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

Yury A. Tarakanov¹, Björn O. J. Johansson¹,², Hans J. Lehmann² and S. Peter Apell¹

¹Department of Applied Physics, Chalmers University of Technology, SE-412 96, Gothenburg, Sweden
²Vitrolife Sweden AB, Box 9080, SE-400 92, Gothenburg, Sweden

The European COMSOL Conference 2009, October 15, 2009, Milan, Italy
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 (length 70 mm, diameter 2.7 mm) 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

The European COMSOL Conference 2009, October 15, 2009, Milan, Italy
Vitrification and warming procedures with Rapid-i™

• “Open straw” vitrification procedure:
  ✔ The Rapid-i™ stick is dropped into the straw floating in LN₂
  ✔ 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 LN₂

  No direct contact between the embryo and LN₂

• 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

The European COMSOL Conference 2009, October 15, 2009, Milan, Italy
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
\[ \frac{\partial \rho}{\partial t} + \nabla \cdot \rho \vec{u} = 0 \]
\[ \rho \frac{\partial \vec{u}}{\partial t} + \rho \vec{u} \cdot \nabla \vec{u} = -\nabla p + \nabla \cdot \left( \eta \nabla \vec{u} + \nabla \vec{u}^T - \frac{2}{3} \eta \nabla \cdot \vec{u} \ T \right) + \vec{F} \]

Equations are solved using COMSOL Multiphysics 3.5a
• 3D
• General heat transfer (transient)
• Weakly compressible Navier-Stokes (transient)
Instant cooling/warming rate:

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

Average cooling/warming rate:

\[ R_{C(W)}^{\text{aver}} = \left| \frac{130^\circ C}{t_{-130^\circ C} - t_{0^\circ C}} \right| \]

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

\[ t_{\text{exp}} = t_{-150^\circ C} - t_{-196^\circ C} \]
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

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
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
• 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?

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

The European COMSOL Conference 2009, October 15, 2009, Milan, Italy
• The warming liquid has a high viscosity (20 Pa·s), hence liquid flow and convective heating are negligible

• Infinite elements to model the infinite domain of warming 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

*The European COMSOL Conference 2009, October 15, 2009, Milan, Italy*
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!