NOVEL STRATEGIES FOR GENERATION AND HYPOTHERMIC STORAGE OF hPSC-DERIVED CARDIOMYOCYTES FOR CELL THERAPY APPLICATIONS

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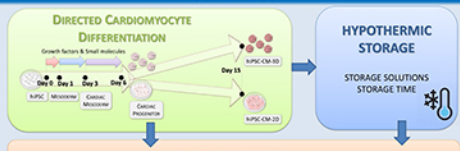
BACKGROUND

Three dimensional (3D) cultures of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) hold great promise for drug discovery and regenerative medicine applications. The transition of CM differentiation protocols from two dimensional (2D) to 3D cultures has been challenging typically resulting in lower CM purities and reduced reproducibility. Moreover, the applicability of hPSC-CMs in the clinic/industry is highly dependent on the development of efficient methods for worldwide shipment of these cells.

AIM

ESTABLISHMENT OF AN INTEGRATED STRATEGY FOR PRODUCTION AND HYPOTHERMIC STORAGE (4º C) OF hPSC-CMs AS 2D MONOLAYERS AND 3D AGGREGATES

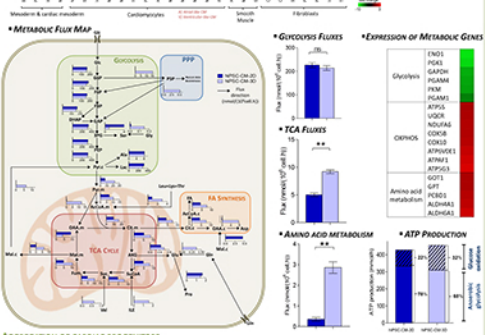
STRATEGY



RESULTS

DIRECTED CARDIOMYOCYTE DIFFERENTIATION

- AT DAY 15 hPSC-CM-3D WERE COMPARED TO hPSC-CM-2D



AGGREGATION OF CARDIAC PROGENITORS...

- ✓ IMPROVED CM ENRICHMENT, DIFFERENTIATION AND COMMITMENT TOWARDS VENTRICULAR-LIKE CM SUBTYPE
- ✓ INCREASED TCA-CYCLE ACTIVITY, AMINOACID METABOLISM AND GENE EXPRESSION OF OXPHOS GENES

HOWEVER...

- AT THE END OF THE DIFFERENTIATION PROCESS BOTH hPSC-CM-3D AND hPSC-CM-2D REMAIN HIGHLY GLYCOLYTIC WITH A FETAL-LIKE METABOLIC PHENOTYPE

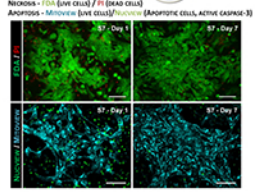
HYPOTHERMIC STORAGE OF hPSC-CMs

- hPSC-CMs WERE STORED FOR 3 (S3), 5 (S5) AND 7 (S7) DAYS AT 4°C IN HYPOTHERMOSOL™ FRI

2D MONOLAYERS

- CELL VIABILITY

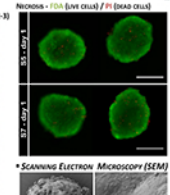
NECROSIS - FDA (LIVE CELLS) / PI (DEAD CELLS)
APOPTOSIS - MITOVIEW (LIVE CELLS)/PACVIEW (APOPTOTIC CELLS, ACTIVE CASPASE-3)



3D AGGREGATES

- CELL VIABILITY

NECROSIS - FDA (LIVE CELLS) / PI (DEAD CELLS)



- SCANNING ELECTRON MICROSCOPY (SEM)

- CELL RECOVERY

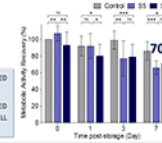
TRYPAN BLUE ASSAY

- METABOLIC ACTIVITY RECOVERY

PRESTOBLUE ASSAY

- METABOLIC ACTIVITY RECOVERY

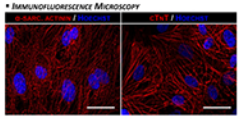
PRESTOBLUE ASSAY



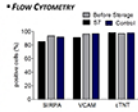
- ✓ 2D MONOLAYERS OF hPSC-CMs CAN BE EFFICIENTLY COLD STORED FOR 3 DAYS (80% OF CELL RECOVERY)
- ✓ hPSC-CMs IN 3D AGGREGATES ARE MORE RESISTANT TO PROLONGED HYPOTHERMIC STORAGE-INDUCED CELL INJURY, SHOWING HIGH CELL RECOVERIES (>70%) AFTER 7 DAYS OF STORAGE

hPSC-CM CHARACTERIZATION

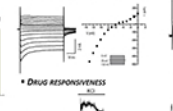
- IMMUNOFLUORESCENCE MICROSCOPY



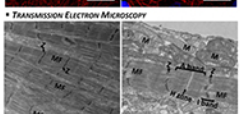
- FLOW CYTOMETRY



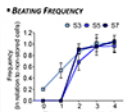
- ELECTROPHYSIOLOGY ANALYSIS



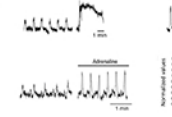
- TRANSMISSION ELECTRON MICROSCOPY



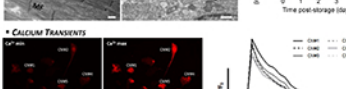
- BEATING FREQUENCY



- 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

- AGGREGATION OF CARDIAC PROGENITORS IMPROVED CM ENRICHMENT AND DIFFERENTIATION
- DEVELOPMENT OF EFFICIENT STRATEGIES FOR HYPOTHERMIC STORAGE OF 2D MONOLAYERS AND 3D AGGREGATES OF hPSC-CMs
- 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] 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, 1113(3):658-669(2016).