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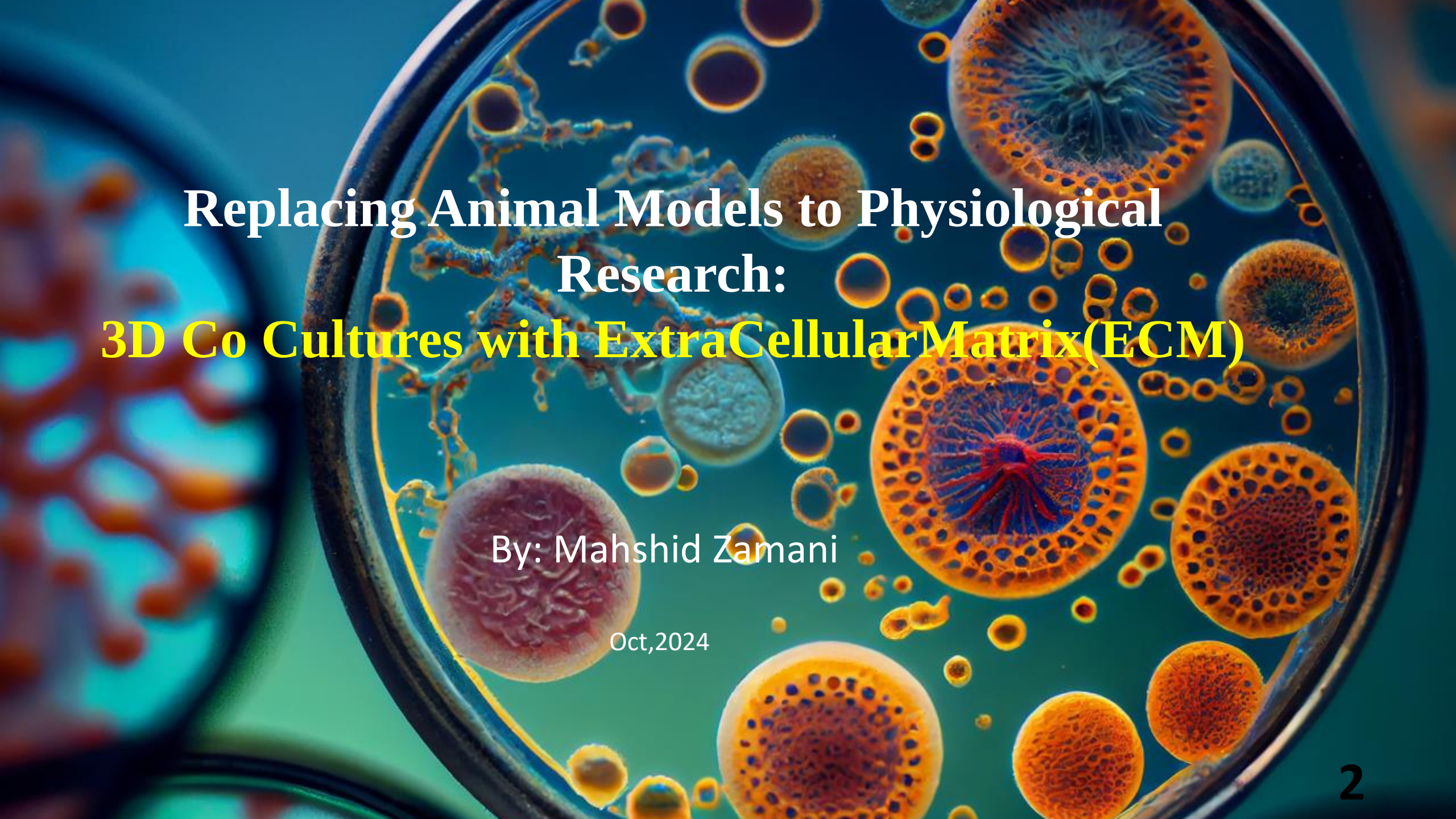


Replacing Animal Models to Physiological Research:

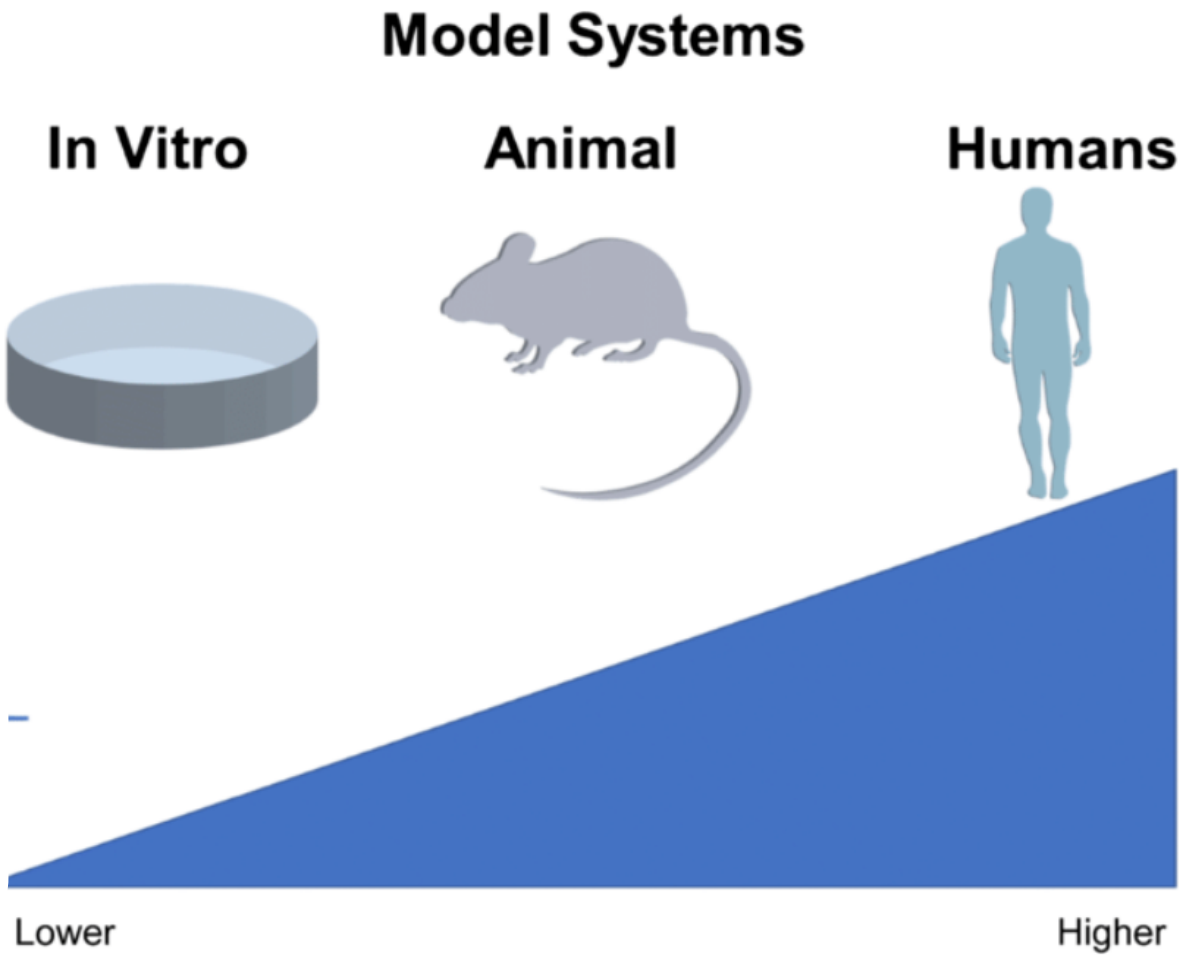
3D Co Cultures with ExtraCellularMatrix(ECM)

By: Mahshid Zamani

Oct,2024

❑ 1.Introduction

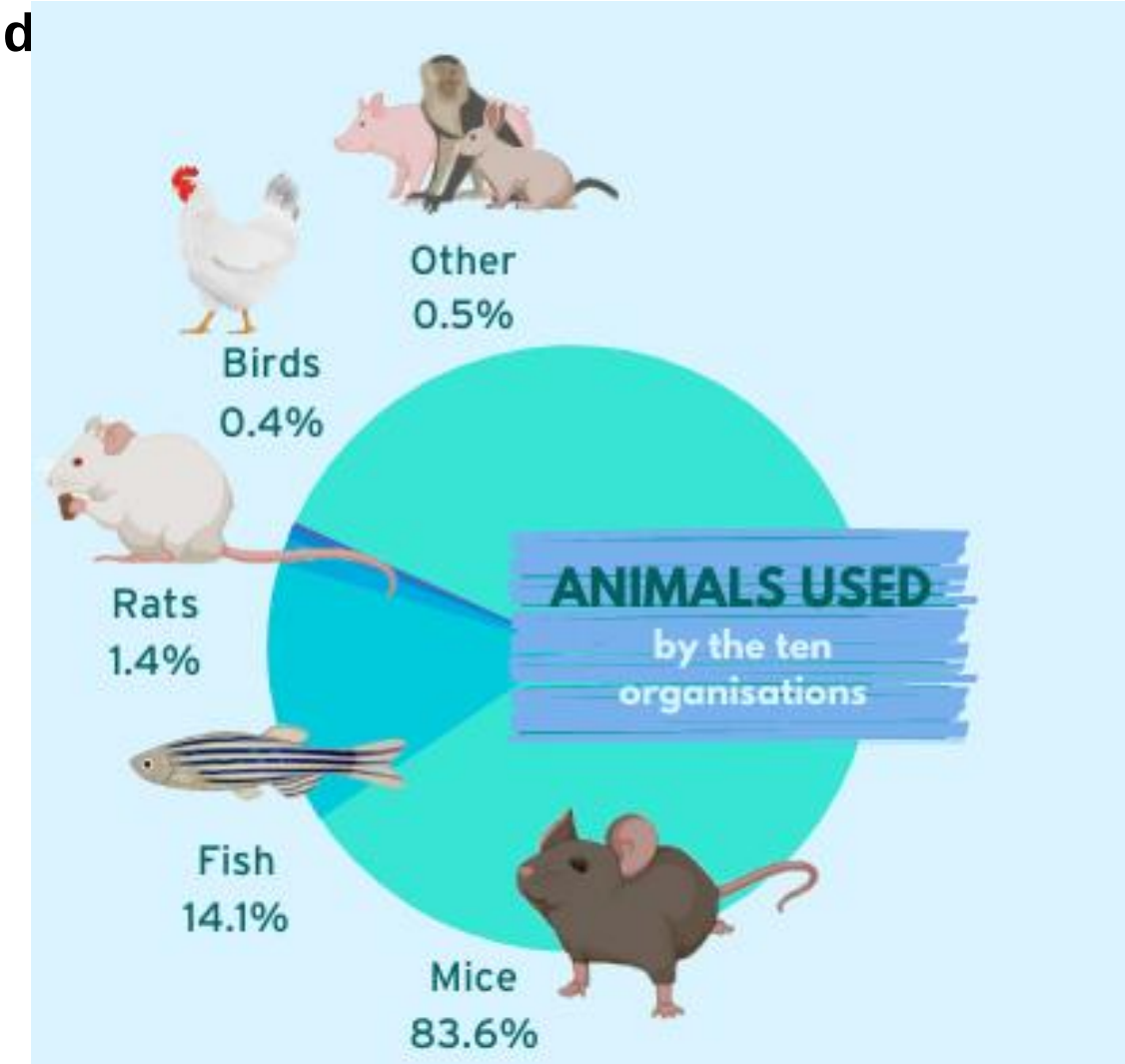
- Over the past few decades, researchers have made remarkable progress in developing accessible and informative **in vitro** models



➢ The reasons for limitations of animals model:

✓ **ethical concerns**

- **More than 110 million animals**, including mice, rats, birds, fish, pigs, cats, and rabbits, are killed annually in **U.S. laboratories** for various purposes such as **medical research, training, and**

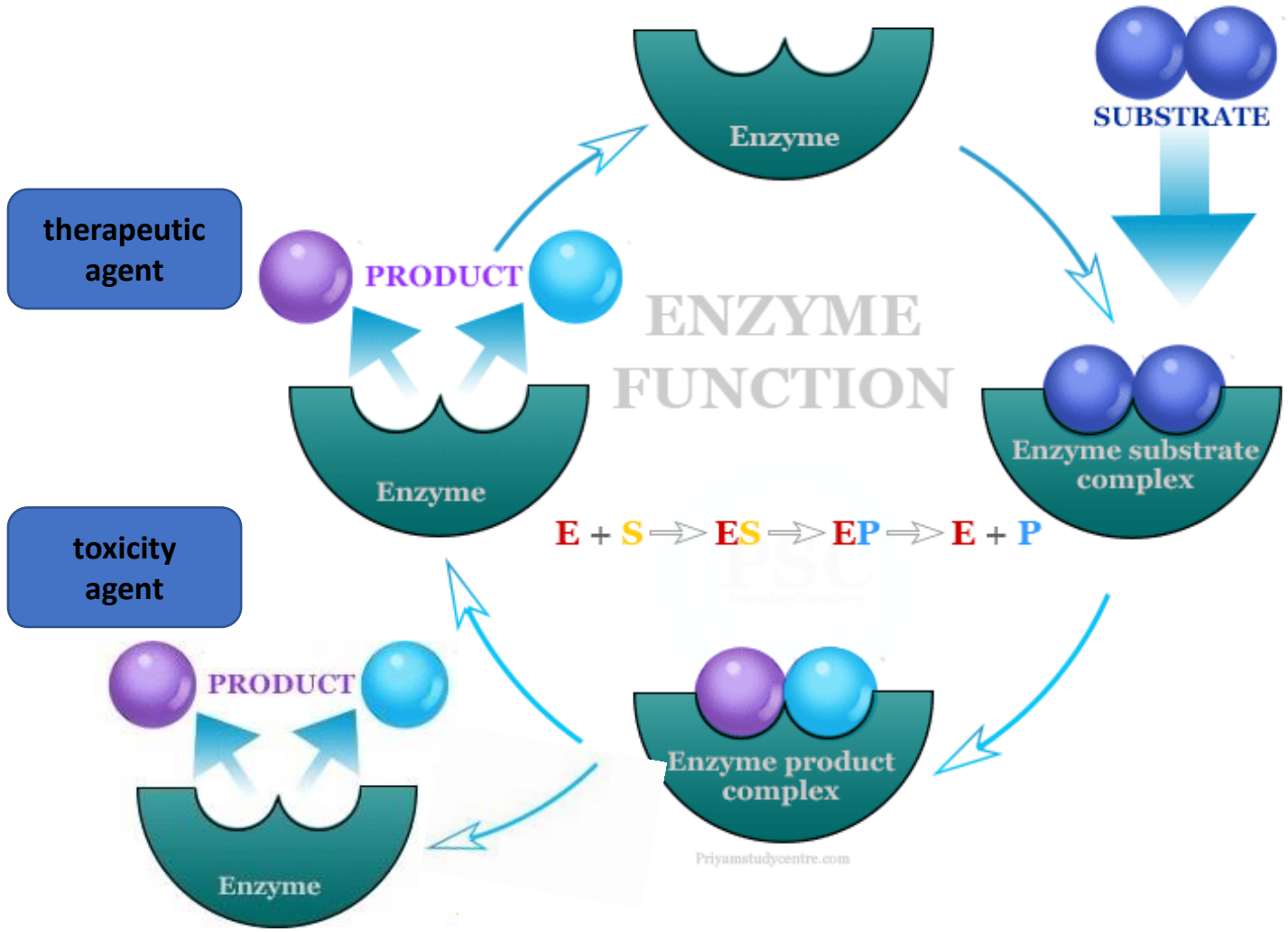


✓**inherent differences** compared to humans:

1. **Variations in drug-metabolizing enzyme activity** among species

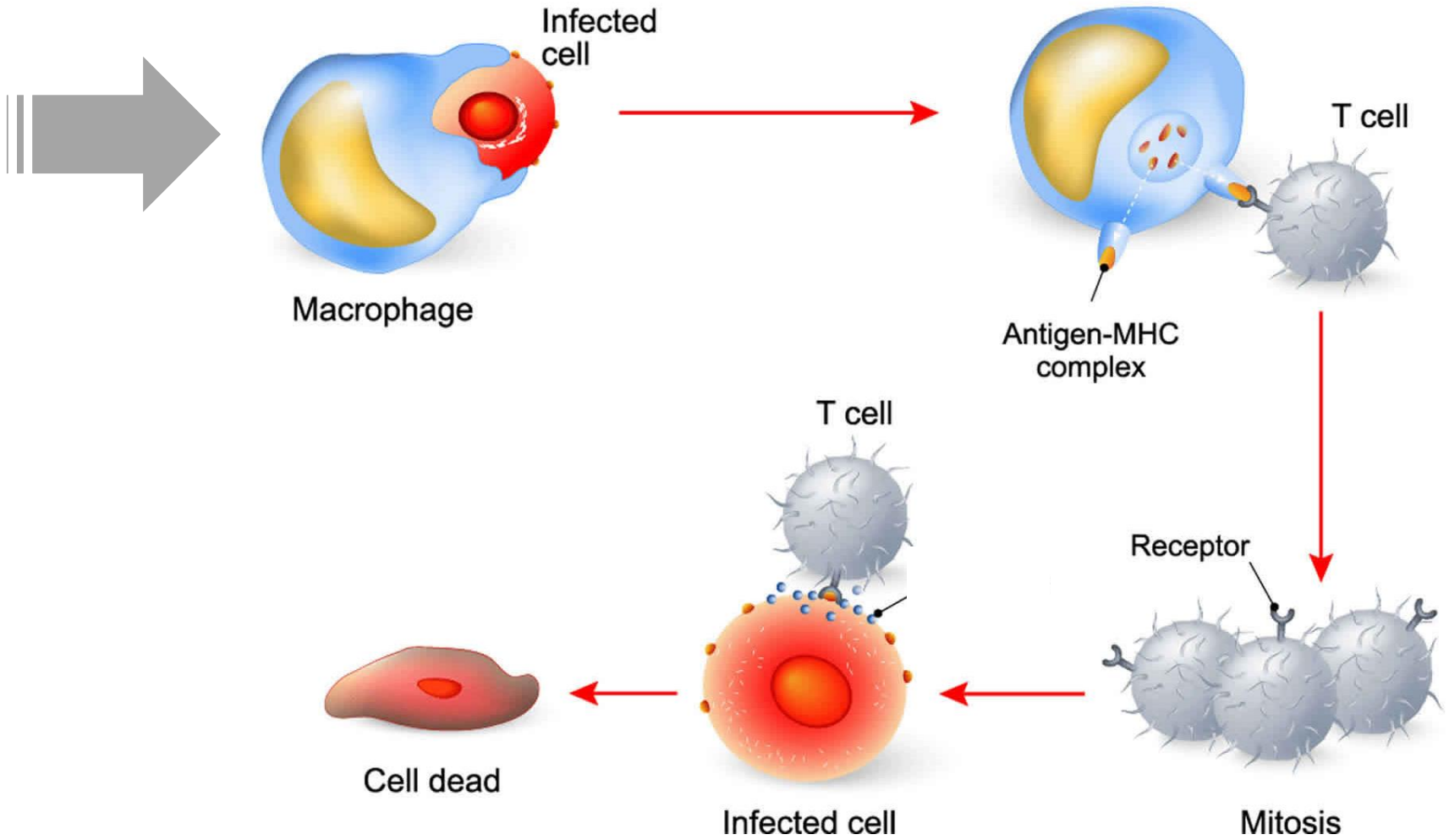
drug

$$\textbf{Efficacy} = \frac{\textbf{therapeutic index}}{\textbf{toxicity index}}$$

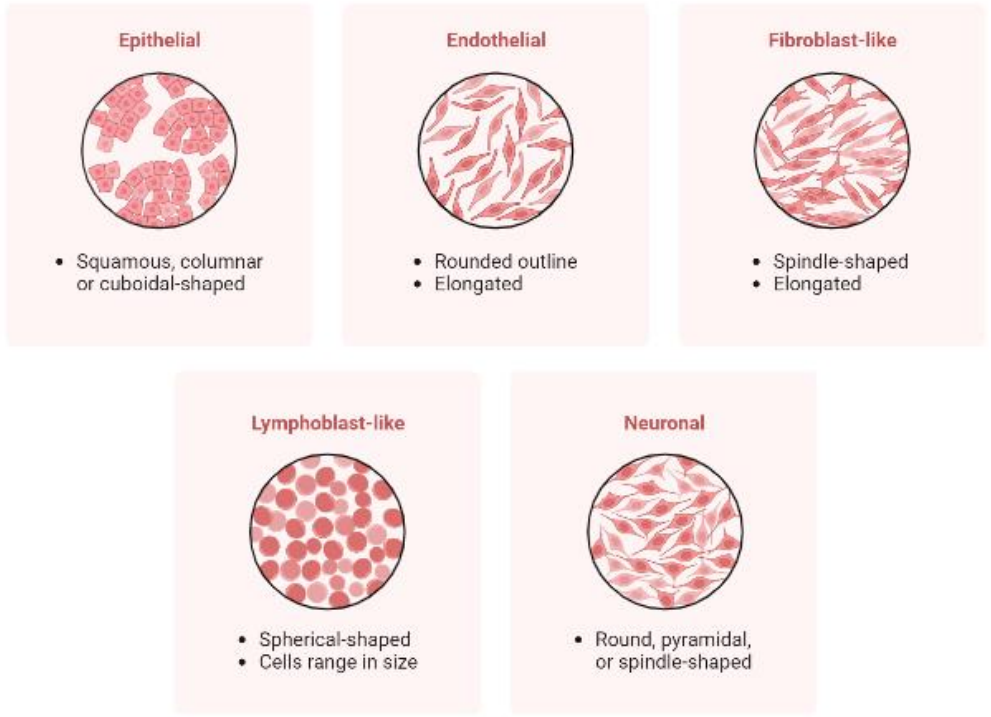


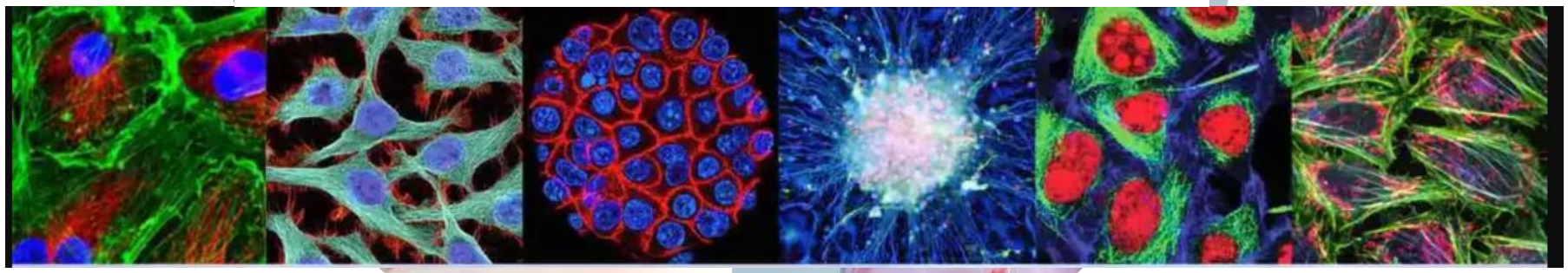
2. When **a model seems to accurately represent a disease**, variations in finer details such as **cellular receptors and immune signaling pathways** can **adversely affect** the assessment of potential therapies

immune signaling pathway

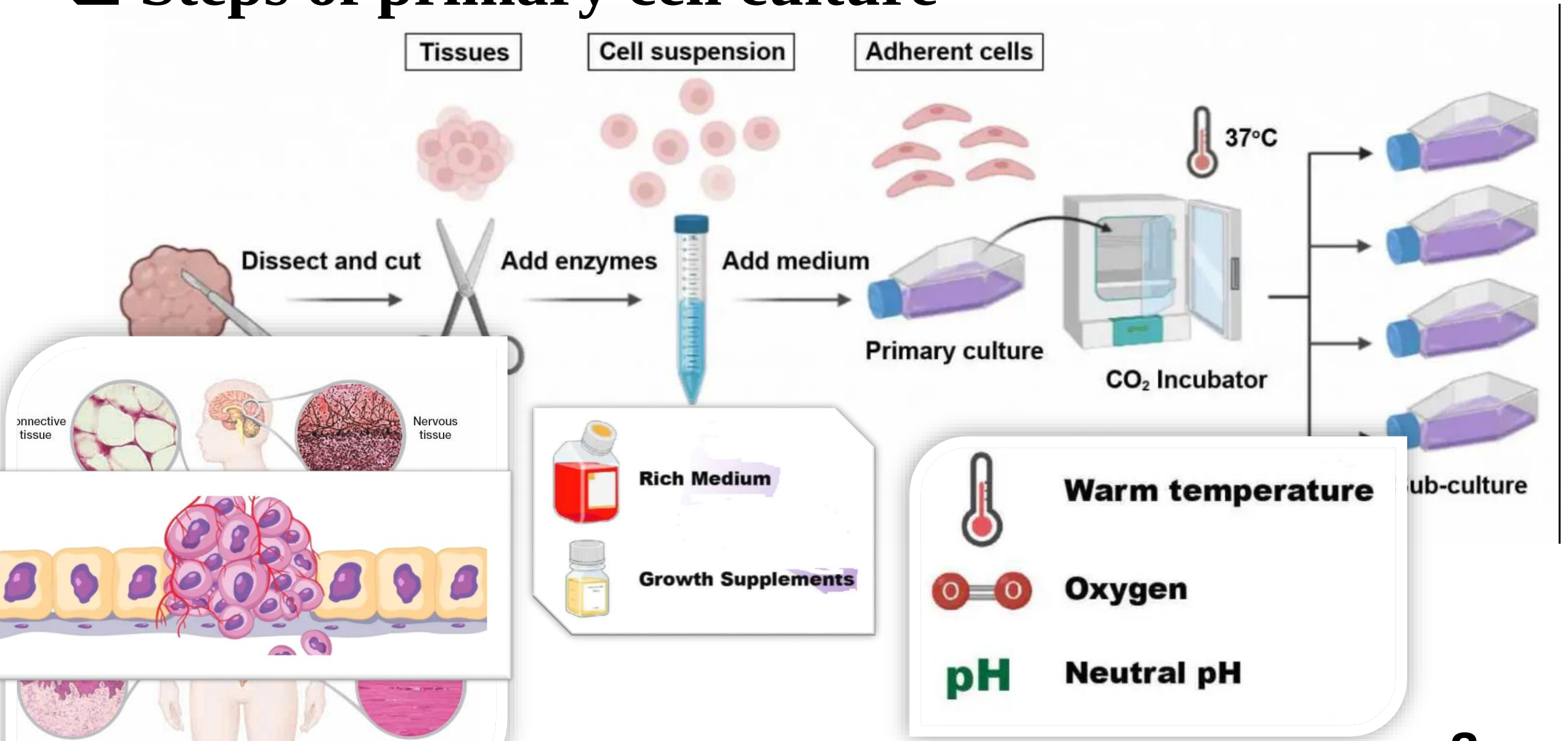


❑ In Vitro-2D


Epithelial
• Squamous, columnar or cuboidal-shaped
Endothelial
• Rounded outline
• Elongated
Fibroblast-like
• Spindle-shaped
• Elongated
Lymphoblast-like
• Spherical-shaped
• Cells range in size
Neuronal
• Round, pyramidal, or spindle-shaped



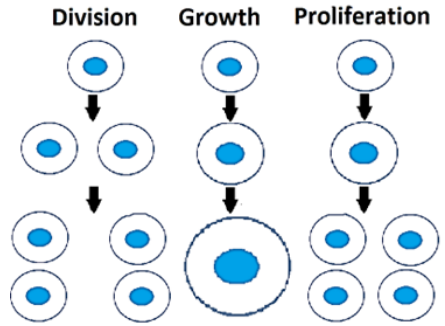
❑ Steps of primary cell culture

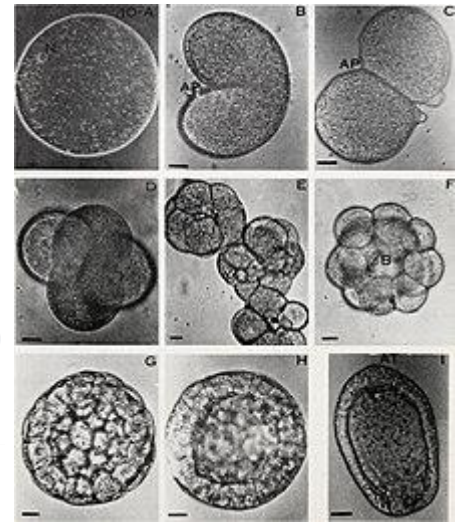


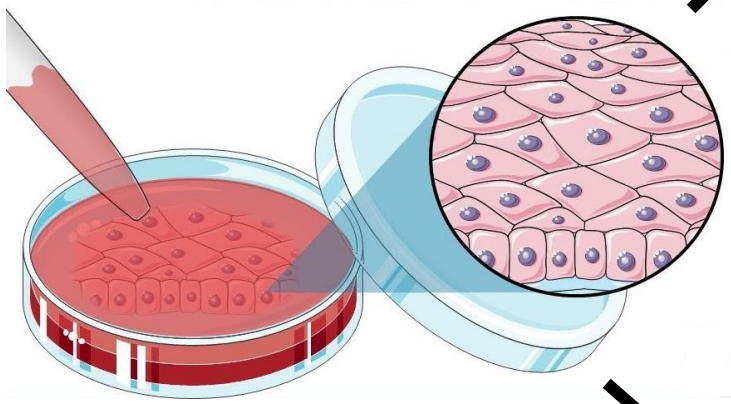
❑ Applications of 2D Cell Culture

effects of chemicals and biosafety of them

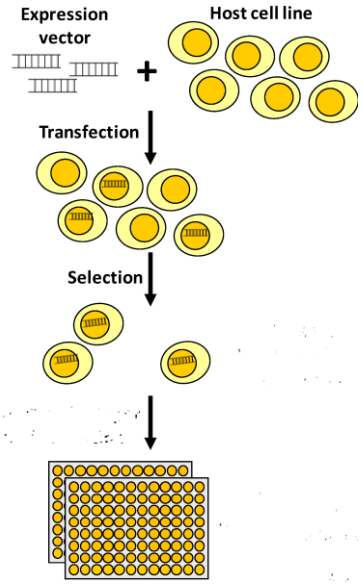
cell growth and proliferation



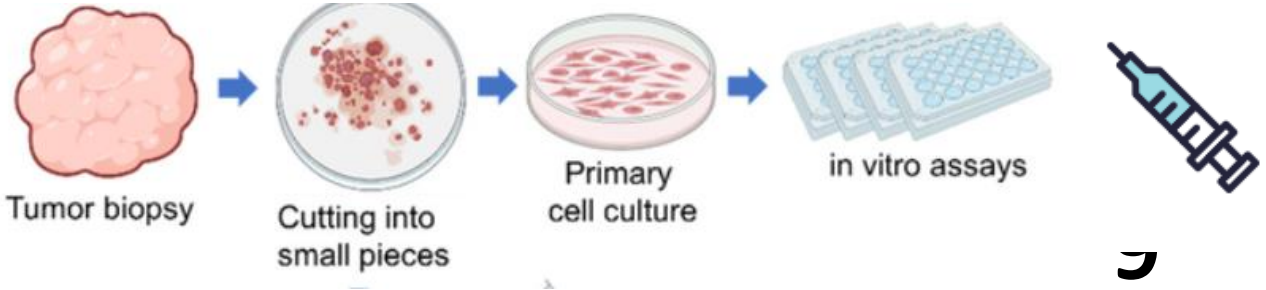




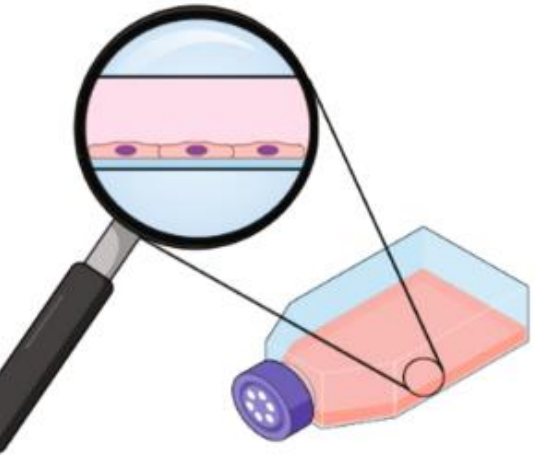
gene and protein expression
&
Vaccine and Antibody production

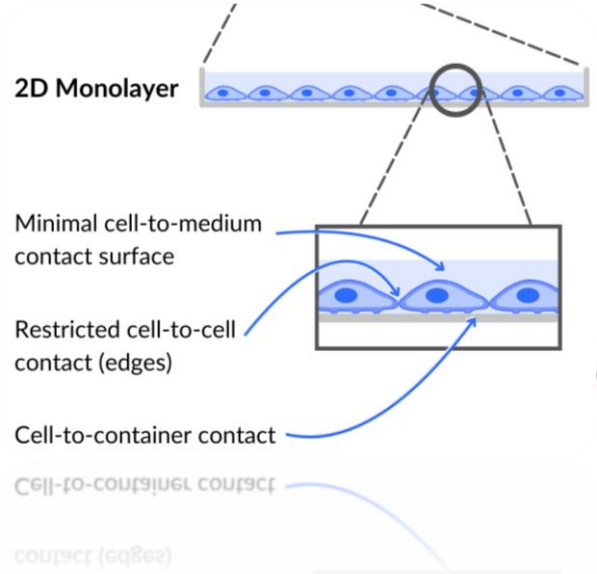


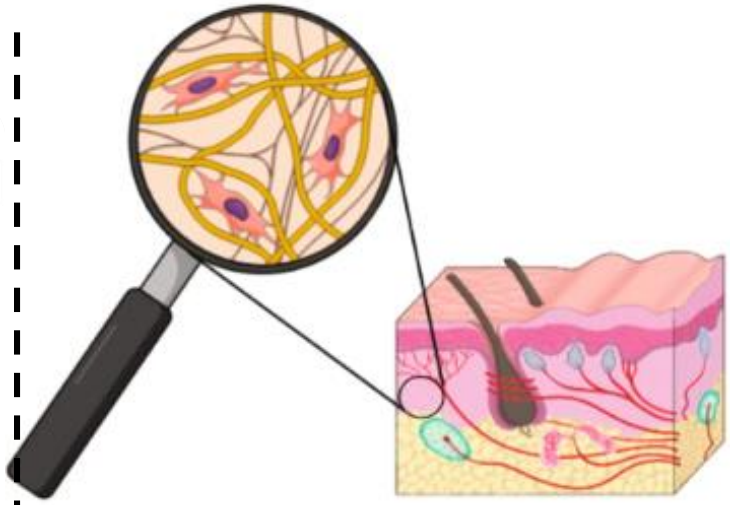
Modeling diseases and Development of new treatments

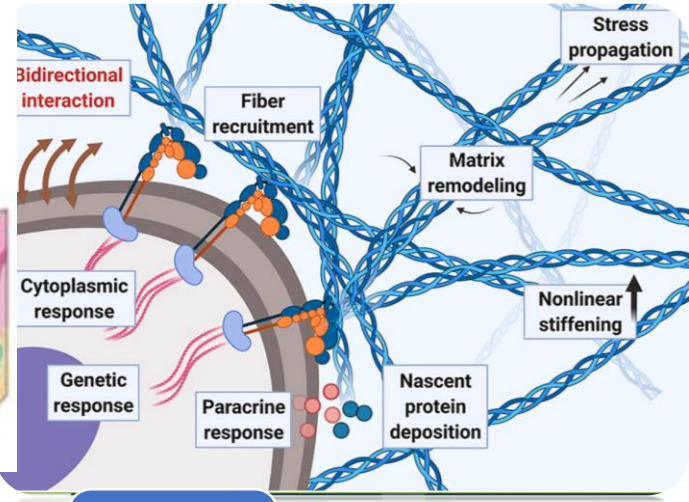


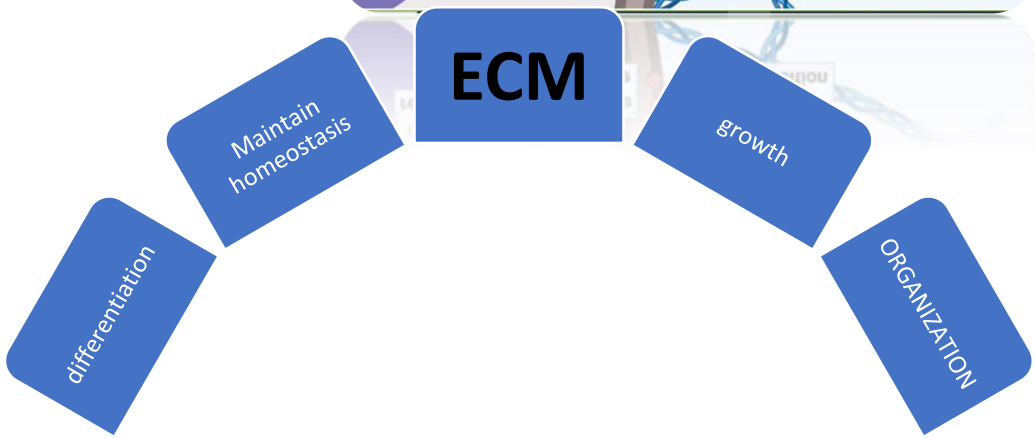
❑ Limitation of 2D cell culture





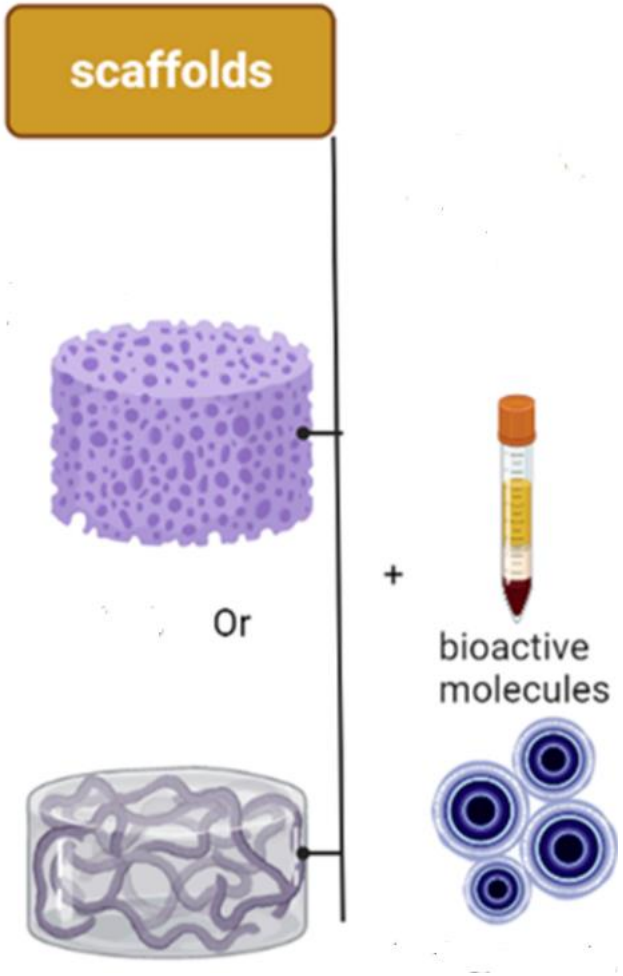


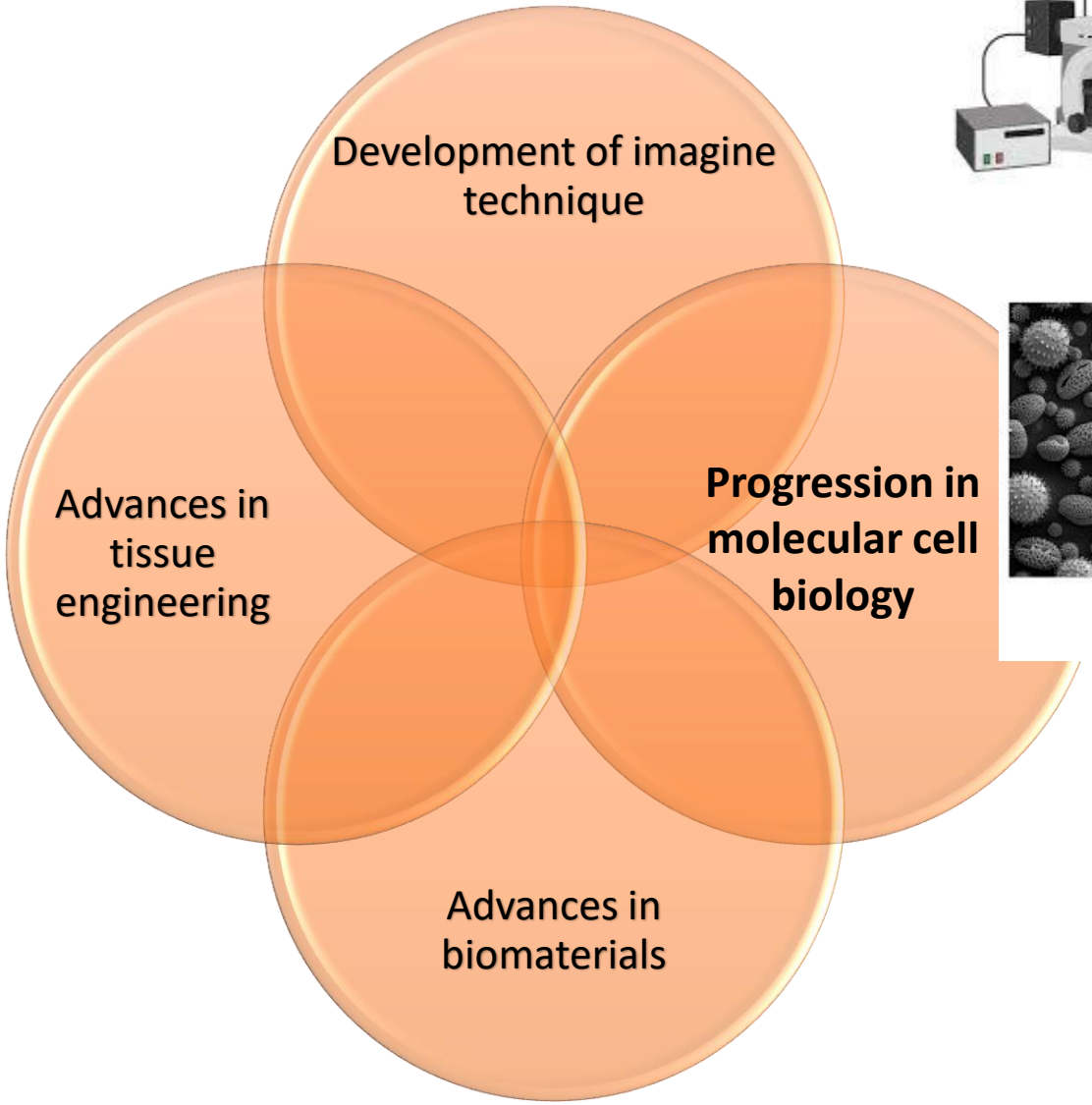


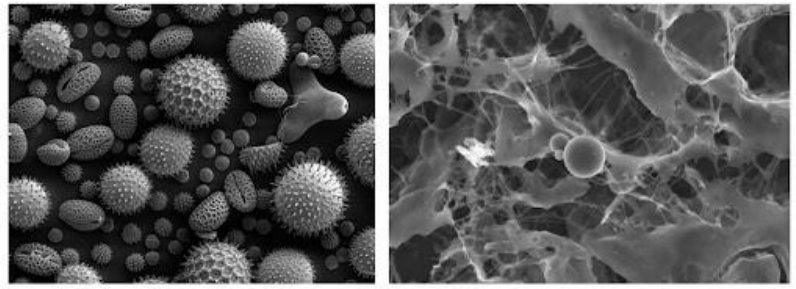


- The flat, two-dimensional surface is one of the most striking differences between 2D cell culture and the in vivo environment
- The absence of mechanical signals is another limitation
- Gene and protein expression patterns in 2D cultured cells often differ from those in native tissues

❑ Emergence of 3D cell culture



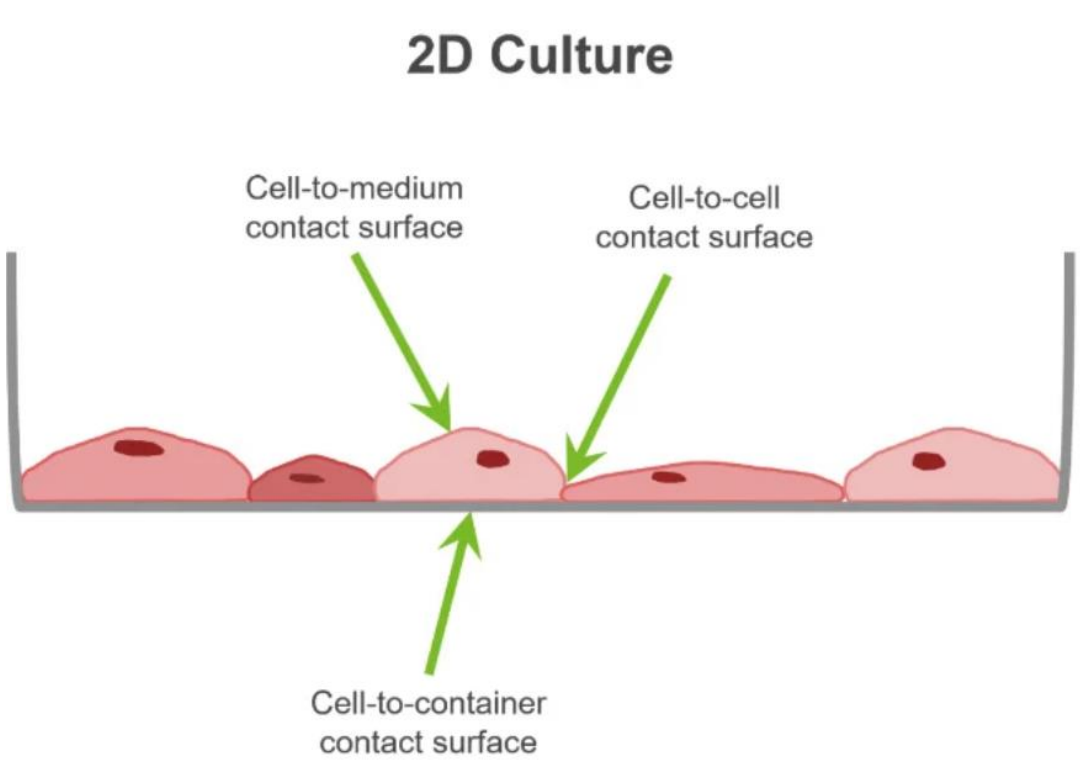


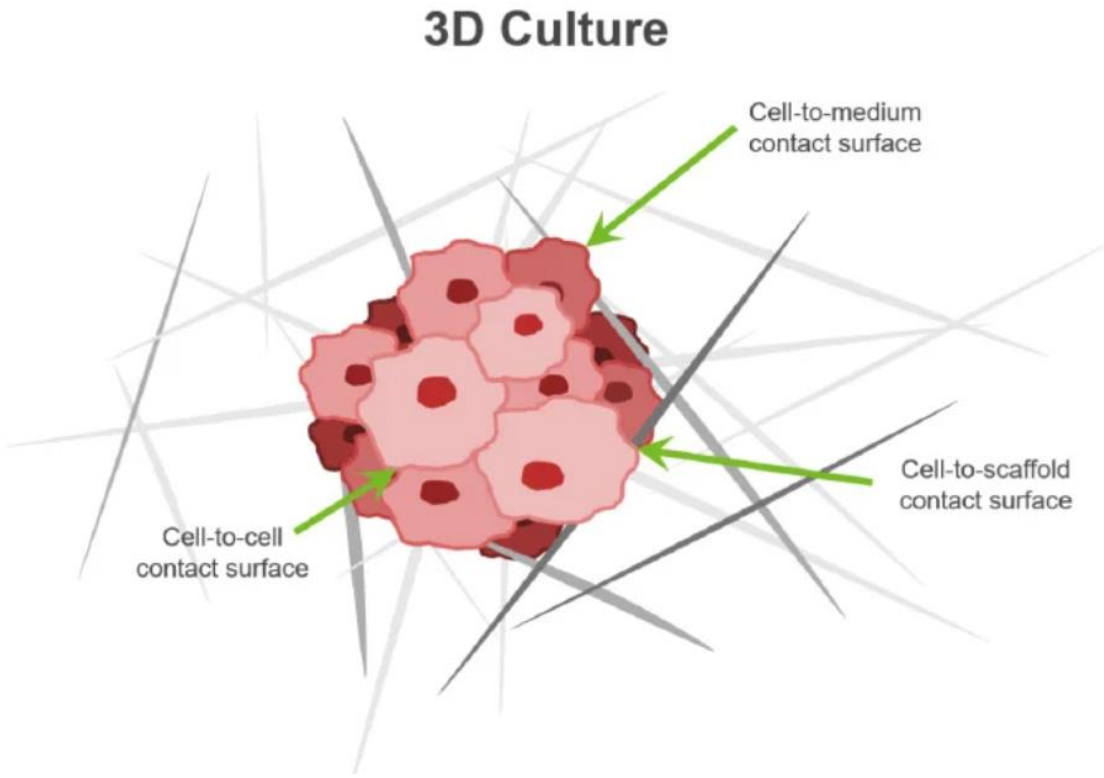


Samples of Electron Microscope

❑ Emergence of 3D cell culture

✓Cell interactions



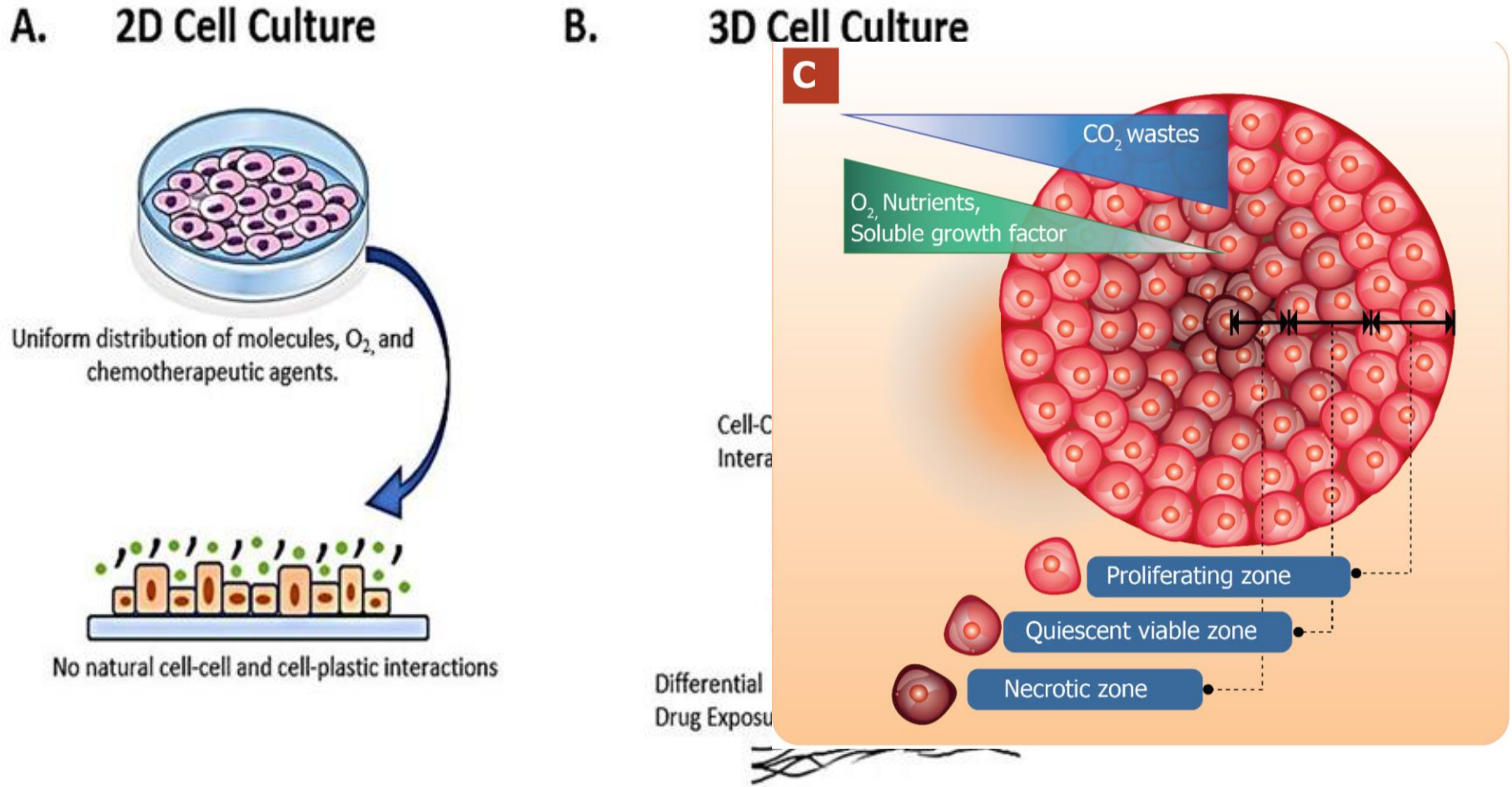


❑ Emergence of 3D cell culture

✓ Access of essential compound

- ➢ Oxygen
- ➢ Nutrient
- ➢ Metabolites
- ➢ Signaling molecules
- ➢ Drug exposure

Same in vivo

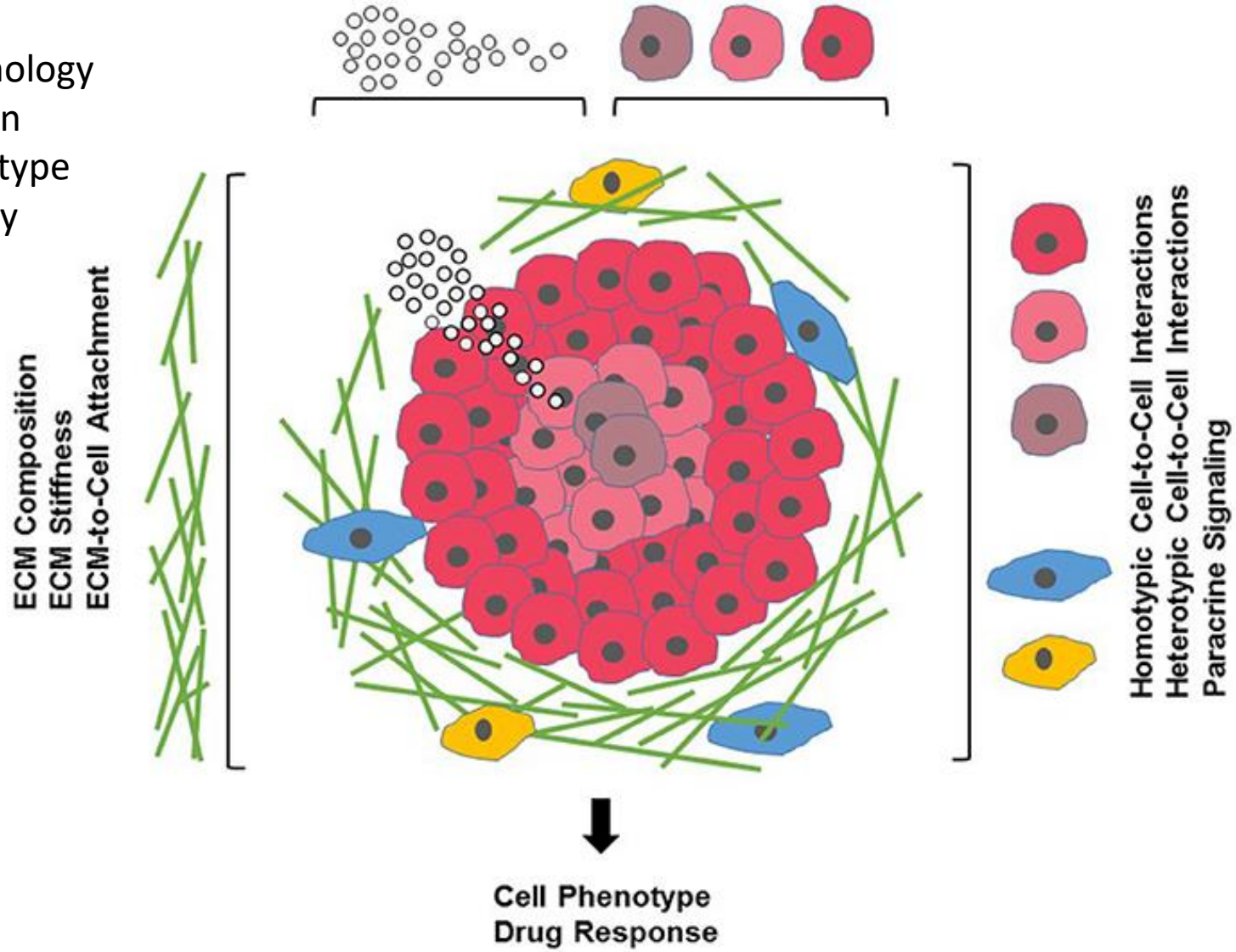


❑ Emergence of 3D cell culture

✓ Characteristic of cell
- ➢ Morphology
- ➢ Division
- ➢ Phenotype
- ➢ polarity

✓ Molecular mechanism

gene expression



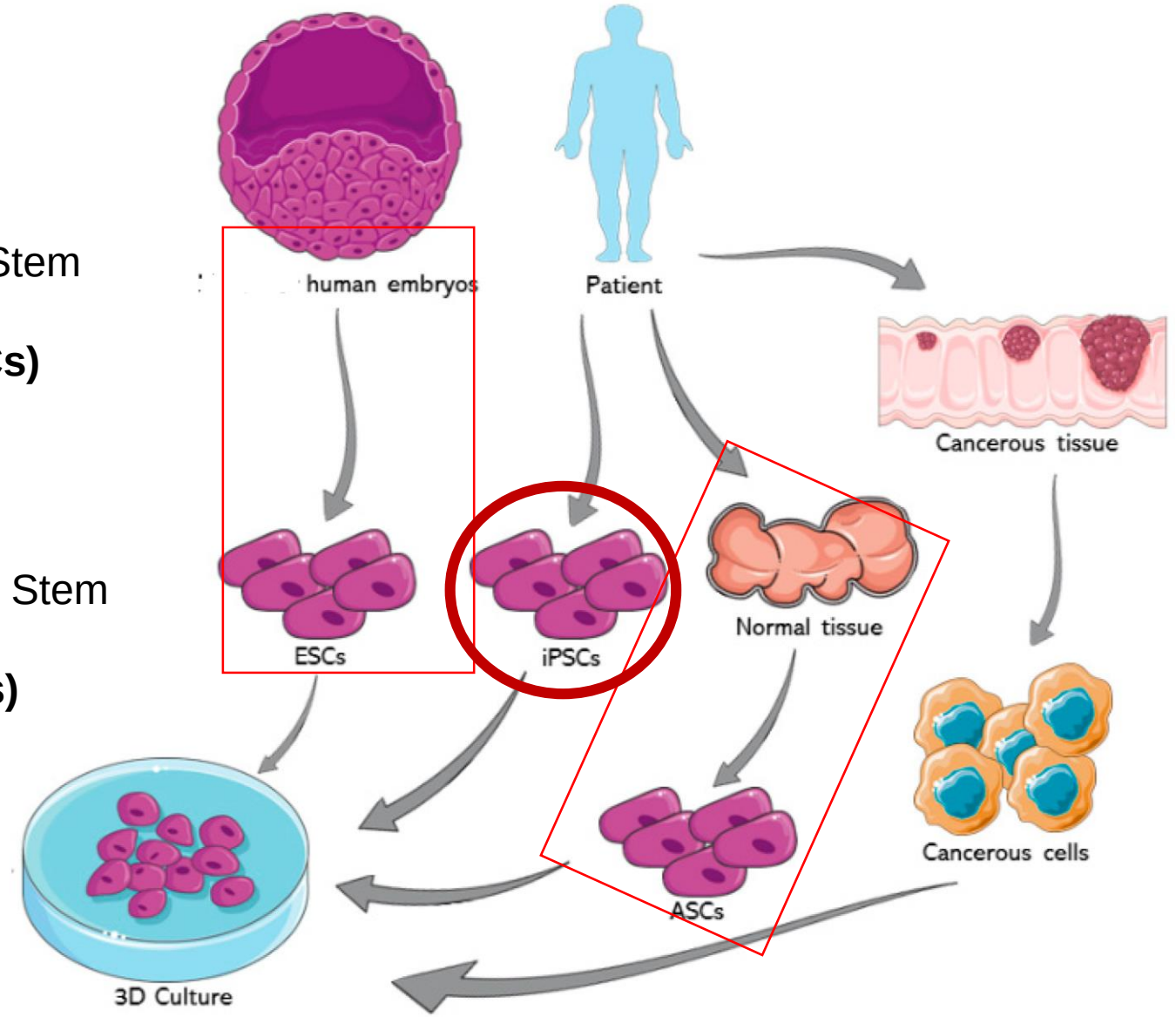
❑ Source of cells used in 3D culture

•Highly pluripotent, able to differentiate into any cell type
•Subject to ethical debates due to their source

Embryonic Stem Cells
(ESCs)

•Adult stem cells derived from fat tissue
•Can differentiate into connective tissue cells such as bone, cartilage and fat.

Adipose-derived Stem Cells
(ASCs)

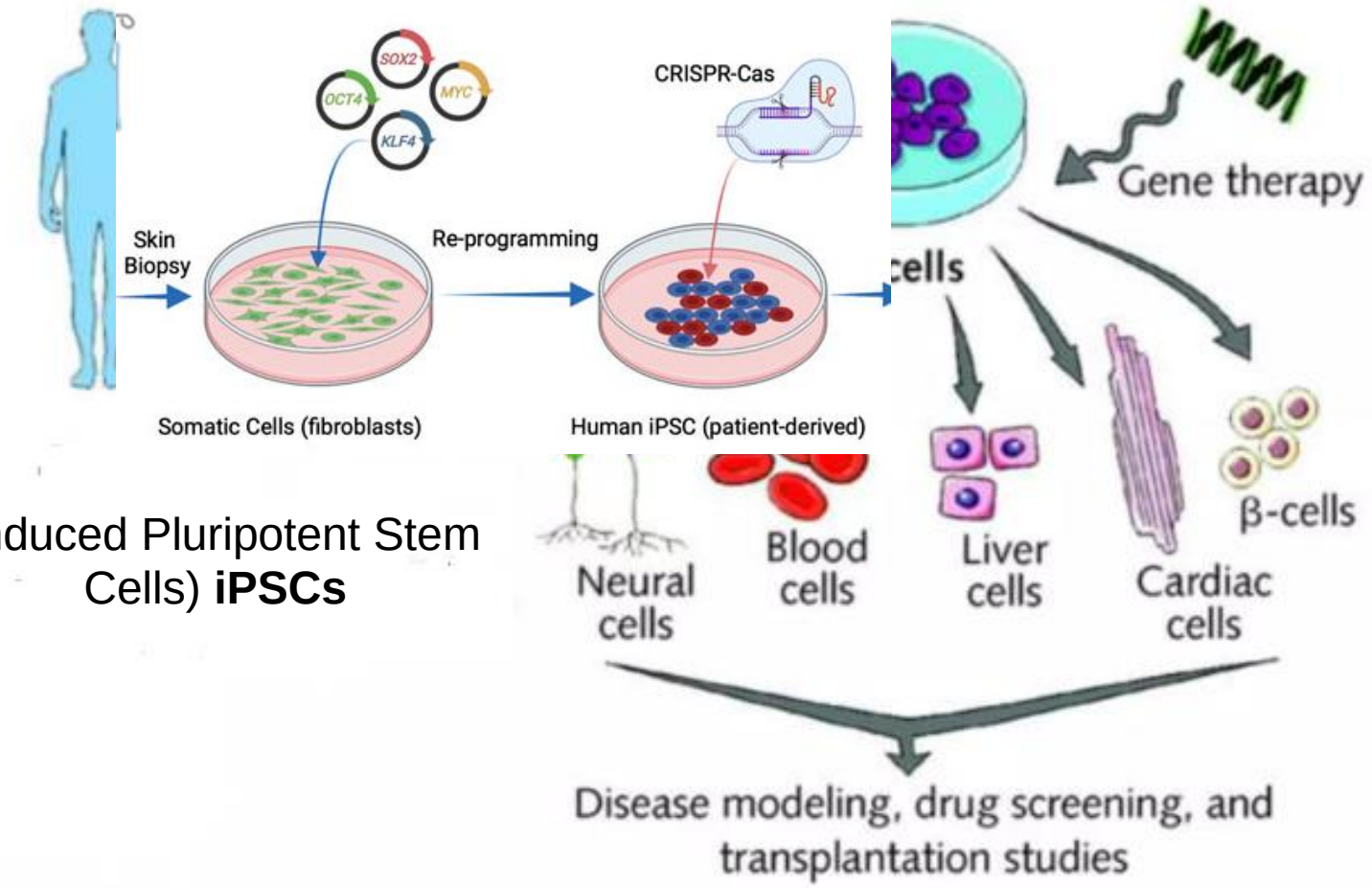


❑ Source of cells used in 3D culture

➢ personalization

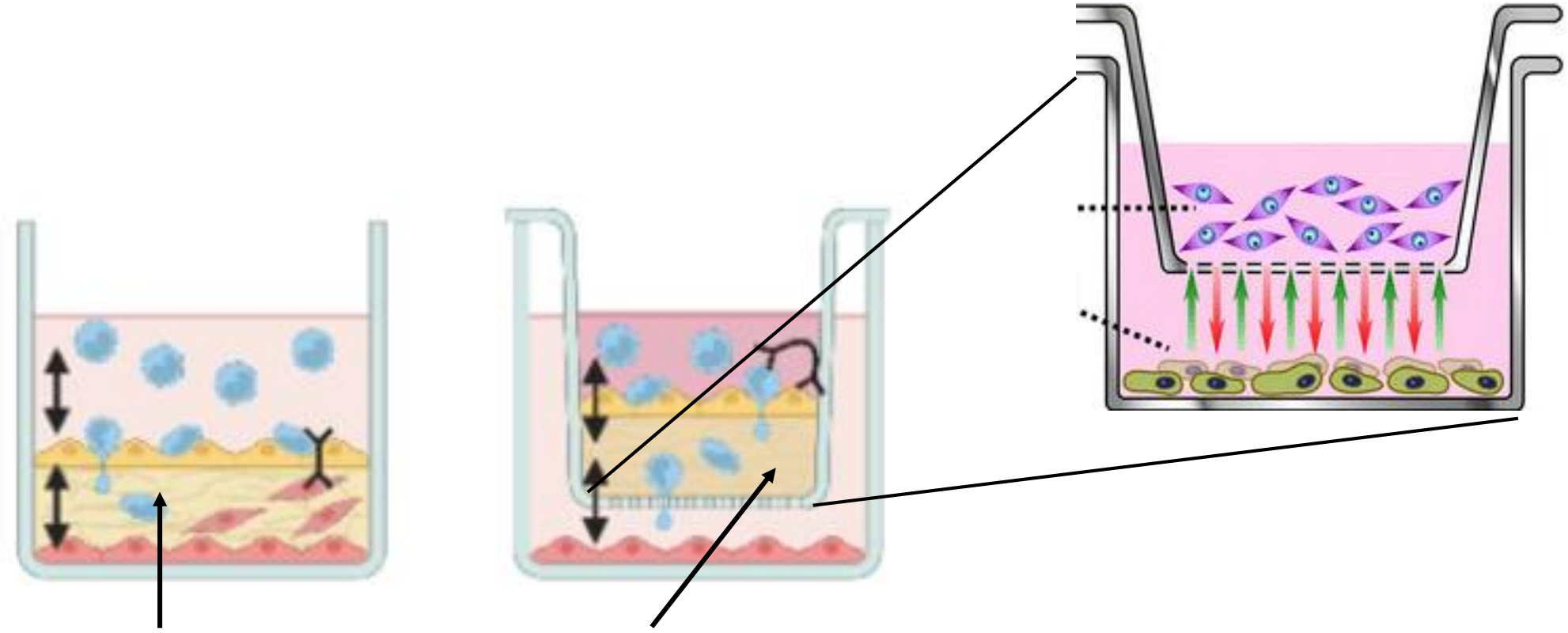
•Created by reprogramming adult somatic cells
•Can differentiate into many cell types
•Avoid ethical concerns associated with embryonic stem cells

(Induced Pluripotent Stem Cells) **iPSCs**



❑ 3D co-cultures with Extra Cellular Matrix(ECM)

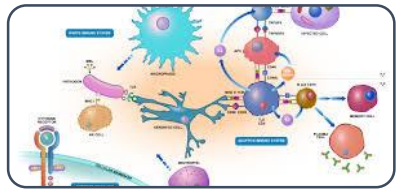
- 3D co-cultures involve growing **two or more different cell types** together in a **three-dimensional environment**, often integrated with **ECM** components



❑ Application of 3D co-cultur

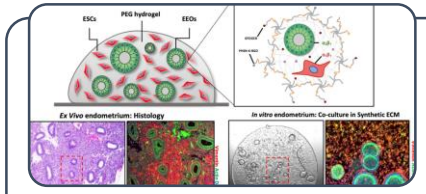
Modeling of diseases

- **Tumorization**
- **Infectious diseases**



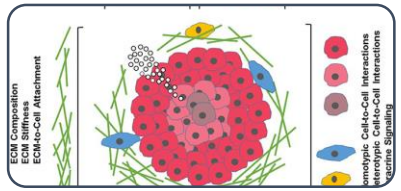
Drug screening

- Discovery of new drugs
- Treatment Personalization



Tissue engineering

- Production of replacement tissues
- Transplant testing



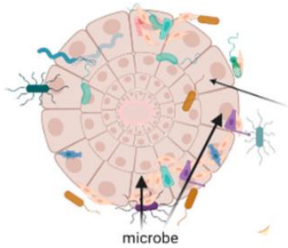
Studying the mechanisms of the disease

- Cell interaction
- Effects of the environment:

Development of new treatment methods

- **Cell therapy**
- **Gene therapy**

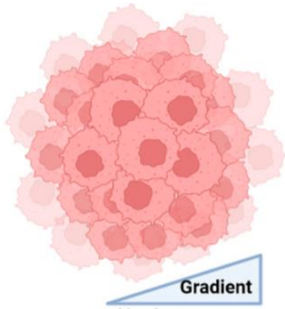
A **Microbe-co-culture**



Applications:

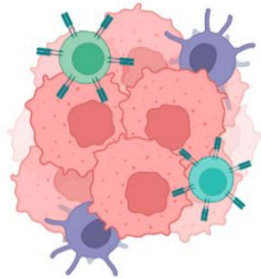
1. Infection model
2. Host-microbe contact
3. Drug screening against to microbe

B **Drug Screening**



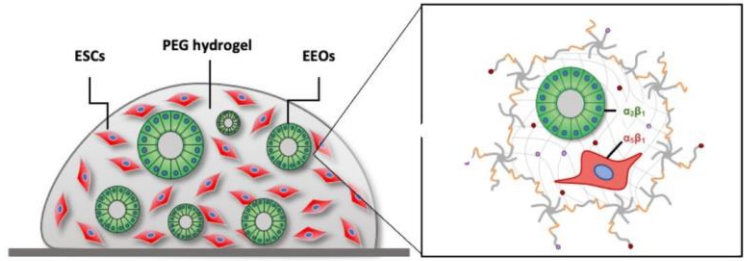
1. Drug model
2. Cell-cell or-ECM interaction similar to in vivo

C **Interaction of immune cells and tumor cells**

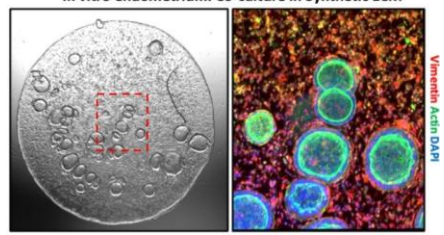


1. Cell-cell imaging
2. Cell-cell labeling
3. Cell-cell function exploitatio

Tissue engineering the endometrial microenvironment in 3D



In vitro endometrium: Co-culture in Synthetic ECM



❑ Applications of 3D Co-Cultures with ECM in diabetes

1. Modeling Pancreatic Function

2. Drug Discovery

3. **Stem Cell Transplantation**

- ✓ **The extracellular matrix (ECM**) is the scaffolding in which cells are located and provides them with the structure and signals they need for proper **growth and function**.

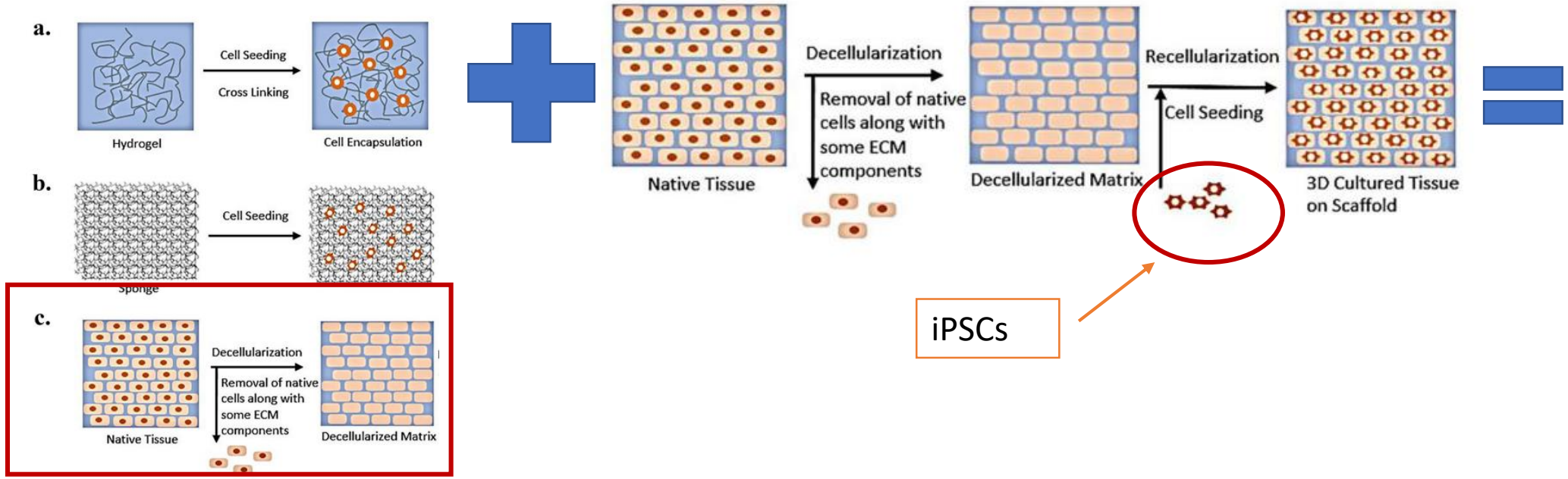
- ✓ In the field of **diabetes treatment**, scientists have realized that using ECM, insulin-producing cells can be cultured in an environment similar to the natural environment of the pancreas, and then these cells can be transplanted into the body of diabetic patients.

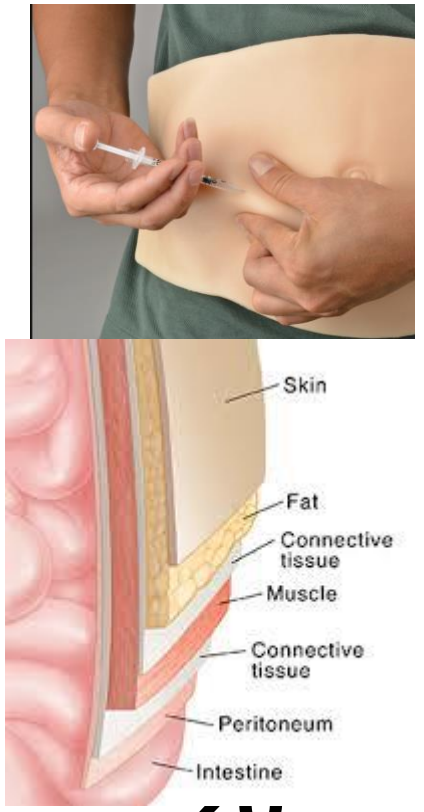
❑ Applications of 3D Co-Cultures with ECM in diabetes

1.ECM(Decellularization of native tissue)

2.Cultivation of beta cells

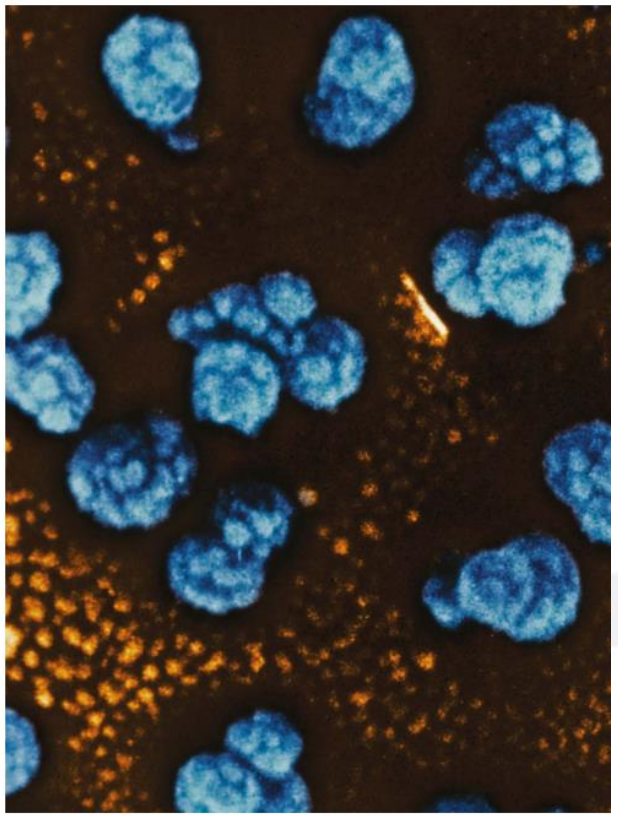
3. Transplantation to the patient's body



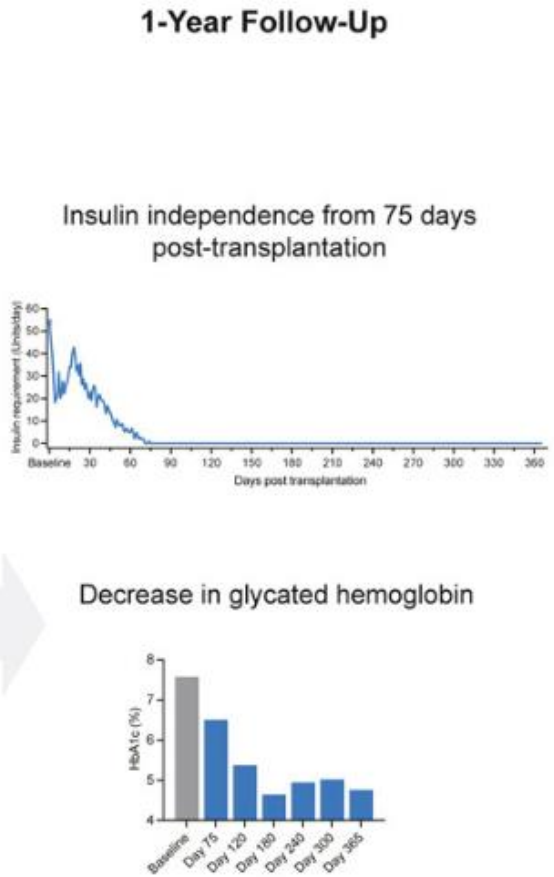


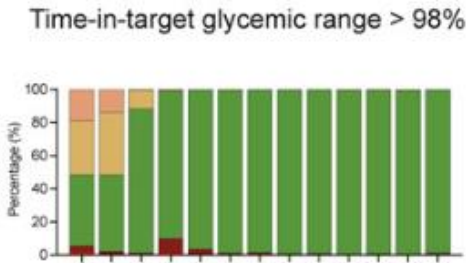
❑ Applications of 3D Co-Cultures with ECM in diabetes

- A groundbreaking case involved a **25-year-old woman** in Tianjin who began producing her own insulin less than three months after receiving a **transplant of reprogrammed stem cells derived from her own body**(Wang et al., 2024)**.**
- This approach utilized chemically induced pluripotent stem (iPS) cells to create 3D clusters of pancreatic islets, which were then transplanted into her **abdominal muscles**.
- The results have been remarkable, with the patient achieving insulin independence for over a year



A woman with type 1 diabetes started producing insulin (blue) after





❑ Applications of 3D Co-Cultures with ECM in MS

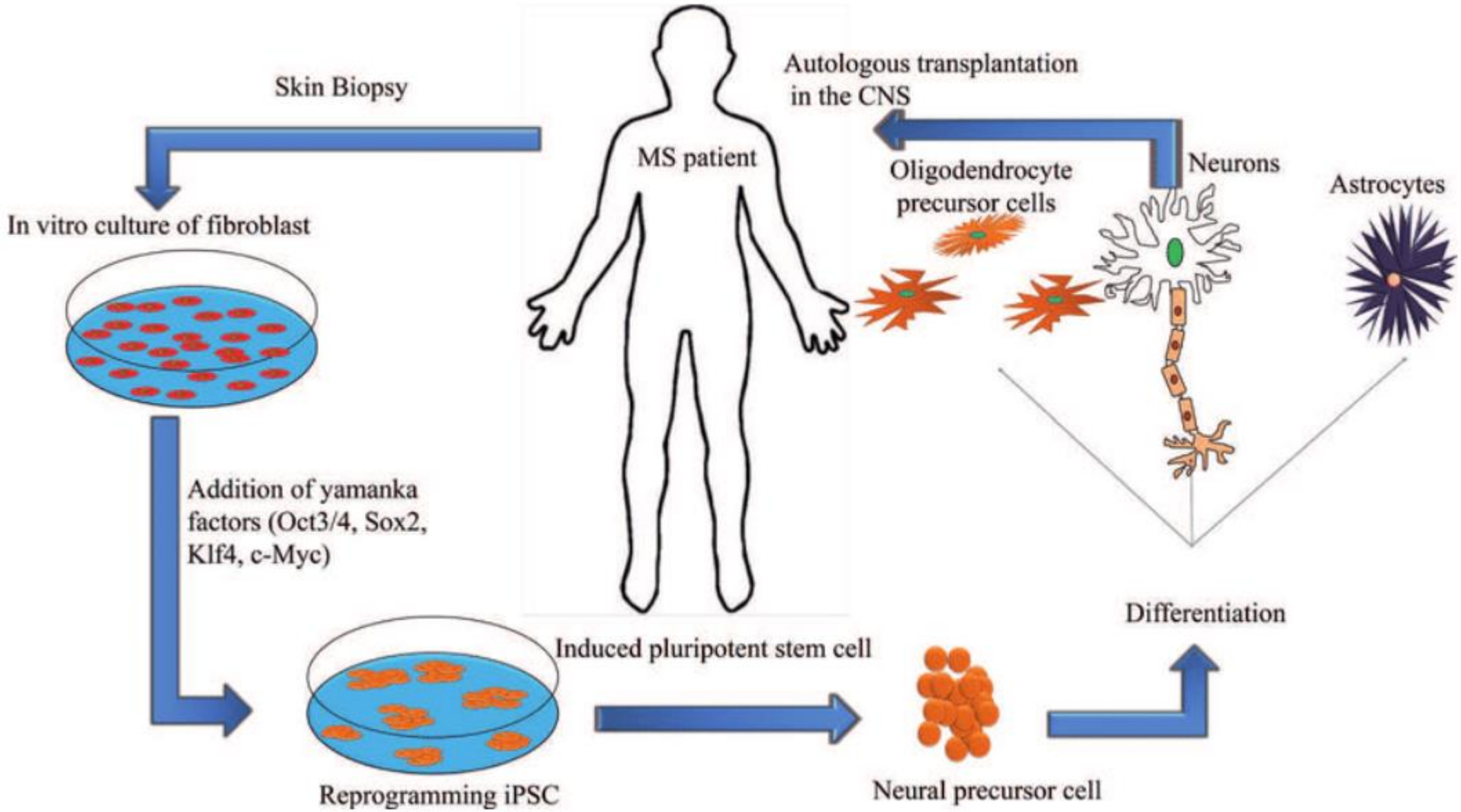
- Suitable cell lines for modeling MS disease in 3D culture:

- **1. Nerve cells**
- **Primary neurons**: These cells are isolated from the central nervous system of animals and can be used to study the direct effects of pathogens on neurons.
- **Neuronal cell lines**: Cell lines such as SH-SY5Y and PC12 are widely used in neuroresearch. These cell lines are derived from neural tumors and although they retain some characteristics of normal neurons, they may have lost some important functions.

- **2.glial cells**
- **Oligodendrocytes**: These cells are responsible for producing myelin, which is a protective covering around nerve axons. In MS, oligodendrocytes are destroyed, causing loss of myelin and impaired nerve transmission.
- **Astrocytes:** These cells play an important role in supporting neurons and regulating their environment. In MS, astrocytes are activated and can contribute to the destruction of myelin.
- **Microglia:** These cells are the innate immune system of the central nervous system and are activated in response to injury or infection. In MS, microglia are overactivated and can contribute to the destruction of myelin and neurons.

- **3.Immune cells**
- **T cells**: Autoimmune T cells in MS attack myelin, causing inflammation and destruction.
- **B cells**: B cells produce antibodies that can attack myelin.

❑ Applications of 3D Co-Cultures with ECM in MS



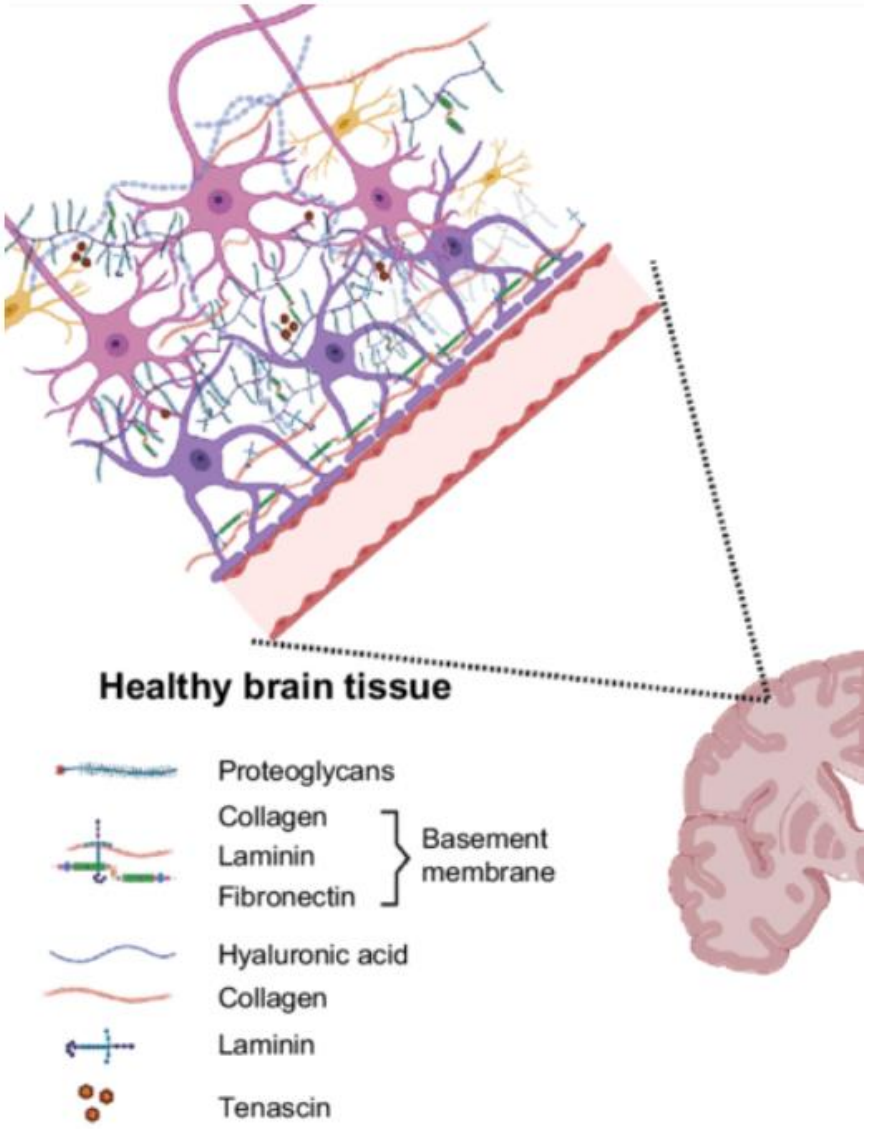
❑ Applications of 3D Co-Cultures with ECM in MS

Promising research results:

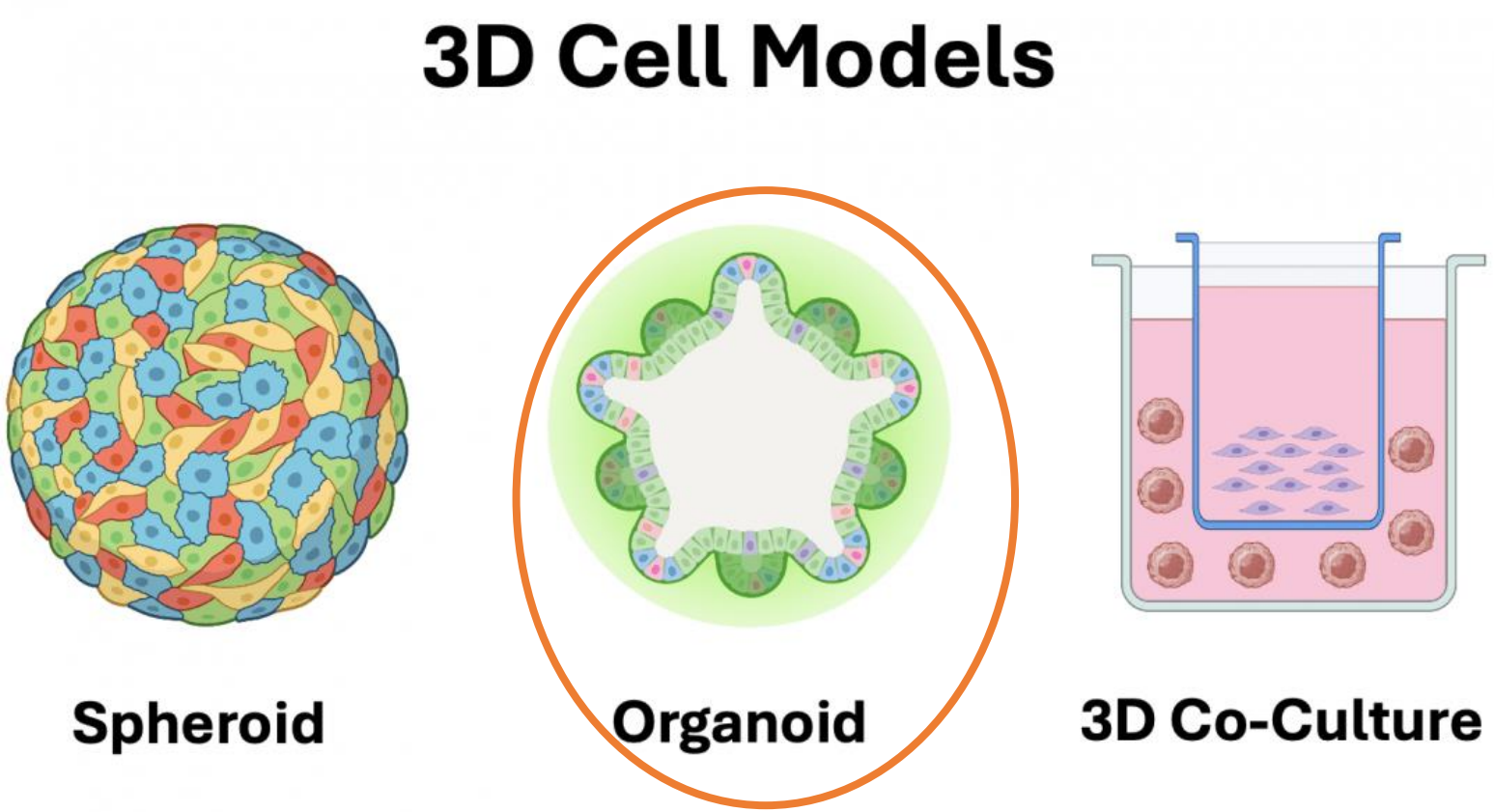
Development of neural scaffolds: Researchers are developing neural scaffolds derived from the extracellular matrix that can serve as a scaffold for the growth and repair of damaged nerve cells in MS patients.

Stem cell transplantation: Using 3D culture, stem cells can be differentiated into nerve cells and then transplanted to the site of damage in the brain(Yoon et al., 2021)(Vagaska et al., 2020).

Stimulation of myelination: Some studies show that 3D culture of myelin-forming cells (oligodendrocytes) can help stimulate remyelination and improve nerve function(Marangon et al., 2021).



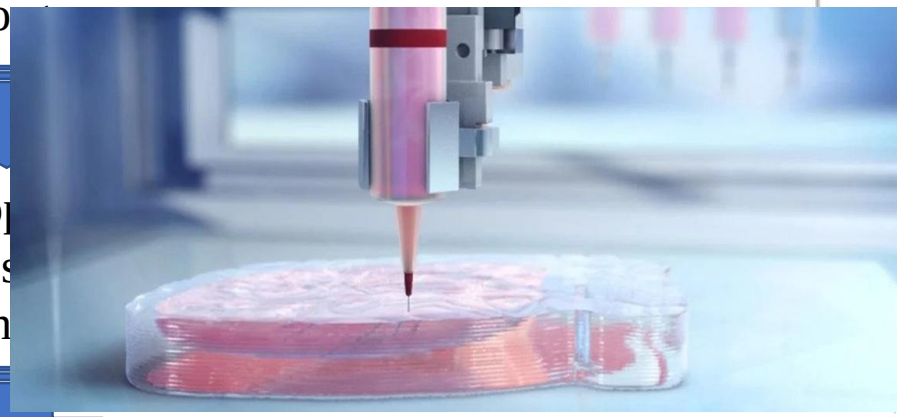
- relationship between 3D culture and Micro-physiological systems (MPS)



❑ relationship between 3D culture and Micro-physiological systems (MPS)

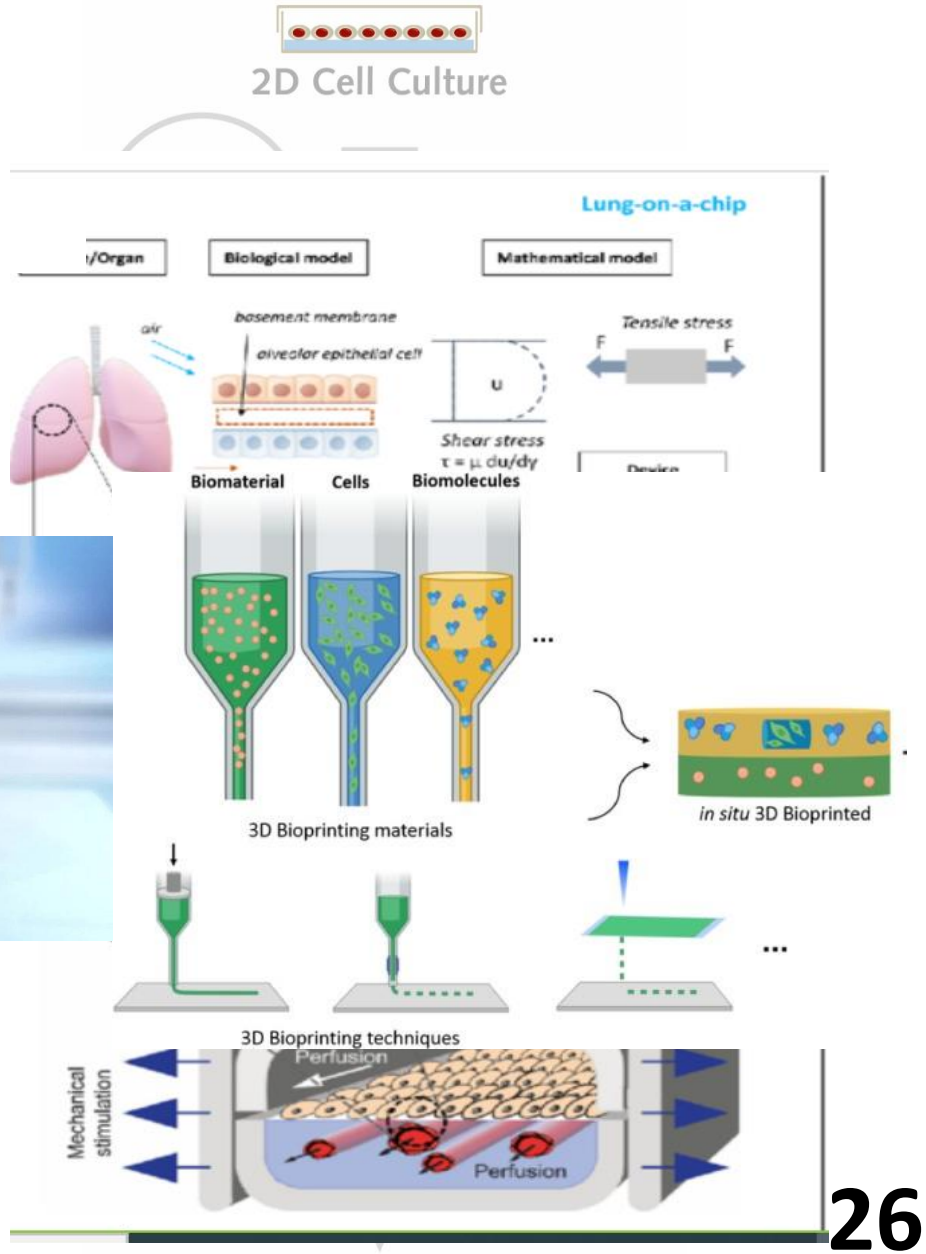
•**3D co-cultures with extracellular matrix(Hydrogel)** : These systems involve culturing cells together with extracellular matrix components to create a more physiologically relevant environment

•**Organoids:** Organoids are 3D cell cultures derived from stem cells that can self-organize into structures resembling specific o

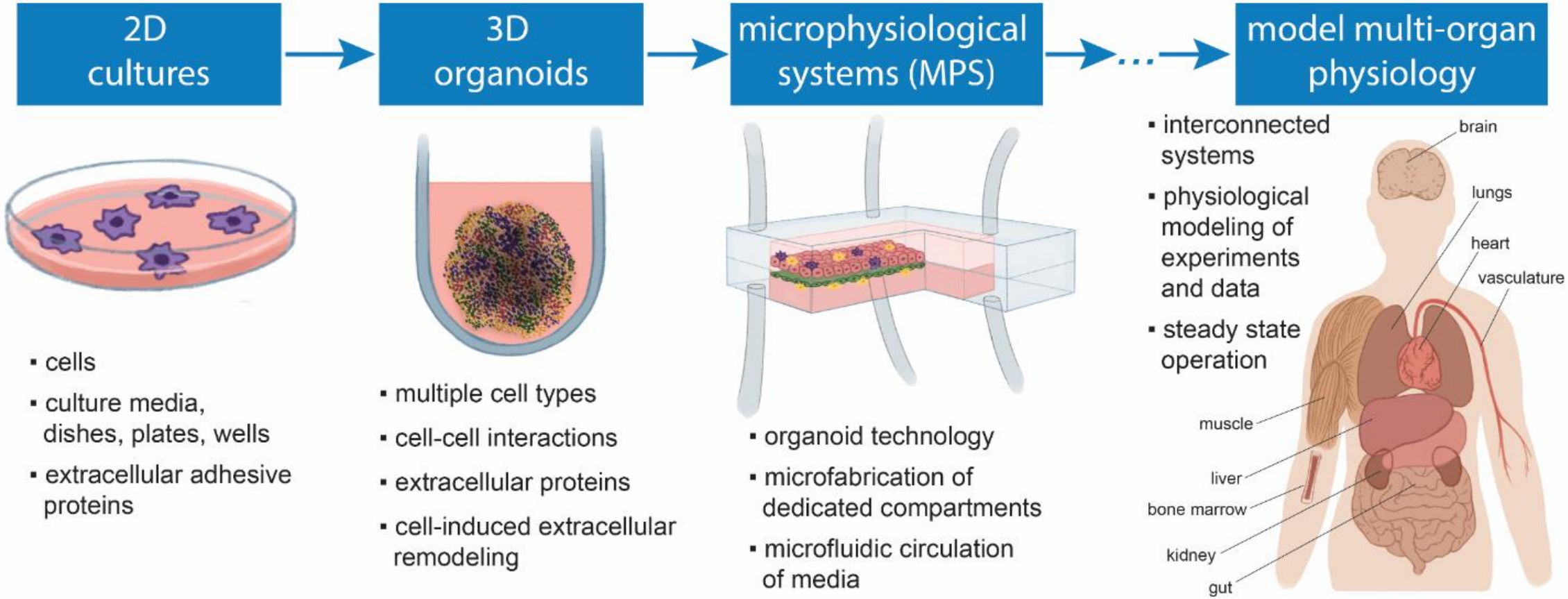


•**3D bio-printed tissues**: Biop
creation of customized 3D tiss
over cell arrangemen

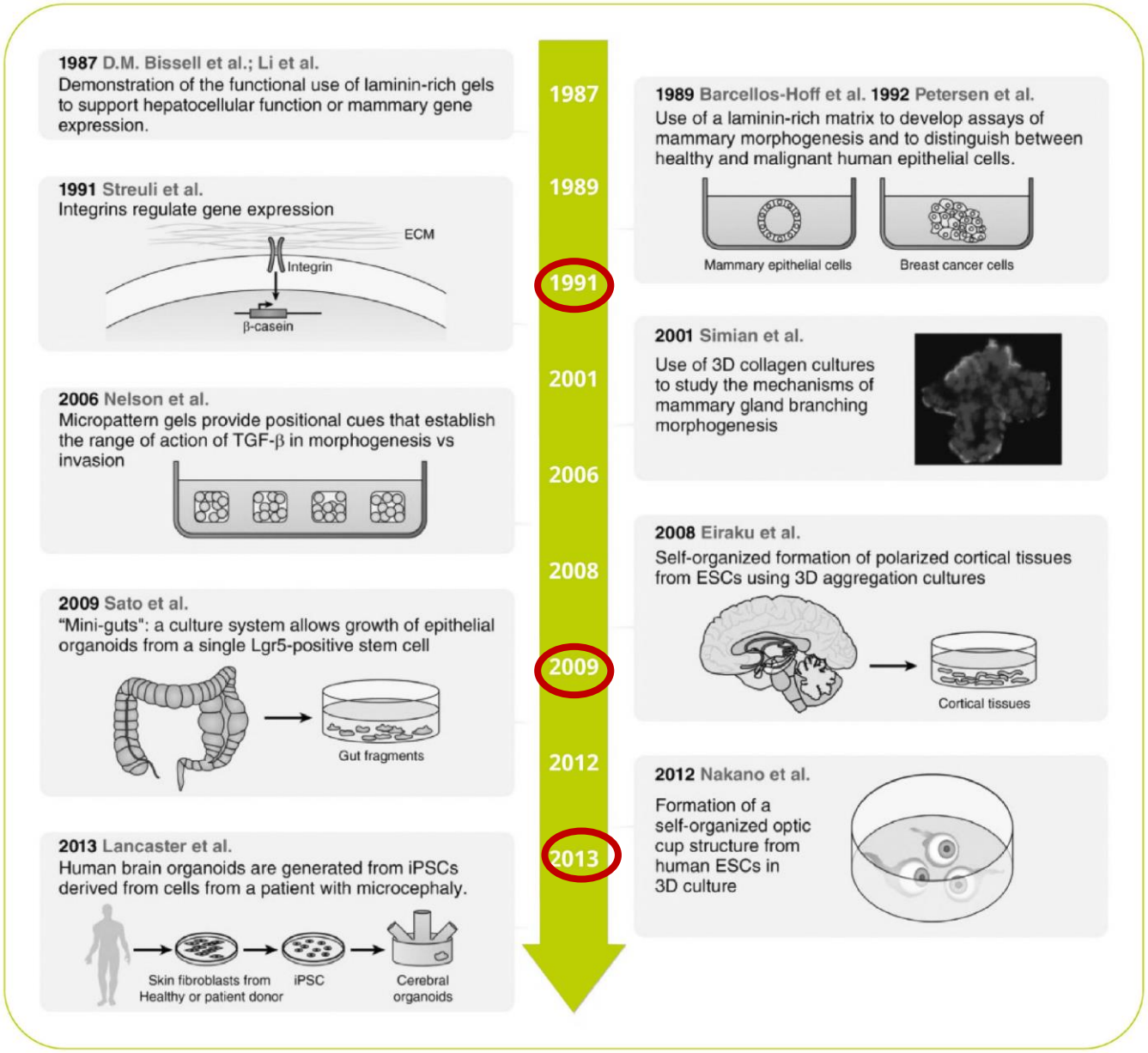
•**Organ-on-a-chip systems**: These systems mimic specific organs, such as the liver, lung, or gut, and are designed to study organ-specific functions and diseases



- relationship between 3D culture and Micro-physiological systems (MPS)



❑ The historical development of microphysiological systems



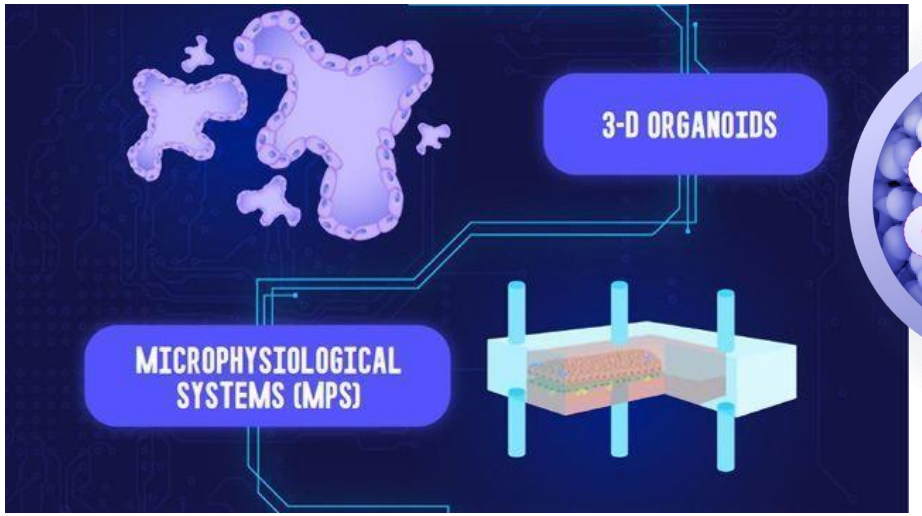
❑ Replacing animal testing with modern technology

- the term “preclinical tests (including tests on animals)” → “nonclinical tests

Cell assay(2Dcell culture)

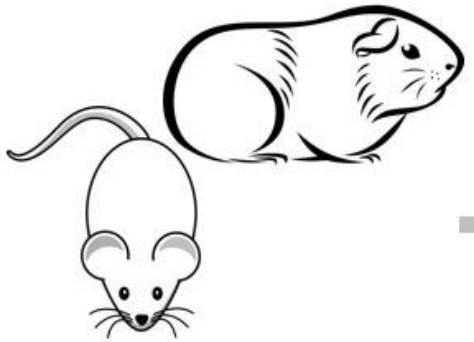
Micro-physiological system(MPS)

Silico testing(Computer models)



The emergence of 3D cell culture and the advent of micro-physiological systems

❑ Replacing animal testing with modern technology

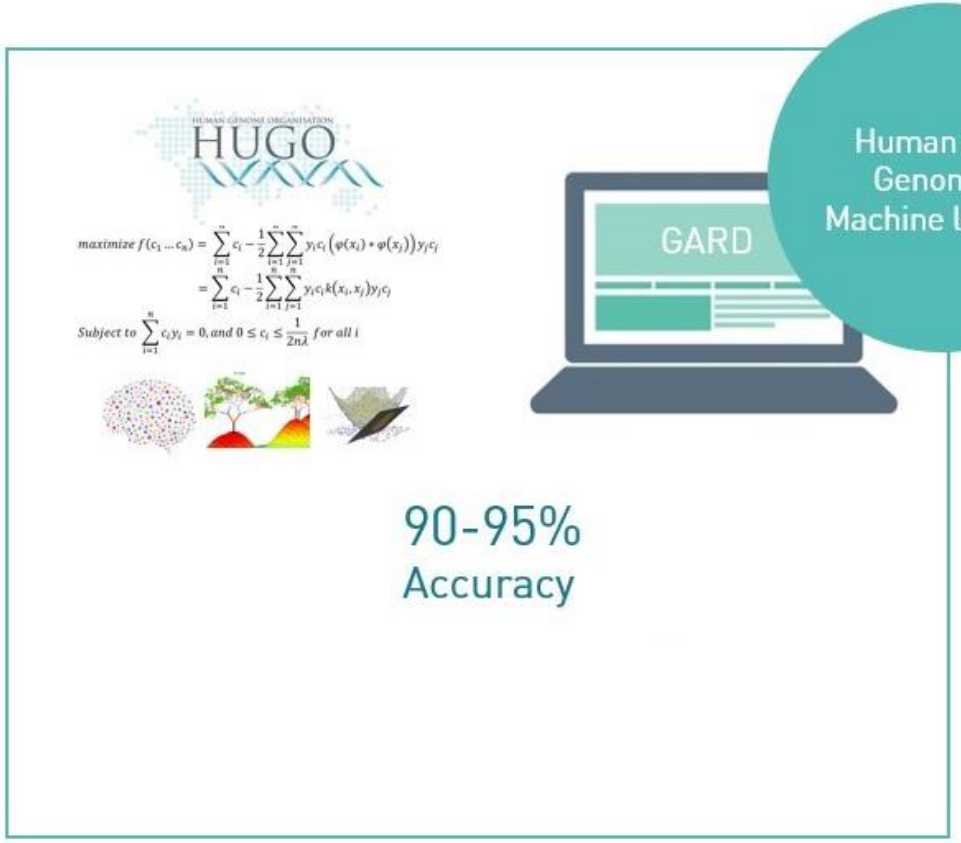


70-75%
Accuracy vs.
Human data

Traditional testing:
In vivo

75-80%
Accuracy

First generation
In vitro



90-95%
Accuracy

Next generation
In vitro

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Thank you for your attention