Johns Hopkins Engineering

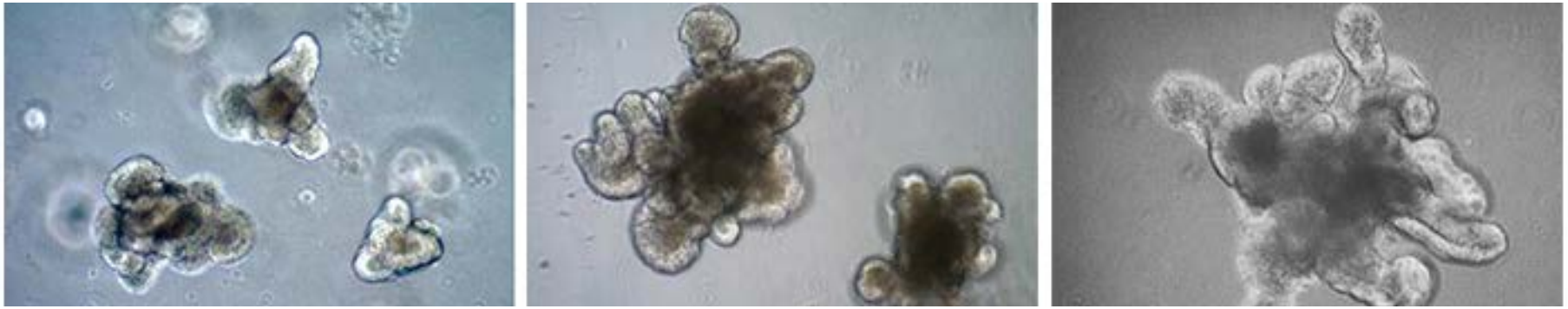
Methods in Neurobiology

Organoids and 3D Cell Cultures

JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

What are organoids?

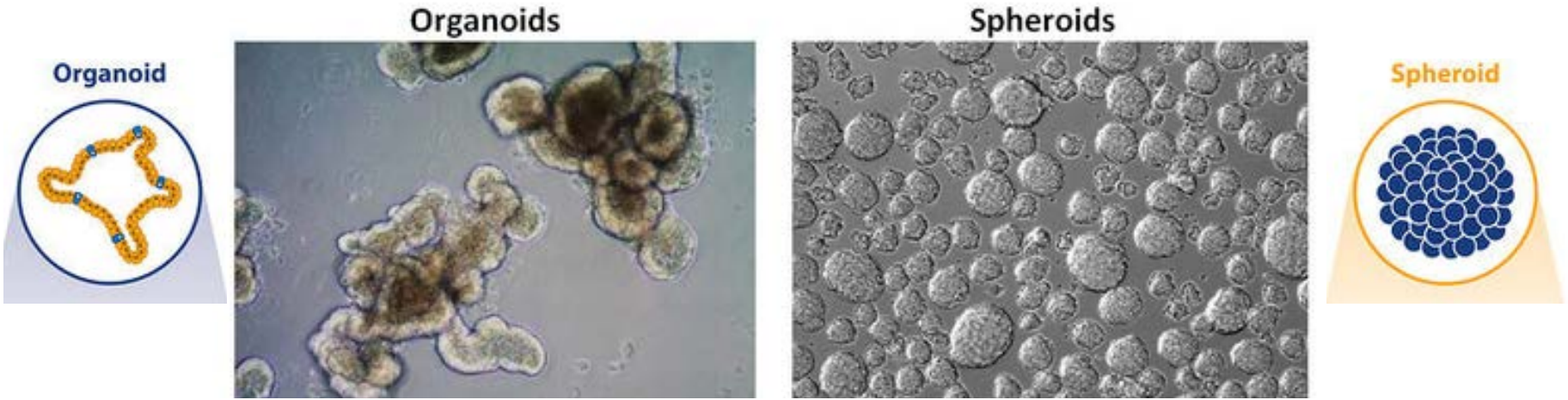
Organoids are *in-vitro* derived 3D cell aggregates derived from primary tissue or stem cells that are capable of self-renewal, self-organization and exhibit organ functionality.



Mouse Intestinal Epithelial Organoids

Organoids v. Spheroids

Organoids and spheroids are both cells cultured in 3 dimensions. Spheroids are often formed from cancer cell lines or tumor biopsies as freely floating cell aggregates in ultra-low attachment plates whereas organoids are derived from tissue stem cells embedded within an ECM hydrogel matrix such as Matrigel. Organoids are highly complex and are more *in vivo*-like when compared to spheroids. Recently, tumor organoids have shown to predict how well patients respond to cancer drugs to aid in personalized medicine.



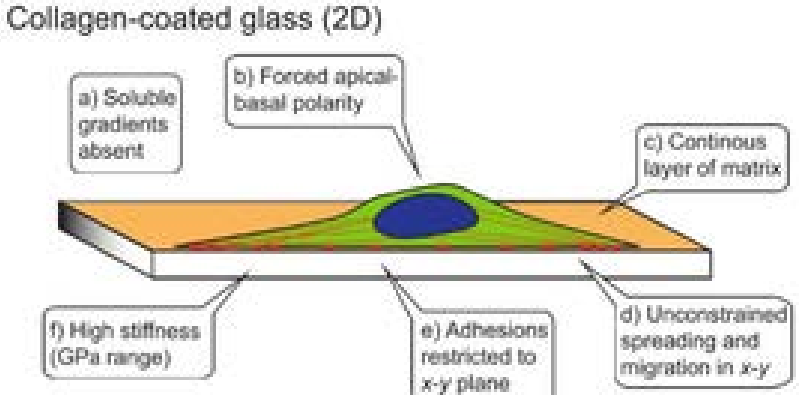
2D cultures v. 3D cultures

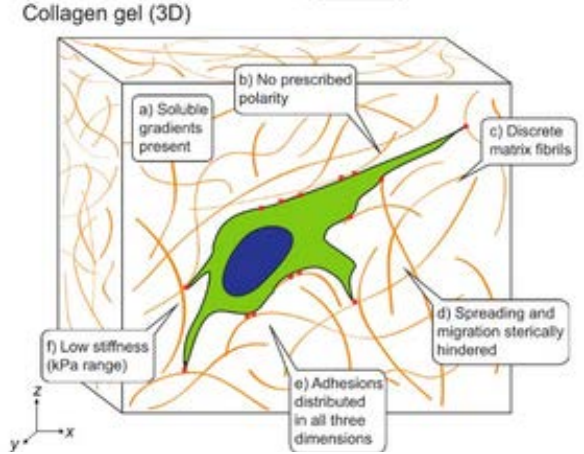
2D Cell Monolayers

- Cells lose their phenotype
- Lack cell-cell and cell-matrix interactions
- Could not mimic cellular functions and signaling pathways as in in-vivo conditions

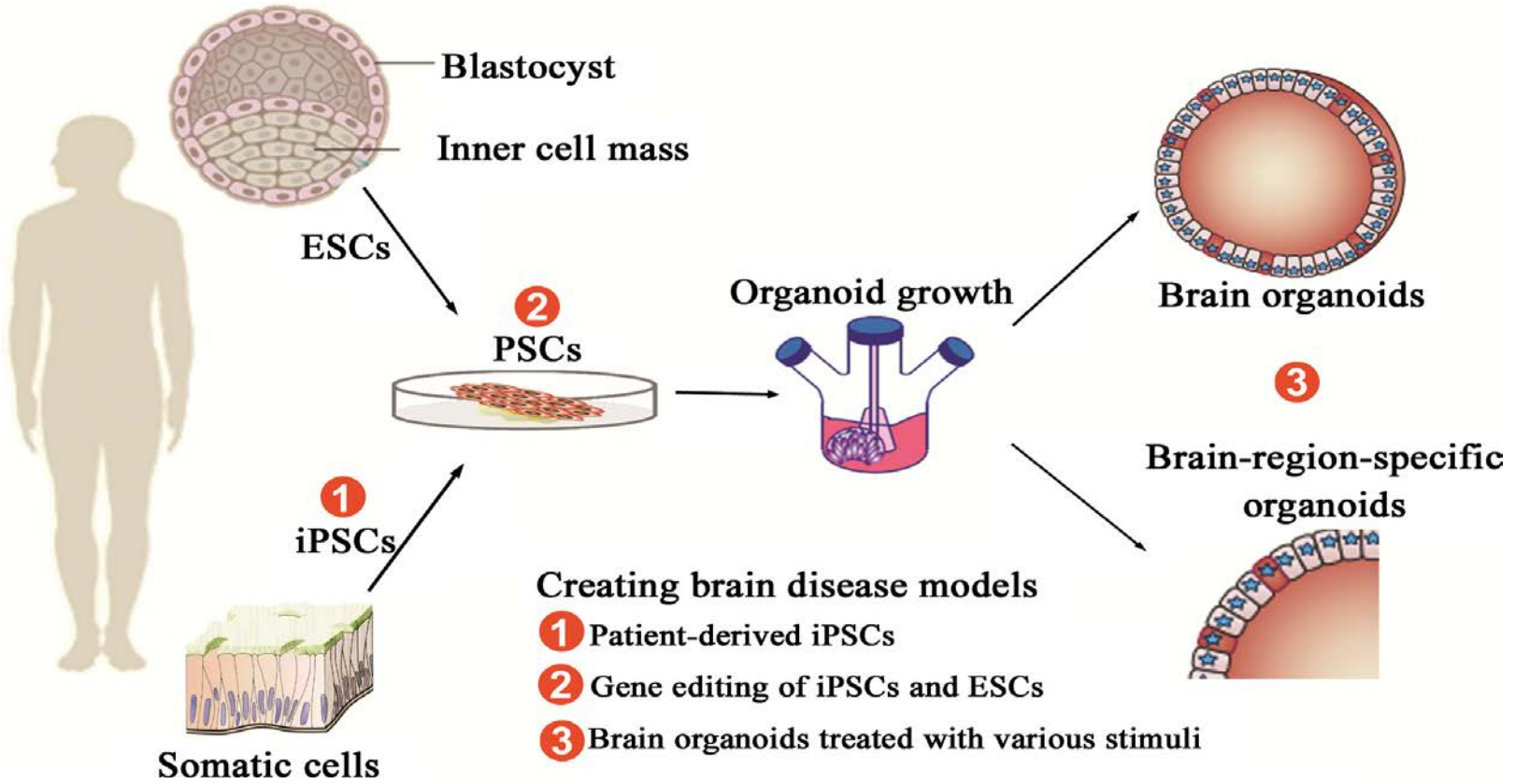
3D Cell Aggregates

- Transiently resemble cell organization and interactions
- Difficult to maintain long term cultures
- Lack potency for self-renewal and differentiation[2]





How to make organoids?



Example of established protocols to develop organoids

Organoids	Source	Culture conditions	Cell types in organoids
Stomach			
	hPSCs	**Endoderm induction:** Rock inhibitor (Y-27632), Activin A, BMP5 **Spheroid generation:** WNT, FGF, Noggin, Retinoic acid **Organoid formation:** Noggin, Retinoic acid, EGF **Maturation:** EGF	LGR5+ cells, mucous cells, gastric endocrine cells
	hAdSC	EGF, Rspondin, Noggin, FGF10, WNT, Gastrin, Nicotinamide and TGFβ inhibitor	LGR5+ cells, pit mucous cells, gland mucous cells, chief cells and enteroendocrine cells
Intestine			
	hPSC	**Endoderm induction:** Activin A, BMP4 **Hindgut differentiation (spheroid generation):** FGF4, WNT3A **Organoid formation:** FGF4, WNT3A **Maturation:** RSpondin1, Noggin, EGF, FGF4, WNT	Enterocytes, Goblet, Paneth and enteroendocrine cells
	hAdSC	**Establishment :** EGF, Rspondin, Noggin, WNT3A, Nicotinamide, Gastrin, TGFβinhibitor, p38 inhibitor **Differentiation :** Without WNT3A, p38 MAP kinase inhibitor and nicotinamide	Intestinal epithelial derivatives and stem cells

- Brain
- Kidney
- Pancreas
- Prostate
- Lung
- Colon
- Liver

References

Slide	Reference
2,3,4,6	MilliporeSigma. (2020). 3D Organoid Culture: New In Vitro Models of Development and Disease. https://www.sigmaaldrich.com/technical-documents/articles/biology/cell-culture/3d-organoid-culture.html
5	Wang, Z., Wang, S., Xu, T., et. al. (2017). Organoid technology for brain and therapeutics research. CNS Neuroscience and Therapeutics. Vol. 23, 771-778.