REVIEW ARTICLE

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Organoids — Preclinical Models of Human Disease

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An organoid is a three-dimensional construct composed of multiple cell types that originates from stem cells by means of self-organization and is capable of simulating the architecture and functionality of native organs. Organoids permit in vivo and in vitro investigation and represent one of the latest innovations in the quest for a model to recapitulate the physiologic processes of whole organisms. (For historical notes on the origins of organoids, see the Supplementary Appendix, available with the full text of this article at NEJM.org.)

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Organoids have advantages over traditional two-dimensional cultures. They can display near-physiologic cellular composition and behaviors. Many organoids can undergo extensive expansion in culture and maintain genome stability,[1-4] which makes them suitable for biobanking and high-throughput screens.[5] As compared with animal models, organoids can reduce experimental complexity, are amenable to real-time imaging techniques, and, more important, enable the study of aspects of human development and disease that are not easily or accurately modeled in animals (Fig. 1).[6-9] Here we review the common platforms of organoid technology and their applications (see video, available at NEJM.org).

An illustrated glossary and a video overview of organoid technology are available at NEJM.org

Organoids can be generated with the use of somatic cells, adult stem cells (including progenitor cells), or pluripotent stem cells. Because of limitations in the availability, expandability, and throughput of tissues needed for somatic-cell organoids,[8] they are less widely used than stem-cell organoids and are not the focus of this article.

MODELING ORGANOIDS FROM ADULT STEM CELLS

MODELING THE INTESTINE

A breakthrough in organoid technology occurred in 2009 when Clevers and colleagues showed that stem cells resident in the adult intestine proliferate and self-organize in vitro.[6] Intestinal stem cells are characterized by the expression of *LGR5*, a gene encoding the receptor for the Wnt agonist R-spondin,[7,8] and they require specific molecules, such as Wnt, epidermal growth factor, and noggin (a bone morphogenetic protein inhibitor) within their environs.[6] Extracellular matrix also has important signaling roles: dissociated intestinal cells undergo anoikis.[9] Sato et al.[6] therefore developed a three-dimensional culture to reconstitute an in vitro, nichelike milieu for intestinal stem cells and obtained organoids, each originating from a fragment of the intestinal epithelium or even a single LGR5$^+$ stem cell and maintaining a crypt–villus architecture with each of the four differentiated intestinal cell types in a self-renewing fashion. These organoids can expand for more than 3 months and remain genomically stable, facilitating the purification of large quantities of organ-specific cell types.

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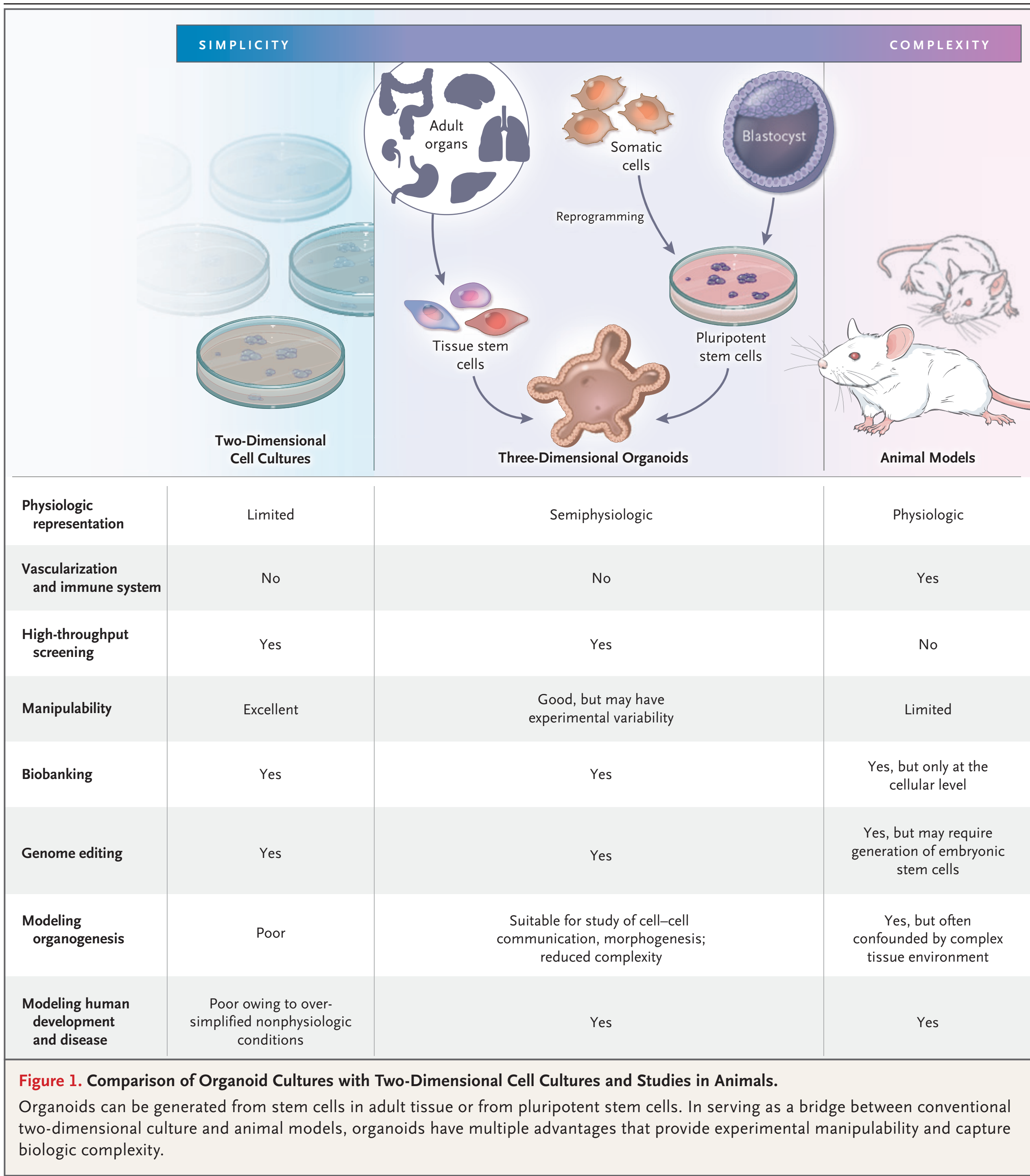

	Two-Dimensional Cell Cultures	Three-Dimensional Organoids	Animal Models
Physiologic representation	Limited	Semiphysiologic	Physiologic
Vascularization and immune system	No	No	Yes
High-throughput screening	Yes	Yes	No
Manipulability	Excellent	Good, but may have experimental variability	Limited
Biobanking	Yes	Yes	Yes, but only at the cellular level
Genome editing	Yes	Yes	Yes, but may require generation of embryonic stem cells
Modeling organogenesis	Poor	Suitable for study of cell–cell communication, morphogenesis; reduced complexity	Yes, but often confounded by complex tissue environment
Modeling human development and disease	Poor owing to over-simplified nonphysiologic conditions	Yes	Yes

Figure 1. Comparison of Organoid Cultures with Two-Dimensional Cell Cultures and Studies in Animals.
Organoids can be generated from stem cells in adult tissue or from pluripotent stem cells. In serving as a bridge between conventional two-dimensional culture and animal models, organoids have multiple advantages that provide experimental manipulability and capture biologic complexity.

The method developed by Sato et al.[6] has since been adapted to generate organoids from epithelial tissues of major organs (Table 1, and Table S1 in the Supplementary Appendix).[37,38,40-46] It is worth noting that *LGR5* expression is undetectable in several tissues,[47-49] and the presence of steady-state $LGR5^{+}$ stem cells is not a prerequisite for organoid generation. The cells of the liver and pancreas do not express much LGR5 under homeostatic conditions, although $LGR5^{+}$ ductal cells are induced during regenerative responses after liver or pancreatic injury.

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Table 1. Summary of Selected Organoid Models.*

Target Tissue and Cellular Input	Species	Applications	References
Intestine			
Intestinal crypts	Human or mouse	Drug screening, transplantation, modeling of genetic disease (e.g., cystic fibrosis and cancer)	Sato et al.,[6] Vlachogiannis et al.,[10] Fujii et al.,[11] Schwank et al.[12]
Embryonic stem cells or induced pluripotent stem cells	Human or mouse	Drug screening, transplantation, modeling of genetic disease (e.g., cystic fibrosis and cancer)	Spence et al.,[13] Múnera et al.[14]
Gastric: embryonic stem cells or induced pluripotent stem cells	Human	Modeling of *Helicobacter pylori* infection and cancer	McCracken et al.[15]
Liver			
Induced pluripotent stem cells	Human	Drug screening, transplantation, single-cell RNA sequencing, modeling of cell–cell communications	Camp et al.,[16] Takebe et al.[17]
Adult tissue	Human	Drug screening, transplantation, modeling of genetic diseases (e.g., Alagille's syndrome)	Huch et al.[1]
Adult tissue	Mouse	Transplantation, gene-expression profiling	Huch et al.[18]
Pancreas			
Adult tissue	Human or mouse	Transplantation, modeling of pancreatic cancer	Huch et al.,[19] Boj et al.[20]
Adult tissue	Mouse	Transplantation, modeling of treatment of mice with diabetes	Takebe et al.[21]
Lung: embryonic stem cells	Human	Modeling of lung differentiation, homeostasis, and disease	Dye et al.[22]
Retina: embryonic stem cells or induced pluripotent stem cells	Human or mouse	Transplantation, modeling therapy for advanced retinal degenerative diseases	Eiraku et al.,[23] Nakano et al.,[24] Assawachananont et al.[25]
Brain: embryonic stem cells	Human or mouse	Single-cell RNA sequencing; modeling of corticogenesis, including folding of cortex; treatment of genetic diseases (e.g., lissencephaly); modeling of Zika virus exposure during human cortical development	Monzel et al.,[26] Mugurama et al.,[27] Sakaguchi et al.,[28] Lancaster et al.,[29] Qian et al.[30]
Pituitary: embryonic stem cells	Mouse	Transplantation rescues in hypopituitary mice	Suga et al.[31]
Kidney			
Induced pluripotent stem cells	Human	Transplantation, transcriptomic analysis, and toxicologic study	Taguchi et al.,[32] Xia et al.,[33] Takasato et al.[34,35]
Fetal kidney tissues	Human or mouse	Transplantation, CRISPR-based genome editing, toxicology study, disease modeling	Li et al.[4]
Uterus: endometrium	Human	Modeling human early pregnancy, endometriosis, and endometrial cancer	Turco et al.[36]
Prostate: adult tissue	Human or mouse	Modeling prostate homeostasis, tumorigenesis, and drug discovery	Chua et al.,[37] Karthaus et al.[38]
Testis: dissociated testis	Human	Modeling spermatogenesis and male fertility preservation and treatment	Baert et al.[39]
Inner ear: embryonic stem cells	Human or mouse	Modeling inner-ear development and disorder, drug screening for hearing and balance disorder, and cell-based therapy	Koehler et al.,[40,41] Liu et al.[42]
Mammary gland: adult tissue	Human or mouse	Drug screening, modeling normal mammary gland development, molecular mechanism of hormonal regulation and intercellular signaling, and breast tumorigenesis	Jardé et al.,[43] Pasic et al.,[44] Zeng and Nusse[45]

* Details on these and other organoids and additional references can be found in Table S1 in the Supplementary Appendix.

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Such LGR5+ cells can form clonal organoids composed of bipotential progenitors (hepatocyte and bile-duct potential for the liver, and ductal and endocrine lineages for the pancreas).[18,19] Bipotent organoids have also been generated from the human liver and pancreas.[1,19,20] Thus, the R-spondin method appears to be applicable to long-term maintenance of adult stem cells in different types of epithelial tissues in the format of organoids.

MODELING THE GENITOURINARY SYSTEM

Several organoid cultures of the components of the genitourinary system have been reported. Organoids have been derived from female and male reproductive tracts as well as the kidney.

In the female reproductive tract, the human endometrium provides the microenvironment for implantation and nutritional support for the early conceptus. Because in vivo study is impractical, long-term culture models are needed to better understand the role of endometrial secretion and endometrial–placental interactions during early pregnancy. To culture isolated endometrial epithelia, Turco et al.[36] started with the R-spondin method and supplemented the medium with growth factors to mimic the in vivo niche of glandular progenitor cells (Table 1, and Table S1 in the Supplementary Appendix). Using specimens from the endometrium and decidua of nonpregnant women, the researchers established genetically stable endometrial organoids that mount an appropriate transcriptional response to sex hormones and that recapitulate characteristics of the gestational endometrium when stimulated with prolactin and placental hormones, signals of early pregnancy. Whether these efforts represent a suitable model for the implantation of in vitro cultured blastocysts has yet to be established. Organoids have also been obtained from malignant endometrium, although further work is required to substantiate their value as a model for endometrial cancer.

There also has been some progress in the development of organoids derived from the male reproductive system. One report[39] showed self-organization of dissociated human testicular cells under conditions similar to those characterizing the organotypic culture[50] of neonatal mouse testes. The dissociated cells formed a condensed spheroid that has been described as a testicular organoid.[39] Despite the absence of native tissue topography, niche cells and spermatogonia persisted in testicular organoids[39] (Table 1, and Table S1 in the Supplementary Appendix). However, the differentiation of spermatogonia, meiosis, and sperm formation were not reported. The testicular constructs do not undergo long-term expansion and are therefore more akin to a primary organ culture.

The functional unit of the kidney, the nephron, which is composed of a glomerulus (renal corpuscle) and a renal tubule, depends on an intricate tissue architecture. During development, nephrogenesis requires reciprocal interactions between two kidney progenitor populations in the intermediate mesoderm — the metanephric mesenchyme and the ureteric epithelium. The spatiotemporally coordinated processes of mutual induction between the mesenchyme and epithelium, cell movement, cell proliferation, and cell adhesion suggest a genetically encoded self-organization program.[51] Indeed, dissociated embryonic kidney cells self-organize into their tissue of origin with spatial fidelity.[52] The developing kidney contains transient nephron progenitor cells that give rise to all nephrons.[53] These progenitor cells have not been found in the adult human kidney, which cannot regenerate nephrons.[54] Although several putative adult kidney progenitors capable of tubulogenesis in organoids have been reported,[55-57] there is disagreement regarding their identity and potential.[55,56]

Embryonic nephron progenitor cells, on the other hand, are better characterized and have been successfully used to create kidney organoids.[32,58-60] However, many hurdles remain: first, the loss of differentiation potential in cultured nephron progenitor cells; second, the limited self-renewal of these cells; third, a lack of evidence for in vivo nephrogenic potential; and, finally, the dependence on transgenic markers of cell identity. On the basis of previous observations,[61-63] we developed a long-term, three-dimensional culture of genomically stable nephron progenitor cells[4] (Table 1, and Table S1 in the Supplementary Appendix). These cells can self-renew only under artificial culture conditions but can be induced to form nephronlike structures with appropriate spatial orientation, indicating an intact nephrogenic potential. We observed that these nephron progenitor cells contribute to nephrogenesis in neonatal mice and chick embryos, generating ectopic, nephronlike structures that connect with the host vasculature. A urinelike filtrate was obtained when these nephronlike structures were transplanted

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into the omentum of immunodeficient mice.[4] Cultures of human nephron progenitor cells with similar properties can be derived from fetal kidneys between 9 and 17 weeks of gestation, and human nephron progenitor cell lines are amenable to genome editing for the purpose of studying human organogenesis and genetic diseases (Table S2 in the Supplementary Appendix).[4]

Pluripotent Stem Cells

Pluripotent stem cells — both induced and embryonic — can self-renew indefinitely and differentiate into any cell type in the body, thus offering an attractive alternative to the use of primary tissues to create organoids. Because organoids derived from pluripotent stem cells are formed through directed differentiation of a homogeneous population, tissue-specific cell types and their microenvironment must be created anew in a dynamic process that is reminiscent of embryogenesis. Accordingly, pluripotent stem-cell organoid culture must provide stage-appropriate niche signals during differentiation. Because this process is neither simple nor straightforward, pluripotent stem-cell organoids often contain cell types that differ from those in the modeled organ, complicating the signaling environment and self-organization of the target tissue.[15,16,64]

Early work in directed differentiation of pluripotent stem cells established signaling requirements for germ-layer formation, patterning, and induction of tissue identity in two-dimensional cultures. Pluripotent stem cells can also spontaneously differentiate as embryoid bodies. These embryoid bodies typically differentiate further along a tissue-specific lineage in the presence of specific cues in two-dimensional culture.

Pluripotent stem cells in two-dimensional culture are known to default to a neural fate in the absence of any inductive signal.[65] The differentiated neuroepithelial cells self-organize into rosettes that resemble neural progenitor cells of the developing neuroepithelium.[66] Building on this knowledge, Eiraku et al. generated neuroectodermal organoids (Fig. 2).[67] Neurons in these organoids showed properties characteristic of neonatal cortical brain tissues, and the organoids recapitulated the spatial and temporal regulation of early corticogenesis (including the organization of distinct zones along the apicobasal axis and the “birth order” of layer-specific neurons in the developing cortex).[67] The neuroepithelia generated by this approach were stimulated to exhibit specific regional identities. For example, when basement-membrane matrix components (e.g., Matrigel) were added to the differentiation culture, the neuroepithelial cells formed a rigid, continuous neuroepithelium that on exposure to the growth factor NODAL self-organized into optic cups composed of a retinal pigment epithelium and a neural retinal epithelium.[23] Similar strategies have been successfully used to generate organoids representing diverse regions of the neuroepithelium, including the retina,[24] adenohypophysis,[31] midbrain,[26] cerebellum,[27] and hippocampus[28] (Table 1, and Table S1 in the Supplementary Appendix).

The growth of cortical organoids is limited by the free diffusion of oxygen, nutrients, and growth factors. Consequently, cells in deep areas of organoids undergo apoptosis.[6,29] A protocol in which the organoid culture is kept spinning in a bioreactor was developed to enhance nutrient exchange, thus substantially improving growth and development of neuroepithelia. These neuroepithelia spontaneously form regions reminiscent of the cerebral cortex in the absence of inductive signals.[29] Morphologic, histologic, and transcriptional analyses showed that these “cerebral organoids” contained interdependent domains recapitulating various regions of the brain.[29] Importantly, human-specific features, including the outer subventricular zone, were observed in cerebral organoids[29,30] (Table S1 in the Supplementary Appendix).

Single-cell RNA sequencing, a powerful tool for studying cellular identity, revealed that cerebral organoids contain neural and mesenchymal cells with progenitor and differentiated phenotypes. A remarkable level of transcriptional similarity was found between the cells of organoids and fetal tissues, lending credence to the notion that the cerebral organoid can be used as a model for human cortical development.[68,69]

Organoids of the mesodermal kidney have been reported. The ureteric epithelium renal progenitors can be generated from human pluripotent stem cells with the use of defined media for intermediate mesoderm induction.[33,70] These progenitors can self-organize into ureteric bud structures when aggregated with dissociated murine embryonic kidney cells.[33] Another renal progenitor, the metanephric mesenchyme, can be differentiated from pluripotent stem cells through

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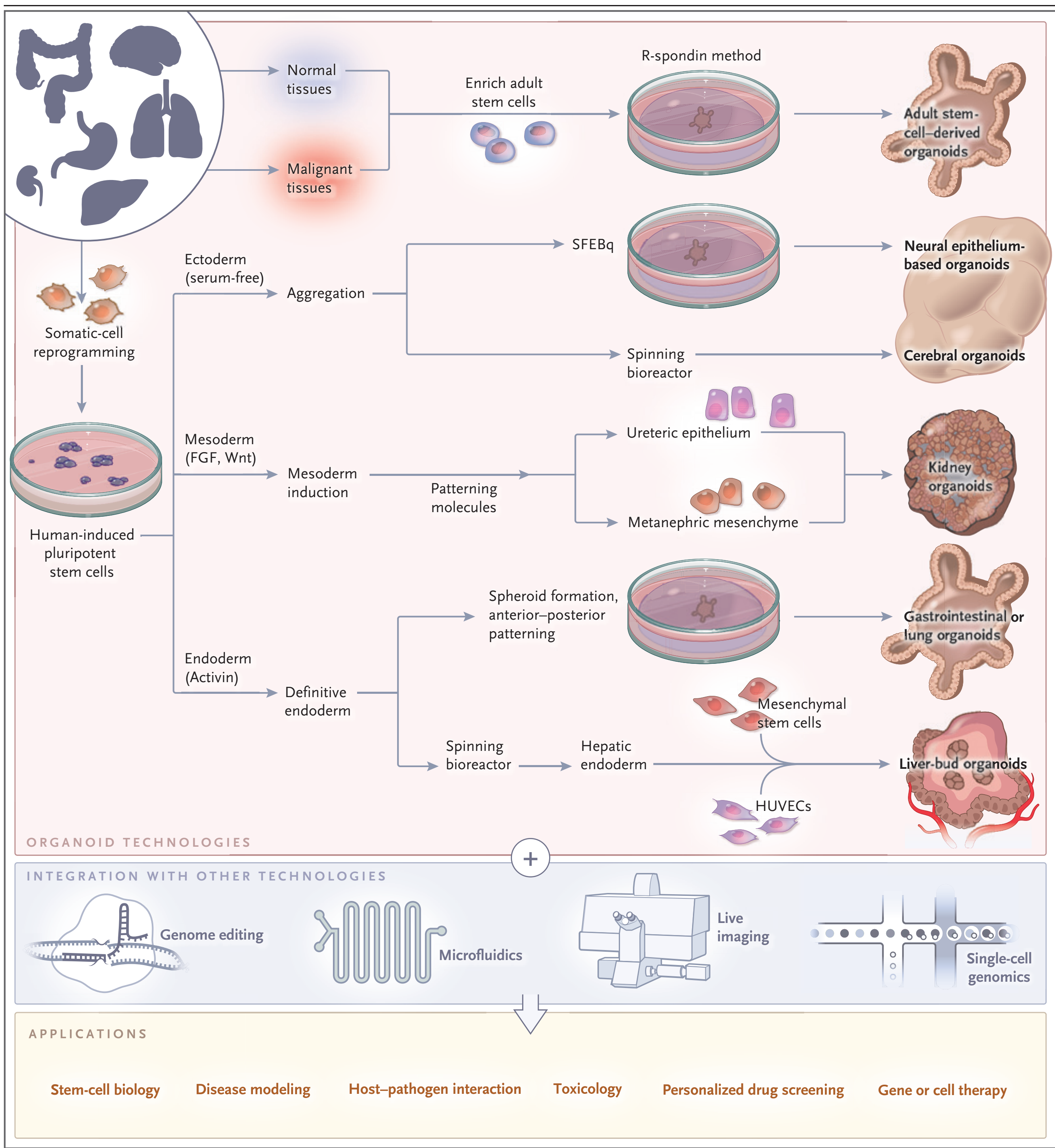

the phasic activation of Wnt and the addition of stage-specific growth factors that promote the formation of the posterior intermediate mesoderm, a key precursor of the metanephric mesenchyme. The resulting metanephric mesenchyme can form nephronlike structures with tubules and glomeruli in three-dimensional culture.[32] It is also possible to simultaneously induce the ureteric epithelium and metanephric mesenchyme through directed differentiation of human pluripotent stem cells.[34] The correctly patterned renal progenitors then self-organize in three-dimensional culture to generate nephrons with defined glomeruli and segmented tubular structures, which are associated with a network of collecting ducts and surrounding interstitial and endo-

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Figure 2 (facing page). Schematics of Mainstream Human Organoid Models and Their Applications.
Human organoids can be generated from normal or malignant primary tissues, most often by the R-spondin method, which is the essential design principle for generating small-intestine organoids, namely, extracellular matrix support (Matrigel), activation of the Wnt signaling pathway (R-spondin1 and Wnt3a), growth factors for organ-specific epithelial proliferation (e.g., epithelial growth factor), and stem-cell self-renewal factors (inhibitors of bone morphogenetic protein (BMP) and transforming growth factor β (TGF-β) (for additional details, see the Supplementary Appendix). Alternatively, somatic cells can be reprogrammed to become induced pluripotent stem cells, which are used as sources of organoids for all three germ layers through directed differentiation. The SFEBq method, which involves serum-free culture of embryoid-body–like aggregates with quick aggregations, entails dissociation of pluripotent stem cells into a homogenous single-cell suspension to minimize any endogenous inductive signals, followed by quick aggregation in a serum-free and growth-factor–free medium. Organoid technologies have integrated well with other technologies, including genome editing, single-cell genomics, live imaging, and microfluidics, thus providing new insights into developmental processes and disease pathogenesis as well as enabling translational approaches to the diagnosis and treatment of disease. FGF denotes fibroblast growth factor, and HUVEC human umbilical vein endothelial cell.

thelial cells.[35] Because it is difficult to distinguish immature pronephric and mesonephric nephrons from mature metanephric nephrons with the use of lineage markers only, further work is needed to ascertain the functional maturity of the organoid nephrons.[71] It would also be desirable to develop cultured conditions for ureteric epithelial progenitors, which have not been propagated as a pure population in culture. Their presence is inferred by means of gene expression and the immunofluorescence of known markers.[33-35] Once isolated, ureteric epithelial progenitors could be tested for the capacity to undergo reciprocal induction with nephron progenitor cells or to reconstitute collecting ducts in embryonic kidney reaggregation assay.[72]

For endodermal lineages, pluripotent stem cells are first differentiated into definitive endoderm through exposure to activin–nodal signaling and low concentrations of serum, and the resulting gut tube can be patterned along the anterior–posterior axis through temporal and spatial manipulation of the Wnt and other signaling pathways.[73,74] Organoids representing tissues that originate from the foregut, including the lung,[22] thyroid,[75] stomach,[15] pancreas,[21,76,77] and liver,[17] as well as tissues from the midgut and hindgut, such as the small and large intestines,[13,14] have been reported[46] (Table 1, and Table S1 in the Supplementary Appendix).

An unusual feature of pluripotent stem-cell organoids is that tissue-specific epithelia are differentiated through a series of progenitors, a process that entails interaction between germ layers. In pluripotent stem-cell organoids, the residual mesodermal cells that are present after endodermal induction become fibroblasts and smooth-muscle cells that develop around the epithelium,[13,15,22] a phenomenon reminiscent of the mesenchymal tissues that develop around endodermal organ primordia during organogenesis.[78] Consistent with their supporting roles in epithelial morphogenesis, mesenchymal stem cells and endothelial cells can be intentionally mixed with human pluripotent stem-cell–derived hepatic progenitors to produce three-dimensional liver buds that become vascularized and functional upon transplantation (Fig. 2). Single-cell RNA sequencing has revealed that communications between different lineages in the liver bud help them become transcriptionally similar to their counterparts in human fetal liver.[16] Such a heterotypic approach to the culture of organoids has been shown to support the formation of vascularized organ buds from the pancreas, kidney, intestine, heart, lung, and brain.[21]

APPLICATIONS OF ORGANOID TECHNOLOGIES

MODELING DISEASE

As compared with two-dimensional cultures, organoids may provide more fundamental insights into development, homeostasis, and pathogenesis and may offer new translational approaches to the diagnosis and treatment of disease (Fig. 2). For instance, cerebral organoids recapitulate human-specific neurogenic processes, thereby presenting an opportunity to study human brain development.[29] Human cerebral organoids have been grown in a microfabricated compartment that allows long-term in situ imaging. This system has been used to model the physics of cortical folding and to study the mechanism underlying lissencephaly, which is caused by mutations in *LIS1*.[79] One study showed that cerebral organ-

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oids generated from induced pluripotent stem cells obtained from a person with lissencephaly have a mitotic defect in outer radial glial cells, which are poorly represented in mouse models.[69] Brain organoids have also been used to show that the Zika virus preferentially infects neural progenitors and reduces their proliferation and viability, which may in turn be a cause of Zika virus–associated microcephaly.[30,80-82]

Screening Anticancer Drugs

Organoids have been successfully grown from primary tumors of the colon, prostate, breast, and pancreas.[46] These "tumoroids" have emerged as preclinical models that have the potential to predict an individual patient's response to treatment (Table S2 in the Supplementary Appendix). For example, a living biobank of tumoroids from patients with metastatic gastrointestinal cancer recapitulated the response of these patients to anticancer agents in clinical trials.[10] Tumoroids can also be used to study the tumor niche. An organoid library representing different grades of colorectal tumors revealed a decreased dependency on niche factors along the transition from normal tissue to adenoma to carcinoma. Niche-factor dependency is found to be primarily associated with the genetic makeup of a tumor.[11] This example and others (Table S2 in the Supplementary Appendix) suggest that tumoroids are a means of linking cancer-related genomic data to tumor biology and can provide a substrate for drug screening and personalized treatment.

Identifying Drug Toxicity

Organ toxicity is the primary reason for failures in drug development and postapproval withdrawals.[83] Current toxicology screens that use cell lines and animal models often do not predict adverse effects in humans, in whom renal and hepatic toxicities are among the most common. Three-dimensional organoids may offer more accurate means of toxicity prediction. Encouragingly, kidney organoids have been shown to recapitulate the nephrotoxic effects of cisplatin[35] and gentamicin.[4] Other advantages of organoids include their genetic stability[1-4] and scalability for high-throughput screens. For instance, human nephron progenitor cells have a nearly unlimited ability to self-renew in three-dimensional culture,[4] which could be a boon for standardization of nephrotoxicity screens. Recently, the Food and Drug Administration has started testing three-dimensional "liver-on-a-chip" constructs to screen for the hepatic toxicity of compounds used in food additives, nutritional supplements, and cosmetics.

Testing Genetic and Cell-Based Therapies

The functional integration of transplanted organoids (or cells from organoids) has been observed in the colon,[84] liver,[17,18] pancreas, retina,[25] and thyroid.[85] In these studies, different levels of evidence were used to support functionality, including morphologic similarity to native tissues,[4,21,25,84] connection to the host (through the vasculature[4,21] or synapses[25]), epithelial permeability,[84] and rescue from a disease or injury.[18,85] Genome editing has also been used to correct mutations in *CFTR* and to restore the functionality of the CFTR protein in colon organoids derived from patients with cystic fibrosis.[12] Such studies suggest that organoids may be a source of cells in future approaches to cell therapy. However, more studies are needed to evaluate the efficacy and safety of such approaches. Efforts to bring induced pluripotent stem cells to the clinic could offer guidance in this area.[86]

Challenges and Future Uses

Despite the progress in organoid research, the field still faces challenges, including the variability and lack of standardization in methods. Fortunately, biologic and bioengineering solutions are being developed at a rapid pace to address these issues.

Extracellular Matrix and Cellular Composition of Organoids

Variability among animal-derived and chemically undefined extracellular matrixes (e.g., Matrigel, from Corning Life Sciences and BD Biosciences[87]) could confound high-throughput screens.[88] Risks from such xenobiotics may also be problematic when translating basic research findings to clinical applications. In addition, the isotropic Matrigel is unable to recapitulate the dynamic changes in biomechanical forces in vivo. To address these issues, synthetic matrixes that can change their biophysical and biochemical properties on demand are being developed.[89] Extracellular matrix engineering may not only replace xenobiotics but also elucidate the ways in which

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tissues are organized, thus improving the reproducibility of organoids.

The lack of representation of important physiological processes, including vascularization and innervation, could be a hindrance to organoid research. Although several types of organoids become vascularized on transplantation,[4,17,21,90] reports of in vitro organoid culture with a vasculature are lacking. Stroma–tumor interaction is important for tumorigenesis and metastasis.[91] Various stromal components, such as immune cells[92] and endothelial cells,[93] have been added to tumoroids for the purpose of studying tumorigenesis. The peripheral nervous system plays an integral role in tissue homeostasis and repair[94] but is rarely represented in organoids. To this end, a recent study generated human intestinal organoids with a functional enteric nervous system by including pluripotent stem-cell–derived neural crest cells.[95]

The source material used to create organoids may introduce variability.[5,29] Small but detectable variations exist in organoids derived from patient-induced pluripotent stem cells,[29,96,97] which differ depending on the age and genetic background of the patient and the culture protocol used by the research group. CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats) technology can be used to engineer organoids with an isogenic background, thus reducing variability (Table S2 in the Supplementary Appendix). Other innovations (e.g., the use of a bioreactor[30]) may minimize variation in culture conditions. Tumoroids generated from patient tumors vary considerably, probably as a result of the heterogeneity of tumors.[5] Medium composition may also affect the growth of tumor and nontumor cells differently.[11,98,99] Systematic studies of a large number of tumoroids (e.g., the TUMOROID trial [NL49002.031.14] and the SENSOR study [NL50400.031.14; EudraCT number, 2014-003811-13]) may answer questions regarding how such variability may affect clinical application.

An audio interview with Dr. Benjamin S. Freedman is available at NEJM.org

TISSUE ARCHITECTURE OF ORGANOIDS

Organoid cultures rely on self-organization that sometimes results in abnormal tissue architectures (e.g., the closed lumen of intestinal organoids[6]). Tissue architecture may be improved by providing a scaffold made of biomaterials or printed with bioinks.[100,101] The latter technique has been used to print three-dimensional renal proximal tubules in a perfusable tissue chip.[102] Such "organ-on-a-chip" systems that combine microfluidics and organoids provide precise control over biomechanical variables and the delivery of bioactive molecules. These systems may facilitate real-time monitoring of single cells, cell–cell interaction, and metabolic processes at the tissue level. However, such real-time monitoring capabilities are generally lacking in current studies of organoids. The development of such techniques will necessitate close collaboration between engineers and developmental biologists. In the future, mature commercial platforms for biomaterial and bioprinting may accelerate the pace of discovery. Organoid technology has been effectively integrated with other cutting-edge technologies (Fig. 2). The resolution of current challenges may further increase the precision with which organoids recapitulate human physiological processes.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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