April 18, 2018
Lab seminar

Design methodology of freeze-thaw processes towards industrial manufacturing of induced pluripotent stem cells

iPS細胞の実生産に向けた凍結・解凍プロセスの設計手法

M2

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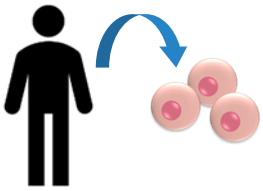
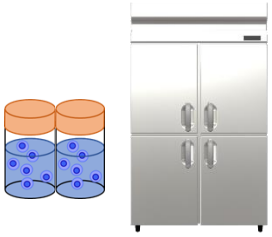
Background

- **Induced pluripotent stem (iPS) cells**
  - Potential to be differentiated into all tissue and organs.
  - Attract much interest in disease modeling, personalized medicine.
  - Standardization of manufacturing process is necessary.

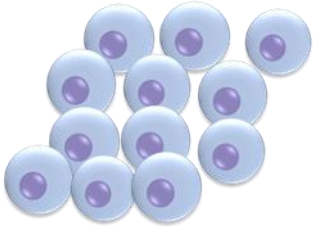


Cell harvest

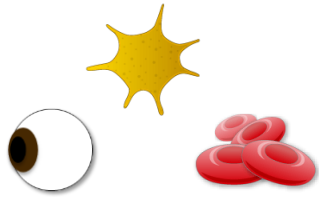
Initialization



Freeze-thaw



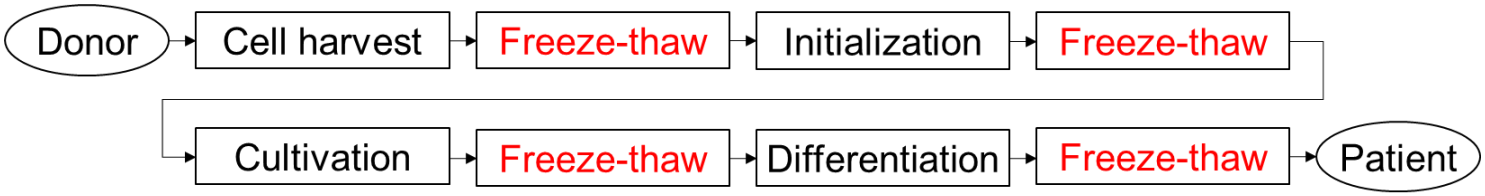
Cultivation



Differentiation

Background

- **Freeze-thaw process**
  - It is required to transport and cryopreserve iPS cells.
  - There are many freeze-thaw processes in manufacturing.



  - iPS cells are known to be vulnerable to freezing and thawing[1].

- **Previous study**
  - Cryoprotective agent study[2]
  - Experimental study[3,4]

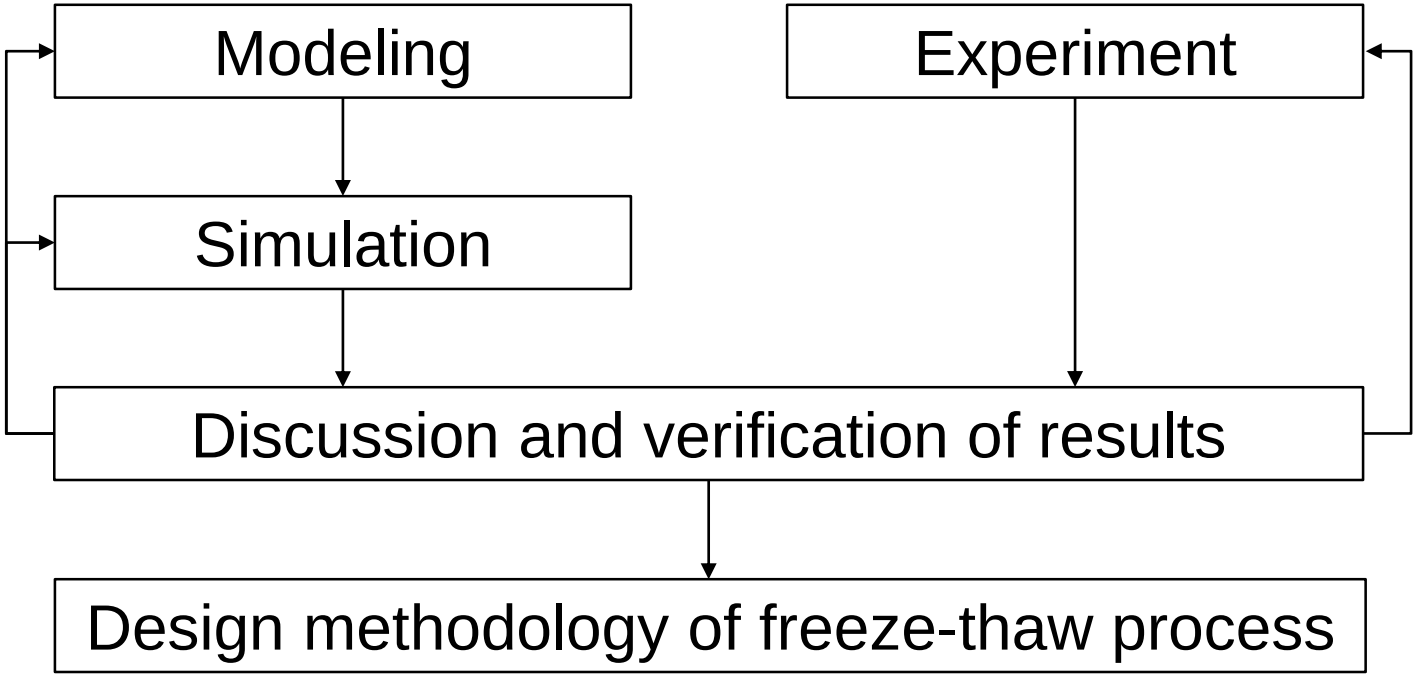
In the freeze-thaw process, it is important to evaluate the impact of heat and mass transfer through the cell membrane.

[1] C. J. Hunt, *Transfusion Medicine and Hemotherapy* (2011), [2] Y. Xu, *et al*., *Arch. Biochem. Biophys.* (2014), [3] R. Li, *et al*., *Tissue Engineering Part C Methods* (2018), [4] Y. Nishimura *et al*. *Neuroscience Research* (2016)

This study

Objective

Development of design methodology of freeze-thaw process for iPS cells considering heat and mass transfer



Experiment (In the future)

- **Program freezer (Freezing)**
  - Cooling rate : 1 K～3 K/min
  - Lowest temp. : 193 K

Program freezer

Cryovial[1]

- **Cryovial**
  - Made of polypropylene
  - Diameter : 12 mm
  - Height : 48 mm

- **Constant temperature water bath (Thawing)**
  - Temperature : 37℃

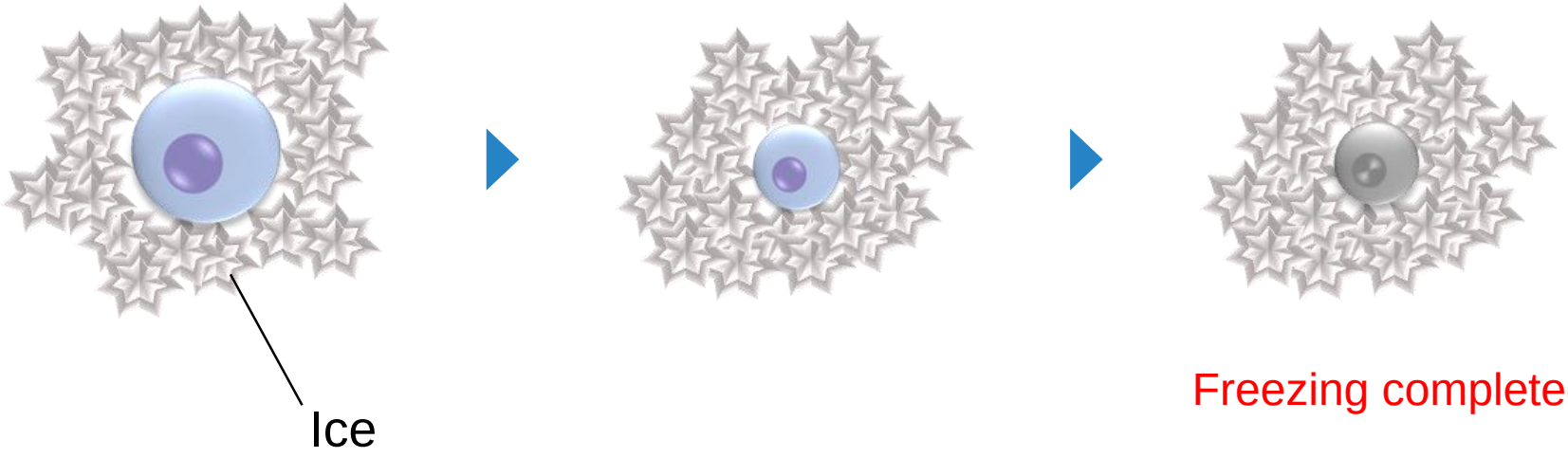

Water bath

[1] Thermo Fisher Scientific Inc.

Freezing of cells

Mechanism

1. Extracellular water is frozen.
2. Water in a cell is transported by osmotic pressure.
3. Depression of freezing point inside a cell occurs.
4. The solute in a cell is frozen at the eutectic point.

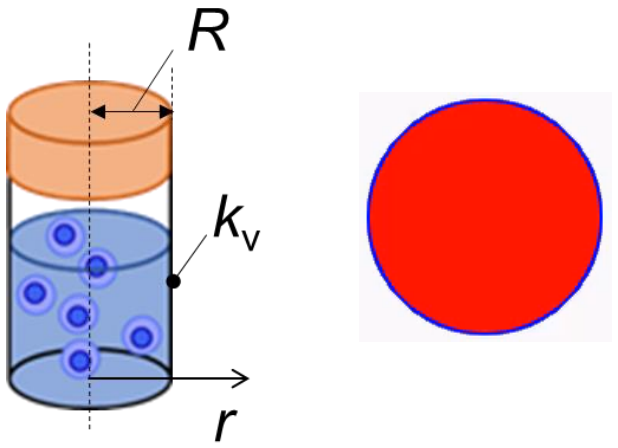


Modeling

Heat transfer

$$\frac{\partial T(r,t)}{\partial t} = \alpha\left(\frac{\partial^2 T(r,t)}{\partial r^2} + \frac{1}{r}\frac{\partial T(r,t)}{\partial r}\right)$$

$$\frac{d\delta}{dt} = \frac{1}{\rho_s H_f}\left[k_s\left(\frac{\partial T}{\partial r}\right)_{\delta} - k_l\left(\frac{\partial T}{\partial r}\right)_{\delta+d\delta}\right]$$

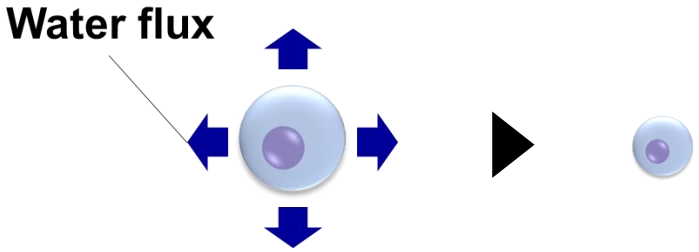


δ : Thickness of ice [m]
H_f : Heat of fusion of ice [kJ kg^{-1}]

Mass transfer

$$\frac{dV_w}{dt} = AL_p\Delta P$$

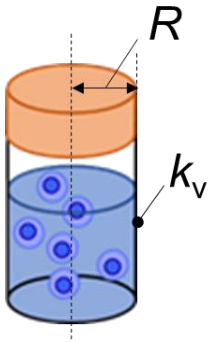
$$L_p = L_{p0}\exp\left[-\frac{E}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right)\right]$$

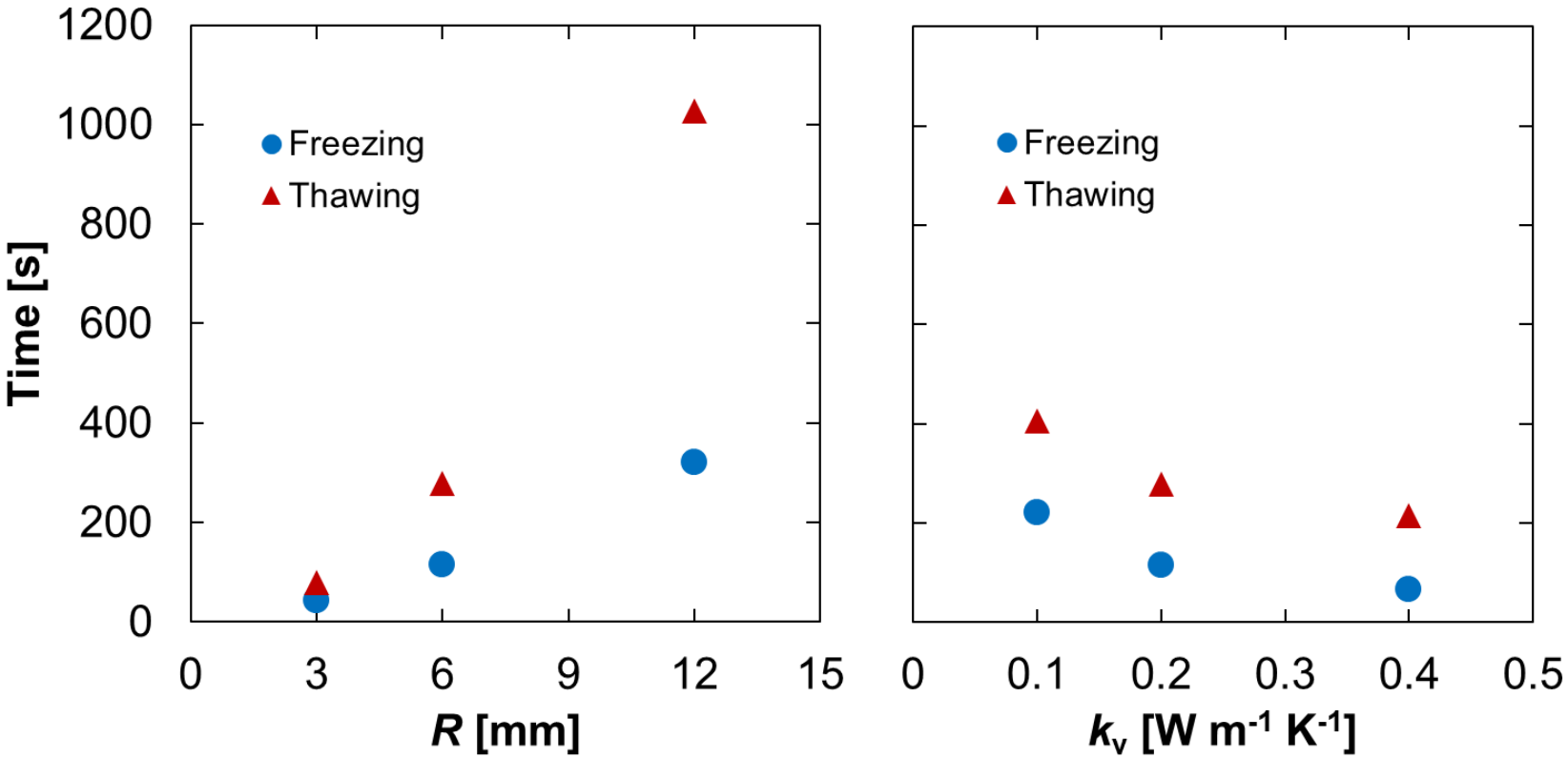


L_p : Membrane permeability [m s^{-1} Pa^{-1}]
ΔP : Osmotic pressure difference [Pa]

Heat transfer simulation

- **Assumptions**
  - Freezer heat transfer : Natural conviction
  - Freezer temperature : 193 K (Constant)
  - Initial vial temperature : 300 K
  - Content of the vial : Water

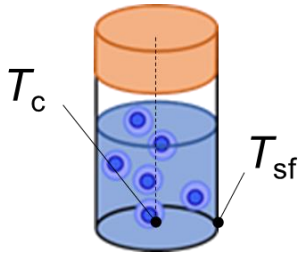




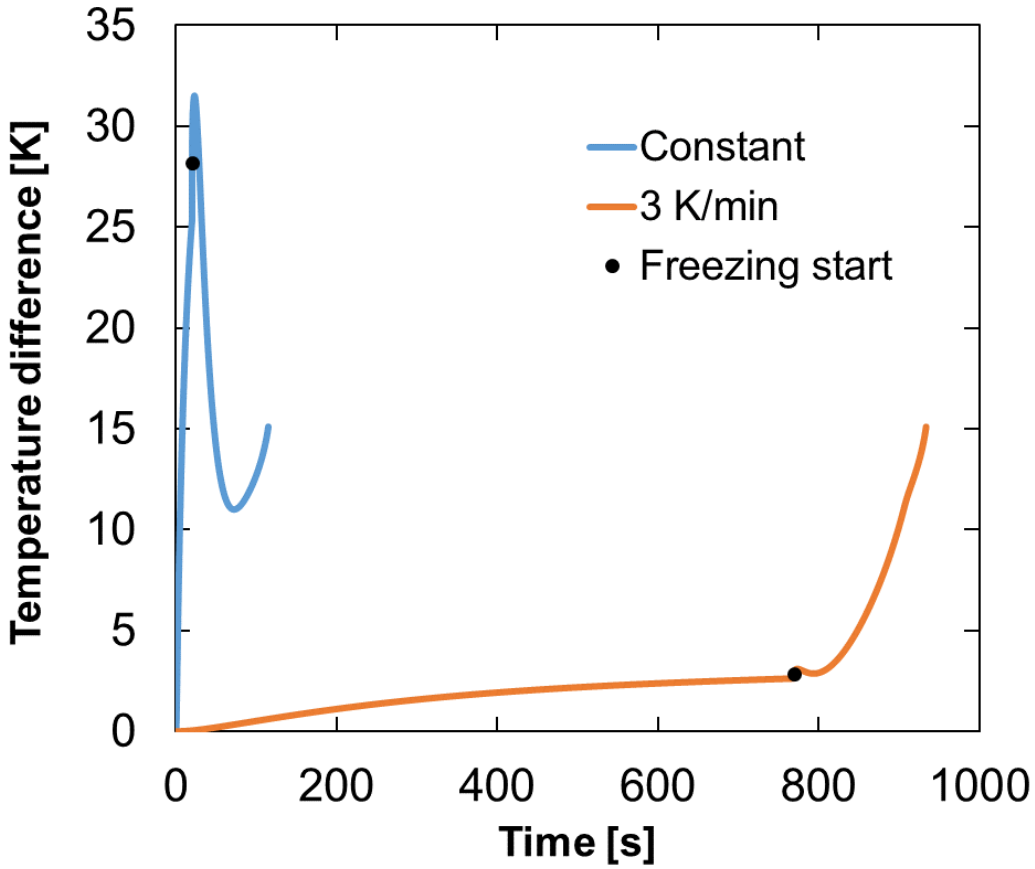
▶ ***R* and k_v are important design parameters.**

Heat transfer simulation

- **Freezer temperature**
  - Constant (193 K)
  - –3 K/min (From 300 K)



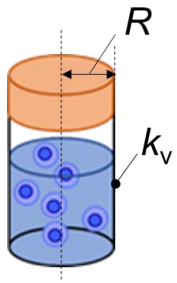
$$\Delta T = |T_c - T_{sf}|$$

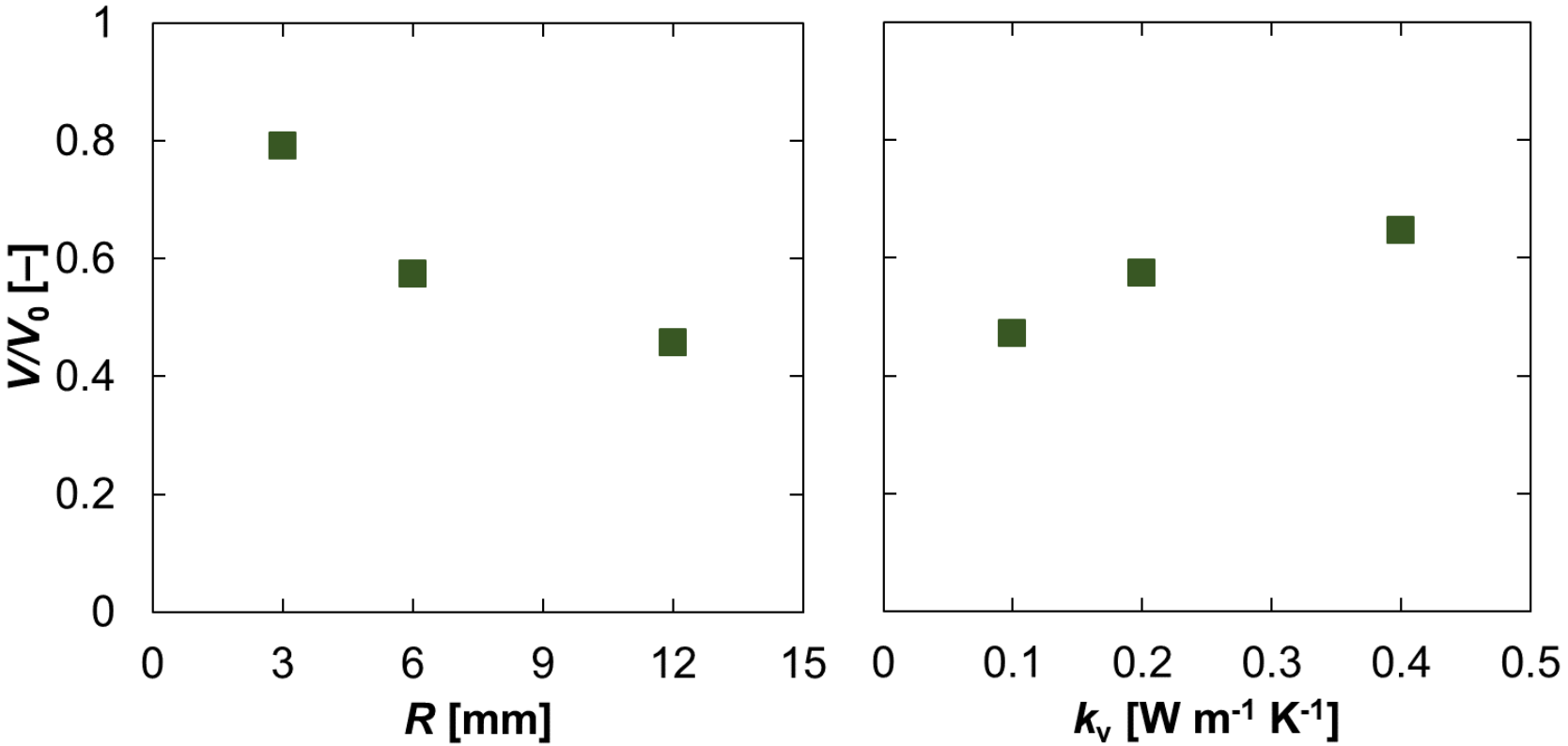


▶ **Freezing procedure is also an important factor.**

Mass transfer simulation

- **Assumptions**
  - Cryoprotective agent : 10%DMSO
  - Initial cell volume V_0 : 2.50×10^{-15} [m^3]
  - Cell surface area A : 8.87×10^{-10} [m^2]
  - Permeable substance : Water





▶ ***R* and k_v are important design parameters.**

Future plan

- **Experiment**
  - Survival rate after thawing
  - Cell membrane permeability
  - Effect of cryoprotective agent

- **Modeling & simulation**
  - Cell damage by osmotic pressure
  - Intracellular ice formation

- **Evaluation**
  - Survival rate
  - Manufacturing cost