Accurate viability assessment and cryopreservation of pancreatic islets

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PANCREATIC ISLET CRYOPRESERVATION

• Low-risk treatment for diabetic patients

• What would be the benefit of cryopreservation

• No efficient method of cryopreservation

Understanding of diffusion properties is key to developing new cryopreservation protocols.
AIMS

1. Determine kinetics of molecule diffusion into pancreatic islets
2. Optimise methods for viability assessment of pancreatic islets
3. Improve cryopreservation of pancreatic islets
Experiments performed on mouse islets isolated in-house

Pre-staining with nuclear dye → Embedding and cryosectioning → Confocal imaging → Image analysis

Original image

Concentric Circles Plugin in ImageJ
DIFFUSION KINETICS IN PANCREATIC ISLETS

20°C

Fluorescence intensity (RFU)

0 10 20 30 40 50 60 70 80 70 60 50 40 30 20 10 0

Distance from the outer edge (µm)

PERIPHERY

ISLET CORE

PERIPHERY

15 min

6 hours

24 hours
DIFFUSION KINETICS IN PANCREATIC ISLETS – TEMPERATURE EFFECT

20°C

Fluorescence intensity (RFU)

Distance from the outer edge (µm)

15 min
6 hours
24 hours

37°C

Fluorescence intensity (RFU)

Distance from the outer edge (µm)

15 min
6 hours
24 hours
EFFECT OF PROLONGED INCUBATION AT 37 °C ON ISLET VIABILITY

Viability after incubation at 37°C

Fluorescein diacetate / Propidium iodide

Fresh

6h at 37 °C
Diffusion of solutes takes up to 6 hours to reach the islet core at 37 °C

- Current viability staining and imaging
- Development of new cryopreservation protocols
CURRENT VIABILITY STAINING AND IMAGING

WIDEFIELD

Focal plane

Lens

Detector

On top of a coverslip

Squashed in between two coverslips

Live/dead

200 μm

200 μm
VIABILITY STAINING AND IMAGING

CONFOCAL

Focal plane

Lens

Pinhole

Detector

nuclei live dead

100 μm
VIABILITY STAINING AND IMAGING

MULTIPHOTON

Focal plane

Lens

Detector

nuclei live

100 μm
VIABILITY STAINING AND IMAGING

LIGHT-SHEET

Focal plane
Lens
Detector

nuclei live dead
DEVELOPMENT OF NEW CRYOPRESERVATION PROTOCOL

Viability immediately post-thaw

% viability

Incubation time with trehalose

Toxicity of DMSO and trehalose

% viability

Concentration of trehalose (mM)
1. Pre-incubation with 200mM trehalose for 1-48 hours

2. DMSO added step-wise in the last 45 minutes

3. Slow-freezing in programmable freezer
DEVELOPMENT OF NEW CRYOPRESERVATION PROTOCOL

Viability immediately post-thaw

![Viability bars](chart1)

Diffusion of nuclear dye into islets at 37 °C

![Diffusion graph](chart2)

* p ≤ 0.05
1 **ATP/ADP ratio**

- Immediately post-thaw
- 24h post-thaw

2 **Glucose-stimulated insulin secretion**

- 3.3mM glucose
- 16.7mM glucose

3 **Transplantation under mouse kidney capsule**

* $p \leq 0.05$
BENEFITS OF NEW CRYOPRESERVATION PROTOCOL

Viability immediately post-thaw

Viability 24 hours post-thaw
CONCLUSIONS

1. Diffusion into islet core increases at 37 °C but still takes up to 6 hours

2. Current viability assessment methods are sub-optimal and disregard islet core

3. Pre-incubation of islets with non-toxic cryoprotectants can improve cryosurvival
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1. Diffusion into islet core increases at 37 °C but still takes up to 6 hours.

2. Current viability assessment methods are sub-optimal and disregard islet core.

3. Pre-incubation of islets with non-toxic cryoprotectants can improve cryosurvival.
SUPPLEMENTARY INFORMATION: DIFFUSION STUDIES

Adding DMSO
1 hour incubation

Increasing hydrostatic pressure
15 min incubation
SUPPLEMENTARY INFORMATION: TREHALOSE CONCENTRATION

![Graph showing % viability across different trehalose concentrations (100 mM, 200 mM, 300 mM, and 400 mM).]