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BRAIN ORGANOIDS - A REVIEW

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ABSTRACT

Brain organoids which is also known Cerebral organoids is a self assembled three-dimensional structure which is composed of millions of cells that resembles the cellular organization, transcriptional and epigenetic signature of a developing human brain. Key features of early human brains development at molecular, cellular, structural and functional levels are mimicked by brain organoids but the formation of distinct cortical neuronal layers, gyrification and the establishment of complex neuronal circuitry are not fully recapitulated. The main aim in this study was that various features of brain organoids, their application and development are analyzed and recent advances in generation of human brain organoids are discussed and reviewed. This Review was done based on the articles obtained from Various platforms like PubMed, PubMed central and Google scholar. They were collected with a restriction in time basis from 2000 - 2020. The inclusion criteria were original research papers, review articles. In vitro studied among various conditions and articles that contain pros and cons. Exclusion criteria came into account for retracted articles and articles of other languages. All the articles were selected based on Brain organoids. Brain organoids help

neuroscientists understand brain development and use of chemical signals to direct stem cells to produce brain-like cells. Stress can be reversed and also access human brain development and disease. Since brain organoids have potential to develop into innovative platforms for pharmacological studies and tissue engineering, more research needs to be done on this in depth for better understanding.

Keywords: Brain organoids, cerebral organoids, three-dimensional structure, brain development and disease, stem cells

INTRODUCTION

Brain organoids which is also known Cerebral organoids is a self assembled three-dimensional structure which is composed of millions of cells that resembles the cellular organization, transcriptional and epigenetic signature of a developing human brain **[1]**. Key features of early human brains development at molecular, cellular, structural and functional levels are mimicked by brain organoids but the formation of distinct cortical neuronal layers, gyrification and the establishment of complex neuronal circuitry are not fully recapitulated **[2]**. Brain organoids can be developed over an extended period, which is more than 9 months and this time period helps in the establishment of relatively mature features including the formation of dendritic spines and spontaneously active neuronal networks **[3]**. Brain organoids are derived from human Pluripotent stem cells (HPSCs) including embryonic stem cells (ESCs) and induced pluripotent stem cells (IPSCs) **[4]**. Growth of large organoids is maintained by spinning bioreactors which generate forebrain, midbrain, Hypothalamus organoids from human induced pluripotent stem cells **[5]**. Brain organoids help in investigating the development and pathogenesis of the human brain **[6]**.

In previous studies done, it is revealed that most of the current understanding of human brain development has been derived from the examination of post-mortem and pathological specimens. However the unique and dynamic features of human brain development cannot be fully captured by these tissue specimens and model systems **[7]**. Human neurological disease including autism and microcephaly are investigated by stem cell derived brain organoids. Light stimulation of photosensitive cells can control neuronal activity within organoids, which may offer a way to probe the functionality of human neuronal circuits using physiological sensory stimuli. Consistent reproducibility in the cell types produced are shown by

organoids derived from different stem cell lines **[8]**. Brain organoids do not have a circulation system with blood Vessels, it mostly depends on simple diffusion from the culture medium for its supply of gas and nutrients. Due to deficiency of oxygen and nutrients, a substantial Number of cells in the organoids undergo apoptosis while culturing occurs over a long period **[9]**.

Over the past years various research done by our team was on osteology **[10-16]**, stature estimation **[17]**, uses and ill effects of electronic gadgets **[18, 19]**, on RNA **[20, 21]**, animal studies **[22]** and in few other fields **[23, 24]**. There is a lack of much information on the current topic of brain organoids, hence the main aim in this study was that various features of brain organoids, their application and development are analyzed and recent advances in generation of human brain organoids are discussed and reviewed.

MATERIALS AND METHODS

This review was done based on the articles obtained from various platforms like PubMed, PubMed central and Google scholar. They were collected with a restriction in time basis from 2000 - 2020. The inclusion criteria were original research papers, review articles. In vitro studied among various conditions and articles that contain pros and cons. Exclusion criteria came into account for retracted articles and articles of other languages. All the articles were selected based on Brain organoids.

They were determined by article title, abstract and complete article. When article holder websites were analyzed on the topic of brain organoids, more than 1900 articles and based articles were found, when it was shortlisted based on the inclusion and exclusion criteria, the number of articles were lowered to 120 articles. When timeline and other factors were quoted only 45 articles came into play. This article is reviewed from the 45 articles collected. Quality of articles used was assessed using a quality assessment tool and graded as strong, moderate and weak **(Table 1)**.

Brain organoids - Definition

Inorder to change our understanding of the development and disorders of the human brain, a new technology, brain organoids has the potential. The spatiotemporal dynamicity of neurogenesis, the formation of regional neural circuitry and integration of glial cells into a neural frame network can be mimicked by brain organoids. Human cortical development is the characteristic progenitor Zone organization with abundant outer radial glial stem cells, where cerebral organoids help to recapitulate its features. In order to model microcephaly, use of RNA interference and

patient - specific induced pluripotent stem cells are helpful **[25]**.

Advances in brain organoids

The development of a miniaturized multi-well spinning bioreactor has enabled efficient optimization of organoid protocols and production. For gaining a better understanding of human brain development, function, evolution and disorders, rapid advances in brain organoid technologies have opened up new avenues. The direct investigation of the etiology and pathological processes associated with inherited and acquired brain disease, drug toxicity and drug discovery can be done by Generation of region-specific cerebral organoids **[26]**.

Application

To predict drug response in a personalized fashion, Patient derived organoids hold promise. For gene therapy, new avenues for regenerative medicine and in combination with editing technology are present. Translational potential of brain organoids help in investigating disorders such as ZIKA Virus, autism - spectrum and glioblastoma multiforme **[27]**.

Challenges

Investigate neural development and disease

Stem cell technologies that enable the generation of human brain organoids from pluripotent stem cells (PSCs) promise to change our understanding of human brain development and study of inherited and acquired brain disease. With implications to personalize therapeutic opportunities for neurological disorders, to understand fundamental features of human neurodevelopment, is provided by the neuroscience toolbox. The specific stages of in Vivo human brain development can be recapitulated by Brain organoids in vitro, thus offering an innovative tool by which to model human neuro-developmental disease as the brain is the most important coordinating center in a person's body **[28]**.

Microcephaly

To model Microcephaly, a disorder that has been difficult to recapitulate, use of RNA interference and patient- specific induced pluripotent stem cells are helpful **[29]**.

On- a-chip to model prenatal nicotine exposure

During gestation, Nicotine exposure elicits impaired neurogenesis in early fetal brain development. Brain organoid - on- a- chip provides a platform to model neurodevelopmental disorders under environmental exposure, which may be of surgical significance to neurosurgeons and radiologists **[29]**.

Understanding Human disease

Cell diversity in photosensitive Human brain organoids

Using light stimulation of photosensitive cells may offer a way to probe the functionality of human neuronal circuits using physiological sensory stimuli which could be used to control neuronal activity within organoids **[30]**.

Understanding ZIKA Virus induced microcephaly

Inorder to mimic the developing human fetal brain, which have been employed to model ZIKV - induced microcephaly is done by engineering brain organoids. Mature cortical neuron subtypes are organized and produced by the cerebral cortex containing progenitor populations **[31]**.

Generation of Human Vascularized brain organoids

In vivo model of brain organoids

Vascularization of brain organoids with patients' own induced pluripotent stem cells - derived endothelial cells are technically feasible. Vascularized organoids are grown in Vitro for 3-5 weeks and in Vivo for 2 weeks **[32]**. Integration of microglia, progressive neuronal differentiation and maturation, gliogenesis and growth of axons to multiple regions of the host brain were shown in organoids graft **[33]**.

3D organoids derived from pluripotent stem cells

Experimental models for developmental disorder and neuro degenerative disorder.

Certain complex human neurological disorders such as microcephaly, autism and Alzheimer's disease, patient- derived brain organoids revealed novel insights into molecular and genetic mechanisms. Application of brain organoids combined with new technologies such as optogenomics for controlling brain development **[34]**.

Building models of brain disorder

The features of the human brain with greater complexity than 2D models and applied to model disease affecting the central nervous system can be recapitulated by stem cell derived 3D human brain organoids. Establishment of terminal cell identity is a highly Constrained process that can emerge from diverse stem cell origins and growth environments **[35]**.

Generation of Human Brain specific region Using a miniaturized spinning Bioreactor

Spinning bioreactors have low throughput and are bulky in size. Forebrain, midbrain and Hypothalamus organoids from human induced pluripotent stem cells are generated by bioreactors. Two receptors regulated specifically in humans: INSR and

ITGB8, are dependent by increased activation of molecular pathways in human radial glia **[36]**.

For modeling ZIKV exposure

Reduced proliferation and increased cell death, resulting in decreased neuronal cell-layer Volume resembling microcephaly are being led by ZIKV injection. Anatomical variations of foramina of the skull can cause problems especially in areas such as neurosurgery which have been the area of interest for the neuroanatomists due to clinical consequences caused by these structures. So anatomical baseline always helps in correlating anatomical findings with surgical and radiological presentations **[37]**.

Fused cerebral organoid model interaction

Complex interactions between different brain regions can be modelled by fusion culture of cerebral organoids. In the nicotine treated organoids identified by the expressions of forebrain, hindbrain and cortical neural layer markers, brain regionalization and cortical development were disrupted **[38]**.

Assembling brain organoids

Formation of neural circuit

Effective brain organoids contain multiple brain regions with defined connectivity yet to be established and therefore it is difficult to analyze diseases that affect neuronal circuits between distant brain regions. complex cell- cell interaction in the brain forms a functional neural circuit **[39]**.

From the embryo to brain organoids

Modeling aspects of human organogenesis in Vitro, even for tissues as complex as the brain, has been advanced by use of 3D brain organoid systems **[40]**. Inductive signaling is an effective organizing strategy to recapitulate in vivo-like topography in human brain organoids **[41]**. Vascularized human cortical organoids form vasculature - like structures that resemble the Vasculature in the early prenatal brain and they present a robust model to study brain disease in Vitro **[42]**.

DISCUSSION

Table 1: Quality of study of articles used

S.No.	AUTHOR	YEAR	TYPE OF STUDY	RESULT	QUALITY OF STUDY
1.	Koo..*et al*	2019	Research article	Due to deficiency of oxygen and nutrients, a substantial Number of cells in the organoids undergo apoptosis.	Strong
2.	Lancaster..*et al*	2013	Systematic review	In order to model microcephaly, use of RNA interference and patient - specific induced pluripotent stem cells are helpful.	Moderate
3.	Qian..*et al*	2019	Research article	Key features of early human brains development at molecular, cellular, structural and functional levels are mimicked by brain organoids.	Strong
4.	liu..*et al*	2016	Research article	Generation of region-specific cerebral organoids allows the direct investigation of the etiology and	Strong

				pathological processes associated with inherited and acquired brain disease, drug toxicity and drug discovery	
5.	**Hansclever**	**2016**	**Systematic review**	**Patient derived organoids hold promise to predict drug response in a personalized fashion.**	**Moderate**
6.	**Chen..*et al***	**2019**	**Systematic review**	**Translational potential of brain organoids help in investigating disorders such as ZIKA Virus, autism - spectrum and glioblastoma multiforme.**	**Weak**
7.	**Lullo..*et al***	**2017**	**Research article**	**unique and dynamic features of human brain development cannot be fully captured by these tissue specimens and model systems.**	**Strong**
8.	**Trujillo..*et al***	**2018**	**Systematic review**	**Cerebral organoids is composed of millions of cells that resembles the cellular organization, transcriptional and epigenetic signature of a developing human brain**	**Moderate**
9.	**Adams..*et al***	**2019**	**Systematic review**	**3D cellular models suffer from the absence of neural cell types such as astrocytes and oligodendrocytes**	**Moderate**
10.	**Lancester..*et al***	**2017**	**Research article**	**Mature cortical neuron subtypes are organized and produced by the cerebral cortex containing progenitor populations**	**Moderate**
11.	**Wang..*et al***	**2018**	**Research article**	**Brain organoid - on- a- chip provides a platform to model neurodevelopmental disorders under environmental exposure**	**Strong**
12.	**Quadrato..*et al***	**2017**	**Systematic review**	**Brain organoids can be developed over an extended period, which is more than 9 months and this time period helps in the establishment of relatively mature features**	**Strong**
13.	**qian..*et al***	**2017**	**Systematic review**	**Neuronal activity within organoids could be controlled using light stimulation of photosensitive cells, which may offer a way to probe the functionality of human neuronal circuits using physiological sensory stimuli**	**Moderate**
14.	**Lancaster..*et al***	**2017**	**Research article**	**If correct culture conditions are met, it is possible to reduce the stress and improve the fidelity of the cells.**	**Strong**
15.	**Pham..*et al***	**2018**	**Research article**	**Vascularized organoids are grown in Vitro for 3-5 weeks and in Vivo for 2 weeks.**	**Strong**
16.	**Mansour..*et al***	**2018**	**Research article**	**Organoids graft showed progressive neuronal differentiation and maturation, gliogenesis, integration of microglia and growth of axons to multiple regions of the host brain .**	**Strong**
17.	**Lee..*et al***	**2017**	**Systematic review**	**Brain organoids are derived from human Pluripotent stem cells (HPSCs) including embryonic stem cells (ESCs) and induced pluripotent stem cells (IPSCs).**	**Weak**
18.	**Chuye..*et al***	**2018**	**Research article**	**Application of brain organoids combined with new technologies such as optogenomics for controlling brain development.**	**Moderate**
19.	**Amin..*et al***	**2018**	**Systematic review**	**Establishment of terminal cell identity is a highly Constrained process that can emerge from diverse stem cell origins and growth environments.**	**Moderate**
20.	**Velasco..*et al***	**2019**	**Systematic review**	**Organoids derived from different stem cell lines show consistent reproducibility in the cell types produced**	**Strong**
21.	**qian ..*et al***	**2018**	**Research article**	**Growth of large organoids is maintained by spinning bioreactors which generate forebrain, midbrain,**	**Moderate**

				Hypothalamus organoids from human induced pluripotent stem cells	
22.	**Pollen..*et al***	**2018**	**Research article**	**Increased activation of molecular pathways in human radial glia, depend on two receptors regulated specifically in humans: INSR and ITGB8**	**Strong**
23.	**Qian..*et al***	**2016**	**Research article**	**ZIKV injection leads to increased cell death and reduced proliferation, resulting in decreased neuronal cell-layer Volume resembling microcephaly**	**Strong**
24.	**Bagley..*et al***	**2017**	**Systematic review**	**Brain regionalization and cortical development were disrupted in the nicotine treated organoids identified by the expressions of forebrain, hindbrain and cortical neural layer markers**	**Strong**
25.	**Yanglei Xiang**	**2017**	**Systematic review**	**brain organoids have the potential to mimic the spatiotemporal dynamics of neurogenesis, formation of regional neural circuitry and the integration of glial cells into a neural network**	**Weak**
26.	**Seto..*et al***	**2019**	**Research article**	**Brain organoids help in investigating the development and pathogenesis of the human brain**	**Moderate**
27.	**Pasca..*et al***	**2019**	**Research article**	**Difficult to analyze diseases that affect neuronal circuits between distant brain regions, as effective brain organoids containing multiple brain regions with defined connectivity yet to be established**	**Strong**
28.	**Arlotta..*et al***	**2019**	**Systematic review**	**Modeling aspects of human organogenesis in Vitro, even for tissues as complex as the brain, has been advanced by use of 3D brain organoid systems**	**Weak**
29.	**Cederquist..*et al***	**2019**	**Research article**	**Inductive signaling is an effective organizing strategy to recapitulate in vivo -like topography in human brain organoids**	**Strong**
30.	**Cakir..*et al***	**2019**	**Systematic review**	**Vascularized human cortical organoids form vasculature - like structures that resemble the Vasculature in the early prenatal brain , and they present a robust model to study brain disease in Vitro**	**Strong**
31.	**Johnson..*et al***	**2020**	**Systematic review**	**Vascular cells are endothelial cells, smooth muscle cells, and fibroblasts**	**Moderate**
32.	**Seppan..*et al***	**2018**	**Research article**	**Ethanolic seed extract has a good effect on increasing reduced number of myelinated fibers, diameter, vacuolization, indentation of the myelin sheath, and degeneration**	**Strong**
33.	**Sekar..*et al***	**2019**	**Research article**	**Hypertension (HTN) or high blood pressure is a predominant noncommunicable disease in the developing and developed world**	**Strong**
34.	**Krishna..*et al***	**2016**	**Systematic review**	**In cases where only facial remains of the skull are bought for medico-legal examination, Stature estimation is an important parameter**	**Moderate**
35.	**Nandhini..*et al***	**2018**	**Research article**	**Anatomical baseline always helps in correlating anatomical findings with surgical and radiological presentations.**	**Strong**
36.	**Subashri..*et al***	**2016**	**Research article**	**For neuroanatomists, Anatomical variations of foramina of the skull have been of interest due to clinical consequences caused by these structures can cause problems especially in areas such as neurosurgery**	**Strong**

37.	**Thejeswar..*et al***	**2015**	**Research article**	**iPad system without carrying heavy textbooks or notebooks, without destroying mammoth trees, iPad are environmentally beneficial and carries a huge aspect on optic defects.**	**Moderate**
38.	**Sriram..*et al***	**2015**	**Research article**	**brain is the most important coordinating center in a person's body**	**Weak**
39.	**Keerthana..*et al***	**2016**	**Research article**	**Our body is highly unique with various abnormal features.**	**Strong**
40.	**Pratha..*et al***	**2016**	**Research article**	**Occurrence of the meningo-orbital foramen in the skull may be of surgical significance to neurosurgeons and radiologists**	**Moderate**
41.	**Menon..*et al***	**2016**	**Systematic review**	**It is very important to make sure that the state of mind and health is given proper care**	**Moderate**
42.	**Samuel..*et al***	**2015**	**Research article**	**Incase of amblyopia conditions both blurry and clear images are formed by the brain at the same time**	**Strong**
43.	**Hafeez..*et al***	**2016**	**Research article**	**Openings in the floor of the middle cranial fossa are important as they allow passage of nerves and blood vessels**	**Strong**
44.	**Choudhari..*et al***	**2016**	**Research article**	**Recognition of posterior condylar foramen and other variant emissary foramina allows a deeper appreciation of the alternative channels of venous drainage from the brain**	**Moderate**
45.	**Kannan..*et al***	**2016**	**Research article**	**The styloid process is also clinically important as it is closely related to important neurovascular structures**	**Strong**

From the studies done previously, it is revealed that 3D cellular models give a complex, multifunctional platform for neurobiology research. These models suffer from the absence of other neural cell types such as astrocytes and oligodendrocytes which together compose the majority of cells in the adult human brain **[43]**. The Viability and growth as neurospheres and brain organoids are reduced using immunocytochemistry and electron microscopy, when ZIKV targets human brain cells **[28]**. Morphology and gene expression patterns can be replicated by 3D tissue in humans early in Vivo brain development up to the end of the first trimester. Unguided and guided methods are the two different types of methodologies used to generate brain organoids. Spontaneous morphogenesis and intrinsic differentiation capacities within HPSC aggregate in unguided method and supplementation of external patterning factors to induce human pluripotent stem cells (HPSC) to differentiate towards desired lineages in guided method Application of brain organoids can be improved by single- all sequencing, genome editing and optogenetics **[29]**.

There are some limitations still which are faced by current organoid protocols including low reproducibility, incomplete cell type diversity and slow maturation. Certain cells generated within organoids and also the extent to which they recapitulate the regional complexity remain

undefined, also its cellular diversity and circuit functionality of the brain also remains unknown. Vascularization of brain organoids with a patient's own ipsc (induced pluripotent stem cells) - derived Endothelial cells is technically Feasible **[32]**. Organoids also have altered gene expression patterns. It is possible to reduce the stress and improve the fidelity of the cells when correct culture conditions are met. Limitations of organoids are potentially reversible **[44]**. The brain organoids have the potential to mimic the spatiotemporal dynamics of neurogenesis, formation of regional neural circuitry and the integration of glial cells into a neural network. This reveals that in order to study the human brain, brain organoids can serve as a representative model system **[41]**. In order to model neurodevelopmental disorders under prenatal nicotine exposure at an early stage, Brain organoid-on - a-chip system derived from human induced pluripotent stem cells is helpful **[29]**. Sonic Hedgehog (SHH) patterned cerebral organoids are used as a tool to study the role of cholesterol metabolism in SHH signaling. To recapitulate in vivo- like topography in human brain organoids, this helps as an effective organizing strategy in inductive signaling. Brain organoids are used for modeling all neural related disorders, therefore helping to elucidate novel aspects of pathogenesis and to generate an innovative reliable drug screening platform **[45]**.

CONCLUSION

Brain organoids help neuroscientists understand brain development and use of chemical signals to direct stem cells to produce brain-like cells. Stress can be reversed and also access human brain development and disease. Since brain organoids have potential to develop into innovative platforms for pharmacological studies and tissue engineering, more research needs to be done on this in depth for better understanding.

REFERENCE

[1] Trujillo CA, Muotri AR. Brain Organoids and the Study of Neurodevelopment. Trends Mol Med. 2018 Dec; 24(12): 982–90.

[2] Qian X, Song H, Ming G-L. Brain organoids: advances, applications and challenges. Development [Internet]. 2019 Apr 16;146(8). Available from: http://dx.doi.org/10.1242/dev.166074

[3] Quadrato G, Nguyen T, Macosko EZ, Sherwood JL, Min Yang S, Berger DR, et al. Cell diversity and network dynamics in photosensitive human brain organoids. Nature. 2017 May 4; 545(7652): 48–53.

[4] Lee C-T, Bendriem RM, Wu WW, Shen R-F. 3D brain Organoids derived from pluripotent stem cells: promising experimental models for brain development and neurodegenerative disorders. J Biomed Sci. 2017 Aug 20; 24(1): 59.

[5] Qian X, Jacob F, Song MM, Nguyen HN, Song H, Ming G-L. Generation of human brain region-specific organoids using a miniaturized spinning bioreactor. Nat Protoc. 2018 Mar; 13(3): 565–80.

[6] Seto Y, Eiraku M. Toward the formation of neural circuits in human brain organoids. Curr Opin Cell Biol. 2019 Dec; 61: 86–91.

[7] Lullo ED, Di Lullo E, Kriegstein AR. The use of brain organoids to investigate neural development and disease. Nature Reviews Neuroscience. 2017; 18(10): 573–84.

[8] Velasco S, Kedaigle AJ, Simmons SK, Nash A, Rocha M, Quadrato G, *et al.* Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. Nature. 2019 Jun; 570(7762): 523–7.

[9] Koo B, Choi B, Park H, Yoon K-J. Past, Present, and Future of Brain Organoid Technology. Mol Cells. 2019 Sep 30; 42(9): 617–27.

[10] Choudhari S, Thenmozhi MS. Occurrence and Importance of Posterior Condylar Foramen. Research Journal of Pharmacy and Technology. 2016; 9(8): 1083–5.

[11] Hafeez N. Thenmozhi. Accessory foramen in the middle cranial fossa Res J Pharm Tech. 2016; 9: 1880–2.

[12] Kannan R, Thenmozhi MS. Morphometric Study of Styloid Process and its Clinical Importance on Eagle's Syndrome. Research Journal of Pharmacy and Technology. 2016; 9(8): 1137–9.

[13] Keerthana B, Thenmozhi MS. Occurrence of foramen of huschke and its clinical significance. Research Journal of Pharmacy and Technology. 2016; 9(11): 1835.

[14] Pratha AA, Thenmozhi MS. A study of occurrence and morphometric analysis on meningo orbital foramen. Research Journal of Pharmacy and Technology. 2016; 9(7): 880–2.

[15] Nandhini JST, Babu KY, Mohanraj KG. Size, Shape, Prominence and Localization of Gerdy's Tubercle in Dry Human Tibial Bones. Research Journal of

Pharmacy and Technology. 2018; 11(8): 3604–8.

[16] Subashri A, Thenmozhi MS. Occipital emissary foramina in human adult skull and their clinical implications. Research Journal of Pharmacy and Technology. 2016; 9(6): 716–8.

[17] Krishna RN, Nivesh Krishna R, Yuvaraj Babu K. Estimation of stature from physiognomic facial length and morphological facial length. Research Journal of Pharmacy and Technology. 2016;9(11):2071.

[18] Sriram N, Thenmozhi, Yuvaraj S. Effects of Mobile Phone Radiation on Brain: A questionnaire based study. Research Journal of Pharmacy and Technology. 2015; 8(7): 867.

[19] Thejeswar EP, Thenmozhi MS. Educational research-iPad system vs textbook system. Research Journal of Pharmacy and Technology. 2015; 8(8): 1158–60.

[20] Johnson J, Lakshmanan G, M B, R M V, Kalimuthu K, Sekar D. Computational identification of MiRNA-7110 from pulmonary arterial hypertension (PAH) ESTs: a new microRNA that links diabetes and PAH. Hypertens Res. 2020 Apr; 43(4): 360–2.

[21] Sekar D, Lakshmanan G, Mani P, Biruntha M. Methylation-dependent circulating microRNA 510 in preeclampsia patients. Hypertens Res. 2019 Oct; 42(10): 1647–8.

[22] Seppan P, Muhammed I, Mohanraj KG, Lakshmanan G, Premavathy D, Muthu SJ, *et al.* Therapeutic potential of Mucuna pruriens (Linn.) on ageing induced damage in dorsal nerve of the penis and its implication on erectile function: an experimental study using albino rats. Aging Male. 2018;1–14.

[23] Menon A, Thenmozhi MS. Correlation between thyroid function and obesity. Research Journal of Pharmacy and Technology. 2016;9(10):1568.

[24] Samuel AR, Thenmozhi MS. Study of impaired vision due to Amblyopia. Research Journal of Pharmacy and Technology. 2015;8(7):912–4.

[25] Lancaster MA, Renner M, Martin C-A, Wenzel D, Bicknell LS, Hurles ME, *et al*. Cerebral organoids model human brain development and microcephaly. Nature. 2013 Sep

19;501(7467):373–9.

[26] Liu F, Huang J, Zhang L, Chen J, Zeng Y, Tang Y, *et al.* Advances in Cerebral Organoid Systems and their Application in Disease Modeling. Neuroscience. 2019 Feb 10; 399: 28–38.

[27] Clevers H. Modeling Development and Disease with Organoids. Cell. 2016; 165(7): 1586–97.

[28] Chen HI, Isaac Chen H, Song H, Ming G-L. Applications of Human Brain Organoids to Clinical Problems. Developmental Dynamics. 2019; 248(1): 53–64.

[29] Wang Y, Wang L, Zhu Y, Qin J. Human brain organoid-on-a-chip to model prenatal nicotine exposure. Lab Chip. 2018 Mar 13; 18(6): 851–60.

[30] Qian X, Nguyen HN, Jacob F, Song H, Ming G-L. Using brain organoids to understand Zika virus-induced microcephaly. Development. 2017 Mar 15; 144(6): 952–7.

[31] Lancaster MA, Corsini NS, Wolfinger S, Gustafson EH, Phillips AW, Burkard TR, *et al.* Guided self-organization and cortical plate formation in human brain organoids. Nat Biotechnol. 2017 Jul; 35(7): 659–66.

[32] Pham MT, Pollock KM, Rose MD, Cary WA, Stewart HR, Zhou P, *et al.* Generation of human vascularized brain organoids. Neuroreport. 2018 May 2; 29(7): 588–93.

[33] Mansour AA, Tiago Gonçalves J, Bloyd CW, Li H, Fernandes S, Quang D, *et al.* An in vivo model of functional and vascularized human brain organoids. Nature Biotechnology. 2018; 36(5): 432–41.

[34] Chuye LB, Dimitri A, Desai A, Handelmann C, Bae Y, Johari P, *et al.* Brain Organoids: Expanding Our Understanding of Human Development and Disease. Results Probl Cell Differ. 2018; 66: 183–206.

[35] Amin ND, Paşca SP. Building Models of Brain Disorders with Three-Dimensional Organoids. Neuron. 2018 Oct 24; 100(2): 389–405.

[36] Pollen AA, Bhaduri A, Andrews MG, Nowakowski TJ, Meyerson OS, Mostajo-Radji MA, *et al.* Establishing Cerebral Organoids as Models of Human-Specific Brain Evolution. Cell. 2019 Feb 7; 176(4): 743–56.e17.

[37] Qian X, Nguyen HN, Song MM, Hadiono C, Ogden SC, Hammack C, *et al.* Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure. Cell. 2016 May 19; 165(5): 1238–54.

[38] Bagley JA, Reumann D, Bian S, Lévi-Strauss J, Knoblich JA. Fused cerebral organoids model interactions between brain regions. Nature Methods. 2017; 14(7): 743–51.

[39] Paşca SP. Assembling human brain organoids. Science. 2019; 363(6423): 126–7.

[40] Arlotta P, Paşca SP. Cell diversity in the human cerebral cortex: from the embryo to brain organoids. Current Opinion in Neurobiology. 2019; 56: 194–8.

[41] Cederquist GY, Asciolla JJ, Tchieu J, Walsh RM, Cornacchia D, Resh MD, *et al.* Specification of positional identity in forebrain organoids. Nature Biotechnology. 2019; 37(4): 436–44.

[42] Cakir B, Xiang Y, Park I-H. Engineering of human brain organoids with a functional vascular-like system. Nat methods. 2019; 16(11): 1169–75.

[43] Adams JW, Cugola FR, Muotri AR. Brain Organoids as Tools for Modeling Human Neurodevelopmental Disorders. Physiology. 2019; 34(5): 365–75.

[44] Lancaster MA, Corsini NS, Burkard TR, Knoblich JA. Guided self-organization recapitulates tissue architecture in a bioengineered brain organoid model. Nat Biotechnol. 2017; 35(7): 659–66.

[45] Xiang Y, Tanaka Y, Patterson B, Kang Y-J, Govindaiah G, Roselaar N, *et al.* Fusion of Regionally Specified hPSC-Derived Organoids Models Human Brain Development and Interneuron Migration. Cell Stem Cell. 2017; 21(3): 383–98.e7.