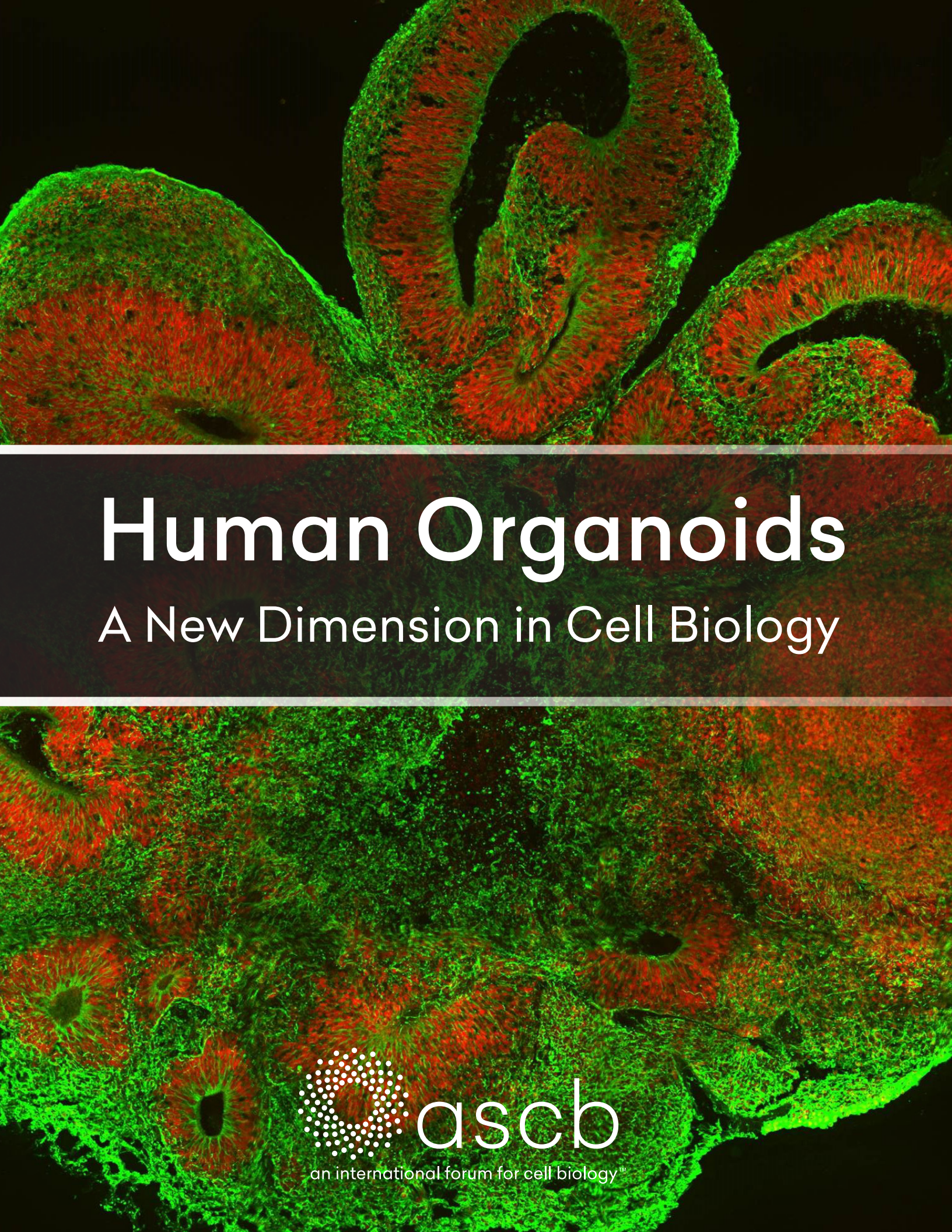

Human Organoids

A New Dimension in Cell Biology

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Human Organoids
A New Dimension in Cell Biology

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Contents

Executive Summary 1

1. Background: Biology and Derivation of Organoids 3
2. Applications for Organoid Research 5
3. Reproducibility in Organoid Research 9
4. Tissue Sourcing and Patient Consent in Organoid Research 13
5. Sharing Materials and Results 17
6. Training in Organoid Research 19
7. Public Outreach 21

Appendices

1 ASCB Organoid Task Force Members 23
2 The Task Force Mission Statement 25
3 ASCB Member Survey 27
4 References 47

EXECUTIVE SUMMARY

Advances in stem cell biology have heralded a revolution in biology and medicine. As these technologies were expanded into human cells, they have paved the way for discoveries in fundamental human biology and for advance in medical care. A major recent step in this revolution has been the development of methods to generate, under controlled cultured conditions, 3D structures known as organoids that recapitulate development and tissue organization and resemble organs in the body. Organoids originate from renewable tissue sources that self-organize in culture to acquire *in vivo*-like organ complexity. Organoids can be generated from human cell sources, including adult tissue specific stem cells, embryonic stem cells (hESCs) and induced pluripotent stem cells (hIPSCs). Therefore, they have the potential to overcome a number of previous limitations in biomedical research aimed at gaining mechanistic insights into human development, produce accurate models of human disease, and generate patient-matched tissue sources for regenerative medicine.

To optimize the potential of these powerful new developments for scientists, the American Society for Cell Biology (ASCB) asked a task force of ASCB members including researchers, several of whom play critical roles in developing organoid systems, ethicists and patient advocates to identify the opportunities of organoid research for biologists, highlight obstacles to progress and challenges, as well as generate recommendations and best practices to increase the impact of this emerging, rapidly expanding and highly promising field. Discussion by the task force, as well as the results of a questionnaire sent to the ASCB membership, acknowledge the enormous potential of these new "model systems," while also demonstrating the challenges for science and society that come with this opportunity. For composition of the task force, see Appendix 1; for summary of questionnaire results, see Appendix 3.

Opportunities

- Organoids offer the possibility to study human tissues at the same level of scientific scrutiny, reproducibility and depth of analysis as has been customarily possible only with non-human model organisms.

- Organoids allow investigators to recapitulate morphogenetic events in human development that lead to tissue and organ formation.

- Organoids can be used to study mechanisms of disease acting within human tissues, generating knowledge and tools applicable to preclinical studies, including drug testing.

- Organoids can be generated from any individual allowing the study of variability between human individuals at the tissue level as well as the cellular mechanisms leading to complex disease phenotypes.

- Organoids resembling the complexity of tissues and organs offer numerous applications for tissue engineering, drug discovery and regenerative medicine.

We propose that human organoids have the potential to provide basic scientists with the opportunity to perform mechanistic studies within a "human model" system, with acceptable ethical constraints.

Challenges and recommendations

- Organoids only recapitulate part of the entire body and may not faithfully capture the stereotypic and complex functions of individual organs. Thus, in contrast to whole animal models, organoids offer only an approximation of the biology of an entire organ and do not mimic the behavior of the complete organism. They lack key *in vivo* features such as defined body axis, functional immune system, and complete physiological networks. Therefore, results from organoids have to be complemented by whole organism studies in model systems and compared to actual human development, tissue organization, and physiology.

- "Gold standards" and best practices must be defined for the study of organoids. The protocols for the derivation and culture conditions of organoids have to provide sufficient details to enable reproducibility. Criteria need to be developed that allow investigators to compare cell types and structures in an organoid to the composition and organization of the respective organ.

- The long-term advance of organoid research relies on the distribution of tissue sources that are renewable and readily comparable between laboratories. Particularly important for the study of disease, is the establishment of tissue banks (biobanks) that distribute hIPSCs from different patients with the same disease. Such biobanks could also be a distribution hub for control samples from both unaffected individuals and genetically modified patient-derived samples.

- For patient-derived tissue samples, patient consent needs to specify the requirement that materials will be shared among different institutions, investigators, and nations.

- Entry of new researchers at different career stages into this field should be encouraged and facilitated by establishing training sites where investigators could acquire and adapt organoid technology. Because of the rapid advance of culture techniques, the intricacy of the materials and the time frame to generate an organoid from a renewable tissue source, either existing facilities or practicing laboratories may offer better opportunities for training than more traditional training courses.

- The potential of organoids for research and medicine brings with it ethical uncertainty and public concern. A clear definition of what organoids are and what they are not, as well as a clear, realistic description of the opportunities they offer should be articulated by scientists and scientific organizations in their communications.

1. BACKGROUND: BIOLOGY AND DERIVATION OF ORGANOIDS

Organoid definition

We study organoids because they represent minimal and reproducible models of complex human tissue dynamics during development, homeostasis and disease. These models can be used by multiple labs, and are easily manipulated, imaged, and subjected to biomolecular analysis without the confounding complexity associated with studies *in vivo*. When grown from human cells, they facilitate the transition from animal models to human biology with acceptable ethical constraints.

What defines organoids is the use of a renewable tissue source that (i) is derived from stem cells or primary tissue, (ii) is cultured in a defined environment, (iii) self-organizes into a structure that mimics the healthy or diseased modeled tissue, (iv) incorporates many aspects of the cellular complexity of the modeled tissue, and that (v) can be propagated and shared either as a culture itself or through a defined stem or progenitor cell population.

A brief history

Organoid culture is firmly based on 3D cell culture methodology developed over the last century. As early as 1906, the so-called hanging drop method allowed cells to be cultured in 3D (Harrison, 1906; Simian and Bissell, 2017 for a detailed historical perspective). The current boom of organoid research results from the ability to grow organoids from cells or tissues derived from individuals, revealing their enormous potential for human biology and medical research (Dekkers et al., 2013; Lancaster et al., 2013). For the purposes of this report, we focus primarily on the opportunities and challenges with regard to human organoids, rather than on organoids generated from animal cells and tissues.

Human organoids as a new model organism for research

For the past several decades, biomedical research was almost exclusively carried out in animal models. While this has led to a deep understanding of many fundamental biological processes, it has left gaps in our mechanistic understanding of human-specific developmental, cell biological, physiological, and disease-related events. Furthermore, the diversity of human individuals is in sharp contrast with the genetic homogeneity of inbred animal models, leading to a deficiency in our knowledge about population diversity. Indeed, this lack of human model systems may have contributed to the low success rate in clinical trials of pharmaceutical compounds developed in animal models.

With the advent of human organoid models, this situation seems poised to change. For the first time, organoids offer the possibility to study the assembly of human tissues in a personalized manner. Organoids allow the recapitulation of many morphogenetic events leading to tissue formation. They can potentially be generated from any individual, whether healthy or diseased. Thus, both the variability between human individuals as well as the human-specific cellular mechanisms that lead to disease phenotypes can be analyzed directly.

However, human organoids have limitations as a model system. In contrast to animal models, organoids offer only an approximation of the biology within a human body. They lack key *in vivo*

features such as a defined body axis, a functional immune or nervous system, or functional vasculature. Therefore, while organoids can teach us about human-specific aspects of organ development and physiology, at present, they are less useful for uncovering aspects of biology that rely on integrated physiological systems and the complex interplay of human organ systems. Whatever results we find in organoids will only be useful relative to the background of knowledge defined in other model systems. In that sense, organoids are themselves a new model system that is complementary rather than superior to existing animal models.

Organoids can be generated in different ways, recapitulating either organ development or organ regeneration. In their simplest form, organoids are generated from adult tissue stem cells cultivated in the presence of growth factors and matrix normally provided by the stem cell niche. Such cultures can contain all cell types derived from stem cells *in vivo*, either during normal tissue turnover or during repair after injury and can recapitulate aspects of their three-dimensional arrangement. Alternatively, organoids can be generated from pluripotent stem cells including embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). This is achieved by culturing them in a specific series of growth factor- or media-cocktails that induce organ-specific cell fates in a sequence that mimics normal development. The final conglomerate of organ-specific cell types can arrange itself in the way found in the actual organ, allowing analysis of morphogenetic and physiological processes “in a dish.”

A survey of ASCB members (see Appendix 3) found that while over 90% of respondents use human cells in culture, less than 30% use human organoids. Among the main bottlenecks that respondents listed were difficulties in growing organoids and the availability of human tissue with which to initiate cultures. A significant number of comments pointed to issues of reproducibility and cost, and many respondents questioned how well organoids actually model human biology. In this report, we will lay out opportunities for organoid research, address concerns, and make recommendations for the use of human organoids to add to the “model organism” repertoire.

2. APPLICATIONS FOR ORGANOID RESEARCH

Organoids offer many exciting experimental applications, ranging from gaining a better understanding of human development to generating clinical models for drug testing and regenerative medicine. Given the rapid progress so far, we believe the applications below will provide a glimpse of what is likely to come in the future.

Human developmental biology

The study of human development has largely been limited to observational studies on preimplantation embryos or progenitor cells and tissue isolated from aborted fetuses. For example, in the latter case, organ specific progenitor cells are isolated from fetal tissues and grown in culture under conditions in which they continue to grow and differentiate (Nikolic et al., 2017). However, the advent of a variety of organoid models derived from iPSCs has provided a path towards dynamic observation and mechanistic studies of human development.

There are two basic approaches to studying human development using organoid models derived from iPSC. In the first, organ-specific progenitors are generated from iPSCs by passing them through a sequence of exposures to defined factors. After further culture the progenitors self-organize into organoids representative of the developing organ. This approach has already provided a wealth of insight into the morphogenesis of several organ systems, and more intriguingly, is beginning to shed light on how human genetics impacts developmental diseases of the brain, lung, and gut (Perez-Lanzon et al., 2018).

In the second approach, iPSCs are coerced to form cellular aggregates that mimic the early pre-implantation embryo itself. These structures, known as embryoids or gastruloids, autonomously undergo the early stages of development. Progress in this area has dramatically accelerated in recent years, even pushing up against the rule which limits the culture of human embryos beyond 14-days, a stage when mesodermal cells are normally generated in the primitive streak (Hyun et al., 2016). These studies have revealed distinctions between the earliest stages of development in humans and model organisms such as the mouse, for example in the establishment of the embryonic axes and the specification of primordial germ cells (Irie et al., 2015; Kobayashi and Surani, 2018; Martyn et al., 2018). They offer the possibility of further insights into early human development, enable evolutionary studies of the species-specificity of early developmental events, and establish a new model system relevant to the underlying causes of early human pregnancy loss.

Human disease modeling

Beyond modeling human development, the cellular complexity and three-dimensional organization of organoids provides a unique platform for identifying the mechanisms of adult human disease. The cellular organization of organoids can be studied at the systems level with advances in functional genomics and proteomics, including single cell analysis and high-throughput transcriptomics, proteomics, and large-scale characterization of chromatin domains and transcription regulatory elements. This level of detailed analysis is difficult to achieve with human tissue taken *in situ* and will provide a more complete understanding of the development and cellular composition of organoids. Moreover, this information would enhance their

relevance as models to study organ morphology, function, and disease and would open up new avenues in drug development and regenerative medicine. For example, the cellular diversity of developing brain organoids was leveraged to model genetic microcephaly (Lancaster et al., 2013), to identify potential mechanisms by which the Zika virus leads to microcephaly (Dang et al., 2016; Garcez et al., 2016), and colon organoids have been used to explore the mutational steps underlying tumor initiation and progression (Drost et al., 2017). Lastly, patient-derived organoids have been used to recapitulate the disease progression of retinitis pigmentosa (Deng et al., 2018), to study the role of neuroglia in neurodegenerative disease (Abud et al., 2017), and to model cystic fibrosis in human airway organoids (McCauley et al., 2017).

Tissue engineering and regenerative medicine

iPSC-derived organoids have tremendous potential for applications in tissue engineering and regenerative medicine. These small tissues have many characteristics of embryonic tissues, which have been previously shown to have regenerative potential when implanted *in vivo*. For example, hIPSC derived intestinal organoids comprising both endoderm and mesoderm differentiate into fully vascularized guts when implanted into immune-compromised mice. Moreover, the implanted organoids incorporate high-level structural features such as villi that are not observed when organoids are cultured *in vitro* (Spence et al., 2011; Munera et al.; 2017). Additional tissues that have been shown to have regenerative potential are the lung, skin, and hair (Hirsch et al., 2017). Other researchers are investigating organoids as regenerative therapies for diseases of the liver (organ buds) and eye (optic cup) (Huch et al., 2017; Mandai et al., 2017).

Personalized medicine

One intriguing application of patient-derived organoids - whether from iPSCs or from adult stem/progenitor cells - is as patient-specific clinical models to aid in identifying drugs or combinations of drugs for treating disease. This concept has already found some success. For example, gut organoids have been used to identify patients who are uniquely responsive to an expensive therapy for cystic fibrosis (Dekkers et al., 2013).

Preclinical disease modeling/drug screens

A report by the Tufts Center for the Study of Drug Development (CSDD) describes the average cost of developing a prescription drug for market approval at $2.6 billion. This expense is driven mainly by the high failure rates (~88%) for drugs that are tested in human subjects (DiMasi et al., 2016). Organoids therefore may provide a unique model for human tissue disease for use in drugs screens or preclinical models. For example, a screen of over 6,000 approved drugs in hIPSC-derived cortical neural progenitor cells identified a handful of compounds that demonstrated protective when tested in human brain organoids (Zhou et al., 2017). In addition to potentially repurposing existing drugs, organoid-based screening can also be used for new drug target discovery.

Several critical challenges must be overcome in order to take full advantage of the enormous potential that organoid research has to offer. These challenges include reproducibility, the development of “gold standards” for different organoid systems, standardized mechanisms of

sharing of tissues and experience, means of patient consent, and transparency in communication with the public. These issues are discussed in detail below.

3. REPRODUCIBILITY IN ORGANOID RESEARCH

The utility of organoids in biomedical research depends to a large extent on how reproducibly they perform in various assays or differentiation protocols. This is true whether the studies are carried out in the same laboratory or in different laboratories, using shared cells or experimental protocols. The factors affecting reproducibility will vary according to the type and complexity of the assay and the source of the initiating cells; for example, whether these are well-characterized cell lines, or are primary stem cells derived from fetal or adult tissues. Quality control to reduce variability is extremely important if organoids are to be grown in large quantities for clinical trials.

It is not possible here to discuss all potential sources of variability in organoid culture, but some of the most important considerations are summarized below. It is assumed that academic investigators will already be following the basic tenets of "rigor and reproducibility" in their experiments. These principles include standardization of nomenclature, number of replicates, statistical analysis, randomization, blinding, sample-size estimates, and transparency in reporting.

Genetic and epigenetic variability in human cells

Genetic variability is less of a concern in the reproducibility of assays using inbred mouse cell lines. The same is true for primary stem cells as long as they are derived from inbred strains. A number of well-characterized human pluripotential embryo-derived stem cell lines (ESCs) are also readily available for studies, e.g., HUES1 and HUES9 (https://grants.nih.gov/stem_cells/registry/current.htm; http://stemcelldistribution.harvard.edu/). To some extent these lines, which were derived from embryos generated by apparently "normal" donors, can be considered as a "gold standard" for testing the reproducibility of organoid assays between different labs.

Potential variability in assays due to genetic background may arise with human derived iPSCs, especially those derived from patients carrying mutations associated with risk for an inherited disorder that has variable severity and penetrance. In these cases, the phenotype of differentiated cells may depend on the genetic background in which the mutation lies. Theoretically, iPSCs derived from different patients with the same mutation may behave differently in organoid cultures. Consequently, organoid studies should use iPSC lines derived from several patients, and, ideally, investigators should be willing to share these different lines with other labs, so that results can be compared. For diseases in which the specific genetic defect is known, "disease model" cell systems could be established and shared where the genetic defect is corrected by CRISPR-Cas9 in patient cells, or the genetic defect is recreated in normal control cells. Proof of principle studies have been provided by patient cell lines from muscular dystrophy and cystic fibrosis patients (Min et al., 2018; Simsek et al., 2016).

Isolation of stem cells from primary tissues

Some organoid assays use stem cells isolated from primary human tissues. Variability may come from the methods used to isolate and purify the cells. For examples, clinical samples may sit around for different times, the proteases used to dissociate the tissue may vary in potency, or

conditions of cell sorting may vary in different ways (such as nozzle size, flow pressure, gating, source of the antibodies to surface markers, reviews in (Hines et al., 2014)). It is therefore important that as many technical details as possible are included in isolation protocols.

Conditions under which cell lines or stem cells are maintained

Investigators new to cell culture and organoid assays need to be aware that cells can change over time in culture, including losing the ability to differentiate. For example, there may be selection of fast-growing variants or changes in behavior due to different compositions of commercially available media (e.g., different glucose and calcium levels), different batches of fetal bovine serum or serum replacement components, and mycoplasma contamination, etc. These potential sources of variability are covered in basic manuals of cell culture. Cell lines should be frozen in aliquots and detailed records kept of numbers of passage, and other variables.

Performance of organoid assays

An important source of variability, both between laboratories and from one experiment to the next, can be the conditions in which organoids are grown. Problems fall into several categories, including batch variability in growth factor purity; differences in exposure of cells to oxygen levels in multiwell trays; and variability in the degree and rate of maturation of differentiated cell types. These variables may be compounded if the organoids are derived from a combination of different cell types, such as "multiplex" organoids in which epithelium, stromal, endothelial, and immune cells are aggregated together. Variability can also come from using different induction protocols. For example, a protocol developed to induce human hindgut endoderm from iPSCs also generates a small amount of mesoderm that is correctly patterned as posterior lateral plate and gives rise to smooth muscle, while protocols to induce anterior endoderm do not generate associated mesoderm (Munera and Wells, 2017; McCauley et al., 2018).

Importantly, organoids are only useful if they come close to recapitulating the actual organ *in situ*. Various criteria have been used for comparison, such as a description of the cell types formed using RNA expression at single cell level, antibodies or genetically encoded markers; a 3D to 4D reconstruction of the complete organoid to determine the overall morphology, layering and regional patterning; and finally, developing assays to evaluate the physiology and function of the specific organoid. The latter may include transplantation and functional integration into a host animal as was shown for intestinal models (Fumagalli et al., 2017; Munera et al., 2017). Robust verification is achieved by comparing these structural and functional measures with the respective organ in another model system, such as mouse, pig, and non-human primate, or in actual human tissue.

Conclusions and recommendations

Given the potential sources of variability discussed above, a number of recommendations can be made:

- It is critical that organoid protocols are described in great detail in initial publications and the sources are provided for the reagents used at every stage (cell isolation;

organoid culture conditions; differentiation induction methods; isolation of differentiated cell types).

- Transfer of knowledge is most efficient by means of lab visits, facilities, and repositories that routinely culture and grow organoids (for further discussion see section 6).
- Criteria (transcriptomic profiles, surface antibody arrays, 3D reconstruction, single cell analysis, behavior after transplantation) need to be established for comparing the differentiated cell types and structures obtained in organoids with cell types and tissue organization present in normal tissues.
- It is likely that genetic background can affect the behavior of iPSCs carrying disease-associated mutations. It is therefore recommended that lines are derived and banked from multiple patients. To maximize the utility of these banks, patient consent should include allowing them to be shared among different institutions and investigators (for further discussion see sections 4 and 5).

4. TISSUE SOURCING AND PATIENT CONSENT IN ORGANOID RESEARCH

It is important to ensure an adequate supply of human tissues while respecting the wishes of the donors and maintaining the public's trust in the integrity of the research and medical application efforts. The process of obtaining consent is an essential part of research with human derived biospecimens, designed to protect the rights and welfare of the individuals participating in research, and to respect the dignity and autonomy of those individuals by allowing them the choice of whether to assist in the research (Huch et al., 2017).

Definition for tissue collection

As has long been the case, work with human tissue triggers a set of concerns and is subject to legal regulations and funding restrictions that vary between countries and at state level in the United States (U.S.)

Tissue still *in situ* is taken from either living or non-living humans

For living humans, taking tissue *in situ* involves intrusion into their bodies and requires voluntary and informed consent. To touch a living person's body without proper consent and in a manner that might be harmful or offensive is an interference with commonly held notions of personal autonomy, as well as a legal wrong in many national systems of law, including that of the U.S.

Tissue taken from deceased individuals may raise questions about whom - if anyone - has the authority to consent to donation. In many legislatures, such as the U.S., a deceased person is not considered a human subject of research for the purpose of triggering federal research ethics rules. Nonetheless, some jurisdictions treat the tissue as under the dispositional authority of someone other than the state or the research community, so that - for example - consent from a near relative might be required. Finally, because of religious, ethnic, or national customs it may be an offense to take tissue from the deceased, or certain types of research (e.g., investigating past population movements and settlement patterns may pose a cultural or even political challenge.)

Ex vivo tissue

Ex vivo tissue includes "abandoned tissue," such as surgical waste, "gifted tissue," when taken pursuant to consent, as above, or fundamentally "altered tissues," such as cell lines. National rules vary on whether it is considered to be some form of property of the person from whom it was taken. Such rules may apply if there is sufficient information embodied in or attached to the sample, such that the original source/donor is identifiable. When that is the case, the source/donor is a subject of study whenever the tissue is studied, and unless the identity is sufficiently obscured, it will trigger approval and protections for the donors' interests.

Regulatory boards

In general, if the tissue is taken specifically for research purposes, national rules for research will apply, and in many systems this will entail not only informed consent but also some form of independent oversight to ensure the research is sound and the interests of participants are protected. In the U.S., for example, it will in most cases be subject to Institutional Review Board

(IRB) oversight and some federal regulations embodied in the Common Rule. Federal regulation and IRB requirements are formally triggered when the research is funded by one of the federal agencies and departments that have adopted the Common Rule, but in many other cases the research is conducted in a setting where voluntary compliance is undertaken.

Even when consent is obtained, it may be necessary to see if the consent extends to all research or, at least, to the particular research being contemplated. Some organoid research might be sufficiently alarming to some members of the public such that this will become relevant. For this reason, the process of obtaining tissue from donors may need to include information about the range of envisioned uses and the possibility of future uses not yet contemplated. For example, working with tissue obtained from a tissue bank may require attention to whether source/donor identities are knowable and, if they are, whether the identities can be obscured. If not, then consent may need to be obtained, unless exceptions have been made for situations involving such minimal risk and unreasonable costs for contact that consent can be waived.

For samples obtained before iPSC or organoid research was established, consent for this specific use was not given. How to deal with the issue of previously consented tissue has been discussed in (Lomax et al., 2015).

For specific research objectives, it may be advisable for institutions to have specialized research boards that have specific knowledge of stem cell technologies and applications. Specific human embryo research now possible with new culturing methods may require additional oversight committees that review, approve, and monitor any research involving organoid studies on early human development or aim to produce human gametes with the implicit goal to study fertilization or use in *in vitro* fertilization.

Several guidelines and discussions of the topic of consent have been published, see for example: Guidelines for Stem Cell Research and Clinical Translation published by the International Society for Stem Cell Research (http://www.isscr.org/docs/default-source/all-isscr-guidelines/guidelines-2016/isscr-guidelines-for-stem-cell-research-and-clinical-translation.pdf).

Elements of consent

When consenting a research participant or a parent/guardian of a research participant, the following should be addressed:

- What are the immediate research purposes and any contemplated future uses, along with known risks and benefits (if any) to this information being collected?
- Does the donor wish to be re-contacted for additional uses in the future, or would s/he prefer to allow the tissue to be used without further consent? The participant should also be asked whether there are any particular uses to which the donor does not consent.
- If the tissue donor is to be identifiable, how will medical and personal information and donor identity be stored? How will it be protected, and what are the rules under which it will be held confidential or distributed to other researchers? In addition, is there any

plan to return research results to participants? If so, the circumstances triggering such a return should be discussed.

- If the tissue donor will be unidentifiable from research on the sample, the donor should understand that de-identified data and biospecimens will be distributed to researchers and/or deposited in central databases and biobanks.
- The participant should be informed that the blood cells or skin biopsies (fibroblasts), or other tissues (i.e., hair) may be used to generate IPSC and organoids, especially if the tissue is collected in the context of a larger study (genetic/genomics for instance) and samples will be frozen for future use. An opt-in consent where the donor or their kin explicitly agrees to the use or, less likely, an opt-out consent where exclusions have to be specified.
- The participant should be asked for permission to link any medical, clinical, and genetic data to the biospecimens and their derivatives. The participant should also be asked to link any medical, clinical, and genetic data to those of their family members, if they are also enrolled in the same research study.
- The participant should be given the possibility to withdraw from the study, with the understanding that material might have already been distributed, been used or be used in the future for research purposes, and communicated in published journal articles or at conferences.
- Organoid research can yield important insight about specific diseases and even treatment. Thus, patients who donated their cells or tissue with broad consent should nevertheless have the option to learn about the results from their organoids.

Guidelines for future collection

Regulations for the need and extent of consent vary broadly based on the source of the tissue. We will only discuss consideration for future collection of tissues.

- For living donors, unidentifiable tissues (often the case of surgical waste, where only information collected is about the disease) should be distinguished from identifiable tissues (where information about the donors can be obtained through studying the tissue).
- For deceased individuals, consent should be based either on prior expressed wishes of the deceased person or consent of next of kin.
- For unidentifiable tissue, no consent should be needed, but consent may be necessary if the type research (e.g., transplantation, generation of embryoids, gastruloids, or germ cells/gametes) might be upsetting if the donor had considered it. For this type of research, it is advisable to discuss with the IRB (or equivalent oversight body, if outside the U.S.) whether it is important to go back to the donors for additional consent.
- For identifiable tissue, consent should be given for use, with preference to broadest possible consent for anticipated and unanticipated uses including sharing broadly among institutions world-wide. Note, that this requires some degree of imagination when describing possible future uses of the tissue.
- If the broadest consent cannot be obtained, tissue should either not be collected, or only distributed noting the specific restrictions. Emerging electronic recording technologies may make it easier to deal with varying restrictions placed by each donor.

- A unique identifier, such as GUID (global unique identifiers), originally developed by the autism research community, generates a common subject identifier for research participants across research laboratories and repositories and is recommended for researchers in other fields as a tool to share data (https://ndar.nih.gov/tools_guid_tool.html)

5. SHARING MATERIALS AND RESULTS

Research progresses by communicating and sharing of results and data. We propose strong guidelines for the sharing and communication of results and materials obtained by organoid research and propose increased use, of federally supported biobanks as centers to enhance and coordinate distribution, training, and sharing. Distribution via data and tissue banks is highly advisable in the future. A few organoid banks and centers already exist in Japan, the U.S. and Europe (Takebe et al., 2018; Yan et al., 2018; Sachs et al., 2018; van de Wetering et al., 2015).

These biobanks can offer several advantages for organoid research:

- Set standards for quality of material preservation.
- Develop standard procedures for organoid culture
- Engage in training.
- Respect and enforce restrictions made by the donor through data depositories.
- Facilitate communications with the donor for special consent (i.e., transplantation, embryoid generation, or germ cells/gamete manipulations).
- Distribute materials with accurate information, detailing quality-control protocols used for cell, tissue, organoid generation, and maintenance.
- Collect and distribute biospecimens for broad distribution that were modified by individual labs, for example to correct a specific genetic defect.

Guidelines for communication and sharing

- Data and samples for research should be available for use by approved investigators without geographical restrictions.
- Data and biospecimens should be distributed after the requesting approved researcher and their institution have agreed to and signed an appropriate Material Transfer Agreement (MTA) and, if needed, submitted an institutional review board approval/exemption from their Institution.
- In the interest of rapid dissemination of data and findings, advancement of knowledge, and replicability of data, the MTA should include language in support of data sharing.
- Investigators should agree to returning generated data and modified biospecimens to a biobank or make materials available within an agreed upon time or by the time of publication, whichever comes first. The sharing language could encourage or require the deposition of results in www.bioRxiv.org or a similar pre-print server to facilitate free access.

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6. TRAINING IN ORGANOID RESEARCH

The success of any research area, technique, or application is directly dependent on the relative experience and training of the scientists carrying out the work. Experimental approaches that are simple to implement are also easier to disseminate, with or without commercialization. More complex protocols - including the generation and characterization of organoids - require extensive experience in enabling procedures prior to mastery. With such technologies, "failure" to replicate prior work does not necessarily mean that the previously published studies were flawed; it is equally likely that the "art" of conducting the procedure was not successfully communicated to and/or mastered by the second set of researchers. This fundamental challenge has severe implications for any field reliant on complicated experimental protocols.

Increasing the number of scientists trained to work with organoids will be essential for several key issues.

- Solving challenges and uncertainties associated with reproducibility. Until a critical mass of researchers at different institutions are trained in dependable and consistent techniques to generate organoids, it will be challenging to understand the extent to which variability arises because of technical differences versus genetic (or biological) ones.
- Alleviating bottlenecks in organoid research. Until training is reliable and easy to come by, the number of labs carrying out research using organoids will remain limited, which directly limits the number of questions that will be asked using this technology.
- Expanding the use of organoids into translational areas, including screening for novel therapeutics and regenerative medicine.
- Using organoids to expand the next frontiers of research. This could include, for example, a comprehensive description of the cell types in the body and their organization into tissues. Tissue-engineering efforts may result in more realistic organoids that more closely resemble the *in vivo* organ including vascularization, innervation, and immune system support.

The field will only thrive once we have reliable and dependable ways to training. We recognize that there are both challenges and opportunities associated with developing and implementing training opportunities. The challenges include the fact that hands-on training in these protocols is difficult and protocols can be challenging to reproduce at different geographic locations. The development of well-structured organoids requires long periods of time (many months to years) and is not easily translated from one type of organoid to the next. Furthermore, derivation and culturing of organoids requires infrastructure, multiple experienced personnel, and institutional buy-in. These challenges make dissemination of detailed knowledge in organoid culturing to new laboratories difficult in typical short-term practical courses such as those presently used to teach experimentation with model organisms.

Thus, alternatives to existing training methods need to be found and indeed already exist in limited locations. These include stem-cell core facilities that could be expanded to provide a teaching platform that might include visiting programs open to people from different institutions. Such programs could essentially solidify "art" into "practice" through the protocols

they choose to teach, and could include topics such as the culture and derivation of human pluripotent stem cells, isolation of tissue-specific stem cells or tissue explants, differentiation into human organoids, genetic manipulations including those based on CRISPR, and functional genomic approaches with single cell resolution. Such facilities would also provide a mechanism and medium for the exchange of knowledge and collaboration by bringing together experts in organ culture, robotics and engineering, microscopy and image analysis, cryopreservation and animal model studies. For examples of collaborative facilities striving towards clinical translation see: (Takebe et al., 2018; Yan et al., 2018).

Conclusions and recommendations

- Training facilities need to drive the dissemination of the “art” of organoid culturing and promote sharing of expertise and knowledge among researchers between institutions.
- Sharing of source material is critical for training and dissemination.
- Best practices for organoid research have to be in place to train and increase the next generation of researchers in this arena.

7. PUBLIC OUTREACH

Since the beginning of medical science, incremental steps forward in understanding the treatment or mechanics of disease have delivered major advances in medical care. These advances, however, have often pushed the envelope of what is considered socially acceptable, and have not always been met with immediate public acceptance.

Public wariness and cultural and religious barriers often stand in the way of immediate acceptance of some of these advances. Examples include organ donation and transplantation, IVF, and other cutting edge medical research procedures. This public uncertainly – sometimes outright opposition – has often resulted in decreased public acceptance of new medical advances and in recent years has led to politically motivated efforts to place policy restrictions (funding bans and in some cases criminal penalties) on scientific research. Examples include recombinant DNA (1970s), stem cell research (2000s), fetal tissue research (1990s, 2016 -), gene editing (2016 -), and mitochondrial replacement therapy (2016 -).

A large fraction of research using organoids is funded by the U.S. National Institutes of Health (NIH), the European Union (EU), Japan's Ministry of Education and other public funders. Scientists, therefore, have a responsibility to be transparent with the public about the results of their research and its implications, without overselling it. Researchers should also be transparent about the sources of material used to generate organoids. Acknowledgement of ethical issues associated with the field is also important.

During the debate in the U.S. about federal support of research using human embryonic stem cells, the pro-research advocates, including scientists, were careful to not promise cures resulting from research that was only in its nascent stages at the time. Scientists were encouraged to discuss the "potential" and the "promise" of the field "if" their research were successful.

It is critical that scientists know their audiences. In general, public opinion of scientists and support for science is quite positive, but can vary widely based on race, gender, age, education, region, and political affiliation. Public audiences are also more likely to be initially wary of new areas of research. Americans became more accepting of human embryonic stem cell research over time as the public and political debate continued between 2000 and 2013 because they had time to learn and become more comfortable with the area of research. Since organoid research is an emerging field and provides investigators with new tools for their research, they should share their excitement about the ways in which these tools will help them reach their research goals. It is also important to assure non-experts about what organoids are not. For example, brain organoids are *not* complete brains. They cannot replace the complete function of the brain and the field is not replicating consciousness in a dish. The same can be said for organoids of other organs of the body. Intestinal organoids do not replicate the intestine and retinal organoids are not eyes and do not have vision. Support will not be gained by one lecture. Like other areas of research, it will take time for the general public to adapt, and the scientific community will have to commit to a continued effort at education and dialogue, particularly as advances continue.

As the research progresses and clinical applications become more apparent and real, organoid research will receive increased attention from the media. This will play an important role in educating the public and will help shape any emerging political debate. Organoid researchers should, therefore, consider educating reporters about this area of research even though they may be hesitant to do so. Researchers without prior experience or media training may be concerned about having their conversation accurately reported by a reporter, particularly one not familiar with the science. The complicated nature of the science surrounding organoids may also serve as a barrier for discussion between scientists and reporters. Too many scientists are not even able to describe their research in the most basic terms let alone show experience in working with reporters or media training. Societies such as the ASCB, or institutional public information and communication offices, are willing and should be consulted.

We suggest that scientists working on organoids consider focusing on the following talking points when communicating with public audiences about organoids:

1. Describe what organoids are and what they are not.
2. Clearly describe the potential opportunities of organoids to your research.
3. Articulate key discoveries that have resulted from organoids that would not have been possible using other approaches.
4. Acknowledge the unknowns and challenges.
5. Avoid talking about unpublished, non-peer-reviewed results.
6. Consult with researchers in other cutting-edge fields about their experiences working with the press and other audiences. What experiences have they had in similar areas of cutting–edge science? What suggestions do they have for you based on those experiences?
7. The members and staff of the ASCB are able to provide you with professional guidance (https://www.ascb.org/advocacy/).

Appendix 1: ASCB Organoid Task Force Members

ASCB Public Policy Committee Organoid Task Force

Committee members

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The American Society for Cell Biology

Kevin M. Wilson
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The American Society for Cell Biology

Appendix 2: The Task Force Mission Statement

Organoid Research Taskforce Mission

Advances in our understanding of the mechanics of disease along with the development of new technologies have delivered major advances in medical care. These advances have often pushed the "socially acceptable" envelope and thus have met with resistance from the general public.

Public wariness as well as cultural and religious barriers often stands in the way of immediate acceptance of new medical technologies and treatments. This public uncertainty – sometimes outright opposition – has often resulted in politically motivated efforts to place policy restrictions (funding bans and in some cases criminal penalties) on scientific research. Examples include recombinant DNA (1970's), stem cell research (2000's), fetal tissues research (2016 - ?), gene editing (2016 - ?), and mitochondrial replacement therapy (MRT) (2016 - ?).

Advances in medical research using organoids are already allowing scientists to begin to explore new areas of research not open to investigation with animal models. One of the most obvious areas is in the use of human brain organoids to study the human brain development and diseases affecting the brain and/or its development. Brain organoids have been useful recently to research the effects of Zika on the human brain, something that cannot be modeled. Additional areas of research that would benefit from the use of organoids include autism, schizophrenia, Alzheimer's disease, and diseases connected with the blood-brain barrier. Research is also being done with organoids of the stomach, mammary glands, pancreas, colon, liver, and others.

Some researchers envision greater future success with the development of organoids using patient-derived tissue and larger organoids. This could be helpful in understanding Downs Syndrome, and why patients with Downs don't develop Alzheimer's disease, etc. In addition, late stage organoids would allow a better understanding of pre- and post-natal development. "Personalized" organoids would also be effective tools in understanding disease and developing treatments.

There is also discussion within the stem cell community that it would be helpful to create embryo versions of organoids – embryoids – which would allow medical science to permit embryos to develop beyond the current 14-day limit.

Public reaction

The current ability of science to develop a human "brain in a dish" has the potential to cause uncertainty with the general public. At the very least, U.S. politicians in search of creating enemies for their base to fear could latch on to possible future areas of research.

Regardless of the potential scientific benefit and medical advances, the public reaction to the harvesting process connected with patient-derived organoids, post-natal organoids, and the

concept of “personalized” organoids would be extreme, particularly within the pro-life community. Without having a full understanding of the science, the public may jump to conclusions about the research itself but also about the general intentions of the life science community.

Much like the self-imposed boundaries on recombinant DNA and gene editing, self-imposed borders on this field of research could be helpful in maintaining public confidence and reducing potential political attacks on the field.

The ASCB is uniquely suited to review the issue and develop recommendations that balance the scientific benefit with the public concern. By examining, and then explaining the science, the ASCB can lead a reasoned discussion of the science, potential benefits and risks behind organoid research, instead of a discussion based on fear, hysteria, and politics.

Some questions the Subcommittee could address include:

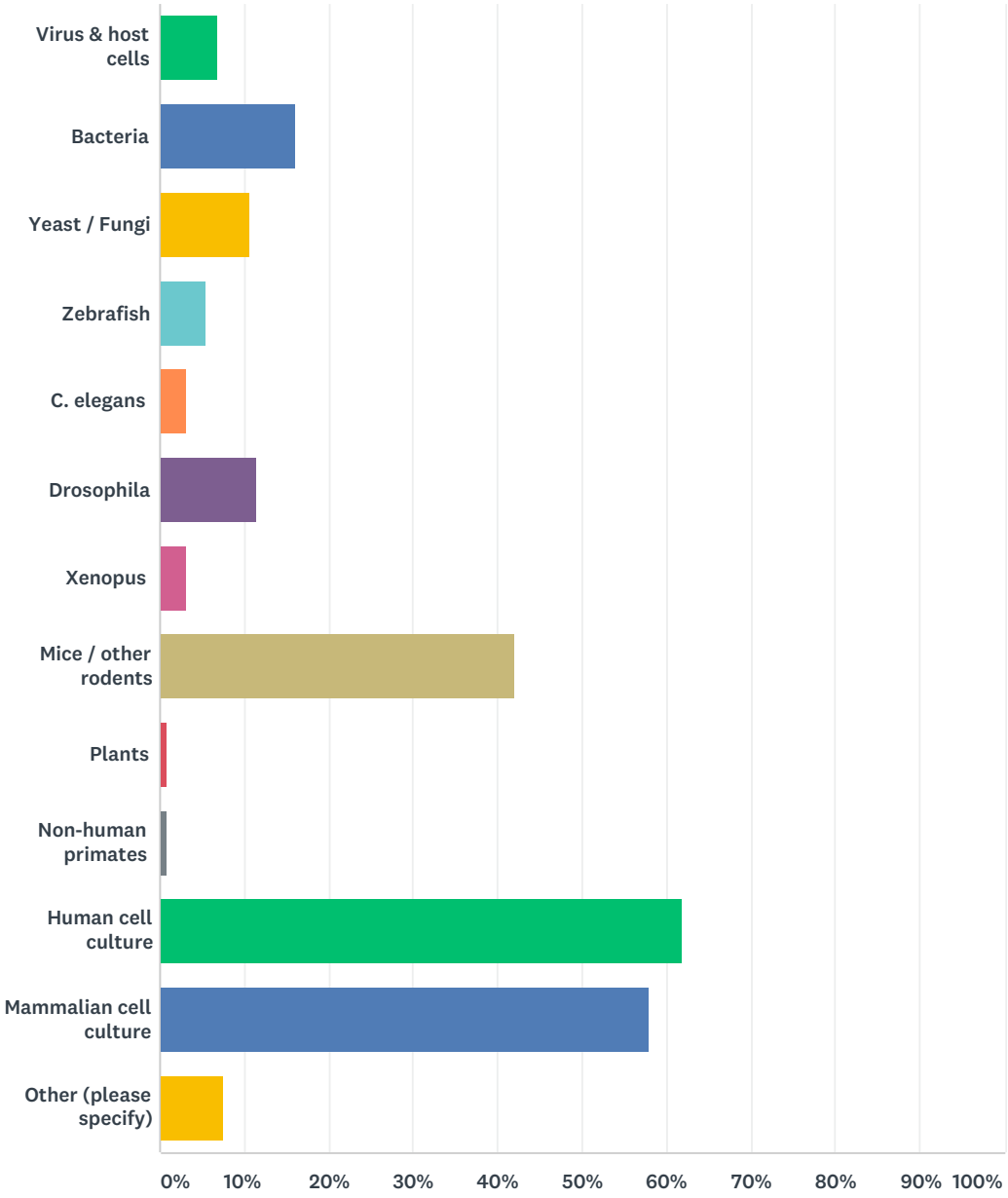
- What are the sources of organoids?
- How are they made?
- What is the scientific value of this field of research? What are the risks?
- Are organoids best used only for basic research or are there currently valuable clinical applications?
- Are there limits to what should be done in this field? If so, could ASCB make recommendations as to what these limits should be?
- How can the scientific community best educate the public about the value of this research?

After considering these and other questions determined to be appropriate and necessary by the subcommittee, it will be asked to draft a white paper that outlines its findings and provides necessary recommendations for future scientific activity. In addition, recommendations on education of the public and policy makers should be included.

Appendix 3: ASCB Member Survey

Q1 Which model systems do you currently use for your research? (check as many as appropriate)

Answered: 131 Skipped: 0



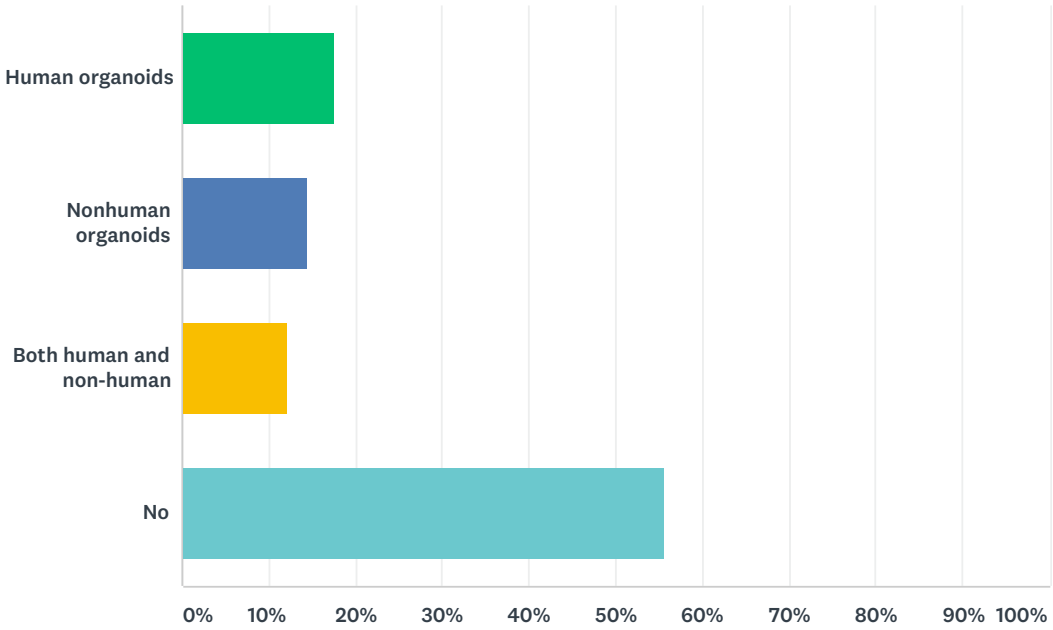
ANSWER CHOICES	RESPONSES	
Virus & host cells	6.87%	9
Bacteria	16.03%	21
Yeast / Fungi	10.69%	14
Zebrafish	5.34%	7
C. elegans	3.05%	4

Drosophila	11.45%	15
Xenopus	3.05%	4
Mice / other rodents	41.98%	55
Plants	0.76%	1
Non-human primates	0.76%	1
Human cell culture	61.83%	81
Mammalian cell culture	58.02%	76
Other (please specify)	7.63%	10
Total Respondents: 131		

#	OTHER (PLEASE SPECIFY)	DATE
1	Retired-no longer using cultures	3/29/2018 12:06 PM
2	chick, reptile	3/29/2018 8:38 AM
3	insect cells	3/28/2018 9:36 PM
4	ORGANOIDS (Melanoma cells)	3/28/2018 7:14 PM
5	human embryonic stem cells and induced pluripotent stem cells	3/28/2018 4:54 PM
6	organoids	3/28/2018 2:54 PM
7	Tetrahymena	3/28/2018 2:32 PM
8	chicken embryos	3/28/2018 2:24 PM
9	Human tissue	3/28/2018 2:12 PM
10	Human platelets	3/28/2018 2:07 PM

Q2 Does your research currently involve the use of organoids?

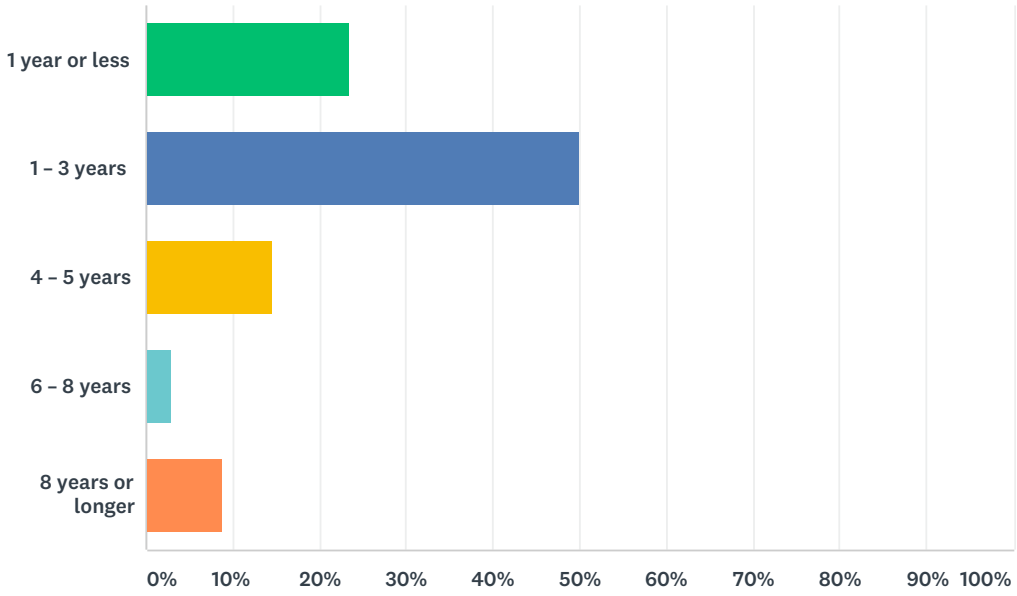
Answered: 131 Skipped: 0



ANSWER CHOICES	RESPONSES	
Human organoids	17.56%	23
Nonhuman organoids	14.50%	19
Both human and non-human	12.21%	16
No	55.73%	73
TOTAL		131

Q3 How long have you been using nonhuman organoids?

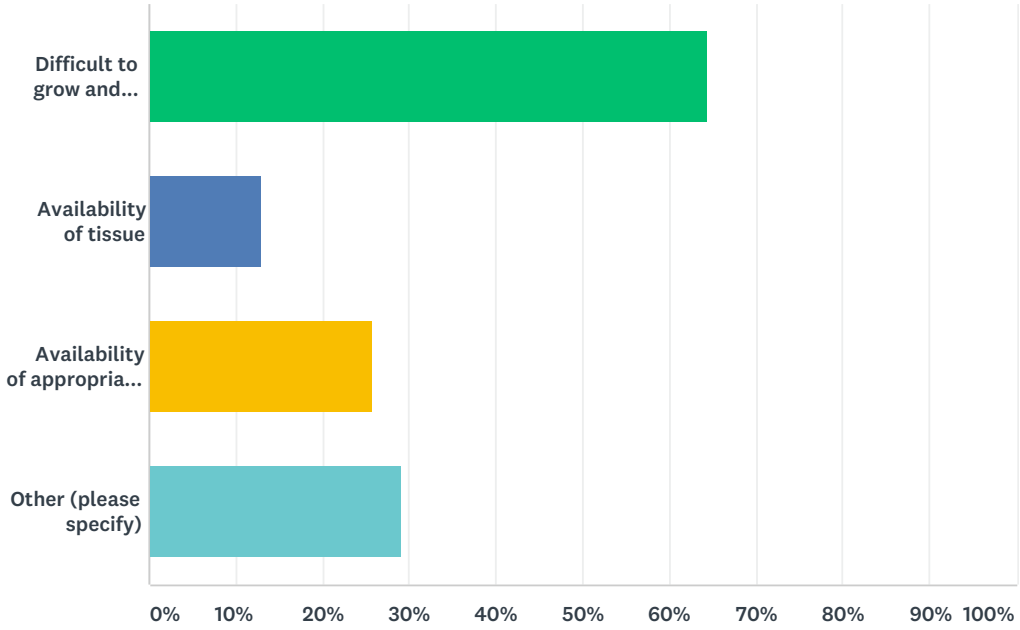
Answered: 34 Skipped: 97



ANSWER CHOICES	RESPONSES	
1 year or less	23.53%	8
1 – 3 years	50.00%	17
4 – 5 years	14.71%	5
6 – 8 years	2.94%	1
8 years or longer	8.82%	3
TOTAL		34

Q4 What bottlenecks have you experienced as you work with nonhuman organoids? (answer as many as appropriate)

Answered: 31 Skipped: 100

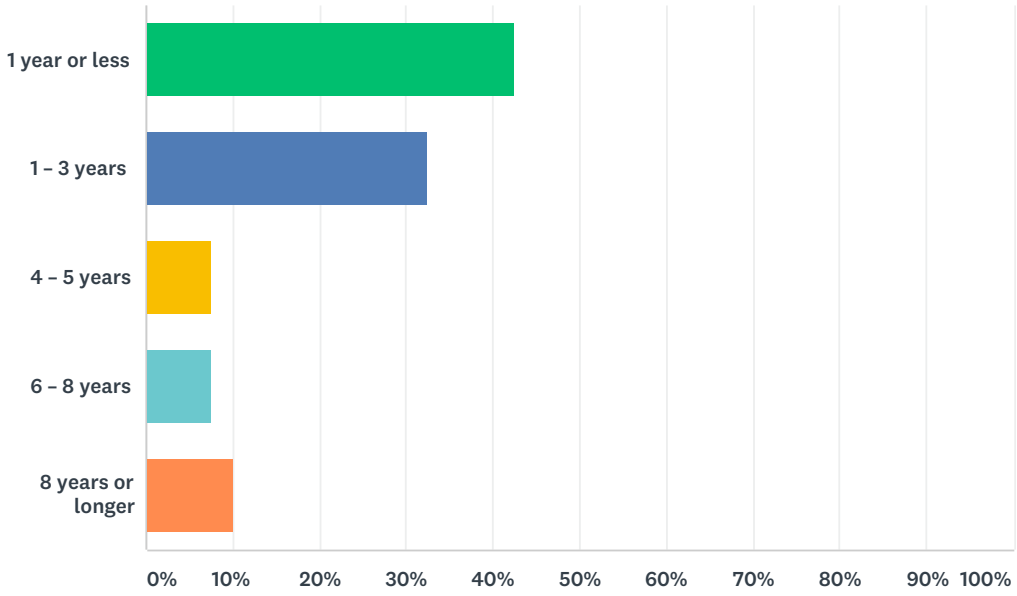


ANSWER CHOICES	RESPONSES	
Difficult to grow and culture	64.52%	20
Availability of tissue	12.90%	4
Availability of appropriate model system	25.81%	8
Other (please specify)	29.03%	9
Total Respondents: 31		

#	OTHER (PLEASE SPECIFY)	DATE
1	necrosis occurs	4/3/2018 3:34 PM
2	expensive reagents (growth factors, Matrigel etc)	3/31/2018 1:04 PM
3	Expensive reagents	3/30/2018 9:10 PM
4	Difficult to adopt imaging assays to organoid systems	3/30/2018 9:47 AM
5	the hype of the field	3/29/2018 1:06 AM
6	Cost	3/28/2018 10:09 PM
7	Sometimes difficult to grow	3/28/2018 7:15 PM
8	reproducibility of phenotypes and high variability	3/28/2018 4:10 PM
9	Variation in matrigel, and growth factors.	3/28/2018 3:16 PM

Q5 How long have you been using human organoids?

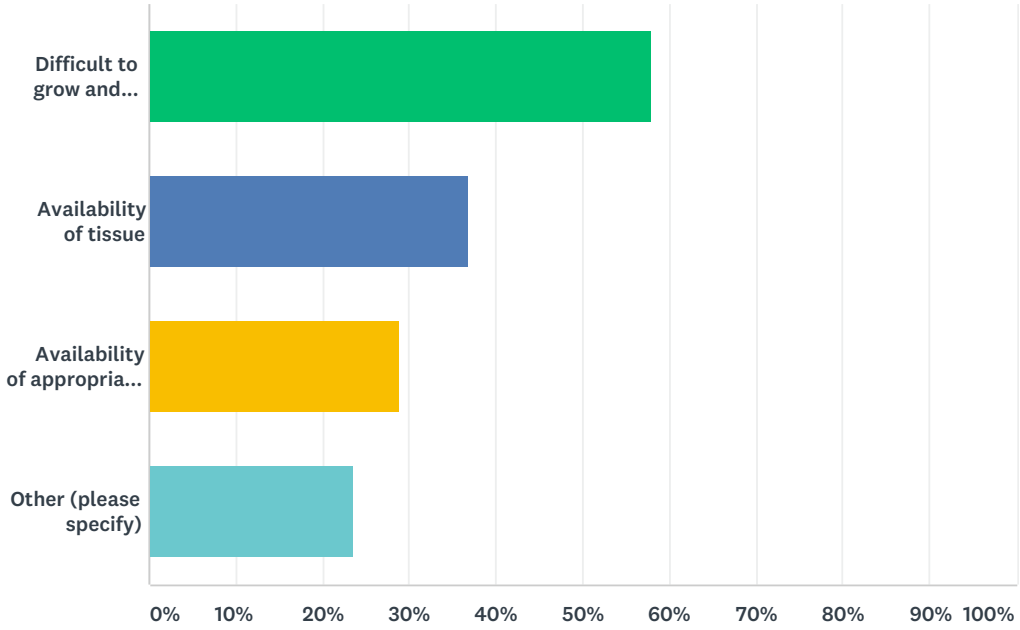
Answered: 40 Skipped: 91



ANSWER CHOICES	RESPONSES	
1 year or less	42.50%	17
1 – 3 years	32.50%	13
4 – 5 years	7.50%	3
6 – 8 years	7.50%	3
8 years or longer	10.00%	4
TOTAL		40

Q6 What bottlenecks have you experienced as you work with human organoids? (answer as many as appropriate)

Answered: 38 Skipped: 93

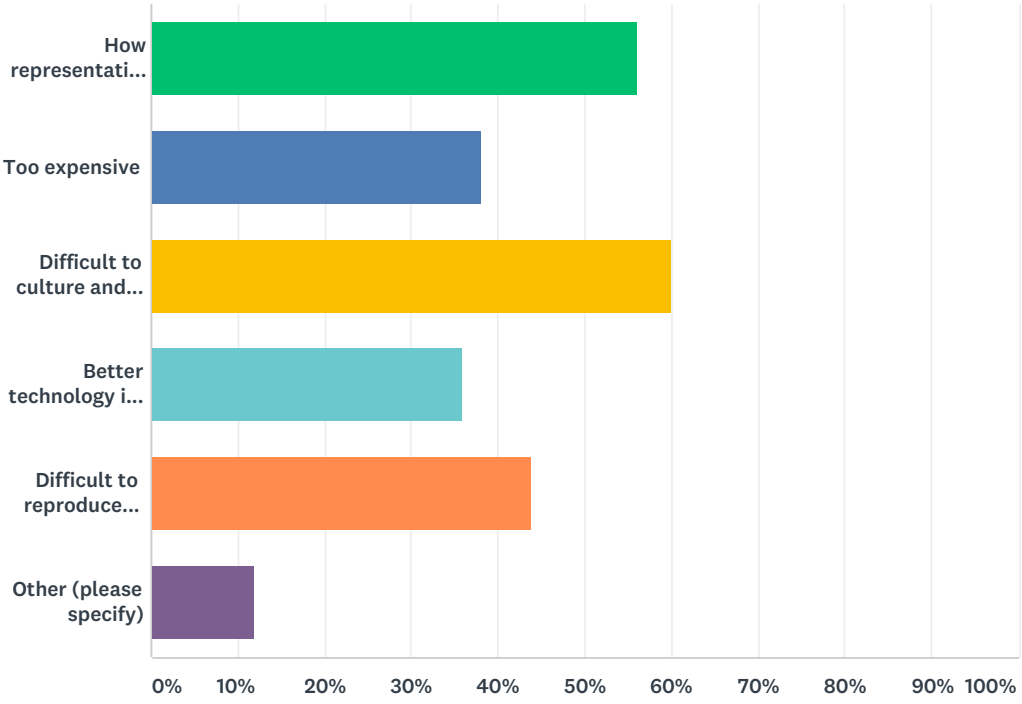


ANSWER CHOICES	RESPONSES	
Difficult to grow and culture	57.89%	22
Availability of tissue	36.84%	14
Availability of appropriate model system	28.95%	11
Other (please specify)	23.68%	9
Total Respondents: 38		

#	OTHER (PLEASE SPECIFY)	DATE
1	difficult for handling for assays	4/3/2018 3:35 PM
2	Imaging the 3d structures	4/1/2018 4:05 AM
3	Appropriate validation and functionality of organoids is very costly!	3/29/2018 3:35 AM
4	Sometimes difficult to grow and culture	3/28/2018 7:15 PM
5	developing and maintaining stem cells is expensive. Organoids take up to 5 months to fully mature, which is also expensive.	3/28/2018 4:56 PM
6	I only work with non-human organoids	3/28/2018 4:44 PM
7	same issues as with nonhuman organoids	3/28/2018 4:10 PM
8	started with organoids directly from in vivo breast, trying to recreate organoids from cultured individual cells; complicated heterogenous tissue hard to recreate	3/28/2018 3:34 PM
9	price of kits, media etc	3/28/2018 2:54 PM

Q7 In your view as a cell biologist, what are some of the outstanding issues concerning organoids?(answer as many as appropriate)

Answered: 50 Skipped: 81

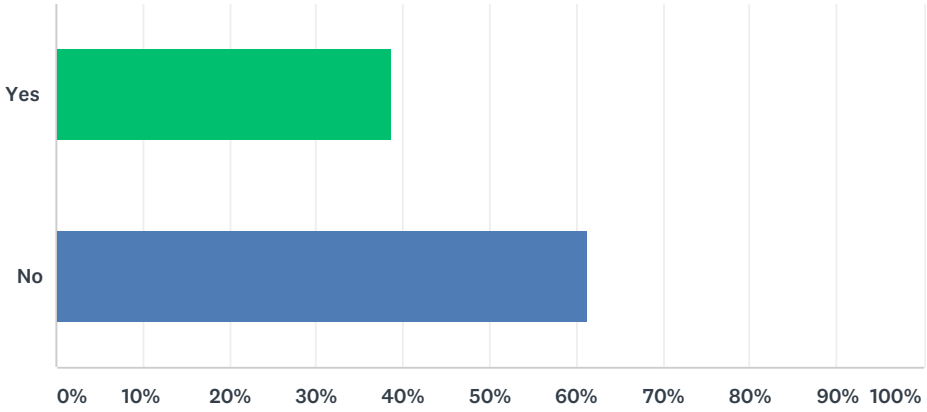


ANSWER CHOICES	RESPONSES	
How representative they are of the tissue they model	56.00%	28
Too expensive	38.00%	19
Difficult to culture and grow	60.00%	30
Better technology is required to scale up	36.00%	18
Difficult to reproduce results	44.00%	22
Other (please specify)	12.00%	6
Total Respondents: 50		

#	OTHER (PLEASE SPECIFY)	DATE
1	Time to get organoids can be very long	4/1/2018 7:29 AM
2	Scaling up and their ability to represent the tissue they model are specifically NOT limitations inherent to organoids. They are limitations imposed by scientists who come to use organoids with rigid ideas already in mind about how they think cell biology should work.	3/30/2018 9:58 AM
3	Maturation is an issue; initial studies suggest that our kidney organoids, for example are akin to very early embryonic tissues	3/29/2018 3:37 AM
4	I take these as challenges rather than criticisms	3/29/2018 1:07 AM
5	Difficult to apply imaging	3/28/2018 5:34 PM
6	TBD	3/28/2018 2:56 PM

Q8 Have you ever been unable to replicate work using organoids from another lab?

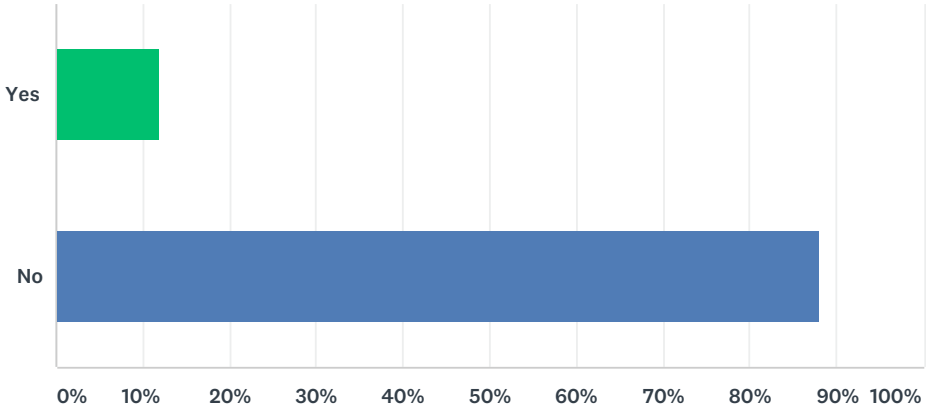
Answered: 49 Skipped: 82



ANSWER CHOICES	RESPONSES	
Yes	38.78%	19
No	61.22%	30
TOTAL		49

Q9 Has another lab ever told you that they have been unable to replicate one of your published experiments that used organoids?

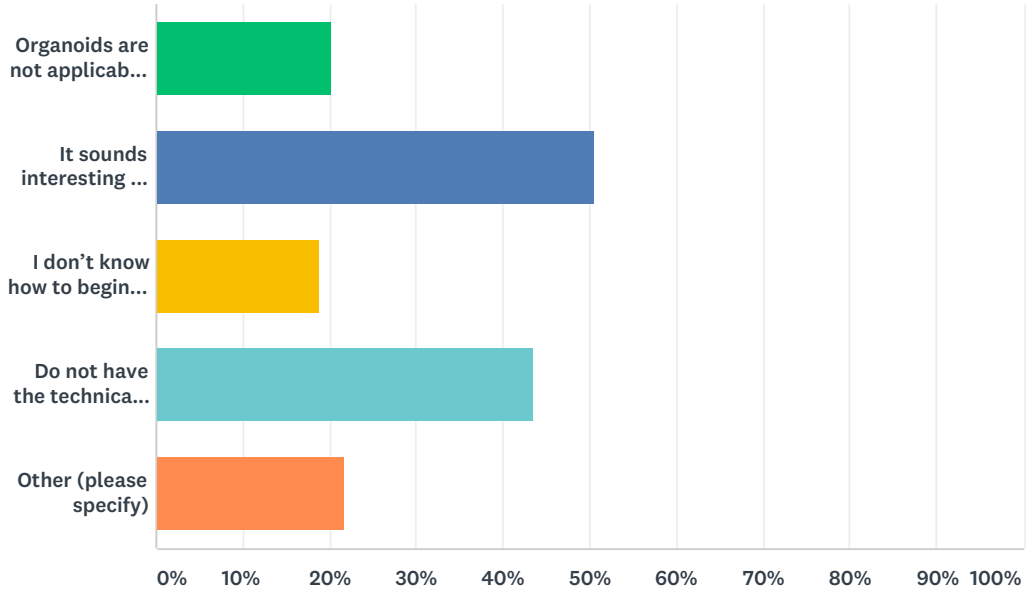
Answered: 50 Skipped: 81



ANSWER CHOICES	RESPONSES	
Yes	12.00%	6
No	88.00%	44
TOTAL		50

Q10 If you don’t use organoids in your research, why not? (answer as many as appropriate)

Answered: 69 Skipped: 62



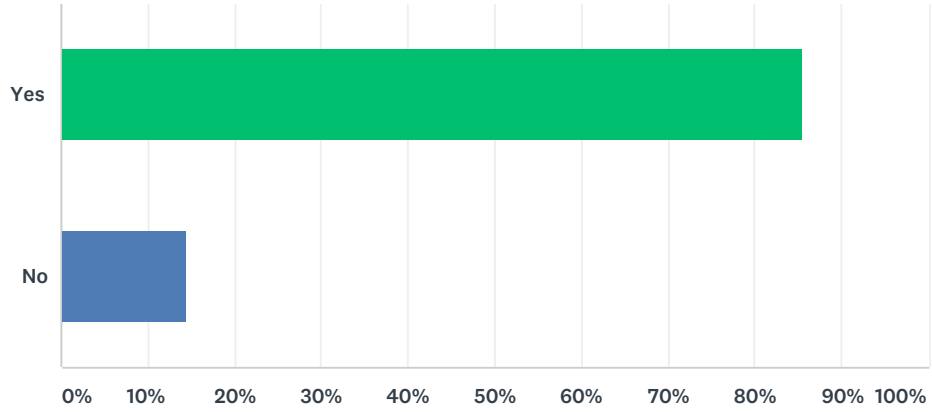
ANSWER CHOICES	RESPONSES	
Organoids are not applicable to my field of study	20.29%	14
It sounds interesting but I need to know more about how they would help my research.	50.72%	35
I don’t know how to begin to incorporate them into my research	18.84%	13
Do not have the technical expertise	43.48%	30
Other (please specify)	21.74%	15
Total Respondents: 69		

#	OTHER (PLEASE SPECIFY)	DATE
1	we are about to use them here. I used organoids previously.	4/4/2018 10:27 AM
2	I think they are a very poor model of development	3/29/2018 2:00 PM
3	Plan to use them in the future	3/29/2018 12:58 PM
4	It's on my "think about it list"--some very cool work is being done and they could be a great epithelial cell model	3/29/2018 12:43 PM
5	I do not know from where to obtain organoids. There is no universal source for this.	3/29/2018 9:33 AM
6	My system needs further characterization/optimization.	3/29/2018 8:29 AM
7	It's not that they're not appropriate to my field of study - they most certainly are - but that work is not part of my remit.	3/29/2018 8:21 AM
8	I am not interested in the ATCC (or other) cell lines. I am ready to use fresh biopsy-based organoids, but the access to tumour samples is not easy	3/29/2018 7:24 AM
9	I want to use it, bit I have no connection to people who are using it and can give it.	3/29/2018 4:04 AM
10	No grant funding. About washed up. :(	3/28/2018 4:36 PM

11	brain organoids are too tricky and slow to culture compared to their potential benefit	3/28/2018 4:28 PM
12	Lung organoids are difficult to work with	3/28/2018 2:38 PM
13	we are just getting geared up. We will start working with human and non-human organoids in a few month time.	3/28/2018 2:20 PM
14	Other labs in our department are using them (kidneys). We can stain them but not grow them as per the MTA	3/28/2018 2:13 PM
15	No money for this type of research right now	3/28/2018 2:08 PM

Q11 If you don’t currently use organoids, are you interested in learning more about them and using them in your laboratory in the future?

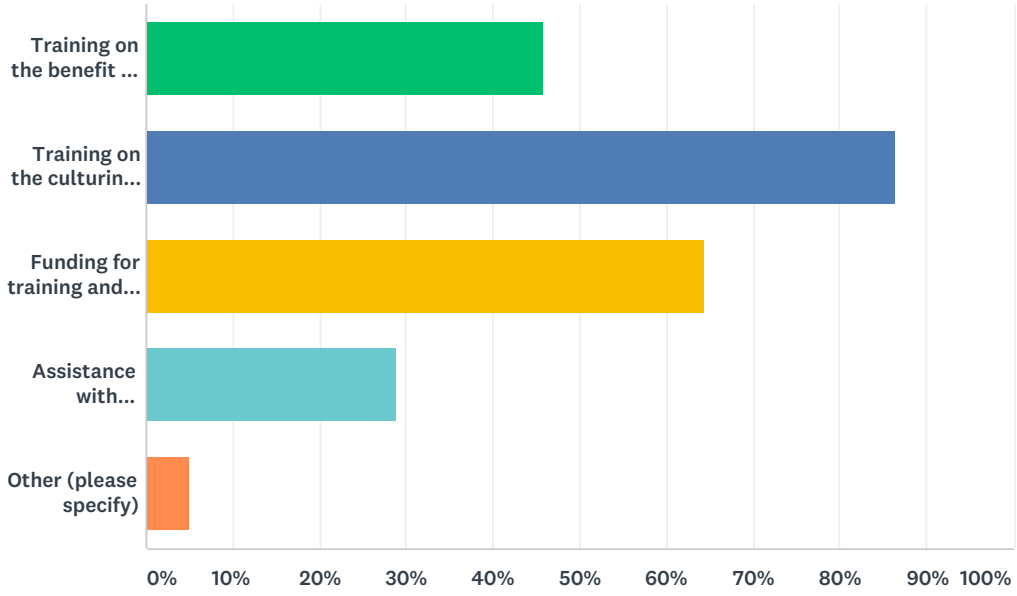
Answered: 69 Skipped: 62



ANSWER CHOICES	RESPONSES	
Yes	85.51%	59
No	14.49%	10
TOTAL		69

Q12 If you are interested, what would be helpful to you to expand your research into this area? (answer as many as appropriate)

Answered: 59 Skipped: 72

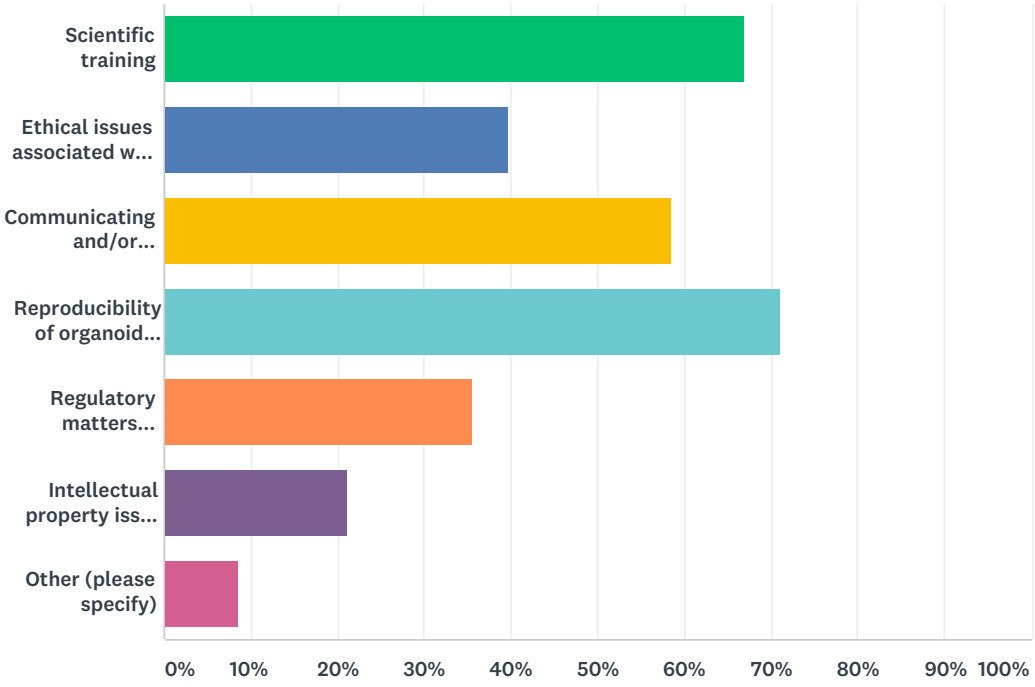


ANSWER CHOICES	RESPONSES	
Training on the benefit and use of organoids	45.76%	27
Training on the culturing and growth conditions	86.44%	51
Funding for training and technology	64.41%	38
Assistance with communicating and talking about organoid research and potential ethical issues	28.81%	17
Other (please specify)	5.08%	3
Total Respondents: 59		

#	OTHER (PLEASE SPECIFY)	DATE
1	training and guidelines on quality controls and standards	4/4/2018 10:28 AM
2	A universal source like ATCC from where we can obtain organoids.	3/29/2018 9:34 AM
3	Better understanding of what the organoids represent - kidney ones seem to be immature but we need differentiated podocytes	3/28/2018 2:15 PM

Q13 Where should the ASCB and the Task Force on Organoids focus their attention (answer as many as appropriate)

Answered: 118 Skipped: 13



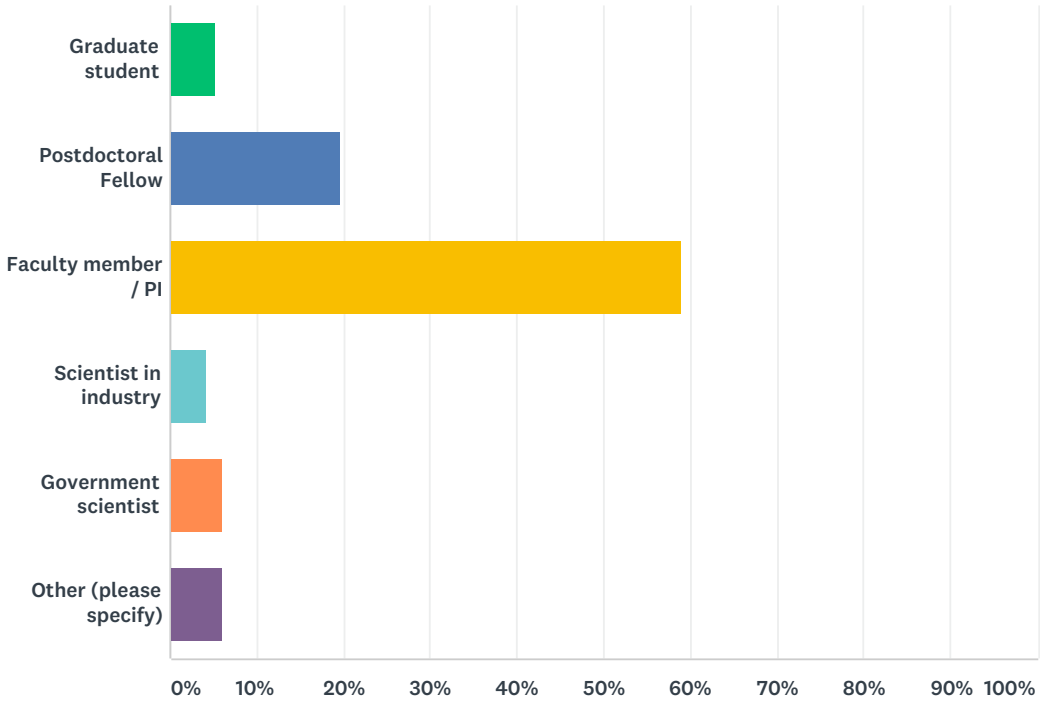
ANSWER CHOICES	RESPONSES	
Scientific training	66.95%	79
Ethical issues associated with organoid research	39.83%	47
Communicating and/or advocating for the value of organoid research	58.47%	69
Reproducibility of organoid research	71.19%	84
Regulatory matters connected with the field	35.59%	42
Intellectual property issues associated with the field	21.19%	25
Other (please specify)	8.47%	10
Total Respondents: 118		

#	OTHER (PLEASE SPECIFY)	DATE
1	The value of organoids must be communicated as one of several model systems that can complement the others; not as a panacea for making cell biology research more relevant.	3/30/2018 9:59 AM
2	Determining if organoids are a model system	3/29/2018 2:01 PM
3	It would be great if the work did not lead to treatments only enjoyable by the rich. Intellectual property and regulatory issues both interface with this "other."	3/29/2018 12:10 PM
4	access to tumour tissue, access to tumour tissue, access to tumour tissue, access to tumour tissue If you are NOT in a hospital, everything is difficult	3/29/2018 7:25 AM

5	I think the Task Force's focus should be on what this survey reveals as to the magnitude of the organoid research axis among respondents. Then these questions could be posed with more meaning. From the standpoint of survey design, this question should have had an entry portal "If you think organoids are important in the current era of cell biology, where do you think the ASCB and the Task Force ..." See what I mean as to the logic matrix/flow chart of the survey?	3/28/2018 7:44 PM
6	Better imaging equipment to facilitate organoid identification and research	3/28/2018 7:26 PM
7	1. Value of 3-D culture systems in cell biology research. 2. Importance of reporting research results in terms of concentrations of cells/cm3.	3/28/2018 7:19 PM
8	Making organoid research more economic	3/28/2018 4:45 PM
9	how accurately they model desired in vivo processes and are useful for studies	3/28/2018 3:36 PM
10	Methods and highly detailed reagent specifications.	3/28/2018 3:17 PM

Q14 What best describes your professional position?

Answered: 117 Skipped: 14

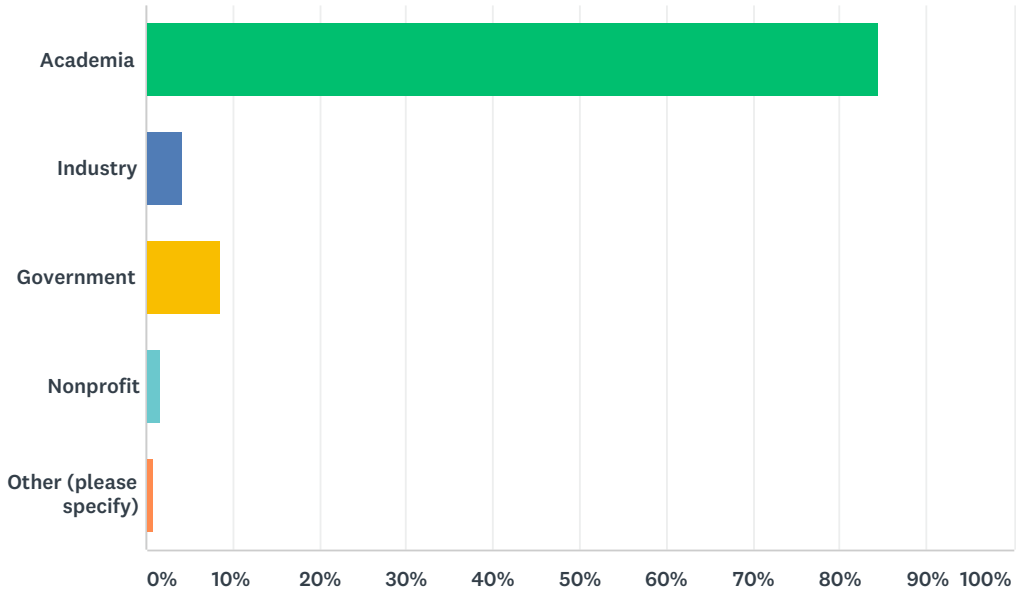


ANSWER CHOICES	RESPONSES	
Graduate student	5.13%	6
Postdoctoral Fellow	19.66%	23
Faculty member / PI	58.97%	69
Scientist in industry	4.27%	5
Government scientist	5.98%	7
Other (please specify)	5.98%	7
TOTAL		117

#	OTHER (PLEASE SPECIFY)	DATE
1	Staff Scientist	4/1/2018 5:46 PM
2	Medical Doctor and research fellow	3/31/2018 7:04 PM
3	professor emerita	3/29/2018 12:10 PM
4	Research Technician	3/28/2018 6:14 PM
5	Senior Lab Specialist	3/28/2018 2:44 PM
6	Research Scientist (Academic)	3/28/2018 2:19 PM
7	academic scientist	3/28/2018 2:13 PM

Q15 In which type of organization do you work?

Answered: 117 Skipped: 14

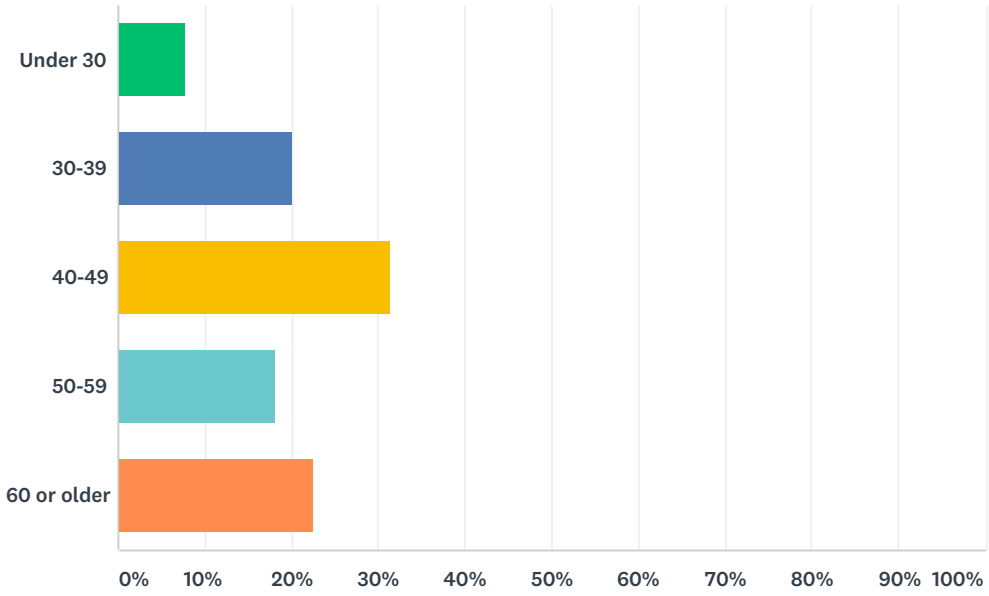


ANSWER CHOICES	RESPONSES	
Academia	84.62%	99
Industry	4.27%	5
Government	8.55%	10
Nonprofit	1.71%	2
Other (please specify)	0.85%	1
TOTAL		117

#	OTHER (PLEASE SPECIFY)	DATE
1	small biotech	3/29/2018 7:25 AM

Q16 Select your age range

Answered: 115 Skipped: 16



ANSWER CHOICES	RESPONSES	
Under 30	7.83%	9
30-39	20.00%	23
40-49	31.30%	36
50-59	18.26%	21
60 or older	22.61%	26
TOTAL		115

Appendix 4: References

References

Abud, E.M., Ramirez, R.N., Martinez, E.S., Healy, L.M., Nguyen, C.H.H., Newman, S.A., Yeromin, A.V., Scarfone, V.M., Marsh, S.E., Fimbres, C.*, et al.* (2017). iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases. Neuron *94*, 278-293 e279.

Dang, J., Tiwari, S.K., Lichinchi, G., Qin, Y., Patil, V.S., Eroshkin, A.M., and Rana, T.M. (2016). Zika Virus Depletes Neural Progenitors in Human Cerebral Organoids through Activation of the Innate Immune Receptor TLR3. Cell Stem Cell *19*, 258-265.

Dekkers, J.F., Wiegerinck, C.L., de Jonge, H.R., Bronsveld, I., Janssens, H.M., de Winter-de Groot, K.M., Brandsma, A.M., de Jong, N.W., Bijvelds, M.J., Scholte, B.J.*, et al.* (2013). A functional CFTR assay using primary cystic fibrosis intestinal organoids. Nat Med *19*, 939-945.

Deng, W.L., Gao, M.L., Lei, X.L., Lv, J.N., Zhao, H., He, K.W., Xia, X.X., Li, L.Y., Chen, Y.C., Li, Y.P.*, et al.* (2018). Gene Correction Reverses Ciliopathy and Photoreceptor Loss in iPSC-Derived Retinal Organoids from Retinitis Pigmentosa Patients. Stem Cell Reports *10*, 1267-1281.

DiMasi, J.A., Grabowski, H.G., and Hansen, R.W. (2016). Innovation in the pharmaceutical industry: New estimates of R&D costs. J Health Econ *47*, 20-33.

Drost, J., van Boxtel, R., Blokzijl, F., Mizutani, T., Sasaki, N., Sasselli, V., de Ligt, J., Behjati, S., Grolleman, J.E., van Wezel, T.*, et al.* (2017). Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer. Science *358*, 234-238.

Fumagalli, A., Drost, J., Suijkerbuijk, S.J., van Boxtel, R., de Ligt, J., Offerhaus, G.J., Begthel, H., Beerling, E., Tan, E.H., Sansom, O.J.*, et al.* (2017). Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. Proc Natl Acad Sci U S A *114*, E2357-E2364.

Garcez, P.P., Loiola, E.C., Madeiro da Costa, R., Higa, L.M., Trindade, P., Delvecchio, R., Nascimento, J.M., Brindeiro, R., Tanuri, A., and Rehen, S.K. (2016). Zika virus impairs growth in human neurospheres and brain organoids. Science *352*, 816-818.

Harrison, R.G. (1906). Observations on the living developing nerve fiber. Exp Biol Med *4*, 140-143.

Hines, W.C., Su, Y., Kuhn, I., Polyak, K., and Bissell, M.J. (2014). Sorting out the FACS: a devil in the details. Cell Rep *6*, 779-781.

Hirsch, T., Rothoeft, T., Teig, N., Bauer, J.W., Pellegrini, G., De Rosa, L., Scaglione, D., Reichelt, J., Klausegger, A., Kneisz, D.*, et al.* (2017). Regeneration of the entire human epidermis using transgenic stem cells. Nature *551*, 327-332.

Huch, M., Knoblich, J.A., Lutolf, M.P., and Martinez-Arias, A. (2017). The hope and the hype of organoid research. Development *144*, 938-941.

Hyun, I., Wilkerson, A., and Johnston, J. (2016). Embryology policy: Revisit the 14-day rule. Nature *533*, 169-171.

Irie, N., Weinberger, L., Tang, W.W., Kobayashi, T., Viukov, S., Manor, Y.S., Dietmann, S., Hanna, J.H., and Surani, M.A. (2015). SOX17 is a critical specifier of human primordial germ cell fate. Cell *160*, 253-268.

Kobayashi, T., and Surani, M.A. (2018). On the origin of the human germline. Development *145*.

Lancaster, M.A., Renner, M., Martin, C.A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., and Knoblich, J.A. (2013). Cerebral organoids model human brain development and microcephaly. Nature *501*, 373-379.

Lomax, G.P., Hull, S.C., and Isasi, R. (2015). The DISCUSS Project: Revised Points to Consider for the Derivation of Induced Pluripotent Stem Cell Lines From Previously Collected Research Specimens. Stem Cells Transl Med *4*, 123-129.

Mandai, M., Watanabe, A., Kurimoto, Y., Hirami, Y., Morinaga, C., Daimon, T., Fujihara, M., Akimaru, H., Sakai, N., Shibata, Y.*, et al.* (2017). Autologous Induced Stem-Cell-Derived Retinal Cells for Macular Degeneration. N Engl J Med *376*, 1038-1046.

Martyn, I., Kanno, T.Y., Ruzo, A., Siggia, E.D., and Brivanlou, A.H. (2018). Self-organization of a human organizer by combined Wnt and Nodal signalling. Nature *558*, 132-135.

McCauley, K.B., Hawkins, F., and Kotton, D.N. (2018). Derivation of Epithelial-Only Airway Organoids from Human Pluripotent Stem Cells. Curr Protoc Stem Cell Biol *45*, e51.

McCauley, K.B., Hawkins, F., Serra, M., Thomas, D.C., Jacob, A., and Kotton, D.N. (2017). Efficient Derivation of Functional Human Airway Epithelium from Pluripotent Stem Cells via Temporal Regulation of Wnt Signaling. Cell Stem Cell *20*, 844-857 e846.

Min, Y.L., Bassel-Duby, R., and Olson, E.N. (2018). CRISPR Correction of Duchenne Muscular Dystrophy. Annu Rev Med.

Munera, J.O., Sundaram, N., Rankin, S.A., Hill, D., Watson, C., Mahe, M., Vallance, J.E., Shroyer, N.F., Sinagoga, K.L., Zarzoso-Lacoste, A.*, et al.* (2017). Differentiation of Human Pluripotent Stem Cells into Colonic Organoids via Transient Activation of BMP Signaling. Cell Stem Cell *21*, 51-64 e56.

Munera, J.O., and Wells, J.M. (2017). Generation of Gastrointestinal Organoids from Human Pluripotent Stem Cells. Methods Mol Biol *1597*, 167-177.

Nikolic, M.Z., Caritg, O., Jeng, Q., Johnson, J.A., Sun, D., Howell, K.J., Brady, J.L., Laresgoiti, U., Allen, G., Butler, R.*, et al.* (2017). Human embryonic lung epithelial tips are multipotent progenitors that can be expanded in vitro as long-term self-renewing organoids. Elife *6*.

Perez-Lanzon, M., Kroemer, G., and Maiuri, M.C. (2018). Organoids for Modeling Genetic Diseases. Int Rev Cell Mol Biol *337*, 49-81.

Sachs, N., de Ligt, J., Kopper, O., Gogola, E., Bounova, G., Weeber, F., Balgobind, A.V., Wind, K., Gracanin, A., Begthel, H.*, et al.* (2018). A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. Cell *172*, 373-386 e310.

Simian, M., and Bissell, M.J. (2017). Organoids: A historical perspective of thinking in three dimensions. J Cell Biol *216*, 31-40.

Simsek, S., Zhou, T., Robinson, C.L., Tsai, S.Y., Crespo, M., Amin, S., Lin, X., Hon, J., Evans, T., and Chen, S. (2016). Modeling Cystic Fibrosis Using Pluripotent Stem Cell-Derived Human Pancreatic Ductal Epithelial Cells. Stem Cells Transl Med *5*, 572-579.

Spence, J.R., Mayhew, C.N., Rankin, S.A., Kuhar, M.F., Vallance, J.E., Tolle, K., Hoskins, E.E., Kalinichenko, V.V., Wells, S.I., Zorn, A.M.*, et al.* (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature *470*, 105-109.

Takebe, T., Wells, J.M., Helmrath, M.A., and Zorn, A.M. (2018). Organoid Center Strategies for Accelerating Clinical Translation. Cell Stem Cell *22*, 806-809.

van de Wetering, M., Francies, H.E., Francis, J.M., Bounova, G., Iorio, F., Pronk, A., van Houdt, W., van Gorp, J., Taylor-Weiner, A., Kester, L.*, et al.* (2015). Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell *161*, 933-945.

Yan, H.H.N., Siu, H.C., Law, S., Ho, S.L., Yue, S.S.K., Tsui, W.Y., Chan, D., Chan, A.S., Ma, S., Lam, K.O.*, et al.* (2018). A Comprehensive Human Gastric Cancer Organoid Biobank Captures Tumor Subtype Heterogeneity and Enables Therapeutic Screening. Cell Stem Cell.

Zhou, T., Tan, L., Cederquist, G.Y., Fan, Y., Hartley, B.J., Mukherjee, S., Tomishima, M., Brennand, K.J., Zhang, Q., Schwartz, R.E.*, et al.* (2017). High-Content Screening in hPSC-Neural Progenitors Identifies Drug Candidates that Inhibit Zika Virus Infection in Fetal-like Organoids and Adult Brain. Cell Stem Cell *21*, 274-283 e275.