The Essential Guide to Implementing Responsible and Reproducible Research on Organoids: MIAOU, ECHOES, and RICOCHECK

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1. What are organoids?

Organoids are products of biotechnology that emerged in the early 2000s, building on decades of research on the potential of human cells to proliferate and renew even outside ofthe body. Intuitively, "organoid" seems to describe an entity that has similarities to an organ in the cellular composition and/or with a similar architecture that reproduces at least some of the features and functions of an organ. However, the term is more generally used by scientists to refer to a family of entities made from various types of natural or engineered stem cells of healthy or pathological origin. Organoids differ from conventional cell cultures in that cells can self-organize into three-dimensional complex structures that share some anatomical and functional properties of developing organs. Organoid research has been accelerated by (i) overall advances in stem cell research and (ii) innovations in culture media reagents and devices that make three-dimensional expansion possible, consistent and reproducible between laboratories, whilst capturing physiological tissue functions.

Here are some examples of entities (among many) currently referred to as "organoids" or related to organoids:

- Intestinal organoid: stem cells issued from patient intestinal biopsies can be cultured in vitro such that small three-dimensional structures adopt the shape of an intestinal crypt. Such organoids can be expanded indefinitely and used to study normalgut physiology or better understand intestinal diseases in patients.
- Neural organoid: skin cells – or other cells of the body – are reprogrammed to induce pluripotent stem cells and then differentiated into neural tissue that can resemble different parts of the immature nervous system. How the tissue self-organizes, or the different forms of neural cells, can provide insights into brain development or neurodevelopmental pathologies.
- Tumoroid: cancer cells taken as a biopsy from tumors in patients can be grown in vitro such that they are a near replica of the original tumor – the (personalized) tumoroid can be used to test drugs and make predictions on whether a drug will be effective in treating the patient or not.
- Embryonic model: pluripotent stem cells can differentiate in vitro such that they replicate some of aspects of early embryo development rather than a specific organ. This kind of laboratory model might help understand why miscarriages occur, improve in vitro fecundation procedures, etc.
- Organoid-on-chip: stem cells or organoids can be introduced into engineered microfluidic devices made of plastic or silicon (the 'chip') that provide them with nutrients, oxygen, or drugs under conditions of fluidic flow or flexible substrates resembling real tissue.
- Assembloid: organoids from different cell types or tissues are grown together (or assembled). For example, a brain organoid might be connected to a muscle organoid to mimic innervation of muscle tissue, or gut and stomach organoids coupled to mimic the gastrointestinal tract. These models do not focus only on a specific organ but give insights into global, physiological or pathological processes in the body.

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- Chimera: namely a mixture of cells originating from two different species. In the case of organoids and related fields, after being grown *in vitro*, human organoids can be transplanted into animals to enable further development and physiological integration including vascularization. This has mostly been reported for neural and kidney organoids. Transplantation aims to mimic normal physiological conditions in tissues resulting in an improved maturation of the human organoids. A main ethical issue is to know if such transplant confers human properties to the engrafted tissue. In example :. engrafted into injured visual cortex in rats, human neural organoids survived for up to 3 months, formed afferent and efferent connections with the host visual network, and responded to visual stimulation.

2. What are organoids used for?

Potential applications of organoids range from fundamental biology (understanding mechanisms of development) and disease modeling (understanding how diseases develop) to use in drug discovery and therapeutic protocols for (personalized) clinical application in medicine.

More specifically, organoids are, or will be, used in:

- *Basic research*. Organoids as models of development offer windows into the physiology and pathology of an organ. They are useful for basic research in developmental biology and in understanding the mechanisms underlying diseases.
- *Preclinical research towards therapies*. Preclinical research tests potential therapies for effectivity prior to clinical trials in patients. Organoids can be used to test whether disease phenotypes are reversed, check toxicity and whether the treatment affects metabolism, etc. Models based on human stem cells could eventually replace some animal models in assessing effectivity and toxicity.
- *Clinical use*. Organoids are increasingly used as tools for personalized treatment selection in the clinic, but could also be a source of biomaterial for regenerative medicine, for example for transplantation as Advanced Therapy Medicinal Products (ATMPs).
- *Bioproduction*. Organoids could be engineered and used for the production of biomaterials, for example virus production for vaccines and gene therapy that already have clinical utility.

With regard to the interests of patients, the most important distinction is between research (fundamental or biomedical) and immediate clinical applications (such as personalized medicine, where a single use model is created for each patient) or bioproduction to allow access to expensive drugs by decreasing the production cost. The vast majority of organoids developed in laboratories today are for research. Clinical applications are still under development although in trial in some advanced research settings.

Basic research	Developmental biology
	Disease modeling
Preclinical research	Drug development and controls
Clinical research	Personalized treatment screening
	Material for regenerative medicine
Bioproduction	Produce molecules, proteins, viruses for treatments, vaccines…

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Applied to the specific field of organoids and related researches, two main areas are considered as priorities for a specific ethical review as they may be subject to measures related to the precautionary principle: i) organoids associated with the dissemination of genetic material in the offspring, and ii) organoids challenging moral values such as embryonic models and complex neural organoids in which higher order brain functions such as consciousness or suffering may emerge.

Four ethical categories are proposed:

- **Cat. 1a**: there is no difference or additional ethical problems in comparison to usual cell cultures: a "simple" approach to organoids (kidney, liver, etc.).
- **Cat. 1b**: specific ethical consideration is recommended to the researcher and certain declarations must be made to the authorities: sexual reproduction organoids, "simple" neural assembloids (interconnected organoids not reaching high order brain functions) and gastruloids.
- **Cat. 2**: cases where specific approval by an Ethics Committee is required: blastoids, complex assembloids such as cerebral cortex neural organoid connected to sensory and possibly motor systems.
- **Cat. 3**: a prohibited research because there is a lack of compelling scientific rationale and/or ethical standpoint concerning: gestating human stem cell-based embryo models, or the transfer of human-animal chimeric embryos to a human or non-human primate uterus

Cat. 1a	General ethical review	"Simple" organoids (kidney, liver, etc.)
Cat. 1b	Specific ethical consideration recommended	"Complex" organoids (neural, sexual reproduction organoids, etc.)
Cat. 2	Specific approval by an ethics committee required	Blastoids, complex neural assembloids, etc.
Cat. 3	Prohibited	Gestating human stem cell-based embryo models, transfer of human-animal chimeric embryos to a human or non-human primate uterus, etc.

3. Why is it important to promote research integrity in the field of organoids?

Biomedical research, especially in the rapidly evolving field of organoids, is complex and multifaceted. To ensure robust and ethically sound progress, researchers need comprehensive guidelines and checklists. The HYBRIDA project has developed such a framework based on two sets of converging principles:

1. The ALLEA Code of Conduct for research integrity, emphasizing reliability, honesty, respect, and accountability.
2. The 2021 WHO expert group recommendations on governance of human genome editing, focusing on openness, transparency, responsible stewardship, inclusiveness, caution, fairness, and social justice.

This resulted in the creation of three essential tools: MIAOU, ECHOES, and RICOCHECK (https://hybrida-project.eu/deliverables/).

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4. How to promote responsible research with organoids and related technologies?

To ensure integrity in science and ensure the interests of all stakeholders are respected, researchers need to comply with general quality standards for scientific integrity and codes of conduct for research integrity in their institute and country of origin. Many procedures are already in place in biomedical research to ensure the ethical conduct of research, especially when it comes to research with human material, including human stem cells. Most researchers are familiar with these prerequisites which also apply to organoid research. Does organoid technology call for additional recommendations or not?

Some forms of organoid research introduce new uncertainties: this may relate to sensitive cell types like germ cells and gametes, being formed, or, as noted above, brain-like structures. Guidelines are one way of coping with uncertainty, especially regulatory uncertainty. The proposed guidelines aim at building trust among the various stakeholders in organoid research by ensuring that scientific knowledge builds upon reliable data and simple tools are available to assess the different aspects of research proposals. Guidelines encourage researchers to report data and metadata in a standardized format that will clarify the methods and purposes of the organoid research that will be carried out.

4.1. A set of requirements to ensure the quality of reporting for research conducted with organoids

To enable a real assessment of the quality of research reporting, each batch of organoids should be associated with standard information (metadata). This includes description of the tissue/cell sources, procurement protocols, validation and conservation of raw material, protocols and databases used, as well as culture and differentiation protocols and quality control criteria for each level of organization such as biobanks. In this way, researchers receiving organoids can rely on the data describing and characterizing the organoid (structural data: omics; morphological data: imaging and functional data). Further, the metadatashould include all regulatory aspects that have been complied with in the relevant jurisdiction, noting that the recipient researcher may be subject to different legislation, so asto avoid ethics dumping: material transfer agreement with provisions for the use of the organoids, prior verification of patient consents, authorization from regulatory agencies for organoids created from patient cells, declaration of collection and of material transfer if applicable.

We call this standard set of requirements the **Minimal Information about an Organoid and its Use for Researchers** (**MIAOU**). It addresses the following: the origin of biological material (including informed consent from cell donors), efficacy/reproducibility, quality of results (size, morphogenesis, cell composition), reliability, genetic integrity, minimization of communication errors (accurate and documented description of materials and methods), compliance with safety, security and research integrity rules, prevention of research misconduct and miscommunication with the lay public. A template questionnaire is provided and will be implemented as a user-friendly webpage.

A second checklist mirrors MIAOU: it is directed to scientific bodies in charge of assessing research proposals on organoids and related technologies. Its goal is to facilitate the work of scientific committees charged with evaluating proposals concerned with building, characterizing and using organoids. This **Evaluator checklist for organoid ethical studies** (**EChOES**) describes how to evaluate the quality of organoid descriptions in a grant application for reproducibility, replicability and rationality of the proposed organoid research.To assess the quality of an application, some elements are mandatory for scientific evaluation, while the

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others are contextual (depending on, e.g., the call requirements, the application domain). It is up to the evaluators to judge whether the answers are acceptable for a given project.

4.2. A practical guide for ethics committees

The Research Integrity Committee Organoid checklist (**RICOCheck**) intends to provide a toolfor Research Ethics Committees (RECs) and Research Integrity Offices (RIOs), that will ensure transparency and anticipate ethical issues. Several principles need to be considered by RECs and RIOs, such as data confidentiality, the societal impact of the research project and its anticipated results, the approval of patient associations and the fair and responsible behavior of ethics committees involved in the evaluation of projects that use organoids. The research should be based on privacy-by-design, incorporating privacy safeguards in all steps of organoid research. Donors and/or the general public should be substantial involved in the RECs and RIOs, societal benefits and any potential harm to donors, patients, and society should be anticipated.

In conclusion, **MIAOU, ECHOES, and RICOCHECK** provide an essential framework to navigate the unique scientific and ethical challenges posed by organoid research. They foster transparency, reliability, efficiency, and ethical integrity, paving the way for a more responsible and collaborative future in this revolutionary field.

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HYBRIDA

CONSORTIUM MEMBERS

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