Effective hypothermic storage of human pluripotent stem cell-derived cardiomyocytes compatible with global distribution of cells for clinical applications and toxicology testing

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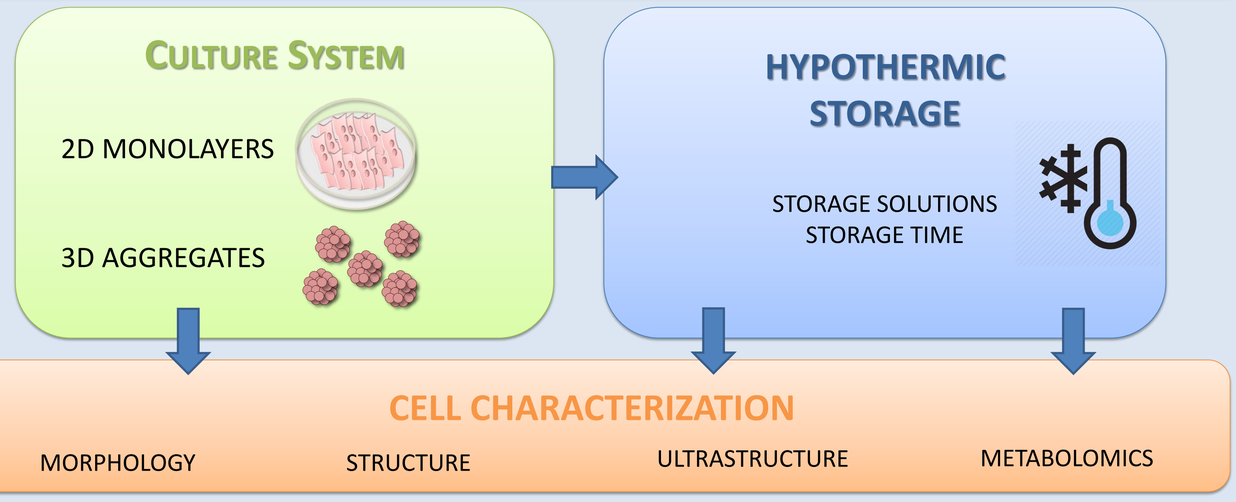
Background

The production of cardiomyocytes (CMs) from human pluripotent stem cells (hPSC) holds great promise for patient-specific cardiotoxicity drug testing, disease modeling and cardiac regeneration [1]. The applicability of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) in the clinic and industry is highly dependent on the development of efficient methods for worldwide shipment of these cells. We evaluated the feasibility to cold store monolayers and aggregates of functional CMs obtained from different PSC lines using a fully defined clinical-compatible preservation formulation and investigated the time frame that hPSC-CMs could be subjected to hypothermic storage [2].

AIM

Establishment of effective clinically compatible strategies for cold (4º C) storage of hPSC-CMs as 2D Monolayers and 3D aggregates

Strategy



Results

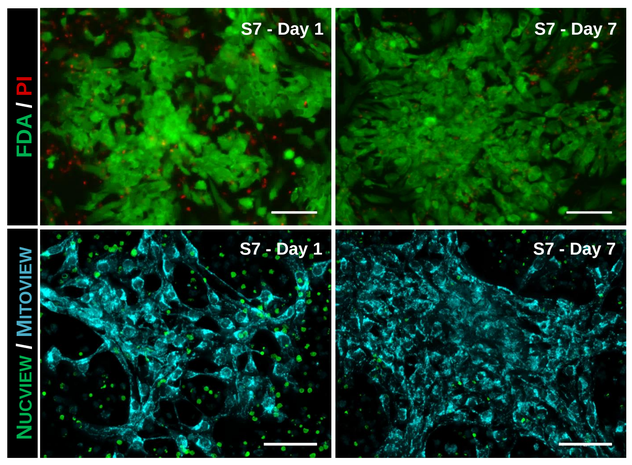
Hypothermic Storage of hPSC-CMs

- *hPSC-CMs were stored for 3 (S3), 5 (S5) and 7 (S7) days at 4°C in HypoThermosol™ [2]*

2D Monolayers

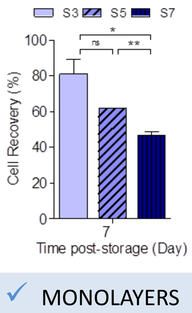
- *Cell Viability*

Necrosis - FDA (live cells) / PI (dead cells)
Apoptosis - Mitoview (live cells)/Nucview (Apoptotic cells, active caspase-3)



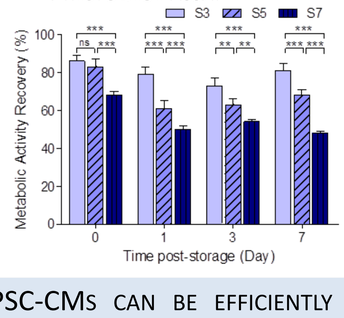
- *Cell Recovery*

Trypan Blue assay

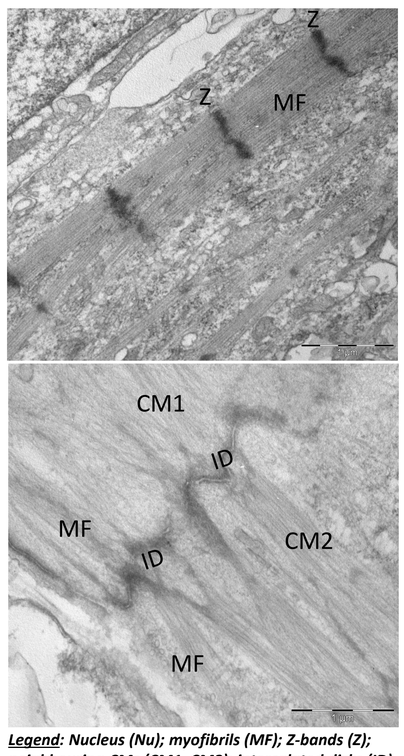


- *Metabolic Activity Recovery*

PrestoBlue assay



- *Transmission Electron Microscopy (TEM)*



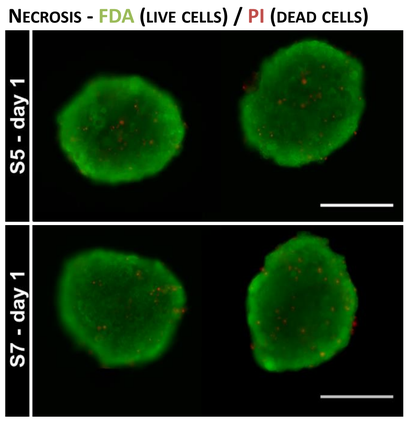
Legend: Nucleus (Nu); myofibrils (MF); Z-bands (Z); neighbouring CMs (CM1, CM2); intercalated disks (ID)

- ✓ Monolayers of hPSC-CMs can be efficiently cold stored for 3 days without compromising cell recovery, metabolic activity and ultrastructure
- ✗ Cell viability decreased when the cold storage interval was extended to 7 days

3D Aggregates

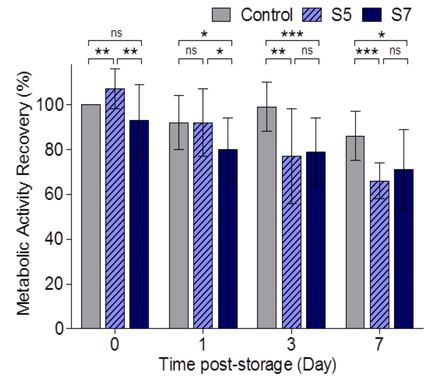
- *Cell Viability*

Necrosis - FDA (live cells) / PI (dead cells)

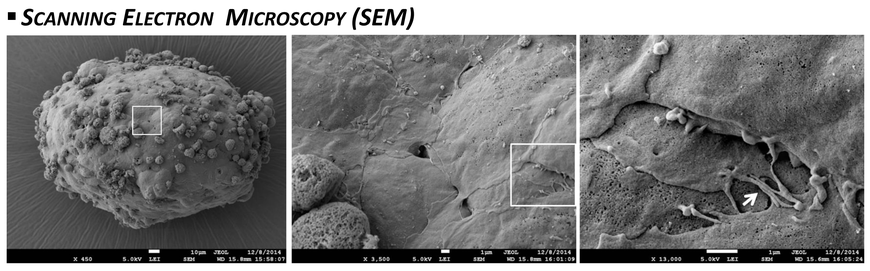


- *Metabolic Activity Recovery*

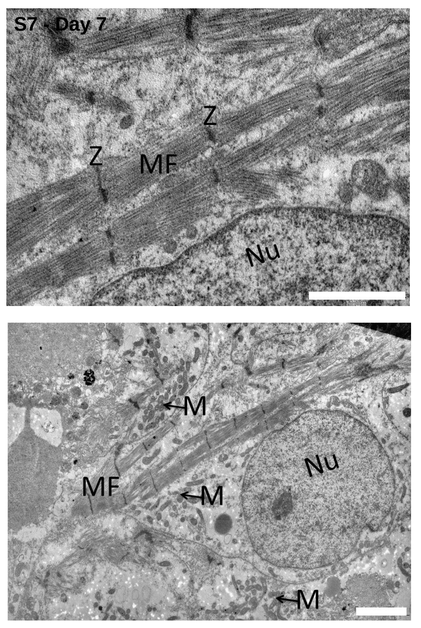
PrestoBlue assay



- *Scanning Electron Microscopy (SEM)*



- *Transmission Electron Microscopy (TEM)*

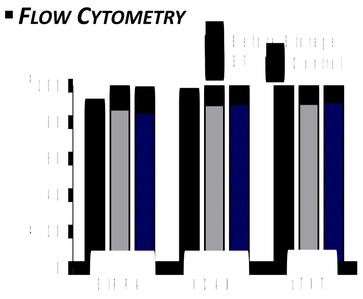


Legend: Nucleus (Nu); myofibrils (MF); Z-bands (Z); mitochondria (M)

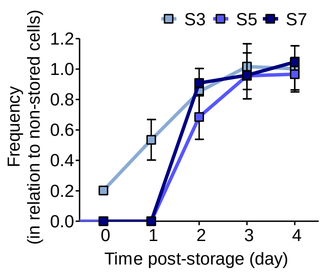
- ✓ hPSC-CMs are more resistant to prolonged hypothermic storage-induced cell injury in 3D aggregates than in 2D monolayers, showing high cell recoveries (>70%) after 7 days of storage
- ✓ Aggregate structure/size and cell ultrastructure were maintained after 7 days of storage

hPSC-CM characterization

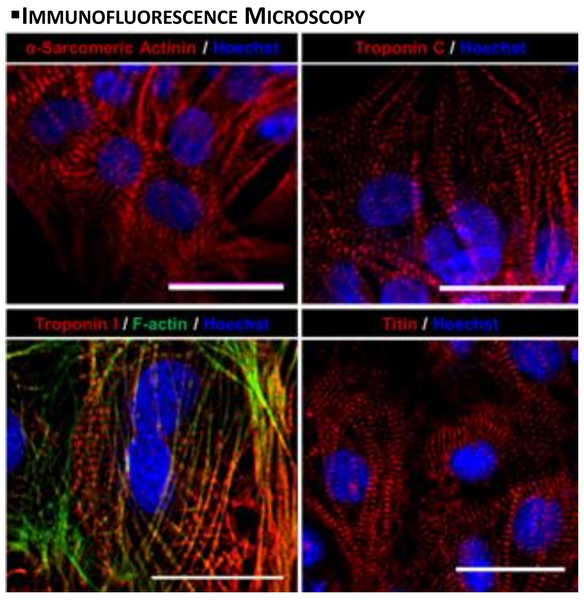
- *Flow Cytometry*



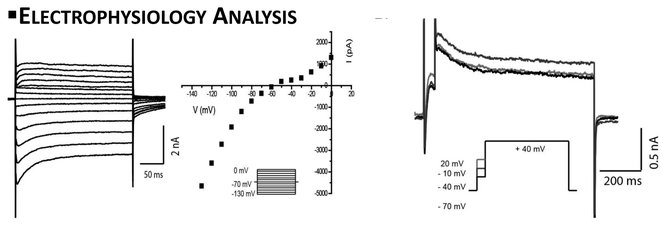
- *Beating Frequency*



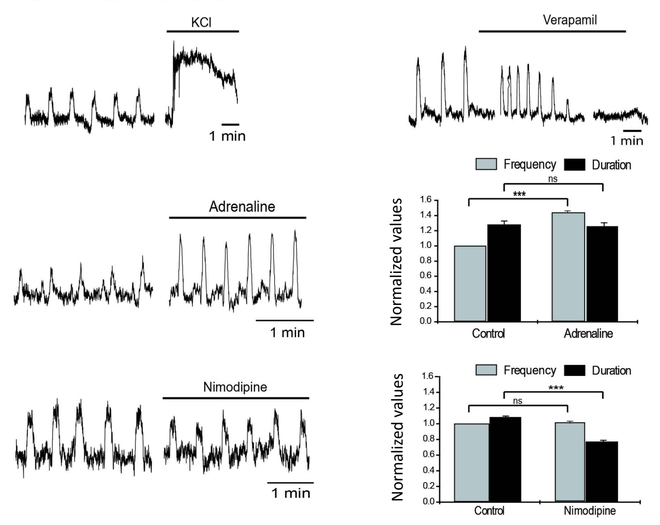
- *Immunofluorescence Microscopy*



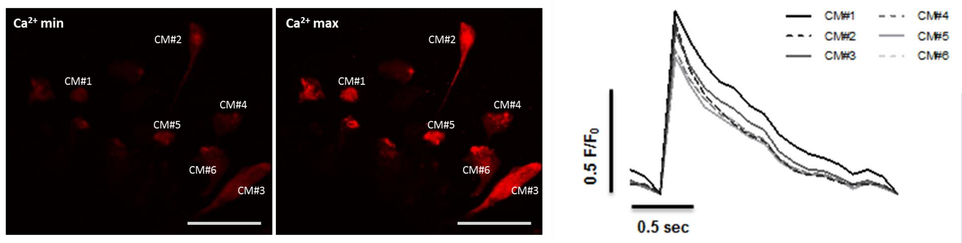
- *Electrophysiology Analysis*



- *Drug responsiveness*



- *Calcium Transients*



- ✓ After 7 days of storage, hPSC-CMs maintained their typical protein expression profile, sarcomeric structure, beating frequency, electrophysiological profiles and drug responsiveness

Conclusion

- **Development of efficient strategies for hypothermic storage of 2D monolayers and 3D aggregates of hPSC-CMs**
- **2D monolayer of hPSC-CMs can be efficiently stored during 3 days (80% of cell recovery)**
- **3D Aggregates of hPSC-CM provide better cell recovery comparing to 2D monolayer after 7 days of storage**
- **7 days of hypothermic storage did not affect the phenotype and function of human PSC-CMs stored either as monolayers or aggregates**

Step forward towards the global commercial distribution of hPSC-CMs

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References: **[1]** Oh, Y., Wei, H., Ma, D., Sun, X. & Liew, R. Clinical applications of patient-specific induced pluripotent stem cells in cardiovascular medicine. Heart 98, 443–9 (2012). **[2]** Correia, C., Koshkin, A., Carido, M. et al. Effective Hypothermic Storage of Human Pluripotent Stem Cell-Derived Cardiomyocytes Compatible With Global Distribution of Cells for Clinical Applications and Toxicology Testing. Stem Cells Trans Med. 5;111(3):658-69 (2016).