries, media, etc...

We can achieve higher quality (cell viability) with more rational design

Please remember SmartFreez if you realize that you need more rational cryosystems



THANK YOU !



www.smartfreez.com