Effect of Aquaporin Translocation on Cryopreservation of Hepatocytes

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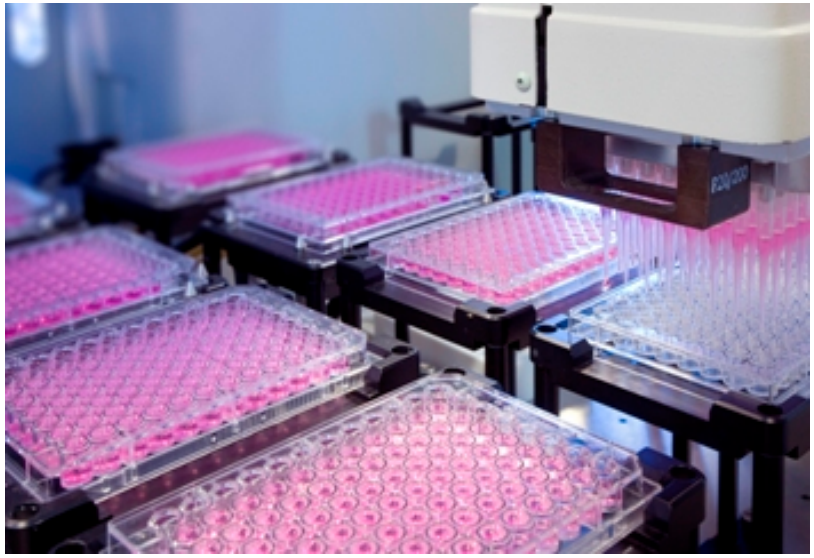
BACKGROUND

- Healthy livers are used for transplantation
- Remaining livers are used for research purposes and drug toxicity testing within pharmaceutical companies using isolated hepatocytes.
  - Hepatocytes: liver cells that potentially imitate the function of a complete liver

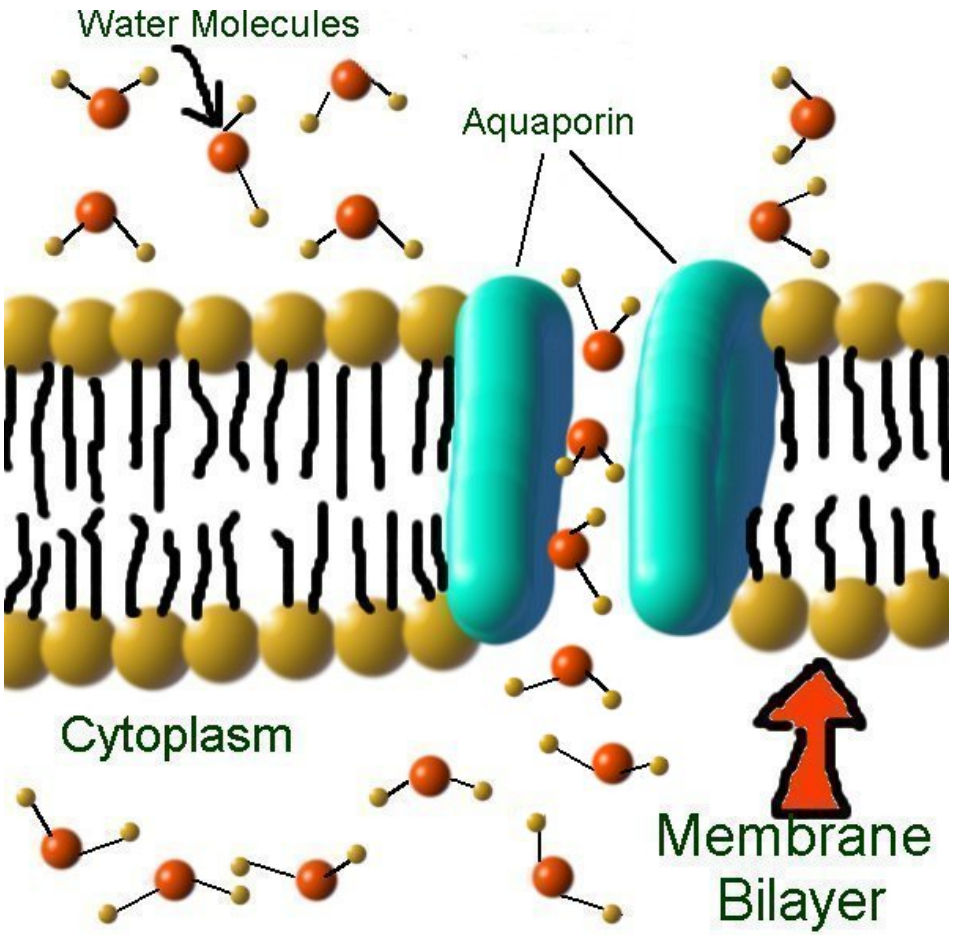


BACKGROUND

- Benefits of Cryopreservation
  - Increased availability, flexibility, and widespread use
  - Ability to be stored for extended periods of time
- Present Challenges of Cryopreservation
  - High incidence of cell death due to formation of intracellular ice

BACKGROUND

- Aquaporins
  - Definition: water transport channels located on the cell membrane
  - Additional are stored within vesicles in the cytoplasm
  - Stored aquaporins can be translocated to the cell membrane to increase water transport out of the cell, resulting in less intracellular ice formation



RESEARCH GOAL

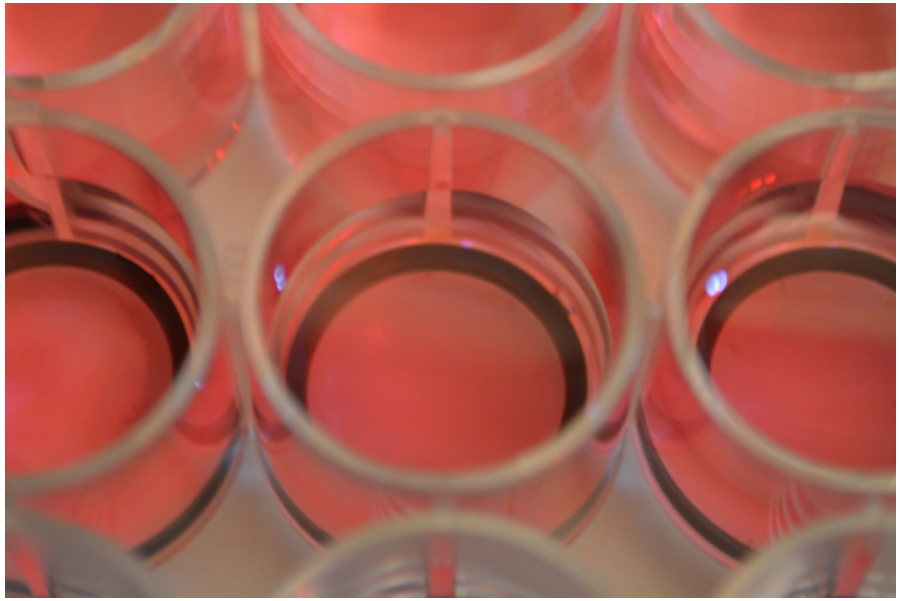
To improve cryopreservation of hepatocytes by the translocation of aquaporins

Methodology

- Induction Agents:
  - DiButyryl Cyclic-AMP (DBcAMP)
    - Choleretic stimulus, which translocates the aquaporins
  - 3-isobutyl-1-methylxanthine (IBMX)
    - Protects DBcAMP from degradation after entering the cell
  - 100µM concentration
- Cryoprotectant:
  - 20% Glycerol
  - 80% 1x Dulbecco's Modified Eagle Medium (DMEM)

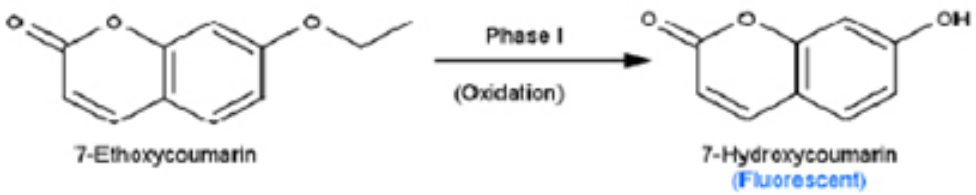
METHODOLOGY

- Digitonin and Propidium Iodide (DI/PI) Assay
  - Assesses plated viability
  - Mixture with optimal concentration of 1x/10x
  - Amount of fluorescence corresponds to number of live cells

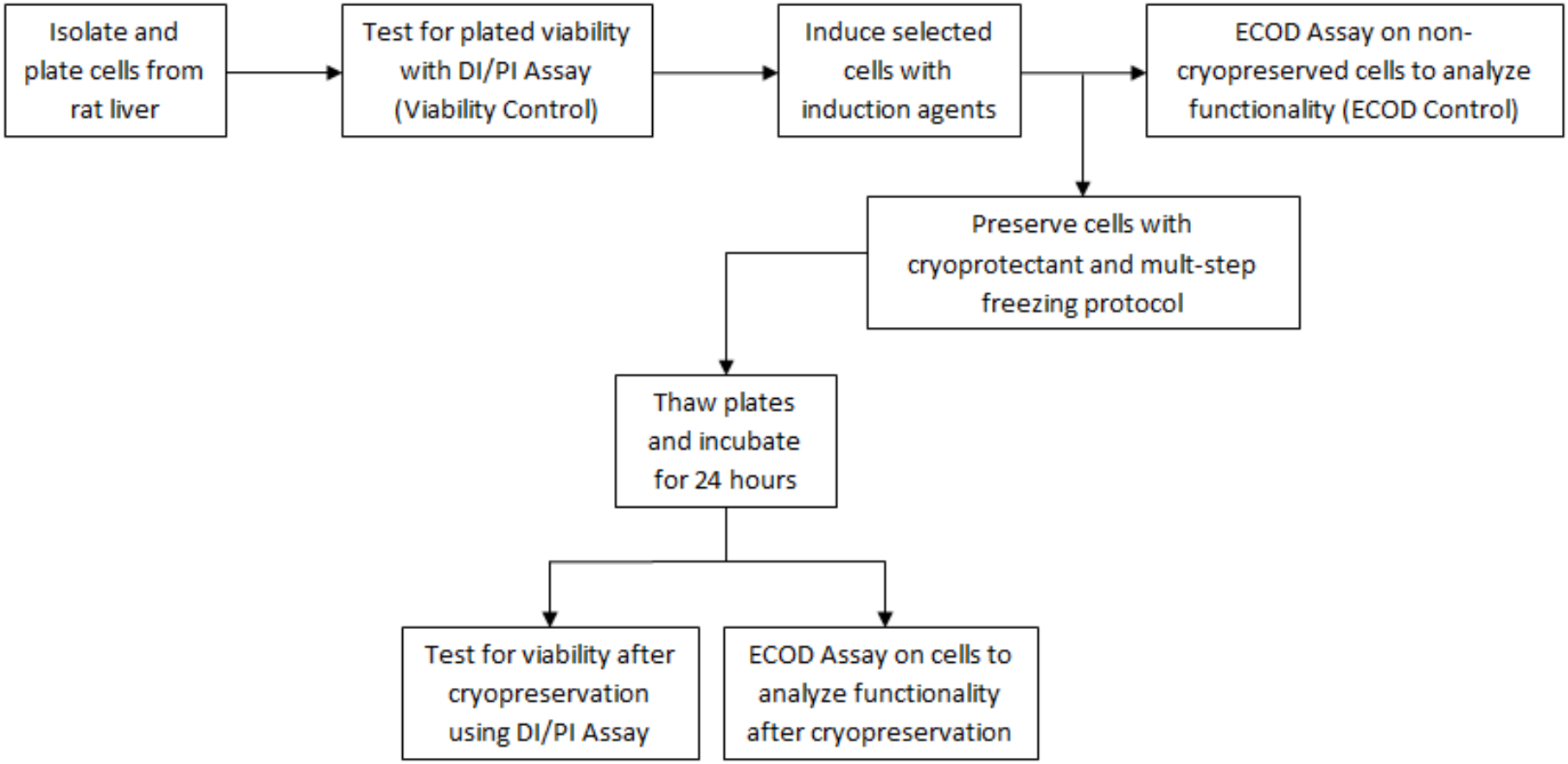


METHODOLOGY

- 7-ethoxycoumarin O-dealkylas (ECOD) Assay
  - Assesses functionality
  - Treated with 1, 2, 3, 4 – Tetrachlorodibenzo-p-dioxin (TCDD) for 24 hours
  - 7-EC applied for an incubation period of 1 hour
  - Supernatant analyzed for fluorescence
  - Positive correlation between fluorescence and ECOD Activity

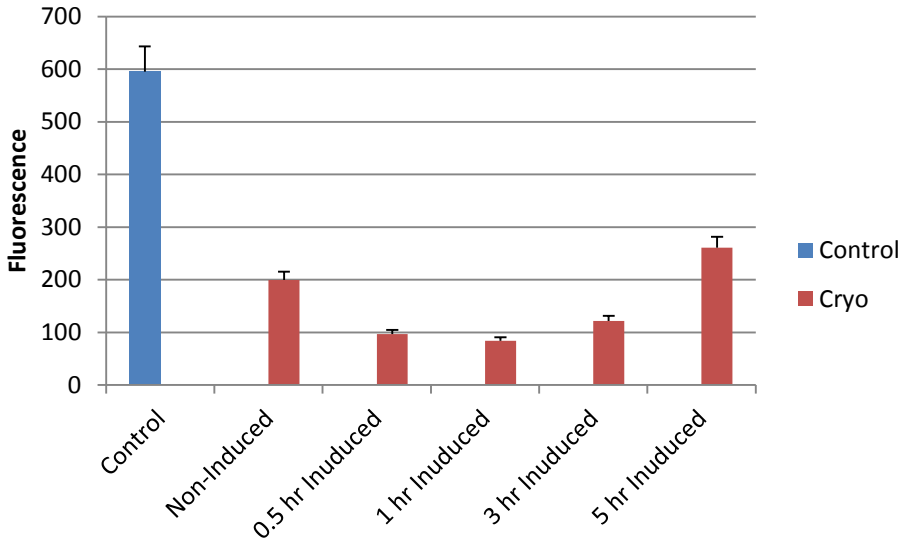


METHODOLOGY



RESULTS

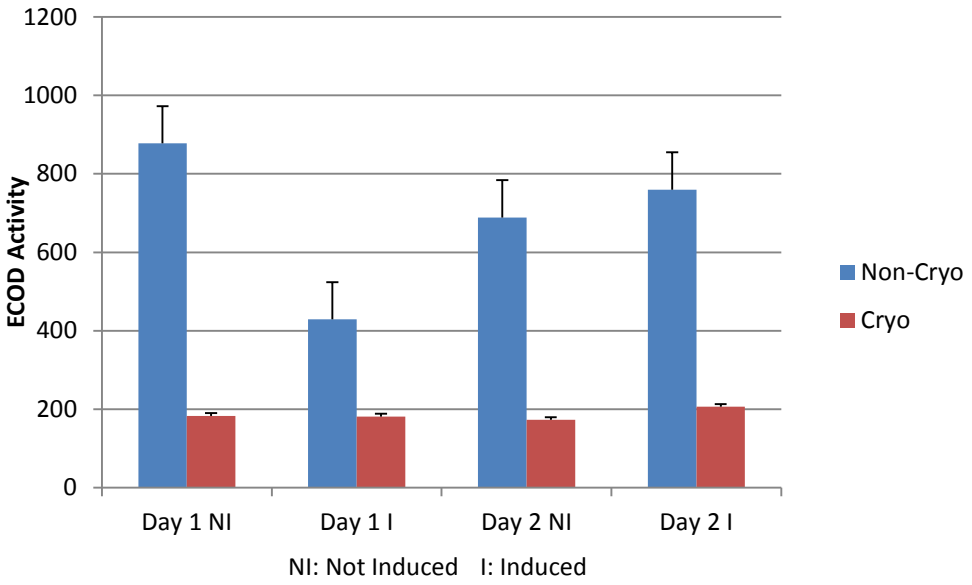
- Viability
  - Inducing for 5 hours before cryopreservation increases viability by about 10% than if non-induced



	Viability After Cryopreservation
Non-Induced	33.51%
0.5 hr Induced	16.29%
1 hr Induced	14.11%
3 hrs Induced	20.44%
5 hrs Induced	43.84%

RESULTS

- Functionality
  - Day 1: Cells were treated with TCDD 24 hours after being plated or thawed
  - Day 2: Cells were treated with TCDD 48 hours after being plated or thawed



	Percentage Functionality Regained After Cryopreservation
Day 1: NI	20.85%
Day 1: I	42.24%
Day 2: NI	25.06%
Day 2: I	27.13%

CONCLUSION

- Inducing the hepatocytes with DBcAMP and IBMX does improve viability and functionality after cryopreservation
- More induction time for the aquaporins to translocate to the membrane
- Additional time to recover from preservation

FUTURE RESEARCH

- Refine DI/PI tests to discover more accurate correlation between fluorescence and live or dead plated cells

- Further analyze ECOD results by performing chloroform extractions

- Apply methods and knowledge to cell suspensions to increase availability