REVIEW SUMMARY

ORGANOID GENERATION

Organogenesis in a dish: Modeling development and disease using organoid technologies

Madeline A. Lancaster *and* **Juergen A. Knoblich***

BACKGROUND: Because of their differentiation potential, pluripotent stem cells can generate virtually any cell type and, as such, can be used to model development and disease and even hold the promise of providing cell-replacement therapies. Recently, structures resembling whole organs, termed organoids, have been generated from stem cells through the development of three-dimensional culture systems.

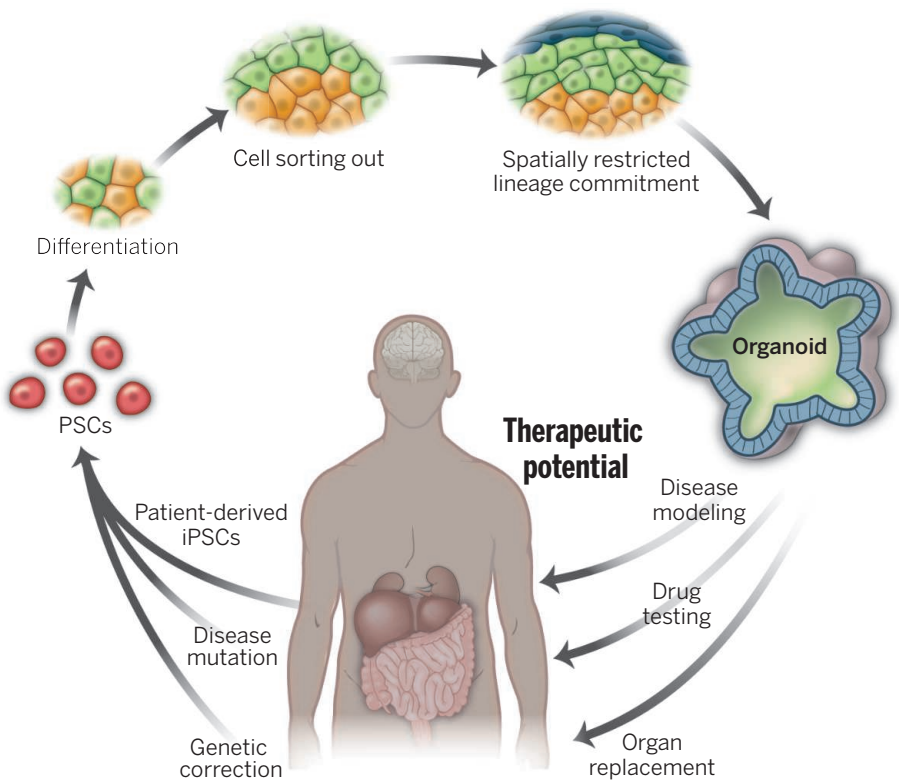
Organoids are derived from pluripotent stem cells or isolated organ progenitors that differentiate to form an organlike tissue exhibiting multiple cell types that self-organize to form a structure not unlike the organ in vivo. This technology builds upon a foundation of stem cell technologies, as well as classical developmental biology and cell-mixing experiments. These studies illustrated two key events in structural organization during organogenesis: cell sorting out and spatially restricted lineage commitment. Both of these processes are recapitulated in organoids, which self-assemble to form the cellular organization of the organ itself.

ADVANCES: Organoids have been generated for a number of organs from both mouse and human stem cells. To date, human pluripotent stem cells have been coaxed to generate intestinal, kidney, brain, and retinal organoids, as well as liver organoid-like tissues called liver buds. Derivation methods are specific to each of these systems, with a focus on recapitulation of endogenous developmental processes.

These complex structures provide a unique opportunity to model human organ development in a system remarkably similar to development in vivo. Although the full extent of similarity in many cases still remains to be determined, organoids are already being applied to human-specific biological questions. Indeed, brain and retinal organoids have both been shown to exhibit properties that recapitulate human organ development and that cannot be observed in animal models. Naturally, limitations exist, such as the lack of blood supply, but future endeavors will advance the technology and, it is hoped, fully overcome these technical hurdles.

OUTLOOK: The therapeutic promise of organoids is perhaps the area with greatest potential. These unique tissues have the potential to model developmental disease, degenerative conditions, and cancer. Genetic disorders can be modeled by making use of patient-derived induced pluripotent stem cells or by introducing disease mutations. Indeed, this type of approach has already been taken to generate organoids from patient stem cells for intestine, kidney, and brain.

Furthermore, organoids that model disease can be used as an alternative system for drug testing that may not only better recapitulate effects in human patients but could also cut down on animal studies. Liver organoids, in particular, represent a system with high expectations, particularly for drug testing, because of the unique metabolic profile of the human liver. Finally, tissues derived in vitro could be generated from patient cells to provide alternative organ replacement strategies. Unlike current organ transplant treatments, such autologous tissues would not suffer from issues of immunocompetency and rejection. ■



Organoid generation and therapeutic potential. Organoids can be derived for a number of organs from human pluripotent stem cells (PSCs). Like organogenesis in vivo, organoids self-organize through both cell sorting out and spatially restricted lineage commitment of precursor cells. Organoids can be used to model disease by introducing disease mutations or using patient-derived PSCs. Future applications could include drug testing and even tissue replacement therapy.

ILLUSTRATION: K. SUTLIFF/*SCIENCE*

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IMBA—Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna 1030, Austria.
* Corresponding author. E-mail: juergen.knoblich@imba.oeaw.ac.at

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REVIEW

ORGANOID GENERATION

Organogenesis in a dish: Modeling development and disease using organoid technologies

Madeline A. Lancaster[1] and Juergen A. Knoblich[1]*

Classical experiments performed half a century ago demonstrated the immense self-organizing capacity of vertebrate cells. Even after complete dissociation, cells can reaggregate and reconstruct the original architecture of an organ. More recently, this outstanding feature was used to rebuild organ parts or even complete organs from tissue or embryonic stem cells. Such stem cell–derived three-dimensional cultures are called organoids. Because organoids can be grown from human stem cells and from patient-derived induced pluripotent stem cells, they have the potential to model human development and disease. Furthermore, they have potential for drug testing and even future organ replacement strategies. Here, we summarize this rapidly evolving field and outline the potential of organoid technology for future biomedical research.

Stem cell technologies hold promise for modeling development, analyzing disease mechanisms, and developing potential therapies. Until quite recently, most stem cell methods focused on pure populations of particular stem cell–derived cell types (*1*), rather than the complete set of cell types of an organ. However, this is beginning to change with the development of three-dimensional (3D) cultures of developing tissues, called organoids.

As organoid technology is on the verge of becoming an independent research field, a precise definition of the term becomes increasingly important. The term organoid, simply defined as resembling an organ, has been used quite loosely for a variety of structures, both in vitro and in vivo (*2*–*4*). The basic definition, however, implies several important features that are characteristics of organs (Box 1). First, it must contain more than one cell type of the organ it models; second, it should exhibit some function specific to that organ; and third, the cells should be organized similarly to the organ itself. This also implies similarity to the manner in which the organ establishes its characteristic organization during development. Thus, we would like to define an organoid as containing several cell types that develop from stem cells or organ progenitors and self-organize through cell sorting and spatially restricted lineage commitment, similar to the process in vivo (Box 1).

Self-organization: The foundation of organoid formation

Organoid methods build upon an extensive foundation of classic developmental biology and cell dissociation and reaggregation experiments (Fig. 1A). Two distinct approaches have been taken to understanding tissue patterning (*5*). In vivo examination of cell movements has revealed mechanisms of cell segregation into discrete domains during tissue morphogenesis (*6*). This process has been extensively examined in, for example, the *Drosophila* wing disc where the anterior-posterior boundary is established through mutually repressive interactions (*7*). A similar process occurs during vertebrate embryonic development at the midbrain-hindbrain boundary, which then acts as an organizer for subsequent tissue morphogenesis (*8*).

The second approach to understanding tissue patterning has been dissociation and reaggregation of tissues to examine relative morphogenetic movements of cells in vitro (Fig. 2A). This approach has been applied to virtually all developing vertebrate organs in a number of classic studies with embryonic chick tissues (*9*, *10*) (Fig. 1A). The results point to a general capacity of cells to reorganize and segregate in a process termed "cell sorting out" to form structures with much the same histogenic properties as those in vivo (*6*, *11*). For example, studies from the early 1960s have utilized dissociated cells from the developing chick kidney (*9*) to form reaggregates that recapitulate virtually complete renal development.

The basis of this organ self-assembly seems to arise from segregation of cells with similar adhesive properties into domains that achieve the most thermodynamically stable pattern (Fig. 2A). Known as Steinberg's differential adhesion hypothesis (*12*) (Fig. 1A), the theory is supported by a range of in vitro cell-mixing experiments (*13*). Differential adhesion is mediated by cell surface adhesion proteins, for example, in separation of vertebrate neural and epidermal ectoderm (*14*, *15*), where differential epithelial and neural and cadherin expression mediates cell sorting out.

A second mechanism that can influence tissue morphogenesis is proper spatially restricted progenitor fate decisions (Fig. 2B). An excellent example is the developing vertebrate retina, where neuroepithelial cells give rise to a complex lineage that generates the various layers of the retina in a temporally and spatially restricted manner.

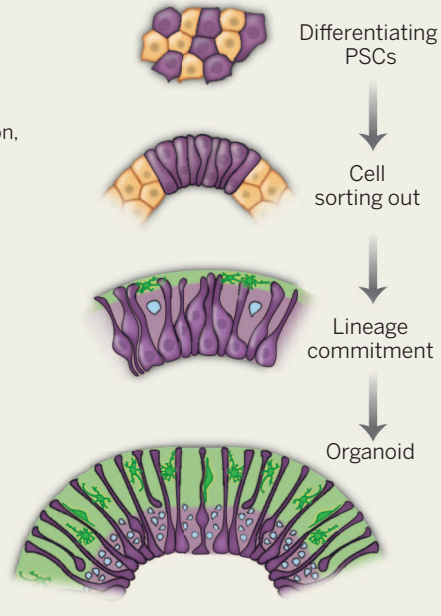
Box 1. Defining organoids.

Organoid *n.* Resembling an organ.

This implies:
1. Multiple organ-specific cell types
2. Capable of recapitulating some specific function of the organ (eg. excretion, filtration, neural activity, contraction)
3. Grouped together and spatially organized similar to an organ

Organoid formation recapitulates both major processes of self-organization during development: cell sorting out and spatially restricted lineage commitment

Definition:
A collection of organ-specific cell types that develops from stem cells or organ progenitors and self-organizes through cell sorting and spatially restricted lineage commitment in a manner similar to *in vivo*.



[1]IMBA—Institute of Molecular Biotechnology of the Austrian Academy of Science Vienna 1030, Austria.
*Corresponding author. E-mail: juergen.knoblich@imba.oeaw.ac.at

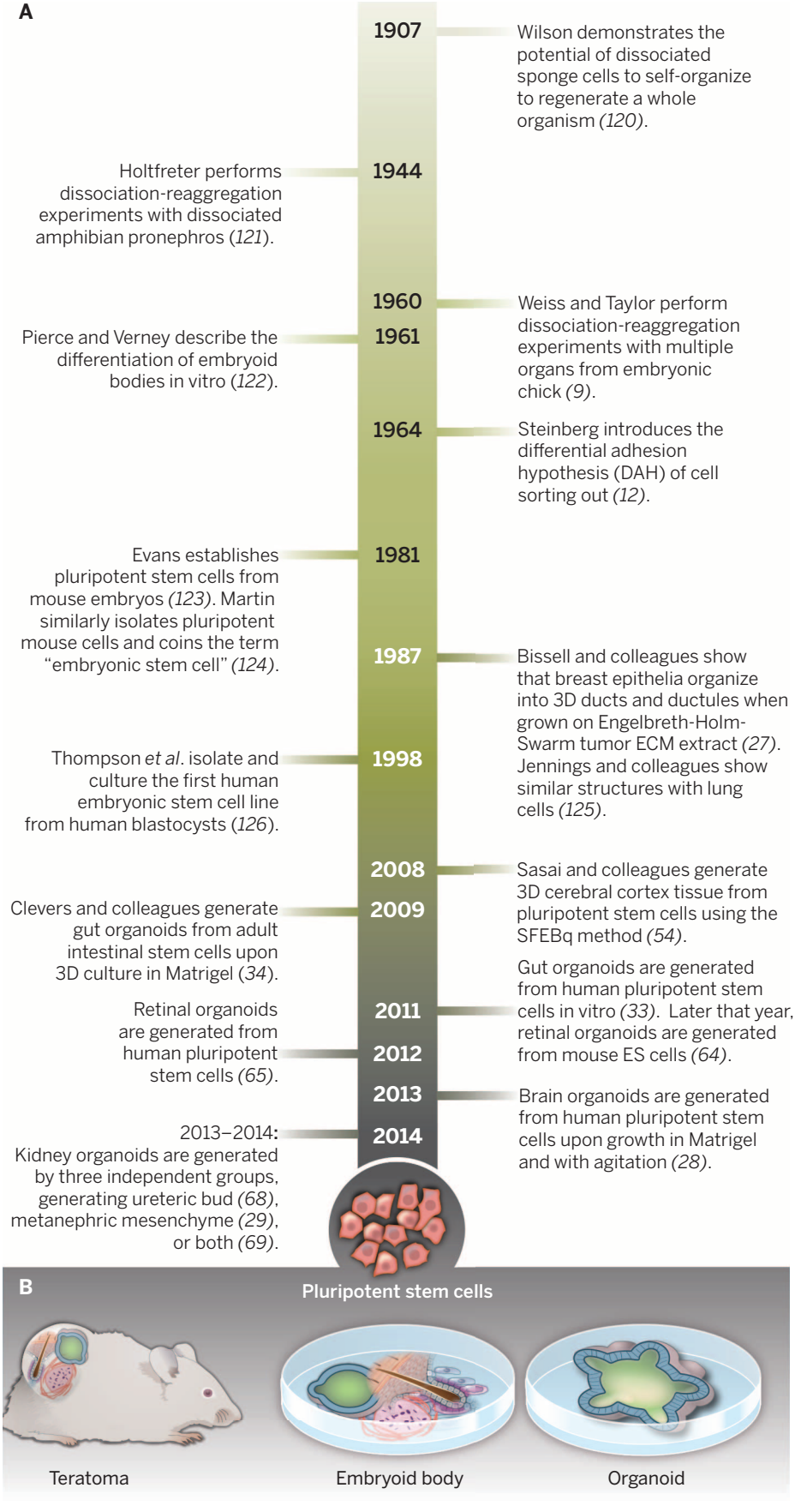


Fig. 1. History of organoid methodologies. (**A**) Key events in the history leading up to various organoid methodologies. (**B**) Comparison of paradigms of self-organization from pluripotent stem cells. Teratomas develop various tissues in vivo, either as spontaneous tumors that can arise in animals and humans or from injected PSCs in a rodent host. Embryoid bodies are 3D aggregates of stem cells that self-organize to develop tissues, similar to teratomas in many ways, but formed in vitro. Organoids are similarly 3D in vitro–derived tissues but are driven using specific conditions to generate individual, isolated tissues.

This stratification depends upon proper stem cell division orientation, the interplay of symmetric and asymmetric divisions, and migration of differentiated daughter cells to defined locations within the tissue (*16*, *17*). Remarkably, this organization can also be recapitulated upon in vitro dissociation and reaggregation (*18*) but only when retinal precursor cells are taken from a chick younger than embryonic day 6 (E6) (*19*, *20*). This suggests that retinal layering depends not only on cell sorting out but also proper execution of lineage decisions by retinal progenitors.

The combination of both sorting out and fate specification in governing self-organization is particularly evident in tumors called teratomas. Teratomas develop from pluripotent stem cells (PSCs) of the germ line and therefore display a variety of organized tissues (Fig. 1B). These include epidermis, nervous tissue, gut, and bone, as well as eyes (*21*) and limbs (*22*). The spontaneous development of these tissues from PSCs is presumably because of a recapitulation of both cell segregation and fate specification. Similarly, these two processes allow for the self-organization seen in organoids (Box 1).

In many ways, organoids represent the methodological evolution of an in vitro system called an embryoid body (EB) that is similar to an early teratoma (Fig. 1B). EBs are 3D aggregates of PSCs (*23*) that undergo initial developmental specification in much the same manner as the pregastrulating embryo (*24*). EBs can further differentiate to form various organized tissues, much like a teratoma. However, their growth in vitro allows for the application of patterning factors to drive particular identities. Not all organoid methods make use of an initial EB stage, but they all involve exogenous tissue patterning and eventually reaggregation to form a 3D self-organized tissue, an organoid.

Self-organization is possible in organoids because of a growing movement away from two-dimensional culture. This movement was triggered by the discovery that epithelial cells, such as kidney (*25*) or breast epithelia (*26*), could develop tubules and ducts when embedded in extracellular matrix hydrogels. Similarly, organoids often make use of such gels, particularly the laminin-rich extracellular matrix secreted by the Engelbreth-Holm-Swarm tumor line (*27*), also called Matrigel.

The resulting self-organizing structures exhibit typical tissue architecture, but note that they are highly heterogeneous. Thus, each organoid

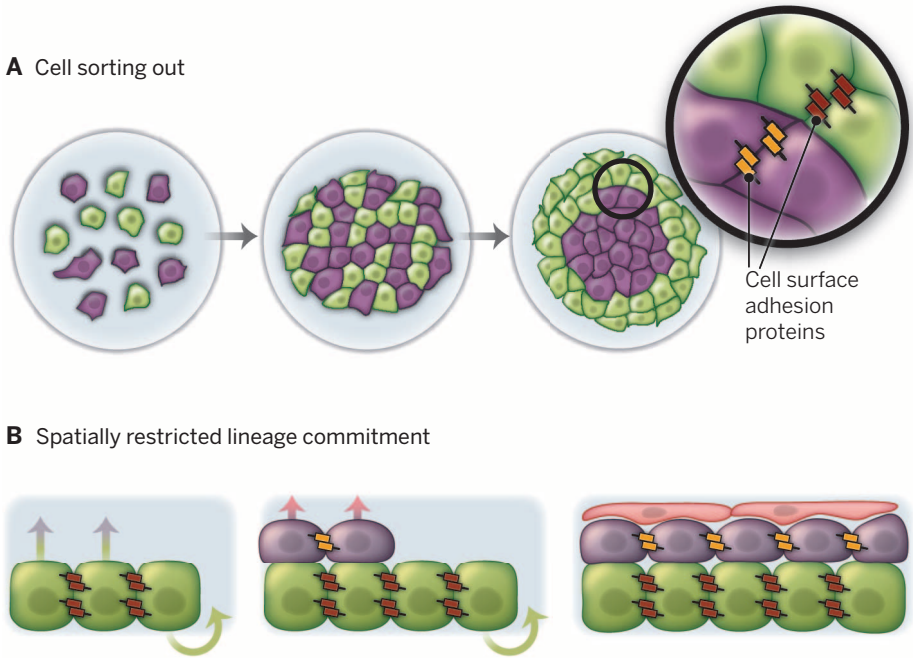


Fig. 2. Principles of self-organization. (**A**) Cell sorting out describes the movement of cells into different domains. Different cell types (purple or green) sort themselves because of different adhesive properties conferred by their differential expression of distinct cell adhesion molecules (shown as brown or orange bars). (**B**) Spatially restricted cell-fate decisions also contribute to self-organization in vivo and in organoids. Progenitors (green) give rise to more differentiated progeny (purple), which, because of spatial constraints of the tissue and/or division orientation, are forced into a more superficial position that promotes their differentiation. These cells can sometimes further divide to give rise to more differentiated progeny (pink), which are further displaced.

is unique and exhibits relative positioning of tissue regions that are often random, possibly because of a lack of embryonic axis formation. For example, brain organoids display various brain regions that individually develop quite similarly to those in vivo (*28*) but are not reliably organized relative to one another because of a lack of anterior-posterior and dorsal-ventral axes. Similarly, kidney organoids develop tubules corresponding to regions of the nephron segment (*29*), but they are randomly positioned rather than displaying medullary and cortical organization. This heterogeneity makes it difficult to generate pure populations of single cell types, but it can be a powerful tool for modeling development and disease on a whole-organ scale.

Current organoid technologies

Organoids derived from human PSCs have so far been established for gut, kidney, brain, and retina, among others (Fig. 3). Many of the organs studied had already been demonstrated to self-organize in reaggregation experiments from embryonic tissues (Table 1), which suggests that organoids could, in principle, be generated if organ progenitors could be derived from PSCs. Below, we will describe the evolution of each of these organoid approaches from this developmental foundation.

Gut organoids

The gastrointestinal (GI) tract develops primarily from the endoderm (*6*), which forms an epithelial tube that develops into three distinct portions, the foregut, midgut, and hindgut (*30*). The foregut gives rise to the oral cavity, the pharynx, the respiratory tract, the stomach, the pancreas, and the liver. The midgut gives rise to the small intestine and the ascending colon. The hindgut gives rise to the remaining portion of the colon, or large intestine, and the rectum. The separation of these three domains involves the combinatorial response to growth factors that have anteriorizing or posteriorizing effects. In particular, Wnt and fibroblast growth factor (FGF) signaling have been shown to inhibit anterior gut fate and instead promote posterior fate, which can lead to midgut and hindgut identities (*31*, *32*).

This knowledge of the posteriorizing effects of Wnts and FGFs provides the foundation on which human intestinal organoids are built (*33*) (Fig. 3). Human PSCs can be driven toward a hindgut identity by initially applying activin A, a nodal-related molecule, to drive mesendodermal identity. The subsequent addition of posteriorizing Wnt3a and Fgf4 then specifies the hindgut, the precursor to the intestine. Originally, this specification was performed in 2D, but surprisingly, the cells spontaneously formed hindgut tubes that budded off to form spheroids. This illustrates the remarkable self-organizing ability of these progenitors, a property that allows them to generate complete 3D organoids when grown in a permissive environment. The laboratory of Hans Clevers had previously shown that adult intestinal stem cells could form organoids when cultured in 3D in Matrigel (*34*). These adult-derived organoids self-organized to form 3D crypt-villus structures that mimicked the physiology and organization of the intestine and could even be transplanted into mice (*35*). Similarly, hindgut spheroids generated from human PSCs can be grown in Matrigel 3D growth conditions to further develop to mature intestinal organoids (*33*).

Intestinal organoids develop crypt-villus structures with stratified epithelium consisting of all the major cell lineages of the gut (*33*, *34*). These include columnar epithelial enterocytes with a brush border of apical microvilli. Furthermore, cell divisions occur at the base of the villus-like protrusions, and intestinal stem cells could be identified by their expression of Lgr5 in more advanced organoids. Finally, these organoids displayed intestinal functions including absorptive and secretory activity.

Although the intestine is the only gut region so far generated from PSCs, other regions of the digestive tract have been developed into organoids from adult stem cells. In particular, gastric organoids have been generated from adult pyloric stem cells (*36*) or chief cells of the stomach (*37*). Lingual organoids have been established from adult tongue epithelium (*38*). These approaches similarly use the 3D Matrigel environment, which suggests that Matrigel is a general requirement in GI tract organoid formation. Furthermore, the use of adult progenitor populations in this manner provides an additional, often more direct, route to the generation of organoids.

Liver organoids

The liver is primarily derived from endoderm, developing from an outgrowth of ventral foregut epithelium that develops into a hepatic bud structure (*39*). This hepatic bud produces the hepatoblasts that generate both hepatocytes and biliary epithelium, whereas adjacent mesoderm-derived mesenchyme contributes liver fibroblasts and stellate cells. The growth of the liver bud involves extensive vascularization and eventually it develops into the major fetal site of hematopoiesis. Thus, liver development represents a complex interplay of both endoderm- and mesoderm-derived tissues.

Early reaggregation studies had shown that dissociated chick embryonic hepatic tissue can reaggregate and organize into secretory units typical of the liver and consistent with formation of functional bile ducts (*9*). More recently, a progenitor population in adult mouse liver that is activated after injury was identified that could generate 3D liver organoids when grown in Matrigel (*40*). These adult-derived liver organoids display cells with biliary ductal identities and can be differentiated to form mature, functional hepatocytes. Finally, liver organoids can be transplanted into mice and were shown to partially rescue mortality in a mouse model of liver disease, pointing to their functionality.

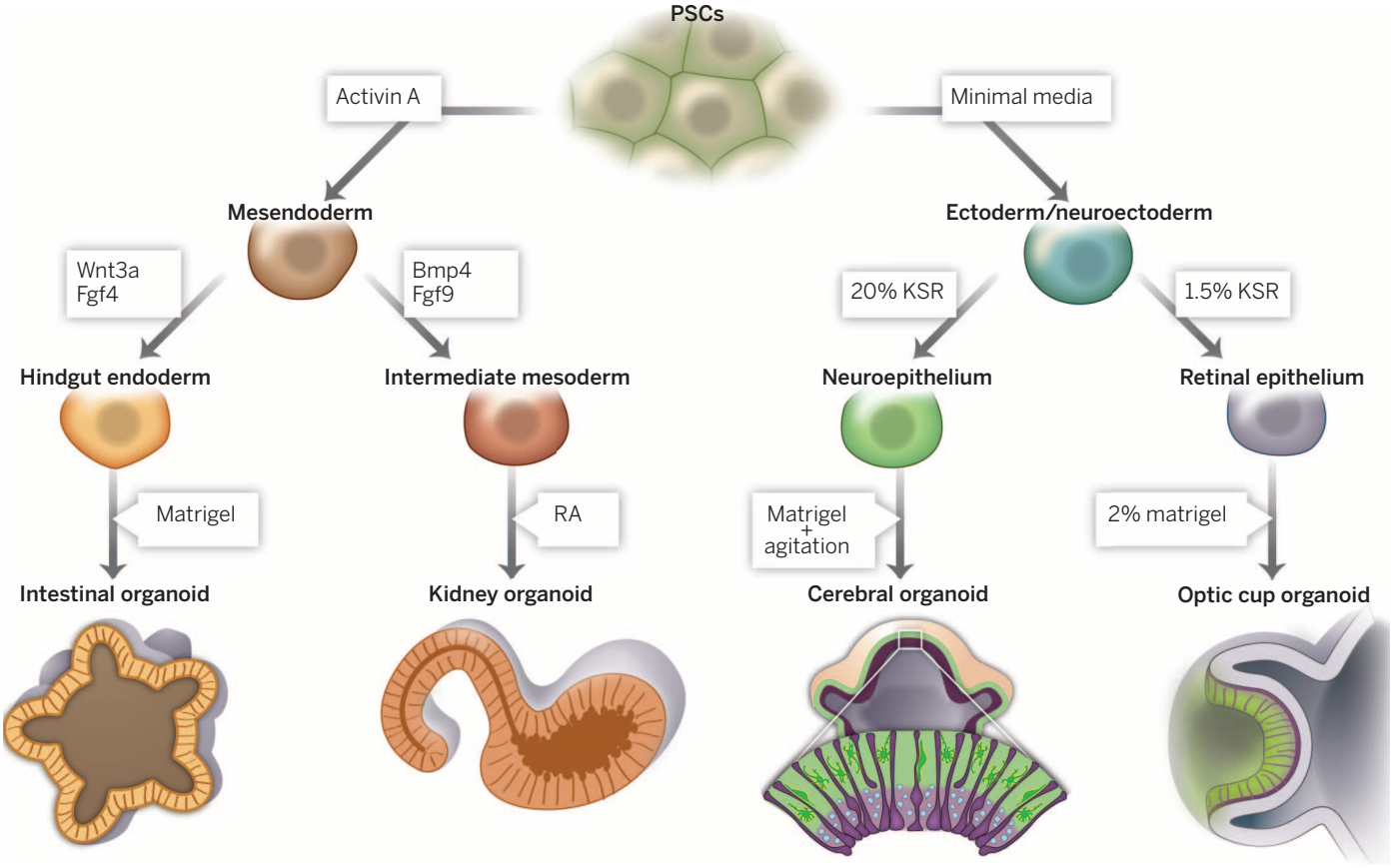


Fig. 3. Overview of organoid methodologies. Organoid differentiation strategies developed so far from human PSCs. Conditions and growth factors are indicated for the derivation of progenitor identities. For neuroectoderm, minimal medium without serum is used. KSR is knockout serum replacement, a serum-free growth-promoting alternative. Limiting its use, along with a low concentration of Matrigel dissolved in the medium, promotes retinal neuroepithelium, whereas higher KSR and embedding in pure Matrigel promotes the formation of various brain regions. Renal organoids have been generated several ways, but growth factors in common are shown.

Although similar human liver organoids have not yet been generated, a very different approach was recently established to generate tissues reminiscent of human liver buds (*41*). Beginning with differentiation of human PSCs into hepatic endodermal cells in 2D, this method mixes three cell populations: the human PSC–derived hepatic cells, human mesenchymal stem cells, and human endothelial cells. This mixed-cell population mimics the early cell lineages of the developing liver. When mixed to a high density on a layer of Matrigel, the cells spontaneously form a 3D aggregate. The liver bud–like aggregates display vascularization and can be ectopically transplanted into mice to allow blood supply. Perhaps most promising is the finding that mice with transplants of these liver bud tissues exhibit human-specific metabolites in the blood. Furthermore, survival of mice subjected to liver injury increased when liver buds were transplanted into them.

Brain organoids

The vertebrate central nervous system derives from the neural ectoderm (*6*). This tissue gives rise to the neural plate, which folds and fuses to form the neural tube, an epithelium with apical-basal polarity radially organized around a fluid-filled lumen that eventually forms the brain ventricles. Axes are established through the concerted action of morphogen gradients, such as the ventral-dorsal Shh-Wnt/Bmp axis, and the rostral-caudal axis influenced by factors such as retinoic acid and FGF (*42*). These axes allow the epithelial tube to subdivide into four major regions, the forebrain, midbrain, hindbrain, and spinal cord. The forebrain gives rise to the majority of the human brain, including the neocortex, hippocampus, and ventral telencephalic structures, such as amygdala and hypothalamus. The midbrain gives rise to the tectum, whereas the hindbrain gives rise to the cerebellum, pons, medulla, and brainstem.

Generally, neurons are generated from neural stem cells that reside next to ventricles (*43*). Neural stem cells initially expand through symmetric proliferative divisions. During neurogenesis, stem cells switch to asymmetric divisions to give rise to self-renewing progenitors and more differentiated cell types, including neurons and intermediate progenitors (*44*). These more differentiated cells migrate outward to generate stratified structures such as the three layers of the medulla, the seven layers of the optic tectum, and the six layers of the cerebral cortex.

Although the final product of neural development is a highly complex interconnected brain, earlier reaggregation studies suggest that this organ has an intrinsic self-organizing capacity (*45*). When taken at early stages of brain development, chick neural progenitors self-organized to form clusters of neuroepithelial cells organized in a radial manner surrounding a lumen, reminiscent of the neural tube. The implication of these classic experiments is that if neuroepithelium can be derived from PSCs, spontaneous self-organization is likely to occur.

Numerous previous studies have made use of in vitro–derived neural stem cells (NSCs) from PSCs to study neural differentiation (*46*). However, these homogeneous NSCs lack the characteristic apical-basal polarity and do not recapitulate the complex lineage of NSCs in vivo. As an alternative approach, neurospheres (*47*) are aggregates of NSCs that can be used to assess their self-renewing capacity. However, neurospheres are likewise not well organized and, therefore, are limited in their capacity to model many aspects of brain development.

More recently, 2D neural tube–like structures called neural rosettes were established from isolated neuroepithelium or the directed differentiation

Table 1. Current state of the art for in vitro self-organizing tissues of various organs. mESCs, mouse embryonic stem cells.

	Organ	Early reaggregation experiments	Identity derivation from PSCs	3D self-organizing structure or organoid
Endoderm	Thyroid	Embryonic chick thyroid (*90*), adult rat thyroid (*91*)	Thyroid progenitors from mESCs (*92*)	Functional thyroid organoid from mESCs (*70*)
	Lung	Embryonic chick lung (*93*)	Lung progenitors from mESCs and hiPSCs (*92*, *94*)	Bronchioalveolar structures from mouse adult lung stem cells (*71*)
	Pancreas	Mouse embryonic pancreas (*95*)	Pancreatic endocrine cells from mESC (*96*) and hESCs (*97*)	Pancreatic organoids from mouse embryonic pancreatic progenitors (*72*)
	Liver	Chick embryonic liver (*9*)	Hepatocytes from mESCs and hESCs (*98*)	Liver organoids from adult stem cells (*40*); liver buds from human iPSCs (*41*)
	Stomach	Chick embryonic gizzard and proventriculus (*99*)	None	Stomach organoids from adult stem cells (*36*, *37*)
	Intestine	Rat embryonic intestine (*100*)	Intestinal cells from mESCs (*101*) and hPSCs (*33*)	Intestinal organoids from human PSCs (*33*)
Mesoderm	Heart	Chick (*102*) and rat (*103*) cardiac tissue	Spontaneous and directed differentiation of mESCs and hESCs (*104*)	Vascularized cardiac patch from hESCs (*105*)
	Skeletal muscle	Embryonic chick leg skeletal muscle (*76*)	Mesoangioblasts from human iPSCs (*106*)	Anchored contracting skeletal muscle in 3D matrix derived from myoblast progenitors (*107*)
	Bone	Skeletal bone of chick embryonic leg (*77*)	Osteoblasts from mESCs (*108*) and hESCs (*109*)	Bone spheroids from human osteogenic cells (*110*)
	Kidney	Chick embryonic kidney (*9*)	Intermediate mesoderm from mouse (*111*) and human (*112*) PSCs	Ureteric bud (*68*) and metanephric mesenchyme (*29*) renal organoids (*69*) from human and mouse PSCs
Ectoderm	Retina	Embryonic chick retina (*61*)	Retinal progenitors from mouse (*113*) and human PSCs (*114*)	Optic cup organoids from mouse (*64*) and human (*65*) PSCs
	Brain	Embryonic chick brain cells (*45*)	Neural rosettes from mouse and human PSCs (*48*, *49*)	Cerebral organoids from mouse and human PSCs (*28*, *55*)
	Pituitary	Chick anterior pituitary (*115*)	None	Adenohypophysis organoids from mouse PSCs (*73*)
	Mammary gland	Mammary gland from adult virgin mice (*75*)	None	3D breast epithelia embedded in Matrigel (*116*)
	Inner ear	Embryonic chick otocysts (*117*)	Inner ear hair cells from mESCs (*118*)	Inner ear organoids from mESCs (*74*)
	Skin	Embryonic chick skin and feather follicles (*9*)	Keratinocytes from mESCs (*119*)	Stratified epidermis from keratinocytes derived from mESCs (*119*)

of PSCs (*48*, *49*). Because neural rosettes recapitulate apical-basal polarity and exhibit spontaneous radial organization similar to that of the neural tube, they are more capable of recapitulating many aspects of brain development. These include the production of intermediate progenitor types, as well as the timed production of layer identities similar to those in vivo (*50*). However, because of the 2D nature of the method, it has many limitations in modeling the overall organization of the developing brain.

Therefore, alternative 3D culture methods with the potential to recapitulate brain tissue organization have been used extensively for investigations in the past several years. In particular, work from the lab of Yoshiki Sasai has focused on developing various isolated brain regions in 3D from mouse or human PSCs (*51*). Beginning with EB formation, particular brain region identities can be generated from neuroectoderm. Specifically, forebrain tissues are generated by plating mouse (*52*) or human (*53*) EBs in 2D and examining adherent cells. However, aggregates develop more complex structures when allowed to continue growing in 3D (*54*), eventually generating dorsal forebrain. This method has further been improved in a recent study (*55*) that also showed neuronal layering reminiscent of early cerebral cortical development.

Other regions can also be generated by mimicking endogenous patterning with growth factors. For example, Hedgehog signaling drives ventral forebrain tissue (*56*). In addition, cerebellar identities can be generated by treatment with either Bmp4 and Wnt3a to generate granule neurons (*57*) or Hedgehog inhibition to generate Purkinje neurons of the cerebellum (*58*). Conversely, minimizing exogenous bioactive factors, such as serum proteins, promotes hypothalamic identity (*59*). Thus, by stimulating neuroectoderm through an EB stage followed by the application of specific growth factors, organoids can be generated for a variety of individual brain regions.

More recently, heterogeneous neural organoids were established, termed cerebral organoids, that contain several different brain regions within individual organoids (*28*) (Fig. 3). The approach similarly begins with EBs, but growth factors are not added to drive particular brain region identities. Instead, the method is influenced by the intestinal organoid protocol, namely, by embedding the tissues in Matrigel. The extracellular matrix provided by the Matrigel promotes outgrowth of large buds of neuroepithelium, which then expand and develop into various brain regions. Cerebral organoids can reach sizes of up to a few millimeters when grown in a spinning bioreactor, which improves nutrient and oxygen exchange. This expansion allows the formation of a variety of brain regions, including retina, dorsal cortex, ventral forebrain,

midbrain-hindbrain boundary, choroid plexus, and hippocampus.

Retinal organoids

The retina is the light-receptive neural region of the eye and is derived from the neural ectoderm. Initially, the optic vesicle forms as an outgrowth of the diencephalon, the most posterior region of the forebrain (60). Like the rest of the CNS, this optic vesicle begins as a pseudostratified neuroepithelium with a fluid-filled lumen. However, concerted movements at two hinge regions force the vesicle to fold in on itself, forming the optic cup. Thus, early in retinal development, two adjacent epithelial layers are established: the outer retinal pigmented epithelium and the inner neural retina. This trend of stratification continues and eventually leads to a fully laminated tissue containing layers of photoreceptors and supportive cell types, such as bipolar cells and amacrine cells.

The retina has a long history of in vitro reaggregation studies and has been used as a model of retinal layer formation for decades (18). The first reaggregates were generated from chick retina in the early 1960s and demonstrated the robustness of retinal self-organization in vitro (61, 62). Subsequent studies have used retinal reaggregates to examine the relations between different cell types and their differentiation and organization (63).

The evolution to PSC-derived retinal organoids, like other organoid approaches, is built upon a foundation rooted in developmental biology (Fig. 3). EBs are derived in minimal medium to generate neuroectoderm (64). A nominal amount of Matrigel is dissolved in the medium at an early stage to allow the formation of more rigid neuroepithelial tissues, a prerequisite of retinal pigmented epithelium formation. This promotes the formation of buds of retinal primordial tissue similar to the optic vesicle. These buds are then cut away from the rest of the neuroepithelial tissues and maintained in a medium that supports retinal tissue identity.

The resulting optic cup organoids very closely mimic early retina. They display proper markers of neural retina and retinal pigmented epithelium, they display retinal stratification with proper apical-basal polarity, and they undergo morphological tissue shape changes that mimic the stepwise evagination and invagination of the optic cup in vivo.

More recently, optic cup organoids were generated from human PSCs (65). These human retinal organoids show many of the characteristics common to mouse retina; however, they display a number of human specific features as well. In particular, the human retinal organoids are larger than mouse organoids, they require more time to develop, and they display certain tissue morphological differences, such as apical nuclear positioning.

Kidney organoids

The kidney arises from an early embryonic tissue called the intermediate mesoderm, a subdivision of mesodermal identity that develops from the primitive streak (66). In vivo, the primitive streak displays opposing gradients of Bmp4 and activin A, which combinatorially specify the endoderm or mesoderm. The intermediate mesoderm is further subspecified through the action of Fgf and Wnt signaling. This tissue then develops into two closely interacting domains, the ureteric bud and the metanephric mesenchyme, which promote each other's growth and branching to develop early renal tubules.

Like many of the tissues for which organoids have so far been developed, evidence that kidney tissue might be capable of self-organization comes from early reaggregation experiments of chick embryonic kidney (9). The resultant tissues displayed various segments of the nephron, including collecting duct, distal and proximal tubules, and glomeruli formed by the interaction with allantoic vessels upon transplantation on the chick allantoic membrane. Furthermore, the tissues could develop the stereotypic organization of the kidney with cortical and medullary region. These experiments suggest that, if kidney progenitors can be made from PSCs, these would, in principle, be capable of forming organized tissues if grown in a permissive environment. This principle is what has now been demonstrated by three independent studies (67) (Fig. 3).

Each of the recently published methods uses various combinations of growth factors to mimic endogenous signaling to drive renal differentiation. Specifically, ureteric bud identity can be generated by exposing human induced PSCs in 2D to Bmp4 and Fgf2 to drive mesodermal identities (68), followed by subsequent application of retinoic acid, Bmp2, and activin A. Such ureteric bud cells can be cocultured with dissociated mouse embryonic kidney to self-organize within the mouse aggregate and populate 3D ureteric bud structures.

The second major renal precursor tissue, the metanephric mesenchyme, can instead be generated beginning with an initial EB stage from mouse and human PSCs (29). Sequential application of activin A followed by Bmp4 and the Wnt agonist CHIR99021 then induces posterior mesoderm, the precursor to the intermediate mesoderm. Finally, application of retinoic acid followed by Fgf9 then stimulates the tissues to take on a metanephric mesenchyme identity. By coculturing with spinal cord tissue, a known nephric inducer, this tissue can produce well-organized nephric tubules and even nascent glomeruli.

Finally, both principal lineages of the kidney can be generated together (69) by applying activin A and Bmp4 to human embryonic stem cells (hESCs) grown in 2D to generate primitive streak identity. These cells transition to an intermediate mesoderm identity upon exposure to Fgf9 and spontaneously develop further into ureteric bud and metanephric mesenchyme in the absence of further growth factors. Although these specification events were initially performed in 2D, the cells take on 3D morphologies by either growing at low density to allow dome-like colonies to form or when cocultured with mouse kidney reaggregates. In both cases, more complex tissues arise in 3D with structures resembling ureteric epithelium and proximal tubules.

Organoids from model organisms

Despite the relatively few human-derived organoids so far described, several others have already been established from mouse PSCs or adult tissue stem cells. These include the endoderm-derived thyroid, lung, and pancreas. Thyroid organoids can be produced by overexpression of two factors important for thyroid specification, Nkx2.1 and Pax8, followed by treatment of EBs with thyroid-stimulating hormone (70). Lung organoids can develop from cocultured adult bronchioalveolar stem cells and lung endothelial cells (71) in Matrigel. And finally, pancreatic organoids can develop from simply plating pancreatic progenitor cells in Matrigel (72). All three systems give rise to self-organized characteristic epithelia and, in the case of thyroid organoids, even synthesis and secretion of functional thyroid hormone.

Organoids have also been derived from ectoderm-derived pituitary and inner ear. Specifically, the two identities of the developing pituitary, the neural ectodermal infundibulum and the adenohypophysis, could be generated in large EBs grown under ectoderm-promoting conditions (73). Remarkably, these pituitary organoids can mature and synthesize pituitary hormones, such as growth hormone, follicle-stimulating hormone, and thyroid-stimulating hormone. Additionally, sensory epithelia of the inner ear can be generated from EBs grown under ectoderm-promoting conditions with subsequent treatment with Bmp4 followed by Fgf2 to drive otic identity (74). The resulting otic vesicles generate functional inner ear sensory epithelia with stereocilia and kinocilia.

The future of organoid technologies

The generation of 3D organoids from human PSCs is currently in its infancy, but the field is rapidly evolving. In the near future, human organoids may be generated for those organs that have already been established in mouse or where a principle of self-organization has already been demonstrated in reaggregation studies. These include skin (9), mammary gland (75), muscle (76), and bone (77), to name a few.

Paradigms of organ development

Because organoids represent an easily accessible model system, they have the potential to open doors to developmental questions that have been difficult or impossible to answer using traditional techniques. This is particularly true for biological principles that are specific to humans. For example, human brain organoids have already been used to examine the unique division mode of human neural stem cells (28). Similarly, retinal organoids have been used to test differences between human and rodent tissue morphogenesis and timing (65). Additionally, organoids for the GI tract can be applied to the study of coordinated development of GI organs, a process

that exhibits important differences in humans compared with laboratory animals (78).

Furthermore, organoids hold the potential to model adult homeostasis as well. Indeed, intestinal organoids have already been used to examine the role of the crypt niche in stem cell self-renewal and differentiation (79, 80). This is particularly true for organoids that have been derived from adult progenitors, such as liver and stomach, that closely recapitulate regenerative events seen in the adult organ.

Therapeutic potential

Disease modeling will likely be a primary focus of future organoid studies (Fig. 4). These can range from developmental disorders, cancer, infectious disease, and degeneration. For example, gut organoids are already being used to examine infectious diseases (81, 82); tumor biology (83, 84); and genetic conditions (85, 86).

Along these lines, patient iPSCs will be a valuable tool in future disease modeling. Recently, kidney organoids were generated from iPSCs derived from a patient with polycystic kidney disease (68). Although the method did not test for a phenotype, this will likely represent an important tool in studying this and other genetic kidney disorders. Similarly, retinal organoids have the potential to model human genetic disorders that lead to blindness, such as retinitis pigmentosa. These types of disorders can be modeled by making use of patient iPSCs or, alternatively, through the introduction of patient mutations into human PSCs using modern genome-editing technologies.

Brain organoids, in particular, have huge potential in this respect. They could, in principle, be used to model various neurodevelopmental disorders that have been difficult or impossible to model in animals. Indeed, brain organoids were the first organoids to make use of patient iPSCs in this manner and to model the developmental disorder microcephaly (28). In the future, cerebral organoids may even have the potential to model disorders such as autism, schizophrenia, or epilepsy, and perhaps even adult-onset disorders like neurodegenerative diseases.

Organoids also have the potential to be used for testing efficacy and toxicity of drug compounds (Fig. 4). This could be applied to organoids that model degenerative conditions—for example, liver fibrosis or cystic kidney diseases—where one could screen for effective treatments. If successful, this approach could even cut down on the use of animal testing, reserving it for studies requiring whole-organism readouts. For this, development of human liver organoids would be of particular relevance (Fig. 4), because the human liver often metabolizes drugs in a manner distinct from animals' metabolism. Drugs can be removed at early stages of screening when they could otherwise be functional in humans, or more drastically, toxic metabolites can be produced specifically in humans but not in tested animals. Methods to screen compounds in an in vitro human liver model are therefore being investigated as an alternative in the drug discovery process (87). Human liver buds have already been shown to produce human-specific metabolites (41), which suggests that liver organoids could represent an ideal system to perform these types of studies.

Finally, organoids have the potential to provide alternative approaches to cell or even whole-organ replacement strategies in the clinic (Fig. 4). Organoids could provide a source of autologous tissue for transplantation. In this respect, renal organoids hold enormous therapeutic potential as this is the organ with the highest rate of end-stage failure leading to the highest organ demand for transplants. Already, Taguchi *et al.* succeeded in transplanting kidney organoids under the renal capsule of adult mice, which led to vascularization, a promising step toward a replacement strategy (29).

Additionally, retinal organoids could be used to treat certain types of retinal degeneration and blindness. Indeed, stem cell–therapy clinical trials are already under way to replace certain degenerating retinal cell types (88). Retinal organoids could provide an alternative approach that may better recapitulate development and, therefore, produce particular cell types of interest for transplantation. Finally, intestinal organoids could provide a treatment option for replacement of damaged colon after injury or following removal of diseased tissue. Remarkably, intestinal organoid transplantation has already been performed in mice and can contribute to colon repair after injury (35, 89). Organoid approaches could even allow for gene correction in the case of genetic defects, using modern genome-editing technologies to replace damaged organ with repaired tissue.

Although it is clear that there are many potential uses for organoids, it is important to keep in mind their current limitations. In particular, all of the organoid systems so far established remain to be thoroughly characterized with regard to the extent of recapitulation of in vivo development. For example, although retinal organoids nicely display typical laminar organization, outer segments fail to form; for example, photoreceptors fail to fully mature to become light-sensitive. Likewise, cerebral organoids recapitulate fairly early events in brain development, but later features, such as cortical plate layers, fail to fully form.

The issue of maturation seems to be a common hurdle in organoid technologies, and it remains to be seen whether this will significantly affect their therapeutic and research potential.

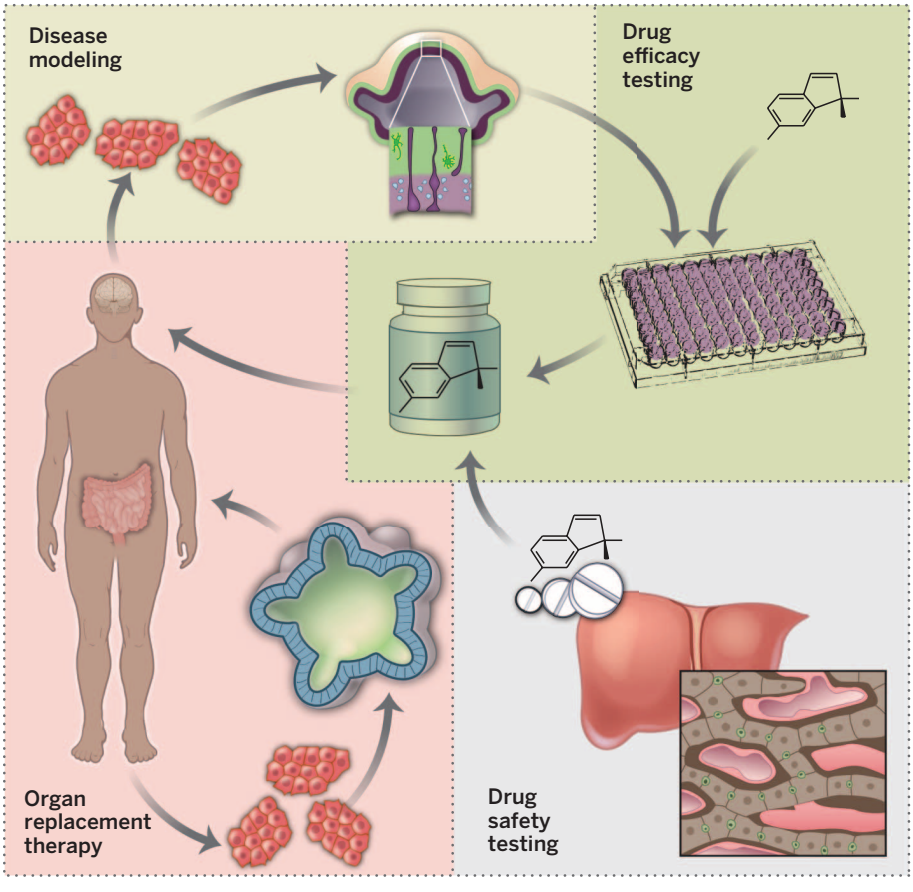


Fig. 4. Therapeutic potential of organoids. Organoids can be used to model diseases (beige box), for example modeling neurodevelopmental disorders with cerebral organoids. These types of disease models can then be used for testing drug efficacy in vitro before moving to animal models (green box). Drug compounds can be tested for toxicity and metabolic profile in liver organoids (gray box). And finally, organoids could be made from patient cells to provide autologous transplant solutions (pink box).

Human intestinal organoids have been shown to display characteristics of mature intestine, producing Lgr5$^+$ adult stem cells (*33*). Other organoids could perhaps be coaxed to fully mature once transplanted, either ectopically or for therapeutic purposes. These studies will likely be a primary focus of future organoid research.

Finally, the lack of vascularization is generally an issue with organoids in vitro. Because of limitations in nutrient supply, organoids have a limited growth potential, which can also affect their maturation. Vascularization is an issue in tissue engineering as a whole, and various approaches have been taken to address it. In the case of organoids, spinning bioreactors can provide better nutrient exchange allowing for sizes of up to a few millimeters (*28*). Alternatively, coculture with endothelial cells can generate vascular-like networks (*41*). Perhaps the most promising solution, however, is the transplantation of these tissues, as has been done for liver buds and kidney organoids, which stimulates invasion from host vasculature (*29*).

Overall, organoids have enormous potential to model development and disease, as a tool for drug testing, and as a therapeutic approach. Future efforts will no doubt bring them closer to reaching that potential.

REFERENCES AND NOTES

1. R. Lanza, J. Gearhart, B. Hogan, D. Melton, R. Pedersen, Eds., *Essentials of Stem Cell Biology* (Elsevier, San Diego, CA, ed. 2, 2009).
2. Y. Hachitanda, M. Tsuneyoshi, Neuroblastoma with a distinct organoid pattern: A clinicopathologic, immunohistochemical, and ultrastructural study. *Hum. Pathol.* **25**, 67–72 (1994). doi: 10.1016/0046-8177(94)90173-2
3. B. Zimmermann, Lung organoid culture. *Differentiation* **36**, 86–109 (1987). doi: 10.1111/j.1432-0436.1987.tb00183.x; pmid: 3446562
4. C. Xinaris *et al*., In vivo maturation of functional renal organoids formed from embryonic cell suspensions. *J. Am. Soc. Nephrol.* **23**, 1857–1868 (2012). doi: 10.1681/ASN.2012050505; pmid: 23085631
5. P. B. Armstrong, Cell sorting out: The self-assembly of tissues in vitro. *Crit. Rev. Biochem. Mol. Biol.* **24**, 119–149 (1989). doi: 10.3109/10409239809086396; pmid: 2651008
6. S. F. Gilbert, Ed., *Developmental Biology* (Sinauer Associates, Sunderland, MA, ed. 6, 2000).
7. C. Dahmann, A. C. Oates, M. Brand, Boundary formation and maintenance in tissue development. *Nat. Rev. Genet.* **12**, 43–55 (2011). doi: 10.1038/nrg2902; pmid: 21164524
8. C. Kiecker, A. Lumsden, Compartments and their boundaries in vertebrate brain development. *Nat. Rev. Neurosci.* **6**, 553–564 (2005). doi: 10.1038/nrn1702; pmid: 15959467
9. P. Weiss, A. C. Taylor, Reconstitution of complete organs from single-cell suspensions of chick embryos in advanced stages of differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **46**, 1177–1185 (1960). doi: 10.1073/pnas.46.9.1177; pmid: 16590731
10. A. Moscona, Rotation-mediated histogenetic aggregation of dissociated cells. A quantifiable approach to cell interactions in vitro. *Exp. Cell Res.* **22**, 455–475 (1961). doi: 10.1016/0014-4827(61)90122-7; pmid: 13773012
11. L. Wolpert, Ed., *Principles of Development* (Oxford University Press, Oxford, ed. 3, 2007).
12. M. S. Steinberg, in *Cellular Membranes in Development*, M. Locke, Ed. (Academic Press, New York, 1964), pp. 321–366.
13. R. A. Foty, C. M. Pfleger, G. Forgacs, M. S. Steinberg, Surface tensions of embryonic tissues predict their mutual envelopment behavior. *Development* **122**, 1611–1620 (1996).pmid: 8625847
14. T. Fujimori, S. Miyatani, M. Takeichi, Ectopic expression of N-cadherin perturbs histogenesis in *Xenopus* embryos. *Development* **110**, 97–104 (1990).pmid: 2081473
15. R. J. Detrick, D. Dickey, C. R. Kintner, The effects of N-cadherin misexpression on morphogenesis in *Xenopus* embryos. *Neuron* **4**, 493–506 (1990). doi: 10.1016/0896-6273(90)90108-R; pmid: 2322458
16. M. Agathocleous, W. A. Harris, From progenitors to differentiated cells in the vertebrate retina. *Annu. Rev. Cell Dev. Biol.* **25**, 45–69 (2009). doi: 10.1146/annurev.cellbio.042308.113259; pmid: 19575661
17. M. Žigman *et al*., Mammalian inscuteable regulates spindle orientation and cell fate in the developing retina. *Neuron* **48**, 539–545 (2005). doi: 10.1016/j.neuron.2005.09.030; pmid: 16301171
18. P. G. Layer, A. Robitzki, A. Rothermel, E. Willbold, Of layers and spheres: The reaggregate approach in tissue engineering. *Trends Neurosci.* **25**, 131–134 (2002). doi: 10.1016/S0166-2236(00)02036-1; pmid: 11852139
19. G. Vollmer, P. G. Layer, Reaggregation of chick retinal and mixtures of retinal and pigment epithelial cells: The degree of laminar organization is dependent on age. *Neurosci. Lett.* **63**, 91–95 (1986). doi: 10.1016/0304-3940(86)90019-4; pmid: 2419803
20. A. Rothermel, E. Willbold, W. J. Degrip, P. G. Layer, Pigmented epithelium induces complete retinal reconstitution from dispersed embryonic chick retinae in reaggregation culture. *Proc. Biol. Sci.* **264**, 1293–1302 (1997). doi: 10.1098/rspb.1997.0179; pmid: 9332014
21. C. Sergi, V. Ehemann, B. Beedgen, O. Linderkamp, H. F. Otto, Huge fetal sacrococcygeal teratoma with a completely formed eye and intratumoral DNA ploidy heterogeneity. *Pediatr. Dev. Pathol.* **2**, 50–57 (1999). doi: 10.1007/s100249900089; pmid: 9841706
22. N. Kuno *et al*., Mature ovarian cystic teratoma with a highly differentiated homunculus: A case report. *Birth Defects Res. A Clin. Mol. Teratol.* **70**, 40–46 (2004). doi: 10.1002/bdra.10133; pmid: 14745894
23. M. Evans, Discovering pluripotency: 30 years of mouse embryonic stem cells. *Nat. Rev. Mol. Cell Biol.* **12**, 680–686 (2011). doi: 10.1038/nrm3190; pmid: 21941277
24. G. Weitzer, Embryonic stem cell-derived embryoid bodies: An in vitro model of eutherian pregastrulation development and early gastrulation. *Handbook Exp. Pharmacol.* **2006**, 21–51 (2006).
25. R. Montesano, G. Schaller, L. Orci, Induction of epithelial tubular morphogenesis in vitro by fibroblast-derived soluble factors. *Cell* **66**, 697–711 (1991). doi: 10.1016/0092-8674(91)90115-F; pmid: 1878968
26. C. Hagios, A. Lochter, M. J. Bissell, Tissue architecture: The ultimate regulator of epithelial function? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **353**, 857–870 (1998). doi: 10.1098/rstb.1998.0250; pmid: 9684283
27. M. L. Li *et al*., Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 136–140 (1987). doi: 10.1073/pnas.84.1.136; pmid: 3467345
28. M. A. Lancaster *et al*., Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373–379 (2013). doi: 10.1038/nature12517; pmid: 23995685
29. A. Taguchi *et al*., Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells. *Cell Stem Cell* **14**, 53–67 (2014). doi: 10.1016/j.stem.2013.11.010; pmid: 24332837
30. J. K. Heath, Transcriptional networks and signaling pathways that govern vertebrate intestinal development. *Curr. Top. Dev. Biol.* **90**, 159–192 (2010). doi: 10.1016/S0070-2153(10)90004-5; pmid: 20691849
31. J. Dessimoz, R. Opoka, J. J. Kordich, A. Grapin-Botton, J. M. Wells, FGF signaling is necessary for establishing gut tube domains along the anterior-posterior axis in vivo. *Mech. Dev.* **123**, 42–55 (2006). doi: 10.1016/j.mod.2005.10.001; pmid: 16326079
32. V. A. McLin, S. A. Rankin, A. M. Zorn, Repression of Wnt/beta-catenin signaling in the anterior endoderm is essential for liver and pancreas development. *Development* **134**, 2207–2217 (2007). doi: 10.1242/dev.001230; pmid: 17507400
33. J. R. Spence *et al*., Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature* **470**, 105–109 (2011). doi: 10.1038/nature09691; pmid: 21151107
34. T. Sato *et al*., Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262–265 (2009). doi: 10.1038/nature07935; pmid: 19329995
35. S. Yui *et al*., Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5$^+$ stem cell. *Nat. Med.* **18**, 618–623 (2012). doi: 10.1038/nm.2695; pmid: 22406745
36. N. Barker *et al*., Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell* **6**, 25–36 (2010). doi: 10.1016/j.stem.2009.11.013; pmid: 20085740
37. D. E. Stange *et al*., Differentiated *Troy*+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. *Cell* **155**, 357–368 (2013). doi: 10.1016/j.cell.2013.09.008; pmid: 24120136
38. H. Hisha *et al*., Establishment of a novel lingual organoid culture system: Generation of organoids having mature keratinized epithelium from adult epithelial stem cells. *Sci Rep* **3**, 3224 (2013). doi: 10.1038/srep03224; pmid: 24232854
39. K. S. Zaret, Regulatory phases of early liver development: Paradigms of organogenesis. *Nat. Rev. Genet.* **3**, 499–512 (2002). doi: 10.1038/nrg837; pmid: 12094228
40. M. Huch *et al*., In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature* **494**, 247–250 (2013). doi: 10.1038/nature11826; pmid: 23354049
41. T. Takebe *et al*., Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* **499**, 481–484 (2013). doi: 10.1038/nature12271; pmid: 23823721
42. T. J. Petros, J. A. Tyson, S. A. Anderson, Pluripotent stem cells for the study of CNS development. *Front Mol Neurosci* **4**, 30 (2011). doi: 10.3389/fnmol.2011.00030; pmid: 22016722
43. M. K. Lehtinen, C. A. Walsh, Neurogenesis at the brain-cerebrospinal fluid interface. *Annu. Rev. Cell Dev. Biol.* **27**, 653–679 (2011). doi: 10.1146/annurev-cellbio-092910-154026; pmid: 21801012
44. M. A. Lancaster, J. A. Knoblich, Spindle orientation in mammalian cerebral cortical development. *Curr. Opin. Neurobiol.* **22**, 737–746 (2012). doi: 10.1016/j.conb.2012.04.003; pmid: 22554882
45. K. Ishii, Reconstruction of dissociated chick brain cells in rotation-mediated culture. *Cytologia (Tokyo)* **31**, 89–98 (1966). doi: 10.1508/cytologia.31.89; pmid: 5959228
46. L. Conti, E. Cattaneo, Neural stem cell systems: Physiological players or in vitro entities? *Nat. Rev. Neurosci.* **11**, 176–187 (2010). doi: 10.1038/nrn2938; pmid: 20107441
47. B. A. Reynolds, S. Weiss, Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255**, 1707–1710 (1992). doi: 10.1126/science.1553558; pmid: 1553558
48. Y. Elkabetz *et al*., Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. *Genes Dev.* **22**, 152–165 (2008). doi: 10.1101/gad.1616208; pmid: 18198334
49. S. C. Zhang, M. Wernig, I. D. Duncan, O. Brüstle, J. A. Thomson, In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat. Biotechnol.* **19**, 1129–1133 (2001). doi: 10.1038/nbt1201-1129; pmid: 11731781
50. N. Gaspard *et al*., An intrinsic mechanism of corticogenesis from embryonic stem cells. *Nature* **455**, 351–357 (2008). doi: 10.1038/nature07287; pmid: 18716623
51. D. Cyranoski, Tissue engineering: The brainmaker. *Nature* **488**, 444–446 (2012).doi: 10.1038/488444a; pmid: 22914148
52. K. Watanabe *et al*., Directed differentiation of telencephalic precursors from embryonic stem cells. *Nat. Neurosci.* **8**, 288–296 (2005). doi: 10.1038/nn1402; pmid: 15696161
53. K. Watanabe *et al*., A ROCK inhibitor permits survival of dissociated human embryonic stem cells. *Nat. Biotechnol.* **25**, 681–686 (2007). doi: 10.1038/nbt1310; pmid: 17529971
54. M. Eiraku *et al*., Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* **3**, 519–532 (2008). doi: 10.1016/j.stem.2008.09.002; pmid: 18983967
55. T. Kadoshima *et al*., Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 20284–20289 (2013). doi: 10.1073/pnas.1315710110; pmid: 24277810
56. T. Danjo *et al*., Subregional specification of embryonic stem cell-derived ventral telencephalic tissues by timed and combinatory treatment with extrinsic signals. *J. Neurosci.* **31**, 1919–1933 (2011). doi: 10.1523/JNEUROSCI.5128-10.2011; pmid: 21289201
57. H.-L. Su *et al*., Generation of cerebellar neuron precursors from embryonic stem cells. *Dev. Biol.* **290**, 287–296 (2006). doi: 10.1016/j.ydbio.2005.11.010; pmid: 16406324
58. K. Muguruma *et al*., Ontogeny-recapitulating generation and tissue integration of ES cell-derived Purkinje cells. *Nat. Neurosci.* **13**, 1171–1180 (2010). doi: 10.1038/nn.2638; pmid: 20835252
59. T. Wataya *et al*., Minimization of exogenous signals in ES cell culture induces rostral hypothalamic differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 11796–11801 (2008). doi: 10.1073/pnas.0803078105; pmid: 18697938

60. W. Heavner, L. Pevny, Eye development and retinogenesis. *Cold Spring Harb. Perspect. Biol.* **4**, a008391 (2012). doi: 10.1101/cshperspect.a008391; pmid: 23071378
61. A. Stefanelli, A. M. Zacchei, V. Ceccherini, Retinal reconstitution in vitro after disaggregation of embryonic chicken eyes. *Acta Embryol. Morphol. Exp.* **4**, 47–55 (1961).
62. A. A. Moscona, Analysis of cell recombinations in experimental synthesis of tissues in vitro. *J. Cell. Comp. Physiol.* **60** (suppl. S1), 65–80 (1962). doi: 10.1002/jcp.1030600407
63. S. Nakagawa, S. Takada, R. Takada, M. Takeichi, Identification of the laminar-inducing factor: Wnt-signal from the anterior rim induces correct laminar formation of the neural retina in vitro. *Dev. Biol.* **260**, 414–425 (2003). doi: 10.1016/S0012-1606(03)00320-8; pmid: 12921742
64. M. Eiraku *et al*., Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* **472**, 51–56 (2011). doi: 10.1038/nature09941; pmid: 21475194
65. T. Nakano *et al*., Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* **10**, 771–785 (2012). doi: 10.1016/j.stem.2012.05.009; pmid: 22704518
66. M. H. Little, A. P. McMahon, Mammalian kidney development: Principles, progress, and projections. *Cold Spring Harb. Perspect. Biol.* **4**, a008300 (2012). doi: 10.1101/cshperspect.a008300; pmid: 22550230
67. B. D. Humphreys, Kidney structures differentiated from stem cells. *Nat. Cell Biol.* **16**, 19–21 (2014). doi: 10.1038/ncb2904; pmid: 24366031
68. Y. Xia *et al*., Directed differentiation of human pluripotent cells to ureteric bud kidney progenitor-like cells. *Nat. Cell Biol.* **15**, 1507–1515 (2013). doi: 10.1038/ncb2872; pmid: 24240476
69. M. Takasato *et al*., Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. *Nat. Cell Biol.* **16**, 118–126 (2014). doi: 10.1038/ncb2894; pmid: 24335651
70. F. Antonica *et al*., Generation of functional thyroid from embryonic stem cells. *Nature* **491**, 66–71 (2012). doi: 10.1038/nature11525; pmid: 23051751
71. J.-H. Lee *et al*., Lung stem cell differentiation in mice directed by endothelial cells via a BMP4-NFATc1-thrombospondin-1 axis. *Cell* **156**, 440–455 (2014). doi: 10.1016/j.cell.2013.12.039; pmid: 24485453
72. C. Greggio *et al*., Artificial three-dimensional niches deconstruct pancreas development in vitro. *Development* **140**, 4452–4462 (2013). doi: 10.1242/dev.096628; pmid: 24130330
73. H. Suga *et al*., Self-formation of functional adenohypophysis in three-dimensional culture. *Nature* **480**, 57–62 (2011). doi: 10.1038/nature10637; pmid: 22080957
74. K. R. Koehler, A. M. Mikosz, A. I. Molosh, D. Patel, E. Hashino, Generation of inner ear sensory epithelia from pluripotent stem cells in 3D culture. *Nature* **500**, 217–221 (2013). doi: 10.1038/nature12298; pmid: 23842490
75. F. Prop, G. J. Wiepjes, in *Tissue Culture: Methods and Applications*, P. F. Kruse Jr. and M. K. Patterson Jr., Eds. (Academic Press, New York, 1973) pp. 21–24.
76. I. R. Konigsberg, Clonal analysis of myogenesis: Its relevance to the general problem of the stability of cell-type in cultured animal cells is discussed. *Science* **140**, 1273–1284 (1963). doi: 10.1126/science.140.3573.1273; pmid: 14034592
77. A. Moscona, H. Moscona, The dissociation and aggregation of cells from organ rudiments of the early chick embryo. *J. Anat.* **86**, 287–301 (1952).pmid: 12980879
78. E. Lévy, E. Delvin, D. Ménard, J.-F. Beaulieu, Functional development of human fetal gastrointestinal tract. *Methods Mol. Biol.* **550**, 205–224 (2009). doi: 10.1007/978-1-60327-009-0_13; pmid: 19495706
79. T. Sato *et al*., Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* **469**, 415–418 (2011). doi: 10.1038/nature09637; pmid: 21113151
80. A. Durand *et al*., Functional intestinal stem cells after Paneth cell ablation induced by the loss of transcription factor Math1 (Atoh1). *Proc. Natl. Acad. Sci. U.S.A.* **109**, 8965–8970 (2012). doi: 10.1073/pnas.1201652109; pmid: 22586121
81. S. R. Finkbeiner *et al*., Stem cell-derived human intestinal organoids as an infection model for rotaviruses. *MBio* **3**, e00159-12 (2012). doi: 10.1128/mBio.00159-12; pmid: 22761392
82. A. Castellanos-Gonzalez, M. M. Cabada, J. Nichols, G. Gomez, A. C. White Jr., Human primary intestinal epithelial cells as an improved in vitro model for *Cryptosporidium parvum* infection. *Infect. Immun.* **81**, 1996–2001 (2013). doi: 10.1128/IAI.01131-12; pmid: 23509153
83. T. M. Yeung, S. C. Gandhi, J. L. Wilding, R. Muschel, W. F. Bodmer, Cancer stem cells from colorectal cancer-derived cell lines. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3722–3727 (2010). doi: 10.1073/pnas.0915135107; pmid: 20133591
84. K. Onuma *et al*., Genetic reconstitution of tumorigenesis in primary intestinal cells. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 11127–11132 (2013). doi: 10.1073/pnas.1221926110; pmid: 23776211
85. A. E. Bigorgne *et al*., *TTC7A* mutations disrupt intestinal epithelial apicobasal polarity. *J. Clin. Invest.* **124**, 328–337 (2013). doi: 10.1172/JCI71471; pmid: 24292712
86. J. F. Dekkers *et al*., A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* **19**, 939–945 (2013). doi: 10.1038/nm.3201; pmid: 23727931
87. C. Luni, E. Serena, N. Elvassore, Human-on-chip for therapy development and fundamental science. *Curr. Opin. Biotechnol.* **25**, 45–50 (2014). doi: 10.1016/j.copbio.2013.08.015; pmid: 24484880
88. C. M. Ramsden *et al*., Stem cells in retinal regeneration: Past, present and future. *Development* **140**, 2576–2585 (2013). doi: 10.1242/dev.092270; pmid: 23715550
89. R. P. Fordham *et al*., Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury. *Cell Stem Cell* **13**, 734–744 (2013). doi: 10.1016/j.stem.2013.09.015; pmid: 24139758
90. S. R. Hilfer, L. B. Iszard, E. K. Hilfer, Follicle formation in the embryonic chick thyroid. II. Reorganization after dissociation. *Z. Zellforsch. Mikrosk. Anat.* **92**, 256–269 (1968). doi: 10.1007/BF00335651; pmid: 4907211
91. J. M. Mallette, A. Anthony, Growth in culture of trypsin dissociated thyroid cells from adult rats. *Exp. Cell Res.* **41**, 642–651 (1966). doi: 10.1016/S0014-4827(66)80115-5; pmid: 4952051
92. T. A. Longmire *et al*., Efficient derivation of purified lung and thyroid progenitors from embryonic stem cells. *Cell Stem Cell* **10**, 398–411 (2012). doi: 10.1016/j.stem.2012.01.019; pmid: 22482505
93. J. W. Grover, The enzymatic dissociation and reproducible reaggregation in vitro of 11-day embryonic chick lung. *Dev. Biol.* **3**, 555–568 (1961). doi: 0.1016/0012-1606(61)90032-X; pmid: 13902340
94. H. Mou *et al*., Generation of multipotent lung and airway progenitors from mouse ESCs and patient-specific cystic fibrosis iPSCs. *Cell Stem Cell* **10**, 385–397 (2012). doi: 10.1016/j.stem.2012.01.018; pmid: 22482504
95. M. R. Bernfield, P. E. Fell, Separation of the proliferating and differentiating cell populations of cultured embryonic pancreatic epithelium. *Proc. Natl. Acad. Sci. U.S.A.* **58**, 2227–2234 (1967). doi: 10.1073/pnas.58.6.2227; pmid: 5242199
96. N. Lumelsky *et al*., Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* **292**, 1389–1394 (2001). doi: 10.1126/science.1058866; pmid: 11326082
97. K. A. D'Amour *et al*., Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat. Biotechnol.* **24**, 1392–1401 (2006). doi: 10.1038/nbt1259; pmid: 17053790
98. H. Shirahashi *et al*., Differentiation of human and mouse embryonic stem cells along a hepatocyte lineage. *Cell Transplant.* **13**, 197–211 (2004). doi: 10.3727/000000004783984016; pmid: 15191158
99. K. Urase, S. Yasugi, Induction and inhibition of epithelial differentiation by the mixed cell aggregates of the mesenchymes from the chicken embryonic digestive tract. *Dev. Growth Differ.* **35**, 33–40 (1993). doi: 10.1111/j.1440-169X.1993.00033.x
100. R. K. Montgomery, H. M. Zinman, B. T. Smith, Organotypic differentiation of trypsin-dissociated fetal rat intestine. *Dev. Biol.* **100**, 181–189 (1983). doi: 10.1016/0012-1606(83)90209-9; pmid: 6617990
101. L. Cao *et al*., Intestinal lineage commitment of embryonic stem cells. *Differentiation* **81**, 1–10 (2011). doi: 10.1016/j.diff.2010.09.182; pmid: 20934799
102. D. A. Fischman, A. A. Moscona, in *Cardiac Hypertrophy*, N. K. Alpert, Ed. (Academic Press, New York, 1971), pp. 125–139.
103. S. P. Halbert, R. Bruderer, T. M. Lin, In vitro organization of dissociated rat cardiac cells into beating three-dimensional structures. *J. Exp. Med.* **133**, 677–695 (1971). doi: 10.1084/jem.133.4.677; pmid: 4926207
104. K. R. Boheler *et al*., Differentiation of pluripotent embryonic stem cells into cardiomyocytes. *Circ. Res.* **91**, 189–201 (2002). doi: 10.1161/01.RES.0000027865.61704.32; pmid: 12169644
105. K. R. Stevens *et al*., Physiological function and transplantation of scaffold-free and vascularized human cardiac muscle tissue. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 16568–16573 (2009). doi: 10.1073/pnas.0908381106; pmid: 19805339
106. F. S. Tedesco *et al*., Transplantation of genetically corrected human iPSC-derived progenitors in mice with limb-girdle muscular dystrophy. *Sci. Transl. Med.* **4**, 140ra89 (2012). doi: 10.1126/scitranslmed.3003541; pmid: 22745439
107. D. W. J. van der Schaft *et al*., Engineering skeletal muscle tissues from murine myoblast progenitor cells and application of electrical stimulation. *J. Vis. Exp.* **2013**, e4267 (2013).pmid: 23542531
108. L. D. Buttery *et al*., Differentiation of osteoblasts and in vitro bone formation from murine embryonic stem cells. *Tissue Eng.* **7**, 89–99 (2001). doi: 10.1089/107632700300003323; pmid: 11224927
109. R. C. Bielby, A. R. Boccaccini, J. M. Polak, L. D. K. Buttery, In vitro differentiation and in vivo mineralization of osteogenic cells derived from human embryonic stem cells. *Tissue Eng.* **10**, 1518–1525 (2004). doi: 10.1089/ten.2004.10.1518; pmid: 15588411
110. S. Kale *et al*., Three-dimensional cellular development is essential for ex vivo formation of human bone. *Nat. Biotechnol.* **18**, 954–958 (2000). doi: 10.1038/79439; pmid: 10973215
111. M. Nishikawa *et al*., Stepwise renal lineage differentiation of mouse embryonic stem cells tracing in vivo development. *Biochem. Biophys. Res. Commun.* **417**, 897–902 (2012). doi: 10.1016/j.bbrc.2011.12.071; pmid: 22209845
112. S. Mae *et al*., Monitoring and robust induction of nephrogenic intermediate mesoderm from human pluripotent stem cells. *Nat. Commun.* **4**, 1367 (2013). doi: 10.1038/ncomms2378; pmid: 23340407
113. H. Ikeda *et al*., Generation of Rx+/Pax6+ neural retinal precursors from embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 11331–11336 (2005). doi: 10.1073/pnas.0500010102; pmid: 16076961
114. D. A. Lamba, M. O. Karl, C. B. Ware, T. A. Reh, Efficient generation of retinal progenitor cells from human embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12769–12774 (2006). doi: 10.1073/pnas.0601990103; pmid: 16908856
115. H. Sobel, The behaviour in vitro of dissociated embryonic pituitary tissue. *J. Embryol. Exp. Morphol.* **6**, 518–526 (1958). pmid: 13575665
116. G. Y. Lee, P. A. Kenny, E. H. Lee, M. J. Bissell, Three-dimensional culture models of normal and malignant breast epithelial cells. *Nat. Methods* **4**, 359–365 (2007). doi: 10.1038/nmeth1015; pmid: 17396127
117. M. F. Orr, Histogenesis of sensory epithelium in reaggregates of dissociated embryonic chick otocysts. *Dev. Biol.* **17**, 39–54 (1968). doi: 10.1016/0012-1606(68)90088-2; pmid: 5642021
118. H. Li, G. Roblin, H. Liu, S. Heller, Generation of hair cells by stepwise differentiation of embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13495–13500 (2003). doi: 10.1073/pnas.2334503100; pmid: 14593207
119. C. Coraux *et al*., Reconstituted skin from murine embryonic stem cells. *Curr. Biol.* **13**, 849–853 (2003). doi: 10.1016/S0960-9822(03)00296-3; pmid: 12747834
120. H. V. Wilson, A new method by which sponges may be artificially reared. *Science* **25**, 912–915 (1907). doi: 10.1126/science.25.649.912; pmid: 17842577
121. J. Holtfreter, Experimental studies on the development of the pronephros. *Rev. Can. Biol.* **3**, 220–250 (1944).
122. G. B. Pierce Jr., E. L. Verney, An in vitro and in vivo study of differentiation in teratocarcinomas. *Cancer* **14**, 1017–1029 (1961). doi: 10.1002/1097-0142(196109/10)14:5<1017::AID-CNCR2820140516>3.0.CO;2-P; pmid: 13735537
123. M. Evans, Origin of mouse embryonal carcinoma cells and the possibility of their direct isolation into tissue culture. *J. Reprod. Fertil.* **62**, 625–631 (1981). doi: 10.1530/jrf.0.0620625; pmid: 7019433
124. G. R. Martin, Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7634–7638 (1981). doi: 10.1073/pnas.78.12.7634; pmid: 6950406
125. J. M. Shannon, R. J. Mason, S. D. Jennings, Functional differentiation of alveolar type II epithelial cells in vitro: Effects of cell shape, cell-matrix interactions and cell-cell interactions. *Biochim. Biophys. Acta* **931**, 143–156 (1987). doi: 10.1016/0167-4889(87)90200-X; pmid: 3663713
126. J. A. Thomson *et al*., Embryonic stem cell lines derived from human blastocysts. *Science* **282**, 1145–1147 (1998). doi: 10.1126/science.282.5391.1145; pmid: 9804556

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Organogenesis in a dish: Modeling development and disease using organoid technologies

Madeline A. Lancaster and Juergen A. Knoblich

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The making of bodies part by part

Mention of organoids—organlike structure growing in a Petri dish—might conjure up images of science fiction. However, the generation of organoids is very real, as is the increased understanding of organ form and function that comes from studying them. Lancaster and Knoblich review organoids as structures that include more than one cell type of an organ that exhibit structural and functional features of the natural counterpart. Knowledge of normal organ developmental pathways guides the formation of these structures. Organoids show great promise for modeling human development and disease and for biomedical research and regenerative medicine.

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