Stem cell-based disease models

Organoids: 3D miniatures are booming

"Organoids" are as tiny as mustard seeds, but many biomedical scientists believe they are the next big trend in health research. Using sophisticated 3D tissue culture techniques, researchers are becoming increasingly skilled at combining stem cells and differentiated cells to create miniature versions of organs such as brains, intestines, and stomachs. The topic is also attracting more and more stem cell researchers in Germany. Organoids are so similar to the full-sized organs on which they are modeled that they have opened up new avenues for investigating principles of developmental biology and disease etiologies. This means that the mini-organs are not only good candidates for testing active substances, they also have enormous potential for diagnostics and regenerative therapies.

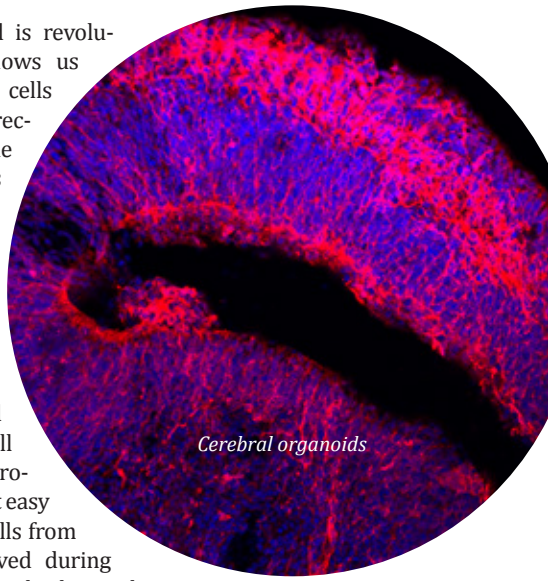
Sina Bartfeld is delighted every time she looks at her mini-stomachs, just days old, through the microscope: "My organoids are so beautiful," says Bartfeld, a biologist who is currently setting up her own group at the University of Würzburg. The delicate, hollow spheres are made of a single layer of cells and measure just under half a millimeter. Smaller bubbles are budding around their edges. "As well as various differentiated cell types, these also contain the stem cells that constantly replenish the stock of cells," says Bartfeld. It takes less than two weeks for a tiny stomach to grow. "The fascinating thing is that the stomach organoids self-organize and keep growing," says Bartfeld.

Bartfeld is one of a rapidly increasing number of scientists whose work with organoids is changing biomedical research. From miniscule intestines and lungs to diminutive mammary glands and brains, tiny versions of almost every organ in the human body are flourishing in laboratories all over the world. As well as resembling the full-sized organs in appearance, the in vitro organoids also carry out astoundingly realistic complex biological processes.

Bartfeld moved to Würzburg from Utrecht in the Netherlands last fall. As a postdoc, she worked at the Hubrecht Institute for Developmental Biology, in the working group of Hans Clevers, a Dutch stem cell researcher who is considered the pioneer and initiator of the recent cell-culture boom. From the elite training center of the Hubrecht Institute, organoid technology is now conquering research laboratories all over the world.

Adult stem cells back in the spotlight

Three-dimensional cell cultures are not a new trend. Researchers involved in the differentiation of pluripotent stem cells are familiar with "embryoid bodies," three-dimensional clumps in which the cells develop much faster than they would in the body. Tissue engineers have also spent many years experimenting with 3D aggregates made from different types of cells.



Cerebral organoids

"Hans Clevers' method is revolutionary because it allows us to isolate adult stem cells from organs and to recreate their niche in the Petri dish," explains Bartfeld. Clevers and Toshiro Sato first accomplished the feat in 2009, with tissue from the small intestine. One of the keys to the team's success was that they had identified an important stem cell surface marker (the protein LGR-5) that made it easy to isolate adult stem cells from intestinal tissue removed during surgery. The marker can also be used to find adult stem cells in other tissues. Sophisticated cell-culture techniques paved the way for another step forward: by adding Matrigel, a gel-like secretion from mouse tumor cells, the scientists made a very good version of the immediate environment ("niche") of adult stem cells. Once they are embedded in this extracellular matrix, the cells find themselves in very comfortable surroundings. Adding growth factors and a few other types of cells was then enough to make the adult stem cells do in the Petri dish what they would do in an actual intestinal wall: renew themselves while simultaneously producing structures like villi and crypts. As a result, a self-organizing, living intestinal system grew in the Petri dish.

Realistic model systems for diseases

"This culture technique provides an inexhaustible source of human cells from a specific type of tissue," says Bartfeld. "And what is more, the cells are unchanged." Bartfeld explains that this means the organoids get much closer to the natural, in vivo situation than cell aggregates, which are obtained using pluripotent stem cells. Another advantage is that it is easy to learn how to obtain the cells and perform the culture technique. The mini-organs are also easy to handle. "Freezing, defrosting, mailing – none of that is a problem," says Bartfeld. Bartfeld explains that the field exploded after Nature published the discoveries in 2009. Adult stem cells, which had retreated into the background in the era of iPS cells, returned to the spotlight. From then on, many of Bartfeld's colleagues in Utrecht began picking out their own organ system.

As cell-based model systems, organoids are not only well suited to studying organogenesis and the developmental biology of tissues. They also open up entirely new scope for investigating disease mechanisms. At the Institute for Molecular Infection Biology in Würzburg, Bartfeld is hoping to

Photo: Helmholtz Zentrum München / Marisa Karow

use her mini-stomachs to recreate the development of peptic ulcers. To do so, she is infecting the 3D structures with Helicobacter pylori bacteria and observing closely how the germs cause cancer to develop. "This means we can simulate and examine in more detail the interaction between the pathogen and the human host cells." Bartfeld's initial experiments have already shown that in organoids infected with the stomach bacteria, key steps involved in the innate immune response occur correctly, although the pattern varies depending on the type of cell.

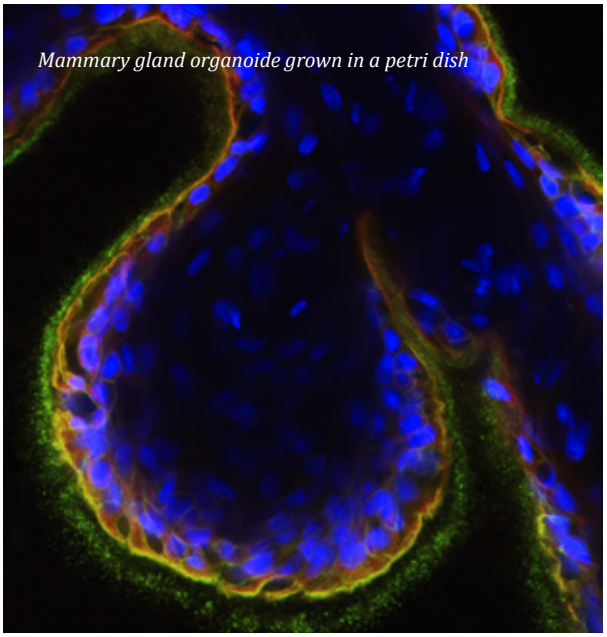
Another potential application is in regenerative medicine. For instance, Bartfeld has succeeded in making mini-stomachs from patients who have had their stomachs removed to treat cancer. "One use for these cancer organoids is to test which medicines are effective and which are not," says Bartfeld. She also explains that the organoids can be used to simultaneously cultivate and compare healthy and diseased tissue from the same patient.

Henner Farin, who since September 2015 has been running a young researchers' group at the Georg Speyer Haus in Frankfurt as part of the German Consortium for Translational Cancer Research (DKTK), is another protégé to come out of Clevers' laboratory and return to Germany. Farin plans to produce intestinal tumor organoids and use them to research the mechanisms of carcinogenesis. He is also planning to use patient-specific organoids to investigate chronic inflammatory intestinal diseases.

News from the organoid workshops

Another reason why health researchers are attracted to working with organoids is that they are easy to combine with other cell technologies. For instance, scientists can equip them with cell types derived from induced pluripotent stem cells (iPS cells). Individual cells in the miniature organs can also be modified or reprogramed using genome editing. This means the 3D structures can be tailored for a specific disease model.

The GSCN annual conference in September 2015 showcased what researchers working on 3D cell cultures are now capable of. Hans-Willem Snoeck of Columbia University in New York reported on his attempts to recreate the development of a human lung as naturally as possible in the Petri dish. With the help of iPS cells and a sophisticated protocol, his team has made remarkable progress. "We have succeeded for the first time in allowing the fine branching and the maturation of the alveoli to occur in the Petri dish," says Snoeck. In addition to producing insights into developmental biology, the models can also be used to study numerous lung diseases, such as the flu, cystic fibrosis, and lung cancer.



Mammary gland organoide grown in a petri dish

Photo: Helmholtz Zentrum München / Jelena Linnemann, Lisa Meixner

A team led by Christina Scheel at Helmholtz Zentrum München, meanwhile, has succeeded in recreating the complex 3D structures of the mammary glands. The Munich researchers isolated stem cell-like cells from tissue removed during cosmetic breast-reduction surgery. In the 3D culture, the cells begin forming complex milk ducts that end in bubble-like structures. The researchers now want to use the mini-breasts to examine the development of breast cancer in more detail.

Reprograming mini-brains

No organoid has caused as much of a stir over recent years as the pea-sized mini-brains that have been grown in Jürgen Knoblich's laboratory at the Institute of Molecular Biotechnology in Vienna. Neural precursor cells derived from iPS cells began, almost of their own accord, combining to form so-called cerebral organoids. The little balls of neural tissue resemble, both outwardly and in terms of their molecular biology, the structures of a young embryonic brain.

"In 2013, this work was definitely an eye-opener for the field and created a great sense of optimism," says Benedikt Berninger of the Institute for Physiological Chemistry at the University of Mainz. His working group is one of many in Germany that have begun focusing on cerebral organoids. "We use the mini-brains as a platform for studying the reprograming of glial cells to form neurons," explains Berninger. His colleague Marisa Karow carries out cell transplants on the tiny brains – a kind of open brain surgery in the Petri dish. It is then possible to follow the consequences of this intervention in real time. The mini-brains have also given the neuroscientists new avenues for deciphering the molecular programs that occur in developmental disorders in the brain.

Berninger believes that the current wave of euphoria surrounding the organoids is absolutely justified, but adds that "we are still in the early phases." He says that the research is expensive and complex, and that key questions relating to the long-term survival of the cell cultures have yet to be answered. The cell-culture experts are still trying to work out how best to look after and feed the organoids over a period of many months. Berninger is campaigning for better networking of the knowledge and expertise within the growing research community in Germany. Julia Ladewig in Bonn, Frank Edenhofer in Würzburg, and Magdalena Götz in Munich are just some of the stem cell researchers whose teams are working intensively on the living 3D brain models.

"We, too, are enthusiastically following and participating in the progress being made with organoids," says Hans Schöler, Director of the Max Planck Institute for Molecular Biomedicine in Münster. The Max Planck researchers are using brain organoids as test objects for the targeted reprograming of astrocytes to form precursor cells. "We hope that this will improve our understanding of neurodegenerative ageing processes," says Schöler. "Right now, organoids are a vast playground."

Text: Philipp Graf

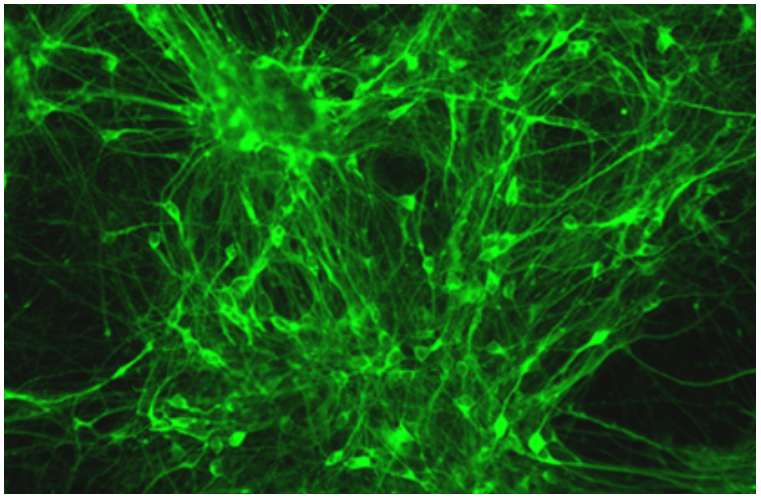
New kits on the block…

ThermoFisher SCIENTIFIC

„Pure, what do you mean, pure?!?!?" Albert grumbled. "It's not so easy to make pure Dopaminergic Neurons for Parkinson's disease research. I always see a certain degree of "contamination" with fore- and hindbrain Dopaminergic Neurons during differentiation from iPSCs and ESCs. For example, I just recently used a kit, which was easy to use and gave me high yields of NSCs from my iPSCs. However when further differentiating these NSCs, I achieved not only midbrain DA Neurons, which Parkinson's researchers are mostly interested in, but I also noticed the presence of a significant number of fore- and hindbrain DA neurons. In this case, the ease-of-use advantage actually turned out to be a disadvantage for this specific application."

"Well, darling, that is interesting to hear, but how can I be of help in this case?" asked Josephine, the lovely wife of Albert, who didn't have a clue about research.

"Dad" said Madeline their daughter, a Ph.D. student in her second year, as she joined the discussion, "I just heard about a new kit, which might fit perfectly with your work. With this new kit you can differentiate midbrain dopaminergic neurons from iPSCs and ESCs. That is what your Parkinson's researchers are looking for, right? I even saw some data on the new kit, which provides a simple to use and reproducible protocol, and is even quicker to use than most of the protocols in common use today. You only need 38 days to get to generate your desired mid-brain dopaminergic cell type, which is 7 days shorter than the 45 days required by most protocols. But much more importantly", said Madeleine as she started to become euphoric, "you can make a break in between! Yes, you can efficiently expand and bank your progenitor cells! This allows you to start your differentiations from an intermediate stage next time around using the same batch cells and avoids you having to go all the way back to the iPSC or ESC stage!"



"Wow, that sounds interesting!" said Alfred, interrupting Madeleine, "Thanks for the tip! Do you recall which company makes this kit?"

"That's the new kit from Thermo Fisher Scientific and it is called PSC Dopaminergic Neuron Differentiation kit or something like that. Just yesterday I received the link from one of my lab mates:

www.thermofisher.com/de/de/home/life-science/stem-cell-research/stem-cell-differentiation/psc-dopaminergic-neuron-differentiation.html

Just check out the homepage! Oh, sorry, but I have to leave now!" and Madeleine ran off in a hurry as The Big Bang Theory had just started on TV.