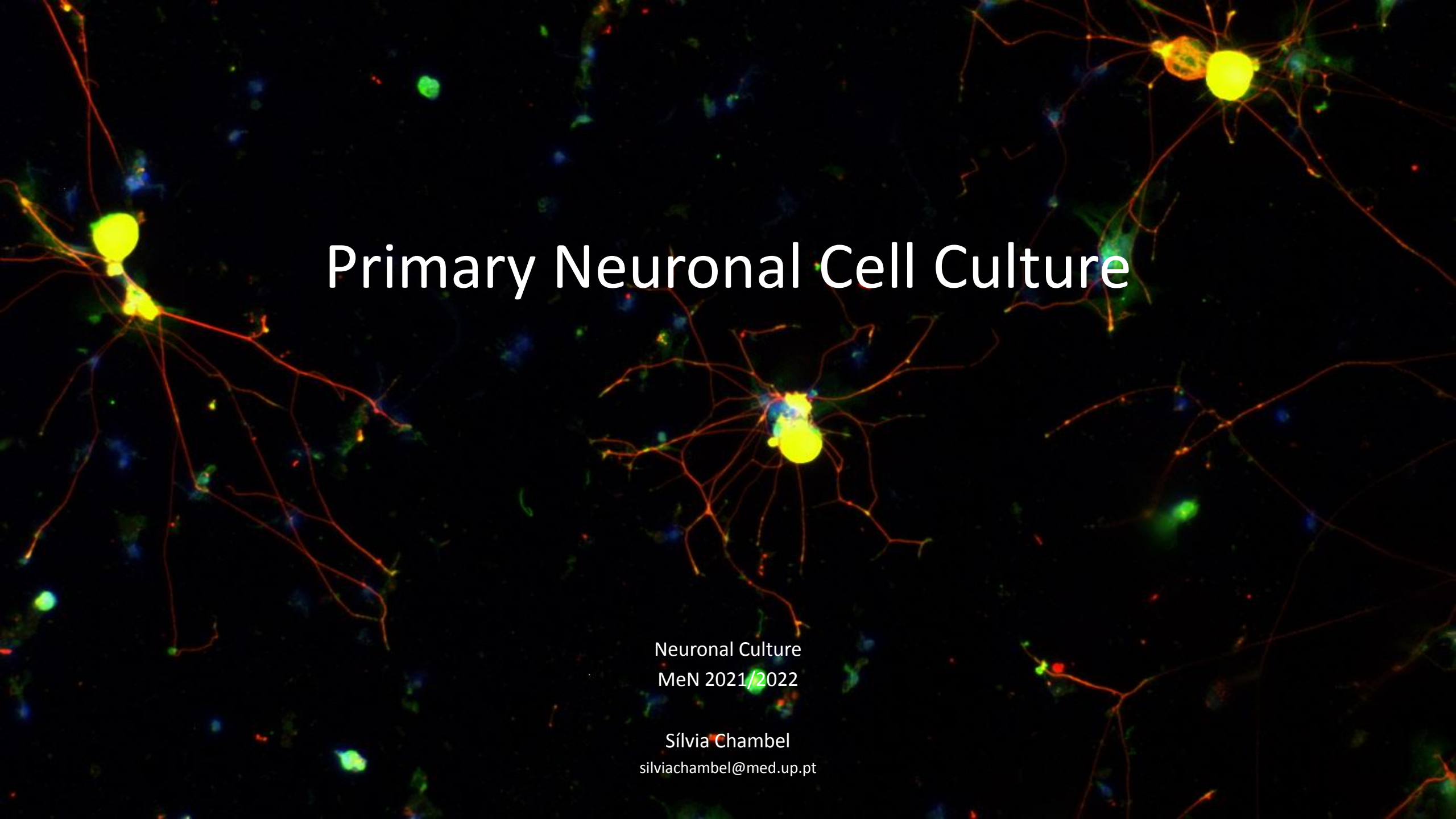


Primary Neuronal Cell Culture

Neuronal Culture

MeN 2021/2022

Sílvia Chambel

silviachambel@med.up.pt

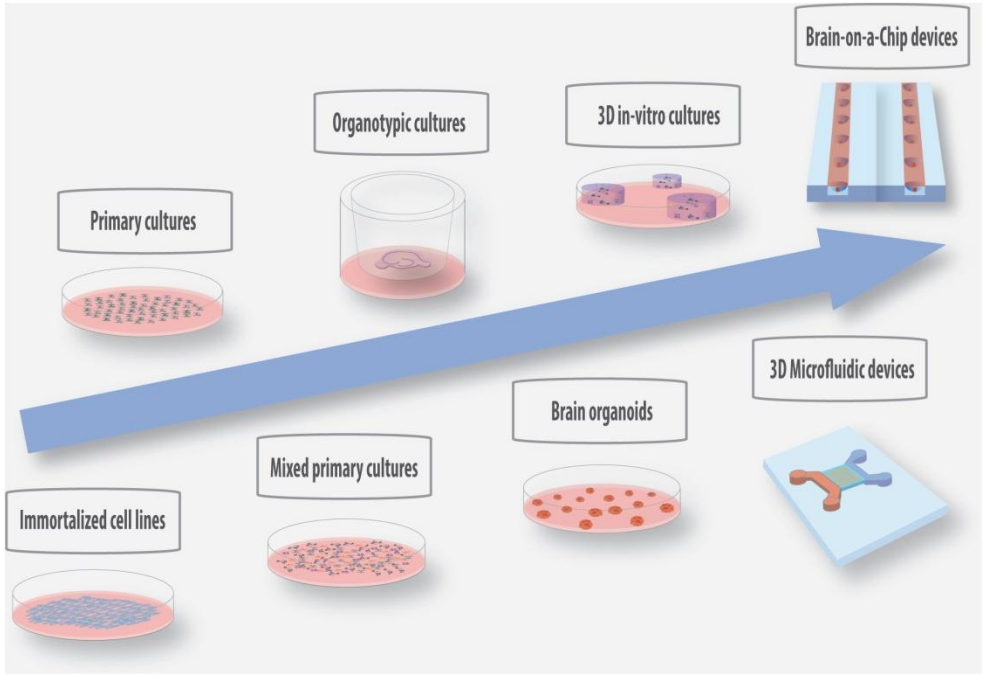
Primary neuronal cell culture

Cells or tissues directly isolated from organs of interest of the central nervous system (CNS) or peripheral nervous system (PNS)

Why are primary cell cultures used?

- Retain morphological, neurochemical, and electrophysiological properties close to those of *in vivo* tissues/organs
- Develop and display properties of mature neurons and spontaneously elaborate neuritis
  - Synapse formation
  - Neurite outgrowth
  - Electrophysiological proprieties

	Advantages	Disadvantages
Immortalized Cell Lines	• Easier to use (grow, transfect, etc.) • Homogeneous • Fairly well characterized • Can create stable cell lines expressing gene of interest	• May not have the same properties as neurons or primary cell type of interest
Primary Cell Culture	• Relevant cell type, physiology, and circuitry	• Heterogeneous populations with high variability



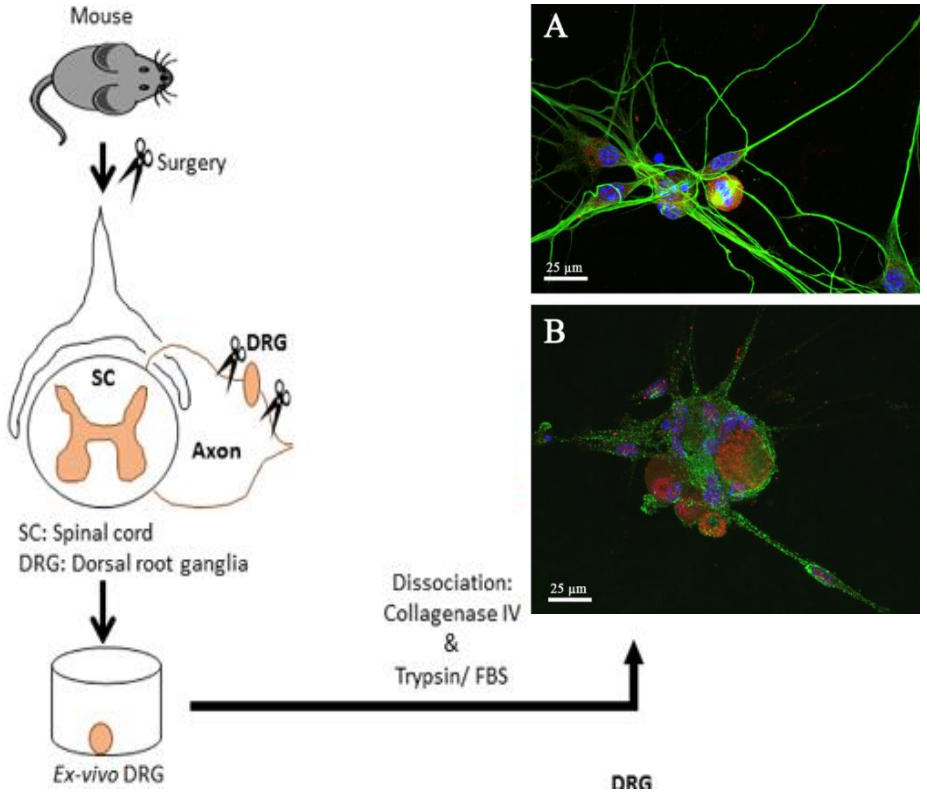
Types of primary neuronal cell culture

Dissociated cell cultures

Excised CNS tissues mechanically and/or enzymatically dissociated into individual cells and cultured on 2D coated glass coverslips

- Directly investigate cells of interest; wild-type vs. transgenic animal
- Cells retain the morphological, physiological and molecular proprieties of the original cell populations – visualize dynamic structures and function; pharmacological studies
- Can be maintained for days/weeks, forming neurites and synapses, expressing receptors and ion channels and produce electrical activity

- Limited lifetime
- Age of the animal influences robustness of culture
- Isolation procedures are challenging
- Small quantity of material – limited number of experiments
- Primary cell cultures are usually heterogeneous
    - **Immunopanning**
    - **AraC**

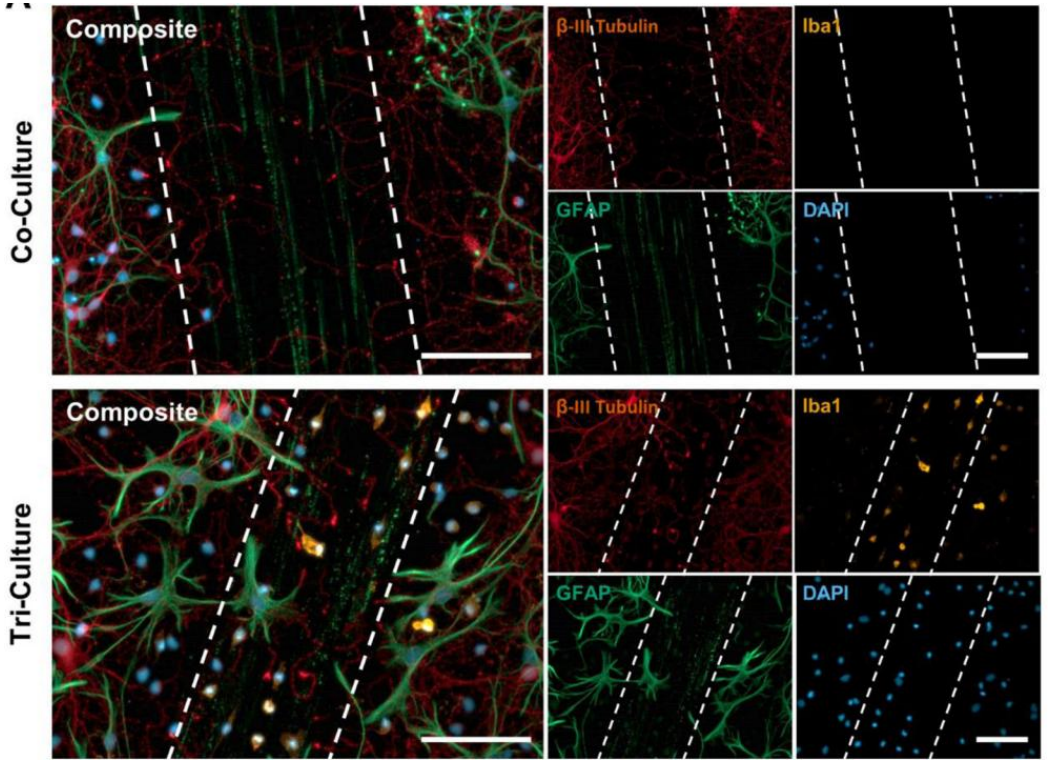


Types of primary neuronal cell culture

Dissociated cell cultures – co-cultures

Variety of cell types are cultured together to examine the effect of one culture system on another

- More representative of *in vivo* conditions than conditioned-media cultures
- Study the attraction, migration, proliferation, and differentiation in neuron-glia crosstalk
- Neuron-glia crosstalk is particularly important to study CNS injury and neurodegenerative diseases
  - Microglia and astrocyte activation



Goshi et al. Journal of Neuroinflammation, 2020.

Types of primary neuronal cell culture

Slice cultures

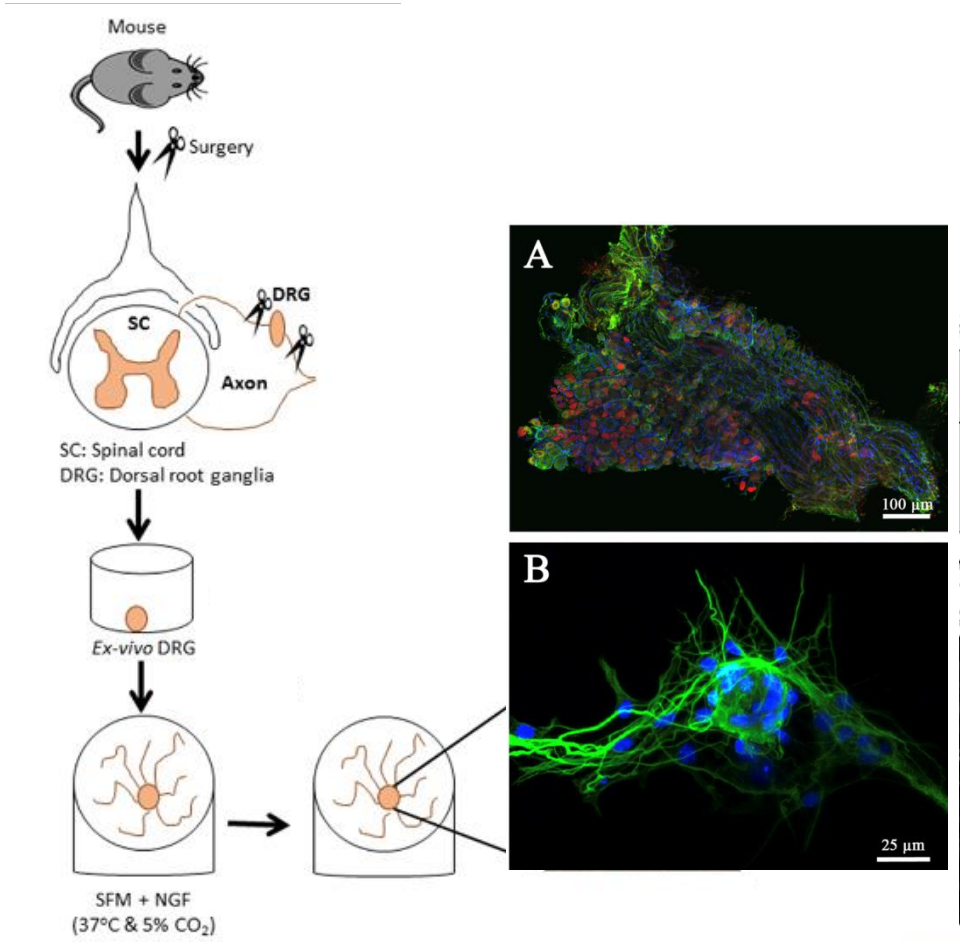
Organotypic primary cultures preserve the native cytoarchitecture and maintain intact neuronal networks

Slices from brain, spinal cord or DRG are obtained using a vibratome (250–400 µm)

- **Acute cultures:** used immediately (e.g. electrophysiological recordings)
- **Organotypic slice cultures:** cultured over longer periods of time to study structural and morphological changes

- Better access and visibility of inner regions
- Possibility to obtain several slices from a single animal
- Study several cell populations and their interactions at once

- Require proper air/liquid interfaces (membrane/gel)



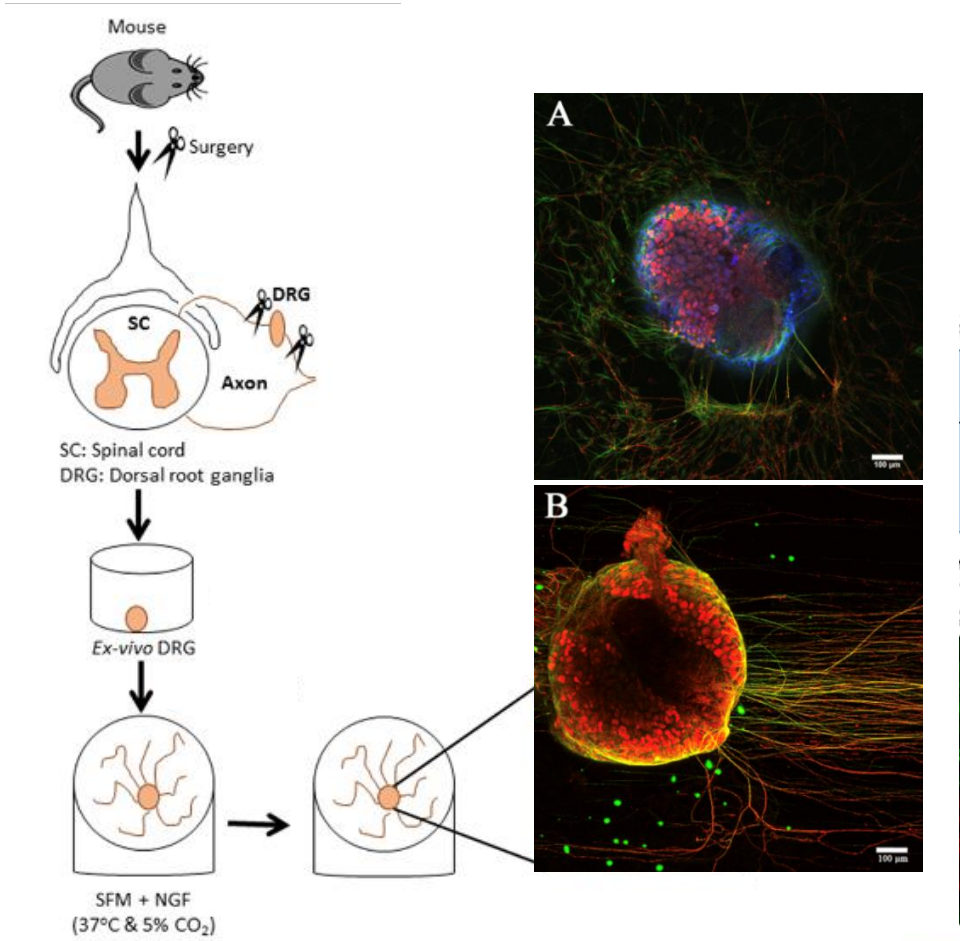
Types of primary neuronal cell culture

Explant cultures

Organotypic primary cultures maintain the structure and organization of the original tissue

Study of cells connected in an organized structure using fragments of tissue with the same cell types as *in vivo*

- Do not require an air/medium interface – explants are submerged in culture media
- Often used in co-culture assays for studies of neurite outgrowth and neuronal migration



Primary neuronal cell culture

Transition from 2D to 3D cell culture models

In the human body, **cells don't grow in 2D** – limitations for study of complex systems

Critical to each microenvironment: extracellular matrix (ECM)

- Structural support for cells
- Regulation of diverse processes: cell migration, proliferation, differentiation and cell-to-cell communication
- Cell-ECM and cell-cell interactions (namely, the neuronal network) are lost

Characteristic or condition	2D	3D
Morphology	Cells grow in a sheet or monolayer	Cells often retain natural shape and proper spatial orientation in aggregates or spheroids
Proliferation	Usually faster than *in vivo* growth	Depending on cell type and system used, may be faster or slower than 2D culture
Gene and protein expression	Often differs from expression *in vivo*	Cells more closely mimic expression *in vivo*
Drug sensitivity	Drugs often appear effective, as exposure is fairly uniform	Cells can have nonuniform toxicity profiles; more closely mimics the true effect *in vivo*
Oxygen tension	High oxygen tension in incubator is not physiological; affects mitochondrial function and development of reactive oxygen species (ROS)	Oxygen tension varies within culture; more closely mimics *in vivo* differences
Genetic drift	Cells in long-term culture undergo genetic drift due to selection following specific growth conditions in individual laboratories; epigenetic and physiological changes may be noted as well	Cells are not in traditional long-term culture, so genetic stability may be improved

Adapted from: Edmondson 2014; Horvath 2016.

Types of 3D cell culture

3D models provide an *in vivo* representation of a tissue or organ in an *in vitro* manner

Spheroids

- Bridge the gap between monolayers and complex organs

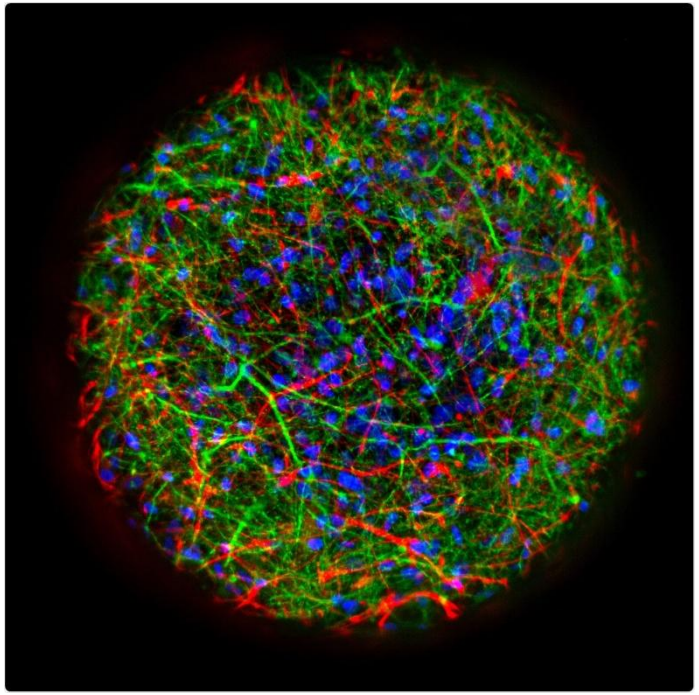
- 3D cellular aggregates, resulting from the growth and aggregation of one or more cell types

- Retain the characteristics of the starting cells and even exhibit enhanced physiological responses

- Rely on the natural tendency of cells to aggregate in the absence of adhesive substract – cell-to-cell contact, proper spatial orientation and ECM production

- Research complex processes such as angiogenesis, drug discovery and tumour development

- Do not undergo differentiation or self-organization



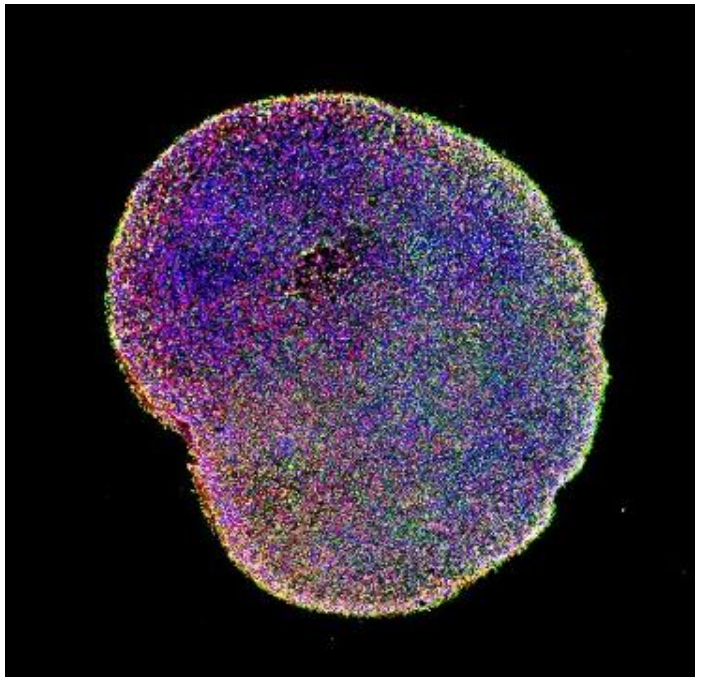
Spheroid showing the presence of neurons (MAP2, green) and astrocytes (GFAP, red). Nuclei are stained blue (DAPI).

Types of 3D cell culture

3D models provide an *in vivo* representation of a tissue or organ in an *in vitro* manner

Organoids

- Highly differentiated 3D structures, commonly derived from stem cells or other progenitor cells, preserving many of the features of the organ from which they derive
- Cells spontaneously self-organize into differentiated and functional structures, recapitulating some function and complexity of their derived organ
- Reproduce organs of origin more closely than spheroid cultures
- Used to study differentiation, physiology and complex multicellular interactions *in vitro*
    - Brain organoids to study human brain development



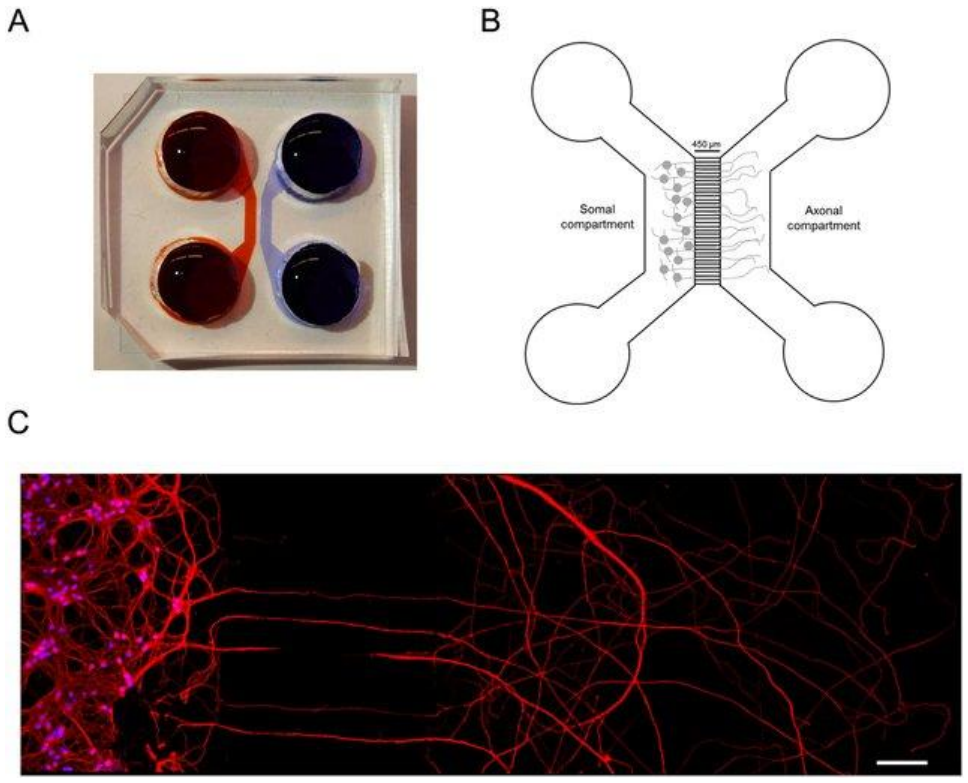
Brain organoid with multiple types of neurons, representing the complexity of the human brain.

Types of 3D cell culture

3D models provide an *in vivo* representation of a tissue or organ in an *in vitro* manner

Organs-on-chips - Microfluidics

- Microfluidics devices that simulate the cellular structure of organs and enable the dynamic flow of media
- Local cell environment is maintained in a predefined and stable environment without abrupt environmental changes
- Spatial control over the placement of cells, ECM, and soluble factors; different cell types; and fluid transport between compartments



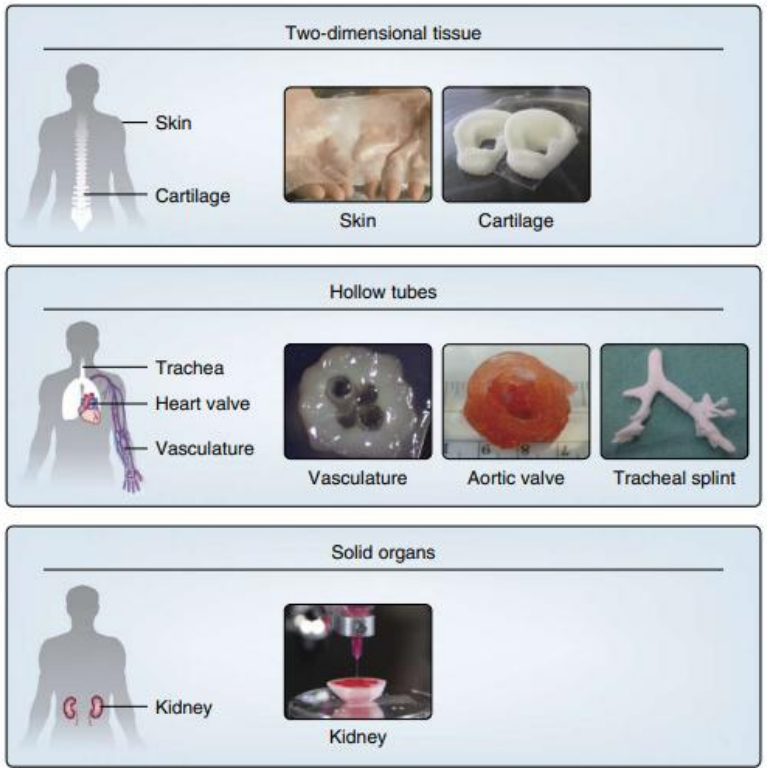
Martins et al, 2017. *Sci Rep* **7,** 4153.

Types of 3D cell culture

3D models provide an *in vivo* representation of a tissue or organ in an *in vitro* manner

Bioprinting

- Cells, scaffolding and supportive materials are printed into a final complex with the architecture, function and topology that resembles the organoid *in vivo*
- Great advances in bone, cartilage, skin and heart bioprinting



- Skin and cartilage substitutes developed using inkjet bioprinting systems;
- Vascular graft construct manufactured using microextruded and fused cellular vascular rods and a microextrusion-bioprinted aortic valve fabricated with dual cell types, aortic root sinus smooth muscle cells and aortic valve leaflet interstitial cells; laser bioprinted bioresorbable airway splint
- Early stage kidney prototype

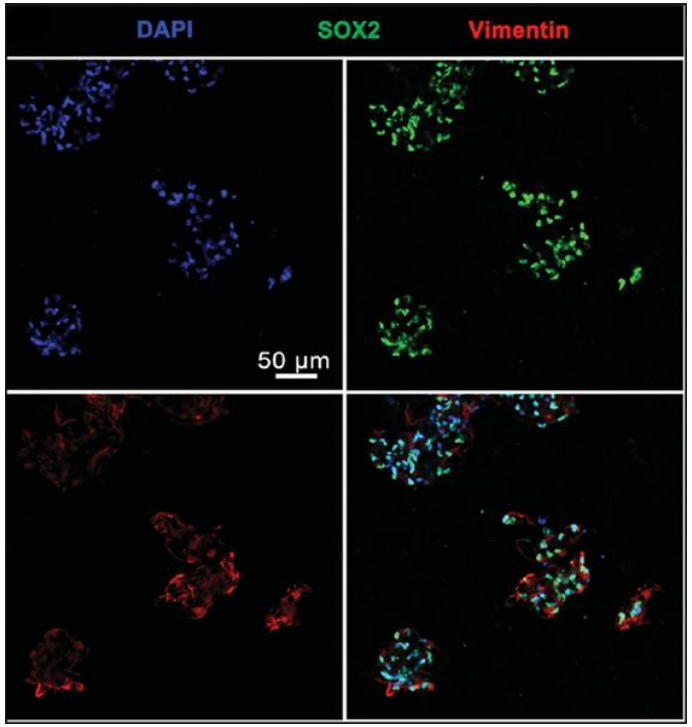
Types of 3D Cell Culture

3D models provide an *in vivo* representation of a tissue or organ in an *in vitro* manner

Bioprinting

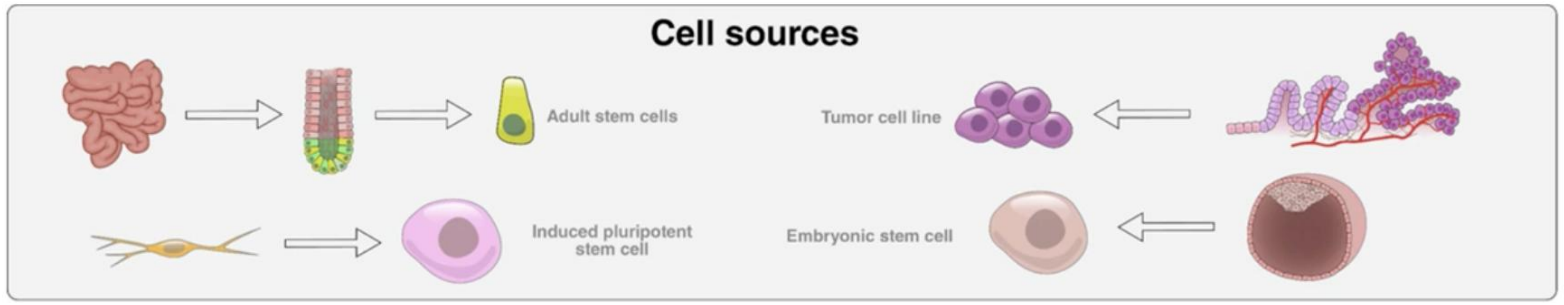
- Cells, scaffolding and supportive materials are printed into a final complex with the architecture, function and topology that resembles the organoid *in vivo*
- Great advances in bone, cartilage, skin and heart bioprinting

- Model complex neural structures to study neurodegenerative diseases
- Still a distant technology in neuronal tissue engineering:
  - Implementation of *in vitro* axon guidance
  - Optimization of printing parameters to increase neural cell viability
  - Incorporation of perfusion systems to achieve long-term cell survival
  - Controlled generation of neural circuitry and systems to monitor their electrical activity



Cells stained with DAPI, vimentin, and SOX2 24 days after printing. Cells largely expressed both DAPI and vimentin, indicating mature neurons

Cell sources in 3D cell culture

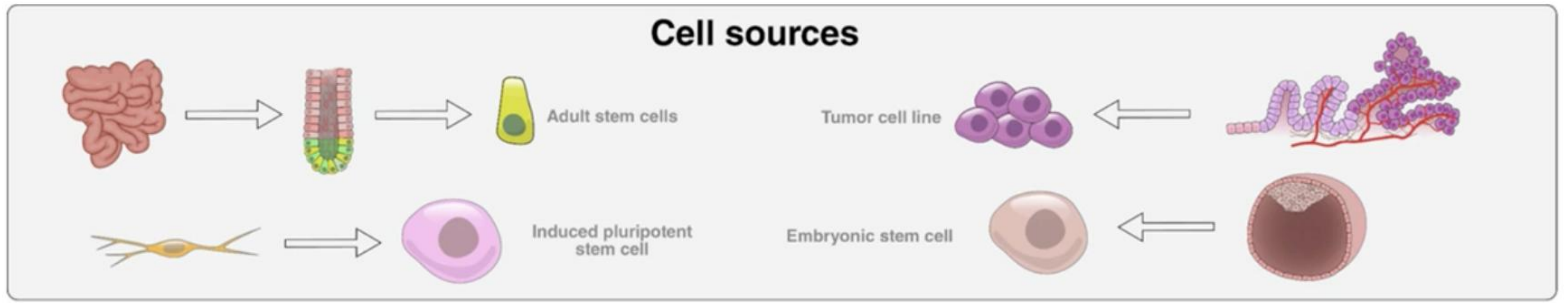


- Cell lines
- Primary cells
  - Somatic cells
  - Pluripotent stem cells
  - Adult stem cells
- Patient-derived cells

Primary cells

- Obtained from volunteer or patient tissue or from research animals
- More representative of the in vivo environment
- Dependent on availability and sample size
- Finite lifespan; when grown in 3D (e.g. Gel matrix), survival is extended
- Terminally differentiated

Cell sources in 3D cell culture



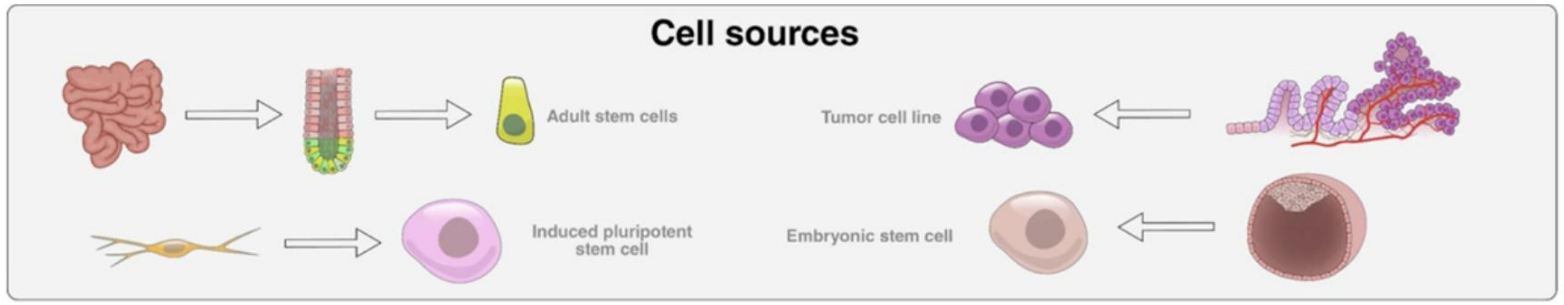
- Cell lines
- Primary cells
  - Somatic cells
  - Pluripotent stem cells
  - Adult stem cells
- Patient-derived cells

Pluripotent stem cells (PSCs)

- Embryonic stem cells (ESCs) (embryos)
- Induced Pluripotent stem cells (iPSCs) (somatic cells reprogrammed to an ESC-like state)
  - Obtained directly from individuals – improve treatment and diagnosis in personalized medicine
  - Differentiate into any cell type
  - Unlimited self-renewal capacity – better to investigate long-term processes
  - PSCs cultured in 3D structures can differentiate into different cell types and undergo morphogenesis to create organoids

To generate iPSCs, somatic cells can be reprogrammed by:
- Cre-Lox
- mRNA or miRNA transfection
- Plasmids

Cell sources in 3D cell culture



- Cell lines
- Primary cells
  - Somatic cells
  - Pluripotent stem cells
  - Adult stem cells
- Patient-derived cells

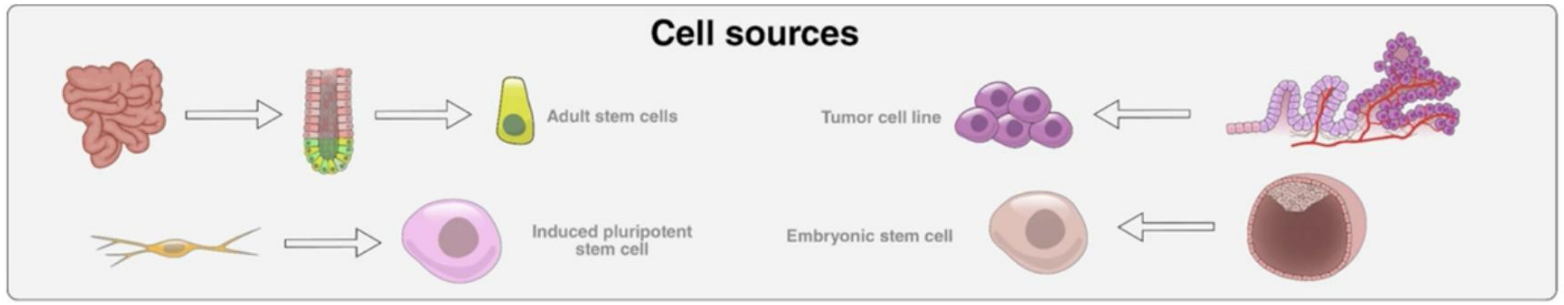
Adult stem cells

Multipotent cells that are committed to the lineage from which they derive

- Intestinal epithelium

- Adult neural stem cells

Cell sources in 3D cell culture



- Cell lines
- Primary cells
  - Somatic cells
  - Pluripotent stem cells
  - Adult stem cells
- Patient-derived cells

Patient-derived cells

- Cells – normal or diseased - obtained from primary tissues
- Important applications in cancer research

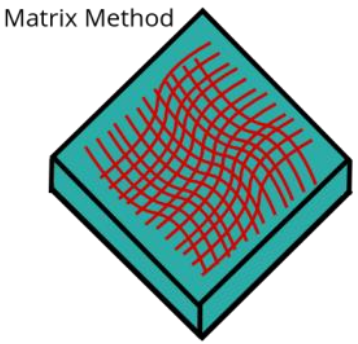
Methods to create 3D cell cultures

Organoids and spheroids can be:

- **Marix-dependent:** used to ease the transition from 2D monolayers to 3D models. E.g.: hydrogels, peptide hydrogels, polysaccharides, collagen, laminin, alginate, commercially available matrices.

  Possible to manipulate and tune scaffold composition through the incorporation of different factors to promote cell survival, migration, and differentiation in a 3D context

Scaffold Techniques



Extracellular matrices (ECMs) are one of the most commonly used matrices – best mimic the *in vivo* environment

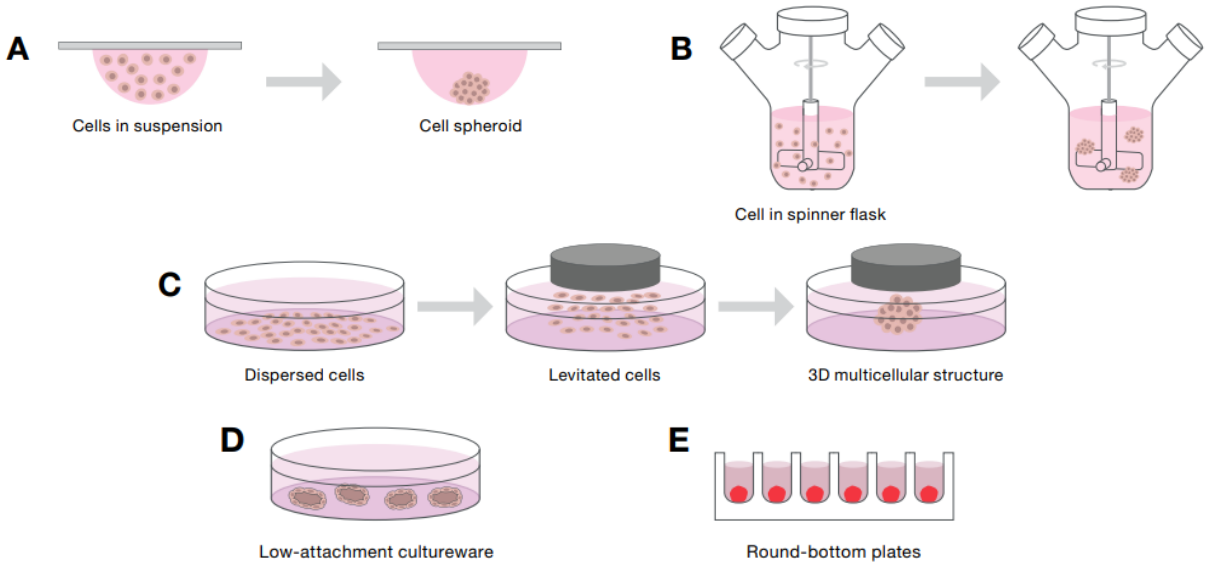
- Preserve biological architecture
- Promote normal biological functions
- Maintain integrin binding sites

- Have inherent lot-to-lot variability – ECMs of animal origin

Methods to create 3D cell cultures

Organoids and spheroids can be:

- **Matrix-independent:** systems provide conditions for cells to create their own ECM



(A) Hanging-drop culture.

(B) Spinner flask culture.

(C) Magnetic levitation of cells preloaded with magnetic nanoparticles.

(D) Low-attachment plates.

(E) Static suspension in round-bottom 3D culture plates.

- Lower cost and ease of use
- Less variability that ECM of animal origin
- Large-scale cultures

-Variability in spheroid size

Bibliography

- Simian M, Bissell MJ. Organoids: A historical perspective of thinking in three dimensions. J Cell Biol. 2017 Jan 2;216(1):31-40. doi: 10.1083/jcb.201610056.
- Gulino M, Kim D, Pané S, Santos SD, Pêgo AP. Tissue Response to Neural Implants: The Use of Model Systems Toward New Design Solutions of Implantable Microelectrodes. Front Neurosci. 2019 Jul 5;13:689. doi: 10.3389/fnins.2019.00689.
- Carter, M., & Shieh, J. C. (2010). Cell Culture Techniques. Guide to Research Techniques in Neuroscience, 281–296. doi:10.1016/b978-0-12-374849-2.00013-6
- Li, Z. (2011). In Vitro Micro-Tissue and -Organ Models for Toxicity Testing. Comprehensive Biotechnology, 551–563. doi:10.1016/b978-0-08-088504-9.00503-1
- Kelava I, Lancaster MA. Dishing out mini-brains: Current progress and future prospects in brain organoid research. Dev Biol. 2016 Dec 15;420(2):199-209. doi: 10.1016/j.ydbio.2016.06.037.
- Chiaradia, I., Lancaster, M.A. Brain organoids for the study of human neurobiology at the interface of in vitro and in vivo. *Nat Neurosci* **23,** 1496–1508 (2020). doi: 10.1038/s41593-020-00730-3
- Hattori N. Cerebral organoids model human brain development and microcephaly. Mov Disord. 2014 Feb;29(2):185. doi: 10.1002/mds.25740.
- Qiu, B., Bessler, N., Figler, K., Buchholz, M.-B., Rios, A. C., Malda, J., Levato, R., Caiazzo, M., Bioprinting Neural Systems to Model Central Nervous System Diseases. Adv. Funct. Mater. 2020, 30, 1910250. doi.org/10.1002/adfm.201910250
- Bhatia SN, Ingber DE. Microfluidic organs-on-chips. Nat Biotechnol. 2014 Aug;32(8):760-72. doi: 10.1038/nbt.2989.
- Murphy, S. V., & Atala, A. (2014). *3D bioprinting of tissues and organs. Nature Biotechnology, 32(8), 773–785.* doi:10.1038/nbt.2958