REVIEW SUMMARY

ORGANOIDS

Human tissues in a dish: The research and ethical implications of organoid technology

Annelien L. Bredenoord, Hans Clevers, Juergen A. Knoblich*

BACKGROUND: Organoids are stem cell–derived structures generated in vitro that display the three-dimensional architecture and physiology of intact organs. They offer unique possibilities for modeling and studying normal development and disease processes and open up innovative approaches to medical research, drug discovery, and toxicology testing. Together with reprogramming technology and gene editing methods, organoids hold the promise to influence the innovation cycle in biomedical research, including fields that historically have been the subject of intense ethical debate. In this Review, we discuss the ethical implications of organoid technology and the impact on biomedical research.

ADVANCES: Owing to their great potential, organoids are likely to affect ongoing ethical debates over subjects ranging from the role of animal experiments and the use of human embryos and tissues in biomedical research to precision medicine, stem cell transplantation, and gene therapy. Most societies have formulated public policies to balance the advancement of biomedical science with the various concerns regarding the use of animals and human embryos and tissues for biomedical research. Organoids may necessitate a recalibration of these ethical and legal policies. However, they should not be presented as a simple solution that can abrogate controversial technologies. We suggest that the use of organoids is complementary to, rather than in competition with, these classical research methodologies.

In addition, organoids should not be seen as a morally neutral alternative. They are grown from cells and tissues obtained from human individuals, and establishing the moral and legal status of human organoids requires ethical discussion and empirical research, particularly for sensitive cases such as brain organoids. The storage and use of organoids in so-called living biobanks raise ethical and governance challenges—for example, questions about the type of donor consent and ethics review needed for long-term storage and use and for feedback of clinically relevant findings to the patient. Personalized drug testing in organoids may close the gap between preclinical drug development and clinical trials. It will further blur the line between research and care and will challenge policies for drug reimbursement by insurance companies. Cautious ethical approaches are needed for the first clinical transplantations of organoids, particularly when organoid technologies are combined with gene editing. Last, the strong public interest in organoids, together with the immature nature of the field, requires particular attention regarding public (media) communication to avoid inaccurate or incomplete representations and excessive expectations.

OUTLOOK: Organoid research holds considerable potential for investigating human development and disease and for advancing precision and regenerative medicine. Despite these promising applications, there are several layers of complexity, not only in a technological sense, but also with regard to the ethical introduction in research, clinical care, and society. By engaging scientists, clinicians, patients, ethicists, policy-makers, and the public in constructive interdisciplinary collaborations and dialog around the challenges discussed in this Review, we strive for responsible research and innovation and long-term acceptance of this exciting technology. ■

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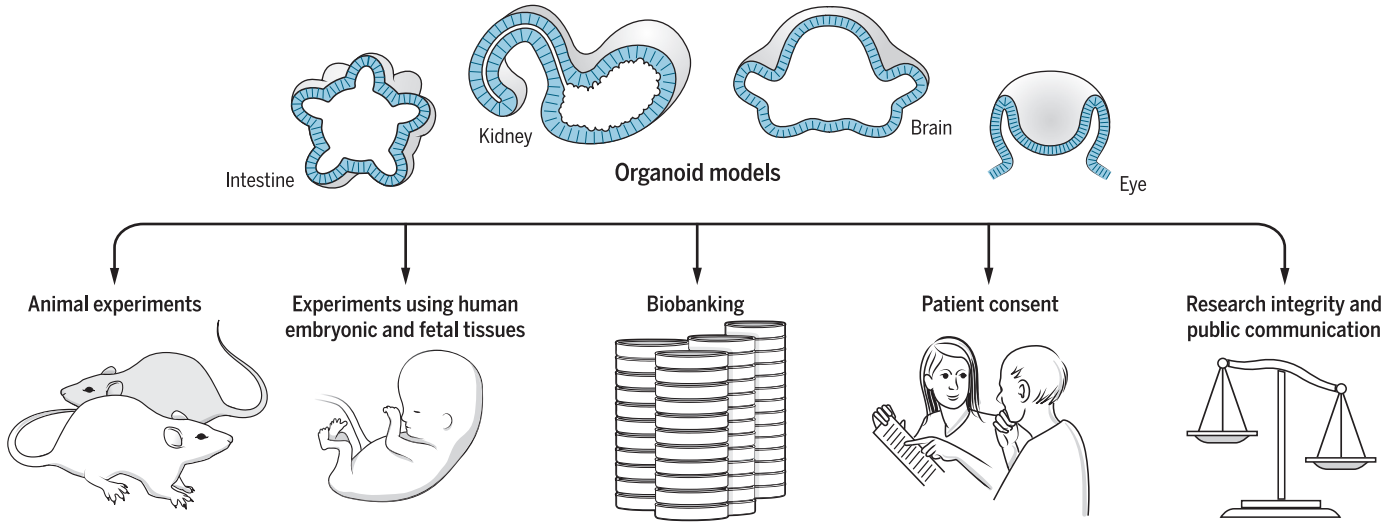
Ethical considerations of organoid models. Organoids are likely to affect policies for research using animals and human embryos. They also have implications for biobanking and patient consent policies and require particular responsibility in communicating results to the public.



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REVIEW

ORGANOIDS

Human tissues in a dish: The research and ethical implications of organoid technology

Annelien L. Bredenoord,[1] Hans Clevers,[2] Juergen A. Knoblich[3]*

The ability to generate human tissues in vitro from stem cells has raised enormous expectations among the biomedical research community, patients, and the general public. These organoids enable studies of normal development and disease and allow the testing of compounds directly on human tissue. Organoids hold the promise to influence the entire innovation cycle in biomedical research. They affect fields that have been subjects of intense ethical debate, ranging from animal experiments and the use of embryonic or fetal human tissues to precision medicine, organoid transplantation, and gene therapy. However, organoid research also raises additional ethical questions that require reexamination and potential recalibration of ethical and legal policies. In this Review, we describe the current state of research and discuss the ethical implications of organoid technology.

Recent years have seen a rediscovery of three-dimensional (3D) cell culture technologies that were originally developed in the 1980s and 1990s (*1*–*3*), when dissociating and re-aggregating cells from developing organs in 3D was used to understand the inductive and adhesion processes guiding organogenesis. In the past 10 years, organoids have reemerged in the scientific literature, yet in a somewhat different form. An organoid is now defined as a 3D structure, grown from stem and progenitor cells and consisting of organ-specific cell types, that self-organizes through cell sorting and spatially restricted lineage commitment (*1*). Most excitingly, many recent studies describe culture technologies allowing human stem cells to self-organize into 3D structures that exhibit key structural and functional properties of a variety of specific organs. Human organoids have been established for a wide range of organs, including the gut, kidney, pancreas, liver, brain, and retina, among others (Fig. 1) (*1*, *2*).

Organoids can be grown from two types of cells: (i) pluripotent stem cells, such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, or (ii) organ-restricted adult stem cells (aSCs) (Fig. 2). Both approaches are critically dependent on the expansion and self-organization potential of cells in culture. In a typical organoid protocol, these stem cells are driven toward particular lineages through combinations of growth factors in culture media. By allowing cells to differentiate in a controlled manner with defined media, most organoid methods follow the endogenous cell fate pathways. They either reiterate organ development starting from pluripotent stem cells or recapitulate adult organ repair from adult stem cells in a culture dish (*2*). Of key importance is a 3D support matrix, typically Matrigel, that allows those events to occur in the same 3D orientation as they would in vivo. The outcome is an in vitro counterpart of the organ that resembles both the architecture and physiology at a striking level of detail (*1*–*3*).

Because organoids model the development and maintenance of a human organ, they have the potential to revolutionize biomedical research and change the drug discovery process. Patient-derived organoids offer possibilities to mimic pathologies of human genetic disorders in a dish and develop personalized treatment, be it for hereditary disease or cancer (*2*–*6*).

We anticipate that organoid technology will affect the ethical dimensions associated with the entire innovation cycle in biomedical research. Some of these ethical challenges are similar to those posed by regenerative medicine, which aims to restore impaired function by repair, replacement, or regeneration of cells, tissues, or organs (Table 1) (*7*). Therefore, the impact of organoids for basic research, clinical research, and precision and regenerative medicine requires a comprehensive assessment of the ethical and societal implications.

The development of organoid technology is reshaping experimental stem cell biology in fields that historically have been subjects of intense ethical discussion, such as animal experimentation and the use of human embryos for research. Below, we describe the current state of research and discuss the ethical issues that are affected by organoid technology. Although many of these issues are common to all types of stem cell research, the public attention that organoids have received and the expectations that are placed on this type of research justify a separate discussion.

How organoids affect animal experimentation

Animal experimentation is widely used as a proxy for understanding human embryonic development and organ function (*8*). Along these lines, disease research strategies commonly involve the generation of animal models. Laboratory animals, typically mice, are genetically modified or otherwise manipulated to develop a comprehensive set of symptoms associated with a specific disease. Histological and biochemical analyses of such disease models yield valuable insights into the underlying disease mechanisms. In addition, animal models can help to identify drug targets, molecules whose inhibition of activation can reduce or abrogate those symptoms. Last, animal models can be used to predict the efficacy and side effects of chemical compounds affecting those drug targets.

Although animal models have been very successful, transferring their results to humans has become a bottleneck in disease research and therapy development (*9*). Profound differences in metabolism and regulation of size and life span all contribute to the fact that most medications developed in animals ultimately fail in human clinical trials. Organoids offer an alternative. They are generated from human cells and therefore exhibit human metabolism and cell turnover. Although none of the currently available organoid models recapitulate the complete physiology of a human organ, organoids have already been used successfully for disease modeling and drug research—e.g., for the development of individualized human cancer models (*4*, *10*) and for the patient-specific evaluation of the therapeutic efficacies of cystic fibrosis drugs (*11*).

The emergence of organoid models has considerable impact on the ongoing ethical debate about animal experimentation. Currently, sacrificing animals for research purposes—and for human consumption—is common practice, yet it is far from ethically neutral (*12*). Diverse views exist on the acceptability of using animals for research (*13*). Strict opponents of animal research believe that any kind of animal experimentation is morally unjustified, regardless of its purpose or potential benefit (*14*). One view endorsed by many is to accept animal experiments under strict conditions. A set of principles, often summarized as the three Rs (replacement, reduction, and refinement), has gained wide international acceptance as a public policy tool to strike a balance between enabling animal experimentation and simultaneously respecting animals. It includes replacing animal experiments with alternative methods where possible, reducing the number of animals needed, and refining procedures so that less

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harm is caused to the animals involved (13). Although commonly accepted, these principles have led to diverging interpretations and multiple reduction-refinement conflicts. Such conflicts may, for example, appear when there is a choice between inflicting less harm on more animals or more harm on fewer animals—e.g., using one animal for every experiment versus reusing animals in different experiments, or using smaller species in larger numbers versus larger species in lower numbers (13). These complex ethical dilemmas raise a need for complementary or alternative approaches.

Organoid technology may be viewed as the long-awaited alternative to animal testing. Currently, however, several limitations common to most organoid protocols restrict their ability to model diseases. First, although organoids often recapitulate small-scale organ histology, their overall shape is commonly variable and lacks the defined reproducible general architecture that is typical of a human organ (1). In the future, this limitation might be overcome by using bioengineered scaffolds that standardize organoid size and establish reproducible polarity axes (15, 16). Second, most current organoid protocols lack blood vessels, limiting organoid size and complexity and generating artifacts due to insufficient oxygen and nutrient diffusion. Again, this problem can potentially be overcome by coculturing organoids with blood vessels generated using one of several in vitro protocols already available. Similarly, the peripheral nervous system innervating most organs is missing from current organoid protocols. Third, organoids currently lack immune cells and thus do not reflect interactions with the immune system. Although this problem might be overcome by the establishment of suitable coculture protocols, organoids are unlikely to recapitulate the detailed understanding of immune reactions that can be obtained from animal models.

In addition to these technical limitations, a number of development and disease aspects are unlikely to ever be modeled in organoids. These include complex and unexpected interactions occurring at the organismal level between multiple tissues and organ systems that can only be discovered in the context of an entire animal. For example, although cancer can be modeled in organoids (4), the same may not be true for the complex organ interactions leading to organ wasting. The recent identification of very complex organ interactions in obesity involving the gut microbiome, the parasympathetic nervous system, and the pancreas (17) provides another illustrative example of complex organ interactions whose identification requires experiments in animals. Thus, organoids are unlikely to ever completely replace animal experimentation. To paraphrase Aristotle, the body is greater, and more complex, than the sum of its parts.

Thus, although animal research will never become entirely obsolete, organoid technology does affect the ethics of animal experimentation. The use of organoids is complementary to, rather than in competition with, this classical research methodology. However, the onus of proof for rationalizing the use of animals might justifiably shift further toward a "comply or explain" paradigm: Either one uses organoids, or one explains why animal experimentation is needed. Justification for the use of animal experiments over organoid models might become necessary on a case-by-case basis. Both the ethical paradigm and legislation of animal research may need ongoing critical scrutiny. In many areas of the world, laws on animal research already encompass proportionality and subsidiarity requirements, which means that researchers should continuously make efforts to reduce, refine, and replace animal experiments. Funding agencies and research institutes should have policies in place to ensure and review whether submitted experiments were indeed necessary and proportionate, and if so, whether the studies were carried out with the smallest possible negative impact on animals. This can be achieved by adding specific paragraphs in grant applications, by asking grant reviewers to specifically take into account these paragraphs, and by having institutional committees on research and animal ethics. Scientific journals may play a role as well. They can increase animal ethics standards by providing authors space in which to define the refinement and animal welfare precautions taken by the research team (18). At the same time, it remains important for organoid researchers to emphasize that basic insights and clinical treatments cannot be developed without animal experimentation.

How organoids affect research on human embryos and fetuses

In addition to their impact on animal experiments, organoids—particularly those generated from pluripotent stem cells—affect the use of research material obtained from human embryos and fetuses (referring to weeks 1 to 8 after fertilization versus weeks 9 and beyond, respectively). Currently, human embryonic and fetal tissue is subject to a variety of techniques, including short-term culture, immunostaining, gene expression studies, and biochemistry. In addition, live-imaging experiments have been performed on human fetal brains and have led to important insights into human brain formation (19). In a recent example, human embryonic brain tissue has been used to elucidate the connection between Zika virus and its associated brain development pathologies (20, 21).

A diversity of views exists on whether it is justified to create and use human embryos for research. Broadly, three positions on the moral standing of human embryos and fetuses can be discerned (22, 23). The first position holds that the human embryo deserves full protection starting with fertilization, either because it is considered a person or because of its inherent potential to become a person. In this view, the destruction of human embryos is considered ethically unacceptable. This absolutist position is particularly defended by orthodox Christian traditions (24). It is not a position common to all religions; even conservative Jewish and Muslim traditions place the ensoulment of the human embryo not at conception but later in development, although the exact moment varies in time and per school of thought. The second position holds that the early embryo has some moral standing that increases throughout development and pregnancy. Support for this position includes some aspects of human developmental biology: The early embryo is nonsentient (not characterized by sensation and consciousness) and may not be considered an individual yet because twinning can still occur (25).

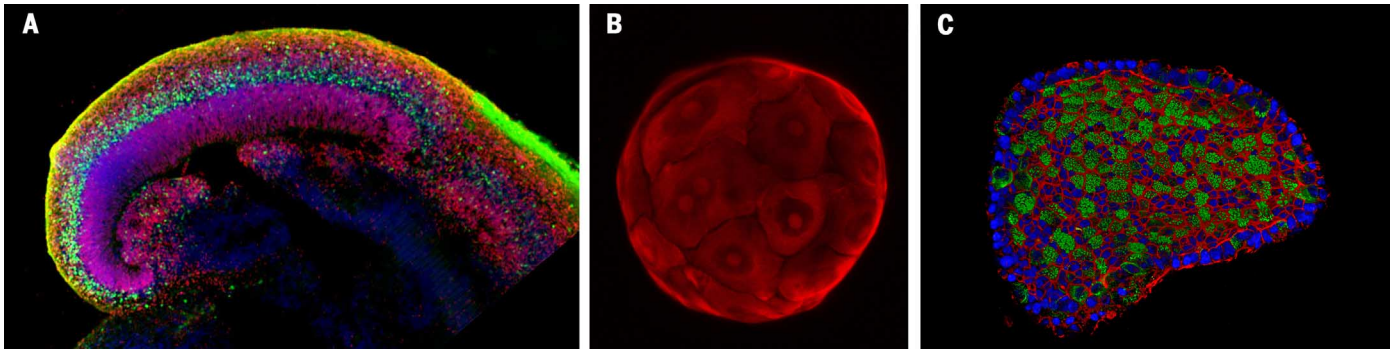

Fig. 1. Examples of organoid models. (**A**) Human brain organoid displaying complex morphology. Heterogeneous regions containing neural progenitors (SOX2, red) and neurons (TUJ1, green) are visible. [Courtesy of M. Lancaster] (**B**) Human liver organoid (actin cytoskeleton in red, blurred). [Courtesy of H. Gehart] (**C**) A human airway organoid (nuclei in blue, actin cytoskeleton in red, and cilia in green). [Courtesy of N. Sachs]

This gradualist or intermediate view considers research on early developmental stages to be acceptable. The position may also be balanced by other moral values such as the potential to prevent or cure disease or reduce human suffering. It has resulted, for example, in the well-known 14-day rule that has permitted human embryo research in vitro in the period before the primitive streak appears (*26*). The third position does not ascribe an independent moral status to the early human embryo. In this position, embryos can conditionally be created and used for research. This position overlaps with the second position in that it may ascribe moral status to the fetus throughout human pregnancy, but it is different in how it values the early embryo. For each of these positions, there is the condition of whether consent is given by the providers of the gametes, developing embryo, or fetus (*22*, *23*).

Many countries in the world have adopted the second, gradualist position, which aligns with the general intuition that the loss of a fetus becomes increasingly problematic during pregnancy. These countries allow embryo research under strict criteria. Nevertheless, most of these countries do not allow the deliberate creation of human embryos solely for research purposes. Typically, research is restricted to embryos that are left over from in vitro fertilization (IVF) procedures. Although this has become a broadly accepted practice, it poses an ethical dilemma in itself, because the IVF procedure involves the creation and discarding of surplus or unsuitable human embryos in vitro solely for a medical treatment—in this case, infertility (*25*). Formally, therefore, this common practice places a higher ethical value on treating infertility than on developing therapies for other more common and often lethal medical conditions.

The emergence of organoid technology might affect the balance between these various views. Nevertheless, international and local guidelines for human embryo research might not need to be adjusted. Most of the rules already encompass proportionality and subsidiarity requirements, which implies that embryos may only be used when no other avenues for research are possible. The International Society for Stem Cell Research (ISSCR) recently revised their *Guidelines for Stem Cell Research and Clinical Translation* to require approval and monitoring by a specialized human embryo research oversight committee (*27*). At the least, researchers will increasingly have to explain what kind of (and how much) tissue or cell source is necessary and proportionate, which means that the expected social value of the research should outweigh the moral harm of using an embryo or fetus for research. This might create a new equilibrium for the necessity and justification of embryo research.

From a purely scientific viewpoint, the emergence of organoid research is unlikely to abolish the need for human embryo material. In fact, it might even increase it. An emerging shift from animal to human models may require increased verification of reagents (for example, antibodies) and research results obtained in "real" human tissue, much like iPS-based observations must currently be compared to the "gold standard" ES cell models. Thus, the validation of newly emerging organoid models may require their comparison to normal human tissue, at least initially, and for this a more detailed analysis of actual human embryology is desirable. The recent elucidation of disease mechanisms triggered by Zika virus has demonstrated how experiments in organoid systems and human tissues can be complementary (*20*, *28*–*31*). Without prior verification of organoid systems by comparison to actual human tissues and without confirmation of results in human embryos (*20*, *21*), the value of these experiments might not have been sufficient for the resulting medical recommendations.

Fig. 2. Growing intestinal organoids. Artistic view of the process of growing organoids from adult intestinal stem cells.

It should also be noted that many individuals may not consider organoids to be a morally neutral alternative to human embryos. Therefore, the moral and legal status of human organoids warrants discussion as well. For example, the value that donors ascribe to "their" organoids has not yet been addressed and should be explored by qualitative and quantitative research methods (*32*). Specific ethical and empirical research is needed to evaluate the creation of increasingly sophisticated cultures that recapitulate human organs, including the brain, in vitro (Fig. 3). Cerebral organoids, also known as mini-brains or cerebroids, will be particularly sensitive, at least in public perception, because they might reveal personalized cognitive features (*32*). Of note, in recent experiments, stem cell–derived embryoid bodies were grown beyond the stage where the embryo normally would implant into the uterus. Although this would potentially have allowed the analysis of this particularly interesting stage of human development, the experiments had to be stopped before 14 days because of the 14-day rule mentioned above (*33*, *34*). It might be valuable to revisit the ethical considerations underlying the 14-day rule (*26*). Last, many of the current developmental organoids start from human ES cells whose establishment requires the use and destruction of embryos derived by IVF or generated specifically for research purposes. In view of the above, we expect

Table 1. Scientific and ethical comparison of animal models, human embryo tissues, and organoid models.

	Biological considerations	Ethical considerations
Animal models	Modeling of complex organ interactions	Animal research is ethically controversial
	Models include immune system, blood vessels	Reduction, refinement, and/or replacement of animal experiments is a commonly accepted goal
	Results often not transferrable to humans	
Human embryos and fetuses	Experimental limitations include reduced numbers because of low availability	Diversity of views regarding the moral acceptability of using and creating embryos for research
	Necessary for verification of organoid results and human reagents (antibodies)	Most countries allow research on embryonic and fetal tissues under strict conditions
	Organoid research might increase rather than decrease the need	
Organoid models	Close to unlimited availability	No animal experiment, no direct use of human embryos and fetuses
	Reprogramming and genome editing techniques allow unprecedented personalization of experiments	Current culture protocols include animal-derived reagents (Matrigel)
	Limited by variability and lack of predefined axis	Some protocols require the use of human embryonic stem cells
	No blood vessels, no immune system	Organoids might require specific patient consent
	Biobanking necessary for some types of organoids	Biobanking raises specific ethical issues
		Frontier science: specific responsibilities for scientists in the field

that organoids will necessitate a reexamination and potential recalibration of ethical and legal policies regarding human embryo research.

How organoids affect human tissue research

Organoids grown from adult stem cells pose their own specific ethical issues. To provide a steady supply of such organoids, human biological samples have to be stored in so-called living biobanks, collections of human biomaterials assembled for medical scientific research purposes (*10*). To collect material, biobanks either use residual tissue left over in the course of clinical care, or they collect tissue specifically for research. Over the past years, an ethical debate has emerged regarding the ethics and governance of biobanks. Organoid biobanking is a promising and exciting new field with considerable potential for scientific research, precision medicine, and regenerative medicine (*10*). The ethical challenges of organoid biobanking are not new, but the storage and use of organoids in biobanks constitute an area of complex converging technology in which several ethical discussions come together (*32*).

First, organoid biobanking raises challenges regarding donor consent. The traditional paradigm for using human tissue for research purposes has been "consent or anonymize": The researcher can either obtain a donor's consent or take measures to de-identify the sample (*32*, *35*). For organoid biobanking, complete de-identification may not always be feasible or preferred, because it disconnects the sample from the patient history and prevents the organoid results from being used for the benefit of the biobank donors. For example, the generation of cystic fibrosis organoids has led to the establishment of an in vitro system for predicting individual patients' response to a specific drug, an experiment that would not be possible in an anonymized situation (*36*). Therefore, the use of human tissue for organoid biobanking requires attention to the patient consent process—for example, an opt-in where the donor gives explicit consent for biobanking, or, less likely in this context, an opt-out, where the tissue is used unless a donor explicitly refuses (*37*). The common procedure to restrict patient consent to a specific research area—for example, a specific disease—does not seem feasible because biobanks are used by a variety of researchers working on diverse problems. It is often the combination of the results that generates the biggest value. We have therefore proposed "broad consent for governance" as a meaningful way of obtaining consent for biobanking (*38*). In this approach, the donor is informed about the governance structure of a biobank, including ethics oversight, the privacy policy, collaboration with public and private partners, data management, data sharing, communication with donors, and the withdrawal policy (*38*). This type of consent enables donors to make autonomous decisions about participating in the organoid biobank and, at the same time, allows the material to be used by multiple researchers for diverse purposes. Establishing this type of consent requires the establishment of a rigid and to some degree invariable governance structure that protects the interests of the wide variety of stakeholders involved and is not driven by scientific needs alone (*39*).

Second, organoid biobanking raises specific ethical challenges of ownership and use. As with cell lines, organoids have a genetic and functional link to the donor (*32*). In contrast to cell lines, however, the owners may relate very differently to a complex organoid model generated from their cells, a fact that should be considered during the patient consent process. Further empirical studies will shed light on how donors relate to their organoids, what interests they may have in organoids, and whether and to what extent they view the donation of tissue for organoids the same way as or differently from donations for other types of cell and tissue research. Such studies will determine whether a separate consent and oversight process for organoid generation is necessary. This may be even more relevant for the many commercial applications of organoid models that are anticipated during the next years (*40*, *41*). Organoids offer unique possibilities for drug development, toxicology, and precision medicine, and in our experience, they are rapidly becoming attractive to pharmaceutical companies (*32*, *41*–*43*). For example, organoids can be used for high-throughput drug screening and will ultimately lead to the commercialization of new drugs (*44*). Thus, the commercial use of patient-derived organoids requires adequate regulation of ownership and fair distribution of rights between the patient donor, the researcher, and the creator of the organoids.

How organoids affect clinical research

In addition to their use as research tools, organoids are expected to be applied in precision and regenerative medicine. 3D organized human tissue

generated from patient iPS cells allows the generation of personalized organoids that enable testing of patient-specific responses to specific compounds (*11*, *36*). In addition, cultured organoids hold great potential for replacing damaged tissue or even complete organs, a potential that has already been demonstrated in animal models (*45*–*47*).

Precision medicine

Personalized drug testing in organoids offers exciting alternatives to the current phased drug testing process but, at the same time, also raises new ethical challenges. Currently, research ethics review committees (or institutional review boards) and legislation strictly distinguish between research and care. Organoids could potentially blur the lines between the two. On one hand, personalized drug testing in organoids may be viewed as research because it provides insight into drug and disease mechanisms. On the other hand, it should be considered care when it becomes part of a treatment strategy for an individual patient. In addition, current policies for drug reimbursement by insurance companies usually depend on evidence generated in large-scale trials. Organoids may necessitate revision to such policies because they potentially provide a new type of evidence in which effectiveness can be demonstrated for individual patients (n = 1) or very small groups (*32*). The possibility to select individual cystic fibrosis patients for selective drug treatment on the basis of an organoid swelling assay (*36*) and the detection of patient-specific responses to various drugs in biobanked colon cancer or pancreas cancer organoids are examples of the power of these new approaches (*10*, *48*).

Last, organoids might profoundly change the drug development process. Current first-in-human studies are designed to examine the maximum tolerated dose of a novel drug. Only if the drug is deemed safe is efficacy determined in phase II and III trials. This approach exposes participants in early phase trials to unknown risks of novel interventions, without the prospect of gaining a therapeutic benefit (*49*). If drugs can first be tested in organoids, future phase I studies might or should combine safety and efficacy more often (*49*). In a new regulatory system introduced in Japan to meet the novel challenges of stem cell biology, the determination of efficacy has shifted from premarket clinical trials to a postmarket mechanism. Although such a fast-track system might give patients earlier access to novel interventions, we emphasize the need for a procedure that both is efficient and scientifically evaluates whether novel interventions are safe and efficacious (*50*).

Organoid transplantation

Transplantation of organoids for repairing and or even replacing damaged organs poses specific ethical challenges. The recent identification of transplantable liver stem cells that can be expanded as organoids, and that thus represent a readily available and lasting hepatocyte source (*46*), has opened the possibility of transplanting those 3D cultures, potentially revolutionizing the prospects for liver disease patients. Translating these and similar results from first-in-human studies to larger randomized controlled trials is essential for testing their long-term safety and efficacy. However, the translational steps to clinical research and the public sphere raise several ethical challenges (*51*, *52*).

First-in-human trials are ethically challenging by nature, because risk and benefit need to be estimated without prior in vivo data from humans (*25*). Next to these so-called hard, quantifiable impacts, organoid technology will also have soft impacts, because it will influence our moral actions, experiences, perceptions, and quality of life (*52*, *53*). Organoids and stem cells are different from traditional pharmaceuticals in several ways (*54*). First, little (pre-)clinical knowledge is available within this emerging field. Second, animal models may not be good predictors of what happens in humans. Third, in contrast to traditional drug trials, transplantation of organoids requires an invasive procedure. Some exceptions exist: Liver organoid transplants may be infused through the portal vein, and this procedure is already being applied in ongoing trials using adult hepatocytes. Fourth, the use of stem cells may pose unexpected risks; effects due to uncontrolled and undirected growth can never be fully excluded. Last, the entire concept of regenerating complete tissues or organs ex vivo is new, and unanticipated events may occur.

For all these reasons, organoid transplantation ethically represents a so-called complex translational trial, in which several invasive interventional and study procedures are combined (*55*). Any such trial raises ethical challenges that need proactive scrutiny. An interdisciplinary, cautious approach is therefore mandatory to enable the first organoid transplantations in an ethically sound way.

Organoids and gene editing

The stem cells that build organoids can be readily modified using genome editing tools such as CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats and associated proteins). The modified organoids can be clonally expanded, the introduced genetic modification can be verified, and off-target effects can be excluded by genome-wide sequence analysis. Indeed, the *CFTR* gene mutation has been corrected using CRISPR-Cas9 in organoids derived from two cystic fibrosis patients (*56*). Thus, the combination of gene editing and organoid technologies appears to provide an ideal vehicle for gene therapy strategies. Although the ethics of gene therapy are beyond the scope of this Review, it is immediately clear that the combination of gene editing with organoid-based regenerative medicine further complicates the considerations outlined here.

Impact of organoid technology on research ethics and integrity

The high potential and public visibility of organoid technology pose ethical issues affecting not only the possible medical applications of organoids, but also organoid research itself. As a result of the ever-increasing efficiency and pace of biomedical research, organoid research is rapidly reaching the stage of medical translation, although it should still be considered "frontier science." In 1992, Bauer proposed the concept of a "knowledge filter" to describe the establishment of commonly accepted scientific knowledge (*57*). At the "frontier," the progress of scientific knowledge is blurred, insights are not sufficiently replicated, and results are often controversial. After multiple rounds of peer review, publication, replication, and scrutiny by others, novel insights have passed many filters and may become textbook knowledge—or obsolete. Frontier science has the potential to transform

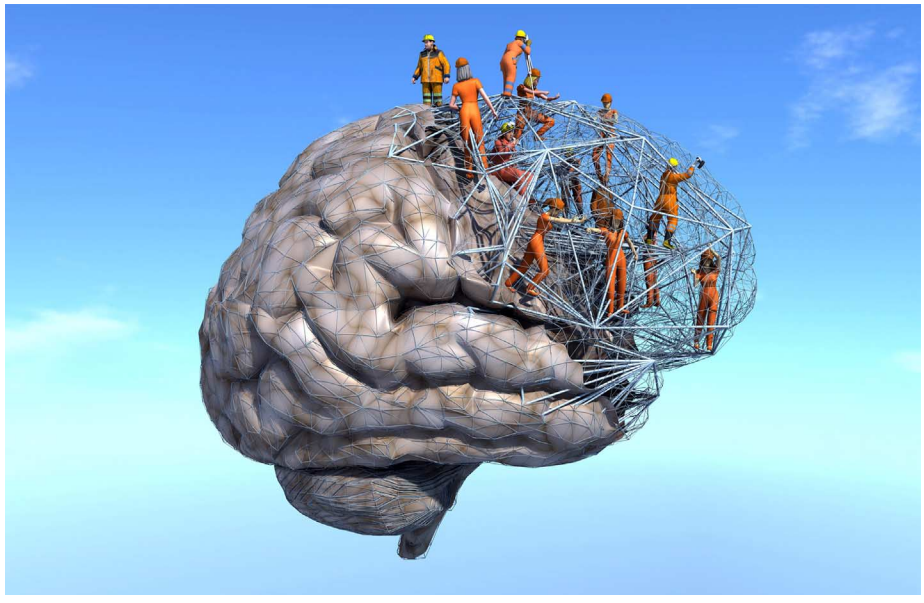

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Fig. 3. Growing brain organoids. Artist's impression of scientists growing human brain tissue in vitro.

existing research paradigms, generating approaches not used in mainstream or "normal" science, according to Kuhn (*58*). In frontier science, researchers are more likely to stress the novelty and opportunities of the innovation to attract the attention of parties required for financial, political, or moral support (*59*).

Despite its relatively immature nature, organoid research has already reached the stage of commercialization and medical application, placing particular responsibilities on scientists working in this field. Biologists working in basic science may not always be used to an immediate medical impact of their work, whereas medical doctors may underestimate the experimental nature of most organoid research. Individual organoids may be highly variable, and very rare morphological features can be made overly prominent by selecting specific images or results. In addition, organoids lack predefined polarity axes, making it harder to interpret individual 2D images without knowing the entire 3D architecture of the organoid from which they were selected.

To avoid these complications, the establishment of organoid-specific quality standards for the analysis of disease phenotypes will be important. Each experiment will have to be accompanied by sufficient information to judge the overall quality of the specific protocol chosen. In addition, 3D reconstruction of entire organoids will clarify how the overall organoid morphology matches the large-scale architecture of the corresponding organ and allow the placement of individual images within the structure. Careful statistics will need to accompany any specific conclusion taken from an organoid experiment. Ensuring these overall quality standards will anchor the long-term trust of the medical community in the newly emerging technology.

Last, the public interest in organoid systems raises particular ethical issues regarding communication with the public, patients, physicians, companies, and the media. The way in which science is represented in public communication can influence expectations and understanding and frame policy debates (*60*). Inaccurate or incomplete representation of research may have various negative consequences—for example, when used by companies exploiting unproven stem cell "treatments" (*27*). In addition, communicating through various (social) media typically requires simplification of scientific results. Although this is perfectly legitimate for commonly accepted scientific results, it becomes problematic when results are preliminary or represent the specific interpretation of individual scientists. Balancing these concerns with the public interest in organoids and their use for analyzing disease may be challenging. Nevertheless, it should be commonly accepted that sharing results with the press before peer review should be avoided, or should at least be done in a very nuanced and cautious way. Although this approach has generally been followed closely during the recent investigation of Zika infection pathology, some previous cases of undocumented scientific results being shared with the press (*61*) have resulted in confusion. The commonly accepted Singapore statement on research integrity, which is the basis for many scientific codes of conduct, is not sufficiently clear on this issue (*62*). Fortunately, the 2016 ISSCR *Guidelines for Stem Cell Research and Clinical Translation* have adopted explicit recommendations on the public responsibilities of stem cell researchers. Hopefully, these will help to promote accurate, balanced, and responsive public representation of regenerative medicine and stem cell research (*27*). The growing interest in organoid research may require research institutions to adopt specific regulations that mediate interactions with the press before peer review and publication. This is particularly true when commercial interests, such as the establishment of companies, are at stake (*63*). In such cases, scientists should refrain from making overly optimistic claims and should openly declare any conflicts of interest.

Outlook

Organoids hold great potential for investigating human development and disease. Organoid technology can reduce animal experimentation and may potentially close the gap between preclinical drug development and human trials. We predict that organoids will become model systems that are complementary to existing animal in vivo and cell-based in vitro models. It is very unlikely, however, that organoid technology will entirely replace animal experiments or experiments on human embryos and fetuses. In this Review, we have summarized ethical challenges arising from this technology (Table 1). Organoids face several layers of complexity (*64*), not only technologically but also with regard to their ethical introduction in research, clinical care, and society. Only by engaging in constructive interdisciplinary dialog around these issues, involving not only scientists but also patients, policy-makers, clinicians, ethicists, and the public, can we ensure responsible innovation and long-term acceptance of this exciting technology.

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Human tissues in a dish: The research and ethical implications of organoid technology

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Editor's Summary

Ethics of organoid research

Growing functional human tissues and organs would provide much needed material for regeneration and repair. New technologies are taking us in that direction. In addition to their use in regenerative medicine, stem cells that grow and morph into organ-like structures known as organoids can be used in drug development and toxicology testing. The potential developments and possibilities are numerous and affect not only biomedicine but also areas of ongoing ethical debate, such as animal experimentation, research on human embryos and fetuses, ethics review, and patient consent. Bredenoord *et al.* review how organoids affect existing ethical debates and how they raise novel ethical dilemmas and professional responsibilities.

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