Disclaimer

The information in this presentation has been reviewed and approved for public dissemination in accordance with U.S. Environmental Protection Agency (EPA). The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or policies of the Agency. Any mention of trade names or commercial products does not constitute EPA endorsement or recommendation for use.

EPA United States Environmental Protection Agency

CSS

Engineering a human organotypic model of osteogenesis and morphogenetic fusion

David G. Belair

Disclaimer: The views expressed in this presentation are those of the speaker and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

Office of Research and Development
US EPA | National Health and Environmental Effects Research Lab

March 11, 2017

Embryonic Tissues Undergo Fusion Events During Development

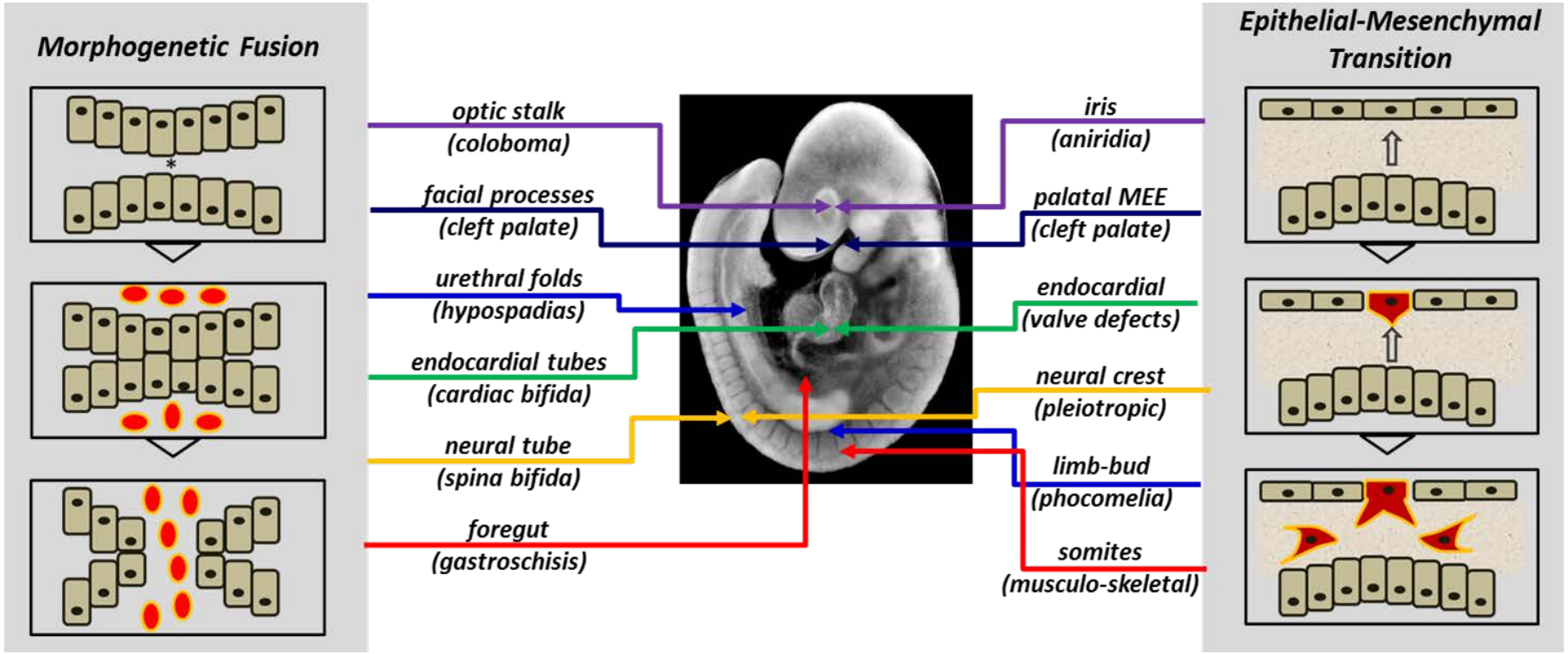
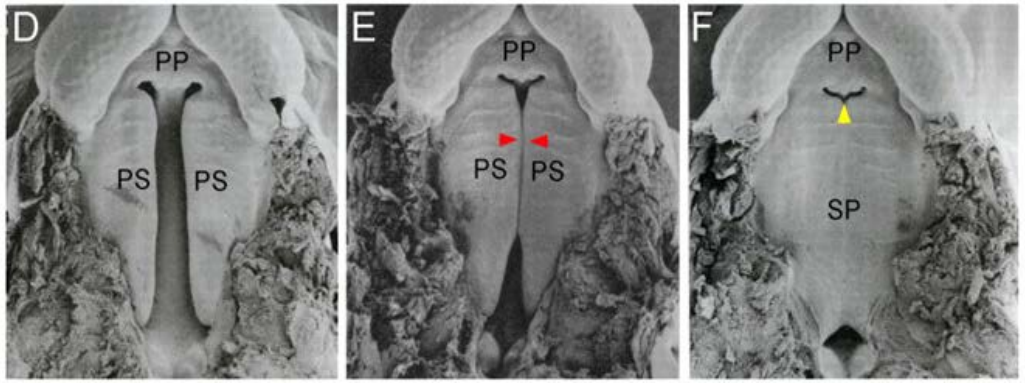


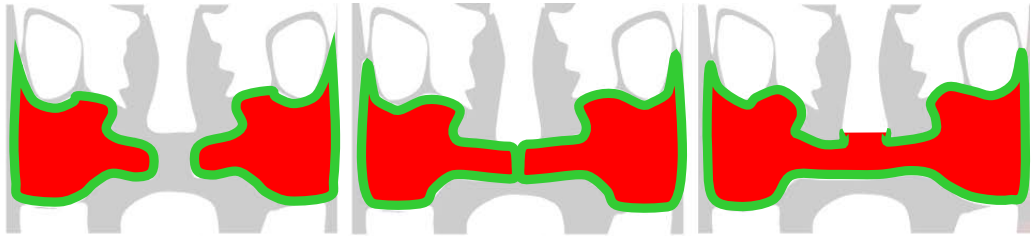
Image Credit: Thomas Knudsen, from Synthetic Biology: 'flipping the switch' on opportunities and challenges with virtual tissues. Presented at CompuCell3D Workshop

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

Morphogenetic Fusion Events in the Embryo Depend on Epithelial-Stromal Interactions

Secondary Palate

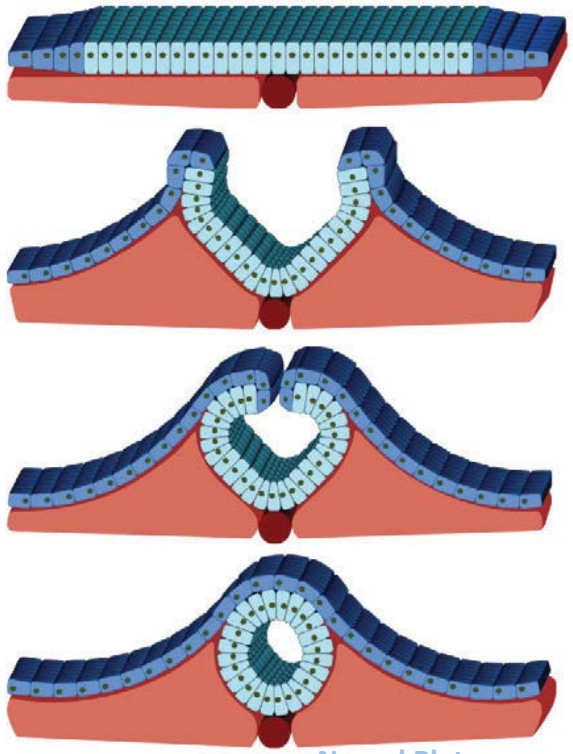




Elevation Adhesion Fusion

Epithelium
Mesenchyme

Neural Tube

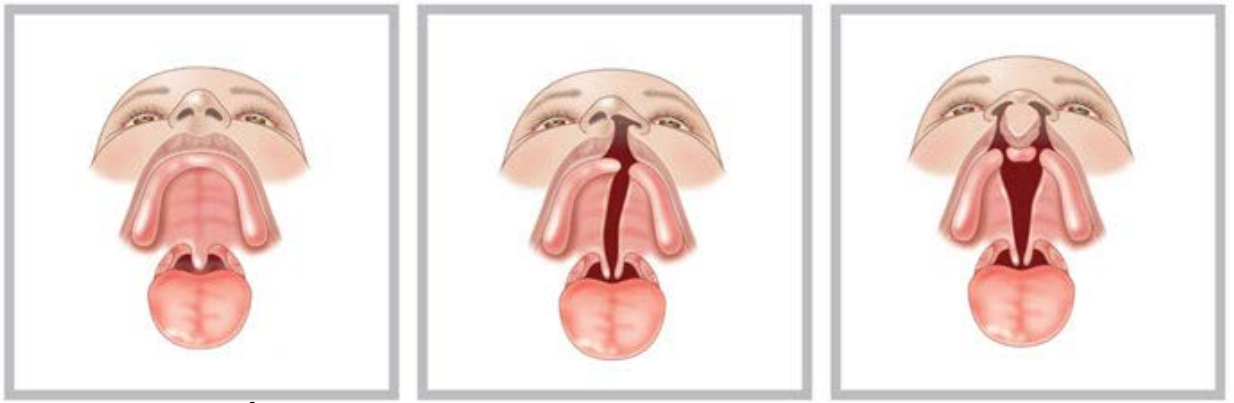


Neural Plate
Non-Neural Ectoderm
Notochord
Mesoderm

[1] Bush *et al. Development* 2012 [2] Ray & Niswander. *Development* 2012

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

Global incidence of orofacial clefting: 0.12%

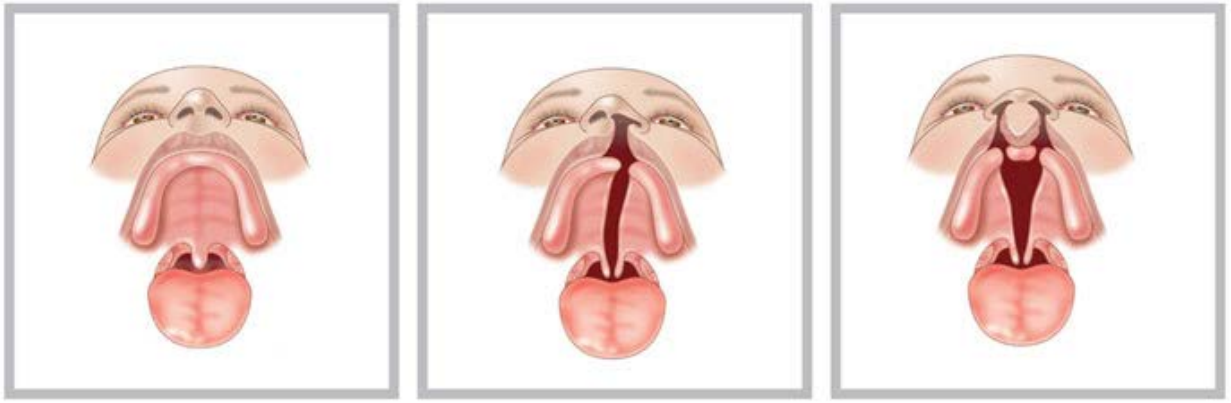


Normal **Unilateral cleft** **Bilateral cleft**

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%

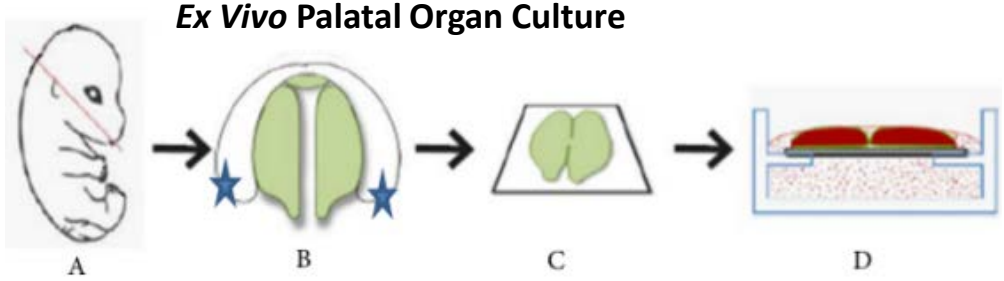


Normal **Unilateral cleft** **Bilateral cleft**

The Royal Children's Hospital Melbourne *Cleft Lip and Palate – an overview*

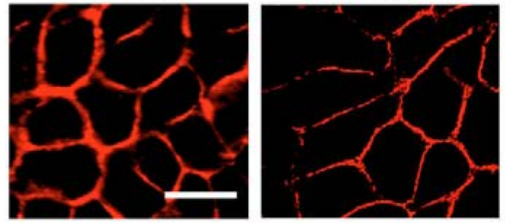
Existing methods for studying palate fusion use animal models, tissue explants, or primary two-dimensional tissue cultures that exhibit a tradeoff between throughput and developmental relevance

***Ex Vivo* Palatal Organ Culture**



Serrano *et al. JoVE* 2015

***In Vitro* Palatal Cell Culture**

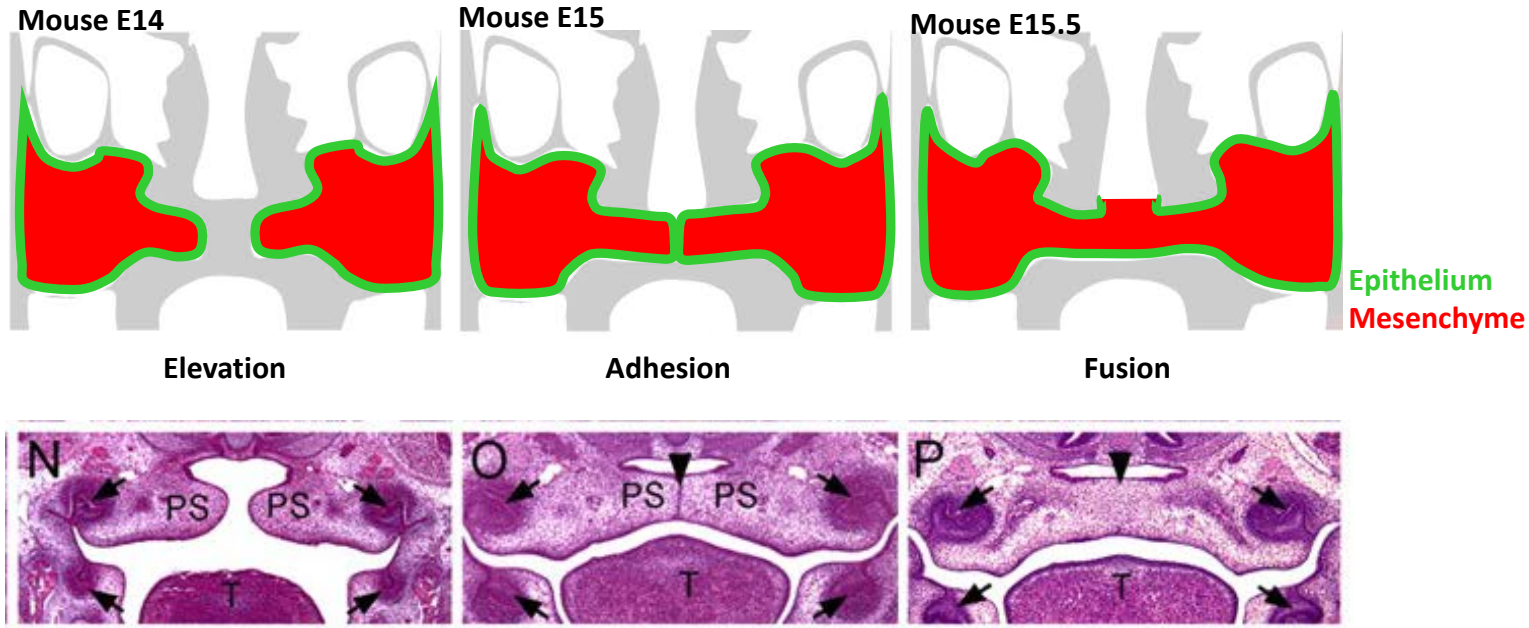


Nawshad *et al. J Cell Sci.* 2007

Throughput

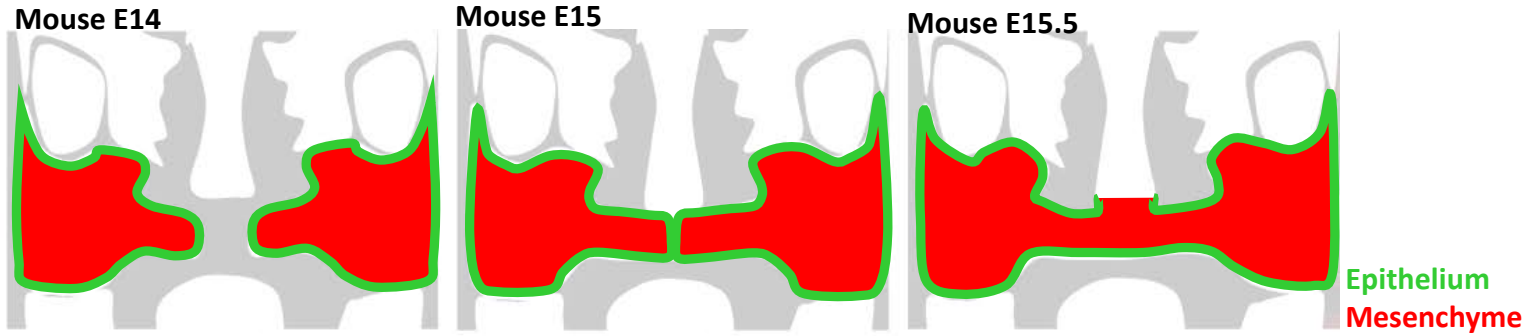
Relevance

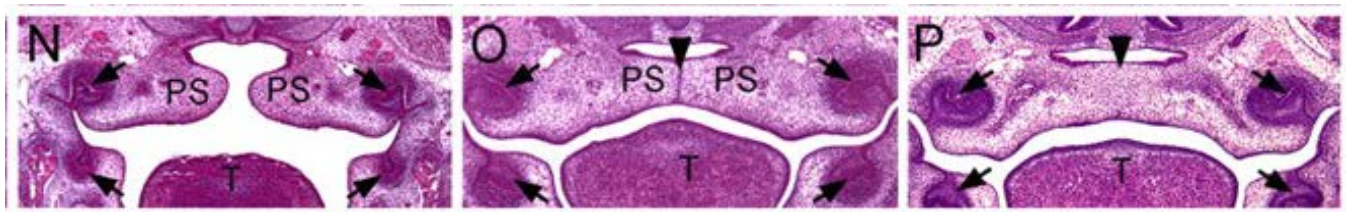
Pathology of Palate Fusion and Cleft Palate



Bush *et al. Development* 2012

Pathology of Palate Fusion and Cleft Palate



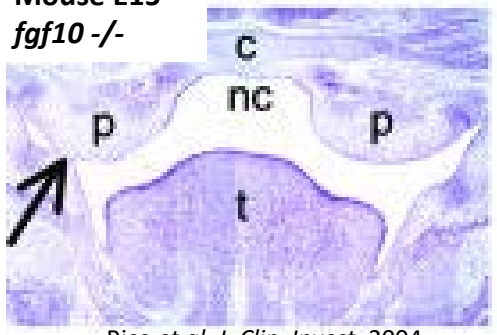


Bush *et al. Development* 2012

Mouse E15.5
egfr -/-

Miettinen *et al. Nat. Genet.* 1999

Mouse E15
fgf10 -/-



Rice *et al. J. Clin. Invest.* 2004

Mouse E15.5

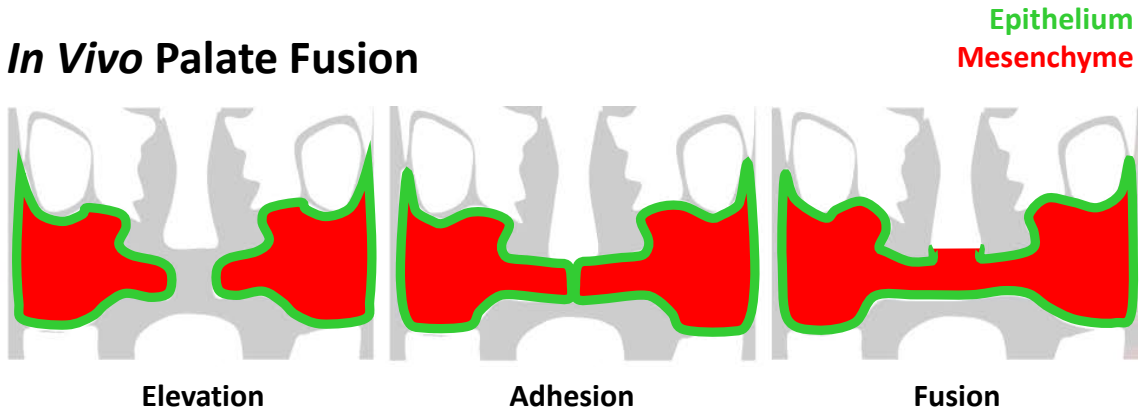
Proetzel *et al. Nat. Genet.* 1995

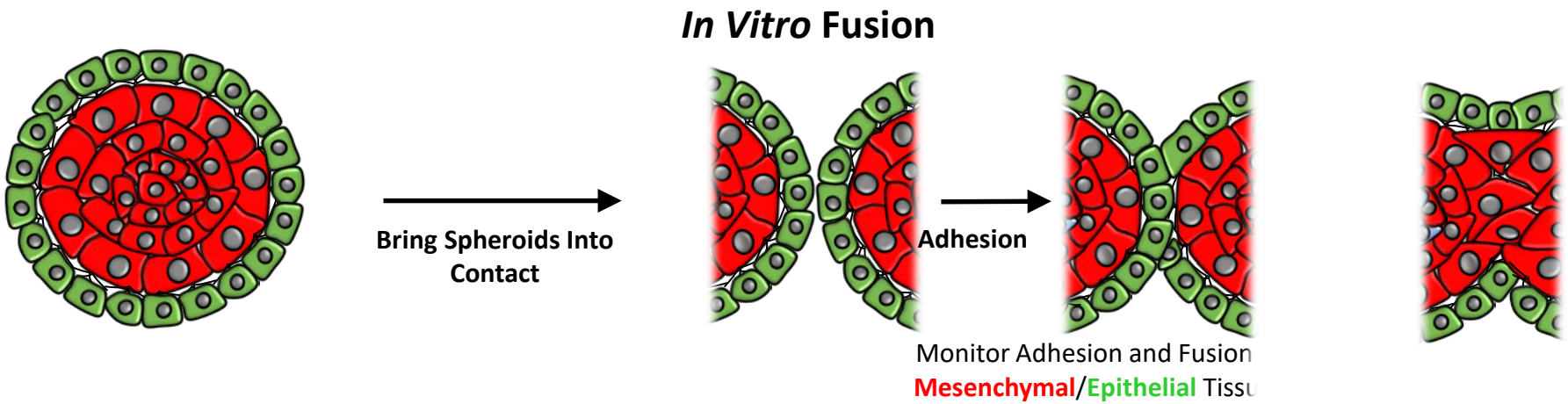
Growth/Elevation Defects

Adhesion/Fusion Defect

Etiology of cleft palate involves genetic, environmental, and genetic x environmental factors

In Vitro Organotypic Model to Examine Morphogenetic Fusion

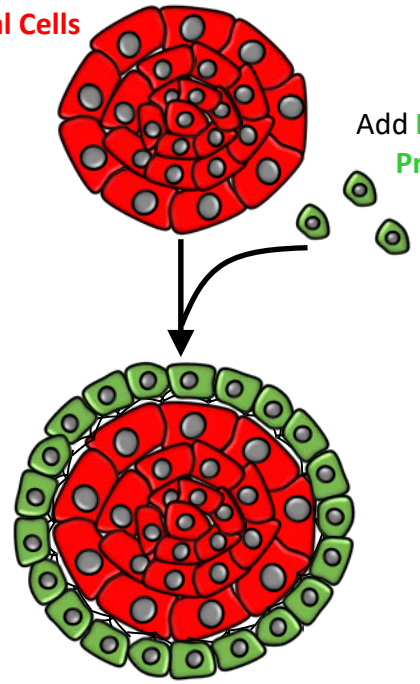




In Vitro Organotypic Model to Examine Morphogenetic Fusion

Stromal Compartment: Human Wharton's Jelly Stromal Cells

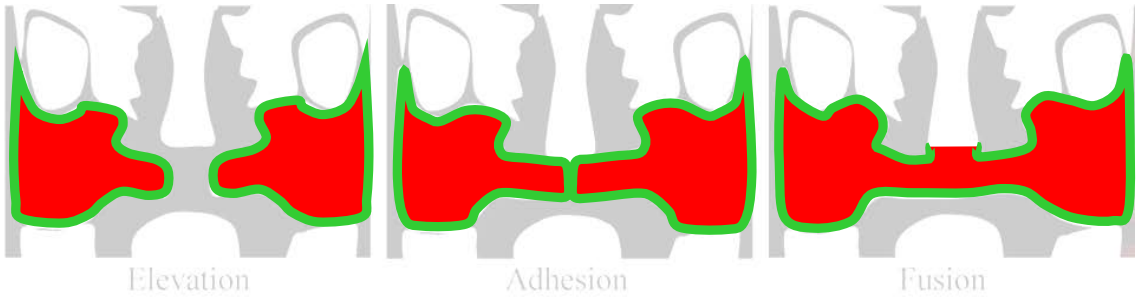
Add Human Epithelial Progenitor Cells



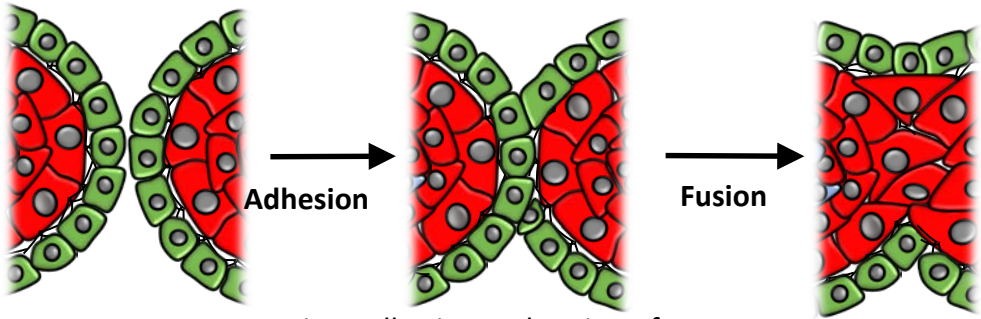
Bring Spheroids Into Contact

***In Vivo* Palate Fusion**

Epithelium
Mesenchyme

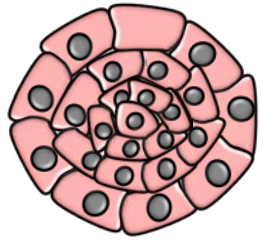


***In Vitro* Fusion**



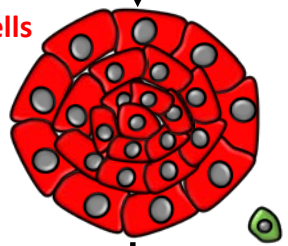
Monitor Adhesion and Fusion of Mesenchymal/Epithelial Tissues

In Vitro Organotypic Model to Examine Morphogenetic Fusion

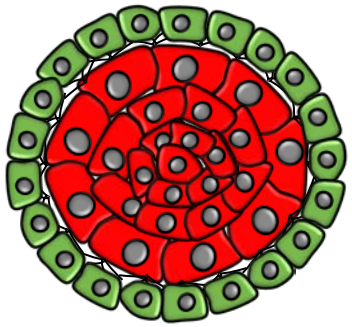


Differentiate to Osteogenic Lineage

Stromal Compartment: Human Wharton's Jelly Stromal Cells



Add Human Epithelial Progenitor Cells

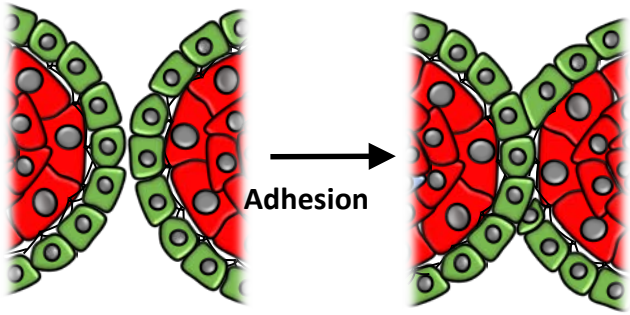


Bring Spheroids Into Contact

***In Vivo* Palate Fusion**

Epithelium
Mesenchyme

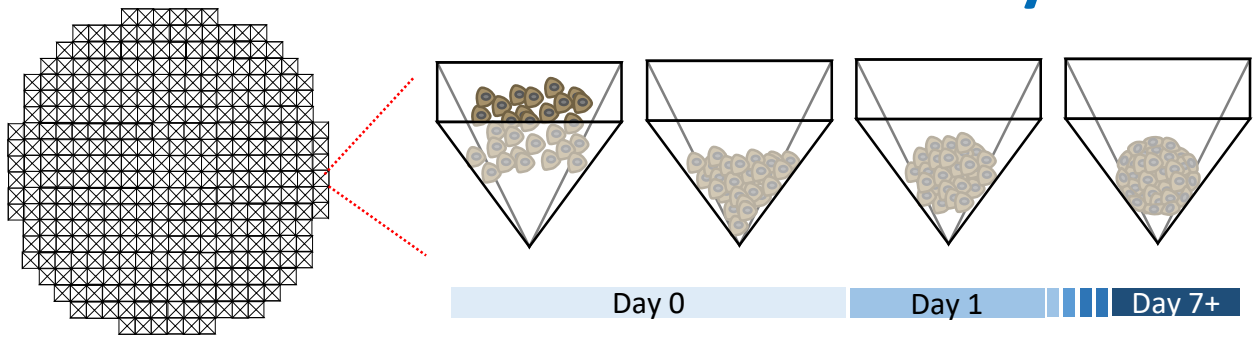
***In Vitro* Fusion**

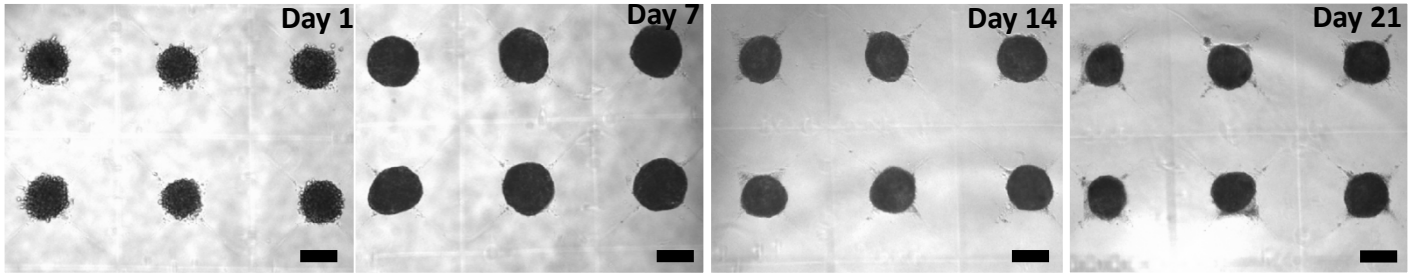


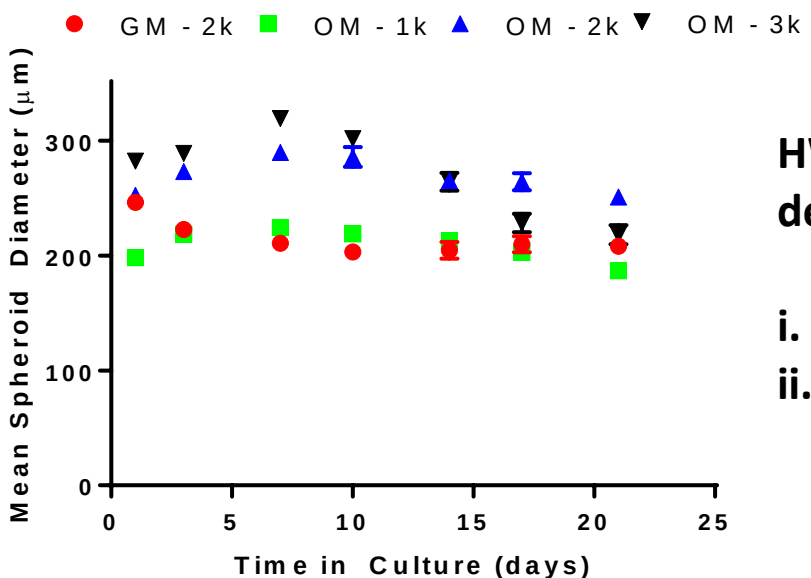
Adhesion

Monitor Adhesion and Fusion
Mesenchymal/Epithelial Tissu

Generating Spheroids of Human Wharton's Jelly Stromal Cells (HWJSCs)





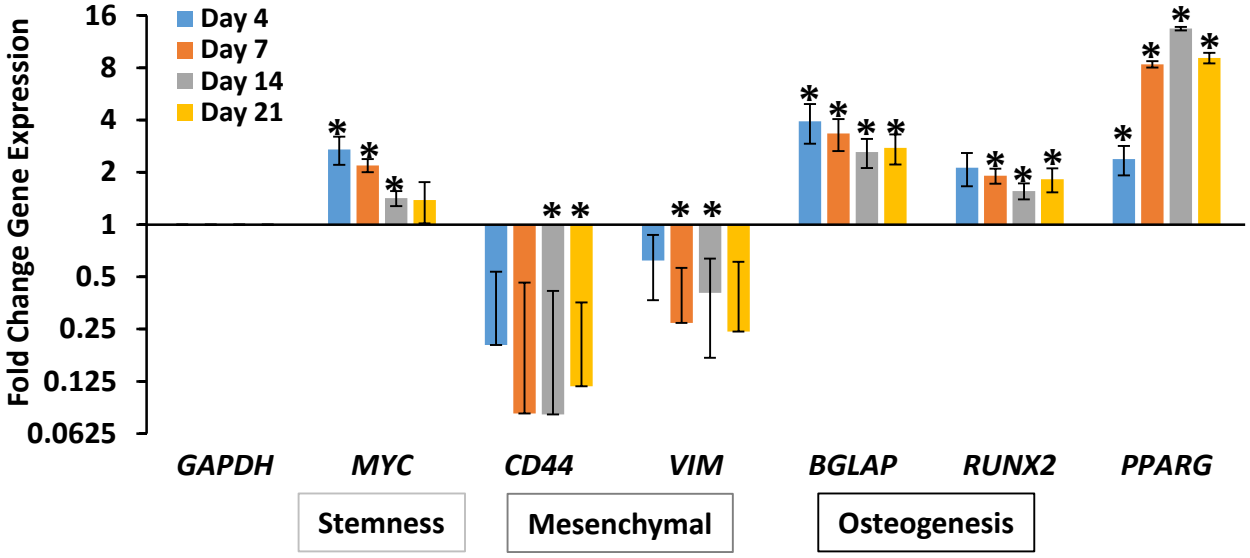


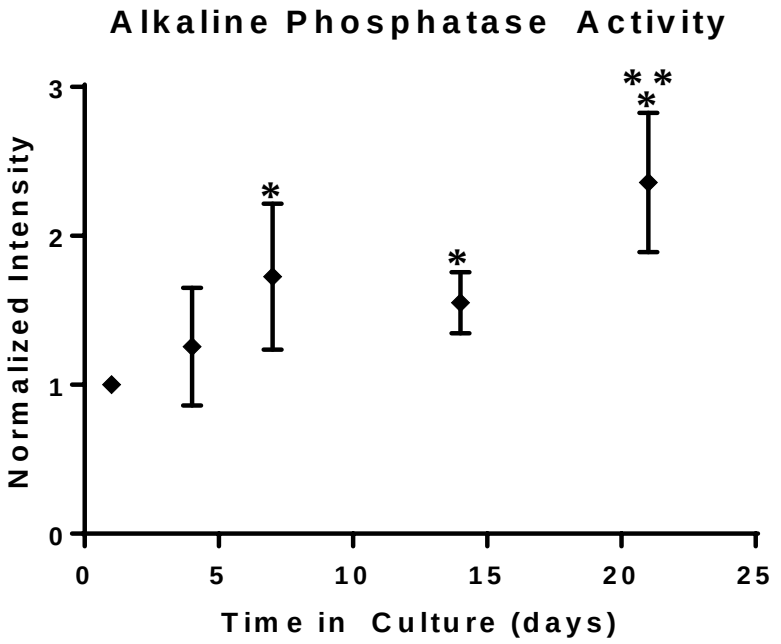
OM: Osteo-induction Medium
GM: Mesenchymal Growth Medium

HWJSC spheroid size is dependent on

i. **culture conditions**
ii. **cell seeding density**

HWJSC Spheroid Osteogenesis Over Time in Culture

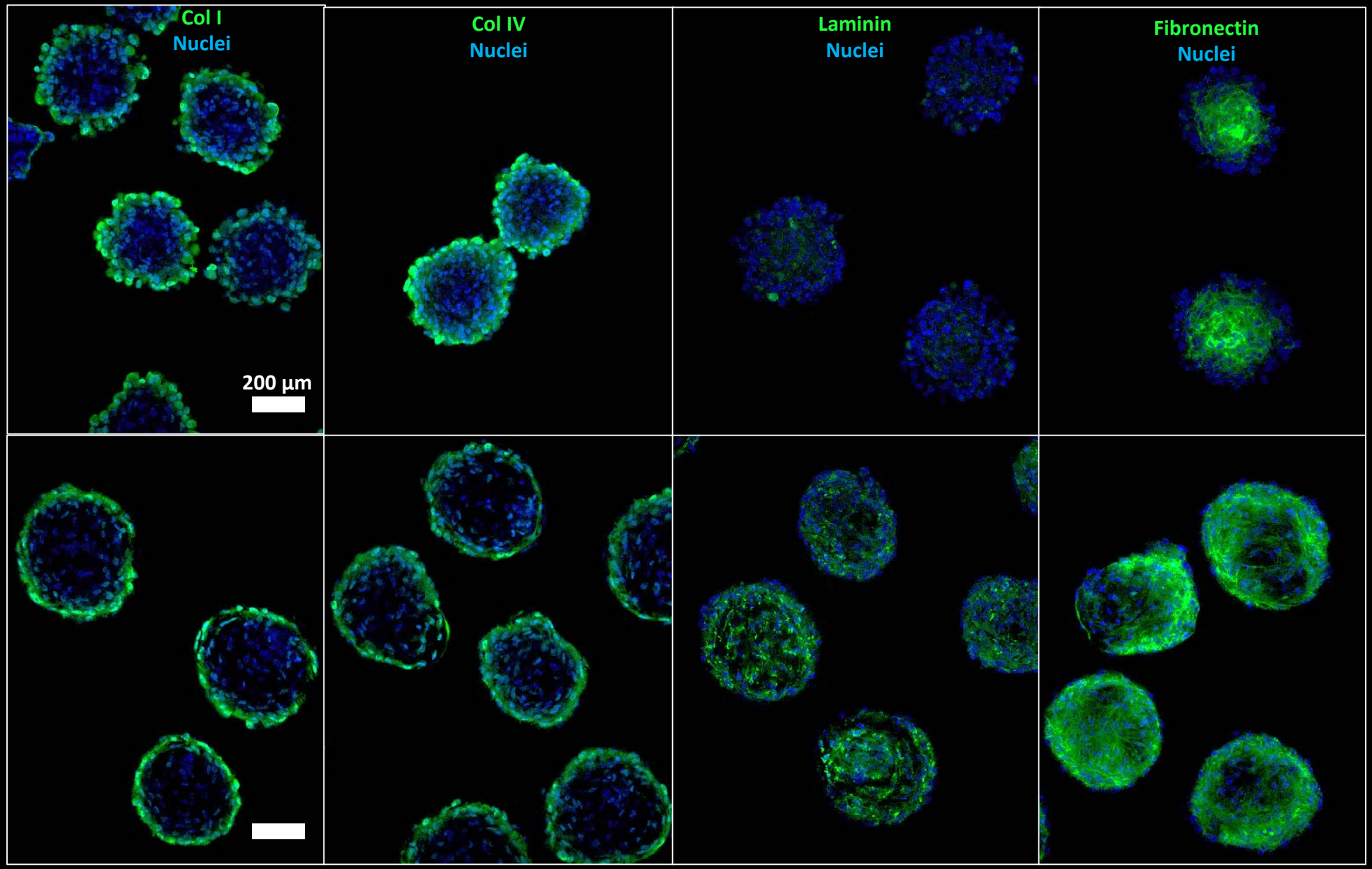




Spheroid culture in osteo-induction medium by day 7 elicits

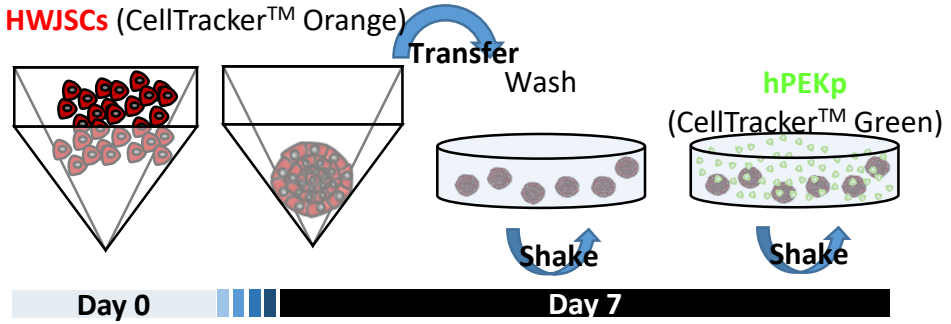
i. Down-regulation of mesenchymal markers
ii. Up-regulation of osteogenic differentiation markers
iii. Increased alkaline phosphatase activity

Phenotypic Characterization of Mesenchymal Cell Spheroids

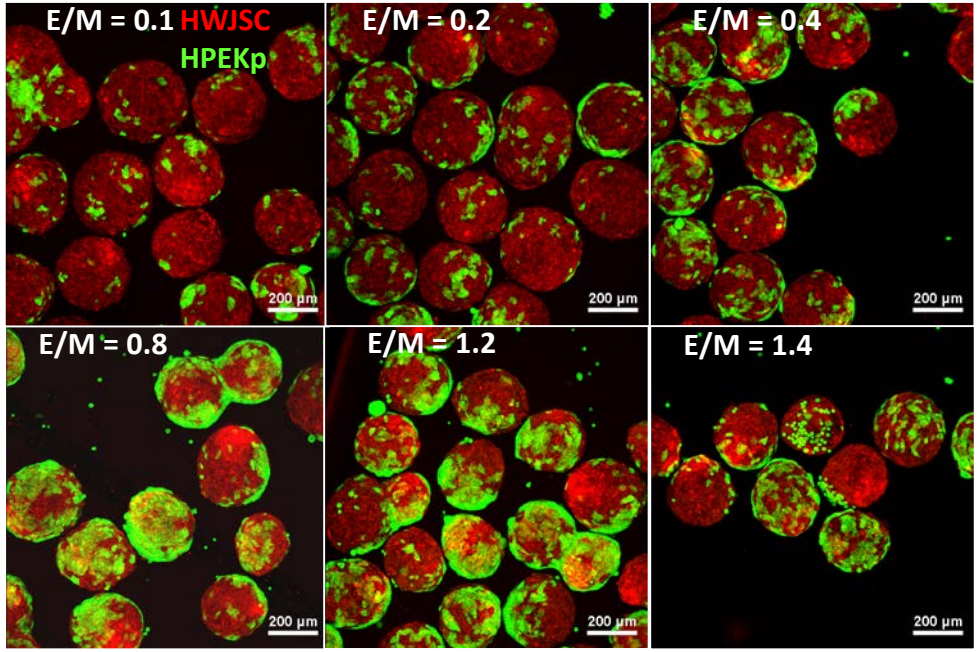


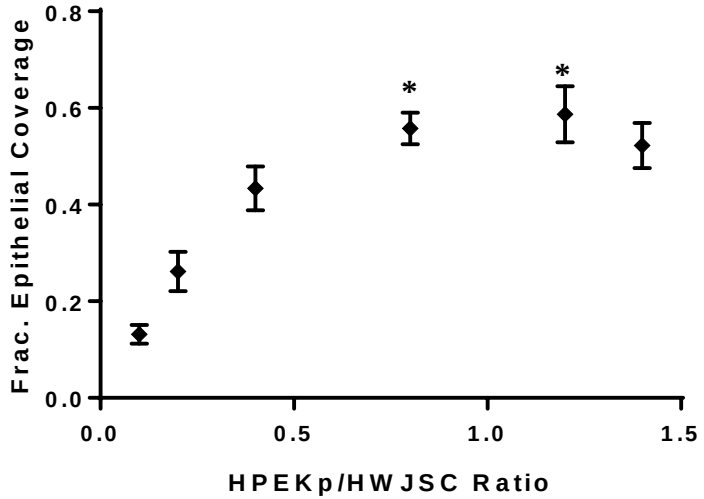
Representative confocal z-slices

Establishing Epithelial-Stromal Co-Culture



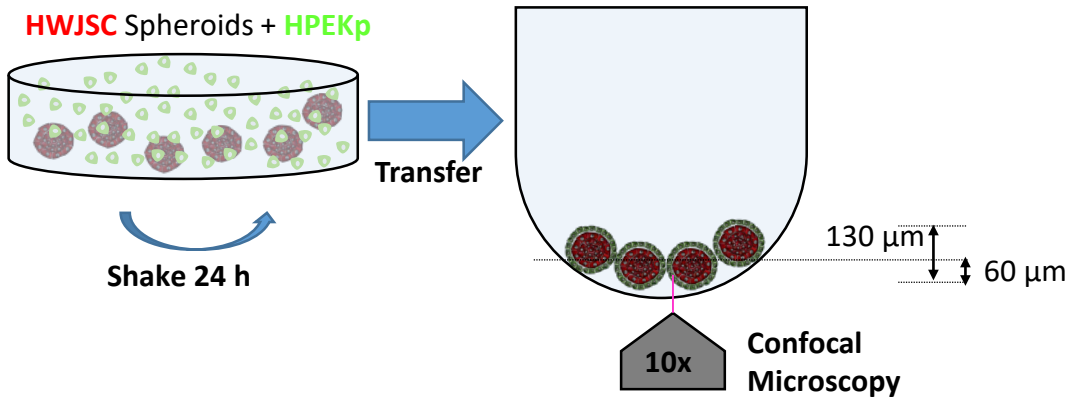
Representative maximum intensity projections



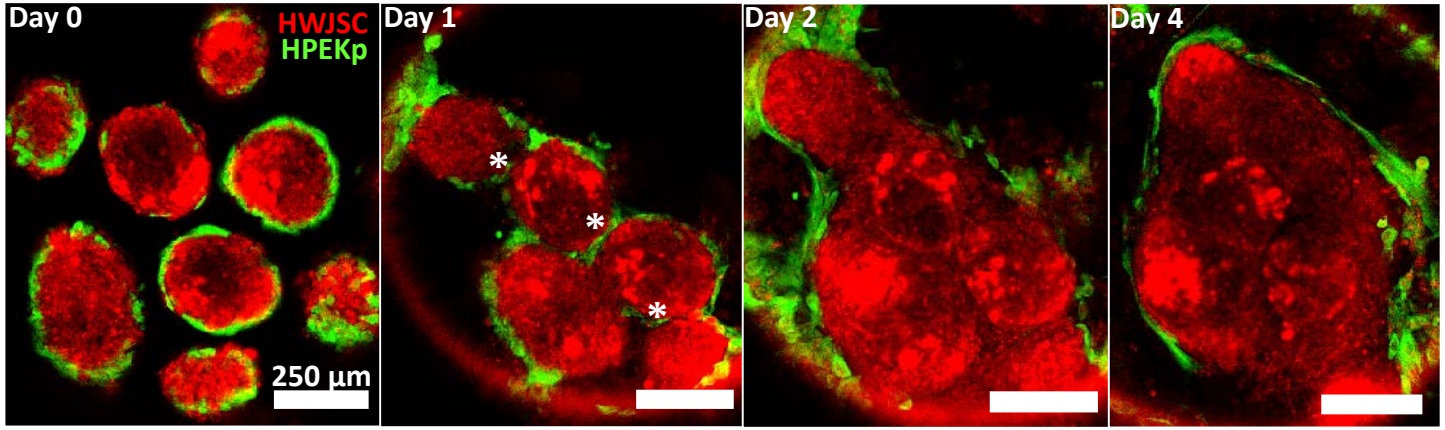


Epithelial attachment to osteogenic HWJSC spheroids is maximal at an epithelial/mesenchymal (E/M) ratio of 0.8

Fusion of Epithelial-Stromal Spheroids

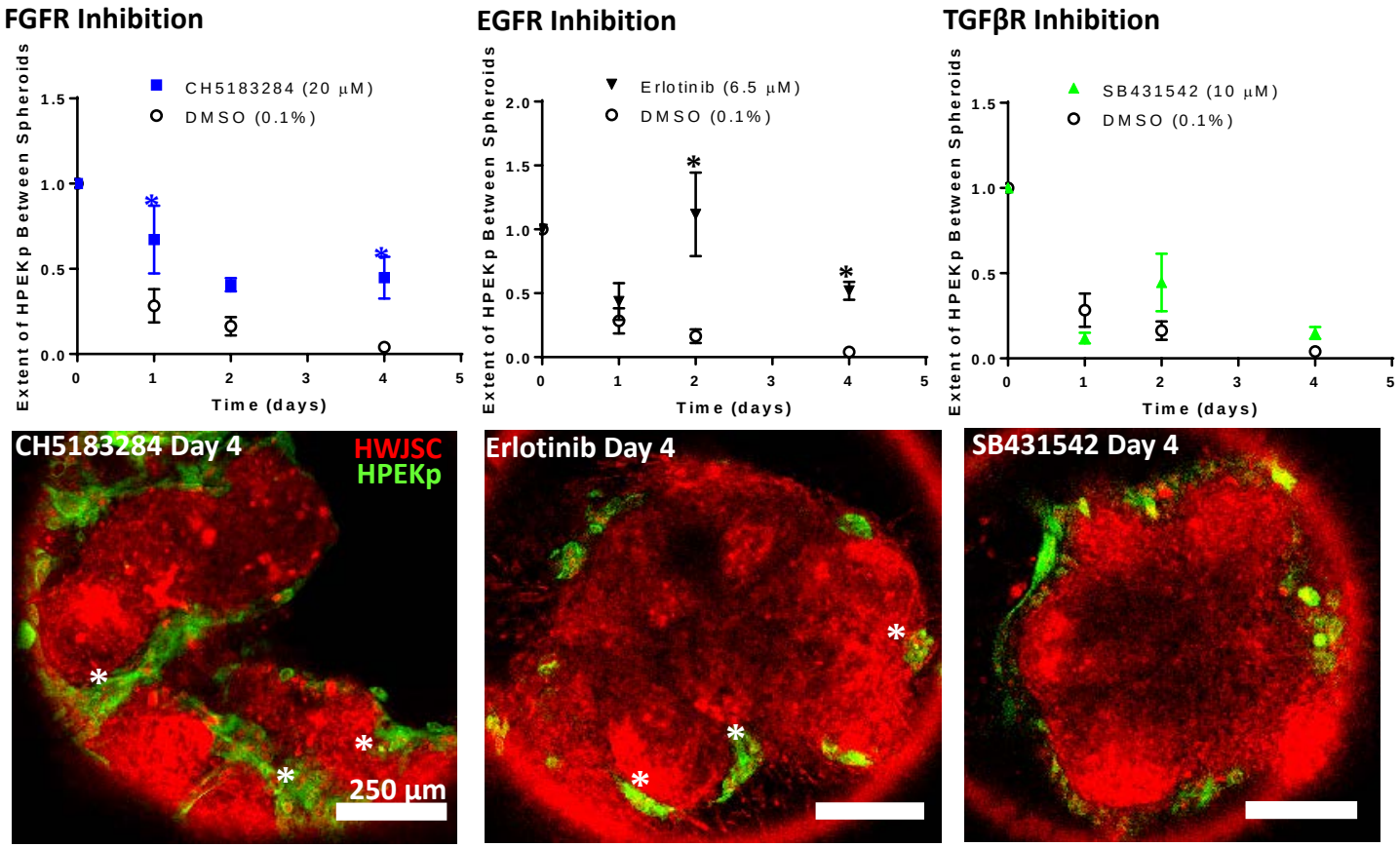


Representative z-slices over time (60 µm from bottom)



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

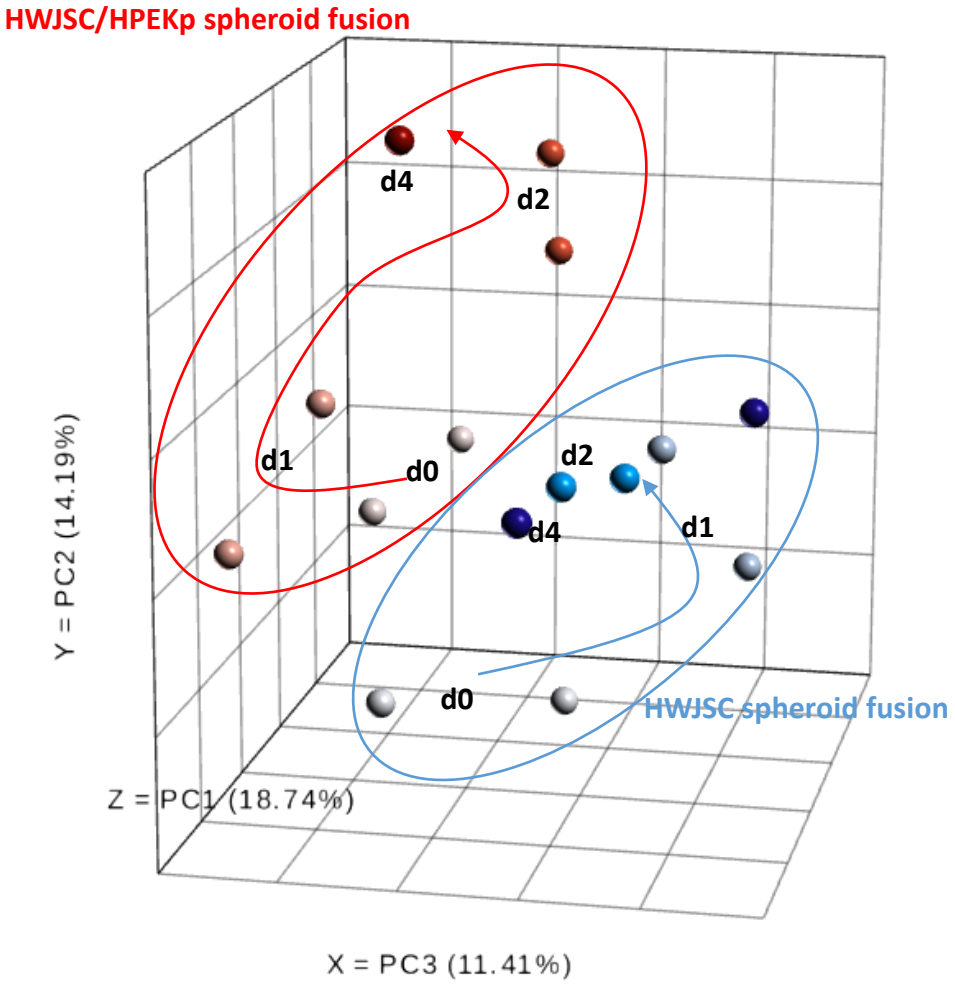
Spheroid Fusion is Dependent on EGF and FGF Signaling

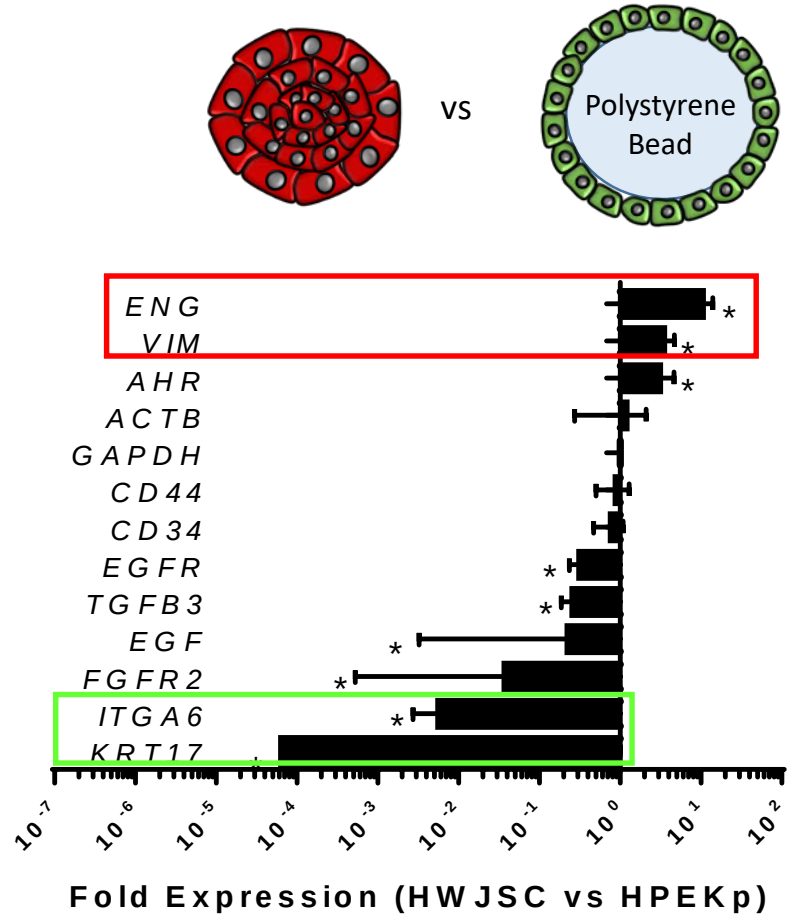


Representative z-slices over time (60 μm from bottom)

Fibroblast growth factor (FGF) and epidermal growth factor (EGF) signaling inhibition interferes with *in vitro* fusion progression in culture

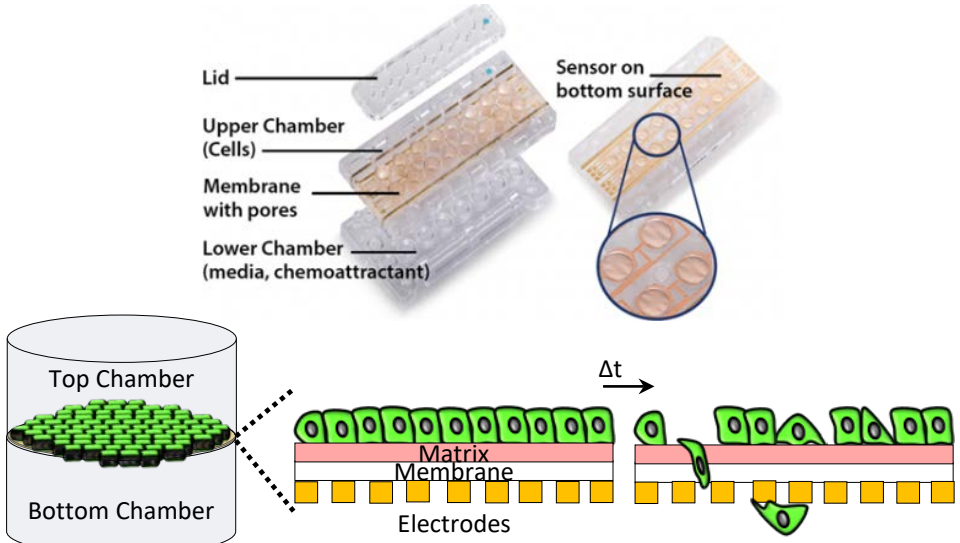
Co-culture Spheroid Fusion Distinct from Mono-culture Spheroid Fusion



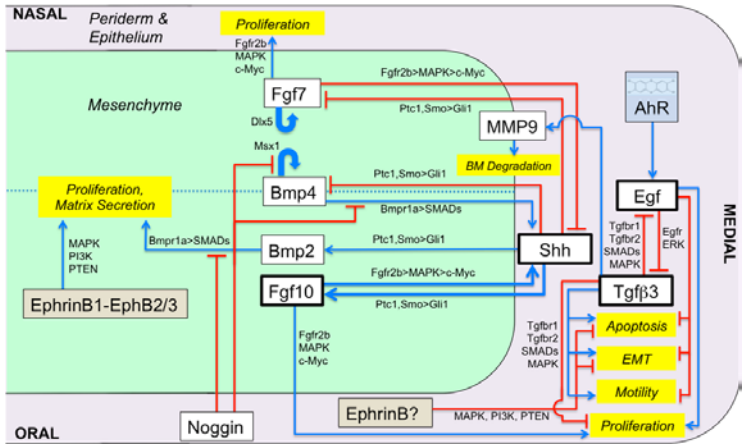


Future Directions

Study Epithelial Morphogenesis in Real-Time

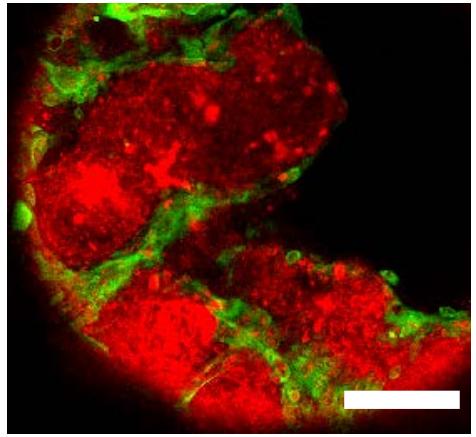


Cross-Validate *In Vitro* Fusion Model with *In Silico* Palatogenesis Model



Hutson *et al. Chem. Res. Toxicol.* 2017

Explore Chemical Effects on *In Vitro* Fusion



Acknowledgements

Toxicity Assessment Division

Barbara D. Abbott (PI)
Cynthia Wolf
Carmen Wood

Virtual Tissue Models

E. Sidney Hunter
Thomas Knudsen
Kyle Grode
Andrew Schwab
Maxwell Leung
Tamara Tal
Nancy Baker

Research Cores Unit

Witold Winnik
Anna Fisher
Rachel Grindstaff
William Padgett
Adam Swank
Hongzu Ren

Transcriptomics Analysis of Day 7 HWJSC Spheroids