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Numerical Simulations Demonstrate Safe Vitrification and Warming of Embryos Using the Rapid-i™ Device

Yury A. Tarakanov[1], Björn O. J. Johansson[1,2], Hans J. Lehmann[2] and S. Peter Apell[1]

[1]Department of Applied Physics, Chalmers University of Technology, SE-412 96, Gothenburg, Sweden

[2]Vitrolife Sweden AB, Box 9080, SE-400 92, Gothenburg, Sweden

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Cryopreservation of living tissues

Cryopreservation:

- Storage of living tissues at cryogenic temperatures
- Ice formation in and around cells is lethal
- Transition of liquids to glassy solid state during freezing - vitrification

Vitrification procedure:

- Dehydration with cryoprotectant (glass-containing) solutions
- Rapid freezing to avoid ice formation

Warming procedure:

- Rapid warming to avoid recrystallization of the devitrified liquids

How rapid should the cooling and warming rates be?

Cooling and warming rates

Conventional requirement:

- cooling rate at least **500 °C/min** (many devices provide 10000-100000 °C/min)
- warming rate has less influence on survival rates

A recent study by Seki and Mazur (*Cryobiology*, 2009):

- quick warming rate **(~3000 °C/min**) is principal for embryos' survival
- cooling rate can be only **200 °C/min**, provided quick warming

Devices should provide quick warming in the 1st place – like the Rapid-i™ from Vitrolife AB

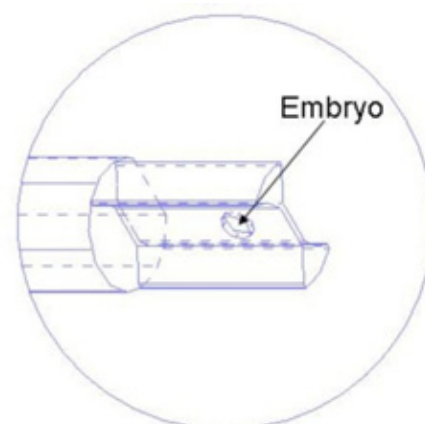
The Rapid-i™ device

- Products and systems for the preparation, cultivation and storage of human cells, tissue and organs
- Assisted Reproductive Technology
  - ✓ cryopreservation of human embryos

The Rapid-i™ device for vitrification and cryopreservation of human embryos

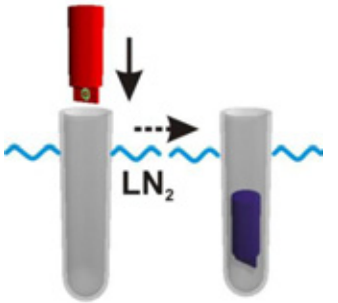
- A PMMA stick (length70 mm, diameter 2.7mm) with a flattened ”holder”
- A small hole where the drop of cryoprotectant solution with an embryo is placed
- A PVC straw with a slightly bigger diameter (3.3 mm) sealed at the bottom

Simple in manufacturing and handling
High survival rates for human and mice embryos
Cooling/warming rates not known

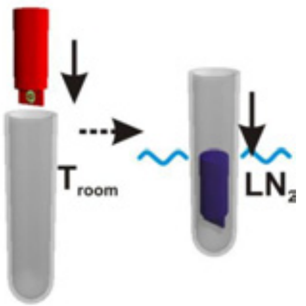


Vitrification and warming procedures with Rapid-i™

- **"Open straw" vitrification procedure**:
  - ✓The Rapid-i™ stick is dropped into the straw floating in LN_2
  - ✓ The stick is cooled down resting in the straw
  - ✓ The straw is sealed

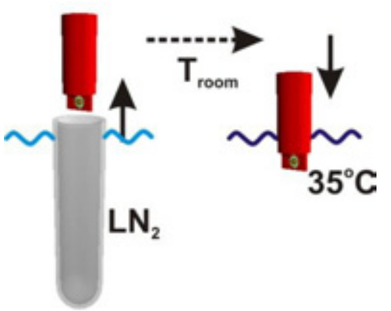


- **"Sealed straw" vitrification procedure**:
  - ✓ The stick is inserted into the straw and sealed at room temperature
  - ✓ The sealed straw (with stick inside) is submersed in LN_2



No direct contact between the embryo and LN_2

- **Warming procedure**:
  - ✓ The straw is cut at the top and the stick is extracted
  - ✓ The stick is quickly submersed into warming solution
  - ✓ Long exposure to air should be avoided



Direct contact between the embryo and the warming medium

Modeling of vitrification and warming: equations

How to obtain the cooling and warming rates?

- Experimentally – tricky and expensive
- Numerical modeling – quick, simple and insight into physics

Heat transfer equation

$$\rho C_p \frac{\partial T}{\partial t} + \nabla \cdot \ -k\nabla T \ = -\rho C_p \vec{u} \cdot \nabla T$$

Weakly compressible Navier-Stokes equations

$$\frac{\partial \rho}{\partial t} + \nabla \cdot \ \rho \vec{u} \ = 0$$

$$\rho \frac{\partial \vec{u}}{\partial t} + \rho \vec{u} \cdot \nabla \vec{u} = -\nabla p + +\nabla \cdot \left(\eta \ \nabla \vec{u} + \ \nabla \vec{u} \ ^{T} \ - \frac{2}{3} \eta \ \nabla \cdot \vec{u} \ \vec{I} \right) + \vec{F}$$

Equations are solved using COMSOL Multiphysics 3.5a

- 3D
- General heat transfer (transient)
- Weakly compressible Navier-Stokes (transient)

Modeling: cooling/warming rates

Instant cooling/warming rate:

$$R_{C(W)} = \left| \frac{dT_{emb}}{dt} \right|$$

Average cooling/warming rate:

$$R_{C(W)}^{aver} = \left| \frac{130^o C}{t_{-130^o C} - t_{0^o C}} \right|$$

Maximum time of the exposure of the Rapid-i stick to air:

$$t_{\exp} = t_{-150^o C} - t_{-196^o C}$$

Modeling: material properties

PMMA and PVC

- Density, heat capacity and heat conductance: independent of temperature, taken from COMSOL Materials library

AIR

- Only gas state considered
- Density: given by ideal gas formula
- Heat capacity, heat conductance and dynamic vicosity: interpolated in wide temperature ranges from experimental data (Lemmon et al., *J. Phys. Chem. Ref. Data,* 2000 and Kadoya et al., *. Phys. Chem. Ref. Data,* 1985)

Vitrification and warming media

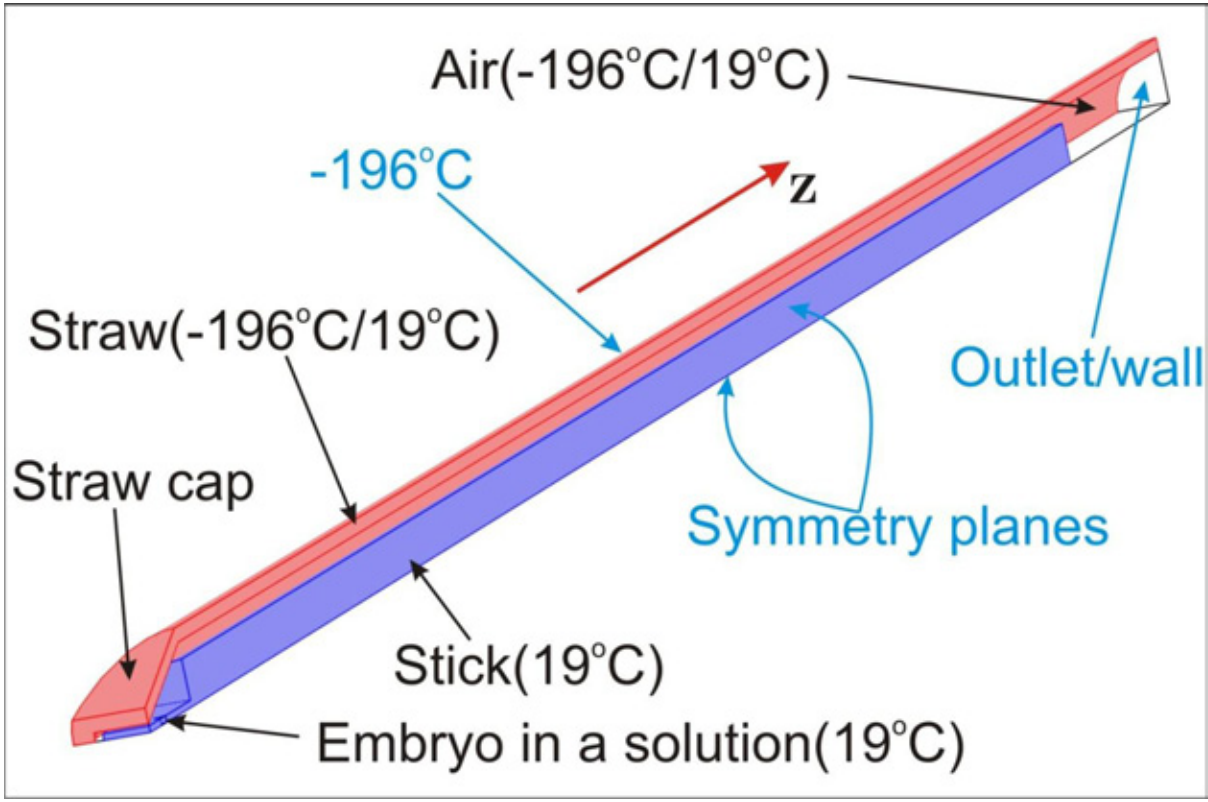
- Liquid flow is not considered
- Thermodynamic properties, especially in phase transition temperature range, are not known
- Taken as average of the constituents at 0°C (CRC Handbook of Chemistry and Physics, 89th edition)

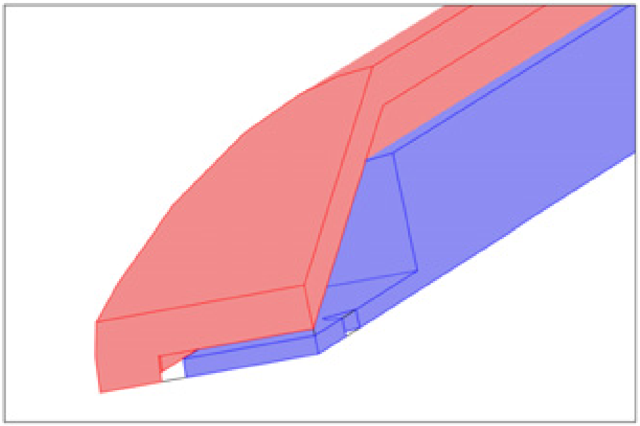
Accurate parameters of the heat transferring medium (air) have the strongest influence on the model accuracy

Modeling of the vitrification: open/sealed straw

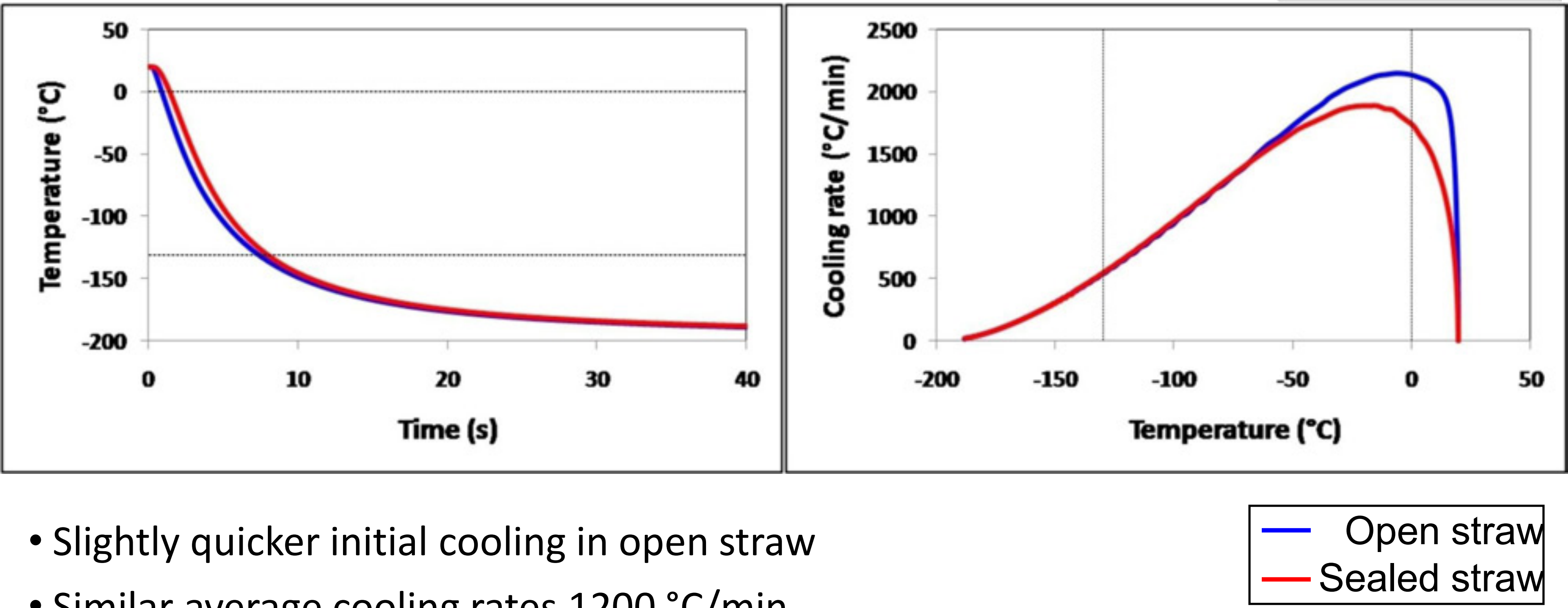
- Dropping phase is too short to change temperature considerably
- OK to simulate from the moment when the stick is resting at the straw bottom

- The same geometry for open and sealed straw procedures
- Different initial temperature conditions: air outlet/wall
- Nonisothermal air flow





Results: vitrification in open and sealed straw



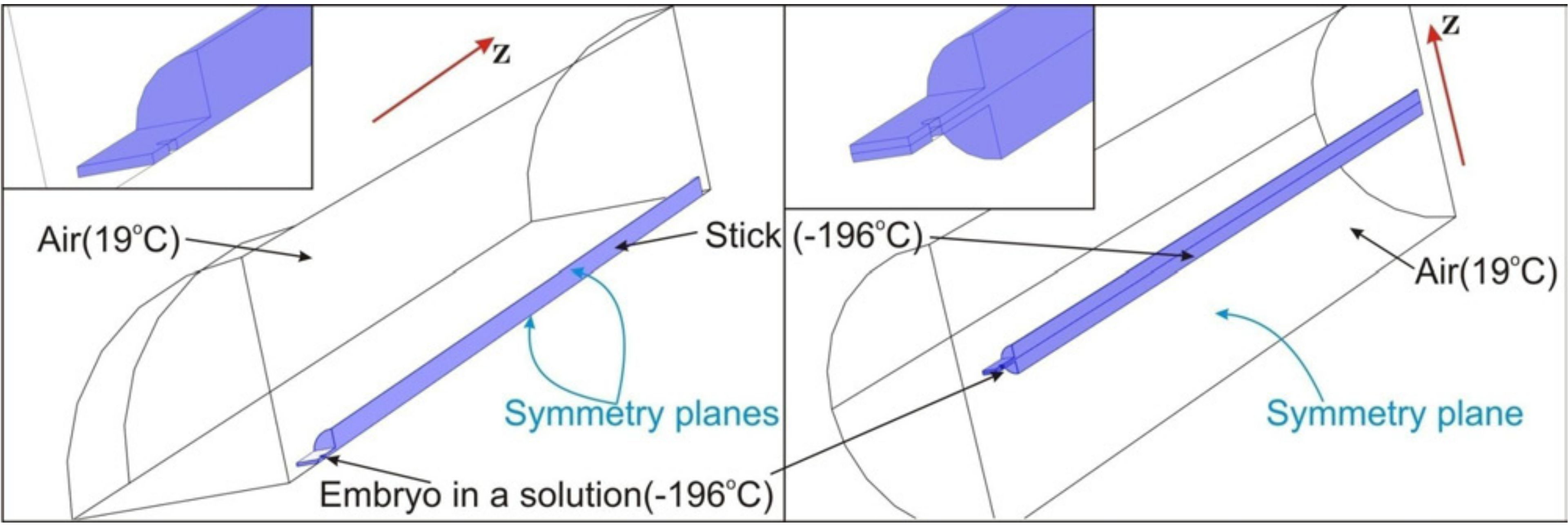
- Slightly quicker initial cooling in open straw
- Similar average cooling rates 1200 °C/min
- Minimum cooling rate 520 °C/min @ -130°C

Cooling rates are rapid enough for safe vitrification

Modeling of the warming: warming in air

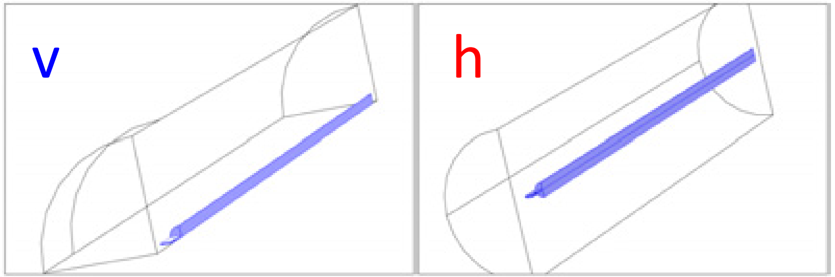
How quickly will the cold stick with embryo be warmed in air

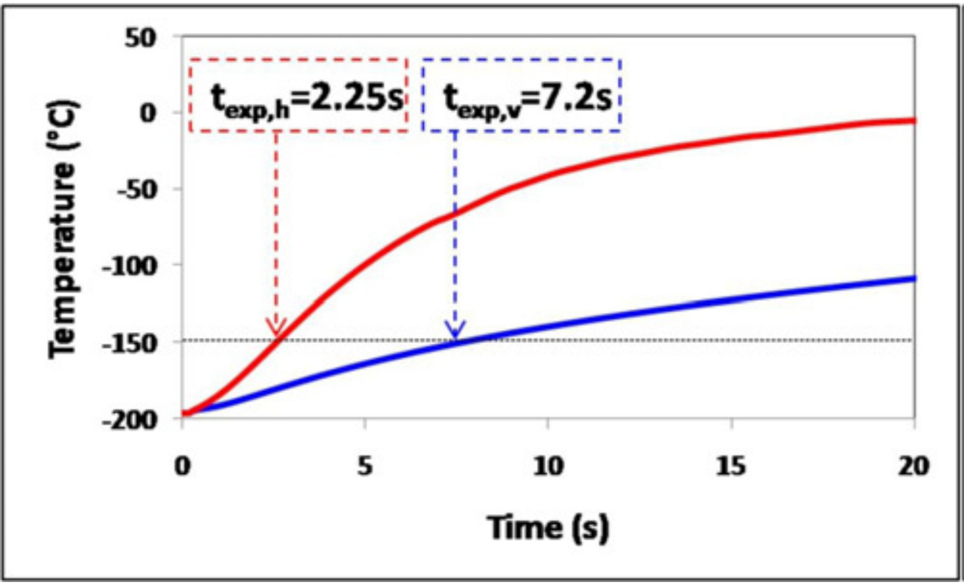
- ✓ if held vertically, with holder down?
- ✓ if held horizontally, with holder in horizontal direction?

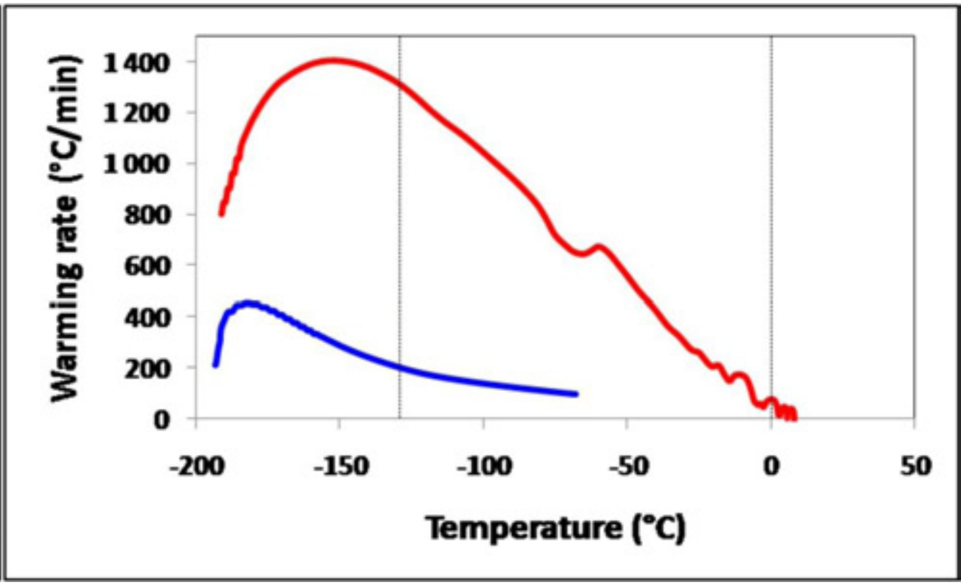


- Nonisothermal air flow is included
- Air domain is truncated to finite volume

Results: warming in air



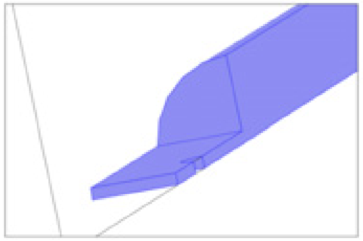


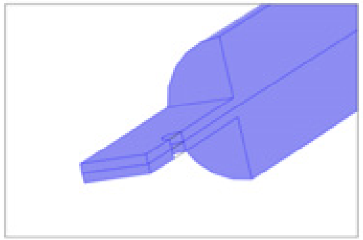


- Low warming rates
- Significantly quicker warming if the stick is held horizontally
- The times to warm up to -150°C are ~2 s (h) and ~7 s (v)

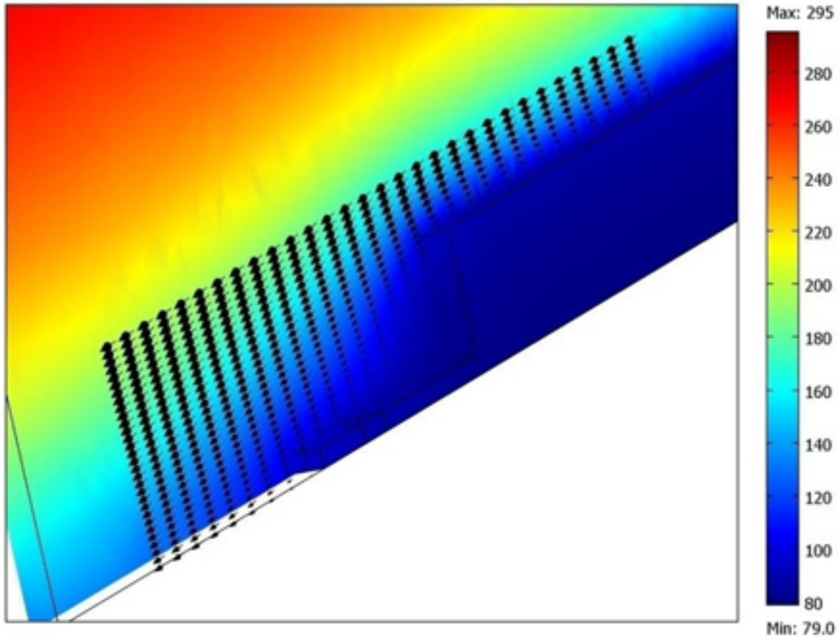
Warming in air is too slow and should be avoided
Maximum safe exposure time is 2 seconds

Warming in air: why different warming speeds?

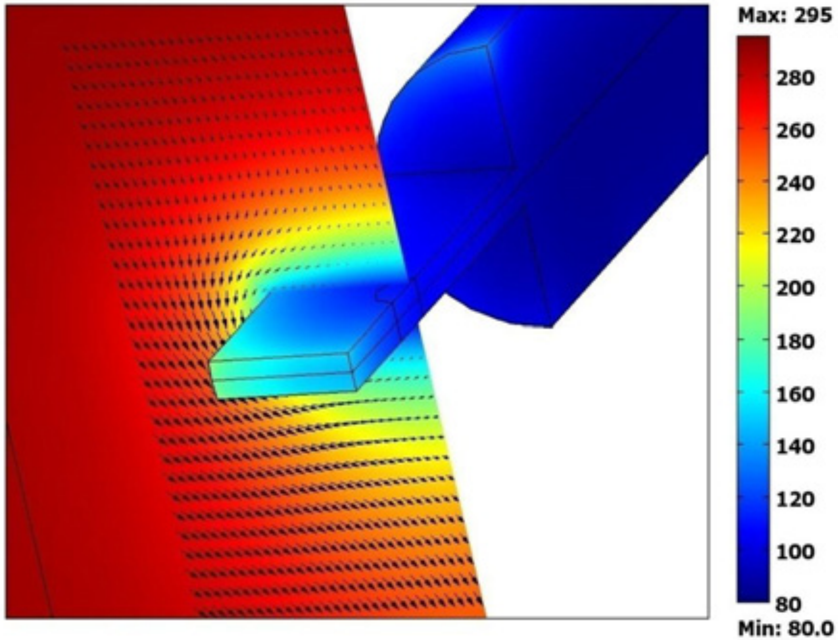




Vertical orientation



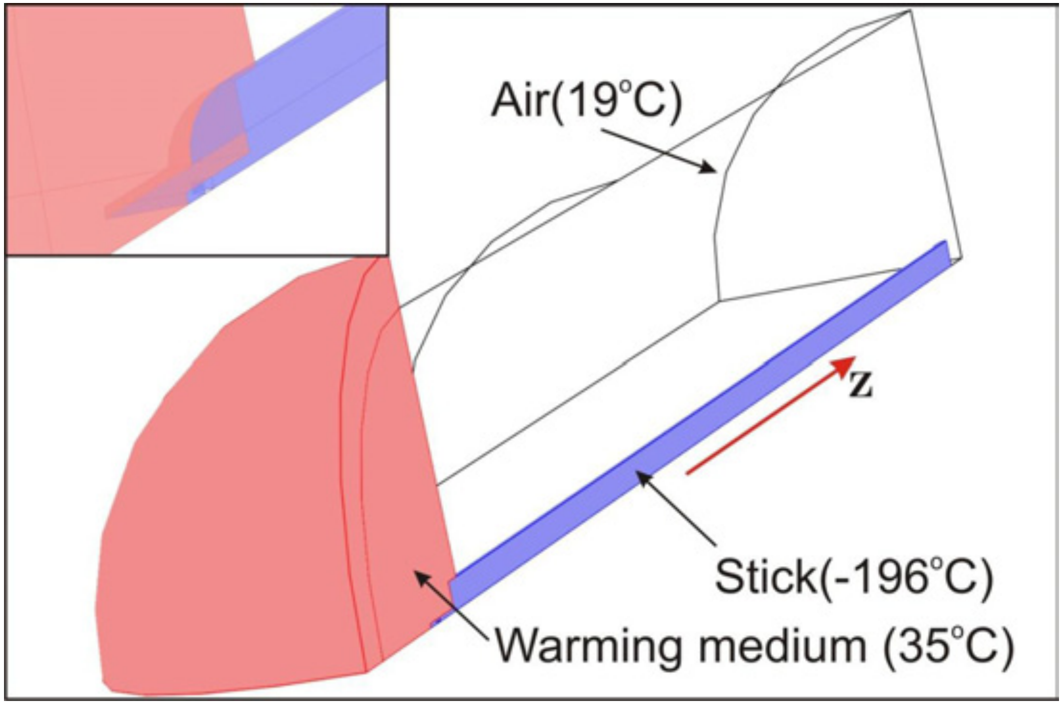
Horizontal orientation



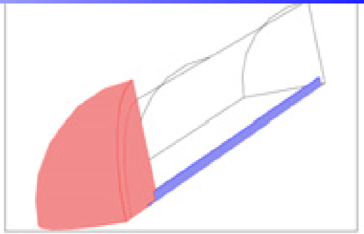
- ”screening” of holder by cold descending air in the vertical case
- effective convective warming of the holder in the horizontal case

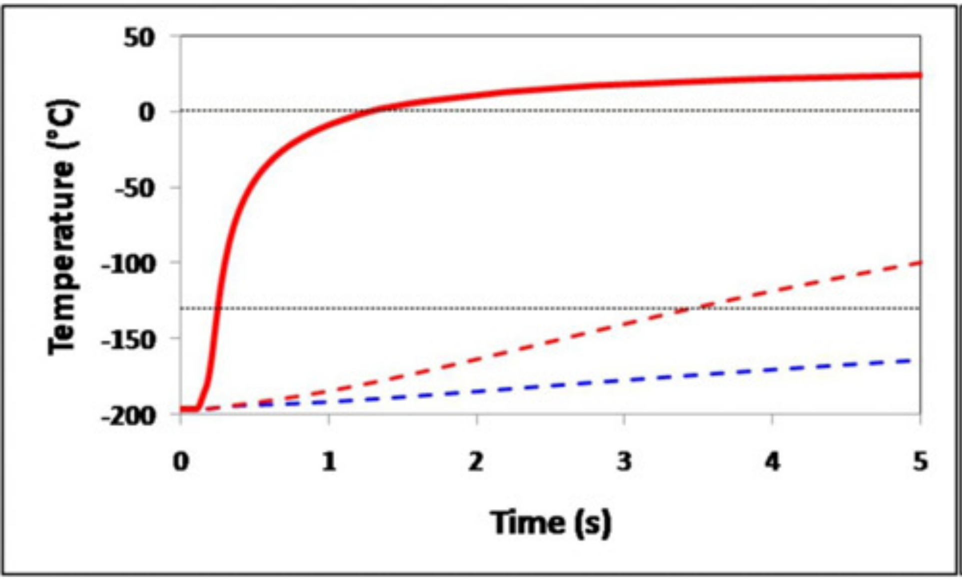
Modeling of the warming: in the warming liquid

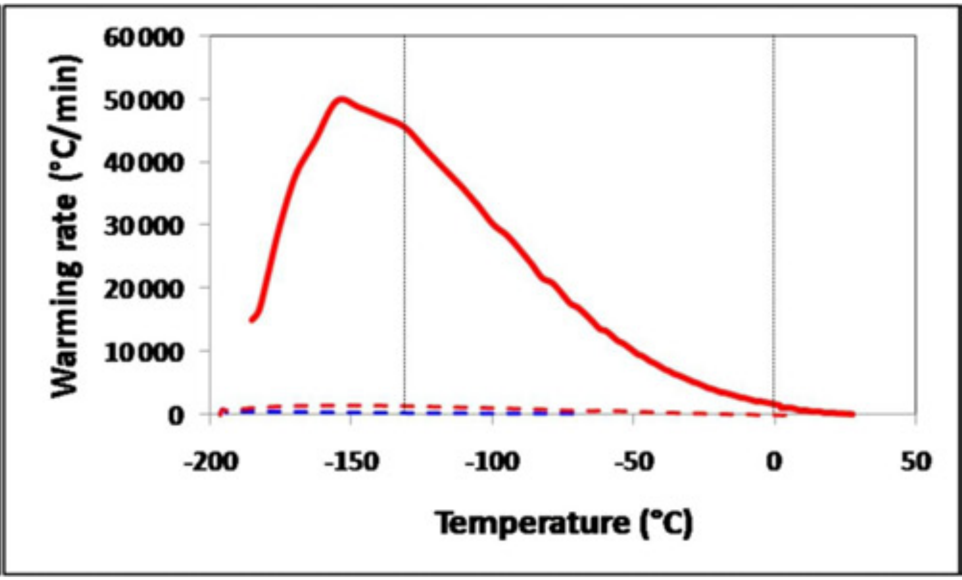
- The warming liquid has a high viscosity (20 Pa·s), hense liquid flow and convective heating are negligible
- Infinite elements to model the infinite domain of warming liquid



Results: warming in liquid







- Direct contact with the solution provides extremely quick warming
- Average warming rate 7700 °C/min
- Minimum warming rate 1600 °C/min @ 0°C

Warming in solution provides a very high warming rate – the key to safe cryopreservation

Conclusions

The cooling and warming characteristics of the Rapid-i™ device

- Average cooling rate 1200°C/min
- Average warming rate 7700 °C/min

Requirements for prevention of ice formation are met

The safe handling of the Rapid-i™ during warming procedure

- Hold stick in vertical direction, holder downward
- Maximum 2 seconds of contact with air

Limitations of the model accuracy

- Phase transitions in vitrification and warming media
- Temperature-dependent properties of the media
- Liquafaction of the air

Thank you for your attention!