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Engineering a human organotypic model of osteogenesis and morphogenetic fusion

David G. Belair

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Embryonic Tissues Undergo Fusion Events During Development

Central Research Goal: Develop a model *in vitro* system that could be used to predict chemical effects on developmental fusion events using human cells

Image Credit: Thomas Knudsen, from Synthetic Biology: ‘flipping the switch’ on opportunities and challenges with virtual tissues. Presented at CompuCell3D Workshop
Morphogenetic Fusion Events in the Embryo Depend on Epithelial-Stromal Interactions

Secondary Palate

Neural Tube

The Need for Fusion-Competent Models of Epithelial-Stromal Interactions

Global incidence of orofacial clefting: 0.12%

Normal  Unilateral cleft  Bilateral cleft

The Royal Children's Hospital Melbourne Cleft Lip and Palate – an overview
The Need for Fusion-Competent Models of Epithelial-Stromal Interactions

Global incidence of orofacial clefting: 0.12%

Normal Unilateral cleft Bilateral cleft

Existing methods for studying palate fusion use animal models, tissue explants, or primary two-dimensional tissue cultures that exhibit a tradeoff between throughput and developmental relevance
Pathology of Palate Fusion and Cleft Palate

Mouse E14  Mouse E15  Mouse E15.5
Elevation  Adhesion  Fusion

Epithelium  Mesenchyme

Bush et al. Development 2012
Etiology of cleft palate involves genetic, environmental, and genetic x environmental factors.

Pathology of Palate Fusion and Cleft Palate

Growth/Elevation Defects

Mouse E15.5
egfr -/-


Mouse E15
fgf10 -/


Adhesion/Fusion Defect

Mouse E15.5
egfr -/-


Mouse E15.5
egfr -/-


Mouse E15
egfr -/-


Etiology of cleft palate involves genetic, environmental, and genetic x environmental factors.
*In Vitro* Organotypic Model to Examine Morphogenetic Fusion

**In Vivo Palate Fusion**
- Elevation
- Adhesion
- Fusion

**In Vitro Fusion**
- Bring Spheroids Into Contact
- Adhesion
- Monitor Adhesion and Fusion of Mesenchymal/Epithelial Tissues
In Vitro Organotypic Model to Examine Morphogenetic Fusion

Stromal Compartment: Human Wharton's Jelly Stromal Cells

Add Human Epithelial Progenitor Cells

Bring Spheroids Into Contact

In Vivo Palate Fusion

Elevation Adhesion Fusion

In Vitro Fusion

Adhesion Fusion

Monitor Adhesion and Fusion of Mesenchymal/Epithelial Tissues

Epithelium Mesenchyme

In Vivo Palate Fusion
**In Vitro Organotypic Model to Examine Morphogenetic Fusion**

- **Stromal Compartment**: Human Wharton’s Jelly Stromal Cells
- **Add**: Human Epithelial Progenitor Cells
- **Differentiate to Osteogenic Lineage**
- **Bring Spheroids Into Contact**

**In Vivo Palate Fusion**

**In Vitro Fusion**

Monitor Adhesion and Fusion of Mesenchymal/Epithelial Tissues
Generating Spheroids of Human Wharton’s Jelly Stromal Cells (HWJSCs)

HWJSC spheroid size is dependent on

i. culture conditions
ii. cell seeding density
HWJSC Spheroid Osteogenesis Over Time in Culture

Spheroid culture in osteo-induction medium by day 7 elicits

i. Down-regulation of mesenchymal markers
ii. Up-regulation of osteogenic differentiation markers
iii. Increased alkaline phosphatase activity
Phenotypic Characterization of Mesenchymal Cell Spheroids

Representative confocal z-slices

Day 1

Day 7

Col I Nuclei

Col IV Nuclei

Laminin Nuclei

Fibronectin Nuclei

200 μm
Establishing Epithelial-Stromal Co-Culture

Representative maximum intensity projections

Epithelial attachment to osteogenic HWJSC spheroids is maximal at an epithelial/mesenchymal (E/M) ratio of 0.8
Fusion of Epithelial-Stromal Spheroids

Representative z-slices over time (60 μm from bottom)

HWJSC/HPEKp spheroids in culture exhibit fusion behavior reminiscent of palatal tissue fusion over 2 days (removal of epithelial cells from seams) that is complete by day 4
Spheroid Fusion is Dependent on EGF and FGF Signaling

Fibroblast growth factor (FGF) and epidermal growth factor (EGF) signaling inhibition interferes with *in vitro* fusion progression in culture.
Co-culture Spheroid Fusion Distinct from Mono-culture Spheroid Fusion

Fold Expression (HWJSC vs HPEKp)

HWJSC/HPEKp spheroid fusion

HWJSC spheroid fusion

Polystyrene Bead

ENG
VIM
AHR
ACTB
GAPDH
CD44
CD34
EGFR
TGFB3
EGF
FGFR2
ITGA6
KRT17

10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}

d0

d1
d2
d3
d4

Y = PC2 (14.19%)
Z = PC1 (18.74%)
X = PC3 (11.41%)

vs

Polystyrene Bead
Future Directions

Study Epithelial Morphogenesis in Real-Time

Cross-Validate *In Vitro* Fusion Model with *In Silico* Palatogenesis Model

Explore Chemical Effects on *In Vitro* Fusion

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Transcriptomics Analysis of Day 7 HWJSC Spheroids

Top 10 Clusters of Upregulated Gene Ontology Terms

- extracellular matrix organization GO:0030198
- response to organic substance GO:0010033
- cardiovascular system development GO:0072358
- cell proliferation GO:0008283
- response to hormone GO:0009725
- locomotion GO:0006928
- regulation of multicellular organismal development GO:2000026
- regulation of anatomical structure morphogenesis GO:0022603
- skeletal system development GO:0001501
- cell-substrate adhesion GO:0031589