a frog, a transplant surgeon, and a mechanical engineer…

The right tissue in the right patient
at the right time

Advances in tissue cryopreservation – strategies to reduce variability in regenerative medicine

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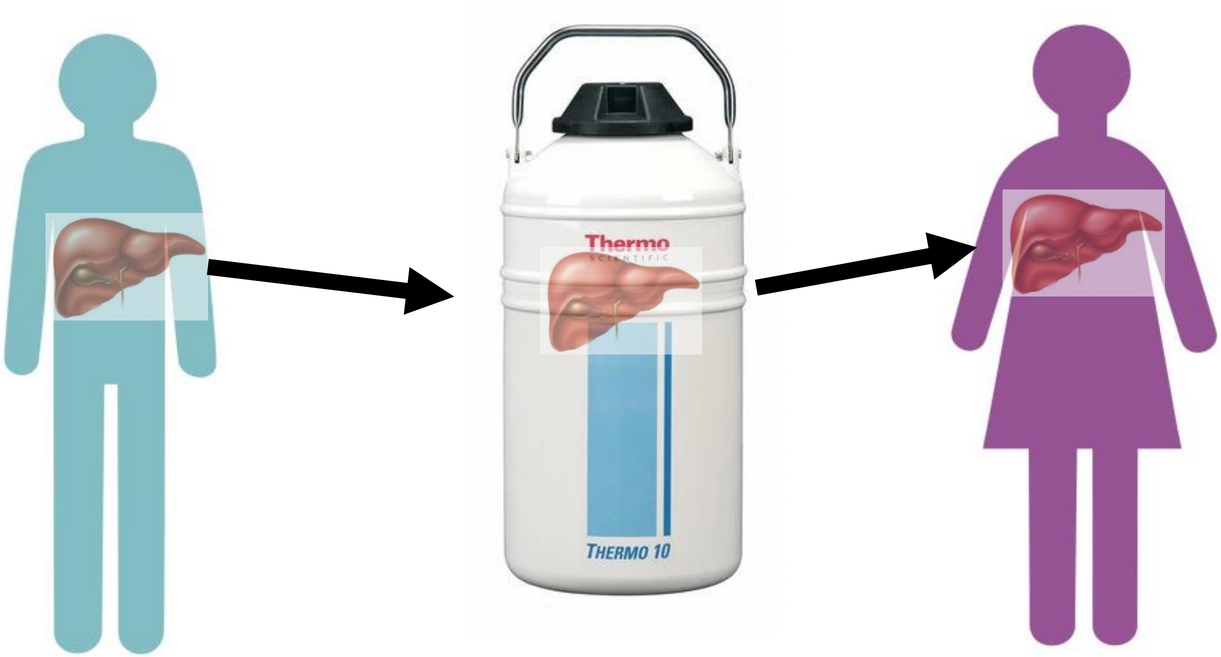
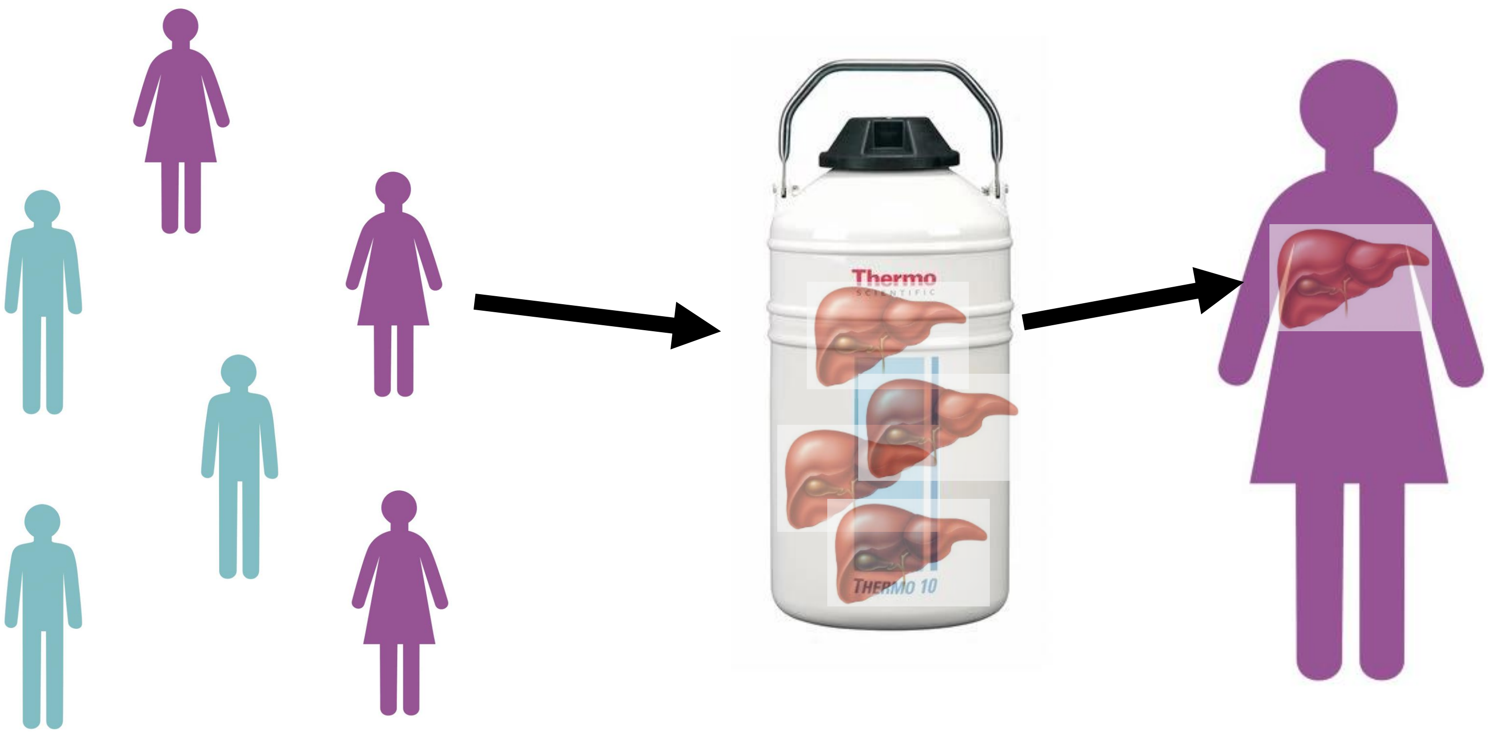
Bottlenecks for testing tissue variability in regenerative engineering products:



One donor One Specimen One treatment

Bottlenecks for testing tissue variability in regenerative engineering products:



Multiple donors

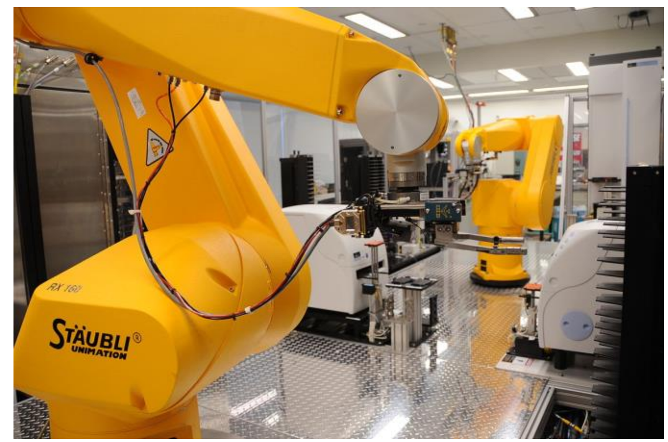
Multiple tissues

Pick the best one

Bottlenecks for testing tissue variability in regenerative engineering products:

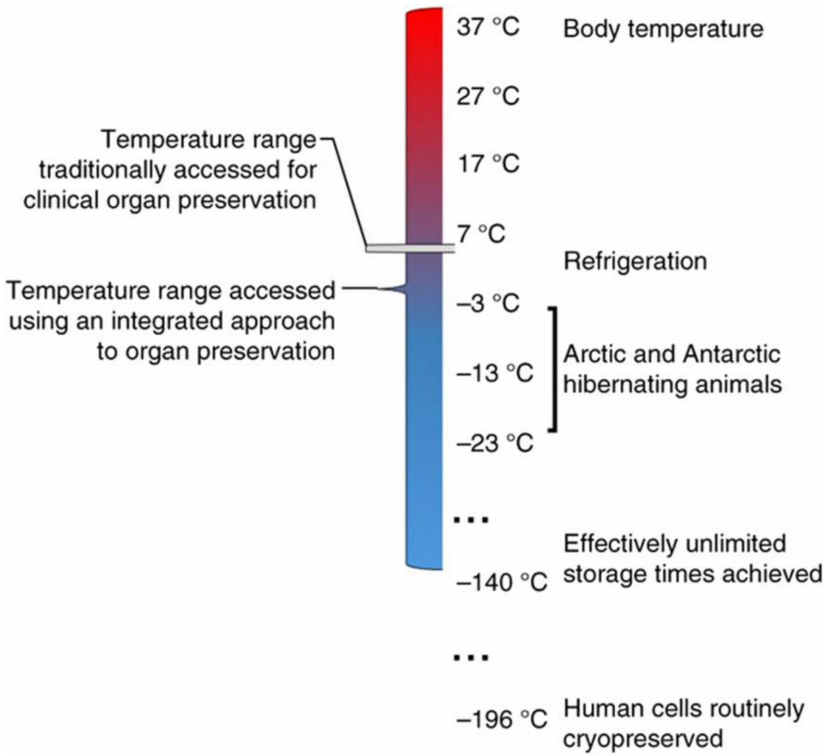
Many donors

Many samples

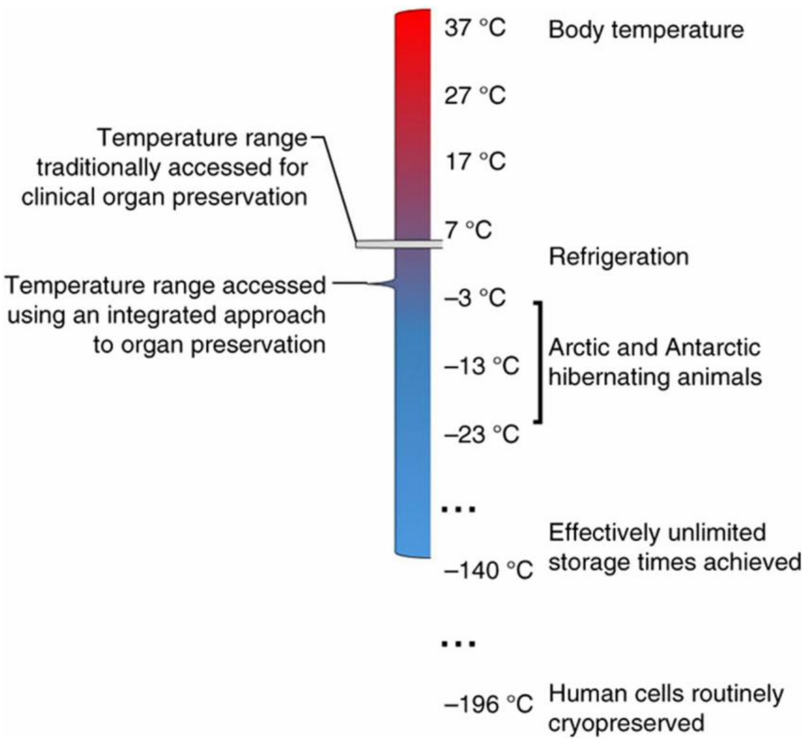


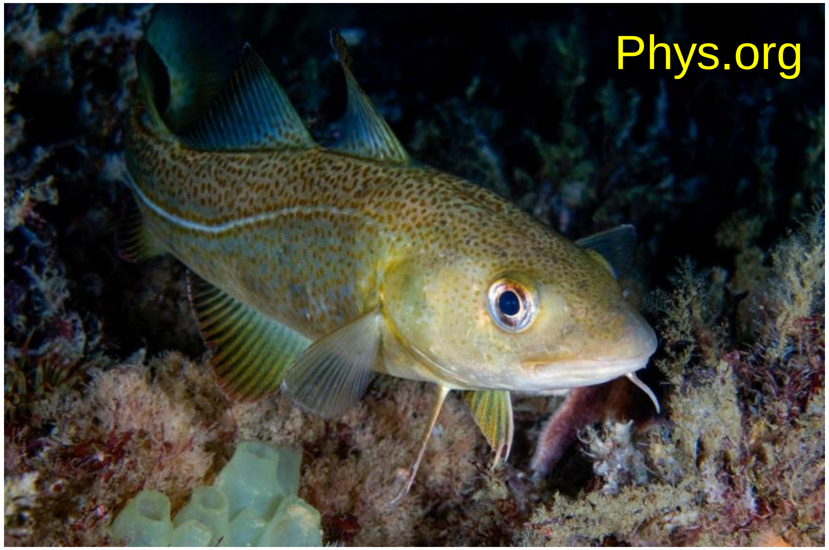
High throughput testing

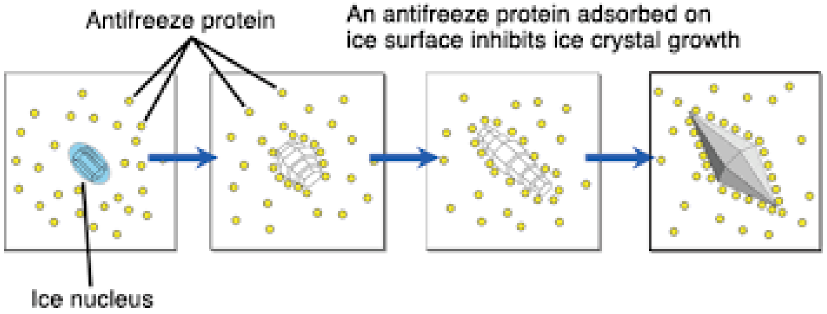
Tissue preservation: pick your temperature



Storage at sub-freezing temperature:





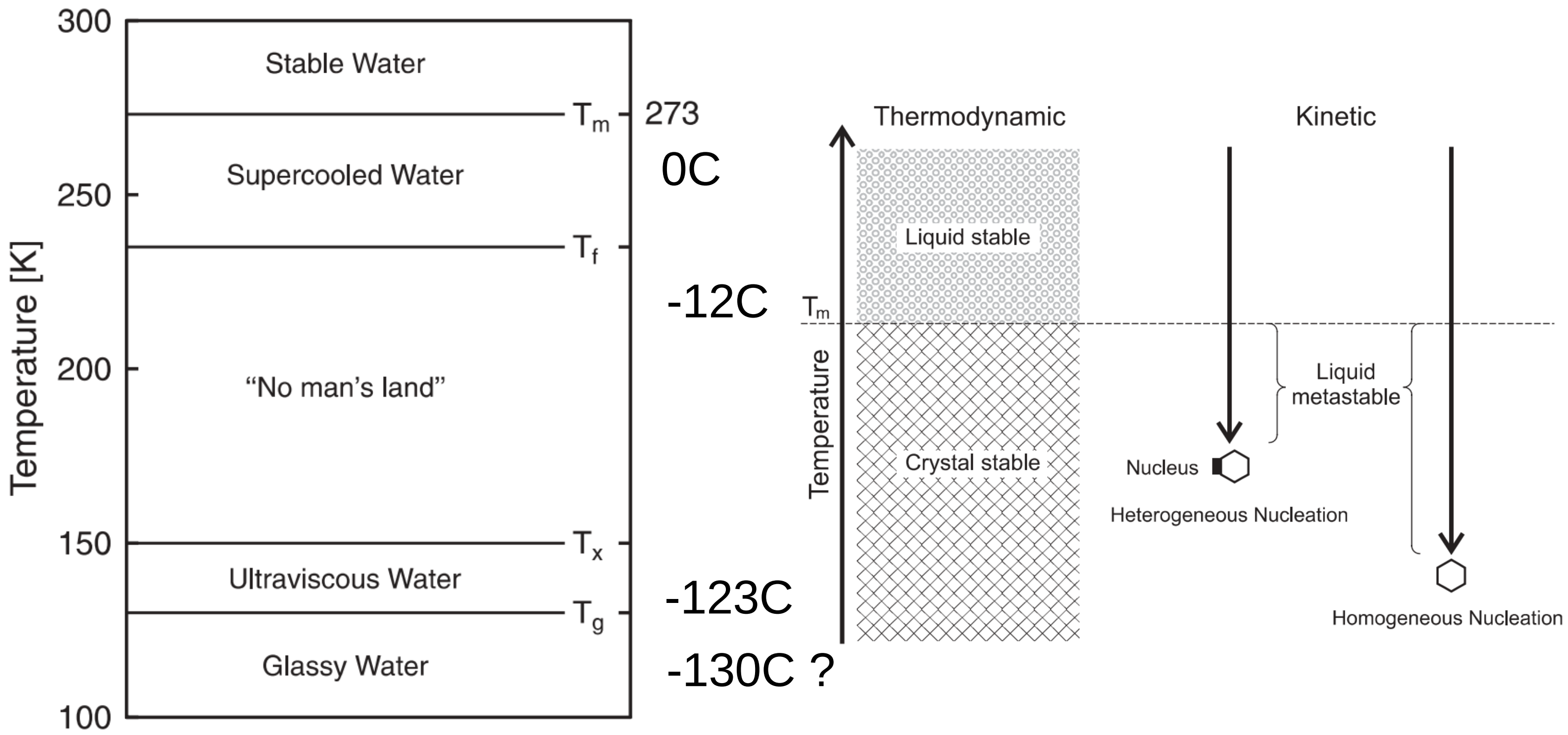


Rana sylvatica – wood frog

During exposure to near 0C temperature glucose and urea levels rise

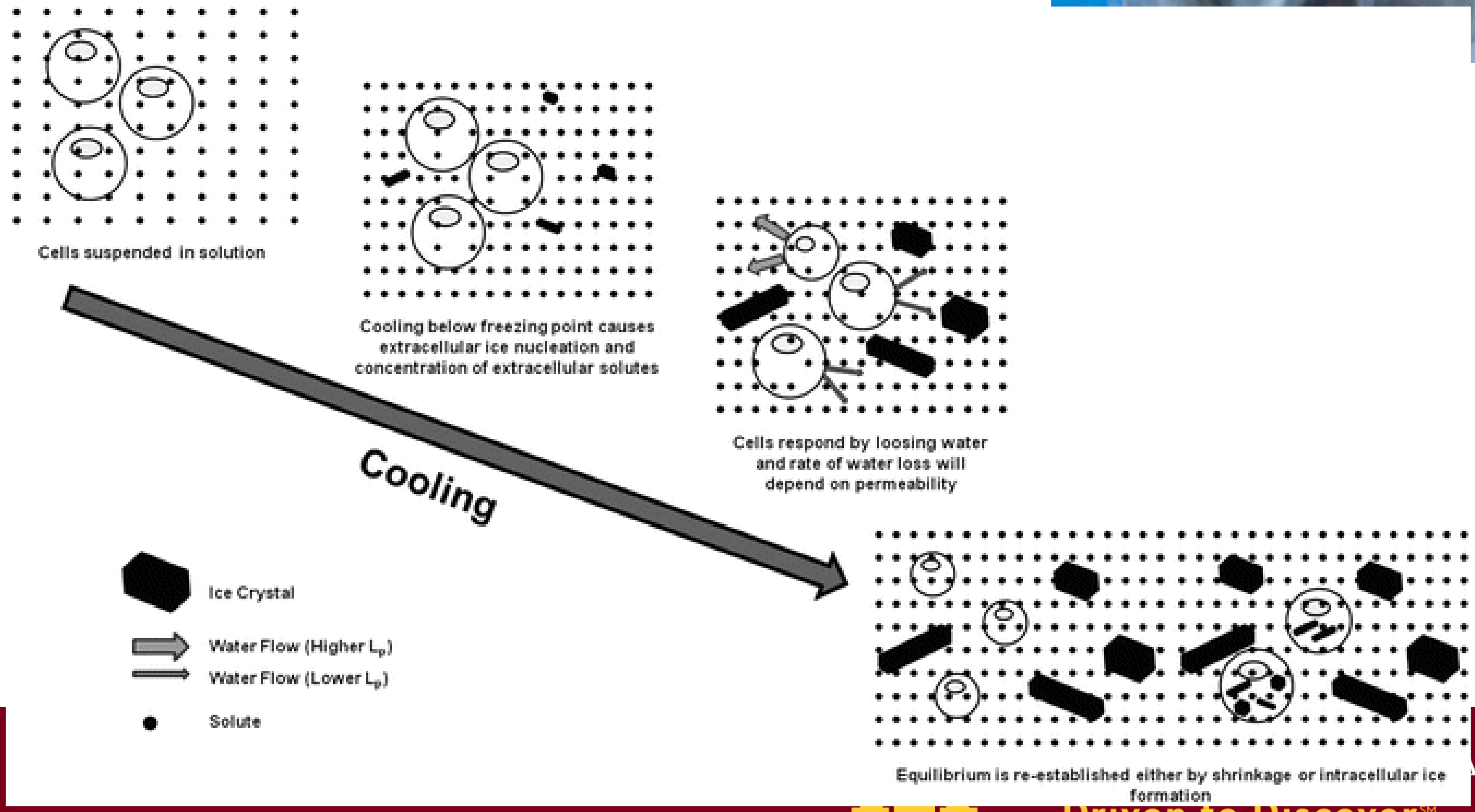
Upon rewarming, the cells rehydrate and circulation and breathing resume

Supercooling: the phase transition of water

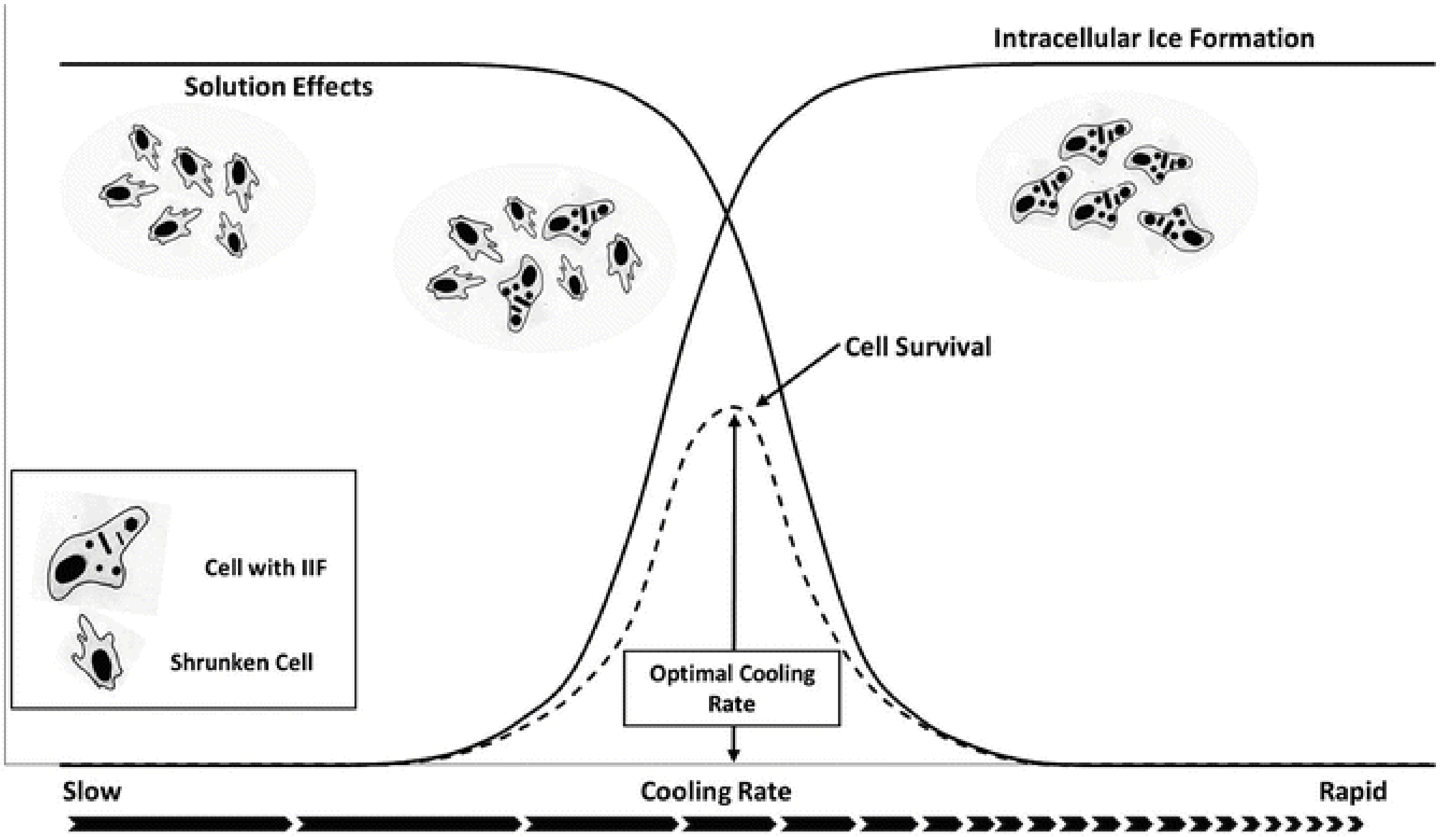


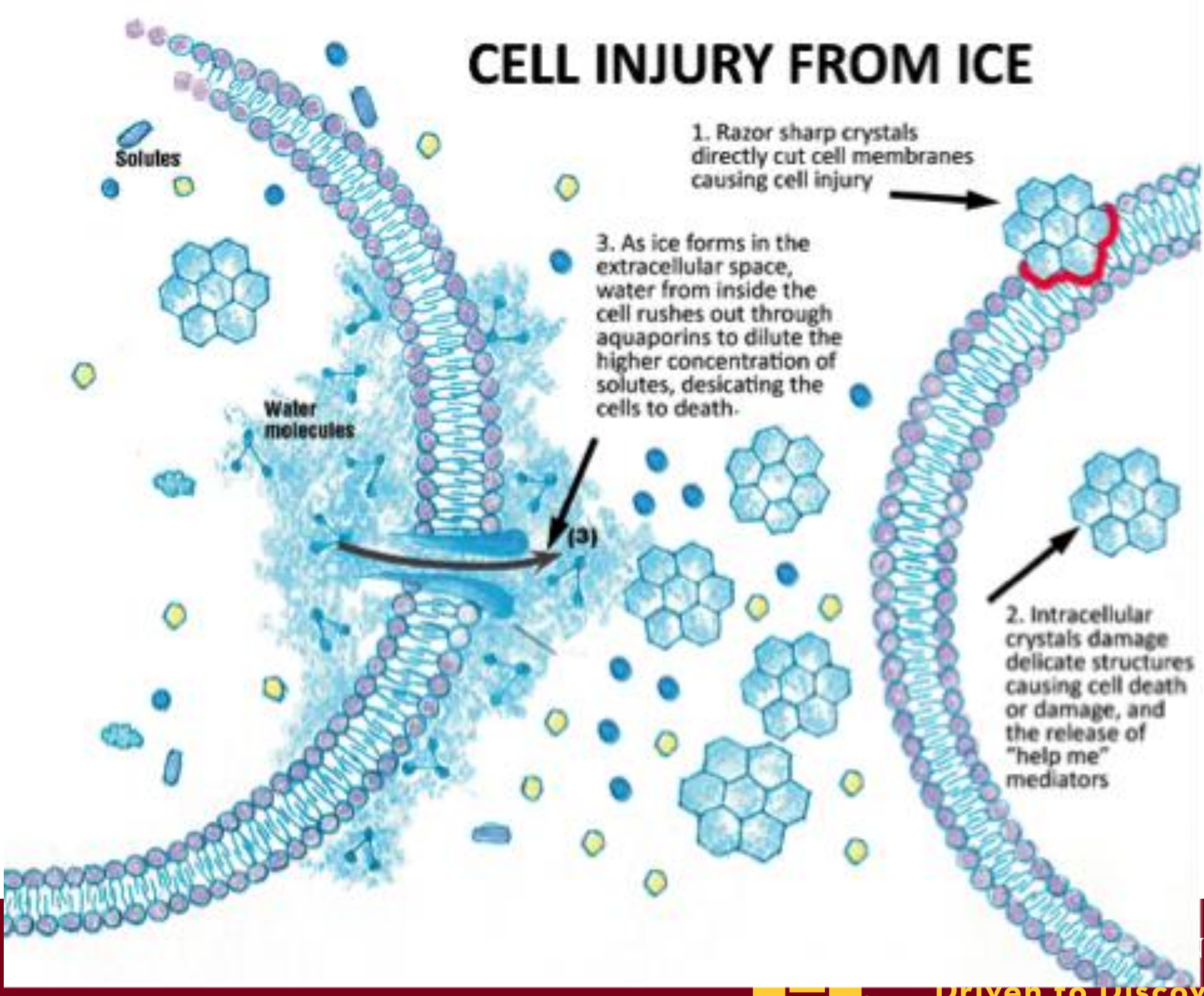
Modified from: Z. Phys. Chem. 218 (2004) 1231–1258

Conventional cryopreservation



Optimal cooling for conventional cryopreservation: -1C/min





CELL INJURY FROM ICE
Solutes
1. Razor sharp crystals directly cut cell membranes causing cell injury
3. As ice forms in the extracellular space, water from inside the cell rushes out through aquaporins to dilute the higher concentration of solutes, desicating the cells to death.
Water molecules
(3)
2. Intracellular crystals damage delicate structures causing cell death or damage, and the release of "help me" mediators

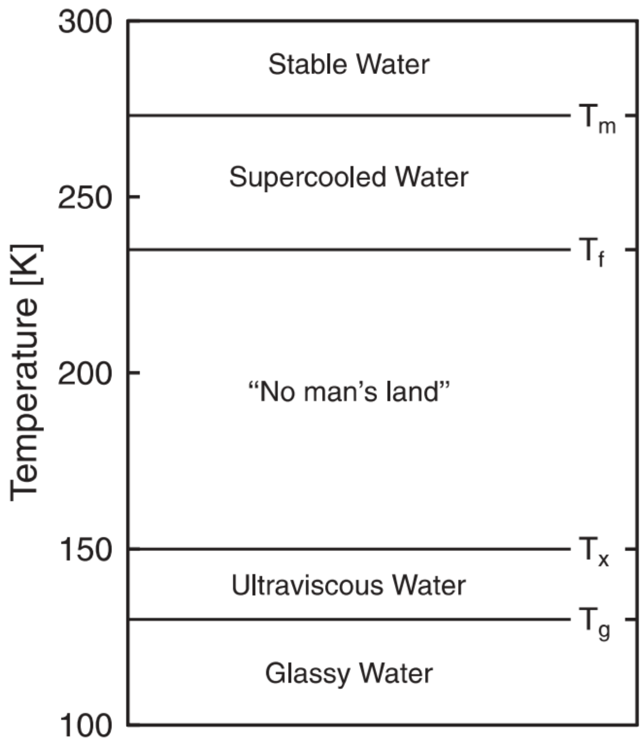
Limitations of conventional cryopreservation

- Ice still forms - cell injury still occurs
- Macroscopic destruction of tissue architecture
- Works for isolated cells in suspension
- Works for very small embryos

- Does not work for larger and more complex biologic specimens -- organs

Ice is the enemy -- how to avoid it, or, how to manage it

Vitrification (from Latin vitreum, "glass" via French vitrifier) is the transformation of a substance into a glass, that is to say a non-crystalline amorphous solid.

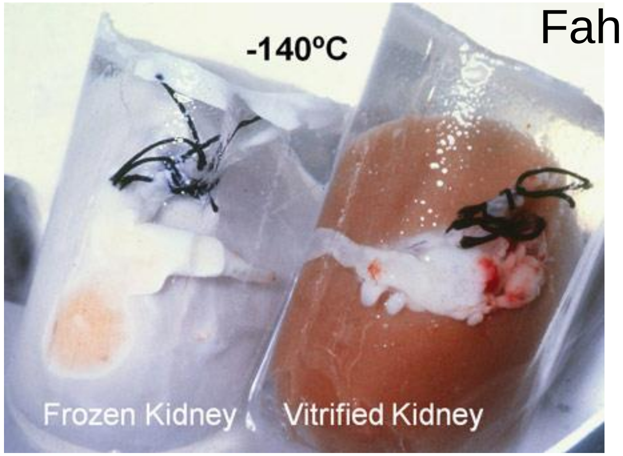


0C

-12C

-123C

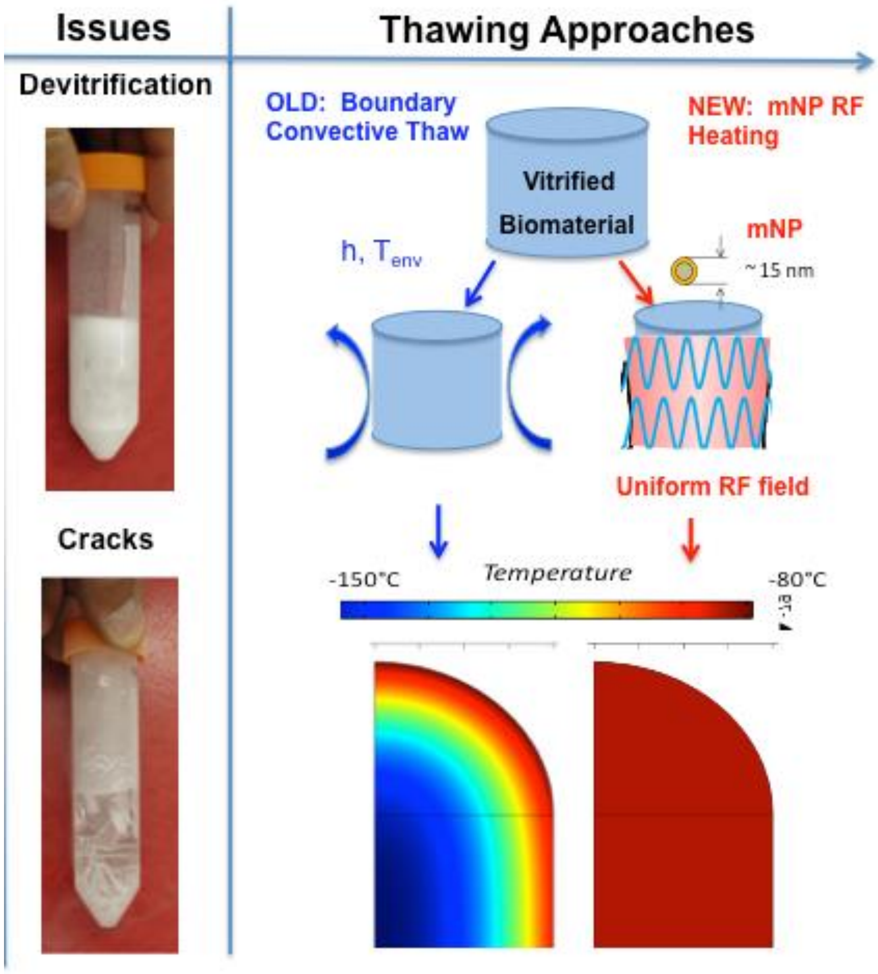
-130C ?



Fahey

Modified from: Z. Phys. Chem. 218 (2004) 1231–1258

Thawing of vitrified tissues must be rapid and uniform



Critical cooling rate, Critical heating rate, and the effect of CPA

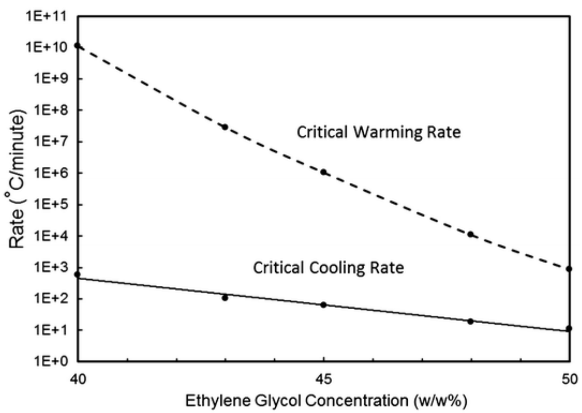


Table 1. CPA Properties with illustration of vitrification and failure modes.

	6M Glycerol	DP6	VS55	M22
Melt temp (T_L)	-26°C	-29.8°C	-38°C	~ -59°C
Glass transition (T_g)	~ -100°C	-119°C	-123°C	~-122°C
Critical cooling rate (CCR)	85°C/min	~40°C/min	2.5°C/min	0.1°C/min
Critical warming rate (CWR)	3.2 x 10^4°C/m	~200°C/min	50°C/min	0.4°C/min
Concentration	6M	6 M	8.4 M	9.3 M

vitrified

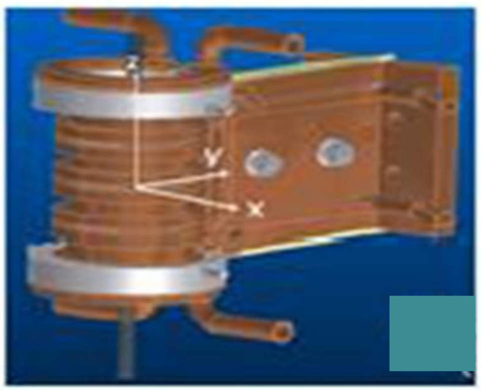
frozen

devitrified

cracked

Basic ingredients for magnetic hyperthermia and nanowarming:

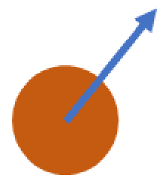
Magnetic field



+

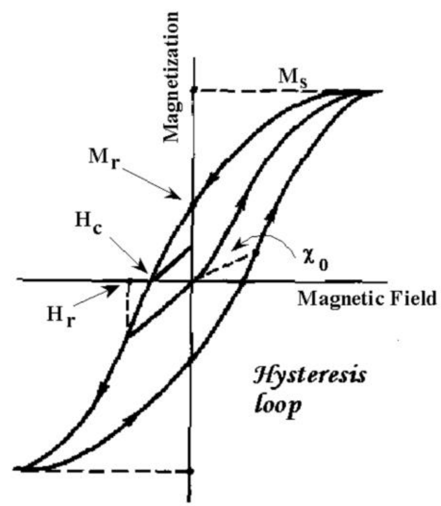
Magnetic nanoparticle

Fe_3O_4



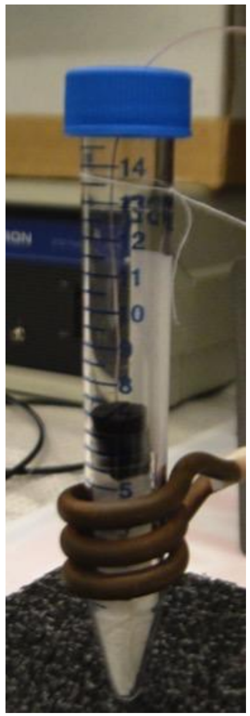
=

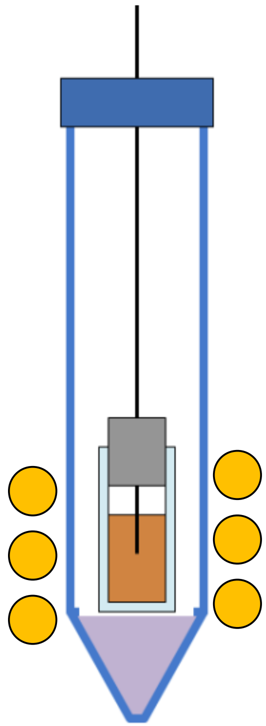
Heat



Manuchehrabadi, N. & Gao, Z. et al. Sci Transl Med 2017
Bordelon, D. et al Ieee T Magn 2012

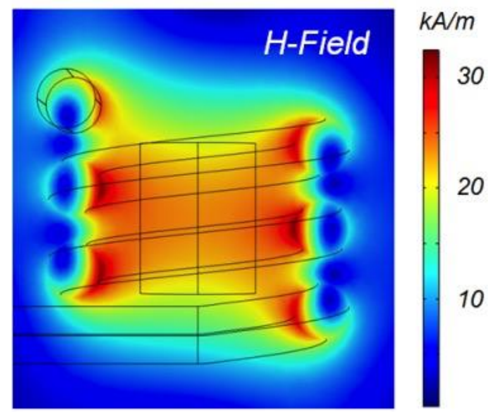
Small RF device





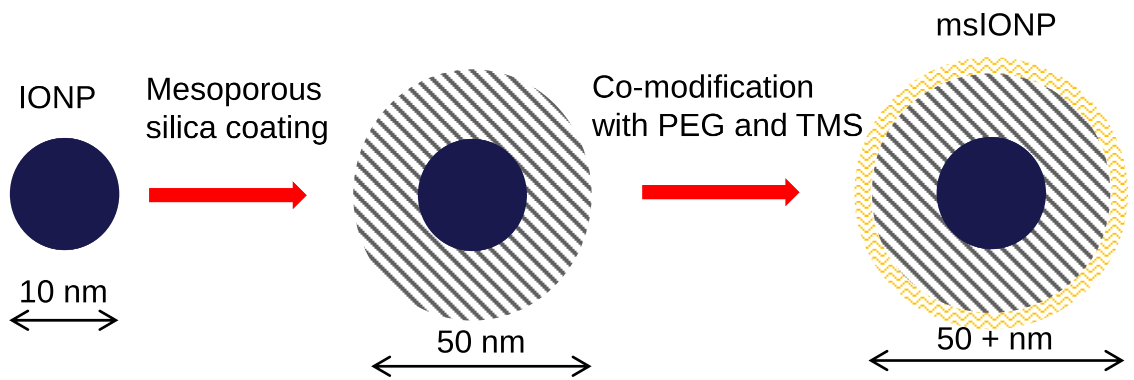
$$\bar{H} = 22.8\, kA/m$$

$$f = 360\, kHz$$

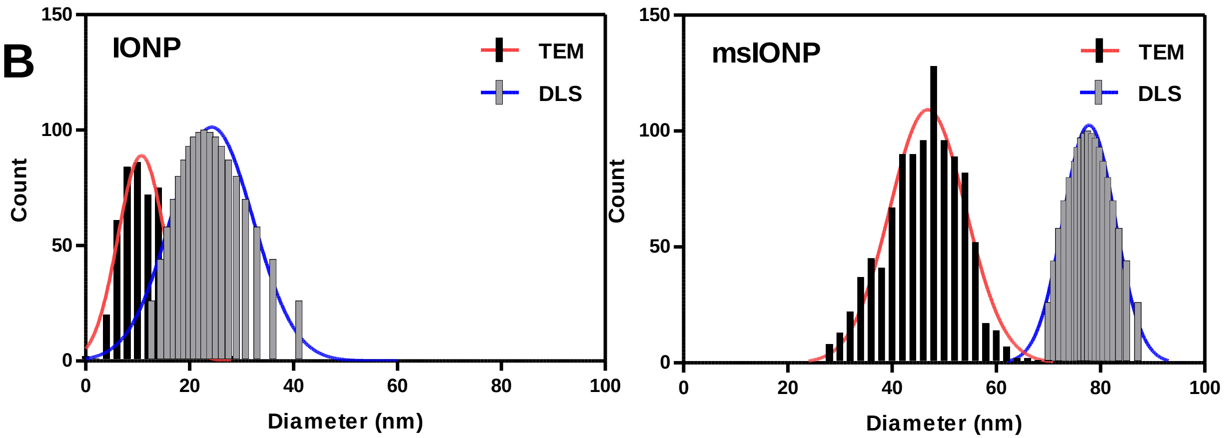


Theoretical and biophysical development -- nanoparticles

A

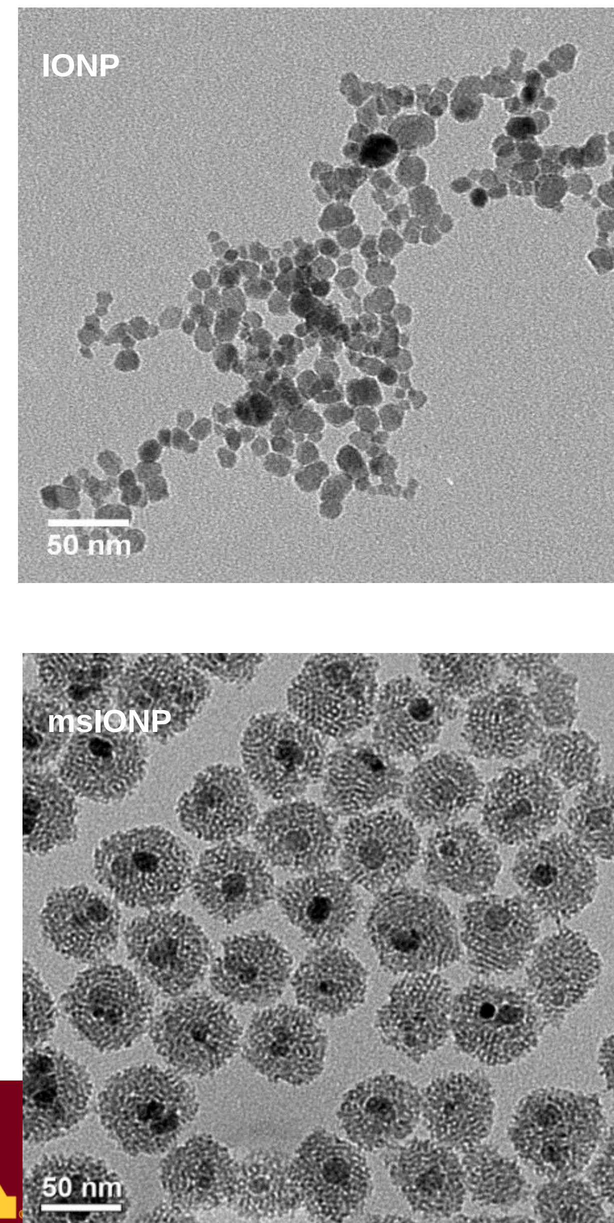


B



C

E



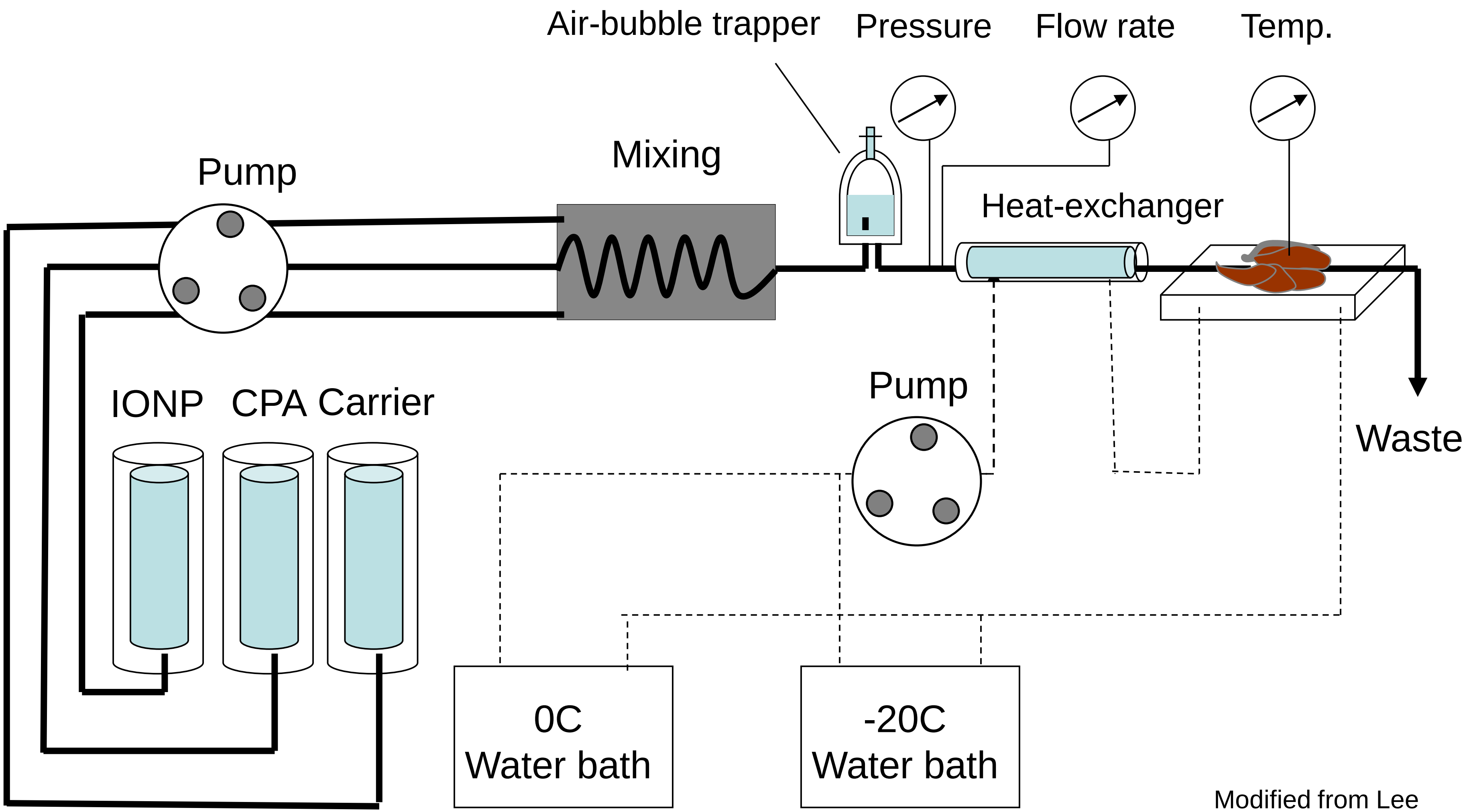
D

	Diameter (TEM) (N=500-1000)	Hydrodynamic Diameter	Zeta Potential
msIONP	46±7 nm	80±1 nm	-36.5±8.9 mV
IONP	12±5 nm	23±1 nm	-35.0±0.8 mV

Critical steps for vitrification:

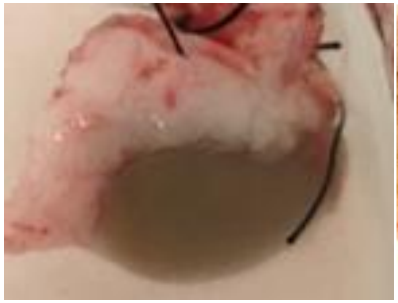
1. Cannulate organ for vascular perfusion
2. Load CPA in stepwise fashion
3. Load nanoparticles
4. Cool in controlled rate freezer (>CCR)
5. Transfer to LN for storage
6. Rapid heating in RF generator
7. Stepwise washout of CPA (and nanoparticles)
8. Assess viability and function

Hypothermic Machine Perfusion Set-up



Modified from Lee

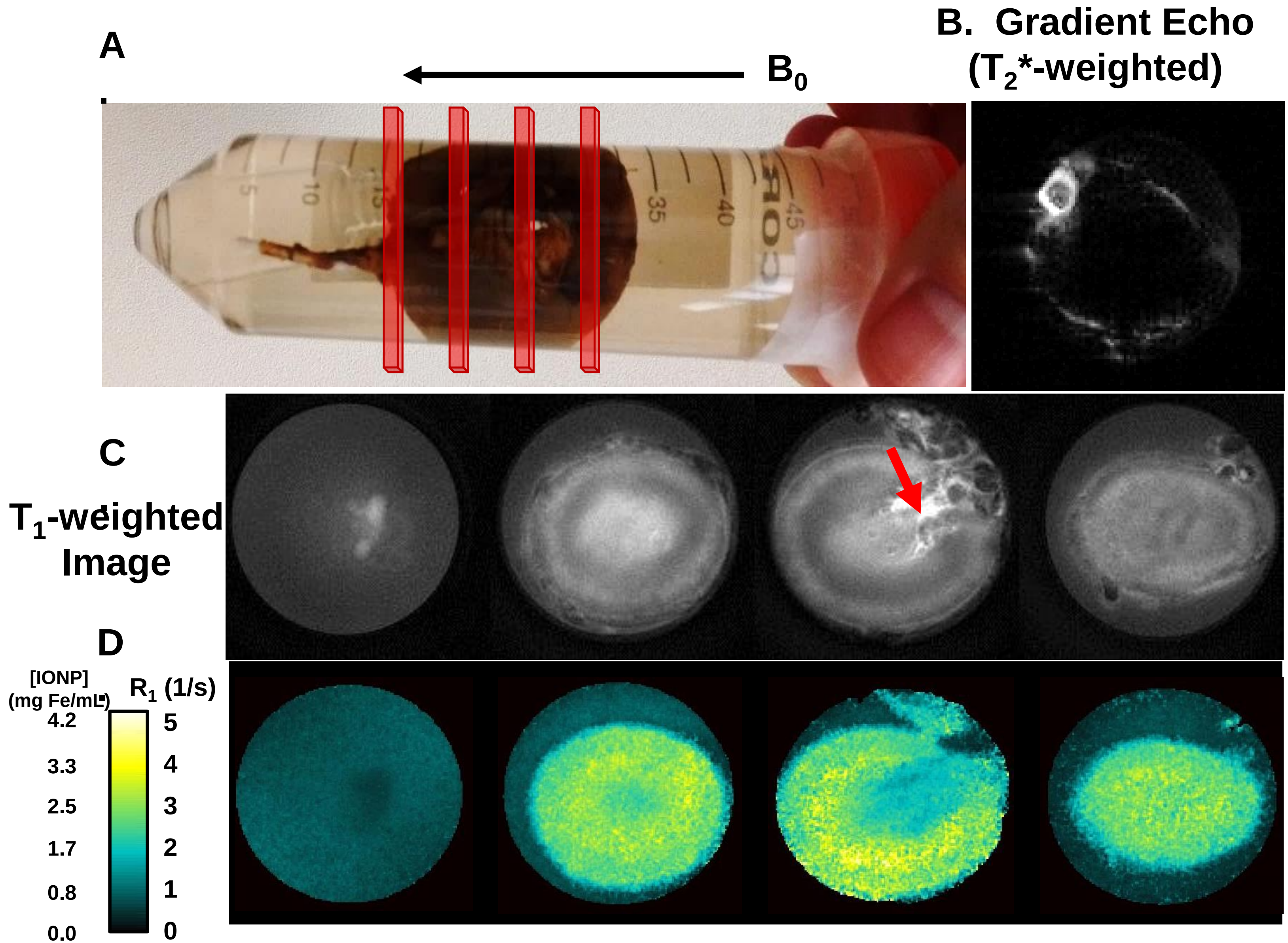
Nanoparticle loading



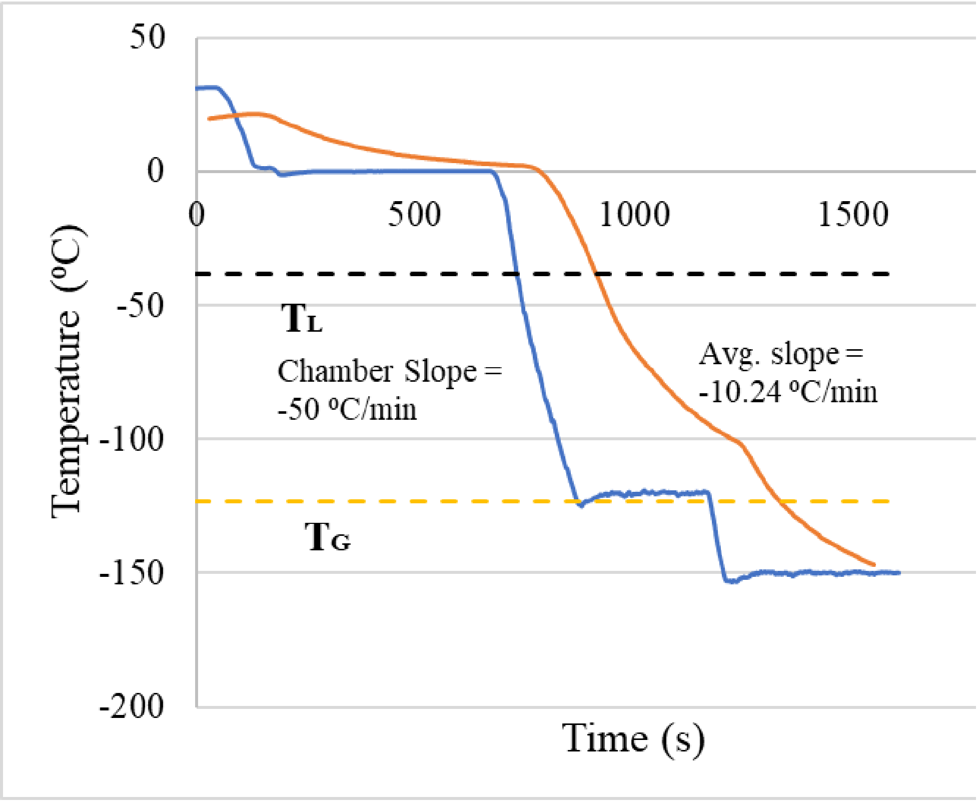
Fresh kidney in EC

sIONP-perfused

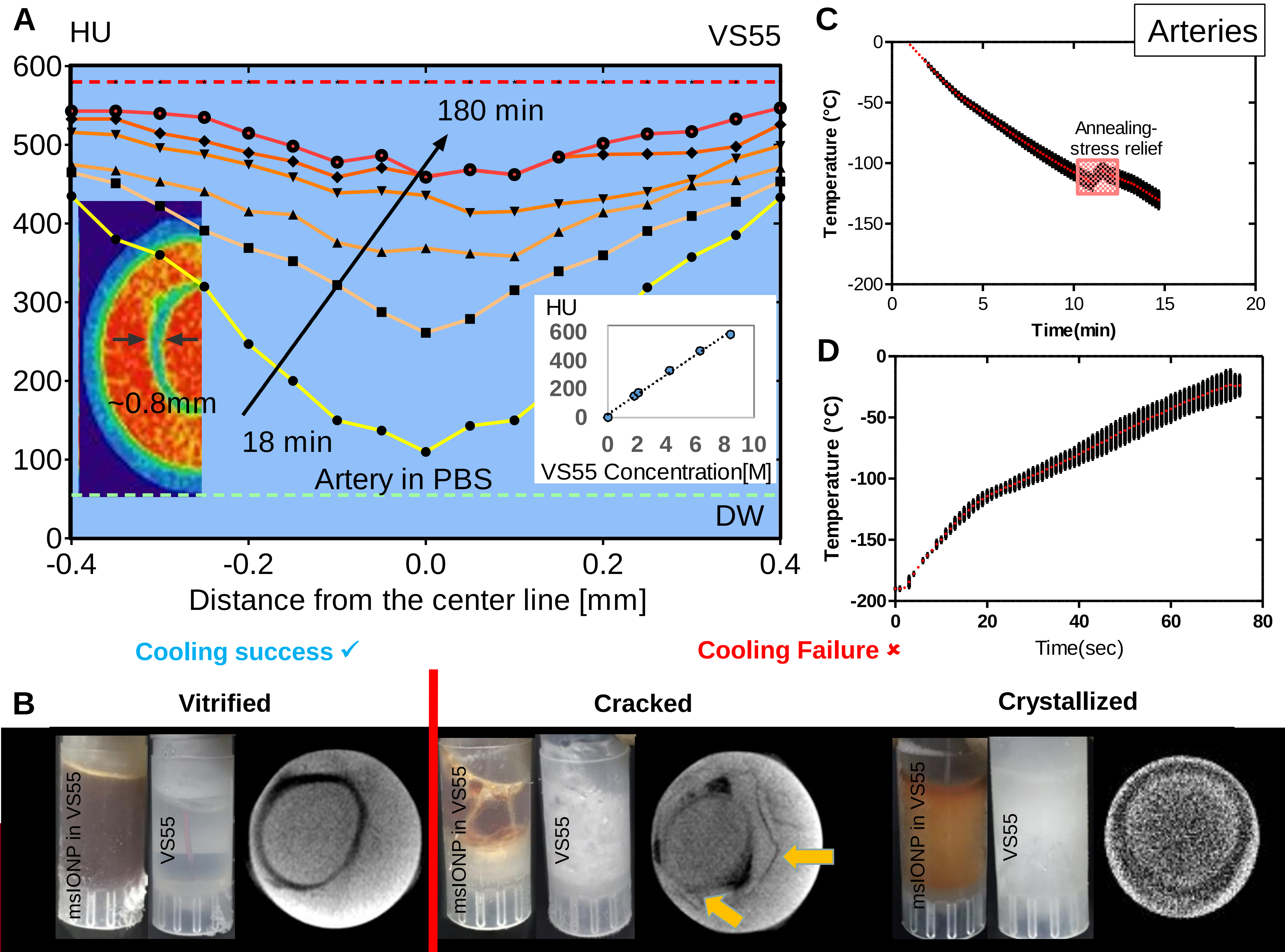
Kidney


A
B. Gradient Echo
(T_2*-weighted)
B_0
5
10
35
40
45
C
T_1-weighted Image
D
[IONP] (mg Fe/mL)
R_1 (1/s)
4.2
3.3
2.5
1.7
0.8
0.0
5
4
3
2
1
0

Cooling -- Successful vitrification of liver

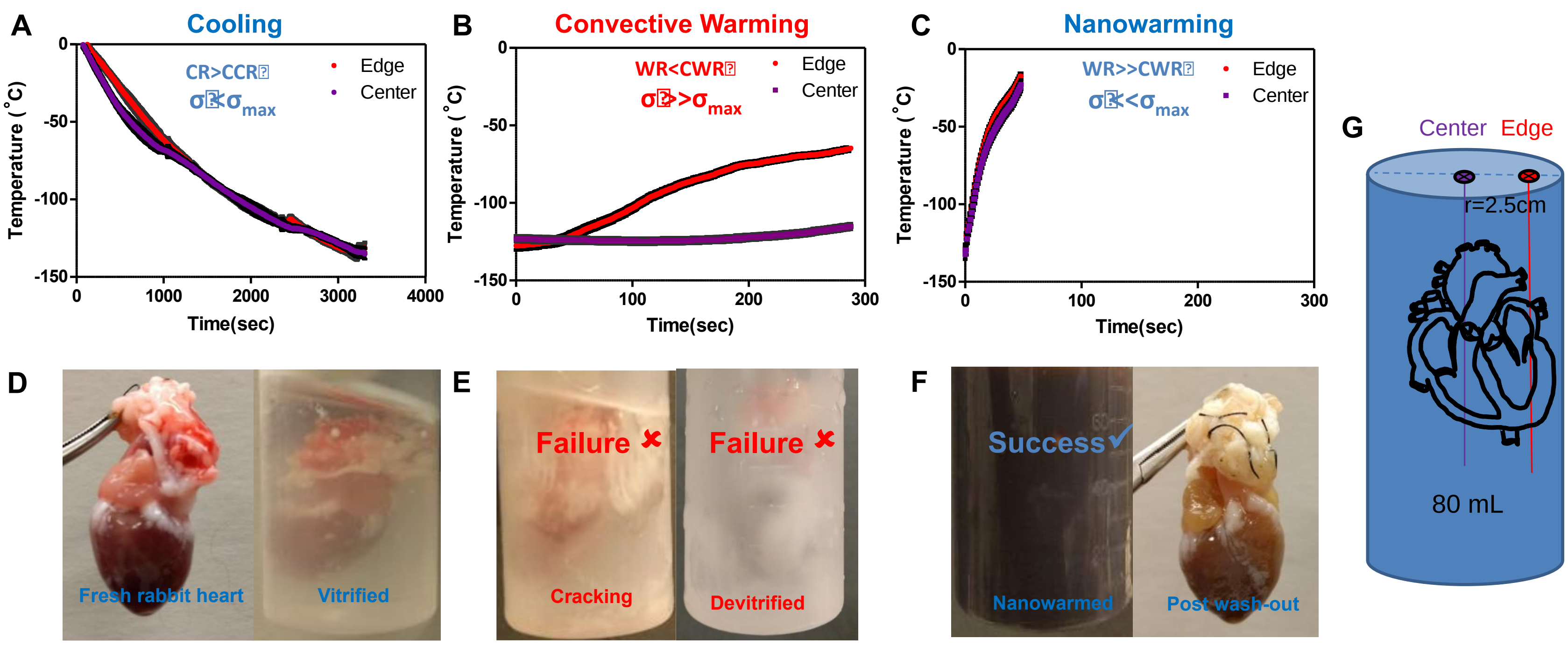


1) Vitrified successfully, no cracks observed initially. Observed cracks in image formed while taking picture.
2) Need to optimize anneal step.

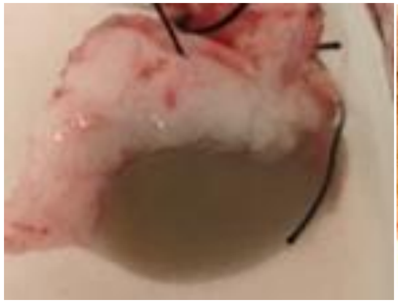


A
HU
VS55
600
500
400
300
200
100
0
180 min
18 min
~0.8mm
Artery in PBS
DW
HU
600
400
200
0
0 2 4 6 8 10
VS55 Concentration[M]
-0.4 -0.2 0.0 0.2 0.4
Distance from the center line [mm]
C
Arteries
Temperature (°C)
Annealing-stress relief
Time(min)
D
Temperature (°C)
Time(sec)
Cooling success ✓
Cooling Failure ×
B
Vitrified
Cracked
Crystallized
msIONP in VS55
VS55

Cooling and heating performance in hearts

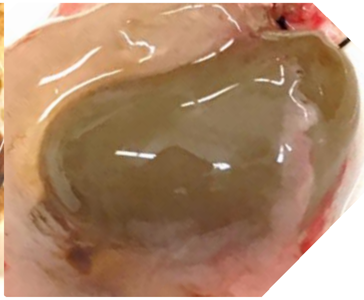


Nanoparticle washout



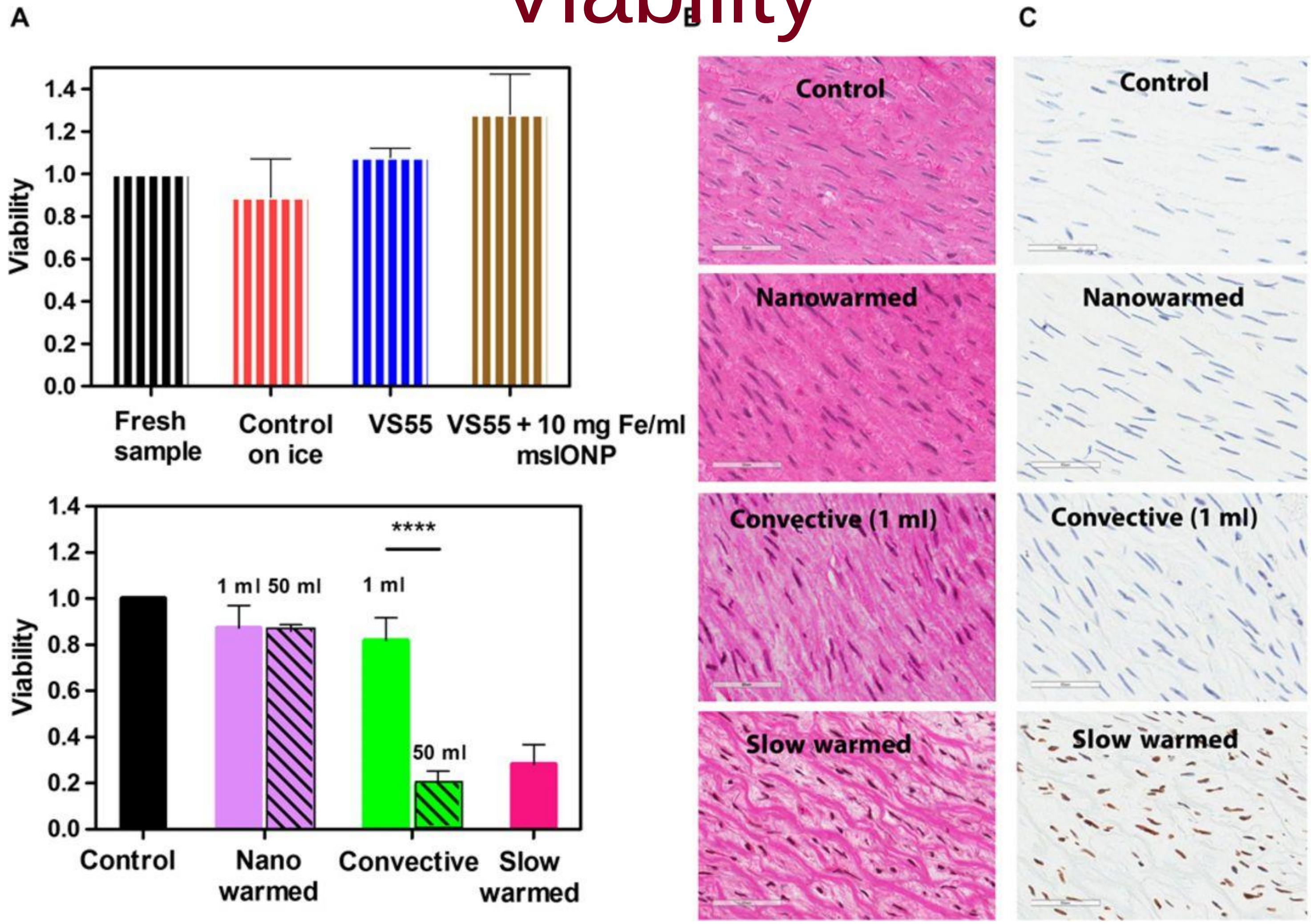
Fresh kidney in EC

sIONP-perfused



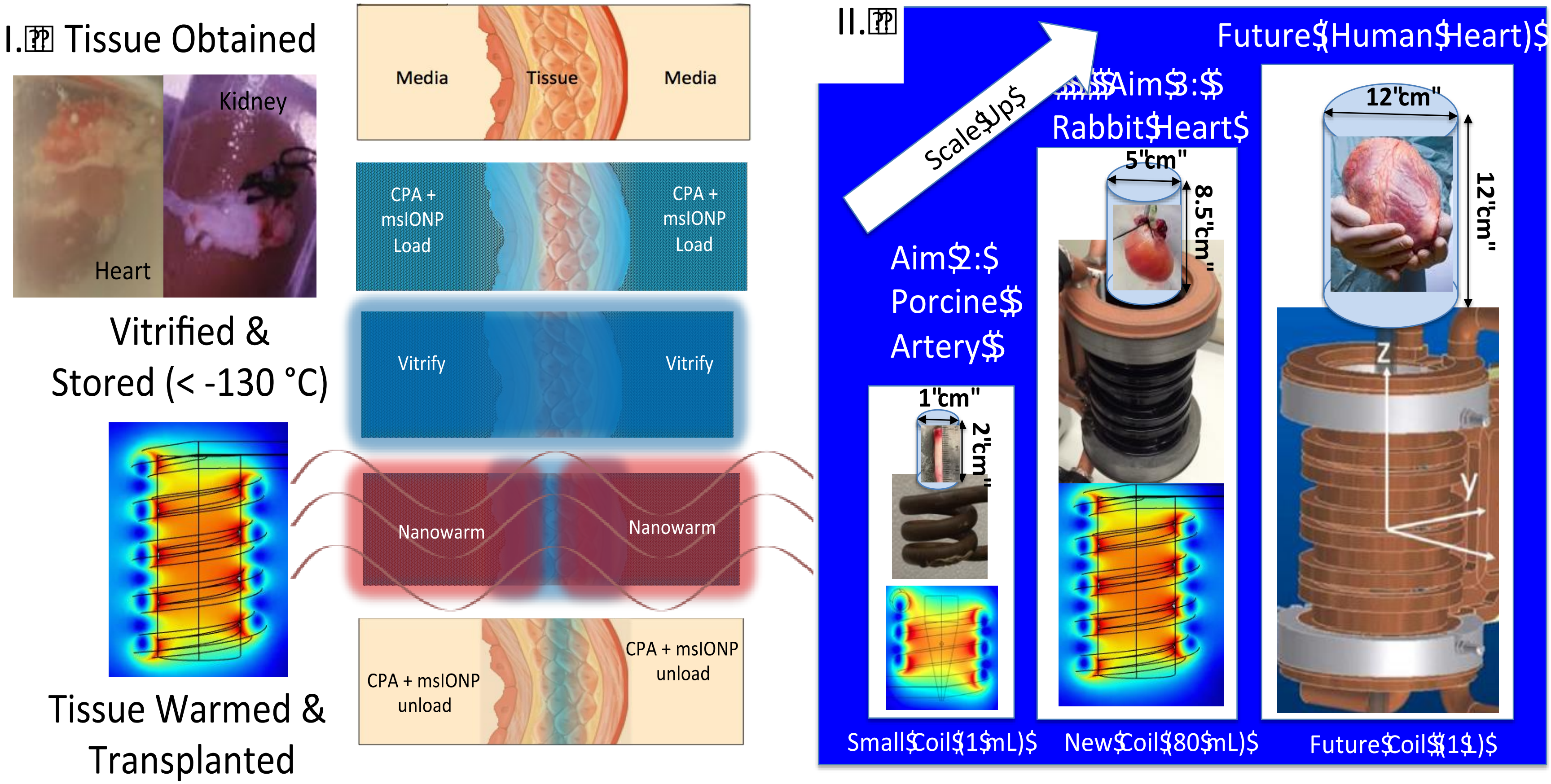
Post-washout

Viability



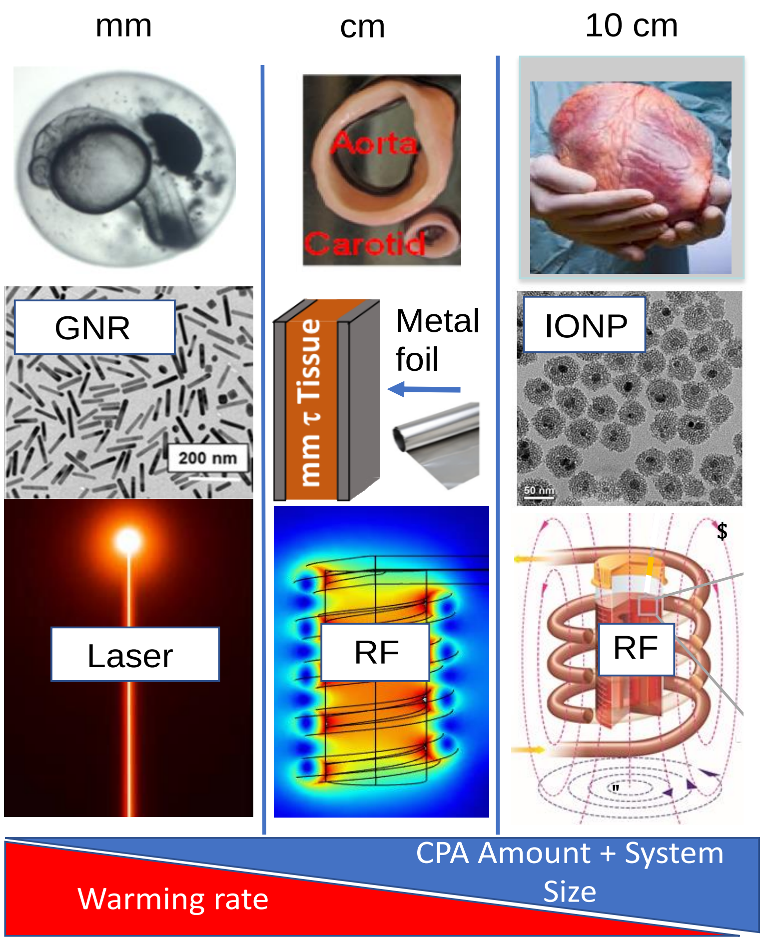
Navid Manuchehrabadi et al., Sci Transl Med 2017;9:eaah4586

Scale up:



I. Tissue Obtained
Kidney
Heart
Vitrified &
Stored (< -130 °C)
Tissue Warmed &
Transplanted
Media
Tissue
Media
CPA + msIONP Load
CPA + msIONP Load
Vitrify
Vitrify
Nanowarm
Nanowarm
CPA + msIONP unload
CPA + msIONP unload
II.
ScaleUp
Future$(Human$Heart)$
Aim$3:$
Rabbit$Heart$
Aim$2:$
Porcine$
Artery$
12"cm"
12"cm"
5"cm"
8.5"cm"
1"cm"
2"cm"
z
y
Small$Coil$(1$mL)$
New$Coil$(80$mL)$
Future$Coil$(1$L)$

Applications



- Organs (Heart, kidney, ovary, liver)
- Flat or annular substances (Blood vessels, skin, cartilage, tissue slices
- Cell clusters (Islets Shrimp, Zebrafish embryos, coral) embryos

Applications for assessing variability in tissue engineering

- High throughput testing: Test many 1,000's of tissue slices from one donor (perfused donor liver)
- Donor variabilty: Test 100's of samples from 100's of donors (liver wedge biopsy)
- Collect samples over time: Test slices all at once
- Thaw samples over time: Multiple assays, retest and confirm
- Large volume samples: Scalable system

- Optimized therapeutic utilization: Use the right tissue at the right time in the right patient

UMN Center for Cryopreservation

Collaborators:

- Erik Finger (Surgery)
- John Bischof (ME)

- Christy Haynes (Chemistry)
- Mike Garwood (Radiology)
- Cari Dutcher (Mech Eng)
- John Iaizzo (Surgery)
- Alena Talkachova (Chem Eng)
- Mike McAlpine (Mech Eng)
- Brian Fife (Medicine)
- Jim Collins (Vet Med)
- Mike Lottie (IEM)
- Jed Lewis (Organ Preservation Alliance)
- Seb Giwa (Sylvatica)
- Charles Lee (UNC Charlotte)

Laboratory Personnel:

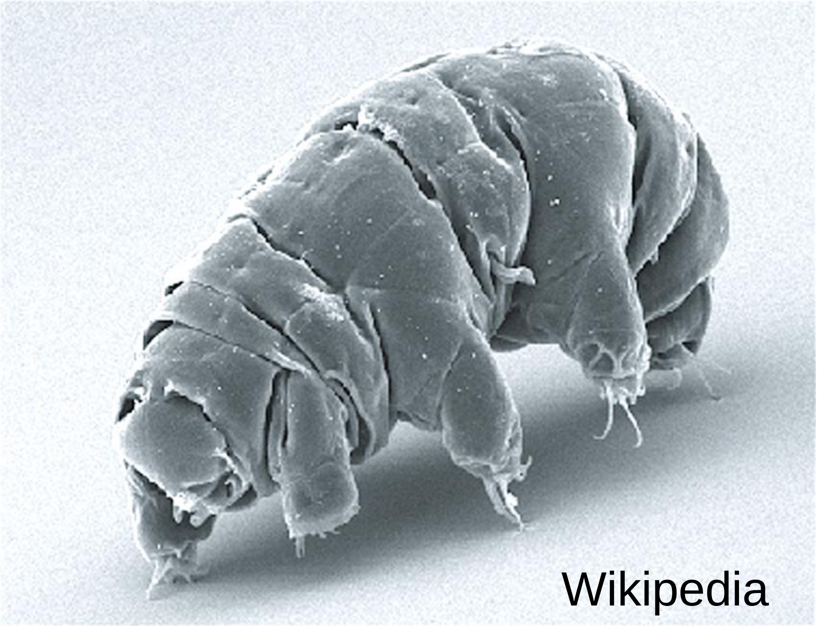
- Anirudh Sharma
- Navid Manuchehrabadi
- Zhe Gao
- Bat-Erdene Namsrai
- Li Zhang
- Zonghu Han
- Hattie Ring
- Nicolle Myers
- Dave Ankarlo

Funding:

- UMN Faculty Research and Development
- NIH HLBI
- NIH NIDDK

Tardigrades (/ˈtɑːrdɪˌɡreɪd/; also known colloquially as **water bears**, or **moss piglets** are water-dwelling, eight-legged, segmented micro-animals.

- Live in hot springs, the Himalaya, deep sea, and polar region.
- Capable of extreme anhydribiosis
- Survival
  - A few minutes at 151 ° C (304 ° F)
  - 30 years at −20 ° C (−4 ° F)
  - A few days at −200 ° C (−328 ° F; 73 K)
  - A few minutes at −272 ° C (−458 ° F; 1 K)


Wikipedia