Engineering an organotypic culture model of endocardial cushion morphogenesis

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Disclaimer

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Congenital heart defects

- Most common type of birth defect.
- Affect 1 out of every 100 infants born in the United States.
- Leading cause of infant deaths due to birth defects.
- Genetic etiology is identified in less than 20% of cases.

Image credit: CDC
Cardiac developmental toxicity

- Due to maternal illness, drug, or environmental exposure.

- Association between exposure to organic solvents (e.g. TCE) and valve and septal defects.

- Endothelial-to-mesenchymal transition (EndMT) is sensitive to the effects of organic solvents.
EndMT during cushion morphogenesis

Embryonic heart

- OFT
- Myo
- Endo
- AVC

Endocardial cushion

- Blood flow (V → A)
- Endocardium
- Myocardium

Key behaviors:
- Mehanosensation
- ECM production

Contraction
- ECM production
EndMT during cushion morphogenesis

Embryonic heart

- Endo
- Myo
- OFT
- AVC
- CJ

Endocardial cushion

- Blood flow
- V → A
- Cardiac jelly

Key ECM components

- Hyaluronan
- Collagen
- Fibronectin
EndMT during cushion morphogenesis

Embryonic heart

Endocardial cushion

Blood flow

Key EndMT signals:
- VEGF
- TGF-β and BMP
- VEGF
EndMT during cushion morphogenesis

Embryonic heart

Endocardial cushion

V Blood flow A

Cardiac jelly

Mesenchyme

Key behaviors

Proliferation Differentiation ECM production
Project goal

To develop a human cell culture model of EndMT to study chemical effects on cardiac septation and valve development.
Key aspects to recapitulate in model

Endocardial cushion

- Blood flow

Cell types
- Endocardial
- Myocardial

Chemical signals
- TGF-β
- BMP
- VEGF

Mechanical signals
- Cardiac jelly
- Blood flow
Key phenotypic changes to measure in model

**Endothelial phenotype**

- Endothelial cells
  - VE-cadherin
  - PECAM-1
  - VEGFR2

**Intermediate phenotypes**

- **End**
- **Mes**

**Mesenchymal phenotype**

- Mesenchymal cells
  - Snail
  - α-SMA
  - Slug
  - Vimentin
  - Twist
  - Fibronectin
Initial approach

To induce endothelial cells cultured on fibronectin (FN)-coated plastic to undergo EndMT using myocardial-derived signals.
Primary and iPSC-derived endothelial cells
Experimental workflow for EndMT induction - 1

Cell seeding

- HUVEC
- hiPSC-EC
- HCAEC
- HMVEC

Serum starvation

1 day

Culture media

- Complete
- Reduced serum
- Reduced serum and lacking growth factors
- Basal

TGF-β1 treatment

5 days

Immunostaining

Cell markers

- VE-cadherin
- Snail
- PECAM-1
- Slug
- VEGFR2
- Twist
- α-SMA
- Vimentin
- SM22a
HMVEC undergo EndMT on FN-coated plastic
Revised approach

To induce endothelial cells cultured on hyaluronan (HA)-based hydrogel to undergo EndMT using myocardial-derived signals.
Experimental workflow for EndMT induction - II

**Cell seeding**
- Cell types: HUVEC, hiPSC-EC

**Serum starvation**
- Culture media: Reduced serum

**TGF-β1 treatment**
- Culture media: Reduced serum and lacking growth factors
- Duration: 5 days

**Immunostaining**
- Cell markers: VE-cadherin, SM22a
HUVEC undergo EndMT on HA-based hydrogel
Next steps in model development
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Questions?