

Application of 3D Organoid Model for Viral Cancer Research

Yeeun (Helen) Oh

Year 1 PhD Student

Supervisor: Dr. Siaw Shi Boon

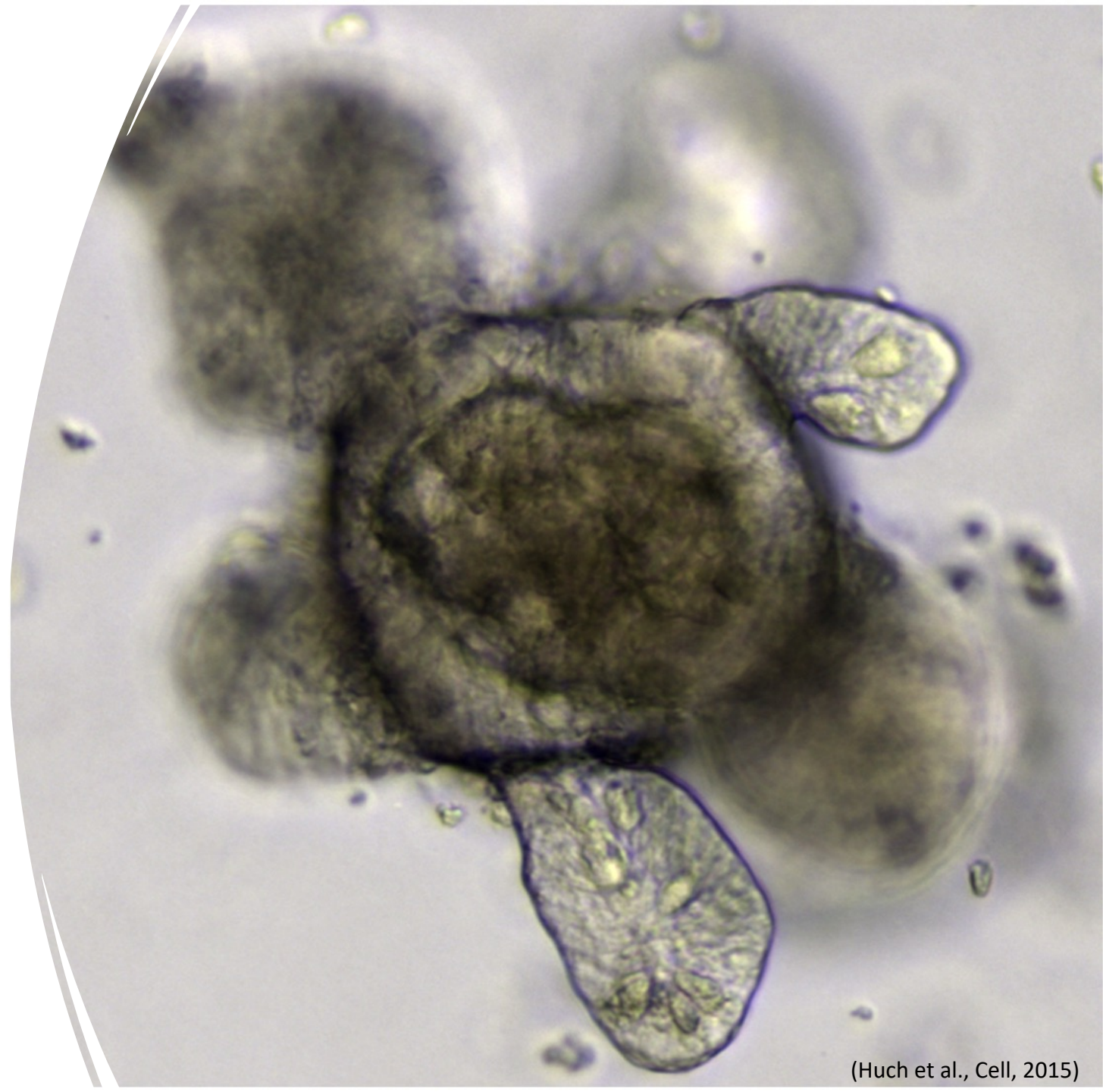


Table of contents

Introduction– What is an organoid?

Types of organoids

Consideration for establishing organoids

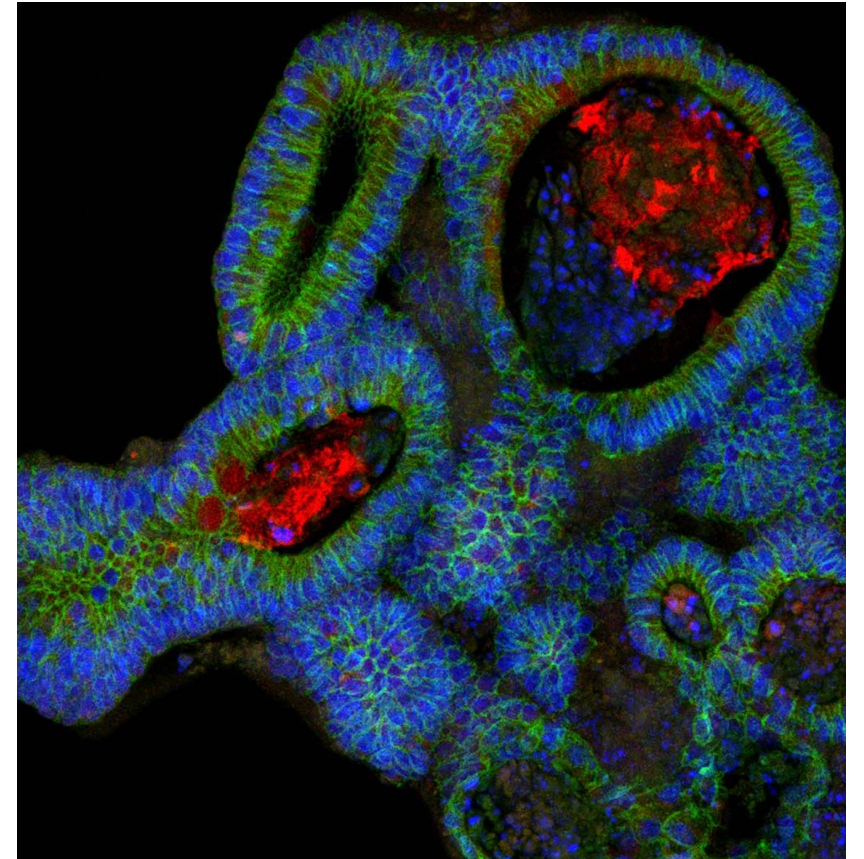
Culturing Process

Applications

Limitations and future directions

Organoids

- Self-organizing 3D structure derived from stem cells
- Imitate the architecture and differentiate into specific tissues
- Examples of established organoid models:
 - Intestines, stomach, esophagus, liver, kidneys, lungs, brain, prostate, pancreas, retina, and ovary



<https://www.corning.com/worldwide/en/products/life-sciences/products/surfaces/matrigel-matrix-for-organoids.html>

Types of Organoids

Adult Stem Cell (ASCs) - derived organoids

- Mimic homeostatic conditions of original tissue
- Suitable for studying adult tissues

Pluripotent Stem cell (PSCs) - derived organoids

- Most resembles fetal-stage tissues, and rarely mature to adult tissues *in vitro*
- Suitable for studying developmental stages of tissue formations

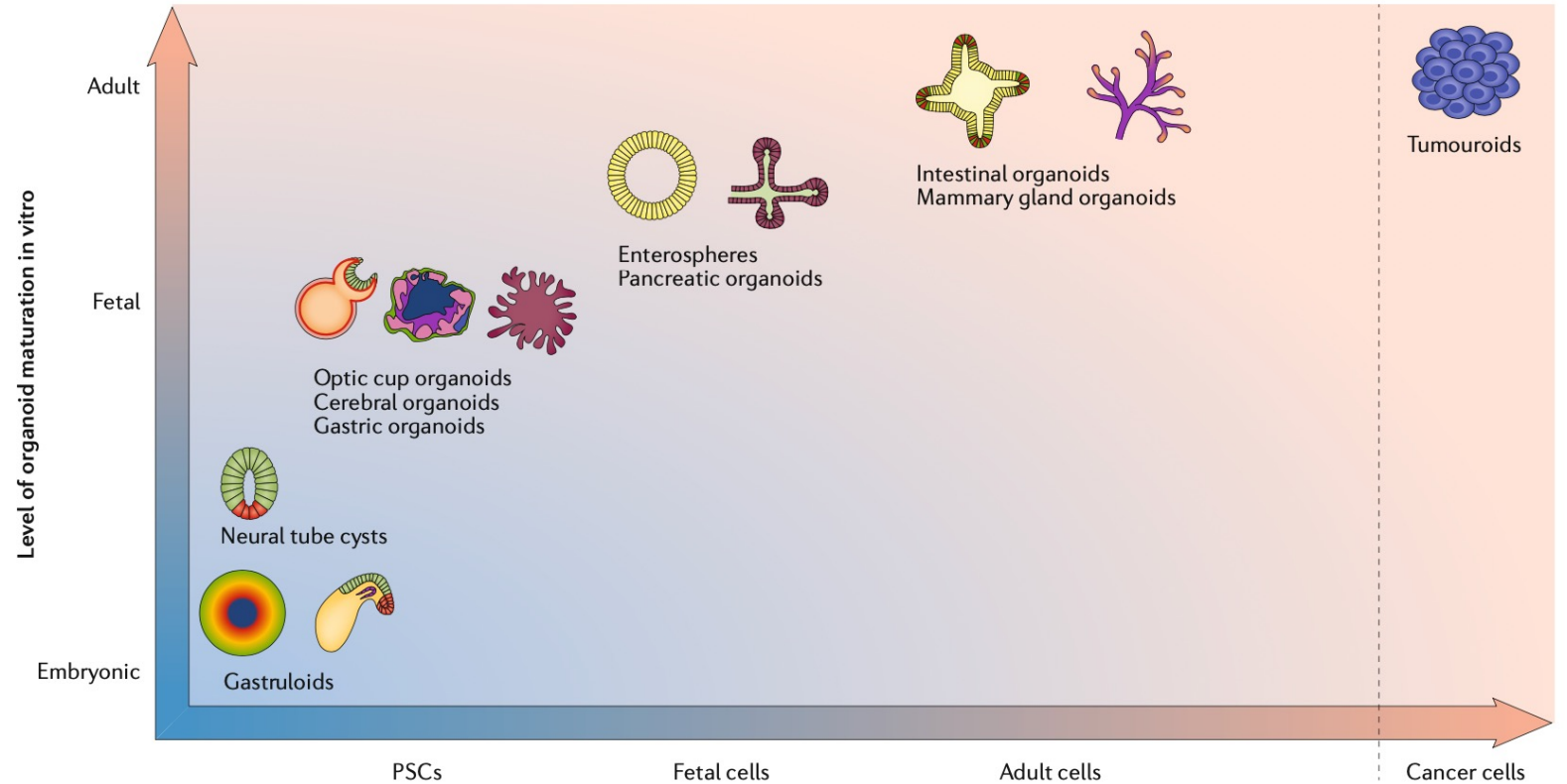
Considerations when culturing Organoids

3 main factors:

1. Cell type
2. Differentiation signaling
3. Culture Environment

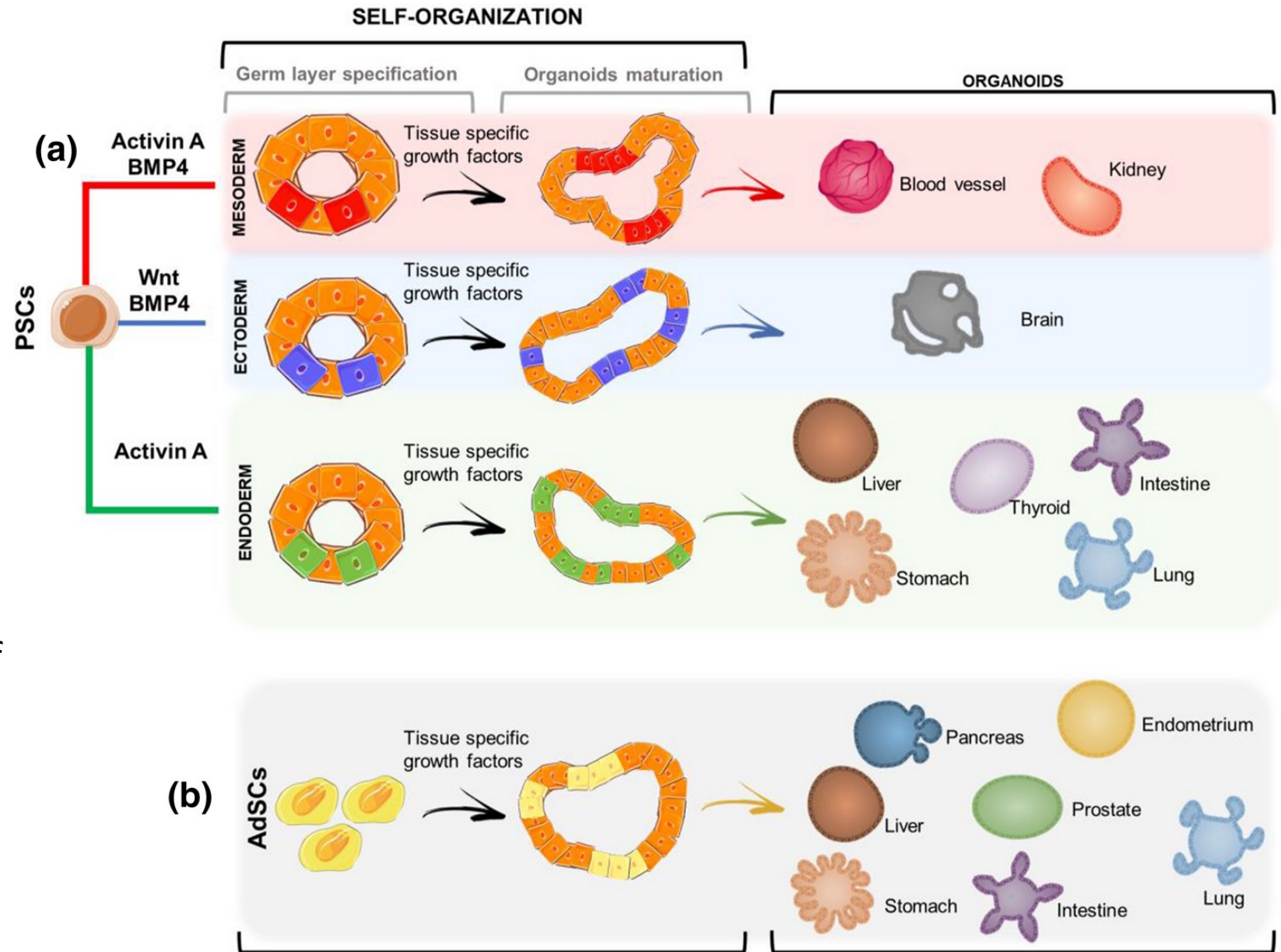
1. Cell Type

- Protocols can vary widely depending on the type of cell used
- Determines the characteristic of the final organoid
 - Maturation stage



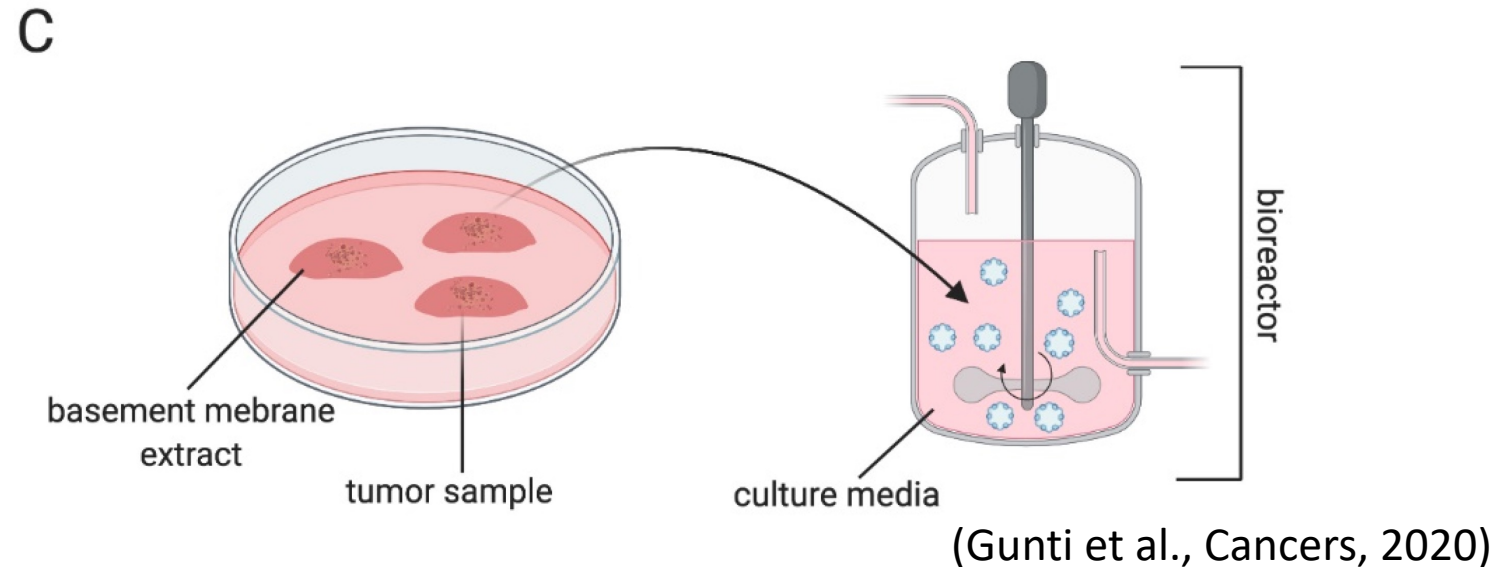
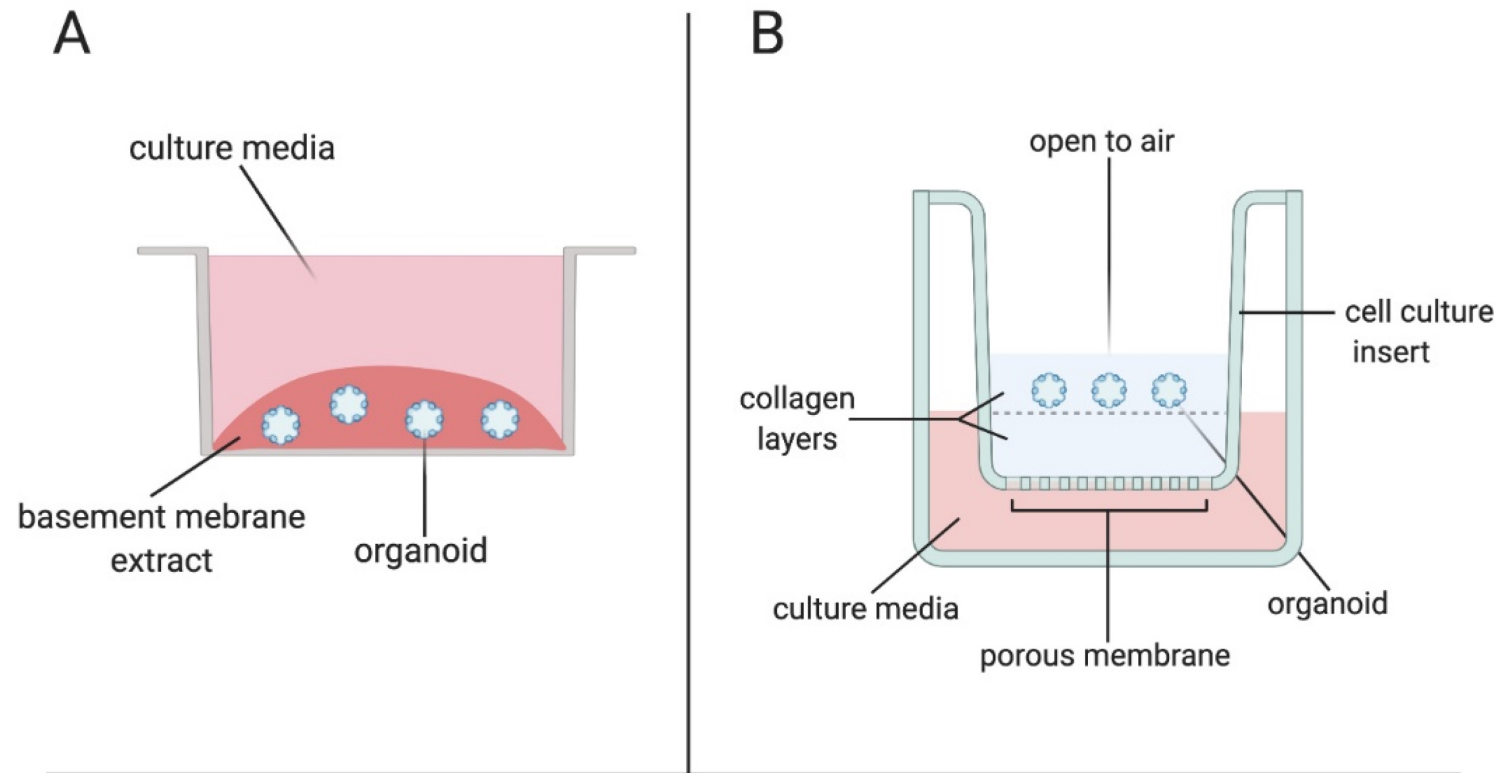
2. Differentiation Signaling

- Isolated ASCs with specific growth factors will differentiate into specified organoids consisting of epithelial layers from tissues of origin
- PSC require additional step of differentiation into primary germ layer (ectoderm, endoderm, mesoderm) before further differentiation



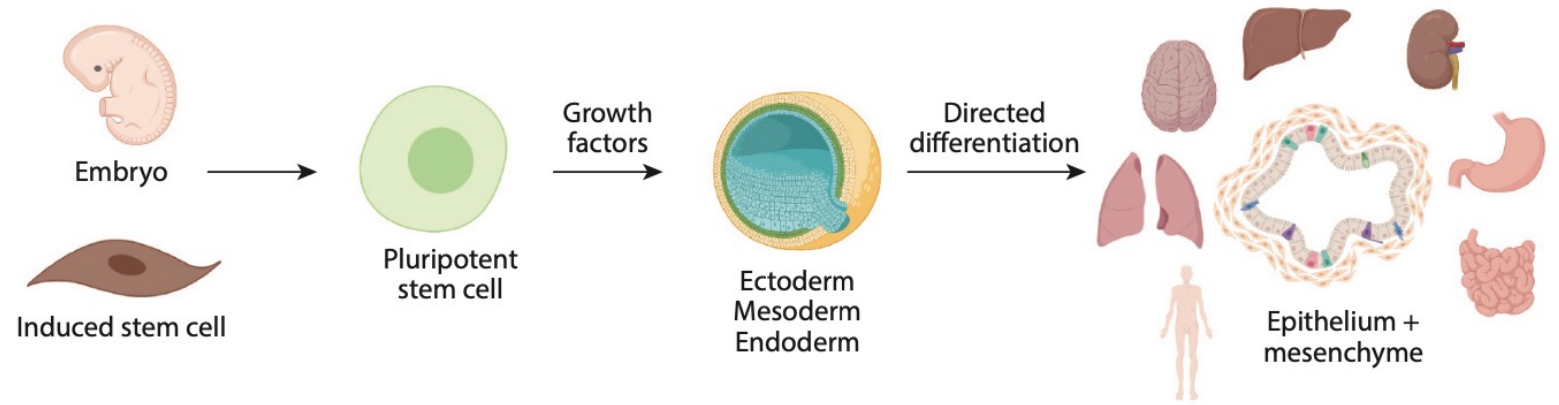
3. Culture Environment

- A. Extracellular matrix (ECM)
 - e.g Matrigel (Engelbreth-Holm-Sarcoma)
 - Chemical-synthetic ECM (PEG)
- B. Air-liquid interface (also known as raft culture)
- C. Suspension using bioreactor

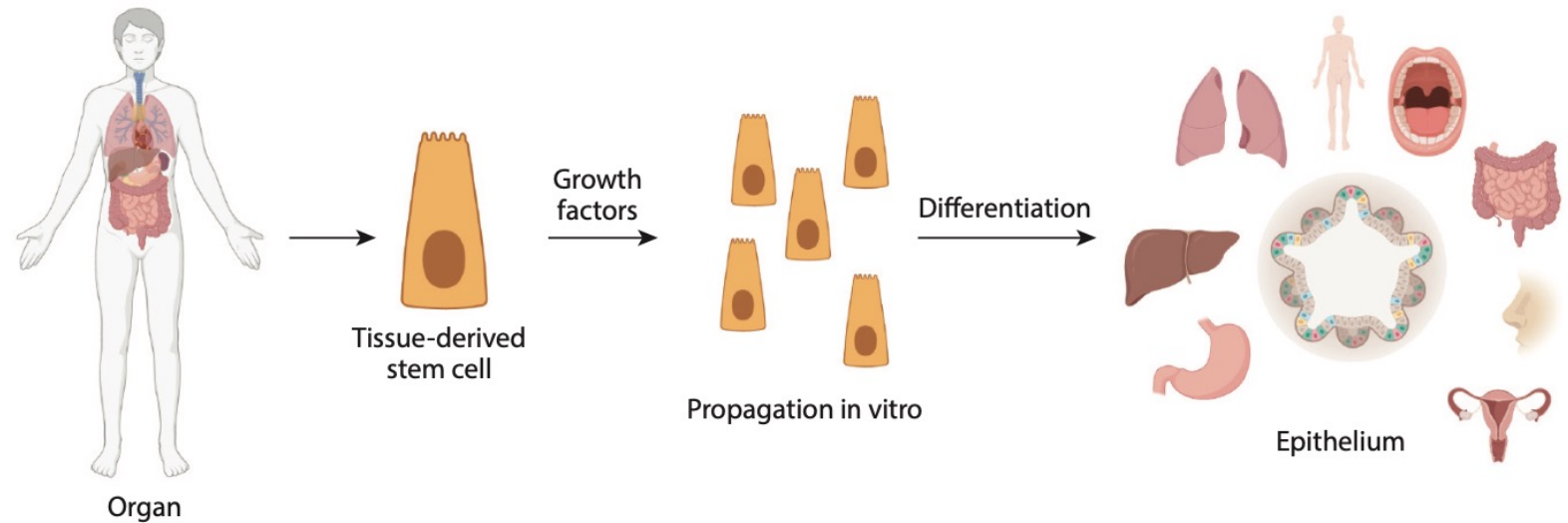


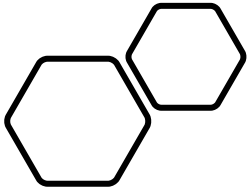
Summary of Organoid Culturing Process

a Pluripotent stem cell-derived organoids





b Tissue stem cell-derived organoids






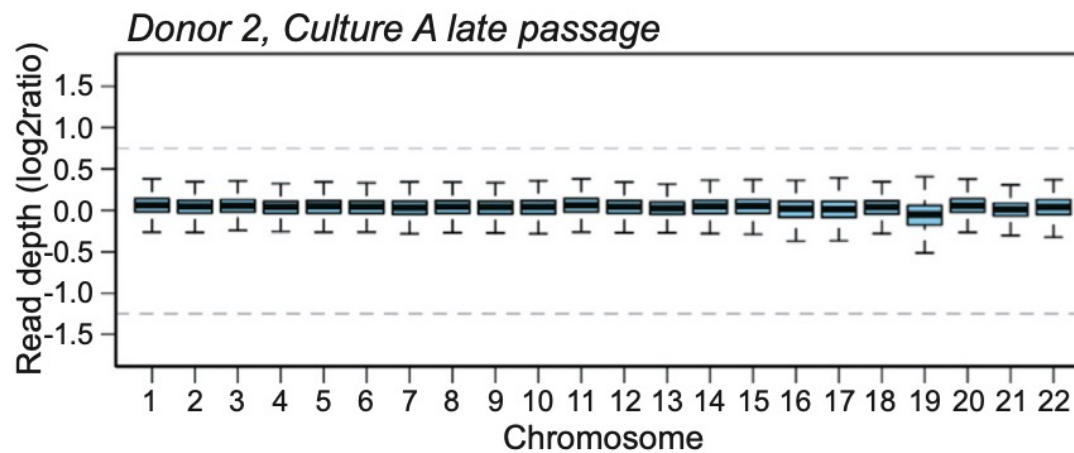
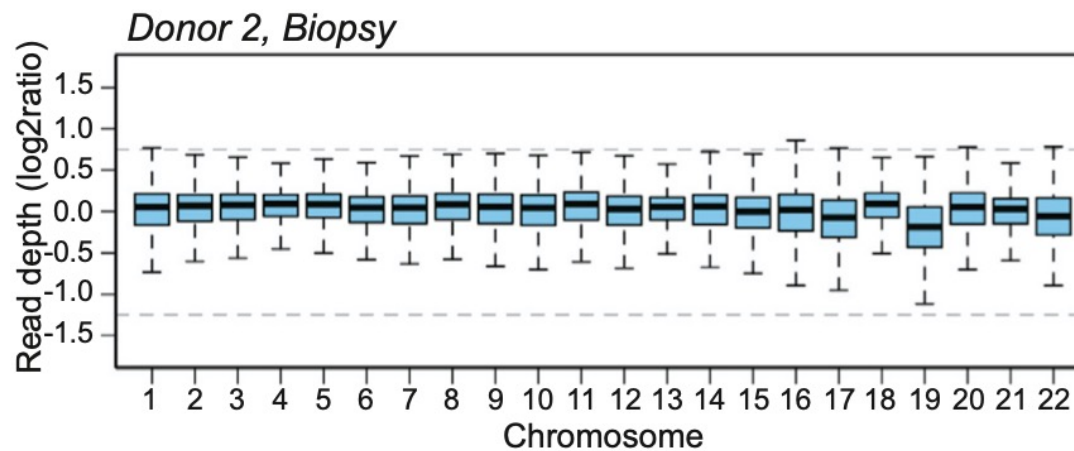
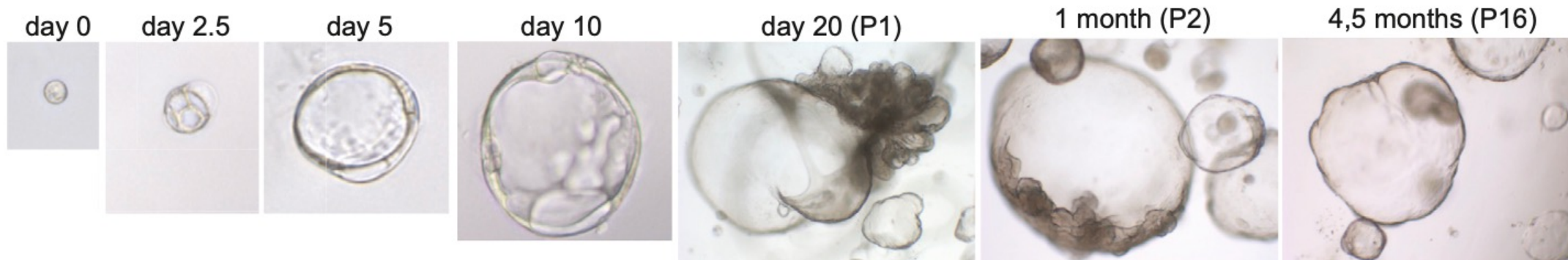
Application in Viral Cancer Studies



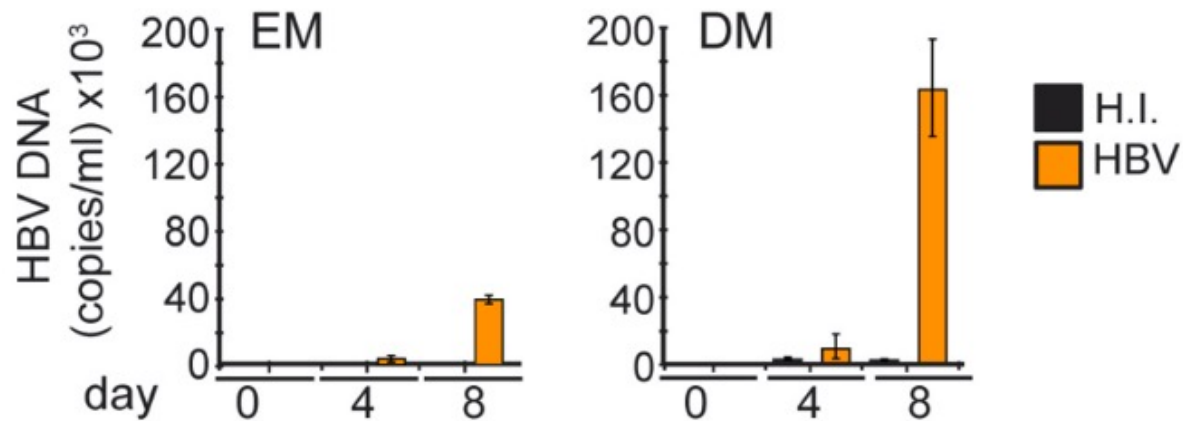
Hepatitis B Virus (HBV) Hepatocellular Carcinoma (HCC)

- Direct mechanism of HBV causing HCC is unclear due to lack of model system that sustain HBV infection.
 - HBV infects mature hepatocytes, thus ASC-derived liver organoids are more suitable
 - Huch et al. was first to derive liver organoids from adult stem cells and observe genetic stability of the models.
 - De Crignis et al. tested the virus susceptibility in organoid models.
- 

(P_) – Passage number

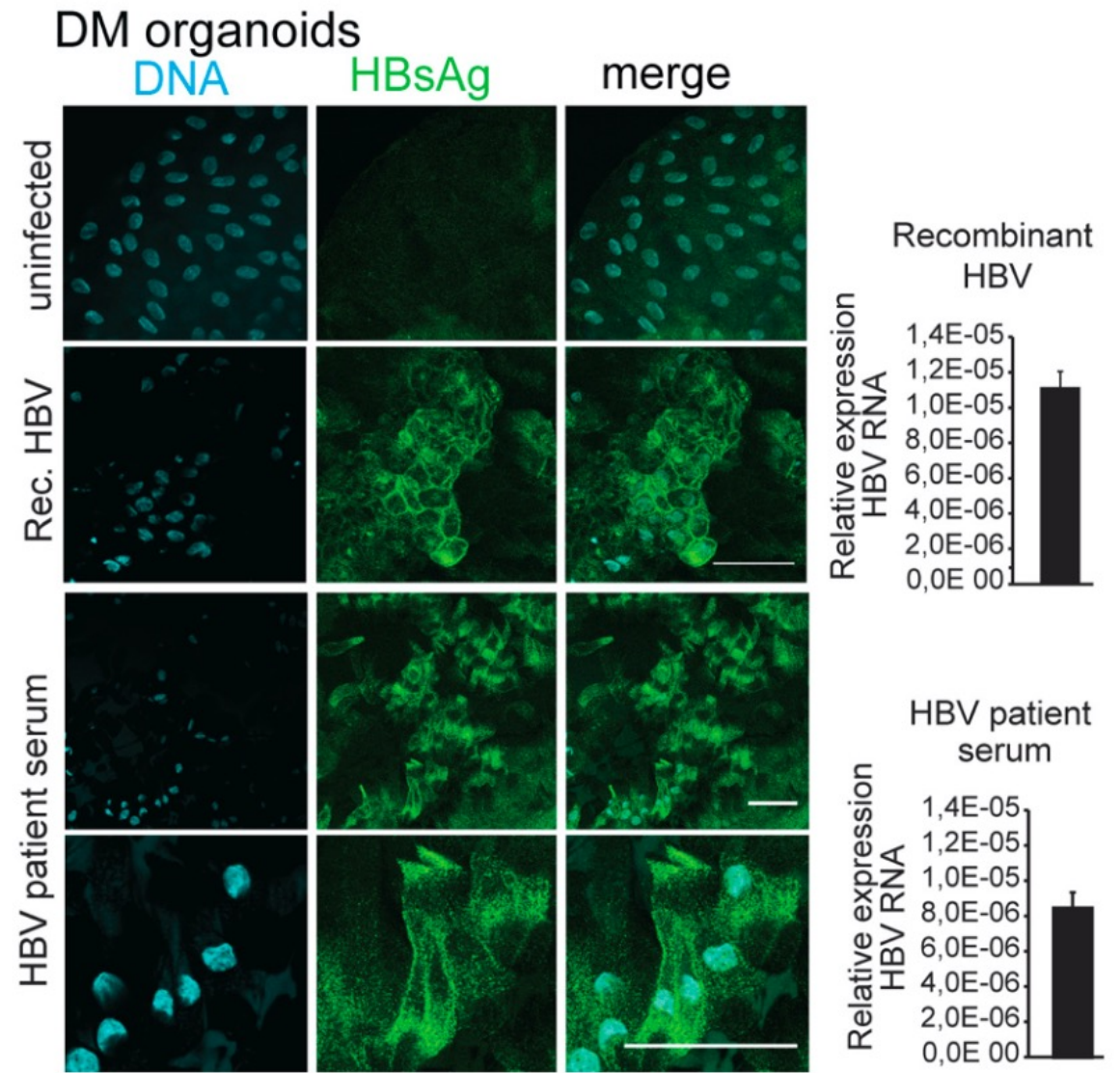


(Huch et al., Cell, 2015)




EM – Expansion Medium
DM – Differentiation Medium

H.I. – Heat Inactivated HBV
HBV – Recombinant HBV



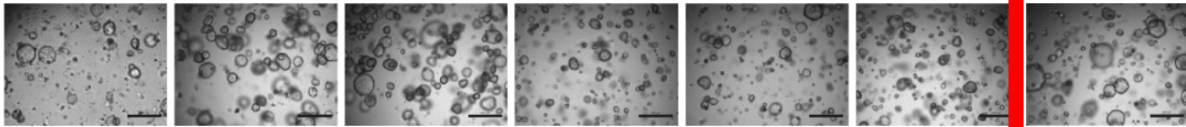
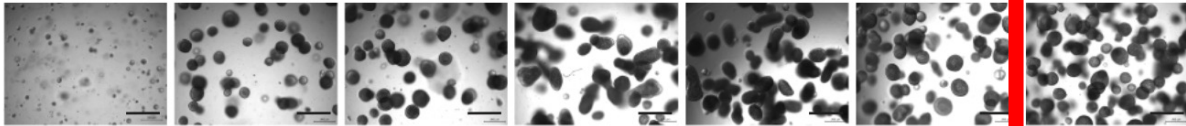


Human Papillomavirus (HPV) Cervical Cancer

- Lowering the cost of vaccinations and effective treatments are still limited.
 - Surgical means to obtain tumor biopsies to establish xenograft models.
 - Lohmussaar et al. report the establishment as well as the dynamic of cervical organoid in recapitulating cervical cancer
- 

B

	M1	M2	M3	M4	M5	M6	M7
BASE	+	+	+	+	+	+	+
FGF7	-	+	+	+	+	+	+
NAC	-	-	+	+	+	+	+
A83	-	-	-	+	+	+	+
FSK	-	-	-	-	+	+	+
FGF10	-	-	-	-	-	+	+
RSPO1	-	-	-	-	-	-	+

Endocervix
P0d14Ectocervix
P0d12

(M_) - Mediums containing different components

FGF7 - Fibroblast growth factor 7

NAC - N-acetyl cysteine

A83 - TGF-B inhibitor

FSK - Forskolin

FGF10 - Fibroblast growth factor 10

RSPO1 - R-spondin1

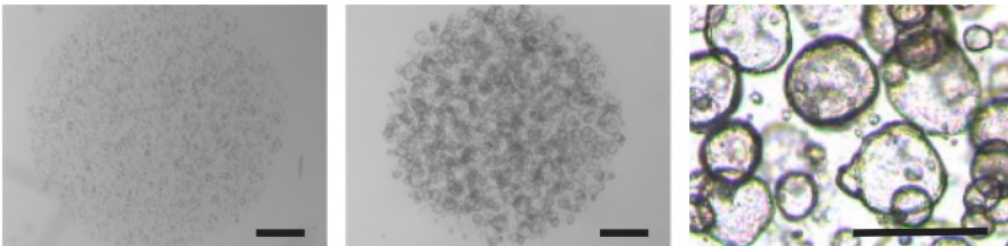
← noticeable growth efficiency

D

P0d7

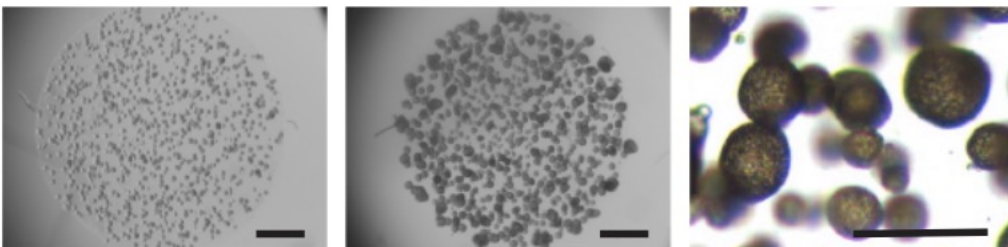
P0d14

Endocervix



← Hollow cystic structures

Ectocervix



← Dense structure

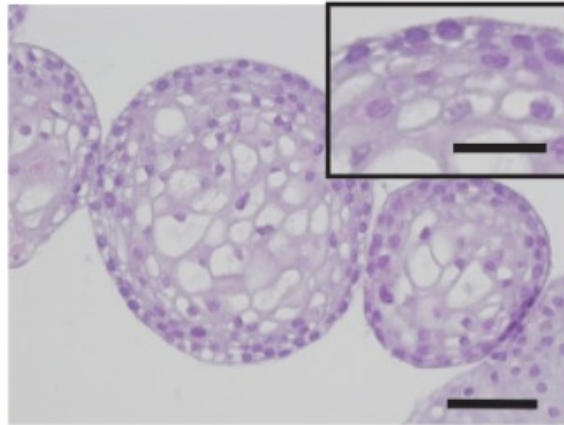
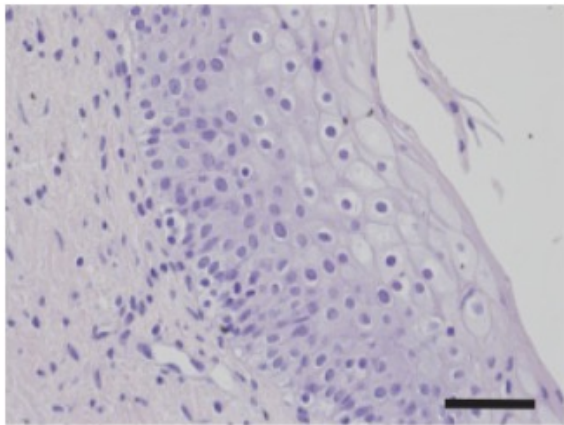
(Lohmussaar et al., Cell Stem Cell, 2021)

D

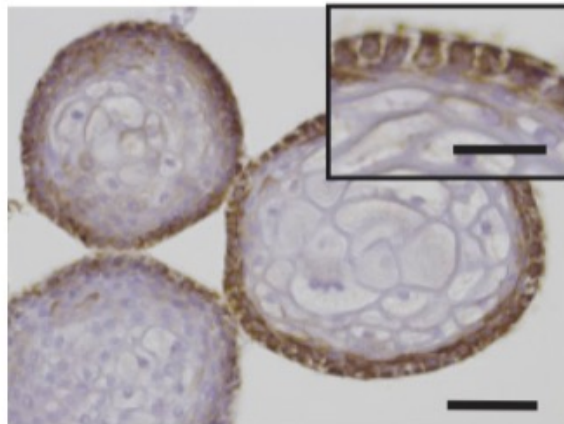
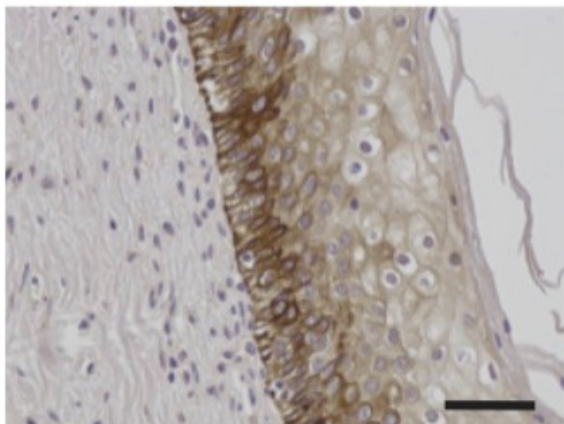
Ectocervix tissue

Ectocervix organoids

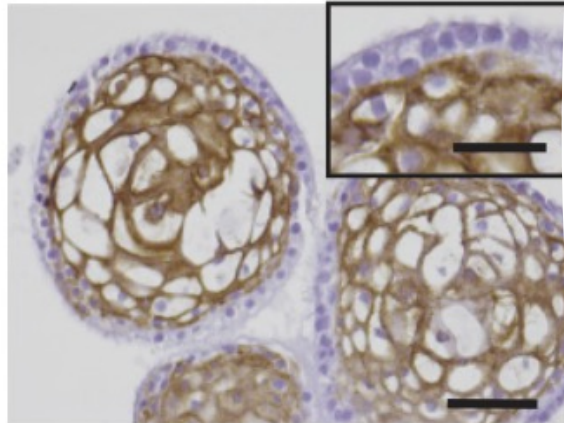
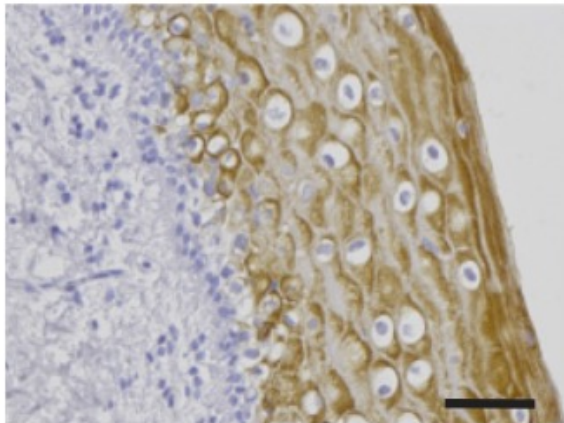
H&E



KRT14

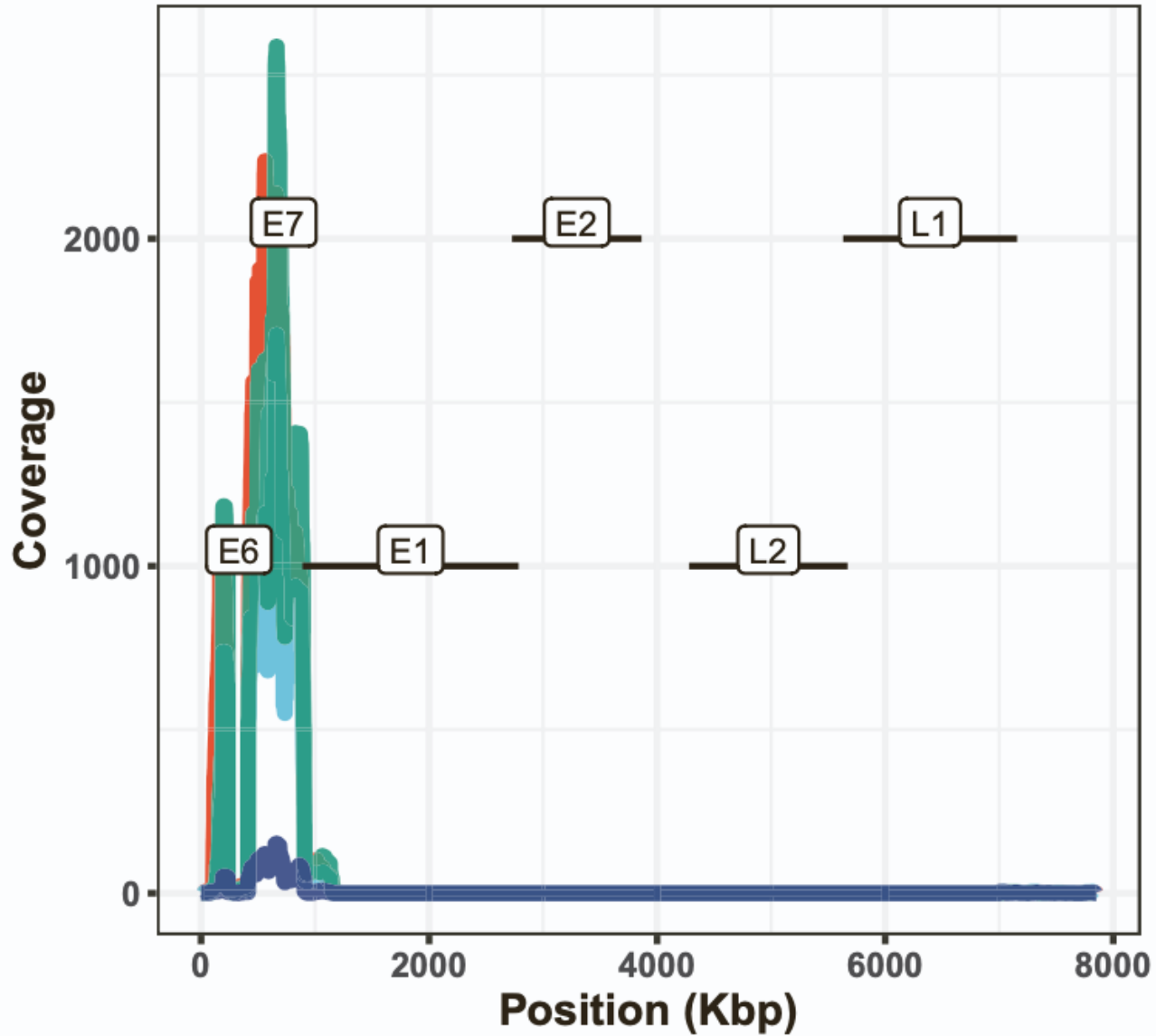


KRT13



- KRT14 – Keratin 14, Basal cell marker
- KRT13 – Keratin 13, Differentiated cell marker

HPV30



Sample

- SqCa-1.1
- SqCa-1.2-early
- SqCa-1.2-late
- SqCa-1.2-tissue

SqCa-1.1 – PAP Brush
SqCa-1.2 – Tissue biopsy

Potential Applications



Disease Modeling

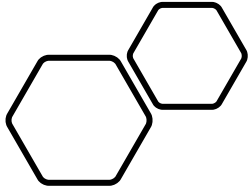
- Host-innate immunity
- Co-infections between viruses
- Pathogenesis
- Biomarkers (Personalized Medicine)

Drug Screening

- Pre-clinical evaluations
- Predictions on patients' response to therapy

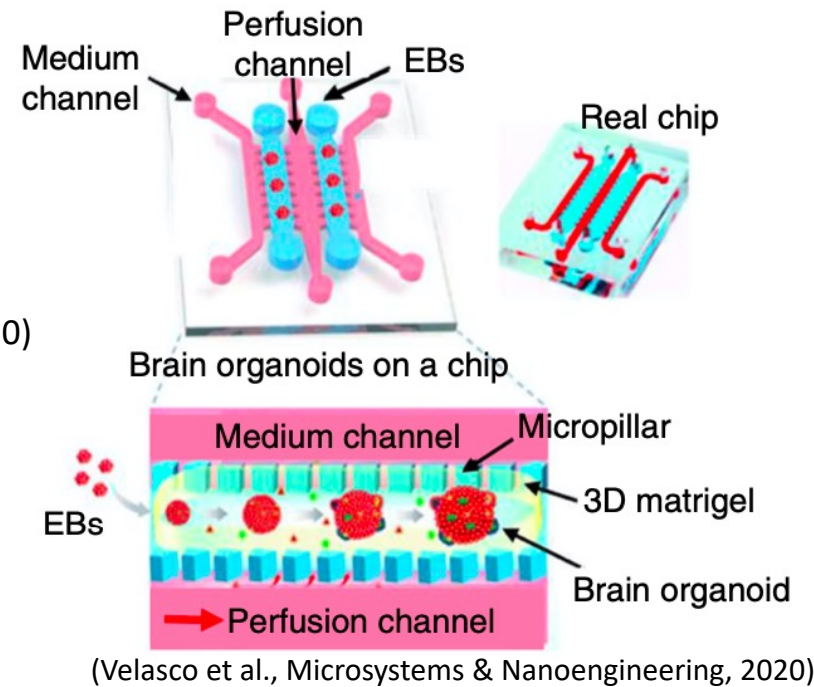
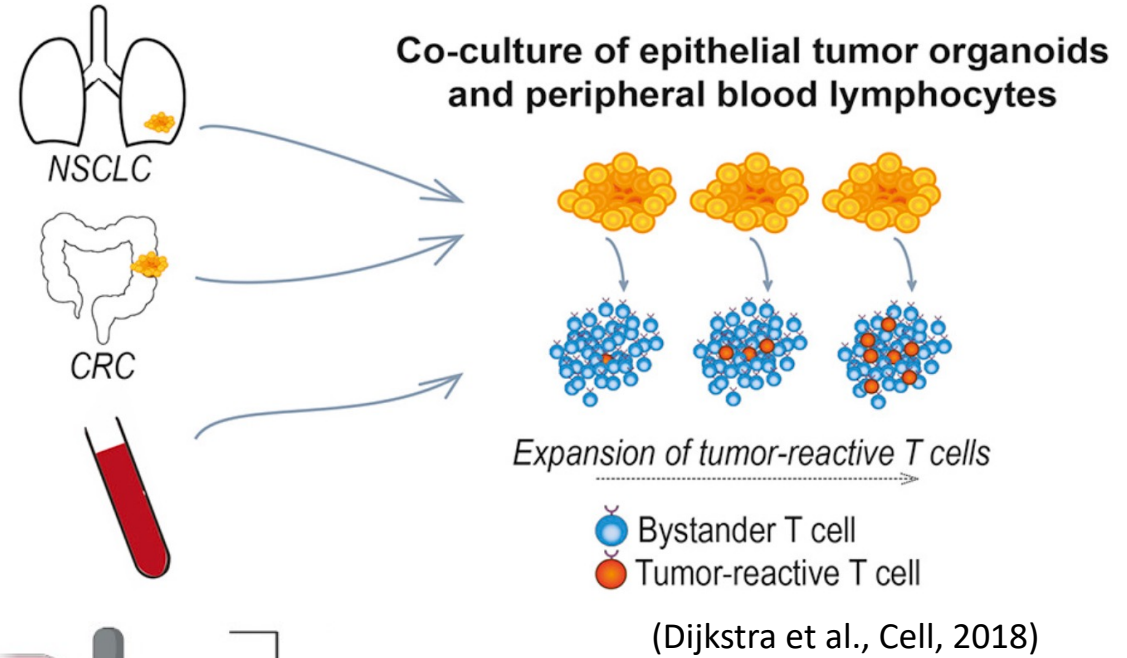
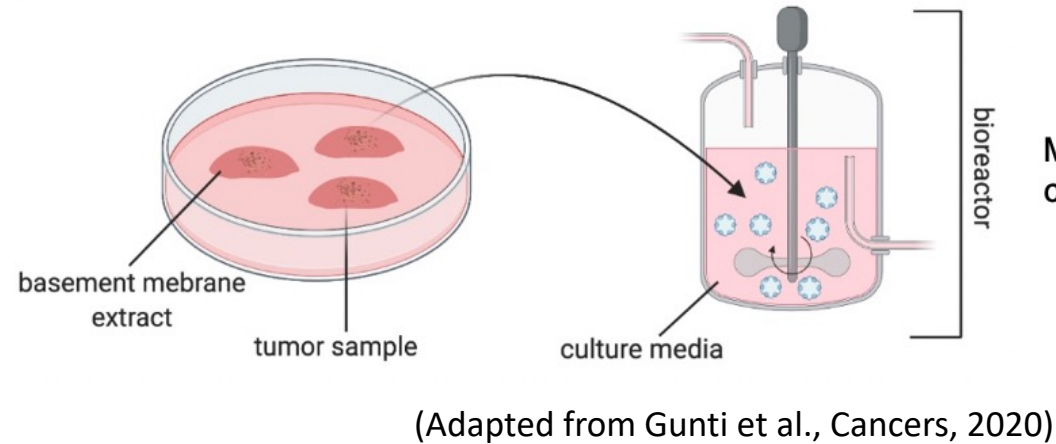
Regenerative Medicine

- Organ Transplantation



Limitations and Future Directions

- Bioreactors – address the size limitation and lifespan
- Organ-on-chip to recreate cross-talk between multiple organs or reproduce precise concentration gradients of signaling molecules
- Co-culturing of multiple tissues



References

- Blutt, Sarah E., and Mary K. Estes. "Organoid Models for Infectious Disease." *Annual Review of Medicine*, vol. 73, no. 1, 2022, pp. 167–182., <https://doi.org/10.1146/annurev-med-042320-023055>.
- Clevers, Hans. "Modeling Development and Disease with Organoids." *Cell*, vol. 165, no. 7, 2016, pp. 1586–1597., <https://doi.org/10.1016/j.cell.2016.05.082>.
- De Crignis E, Hossain T, Romal S, Carofiglio F, Moulos P, Khalid MM, Rao S, Bazrafshan A, Verstegen MM, Pourfarzad F, Koutsothanassis C, Gehart H, Kan TW, Palstra RJ, Boucher C, IJzermans JN, Huch M, Boj SF, Vries R, Clevers H, van der Laan LJ, Hatzis P, Mahmoudi T. Application of human liver organoids as a patient-derived primary model for HBV infection and related hepatocellular carcinoma. *Elife*. 2021 Jul 30;10:e60747. doi: 10.7554/eLife.60747. PMID: 34328417; PMCID: PMC8384419.
- Dijkstra, K. K., Cattaneo, C. M., Weeber, F., Chalabi, M., van de Haar, J., Fanchi, L. F., Slagter, M., van der Velden, D. L., Kaing, S., Kelderman, S., van Rooij, N., van Leerdam, M. E., Depla, A., Smit, E. F., Hartemink, K. J., de Groot, R., Wolkers, M. C., Sachs, N., Snaebjornsson, P., ... Voest, E. E. (2018). Generation of tumor-reactive T cells by co-culture of peripheral blood lymphocytes and tumor organoids. *Cell*, 174(6). <https://doi.org/10.1016/j.cell.2018.07.009>
- Dong, Renshun, et al. "Liver Organoids: An in Vitro 3D Model for Liver Cancer Study." *Cell & Bioscience*, vol. 12, no. 1, 2022, <https://doi.org/10.1186/s13578-022-00890-8>.
- Dutta, Devanjali, and Hans Clevers. "Organoid Culture Systems to Study Host–Pathogen Interactions." *Current Opinion in Immunology*, vol. 48, 2017, pp. 15–22., <https://doi.org/10.1016/j.coi.2017.07.012>.
- Dutta, Devanjali, et al. "Disease Modeling in Stem Cell-Derived 3D Organoid Systems." *Trends in Molecular Medicine*, vol. 23, no. 5, 2017, pp. 393–410., <https://doi.org/10.1016/j.molmed.2017.02.007>.
- Duzagac, F., Saorin, G., Memeo, L., Canzonieri, V., & Rizzolio, F. (2021). Microfluidic organoids-on-a-chip: Quantum Leap in Cancer Research. *Cancers*, 13(4), 737. <https://doi.org/10.3390/cancers13040737>
- Gunti, S., Hoke, A. T. K., Vu, K. P., & London, N. R. (2021). Organoid and spheroid tumor models: Techniques and applications. *Cancers*, 13(4), 874. <https://doi.org/10.3390/cancers13040874>
- Huch, Meritxell, et al. "Long-Term Culture of Genome-Stable Bipotent Stem Cells from Adult Human Liver." *Cell*, vol. 160, no. 1-2, 2015, pp. 299–312., <https://doi.org/10.1016/j.cell.2014.11.050>.

References

- Iakobachvili, N., & Peters, P. J. (2017). Humans in a dish: The potential of organoids in modeling immunity and infectious diseases. *Frontiers in Microbiology*, 8. <https://doi.org/10.3389/fmicb.2017.02402>
- In, Julie G., et al. "Human Mini-Guts: New Insights into Intestinal Physiology and Host–Pathogen Interactions." *Nature Reviews Gastroenterology & Hepatology*, vol. 13, no. 11, 2016, pp. 633–642., <https://doi.org/10.1038/nrgastro.2016.142>.
- Löhmußaar, Kadi, et al. "Patient-Derived Organoids Model Cervical Tissue Dynamics and Viral Oncogenesis in Cervical Cancer." *Cell Stem Cell*, vol. 28, no. 8, 2021, <https://doi.org/10.1016/j.stem.2021.03.012>.
- Ramani, Sasirekha, et al. "Human Organoid Cultures: Transformative New Tools for Human Virus Studies." *Current Opinion in Virology*, vol. 29, 2018, pp. 79–86., <https://doi.org/10.1016/j.coviro.2018.04.001>.
- Rossi, Giuliana, et al. "Progress and Potential in Organoid Research." *Nature Reviews Genetics*, vol. 19, no. 11, 2018, pp. 671–687., <https://doi.org/10.1038/s41576-018-0051-9>.
- Torresi, Joseph, et al. "HBV-Related Hepatocarcinogenesis: The Role of Signalling Pathways and Innovative Ex Vivo Research Models." *BMC Cancer*, vol. 19, no. 1, 2019, <https://doi.org/10.1186/s12885-019-5916-6>.
- Tortorella, I., Argentati, C., Emiliani, C., Martino, S., & Morena, F. (2021). The role of physical cues in the development of stem cell-derived organoids. *European Biophysics Journal*, 51(2), 105–117. <https://doi.org/10.1007/s00249-021-01551-3>
- Velasco, Vanessa, et al. "Microtechnology-Based Methods for Organoid Models." *Microsystems & Nanoengineering*, vol. 6, no. 1, 2020, <https://doi.org/10.1038/s41378-020-00185-3>.
- Yuki, Kanako, et al. "Organoid Models of Tumor Immunology." *Trends in Immunology*, vol. 41, no. 8, 2020, pp. 652–664., <https://doi.org/10.1016/j.it.2020.06.010>.



Thank you