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# ORGANOIDS: A REVIEW ON ITS CREATION AND APPLICATIONS

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**Abstract:** The 3D structures that can replicate in - vivo organs and in -vitro are called Organoids. Cell lines like that are pluripotent or mature cell can be used to create these organoids. In the past decades since organoid research is going on mainly through isolation and reorganization of cells. Research on organoids traced back as far as 1907. Due to recent developments in organoid technology, strong three- dimensional (3D) models that accurately replicate the cellular heterogeneity, structure and functions of primary tissues have transformed the in- vivo culture tools for biomedical research. With the ability to replicate human organs and disorders in a dish, organoid technology has enormous potential for a variety of translational applications, including precision medicine, drug discovery and regenerative medicine. Organoid culture is an emerging 3D technology and organoids derived from various organs and tissues such as brain, lung, liver and kidney as being generated. Process of developing organoids, elements of the engineering process of organoids such as cell source, matrix, soluble ingredients, integrating cues and physical cues, it encompasses significant occasions in the evolution of organoids. Methods of organoids and information on 3D models, organoid applications and advantages over the 2D models are being studied in terms of their strengths and limits are included in this article.

**Keywords:** Organoids, Pluripotent stem cells, Adult stem cells, 3D models, Organoid Culture.

**History and Introduction:**

During past decade's most of the research on human cells, focused on 2D cultured cell lines. Handling of these classical cell lines is easy and relatively cheap. But, their establishment initially inefficient and it requires extensive phenotypic and genotypic adaptations to the condition of culture. Thus, the cell lines are almost invariable eg., which were derived from tumors or and have oncogenic acquired potential in- vitro. There are drawbacks in 2D cultured cell lines such as when we were used to represent the any disease cells there is lacking of matching normal cells or if not all differentiated cell types present in the original tissue. [1]

In an effort to avoid many of the issues associated with employing these cell lines, organoid cultures or cultures have been created recently. Organoids are in-vivo cultivated 3D structures that resemble certain aspects of biological organs. These organoid cultures can be produced using pluripotent or adult stem cells and organoids which are derived from pluripotent cells need extracellular matrix to serve as the base layer for the cells in cultures. The majority of examples that are high in collagen and laminins, like matrigel and basement membrane extract. [1]

Organoids made from pluripotent stem cells feature a variety of structural characteristics, such as mesenchymal, endothelial and epithelial components. The brain, liver, colon, lung, stomach and kidney were the next organs to be created from pluripotent stem cells. Throughout several months, pluripotent stem cells (PSC) generate organoids, which are derived from the original stem cells are more basic. Not only that, but the organoids solely depict the tissue's epithelial areas. Researchers using adult stem cells began their investigation of the intestines. Owing to the proliferation of normal epithelium, cultivating organoids like tumors is frequently more difficult. In other cases, however, this can be avoided by utilizing cancer- specific selection technique. [1]

**Process of Organoid development:**

It is comparable to the process via which zygote becomes an adult, fully formed creature. Regulation of differentiation, apoptosis and proliferation, patterning and organization culminate in mature tissue. The stem cells will go through the tissue- specific processes of differentiation, culture and organization. Figure 1 shows the process of creating organoids from pluripotent and adult organoids from pluripotent and adult stem cells. Modified cells are used to create organoids, which are created in- vitro and intended to replicate different aspects of in- vivo tissue, such as complex architecture and related functions. Stem cells including adult and pluripotent stem cells are necessary to maintain the organ's growth, function or structure. A cell's niche is as specific habitat that is necessary for stem cells. [17]

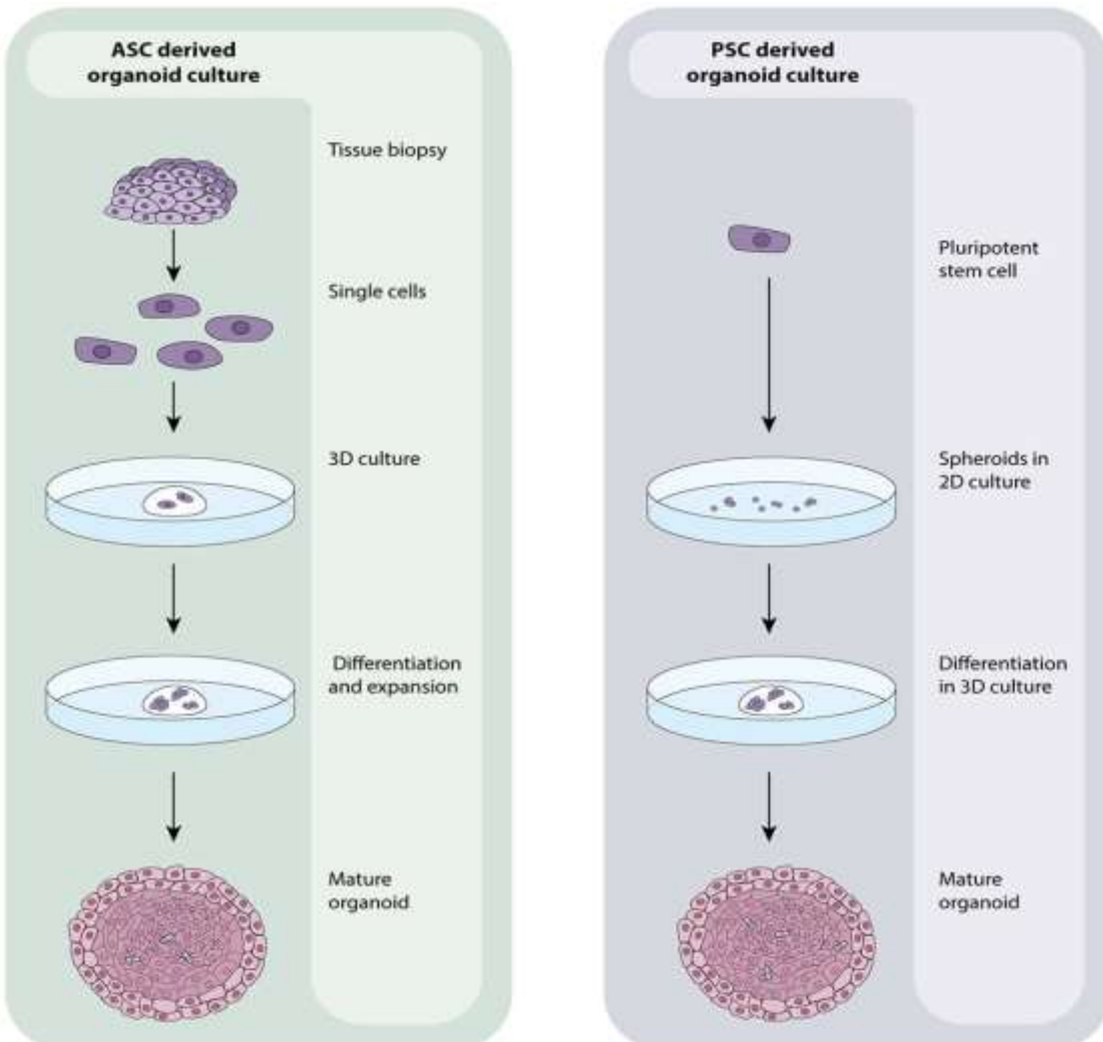


fig 1: process of development of organoids from adult stem cell and pluripotent stem cell. [16]

**Components of Organoid engineering process**

1. Cell Source
2. Soluble factor
3. Matrix
4. Physical cues
5. Integrating cues.

**1. Cell source:** Pluripotent stem cells, adult stem cells or differentiated cells collected under the right circumstances from a variety of organs such as the small intestine, stomach, colon, liver, tongue, kidneys and so on, are the examples of cell sources. Starting a cellular population is the primary role of organoid. These artificially generated stem cells are placed in a matrix resembling stem cell niches. Organ biopsy samples from humans and animals are obtained in order to create tissue-derived organoids. [4]

**2. Soluble factors:** The organoid cultures basically based on the knowledge of biology. Soluble factors are those factors which are used to differentiate induced pluripotent stem cells and tissue derived cultures. The factors like growth factors, fibroblast growth factor. Some factors such as L-wnt-3A, are commonly used to replace the growth factors like such as WNT3A ligands. [4]

**3. Matrix:** After isolation of the cells. These are seeded into natural ECM like collagen or matrigel or hydrogel which is synthetic matrix> Matrix support the organoid culture. The matrix such as matrigel is poorly defined composition of this matrix have control over the biochemically and biophysical etc. Which are necessary for improving the organoid (ie., Organoid culture). Alternative to matrigel like such as fibrin, human collagen. The matrix is produced from polysaccharide or proteins. The synthetic matrix are powerful tools that independently able to control the features of the organoids and also improve or enhance the functionality of organoids. In the matrix the gels are composed of components of ECM. The gels of synthetic matrix can decouple stiffness and variability increases. [4]

**4. Physical Cues:** In addition to the growth factors (biological cues), physical cues should also be taken into account to know how the body should respond to the physical cues given to organoid culture. During the formation of organoid tissue, the diffusion-dependent processes of waste removal and nutrition delivery are less effective. It is one of the causes for the requirement of regular reseeding and fragmentation in organoid culture of the gut. Necrosis within the inner core of certain organoid cultures, such as the brain, is frequently caused by the diffusion- dependent processes of waste clearance and nutrition delivery. Bioreactors can be spun to solve the problem or the cultures can be shaken with carefully managed agitation. These bioreactors can be used to track pH, temperature and oxygen levels.

To encourage the long-term culture of organoids, perfusable micro-fluid chips have also been produced in this respect. Providing topographical signals to regulate organoid culture in-vitro in the final step is useful consideration. Cell area, form and interactions are known to be modulated by the substrates topography, which produces biochemical signals which may affect the destiny of stem cells as well.

Organoids such as intestine organoids growing on the soft hydrogels can be used to illustrate their development. [4]

**5. Integrating cues:** Tissue engineering is the field that uses cue integration most frequently to create tissues both in-vivo and in-vitro. Certain engineering techniques can be used to regulate the morphogenesis, differentiation and proliferation of cells. Bio-printing is a common bio-engineering technique used to recreate tissue. The use of this technology utilizes as in layers method to generate 3D geometric forms with bio-ink that resemble natural tissue. The micro- fluidic platform is another method for reconstructing the tissue. It is utilized to build a cellular model of a reduced organ that replicates important elements of the organs functioning.

Integrating is essential to reproduce organs anatomical and physiological characteristics. Using cardiac and hepatic organoids, two organoid micro- fluidic devices with various read-outs were recently constructed. Additionally, induced stem cells from the stomach, liver, kidneys, etc., have better properties than OOC (Organ on a Chip) devices. [4]

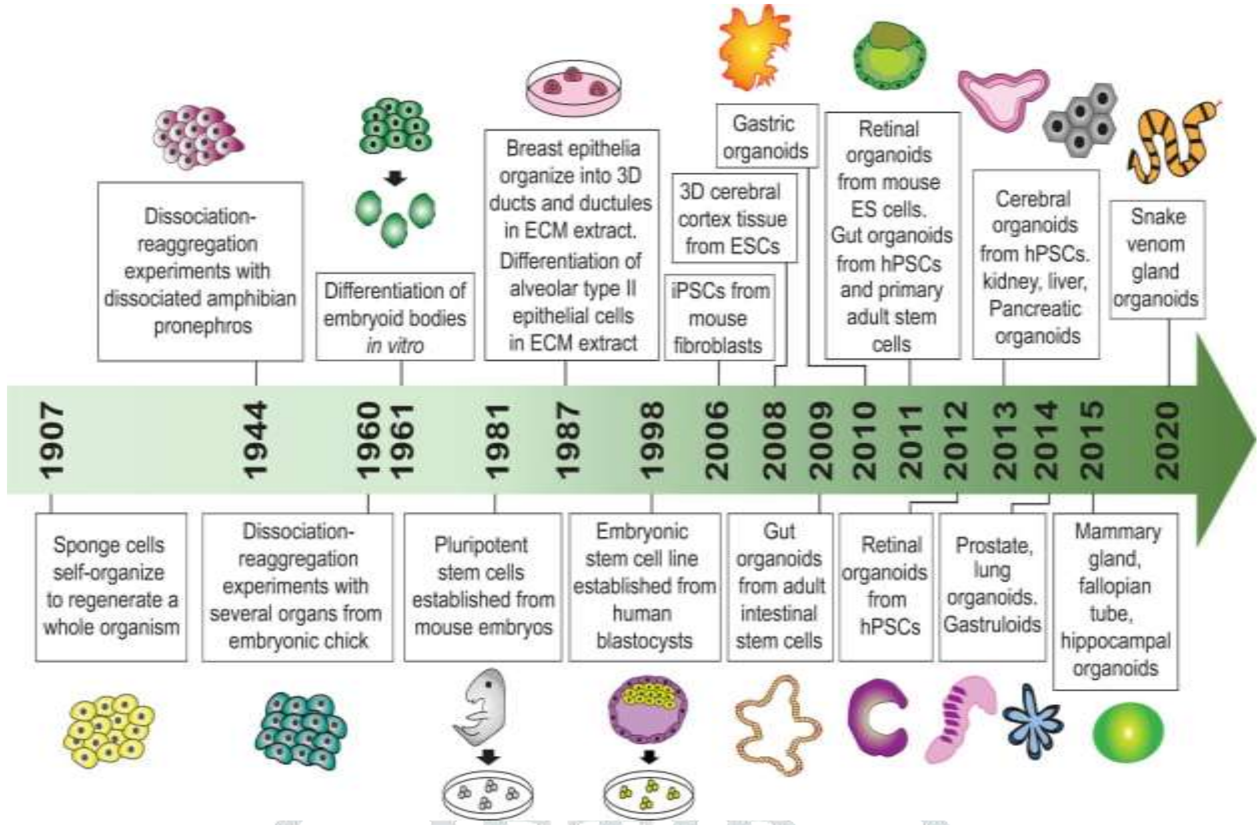


fig2: land mark events in the development of organoids over the year are shown.

The above fig.2 represents land mark events of development of organoids over the years from 1907 to 2020. From sponge cells self- organize to regenerate in whole organism to mammary gland, fallopian tubes, hippocampal organoids. [21]

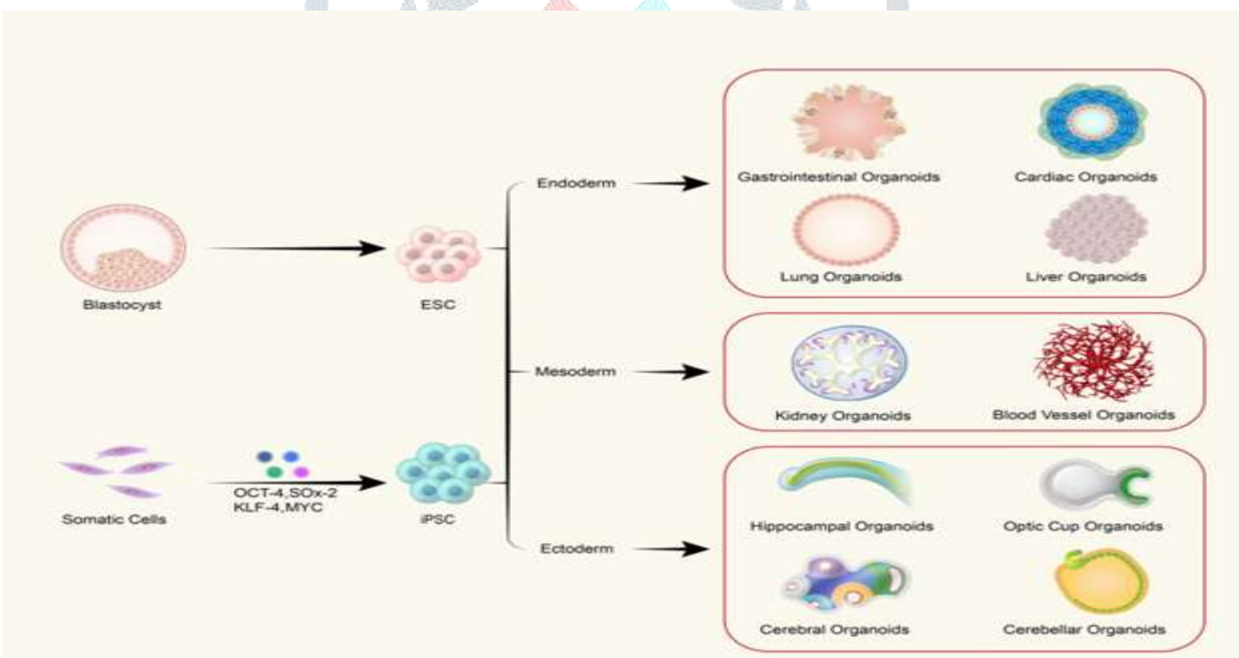


fig 3: schematic representation of the generation of organoids in above

The above fig.3 represents about generation of organoids from blastocyst and somatic cells of embryonic stem cell and pluripotent stem cell ectoderm, mesoderm and endoderm. [44]

**Strategies for growing organoids:**

It provides minimal information on cell differentiation and leaves the cells alone aside from providing nutrients and growing circumstances. Other mechanisms, like stochastic processes that shape the tissues, also allow for intrinsic self-organization such as in brain organoids. Human pluripotent stem cells are used to create brain organoids from their neuro-ectodermal embryonic bodies in a matrix (similar to matrigel), however these organoids do not provide any further external cues to the cells. The cortex, mesoderm and endoderm are only a few of the structures of the brain regions that are produce by this method or process. Alternatively the cells are patterned towards specific regions of the CNS based on knowledge that exists before signal control development, with additional contribution from the self- organizing process. For example, the specific region organoids of the brain are developed from the PSC of humans by patterning the structures that primarily consist of the dorsal or ventral forebrain.

Organoids generated from adult stem cells replicate typical signaling pathways that drive tissue repair following steady state or maintenance tissue injury. Organoids produced from adult stem cells of epithelial organoids in the numerous GI tract organs include: nearly all cell complement types require Wnt signaling, matrigel etc in order to create and maintain the cell in-vivo. [20]

**3D Organoids Models:**

Organoids are developed by the suspension culture to avoid the contact with plastic dish. It can done or achieved by using a technique called scaffold- free- technique. The scaffold is a synthetic one that is similar natural matrix (Extracellular matrix). As we know that most commonly used one is matrigel. Matrigel is a heterogeneous protein secreted from the mouse sarcoma cells of Engel breth-Holm-swarm. It mainly consists of proteins which are adhesive such as the laminin, entactin, collagen etc., which have capability to resemble like extracellular conditions for to provide a support structurally and signals to cells. In the technique of scaffold, the cells are cultured in form of droplets in a medium and it is hanged by gravity and surface tension. There is another technique or a method to establish or develop the organoids by air-liquid interface. By this technique/method the basal layer of fibroblast or matrigel cell are cultured. The matrigel and basal layer of fibroblast is submerged initially in the medium which gradually evaporates and it exposes the cell layer upper surface to the air for to allow differentiation and polarization. [21]

**Organoids Research Progress:**

**1. GI Organoids**

**2. Intestinal organoids**

**3. Gastric Organoids**

**4. Tongue and the salivary gland Organoids**

**5. Liver and pancreatic Organoids**

**6. Brain Organoids**

**7. Ophthalmic or Retinoid Organoids**

**8. Kidney Organoids**

**9. Other Organoids types**

**1. GI Organoids:** During developmental process the GI tract is developed from the region of endoderm. It is in the form of tube which have 3 distinct regions namely foregut, mid- gut and hindgut. The foregut which gives rise to oral cavity, respiratory tract, stomach, liver, pharynx and pancreas etc., and the mid-gut which gives rises to the small intestine and colon ascending part and remaining part of colon and rectum gives rise to by hindgut. Knowing about mechanism, signaling regulation of GI development and their homeostasis is important for maintenance and establishment of any stem cell derived organoids of these regions for GI tract. [21]

**2. Intestinal Organoids:** As we know that intestine is a major organ in our body which involves in functioning of digestive system and for nutrients absorption process. Intestine is structured with a crypts and finger like projections and protrudings called villi. Intestinal organoids can be developed by different stem cells such as PSC and/or from the isolated multicomponent stem cell and also from cells of progenitor which is present in-vivo crypts of intestine. Intestinal organoids can be grown in the Egf, Bmp, Wnt- agonist and R- spondin etc. Inhibitor Noggin binds to crypts-villus of intestine which were able to differentiate and recapitulating the organization and function of intestine. [21, 44]

**3. Gastric Organoids:** The epithelium of stomach and intestine share many similarities at molecular and physiological level. There is presence of proliferating stem cells such as Lgr5+ at the base of the crypts. Organoids of gastric are produced from pyloric cells of mouse and subsequent human gastric organoids are developed from PSC. By the addition of wnt3a, Noggin and retinoic, there is growth of posterior part of foregut and 3D culture for maturation in matrigel. These Gastric organoids which were developed are believed predominantly adopt pyloric lineage. [21]

**4. Tongue and salivary gland Organoids:** Organoids of the tongue and salivary glands have also been created from the adult tongue epithelium, which lacks taste buds. Later employing CD44++, LGR6+ etc., taste buds and acinar cell organoids were also produced. Taste buds and papilla tissue respectively were used to produce stem cells. [21]

**5. Liver and Pancreatic Organoids:** Using induced pluripotent stem cells of adult human, it is possible to create liver and pancreatic organoids. These were first differentiated into 2D structure and then grown on the ECM into a 3D structure before being further engrafted in- vivo to generate a liver that vascular network. After transplantation, the endocrine and ductal lineages of the pancreatic organoids were differentiated from the adult pancreas in a manner similar to that described above. [21]

**6. Brain Organoids:** The brain is part of central nervous system, during the process of development it is derived from neuro-ectoderm. As we known that the brain is divided into 3 regions- forebrain, midbrain and hindbrain. Regions of the brain were composed of neurons and cell glia. Brain organoids are those organoids which were developed from the PSC which resembles the human brain of embryonic stage. The brain organoids can be developed by 2 methods. They are

1. Guided development of brain organoids

2. Unguided development of brain organoids

The organoids which were developed from the unguided methodology consists of heterogeneous tissue having different regions of the brain. The organoids which were developed from this is also called as cerebral organoids. These are developed by the capacity of differentiation and by signaling intrinsically they will mimick the human brain. The organoids which were developed from the guided methodology have no information of heterogeneous tissue type. This methodology involves use of molecules which are small and growth factors to generate the organoids called spheroids of one type of tissue. Guided methodology is also used to develop two or more types of spheroids of different regions of brain which were later fused as assemoids and which helps to study different regions of brain interaction. [21,40]

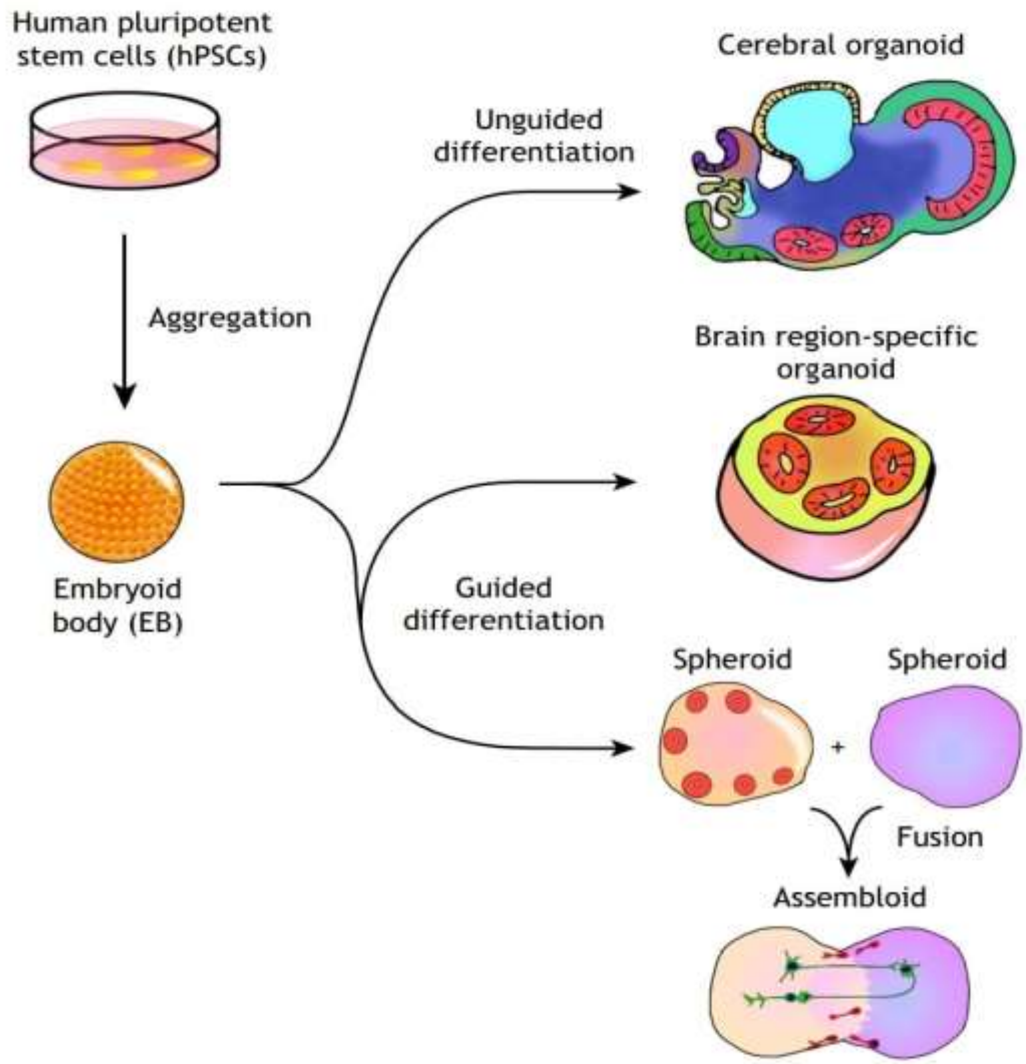


fig 4: unguided and guided approaches for making brain organoids- shows about the development of brain organoids by two methods. [40]

**Applications of brain organoids:** Brain organoids are used in a wide variety of applications.

1. Brain organoids are utilized to detect infections and genetical-based brain abnormalities.

2. Screening and later validation procedures can be carried out using the produced brain organoids.

3. By using human organoids to other species, it is possible to learn and understand the human brain from the perspective of evolution.

4. Psychiatric diseases like schizophrenia and autism can be modeled using brain organoids.

5. The ability to depict disease relevant brain organoids in prospective organoids is enabled by in-vitro aging.

6. The growth of functional brain organoids is essential for modeling. [40]

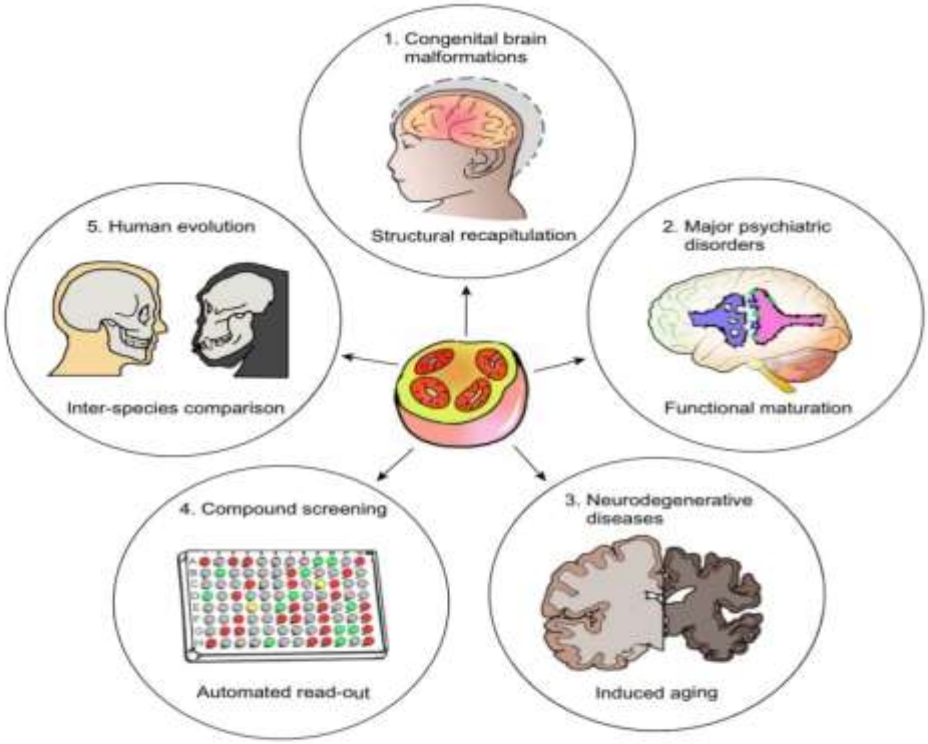


fig: 5 shows the applications of brain organoids. [40]

**7. Ophthalmic or Retinoid Organoids:** The retina which is neuro-ectoderm develops from the optic vesicle during development. Two epithelial layers, which are the outer retinal layers of the neural retina, are formed in front of the vesicle. From PSC's, these retinal organoids are bigger and are able to develop into multilayered issues that include both rods and cones respectively. [21]

**8. Kidney Organoids:** The kidney is a key organ in the body for elimination of waste products. Wnt and Fgf signaling induces kidney development from intermediate mesoderm. It develops into the uteric bud and metanephric-mesenchyma to create the kidneys' early renal tubes. Nephrons are found in kidneys and they are linked to a collecting duct that is encircled by an interstitial network of endothelial cells. In recent years, it has known that long-term culture of kidneys organoids and tubular renal organoids has been further established in adult humans as well as from human urine which forms PCT and DCT of the nephron. [21]

**9. Other Organoids:** Other organoids include mammary glands, prostate organoids, lung organoids and fallopian organoids. From the specific cells, these organoids can be developed. The technology of organoids has also been extended to other animal models such as mice, porcines, ovines, chickens, felines and calves eg., liver organoids, mammary organoids, keratinocyte organoids, etc. Organoids of snake venom has been recently developed and could be used to study toxicology. [21]

**Strengths and limitations of Organoids of 3D culture over the 2D culture (conventional model):**

Due to their lack of tissue complexity and architecture, cell lines from 2D cultures are regarded as non-physiological. These 2D cellular or tissue cultures have been used for many years to represent human development and disorders. Alternatives to biological processes model includes Patient-Derived Xenografts (PDX) and genetically altered mice models. However, it takes time for the establishment or formation (ie., Initiation) of genetically engineered mouse models and these models are unable to accurately reproduce human conditions such as those related to genetics, physiology and other factors or to demonstrate how they might affect the assessment of clinical outcomes.

The GEMM's (Genetically Engineered Mouse Models) are currently being employed in cancer research to facilitate the generation of PDX's and to accelerate the process of xenotransplantation in human cancer models. On the other hand xenograft production from patient samples is a time consuming and ineffective process. An organoid produced from patients can be used to stimulate diseases instead of cell lines and it performs better than both xenografts and GEMM's.

PDO's (Patient Derived Organoids) which can be generated from primary tissue like urine, needle biopsies or bronchial lavage materials are easier to develop and require less time than both GEMM's and PDX's. Comparision of the cell lines generated from the original tissue materials was found to be inefficient and require a significant amount of adaptation to 2D conditions, leading to noticeable or extensive alterations.

Organoids are accepted mostly by comparing with the cells lines which are immortalized. Organoids are the one which is superior in recapitulating the 3D organization. Cell functions of tissues which are primary related ones, heterogeneity and also physiologically applicable for disease modelling for humans and determining the response of drug. Other models like genetically engineered mouse models and patient derived xenografts are better in recapitulating of diseases in-vivo and these are costly, labourious and time consuming. Therefore, these are not suitable for high screening.

The organoid technology is reach over the gap between the cell lines and models of in-vivo. But there are still many limitations in the present system. Most of the patient derived organoids despite of heterogeneity they lack the stromal cells in the culture which involves fail in the process of reconstitute of tumor microenvironment.

The clinical outcomes from the microenvironment which consists of immune cells in addition to endothelial cells and ECM are unpredictable. The most important application of an in-vitro screening platform might be to determine a drugs immunotherapy for individualized care.

Studies of PDO's revealed the formation of patient derived organoids from different types of cancer using an air-liquid interface that will retain cells like insusceptible cells and fibroblasts, which can be used for personalized testing of immunotherapy. But the immune cells and fibroblasts of patient- derived organoids decrease over a period of 1-2 months. So, these are used for the disease modelling of short- term disease.

Organoids produced from patient possess the potential and ability to be used in studies such as immuno-oncology. As far as we are aware, organoids are cells grown in a medium that is saturated with growth factors and suspended in matrigel extracellular matrix. In contrast to 2D culture cell lines, the ECM can complicate cell harvesting and passaging as well as impact assessments of biochemical and functional characteristics. The growth factors could jeopardize natural morphogens tissue gradients.

Some of the issues may be solved or resolved by bioreactors for spinning, which are optimised for organoid brain culture. Some organoids do not have the ability to recapitulate cellular subtypes distinctly. It means they suggest that more evaluation is needed for the mini-brain organoids. Beside the limitations mentioned above, there are some issues to consider such as high cost of reagents required to generate patient derived organoids, which is not affordable by the healthcare system or patients and production of patient derived organoids is not easy because the cell lines are complex 3D systems of culture. Finally, in order to accurately forecast the patient's response to treatment, it is essential to design consistent and standardized drug screening procedures and readouts. [21]

**Advantages of Organoids:**

1. Organoids have many advantages in mimicking organ.

2. Organoids also provide an advantage in studying interactions such as those between the cells and ECM.

3. Help study human organs, which serves as a substitute for animal models.

4. Organoids will preserve the cellular mechanism of the cells of humans.

5. It also provides an opportunity for mechanistic study within the model of human system. [41]

**Applications of Organoids:**

Organoids present potential uses and breakthrough in the domains of fundamental investigation, biology of development, tissue modification, pharmaceutical testing and assessment, given the benefits outlined above.

Organoids derived from patients may have benefits in regenerative therapies and personalized medicine via therapy with cells. [41]

1. Drug Research

2. Disease stimulation

3. Evolutionary Biology

4. Customized medications.

**Conclusion:**

1. Stem cells that are adult (ASC's) or stem cells that are pluripotent (PSC's) can be used to create organoids, which are 3D epithelial in- vitro cultured structures.

2. Innovations in disease modelling and in-vitro organogenesis have been made possible by the use of organoid platforms, which has opened up promising development of novel therapeutics.

3. In addition to being able to stimulate disease states, organoid models may also be utilized to develop specific to patient remedies and more predictive drug screening systems.

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