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**ABSTRACT**

Over the past decade, the development of three-dimensional mammalian cell organization—called human organoids—from stem cells has provided a framework for future clinical therapies. As human organoid research progresses, we also need to keep in mind the cross-cultural and ethical dimensions of human organoids research. Our review article aims to examine the ethical dimensions of cerebral human organoids and provide an ethical framework guide within human organoids research.

Key Words: cerebral organoids; ethical dimensions; clinical therapy; cystic fibrosis.

○ Introduction

Organoids are three-dimensional clusters of cells grown *in vitro* from stem cells that are able to mimic real organs by forming the specific cells found within said organs (Table 1). Scientists have been able to grow organoids containing cells found in different organ structures, including brain, kidney, epithelium, and cardiac tissue, among many others. One of the current dilemmas facing organoid research is what type of ethical considerations should be examined when conducting this type of research. This question is especially relevant surrounding cerebral organoid research in which scientists are examining the possibility of consciousness arising from the three-dimensional models. In our review article, we wish to use the example of cerebral organoid research to explore an in-depth ethical analysis of organoid research (Figure 1). Table 1 includes key definitions used within this article.

“The development of three-dimensional mammalian cell organization—called human organoids—from stem cells has provided a framework for future clinical therapies.”

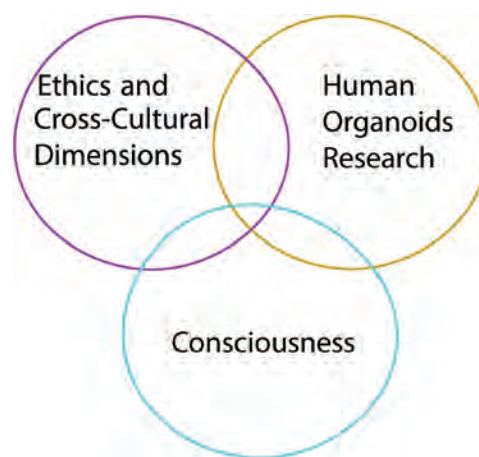


Figure 1. Interconnected Circles of Human Organoids, Consciousness, and Cross-Cultural and Ethical Dimensions.

○ Background

Organoid research was pioneered in 2005 when a team led by Yoshiki Sasai team grew a mouse cerebrum using mouse embryonic stem cells (Watanabe et al., 2005). In 2008, the same team succeeded in mimicking human neural tissue after refining their differentiation method. Since the first Sasai team organoid development, different studies have resulted in models mimicking the hypothalamus, optic cup, and thalamus, among a handful of other types of neural tissues. In 2013, Lancaster and colleagues demonstrated that organoids could be derived from neuroepithelium (Lancaster et al., 2013).

Table 1. Definitions in Organoid Research.

Term	Definition	Reference
Organoid	Three-dimensional tissue cultures grown from stem cells.	https://hsci.harvard.edu/organoids Javier Barbuzano, "Organoids: A new window into disease, development and discovery," Harvard Stem Cell Institute, November 7, 2017.
Cerebral Organoid	Three-dimensional tissue cultures that mimic the growth process of a developing human brain.	https://www.stemcell.com/technical-resources/area-of-interest/organoid-research/neural-organoids/overview.html STEMCELL Technologies, "Neural Organoids," 2020.
Cell Assembly Hypothesis	A concept characterized by the idea that when a network of neurons is repeatedly activated in association with specific mental activities, the network becomes stronger, resulting in more rapid synaptic firing.	http://www.scholarpedia.org/article/Cell_assemblies Moshe Abeles, "Cell Assemblies," Scholarpedia, April 9, 2011.

Table 2. Key Discoveries in Cerebral Organoids Research.

Highlighted Discoveries in Cerebral Organoids Research	Reference
Directed differentiation of telencephalic precursors from embryonic stem cells	Watanabe et al. (2005)
Self-organized formation of polarized cortical tissues from embryonic stem cells (ESCs) and its active manipulation by extrinsic signals	Eiraku et al. (2008)
Cerebral organoids model human brain development and microcephaly	Lancaster et al. (2013)

○ Usage of Organoids in Clinical Therapy Applications

Most current research using organoids is centered around the study of difficult-to-explore diseases like Alzheimer's, brain tumors, and autism. Organoids may also benefit neuropharmacology, where methods used to detect the binding of Ca^{2+} ions may be able to detect irregular neural activity when assessing toxicity in drug candidates. Here, we provide the molecular and cellular physiology of cystic fibrosis and then present how the clinical therapies of organoids provide a platform for ethical discussions on organoids research.

In 2019, research published by Berkers and colleagues discussed how organoids derived from rectal tissue were used to study individual drug responses in subjects with cystic fibrosis (Berkers et al., 2019). Researchers closely examined several mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel allele that include the serine to asparagine amino acid mutation (S1251N), and then they observed the response to different molecular therapy treatments (Berkers et al., 2019). These treatments, such as genistein plus curcumin and Lumacaftor with

ivacaftor, also varied in timing of treatment. One of the key outcomes from the study was the identification of a special molecular readout (or biomarker) of CFTR function called forskolin-induced swelling (FIS) that was used in the organoid model system for cystic fibrosis. Furthermore, the authors demonstrated that FIS is associated with two additional parameters to measure CFTR function: sweat chloride concentration (SCC) and percentage of predicted forced expiratory volume in one second ($ppFEV_1$) to assay or measure for clinical outcomes in patients with cystic fibrosis. Although the *in vitro* rectal organoid approach still requires more improvements to address the challenges of developing additional testing conditions of organoids, there is a lot of interest in developing more cost-effective therapies using transplantable human organs from organoids, and these deserve further investigations.

More recently, organoids research has enabled scientists to examine the molecular and cellular features of alveoli in lungs (or alveolospheres) to gain insight into how the SARS-CoV-2 virus infects lungs and how lungs respond to the infection. This real-world example can be used as a platform to discuss important ethical dimensions of organoids research and example framework question and discussion topics presented in Table 3. In light of the Association of American Colleges and Universities (AAC&U) VALUE rubric for ethical reasoning (AAC&U, 2009), we envision that the example open-ended questions can be used in both virtual/online and in-person class discussions in undergraduate class settings. As mentioned later in our article, we also recommend two review articles on nursing and medical ethics by Heale and Shorten, 2017, and Taylor, 2013, respectively.

○ Limitations and Ethical Concerns

One of the biggest hurdles affecting cerebral organoid research is the lack of supporting neural tissue. For example, Lavazza and Massimini (2018) raised important ethical dimensions in cerebral organoids: "Consciousness relies on the joint presence of integration and differentiation in neural circuits." The ability to grow cerebral tissue has been a great start, but without the surrounding endothelium, microglia, vasculature, and other types of neural cells, contemporary

Table 3. Example ethical dimensions of organoids research.

<p>Example question:</p> <p><i>Current clinical research uses special mini-organs in a petri dish (called organoids) to study how organs develop over space and time.</i></p> <p><i>Organoids are also used to identify new therapies for human diseases, including viral infections in host organs related to novel coronavirus disease 2019 (COVID-19).</i></p> <p>Mini lung organoids replicate COVID-19 pathology in a dish (https://www.drugtargetreview.com/news/76700/mini-lung-organoids-replicate-covid-19-pathology-in-a-dish/)</p> <p><i>Why are ethical dimensions important in organoids research settings? Write down all your responses, and we will discuss as a community of scholars.</i></p>
<p>Example topics for discussion:</p> <p>Importance of patient consent and HIPAA; making sure people’s rights and religious beliefs are not violated</p> <p>Ensuring no harm comes to the subject or their environment for the sake of research</p> <p>Importance of informed consent and environmental awareness</p>

organoids are very different from human brains. Organoids average about 4 mm in size when grown *in vitro*. Still, there exists optimism that more advanced organoids are possible in the near future. An example is research that intends to fuse three-dimensional models of the thalamus to models of the cerebrum: “As the thalamus is the gate of all sensory input to the cerebrum, such a finding might have the potential to transmit sensory information to the neuronal tissue of the organoid” (Sawai et al., 2019). If the research is taken a step further and the thalamocortical model is fused to spinal cord and peripheral nerves, it could be possible for the organoid to have a “somatic sensory experience” (Sawai et al., 2019).

This is where ethical concerns begin to materialize. Some predict that once the development of organoids reaches a point where entire neural networks are developed, they may begin to develop human sentience. Although it is highly improbable that organoids scientists are working with today have consciousness, research suggests that they may in the future. An example of this possibility is research by Hideya Sakaguchi and his team to complete a functional assessment on neural activity in cerebral organoids. The team used calcium imaging to create a visual representation of cellular activity within an organoid. What they uncovered was the presence of both synchronized and non-synchronized electrical gradients. Considering the Cell Assembly Hypothesis, “This kind of neural activity can be the basis for various brain functions including perception and memory, and, from there on, higher brain functions, such as cognition and consciousness” (Sawai et al., 2019).

Another argument for the establishment of ethical guidelines in organoid research is connected to the use of non-human animals in cerebral organoid research. A recent experiment claims to have successfully implanted a whole-brain organoid into an adult mouse. This is relevant because skeptics of organoid consciousness argue that ethical considerations surrounding organoid research are not necessary until we find a way to provide organoids with sensory inputs. This could lead to the vascularization of the mini-brains,

which is predicted to lead to growth, potentially increasing their size and sophistication. The biggest concern with this method of research is the host animals becoming more “human” and potentially developing an understanding of their captivity.

Furthermore, cerebral organoid research intersects with health care, and we will need to reflect on nursing research and medical ethics (Heale & Shorten, 2017; Taylor, 2013). For instance, in ethics of nursing research (Heale & Shorten, 2017), principles of justice, minimizing harm, and informed consent are important dimensions to consider in organoids research and clinical applications. Moreover, in theories of medical ethics (Taylor, 2013), consequentialism, deontology, virtue, and normative ethics are each important to consider in ethical analysis regarding the use of specific brain or lung cell types in organoid research for new clinical care therapies. Additionally, alternative pathways in medical ethics—including narrative, case-based, and feminist ethics—are also important dimensions to consider in all aspects of organoids research and informed consent. As outlined in Table 3, continuing community dialogue and reflections on the ethics of organoids research and its clinical applications will remain paramount as animal and human organoids research unfolds in years to come. In light of the ethical reasoning in AAC&U’s VALUE rubric, it will be important to monitor how different ethical perspectives and concepts connect with organoids research.

○ Conclusion

Based upon review of cerebral organoids research, it will be important to continue ongoing discussions on the ethical dimensions of cerebral organoids research and consciousness. While there are already ethical guidelines for conduct in the use of animals in research, it will be crucial to include discussions of ethical principles, including justice, nonmaleficence, patient autonomy, and beneficence. That being said, it is important that we continue

to explore this topic and collect data on how cerebral organoids research and consciousness are emerging.

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