Organoids: the body builders

Stem-cell-derived organoids offer increasingly sophisticated models for studying human development and disease, as well as powerful emerging tools for precision medicine.

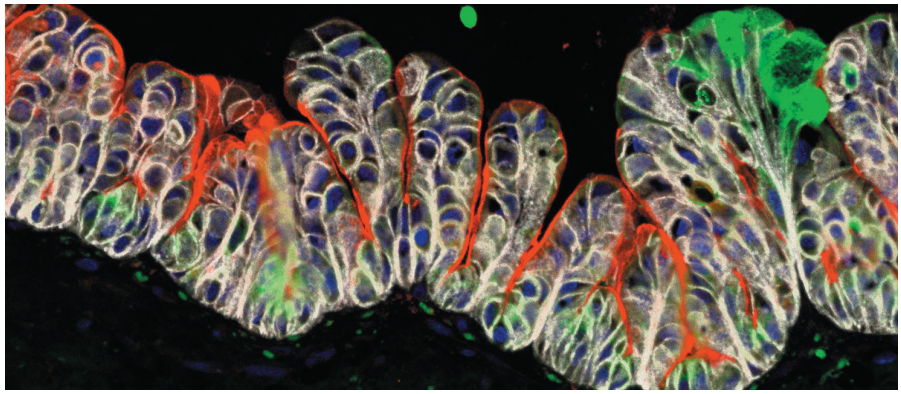
Inside every stem cell is an organ waiting to happen—biologists have known this for generations. But only recently have they learned how readily that potential can be unlocked in culture. With relatively minimal coaxing, researchers around the world are now converting stem cells into organized three-dimensional (3D) 'organoid' assemblies that mimic the structure and function of organs ranging from the colon to the brain. "The power that these cells have after hundreds of millions of years of evolution to build a higher-order structure is just incredible," says Hans Clevers, a leader in the field, based at the Hubrecht Institute in the Netherlands.

Many researchers stumbled upon these capabilities through a process they describe as 'chance' or 'serendipity'. For example, Madeline Lancaster's cerebral organoids were born from a happy accident while cultivating mouse neural stem cells as a postdoc in Jürgen Knoblich's lab at the Institute of Molecular Biology in Austria. "We had some leftover reagents for making the cells stick to the dish, but these had probably gone bad because the cells didn't stick," says Lancaster. "Instead, they formed these really interesting spherical 3D structures." When she transferred these spheres to a solid substrate, they formed simple cortical structures with virtually no further intervention.

Of course, these primitive assemblies are only starting points, and researchers in this field are working hard to make organoid production more robust, reproducible and representative of *in vivo* tissues. But the rewards have already been considerable, with early efforts clearly demonstrating the power of organoids as tools for developmental biology, disease research and regenerative medicine.

DIY development

While 3D cell culture is at least a century old, foundational work by pioneering researchers such as Mina Bissell more recently demonstrated that cells can assemble into 3D structures that reflect *in vivo* organization and behavior. Organoids as typically described today are constructed from adult or pluripotent stem cells, yielding models that can recapitulate development or tissue homeostasis and also reflect the genomic makeup of the donor.



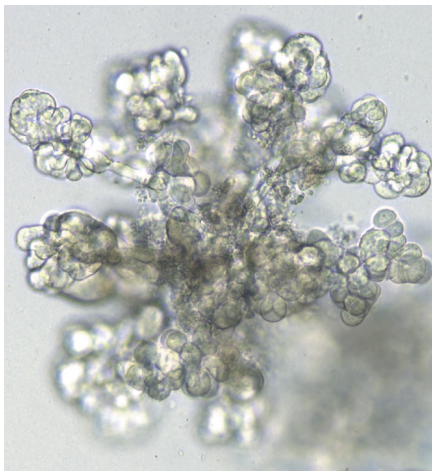
Jason Spence's lab is cultivating bacteria within iPS-cell-derived intestinal organoids to understand the physiological influence of the gut microbiome. Image courtesy of David R. Hill.

Much recent work on organoids has focused on relatively simple tissue structures, such as the digestive tract. In a seminal 2009 paper, the Clevers group coaxed individual adult stem cells into forming 3D structures that mirrored the crypt and villus structures of the small intestine[1]. Jason Spence's lab at the University of Michigan has likewise shown that they can generate organoids that replicate the structure and function of the duodenum and ileum from embryonic stem (ES) or induced pluripotent stem (iPS) cells. "They differentiate into goblet cells that make mucus and secrete that mucus into the lumen of the organoids," says Spence, "and make enterocytes that can absorb amino acids and small peptides."

Another important early demonstration came from the late developmental biologist Yoshiki Sasai, whose team showed that they could induce mouse ES cells to form an optic cup-like structure[2]. This primitive tissue, which ultimately gives rise to the retina, is relatively simple from a structural perspective but nevertheless possesses a specific cellular organization and composition, which these organoids recapitulated.

Organoid models can also form more highly patterned tissues, such as the branched airways of the lung and the ducts and tubules within the liver and pancreas. For example, by creating conditions that simulate tissue injury, Meritxell Huch's team at the University of Cambridge in the UK can reactivate latent regenerative programs in individual adult liver cells that promote organoid growth. "They form this network of bile ducts with a beautiful epithelial structure," says Huch. With additional coaxing, these cell assemblies can even spawn hepatocytes, which mediate the liver's metabolic and detoxifying functions.

Perhaps the most impressive demonstrations are those organoids replicating tissues in which many different cell types must be spatially organized in a specific manner. Melissa Little's team at Australia's University of Queensland is making headway in generating iPS-cell-derived nephrons—the



Bright-field image of a tumor organoid derived from a patient with liver cancer. Image courtesy of Laura Broutier.

sophisticated structures responsible for blood filtration within the kidney. "There are probably ten easily identifiable cell types in those organoids," she says. "We had nephrons that were patterning and elongating and segmenting, and podocytes in the glomerulus that were making primary and secondary foot processes and interdigitating with each other."

As a group leader at the Laboratory of Molecular Biology in Cambridge, Lancaster is developing ever more sophisticated and anatomically accurate human cerebral organoids, and many other groups are also making progress in reconstructing this pinnacle of biological complexity. "The advances have really been quite dramatic—one could almost say stunning, even if there is still a long way to go to truly mimic normal brain development," says Arnold Kriegstein, who works with cerebral organoids at the University of California at San Francisco. In one recent study, Paola Arlotta and colleagues at Harvard University showed that they could reproducibly generate organoids containing nearly a dozen different brain cell types, including rudimentary circuits incorporating retinal neurons that could be selectively activated by external stimulation with light[3].

Most organoids are formed by simply cultivating stem cells in a 3D bed of extracellular-matrix-derived proteins and exposing them to a handful of well-chosen signaling factors. For example, Clevers has found that many epithelial organoids—including prostate, breast and liver—can be generated by treating adult stem cells with signaling proteins belonging to the Wnt family in combination with one or two other biomolecules. Finding the right recipe requires a careful reading of the developmental biology literature. "We try to identify a minimal set of factors to initiate the process, and then the cells seem to know what to do through a genetically encoded program that takes over," says Spence. However, there is also considerable trial and error involved in getting the timing and dosages correct. "There's an endless number of possibilities," says Little. "You've got to ask when do I need to change what's in the media to get a particular outcome."

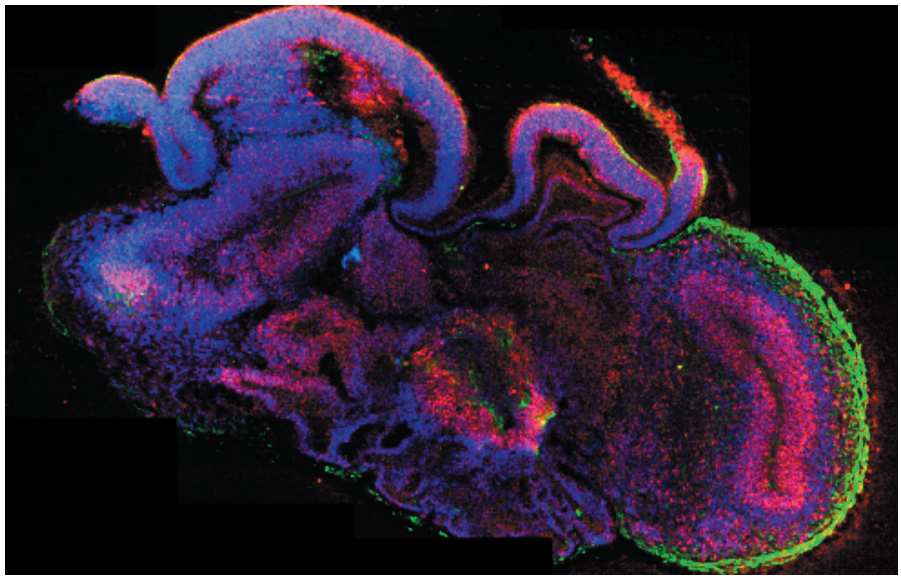
Once researchers find the magic formula, the process that unfolds can offer an unprecedented front-row view of organ development. "Animal models don't fully recapitulate what goes on in the normally developing human cerebral cortex," says Kriegstein. "We've been very interested in looking into what those differences are." In some cases, organoids have even revealed insights into organ composition that previously eluded *in vivo* discovery, as demonstrated in 2015 by Clevers and his colleague, Alexander van Oudenaarden[4]. "Alexander single-cell sequenced our mouse mini-guts, and detected two cell types that had never been seen before, and we then confirmed that they exist *in vivo*," says Clevers.

Deconstructing disease

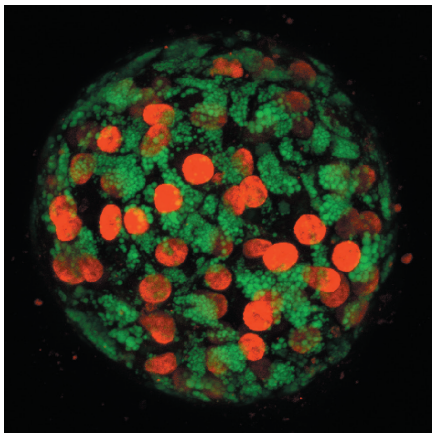
Under well-controlled conditions, organoid development from pluripotent cells strongly resembles natural fetal organ formation, although the process stalls well before full maturity. "We used gene expression profiling and matched our organoids to human trimester one kidneys," says Little. Other researchers report similar endpoints, with maturation proceeding no further than midgestational human development.

This makes organoids a powerful tool for investigating genetic disorders that begin exerting their malign influence long before birth, especially for diseases that may not be accurately modeled in nonhuman species. For example, Kriegstein's team has used iPS-cell-derived organoids to study Miller–Dieker syndrome[5], a severe neurodevelopmental disorder in which the cerebral cortex fails to form its familiar folds. "We saw an abnormality in the outer radial glial cells, which are highly enriched in primates and humans, but are almost not present in all in rodents," he says. "But in humans, these are a major source of cortical neurons." Lancaster's team is likewise using brain organoids to study disorders associated with microcephaly and macrocephaly, which respectively correspond to undergrowth and overgrowth of neurons within the cortex and arise from genes that may have played a prominent role in the evolution of the human brain. "Often these genes are also involved in human brain disorders," says Lancaster. "There is also a link between autism and brain size, with particular parts of the brain likely to be enlarged."

Organoids may also help match patients with appropriate treatment. For example, there are multiple cystic fibrosis drugs



Cerebral organoid derived from iPS cells, with neural stem cells labeled red and neurons labeled green. Image courtesy of Madeline Lancaster, IMBA.



Live-cell imaging of a fatty liver organoid (nucleus, red; fat, green). Image courtesy of Rie Ouchi and Takanori Takebe.

available that are effective in individuals with certain disease mutations. But the benefits of these ultraexpensive medicines are uncertain for up to 45% of patients, who lack clear genetic signatures of sensitivity. Clevers and collaborators have demonstrated that patient-derived gut organoids offer a powerful model for predicting treatment response, and they have embarked on a nationwide effort to screen every cystic fibrosis patient in the Netherlands. "We now have 40 or 50 patients with rare mutations who would otherwise not have access to these drugs but they're positive in the organoid test and they actually respond well clinically," says Clevers. "Now, they can get the drug." Takanori Takebe of the Cincinnati Children's Hospital is likewise partnering with Takeda Pharmaceuticals and other drug companies to test safety and efficacy of drug candidates on patient-derived liver organoids that manifest scarring damage from nonalcoholic steatohepatitis. "We have already proven high fidelity to clinical testing approaches," says Takebe. "It can really predict liver toxicity."

Modeling a natural developmental process refined throughout evolution is one thing, but organoids can also recapitulate the warped and deviant growth of cancerous tissue. This past November, Huch's team showed that they could generate organoids replicating patient tumors from three common subtypes of liver cancer with remarkable fidelity[6]. "If you show me pictures of my tumor organoids, I can tell you which patient they belong to because they are that specific," she says. "We can also recapitulate the mutations and alterations in gene expression that each tumor has." Genome editing tools like CRISPR–Cas9 offer the possibility to selectively manipulate candidate tumor suppressors and oncogenes and identify pathways that drive tumorigenesis. "You can do all sorts of combinations in one try—four, five or six different oncogenes in the same organoid batch," says Clevers.

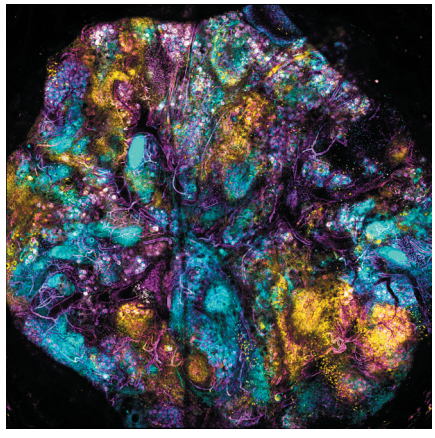
Clinical researchers are exploring organoids as a faster, simpler alternative to xenograft models, in which patient tumors are transplanted into mice and tested against different treatments. Senthil Muthuswamy's team at the Beth Israel Deaconess Medical Center in Boston is investigating how well pancreatic tumor organoids predict real-world patient response and is developing a similar model for metastatic breast cancer. "We have set it up so that within 11 to 12 weeks from a patient's tissue coming into our lab, we will be able to test anywhere from 10 to 12 different drugs and see if there is a difference in efficacy in terms of how they respond," says Muthuswamy. He notes that his team is especially focused on highly aggressive and hard-to-treat cancers, where time is of the essence. Clevers' team is likewise modeling roughly half a dozen different cancers and has spearheaded a 'Living Biobank'—a repository of patient-derived organoids that can be accessed by researchers in academia or industry for testing experimental drugs.

Organoids also offer a window into infectious disease. In the midst of the Zika outbreak in Latin America, multiple research teams explored the use of brain-derived organoids to characterize the link between viral infection and microcephaly in the developing brain. Clevers, Spence and others have also used gastrointestinal organoids to model infection of pathogens ranging from *Cryptosporidium* parasites to norovirus to *Clostridium difficile* bacteria. The same approach is also proving useful for exploration of the 'helpful' bacteria that comprise the gut microbiome, a vast ecosystem of poorly characterized microbial species that are otherwise difficult to cultivate. Spence is particularly interested in understanding how these bacteria facilitate healthy gut development during infancy. In one recent study, his team examined the protective influence of commensal, gut-derived *Escherichia coli* in the immature intestine[7]. "We simulated inflammation in the intestine by adding cytokines in the media, and showed that organoids colonized with *E. coli* were less susceptible to that inflammatory insult," he says.

Room to grow

The current crop of models is still a far cry from fully functional organs. This is due in part to the 'maturation barrier', which leaves constructs stalled at fetal stages. One of the main obstacles in this regard is the lack of a circulatory system to sustain further growth and development. "If you transplant intestinal organoids into mice, they get vascularized and then they gain a more complex architecture and appear much more like the adult intestine," says Spence. Researchers are still grappling with how to introduce functional blood vessels into organoids *in vitro*. Takebe's team has found that they can establish some primitive vasculature in their 'liver bud' organoids by starting with a stem-cell-derived mixture of liver cells, connective tissue and endothelial cells, but a proper circulatory system is only established after grafting into a live animal.

Similar cellular 'cocktails' are also enabling more sophisticated organoids with functional elements that would normally arise from distinct progenitor cells within a developing embryo. For example, by combining gut organoids with neuronal progenitor cells, a team led by James Wells at Cincinnati Children's Hospital produced intestinal organoids with a functional enteric nervous system—networks of nerves that control functions such as peristalsis[8]. Other groups are trying to introduce elements of the immune system into their organoids. "We could use these as models for immuno-oncology, for example," says Clevers. Along the same lines, several teams have found that they can integrate multiple organoids into more sophisticated superstructures. Two different teams of research-



In vivo imaging of a kidney organoid derived from multicolor-labeled embryonic cells after transplantation into a mouse. Image courtesy of Emi Yoshizawa and Takanori Takebe.

ers, led respectively by Knoblich and Sergiu Pașca of Stanford University, have fused pairs of organoids representing distinct regions of the forebrain, giving rise to an interconnected whole that more faithfully recapitulates the cerebral cortex[9,10]. "You really see this migration of cells from one region to another, showing that they really do communicate," says Lancaster, who was not involved with this work.

Eventually, organoids could offer patient-specific 'spare parts' for the repair of damaged or diseased tissues. Although this is far over the horizon for complex tissues such as the brain, simpler organs like the gut or liver may be within reach. Takebe notes that his team's liver buds begin to functionally integrate into recipient organs within a day or two after transplantation into mice, and his group has shown that such implants can effectively remedy liver disease in these animals. His hospital has made clinical translation a priority, with a newly launched Center for Stem Cell & Organoid Medicine, and Takebe is already preparing for first-in-human testing in the near future. "We are planning on clinical trials in Japan by 2020," he says.

Little is one of several investigators affiliated with the (Re)Building a Kidney consortium, an effort from the National Institute of Diabetes and Digestive and Kidney Diseases that has brought together experts from across the world of regenerative medicine to identify strategies for repairing and replacing damaged organs. Organoids themselves may not offer the ultimate solution, but Little notes that they provide a powerful way to generate specialized cell types that might otherwise prove hard to cultivate.

For now, organoid researchers are still trying to understand how far these models can be pushed—and with progress happening at a dizzying pace, it is already clear that these tiny lab-grown tissues can offer detailed insights into human biology that would be all but inaccessible with conventional cell culture or rodent models. Nevertheless, the experts caution against viewing them as a panacea for every research problem. "I think they're going to allow us to really dive into more mechanistic questions of tissue development, tissue homeostasis and disease…but it's always good to have the ability to interrogate a phenotype in a live animal," says Spence. "I look at them as a beautiful complement to all the other systems."

Michael Eisenstein

Michael Eisenstein is a freelance science writer based in Philadelphia.
e-mail: michael@eisensteinium.com

1. Sato, T. *et al. Nature* **459**, 262–265 (2009).
2. Eiraku, M. *et al. Nature* **472**, 51–56 (2011).
3. Quadrato, G. *et al. Nature* **545**, 48–53 (2017).
4. Grün, D. *et al. Nature* **525**, 251–255 (2015).
5. Bershteyn, M. *et al. Cell Stem Cell* **20**, 435–449. e4 (2017).
6. Broutier, L. *et al. Nat. Med.* http://dx.doi.org/10.1038/nm.4438 (2017).
7. Hill, D.R. *et al. eLife* **6**, e29132 (2017).
8. Workman, M.J. *et al. Nat. Med.* **23**, 49–59 (2017).
9. Bagley, J.A. *et al. Nat. Methods* **14**, 743–751 (2017).
10. Birey, F. *et al. Nature* **545**, 54–59 (2017).