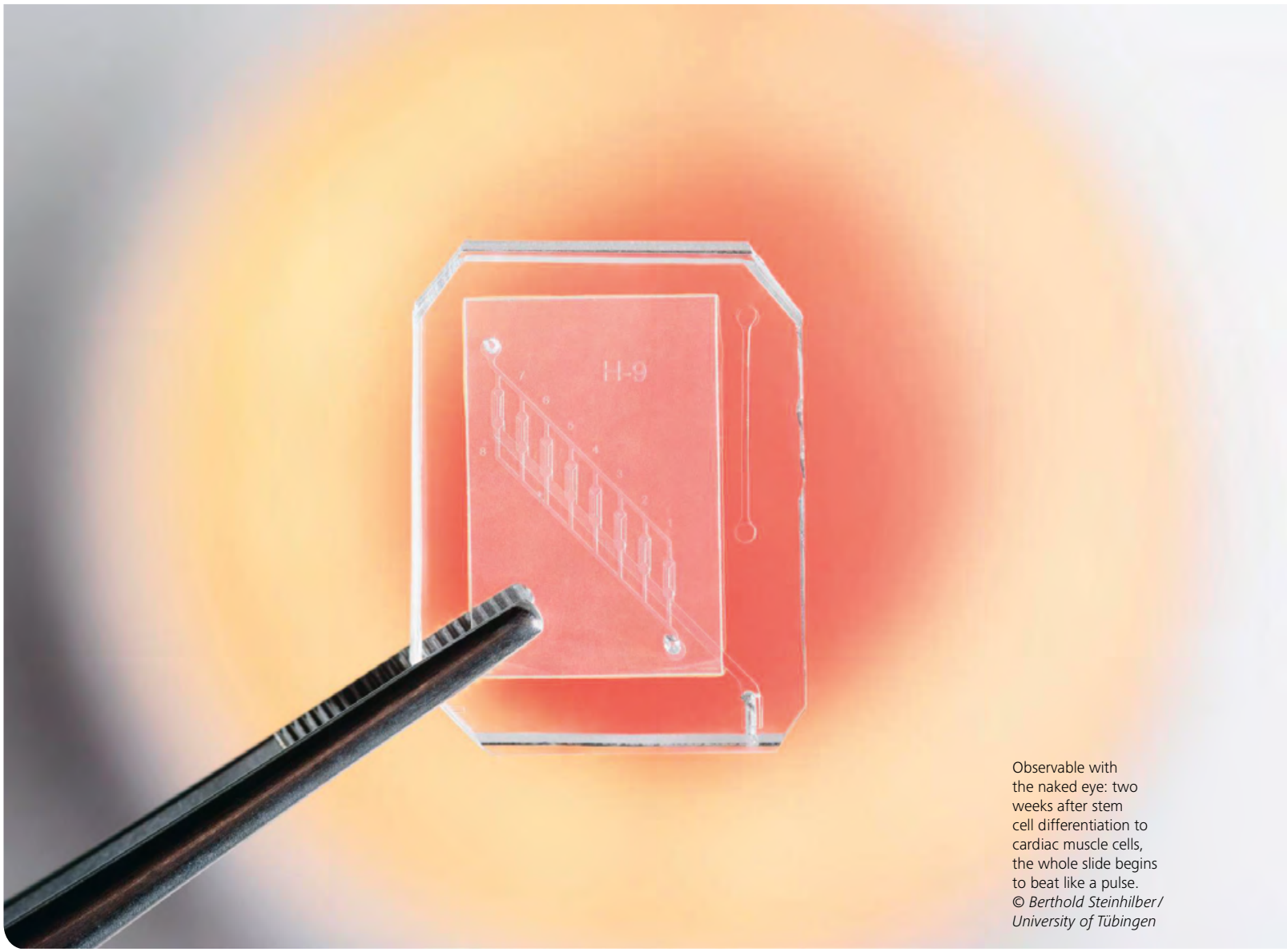


Observable with the naked eye: two weeks after stem cell differentiation to cardiac muscle cells, the whole slide begins to beat like a pulse.
© Berthold Steinhilber/ University of Tübingen

Cells that beat like a heart

More than meets the eye: organ-on-a-chip technology could lead drug research toward the development of gender-specific medicine.

Text: Christine Broll

It seems like an act of creation. For Dr. Christopher Probst, however, it's all part of the job. Probst has just taken a dish with cell cultures from the incubator. He slides the cultures carefully under the microscope and observes how the thin tissue of transparent cells pulsates. The stem cells have differentiated to cardiac muscle cells. "By the time the cells are two weeks old," Probst explains, "you can see, with the naked eye, the whole slide beating like a pulse!"

Probst is a research fellow at the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB in Stuttgart. He is part of the Attract working group, which is led by Prof. Peter Loskill. If successful, the group's research may well substantially reduce the number of animal experiments. **(see interview, right)**.

Doctoral student Oliver Schneider will use the cardiac muscle cells to create a so-called organ-on-a-chip (OOC): postage-stamp-sized polymer chambers in which minuscule tissue cultures and organoids are fed with nutrients via a system of microchannels. Back in 2016, OOC was declared one of the top emerging technologies by the World Economic Forum. Today, there are OOC systems for a whole range of tissue types, including cardiac muscle, liver, kidney and even brain tissue. The working group at Fraunhofer IGB has helped spearhead this technology in Europe, pioneering a number of unique developments that include the recreation of human fatty tissue and human retinal tissue on a chip.

Loskill is pursuing an ambitious goal. He first began working on OOC technology in the USA back in 2013, where a huge funding program had just been launched. This saw a number of research organizations team up with federal and public bodies for a variety of projects. The Food and Drug Administration (FDA), for example, saw OOC as a means of speeding up drug development, whereas the U.S. Department of Defense was interested in tests for chemical and biological weapons. The Environmental Protection Agency (EPA), meanwhile, wanted ways of investigating pollutants. Buoyed by hundreds of millions of dollars in funding, U.S. researchers soon forged ahead of the rest of the world, including Europe. Loskill is now seeking to redress the balance. Together with three fellow researchers from the Netherlands, he set up the European Organ-on-Chip Society in November 2018. This was followed by an inaugural conference in Stuttgart. Furthermore, EU funding has now been secured for two undertakings: a Marie Curie project involving 21 European partners, and an initiative to draw up a road map for OOC technology in Europe.

This collaboration between European research groups will help pave the way for a broad-based application of this technology. And now that scientists have mastered the technique of placing a whole variety of tissue cultures on a chip, the next challenge will be to increase the throughput of the various substances being tested. Doctoral student Schneider is already working on ways of scaling up this technology. In the future, so-called organ-on-a-disc systems will combine hundreds of human tissue samples in one handy format, thereby helping to turn this technology into a routine procedure.

"Many diseases manifest themselves in different ways in male and female patients," Prof. Peter Loskill explains. His research may well lead to the development of gender-specific therapies. © *Bernd Müller/Fraunhofer IGB*

Teaching a chip how to see

The latest breakthrough to emerge from Loskill's lab is a retina-on-a-chip system, featuring the complex stratified tissue of the human retina as an organoid. Right now, Loskill and his team – which includes doctoral student Johanna Chuchuy – is busy endowing it with the capacity to see. Working with partners from the University of Tübingen, they have been able to differentiate stem cells and incorporate them in a chip in such a way that they recreate a multilayer tissue. This tissue comprises, among other things, light-sensitive rods and cones, retinal pigment epithelium and ganglion cells, which make up the optic nerve. "When we shine light on the retina-on-a-chip, we register an electrophysiological signal in the rods and cones," Loskill explains. "And now we're working on a system with which we can quantitatively measure this signal."

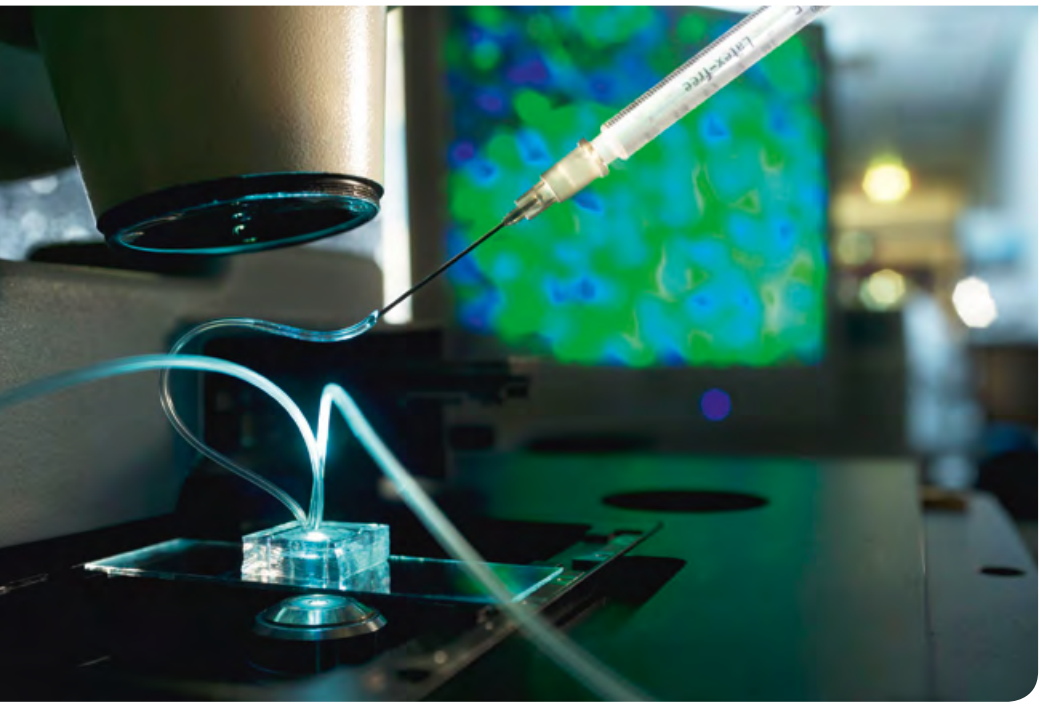
Such a system will make it possible to measure the extent to which a substance influences the "visual capacity" of the retina-on-a-chip. "The pharmaceutical industry is showing a big interest in retina-on-a-chip technology," Loskill adds. "Lots of modern drugs have retinopathic side effects." To date, model systems are rare in this field. Animal models, for example, are of only limited use since the retina of animals has a different structure than that of the human retina. Moreover, retina-on-a-chip technology will also facilitate research into diseases of the retina and the development of drugs to treat conditions such as age-related macular degeneration and diabetic retinopathy. ▶

"Substantially fewer animal experiments"

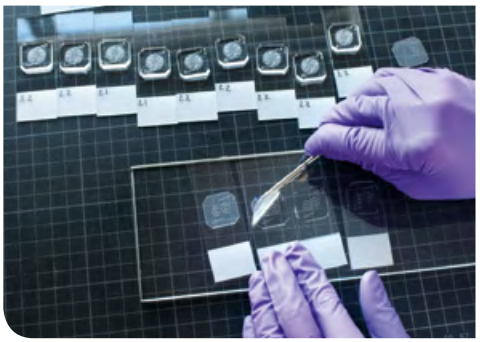
Prof. Loskill, will organ-on-a-chip technology help reduce large-scale animal experimentation? For sure. We're already seeing a big interest on the part of the pharmaceutical industry. The technology can be used right across the board: screening for new active ingredients, carrying out toxicity tests and backing up clinical studies.

Will the regulatory authorities accept organ-on-a-chip data in place of results from animal experiments? The regulatory authorities are very open to this new technology. I'm currently working with regulatory bodies on two EU projects. These include the German Federal Institute for Drugs and Medical Devices (BfArM).

Will the technology make animal experiments completely redundant in the long term? No technology alone will ever be able to completely replace animal experimentation. But our chips do provide a source of data that will enable us to gain requisite certainty with significantly fewer animal experiments.



WAT-on-a-chip, under a microscope: a syringe is used to introduce an active ingredient in order to investigate how the tissue reacts. © *Bernd Müller / Fraunhofer IGB*



WAT-on-a-chip preparation at Fraunhofer IGB in Stuttgart. © *Berthold Steinhilber / University of Tübingen (top), Bernd Müller / Fraunhofer IGB (bottom)*

Fat makes up one quarter of the weight of a healthy person – but we're only just beginning to understand its role in the body.

▶ **Of even greater medical significance** are so-called WAT-on-a-chip systems. WAT is the abbreviation for white adipose tissue – a key factor in human health, not least because it makes up such a significant proportion of body mass. Adipose tissue comprises a quarter of the body mass of a healthy person and up to half the body mass of a clinically obese person. It has only recently become clear that adipose tissue is responsible for secreting a whole variety of hormones and other chemical messengers into the blood stream. Much of this field is still not properly understood, but WAT-on-a-chip systems should help deepen our understanding of the role that white adipose tissue plays in the body and enable researchers to develop more selective treatment for associated diseases such as diabetes.

In addition, WAT-on-a-chip systems help reveal the processes by which substances are stored in adipose cells. To demonstrate this, doctoral student Julia Rogal uses a fatty acid marked with a green fluorescent dye. She injects the fatty acid into the chip and places it under the microscope. Within a few minutes, the green fatty acid can be observed penetrating and accumulating in the round adipose cell. Similar methods can be used to investigate whether herbicides, for example, or microplastics accumulate in fatty tissue.

Loskill also expects OOC technology to open up a further field of research – that of sex-specific medicine. "Many diseases manifest themselves in different ways in male and female patients," he explains. "It's an aspect that has not yet received enough attention in medical research and drug development." In the future, OOC systems will enable researchers to investigate male and female tissue separately. For example, it would be possible to use a female organ-on-a-chip system to simulate the menstrual cycle and observe whether it has an impact on a specific disease and potential drug therapies. Such projects form part of his work as junior professor at the Institut für Frauengesundheit (Institute for Women's Health) at the University of Tübingen, where he is also producing chips containing the tissue of breast and cervical tumors.

Collaboration with a working group under the leadership of Dr. Frank Sonntag at the Fraunhofer Institute for Material and Beam Technology IWS in Dresden has yielded further breakthroughs. Building on Fraunhofer IWS' expertise in microfluidics, the team has developed a multi-organ chip that simulates the way in which the organs in the human body are supplied with blood. Using a smart combination of pumps, valves and control technology, the chip is able to replicate the differing rates of blood flow to the organs. Last October, the multi-organ chip from Fraunhofer IWS was presented with an Innovation Award from the European Association of Research and Technology Organisations in Brussels. The two Fraunhofer research groups have now submitted a joint patent application with a view to combining the two systems. ■