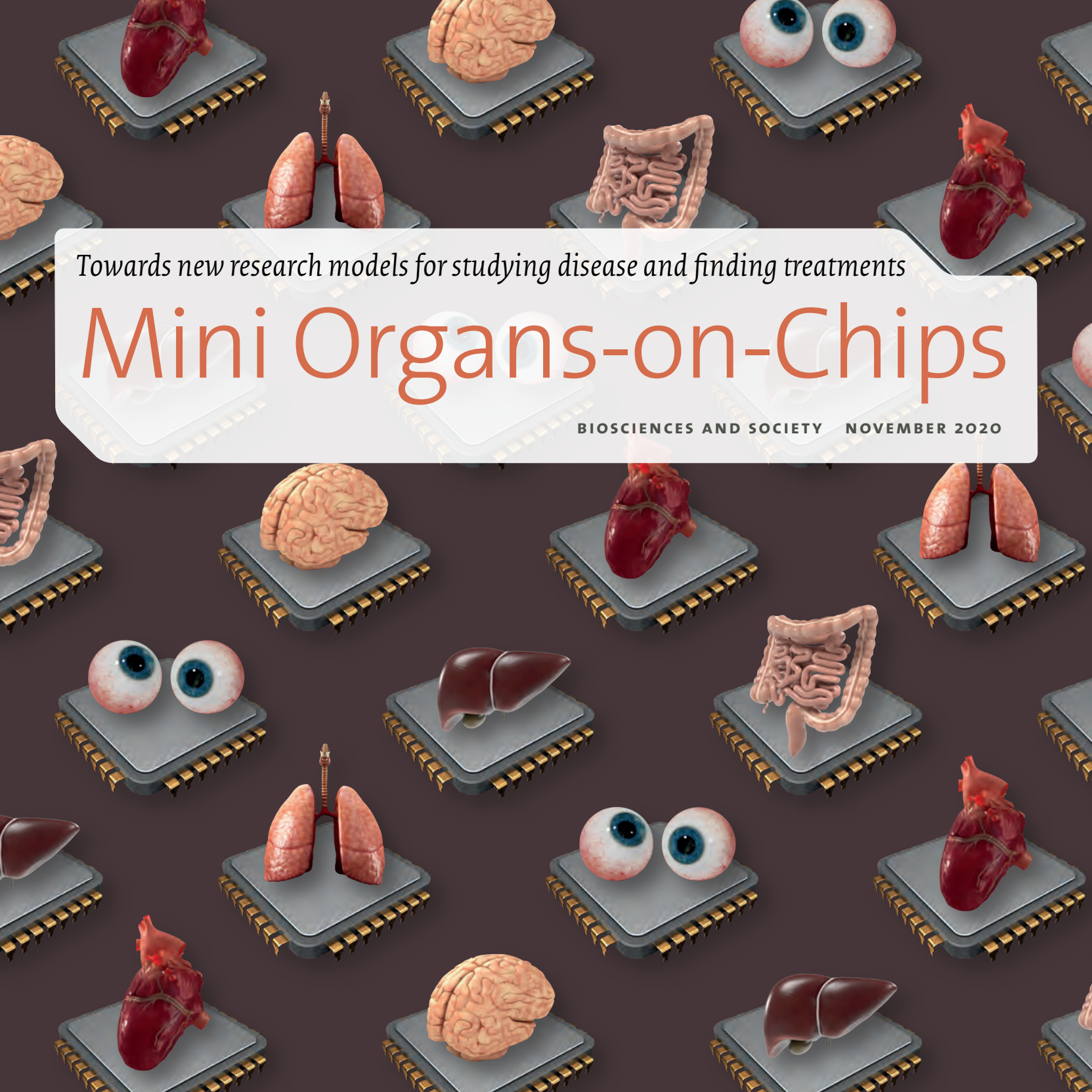


Towards new research models for studying disease and finding treatments

Mini Organs-on-Chips

BIOSCIENCES AND SOCIETY NOVEMBER 2020

Cahier 3 | 2020 | 39th volume

Mini Organs-on-Chips

This booklet is a publication of Dutch Foundation for Biosciences and Society (BWM). Booklets are published three times per year. Each booklet is dedicated to a theme from the life sciences with a particular focus on its societal consequences.

Stichting BWM is a unit within the Netherlands Organisation for Health Research and Development (ZonMw).

PRINTED BY
Drukkerij Tesink, Zutphen

INFORMATION, SUBSCRIPTIONS AND ORDERING OF SEPARATE ISSUES
Dutch Foundation for Biosciences and Society
Laan van Nieuw Oost-Indië 334
2593 CE The Hague
The Netherlands
Telephone: +31 70 349 5402
Email: info@biomaatschappij.nl
www.biomaatschappij.nl

ISBN/EAN 978-94-93232-01-3

bio wetenschap+ maatschappij

Contents

Preface

Exactly ten years ago, we were talking to each other at a meeting of the Royal Netherlands Academy of Arts and Sciences (KNAW). One of us is an expert in stem cell biology, the other, hard-core micro-nanotechnologist. While discussing each other's work, the stem cell biologist thought microfluidic chips were great but were being studied using the "wrong" cells and the engineer thought human stem cells had fantastic possibilities but why did we grow them on plastic, which is even harder than bone. We felt that combining the best cells with the finest technology could create excellent opportunities to produce new age Organs-on-Chips. Our enthusiasm resulted, among other things, in one of the 49 questions on the Dutch Research Agenda: "Can we build Organs-on-Chips?". It also led to a broad national collaboration through the establishment of the virtual Institute for human Organ and Disease Model Technologies (hDMT).

In hDMT, nearly all Dutch universities and medical centres as well as several companies are collaborating in a unique manner to develop Organ-on-Chip test systems. Since hDMT was founded in 2015, we have started many joint research projects, all of them including hDMT researchers. One is a large 10-year programme with a grant from the Dutch Research Council (NWO) to develop new human disease models for gut, heart and brain. We held the first international Organ-on-Chip conference and founded the European Organ-on-Chip Society (EUROoCS).

Many students have now heard of Organ-on-Chip and we are frequently contacted by those

keen to join this multidisciplinary research area, not least because it has the potential to accelerate drug development whilst contributing to the development of alternatives to animals in the drug development pipeline and assessing drug safety. We think it is important that more people understand what we can (and cannot) do with this technology and how it might impact the health and well-being of society at large.

This booklet has contributions from many of the top research groups in the Organ-on-Chip field in the Netherlands and we hope and believe it provides a state-of-the-art overview of what we can expect now and in the future.

Happy reading!

Christine Mummery and Albert van den Berg,
Stem cell biologist and micro-nanotechnologist

Introduction

In recent years, the term Organ-on-Chip has increasingly appeared in the lay literature in addition to specialist journals, so many of our readers will know this term. But what are Organs-on-Chips? Of course, they are not complete organs as in our body: a whole heart, lung or liver would not fit in a chip which may only be a centimetre or two in size. They are in fact pieces of organ tissue that exhibit essential functions of the organ but on a very small scale. Examples are pieces of heart muscle or groups of cells that beat as in a real heart, or lung alveoli that expand and contract in a way that simulates breathing. They can be used to carry out experiments at the microscale such that they simulate organ function as closely as possible and provide much more realistic mimics of tissue than used in most laboratories right now: the plastic Petri dish.

An important reason for developing Organs-on-Chips is the considerable need for better test systems for human health and disease. After cardiovascular diseases and cancer, unintended side-effects of drugs are among the most common causes of death. Whilst this can be due to incorrect drug use, in many cases it can also be caused by toxic effects on the heart, liver and kidney. The unwitting patient taking a drug to treat his illness may also be exposed to life-threatening side effects.

Drugs are usually developed to treat the "average patient" – a Caucasian, young- to middle age male – and it is this group of (healthy) individuals that are included in "first-in-man" studies for safety. Drugs are almost never produced or prescribed in a "personalised" manner. For example, a recent study revealed that the ten most sold drugs in the United States were only effective for, on average, 15% of the entire population: waste of money, you would say, but also potentially very precious time and disappointment for the patient?

Organs-on-Chips combined with human cells have the potential to allow pre-testing of a drug to check its suitability for a certain patient group – or, in the future, even one specific patient – which could result in greatly enhanced efficiency in drug use.

Why do we need Organs-on-Chips?

There are broadly three important reasons for using Organs-on-Chips:

- Drug development: candidate drugs can be quickly tested on human cells using Organ-on-Chip devices which increases the chance that they are effective.
- Personalised drugs: Organs-on-Chips containing a single patient's cells can provide specific information on the right drug for that patient and can indicate the right dosage.
- Research on diseases: by developing "diseased" Organs-on-Chips, much can be learned about disease mechanisms as a basis for possible therapies.

One good example is a recent study on atherosclerosis. This "disease of aging" often leads to thrombosis (blood clots) which are very dangerous because they can cause stroke. A Blood Vessel-on-Chip with a narrowing of the microfluidic channel as in a blood vessel with atherosclerosis simulates this blood clotting and has been used to test

The "average patient" or test person is often a caucasian man of young to middle age. Do you recognise yourself in that image?

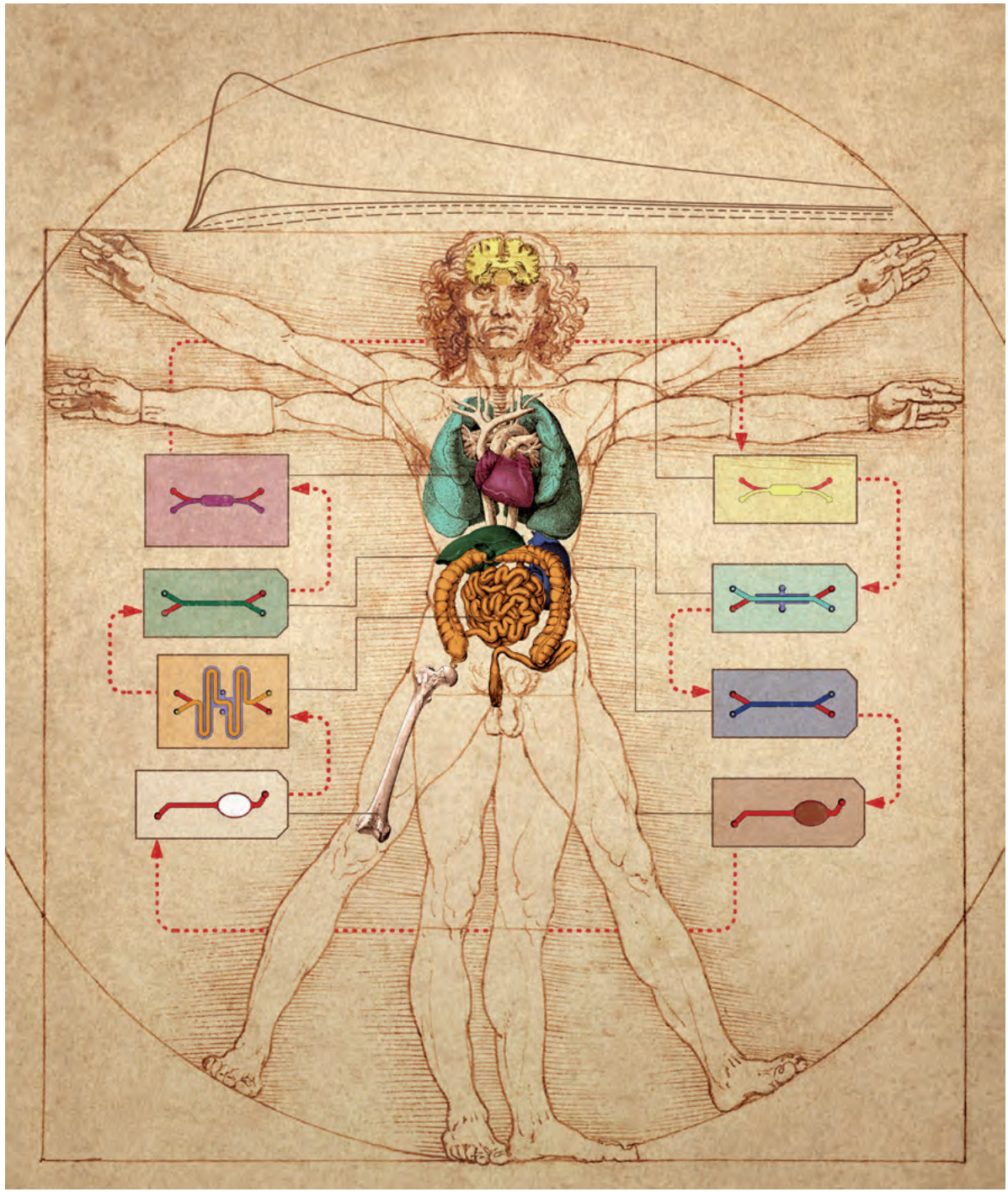
antithrombotic drugs. All done on a chip and not a patient or laboratory animal. Currently Lung-, Heart- and Blood Vessel-on-Chip models are also being examined for their suitability to test new treatments for Covid-19 patients both early in the disease to prevent virus spread and for those in intensive care with organ failure and sepsis.

During drug development, not only the effectivity of a drug but also stressful- or even life-threatening- side effects are being studied. One high-risk side effect of some drugs is for example, the occurrence of irregularity in the heart rhythm (arrhythmia) that can result in sudden cardiac death through a heart attack. However, these risks can be increasingly well-predicted for the human heart by research on Heart Cells-on-Chip or heart "organoids"(see later) that have been derived from human stem cells. Again, for Covid-19, several drugs being developed, such as antivirals, can have side effects on the heart, especially in combination with other treatments. Heart-on-Chip can help to detect those side effects.

Symbiosis

The interesting thing about the Organ-on-Chip field is that it requires a combination of micro-nanotechnology and cell biology. Cell biology is essential because tissue cell types to put into the chips must be derived and cultured. One of the most promising sources of cells for this are stem cells. Stem cells can be derived from many (but not all) organs in the body (like the intestine or bone marrow); they are then called "adult stem cells". They can also be derived from early human embryos:

Organs-on-Chips that are interconnected to produce a Body-on-Chip.



they are then called "human embryonic stem cells". Or they can be derived by what is called "reprogramming". This stem cell technology enables just a few cells from anywhere in the body (say the skin) to be turned into stem cells by small alterations in the cellular DNA. These stem cells (and embryonic stem cells) can change (differentiate) into every cell type in the body of the individual from whom they were obtained while retaining their genetic characteristics. In addition, when the three-dimensional structure, environment and fluid flow are very precisely controlled, which requires micro- and nanotechnology, and the cells from (say) a patient are put in the chip, aspects of the disease can be recapitulated. This can be measured using sensor technology. Sensors are essential to measure what happens to the cells in the chip.

In the body, all organs are linked to each other via the vascular system. It is for good reason that the body contains about 60,000 kilometres of blood vessels: every cell is located close to a blood vessel so that it has access to nutrients and oxygen and can dispose of waste products such as carbon dioxide. But it is more than just a transport system. Via the blood vessels, there is a continuous dialogue between organs, which provide each other with nutrients or convert substances into forms that can be used by the body. This conversion is referred to as metabolisation and it mainly takes place in the liver. Besides supplying the body with useful products, the liver can also break down toxic substances such as alcohol. The liver can also break down a drug, resulting in loss of its effectivity, or, worse, its conversion into a toxic product. For a drug test with a specific Organ-on-Chip it is sometimes important to also include liver cells or Liver-on-Chip where flow through the liver is required; in this way, two Organs-on-Chips are interconnected.

Going one step further: when a drug enters the body via the gut, skin or lung and goes to the circulatory system, it travels via the liver to the target organ and is ultimately excreted via the kidney and urine. Studying the entire pathway of a drug in a chip system would require interconnection of multiple Organs-on-Chips. In the United States, researchers recently managed to develop such complex Organ-on-Chip systems and to connect eight different Organs-on-Chips. The first steps towards a whole Body-on-Chip? Or just a useful laboratory exercise?

Bonus: fewer animal experiments?

It is very likely that animal experiments will always be needed in biomedical science but a helpful *credo* might be: "*As many as necessary, as few as possible*." Human Organs-on-Chips can contribute to reduction in the use of laboratory animals and improvement of research outcomes, since evidence increasingly shows that animal models are not always ideal in predicting how certain diseases will manifest in humans or how humans will respond to certain drugs. Although there is a long way to go before the regulatory bodies are convinced that there is sufficient evidence to replace laboratory animals, human Organ-on-Chip models can already contribute to the drug development process. In particular, by indicating early in the process which drugs are potentially high-risk and which drugs will be most effective.

From an anatomical, metabolic and cellular perspective, the mouse is different from humans. For this reason, the results of animal experiments cannot always be effectively extrapolated to humans.

1

Why many medicines fail

PROF. CHRISTINE MUMMERY AND DR. BEREND VAN MEER

Today, cells, tissues and laboratory animals are the most widely used models for drug development. The same is true for research on the onset of diseases. Although certainly able to deliver results on drug distribution in the body or how a signalling pathway is affected by a drug for example, the problem with these methods is that outcomes of these systems cannot always be effectively translated to humans, which are more complex or have different physiology. But even "a human" is not always a good model for the patient. For clinical studies on drugs, healthy men are often still selected: no women, the elderly or children are included. Researchers are therefore looking for alternatives; among these are Organs-on-Chips.

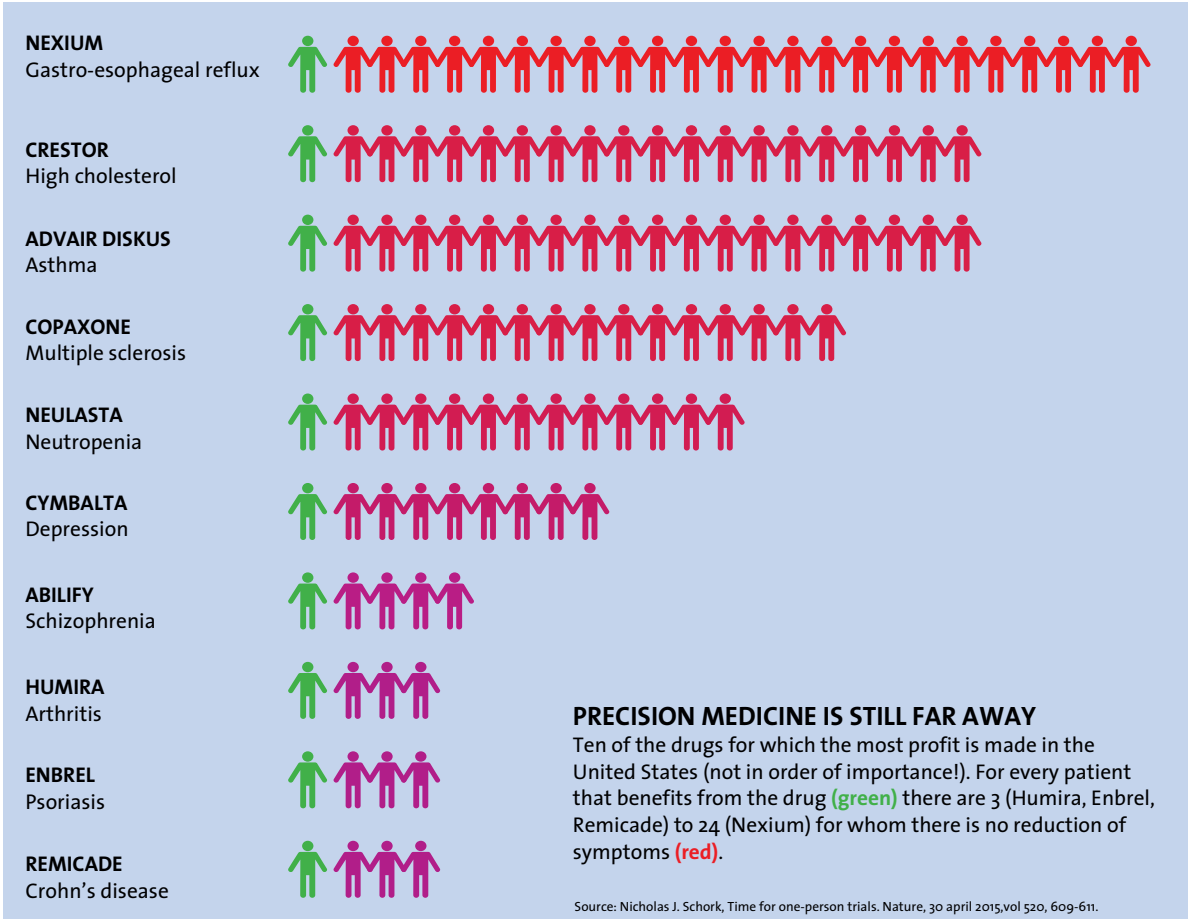
Targeting the disease and the individual

Each day, millions of people worldwide use drugs that will not cure them. For the top ten most profitable drugs for the pharmaceutical industry in the United States, at best only 1 in 4 people benefit from the prescribed treatment. In the worst case, this is 1 in 25. This is an enormous waste of healthcare resources. Furthermore, for many patients there is no effect of the treatment but they rather suffer from the side effects. In addition, some drugs can be very harmful for women or certain ethnic groups because the medical studies mainly include western, Caucasian, male test individuals.

Individual variation

A fundamental problem in drug-based therapy, is the lack of precision in prescribing a drug to the individual patient. This is due to the fact that research models used to test the efficacy and safety of drugs do not take into account individual variation. In practice, doctors often prescribe a medicine – for example, to reduce blood pressure – and then see whether it is effective. If there is no improvement after several weeks, the doctor will prescribe a different drug.

Twenty years ago, it was thought that as soon as the complete human DNA profile had been mapped, it would become clear why a certain disease manifests differently in one patient versus another, and why patients respond differently to drugs.



PRECISION MEDICINE IS STILL FAR AWAY

Ten of the drugs for which the most profit is made in the United States (not in order of importance!). For every patient that benefits from the drug (green) there are 3 (Humira, Enbrel, Remicade) to 24 (Nexium) for whom there is no reduction of symptoms (red).

Source: Nicholas J. Schork, Time for one-person trials. Nature, 30 april 2015,vol 520, 609-611.

Although some evidence for possible causes of this was obtained from the DNA profiles, the differences between patients have never been fully explained.

Tailor-made treatments for the individual require other ways to test the efficacy of a drug. In addition to genetic differences, sex, environmental factors, the condition- and "memory" of the immune system, allergens and other drugs can also have impact and should be taken into account. All these factors jointly determine how an individual responds to a drug in the case of disease. In fact, it is thus hardly surprising that drugs do not work in all patients. The question is: how do we solve this?

Improved biological insight

Insight into how the human body functions and how it responds to a drug also helps in predicting a patient-specific response. However, in medical studies only the efficacy and safety of new medicines is tested. Preclinical models are used to obtain biological insight into the drug effect. These models may be too simple or based on small animals with different physiology, thus hampering full understanding of the mode of action of the drug in humans.

The question is if and how these preclinical models can be humanized and improved such that they

take into account the condition of the individual patient and factors, like sex and genetic make-up. Also important is the question of how these human models perform in comparison to real life. Is it possible to compare healthy and diseased tissues? How will those differences be measured? Are these differences large enough to demonstrate that new drugs are indeed effective? Can these models simulate the environment of these tissues in the body? In principle, this would require recapitulation of the natural environmental factors in the laboratory: local pressure (as in blood vessels), elongation and stretch (as in muscles), periodic contraction or peristalsis (as in the heart and gut, respectively) and electrical stimulation (as in the heart and brain). New chip technology for simulating these conditions in combination with human stem cells for specific tissues – the development of Organs-on-Chips – might contribute to personalised treatment in the future.

A "hit" in screening for possible drugs refers to a substance inducing a cellular response

What are the current test models?

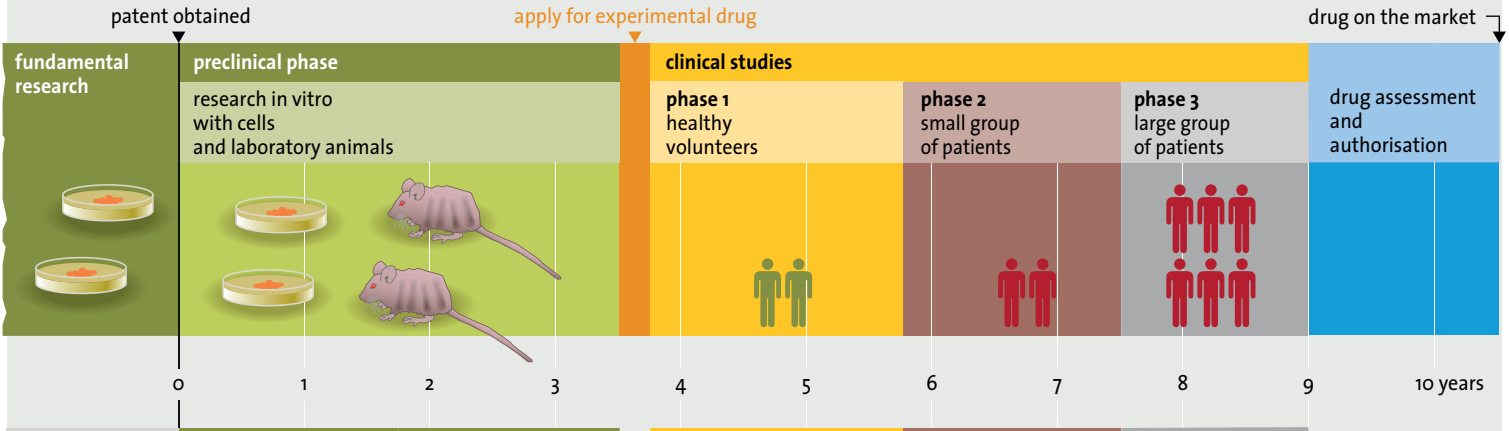
During the search for new drugs, pharmaceutical companies start with the simplest test system in order to test millions of different substances from their collection (or "library") as quickly and cheaply as possible. For this purpose, they need to know which factors in a body cell are involved in a patient's disease. That type of information mostly comes from university laboratories that carry out fundamental research on the onset of a disease.

A well-known example is cystic fibrosis: this disease is caused by a defect in a gene (a piece of DNA that codes for proteins), that is responsible for the development of a specific ion channel in body cells. This gene is expressed in the epithelium (or lining) of many organs like the lung and intestine. As a result of this gene defect, the transport of water by the affected cells is inefficient. Consequently, the mucus in the lungs and gut becomes so viscous through the lack of water that the organs can no longer function properly.

Cells in a Petri dish

In order to test drugs in a laboratory, the defective piece of DNA is inserted in a cell type that can easily be cultured: this gives rise to what is called a mutant cell line. Subsequently, millions of cells with the DNA defect can be cultured from this cell line. These cells are placed in separate wells (96, 384 or even 1536 wells) of a large plastic Petri dish. Robots subsequently add the different substances from the enormous collection (candidate drug bank or library) to the wells. If cells respond to the substance, this is called a "hit". With these hits, varying in number from 20 to 100 substances per disease, an increasing number of tests can be done, that become more and more complex and expensive.

TRADITIONAL DRUG DEVELOPMENT PROCESS



study aim	find new/ better drugs	test safety for humans	test if drug is safe	test if drug works	test which dose is optimal
model used	simple cell lines	animal experiments	healthy volunteers	patients	patients
number of cells	few cells	billions of cells	trillions of cells		
number of organs	from one organ	all organs	all organs		
biological species	human	animal (sometimes humanised)	human		
genetic variation	no variation	no variation	10-300 different people, but often just from a single ethnic group		
physiological environment	lab material, controlled	animal body, uncontrolled	human body, uncontrolled		

WISHES FOR IMPROVING THE CURRENT PROCESS

Development of a drug usually starts in the laboratory, testing a collection of drug candidates on (human) cells cultured in a plastic Petri dish. When some of these induce the expected response, they are usually tested next in laboratory animals, often mice. The last step involves humans, where tests are in three phases (phase I (safety and feasibility), phase II (effectivity and dose) and phase III (blinded trial in a large patient group). Only after these are complete, can the drug be considered by the regulatory authorities for market entry.

At the end of the procedure, only two or three substances, the potential drugs, appear to be effective in the body cells that are affected by the disease. All of this takes place using *in vitro* laboratory studies. *In vitro* literally means "in glass" because in the past Petri dishes were made of glass, whereas nowadays they are made of plastic.

Animal experiments

The next step is to test the substances in animals. This is usually a mouse with the same or similar disease. Researchers create this disease by inserting the same DNA defect (or mutation) in the (live) mouse so that the disease resembles that in the patient as closely as possible.

The entire set of animal experiments, required by the regulatory bodies of the drug market, takes several years: they want to know what happens in the body with the possible new drug, where it ends up, how it leaves the body, what its effect is on vital organs such as the heart, liver and kidneys, and what the effect is on the reproductive organs.

Clinical trials

As soon as the studies in mice have been satisfactorily completed, the pharmaceutical company needs to carry out increasingly expensive medical studies: first with larger animals, and then for the first time in humans – first-in-human studies – with healthy young male volunteers, followed by phase I studies (is the drug safe and has it an effect?), phase II studies (is it effective in a small group of patients who do not know if they receive the drug or a *placebo* (fake drug)?) and, finally, phase III studies (a double-blind study with *placebo* controls, being a group of patients that receive a *placebo* drug or *placebo* treatment) in which hundreds of patients can participate. Only when these medical studies have been completed, can a pharmaceutical company request permission from the regulatory bodies to bring the drug to the market. In Europe, the regulatory body is the European Medicines Agency (EMA) and in the United States, it is the Food and Drug Administration (FDA).

Human cell cultures in a Petri dish are a considerable simplification of reality.

By the time that permission is requested, the costs for the pharmaceutical company have risen considerably – estimates vary from 1 to 3 billion euros – and many years have passed. During the last phase in particular, it is a race against the clock for the pharmaceutical company; what are the competitors doing and will they obtain permission earlier? At this stage, the patent for the drug has already been submitted for years. Would it be possible for the company to generate enough return of investment before the patent expires? Thus, there are many considerations, often from an economic or commercial-strategic perspective, that determine if the drug will be tested in clinical trials and will actually ever be prescribed to real patients.

Why are these test models not good enough?

Cultured cells in a Petri dish oversimplify reality

During the development of new drugs, the process can fail at various points, resulting in considerable loss of time and money. An important cause of this failure is that the current models are not always capable of predicting the complexity of patients, human biology, or variations between population groups.

Cell cultures

Cultured cells on a plastic substrate are relatively cheap test models that can be used on a large scale. However, these test models are often an oversimplification of reality towards reliable identification of promising drugs. Although this is done on purpose to keep the costs as low as possible in this phase of drug development, this approach usually reduces the complexity of the disease to a single chain of biological steps: a part of a metabolic pathway or a signal pathway.

However, the reality is that many diseases result from a combination of several pathways, genes and conditions, so that a possible hit in a test might have no effect at all in the patient. Or even worse, that a hit is missed that could have been a promising candidate for treatment. A hit that does not seem to work is filtered out in the next research phases, but missing a hit could mean missing a successful drug.

Laboratory animals

The use of animals as a model is much more expensive and time-consuming. And laboratory animals have a clear limitation: they do not always accurately reflect the response of humans to drugs because their physiology and genetic background are significantly different. This means that drugs can be very effective in a mouse model with an inbred genetic background but do not have an effect in humans. Furthermore, there is sometimes a problem with animal experiments if the human gene does not occur in a mouse (as is the case for a certain muscular disease) or if a pathogen for humans is not recognised in the mouse body. An example here is the coronavirus, which causes the Covid-19 disease and enters a human cell via the so-called ACE2-receptor (virus recipient); the virus that makes people sick is not being recognised by the ACE2 receptor in mice at all. Mouse cells thus are not infected and are not representative for how Sars-CoV-2 affects humans.

For specific research purposes, animals are very valuable models, for example for studying cancer, neurobiology, effects of radiation and the distribution of drugs in the body. For heart research they are less so. The heart of a mouse beats 500 times per minute, whereas the human heart rate is about 60 times per minute. Also, the physiology of the heart is very different and drugs that influence the human heart rhythm often have no effect on the mouse heart at all.

Besides ethical objections and the strong wish from society to reduce the number of animal experiments, large animals and even primates (which are evolutionarily closer to humans than mice), are not always the right research model for human diseases or for studying drug effects. Moreover, 90% of the promising drugs that are tested for the first time in humans following animal experiments, immediately fail due to unexpected side effects or because they have no effect at all.

Test persons

Does this mean that the best test model for a human is a human? (see also: Who is the ideal test person?) In principle, that is the case, but only those drugs with estimated minimal risk for the test person can be tested in humans. But you

Life-threatening drugs

DR. BEREND VAN MEER

Models that are used in drug research do not always accurately predict what will happen in a human. It can turn out that a drug does not work or even can have life-threatening side effects, whereas the disorder for which the drug was initially taken maybe was not life-threatening at all. Some well-known examples:

Softenon (thalidomide) – 1957
Softenon was a sleeping medication and painkiller that was popular for controlling morning sickness in pregnant women. Unfortunately, it was not known that this drug caused severe abnormalities in unborn children. Four in ten babies died and the other children had shortened or absent arms and legs and/or defective eyes and ears. It is estimated that about 100,000 babies were affected by Softenon and about 3000 of them are still alive today. These side effects were missed in the animal experiments because, unlike in humans, Softenon could not pass through the placenta of laboratory animals. Since 1961 the drug is no longer available, except for very specific diseases or as an alternative for the treatment of specific forms of cancer and leprosy.

Prepulsid (cisapride) – 1988
Prepulsid was mainly used to reduce nocturnal heartburn. Unfortunately, this drug also caused fatal cardiac arrhythmias. Worldwide, 130 cases are known. The drug was withdrawn from the market in 2000 and is now only available for very specific diseases. These side effects were not observed in the animal experiments on rats, mice and dogs because the effect of the drug occurs by interaction with an ion channel that is exclusively present in humans.

Vioxx (rofecoxib) – 1999
Rofecoxib, a painkiller for arthritis or menstruation, was used by more than 84 million people worldwide. Although popular and successful, long-term use of this drug appeared to cause heart attacks. In five years time, it might have caused 88,000 to 140,000 life-threatening cardiovascular conditions resulting in an estimated number of 27,285 deaths. In 2004 the drug has been withdrawn from the market.

BIA 10-2474 – 2016 (test phase)
Recently, things went wrong with the testing of the new drug BIA 10-2474, developed to stop nerve pain. The experimental drug was tested in France on healthy test persons until five of them developed severe brain symptoms and were hospitalised. One of them died and the study was closed.

In all cases mentioned above, it was thought that, based on the results of the animal experiments, the drug could be safely administered to patients. Unfortunately things worked out differently in practice. This does not mean that experiments with human Organs-on-Chips can completely eliminate this risk. But it is important to test drugs also in human models, because the response in humans might be different from that in animals. And that could save lives.

While the claims against the pharmaceutical company Merck about the arthritis drug Vioxx increased, lawyers firms advertised for claimants in Texas with advertisement boards like this one on Highway 44.

never know for sure (see: Life-threatening drugs). Therefore, people cannot be asked to participate in large-scale tests with many substances. Exceptionally, this is what is happening in the tests for possible Covid-19 vaccines. Furthermore, clinical trials, which are representative for the worldwide variation in genetic background, age, sex, environmental factors and diet, would have to be set up on such a large-scale that this is practically impossible. Thus, even a human is not the perfect model for another human.

Alternative models

Can the process of drug development be made more predictable? In other words, are there alternatives for the current models? The most important candidates can be divided into two categories: *in silico* models and advanced human (stem) cell *in vitro* models, including organoids and Organs-on-Chips.

In silico models

Computer models used to describe biological systems and to predict the effects of drugs are called *in silico* models. *In silico* literally means "from silicon", the material used to make computer chips. These models are developed on the basis of data and insights from laboratory studies, medical studies and, in some cases, animal experiments too.

The exponential increase in computer power in recent years has greatly improved the possibilities and applications of these systems. Bioinformaticians can now model many different organs and organ-organ interactions of the human body. And subsequently, they can also vary a large number of different parameters to quickly test what the effect is of different circumstances. Although *in silico* models are promising and powerful, the disadvantage is that they are dependent on existing knowledge of human physiology, which is far from complete.

Based on data and insights from laboratory studies and medical studies, computer models are developed that can describe biological systems and predict the effect of drugs.

In vitro models

Human cells from biopsies or tissue left over from surgery (primary cells) are used to study diseases or the effects of drugs. However, this material is only available in limited amounts. Therefore human (stem) cells are made that serve as a permanent source for alternative models to improve drug development. As stem cell models can be made for different ethnic groups and sex (see: Where do the cells come from?), these models can contribute to personalised medicine.

Nowadays, many different cell types of the human body can be derived from stem cells (see: How does a body function?). Since these cells come from humans, their physiology is very similar to those in the human body. The culture conditions for human stem cells depend on the user's question. Sometimes two-dimensional cell cultures (cells in a single layer in a Petri dish) are sufficient for a laboratory experiment. Sometimes it is better to culture the cells in three-dimensional structures called "organoids" that may additionally have a protein-like scaffold. And sometimes it is useful to culture cells under conditions that simulate the physical and mechanical dynamics of the body (like fluid flow), as in Organ-on-Chip systems. Organs-on-Chips are the focus of this booklet. The following chapters explain what exactly Organs-on-Chips are, how they can be used and what the latest developments are.

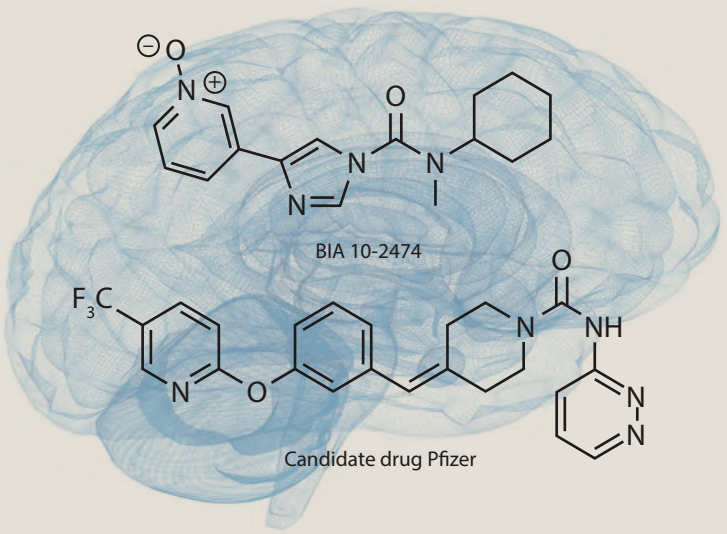
How a clinical test could go wrong

PROF. STEVEN KUSHNER M.D.

The drama took place in the French city of Rennes in January 2016. An experimental drug to treat chronic pain, anxiety or symptoms of Parkinson's disease was tested on healthy volunteers on behalf of the Portuguese pharmaceutical company Bial. Of the six who received the highest dose, five ended up in hospital with severe neurological symptoms; one of them died.

Could this have been prevented?
The drug, known under code BIA 10-2474 (BIA in short), was tested for safety in a phase I study. BIA inhibits the enzyme FAAH *(fatty acid amide hydrolase)* that breaks down cannabis-like substances that are naturally present in the brain, the endocannabinoids. Other pharmaceutical companies, in particular Pfizer, had postponed the development of their FAAH inhibitors (other molecules). This was mainly due to disappointing research on the effectiveness during late phase II studies. According to large clinical trials submitted to the American Food and Drug Administration nothing was wrong with the safety of FAAH inhibitors. Therefore, the French authorities found no evidence that the rules had been violated regarding the performance of the clinical experiments.

What went wrong during the BIA study?
Could the fatal neurotoxicity of BIA have been due to a previously unknown (side) (or off-target) effect on other critical enzymes in the brain instead of acting only as an FAAH inhibitor? A team led by researchers from Leiden University and Erasmus Medical Centre investigated this. They used a technique called *activity-based protein profiling* to screen the activity of BIA on a large group of enzymes in a human Brain-on-Chip model. They discovered that if BIA is administered at high concentrations, the activity of various brain lipases, enzymes that break down fatty acids, is disrupted. This means that BIA can disrupt how brain cells convert lipids, with all its consequences for the brain as a whole. The exact mechanism could not be studied because samples from the volunteers from the Bial study were not available. The drug developed by Pfizer that was found to be safe in large clinical trials only had an effect on FAAH and not on the other enzymes.
It is known that the off-target effects described above can depend on the (animal) species. That would explain why studies with rats and mice could not identify the neurotoxic effects of BIA in humans. Consequently, the European Medicines Agency has now developed stricter rules for *first-in-human studies*. Drug developers first have to perform extensive preclinical tests with a new drug, including how the drug binds to the target protein and whether there are off-target effects, preferably in validated human Organ-on-Chip models, before the step to humans is made.



The chemical structure of BIA 10-2474 and the candidate drug from Pfizer. Both inhibit the enzyme FAAH and only BIA had a neurotoxic effect on the human brain.

Who is the ideal test person?

PROF. JOS VAN DER MEER

That we can now develop and use Organs-on-Chips is the realisation of a dream of generations of scientists in biology, biochemistry and medicine. It began in 1907, more than a century ago, when the initial step was made by embryologist Ross Granville Harrison who developed the first cell and tissue cultures. Since then, enormous progress has been made, with important contributions from researchers in the Netherlands using cell culture techniques that have considerably increased our knowledge of (human) biology and provided new insights into disease processes in the human body. Predictions about the disease progression obtained in these culture systems are also gradually becoming important for finding therapies. By culturing stem cells from sick animals and humans as "mini organs", we come closer to treatments tailored to specific characteristics of the patient. This is often referred to as personalised or precision medicine.

Influence of climate, nutrition and behaviour

In spite of fine-tuning and refining of these cell culture techniques, we still need laboratory animals and test individuals. The form and dynamics of physiological processes in animals and humans are so complex that even the most advanced culture systems are still far from providing a complete picture of the human body. Interactions between organ systems and with the bacteria that populate our organs cannot be entirely simulated. Although we can try to answer questions about the role of sex and certain genetic backgrounds (and for example of ethnicity) in the culture systems, it is much more difficult, if not impossible, to involve factors such as living environment, lifestyle and the influence of nutrition and behaviour in laboratory research. Such influences are intensively studied in what is currently called systems biology and systems medicine.

Searching for the cause of death

Who the ideal test person is depends on the particular scientific question. In the third century BC, people started carrying out "obductions", now referred to as *post mortems* or autopsies. The word autopsy comes from "auto" and "opsis", which means "seeing for yourself". People wanted to know what the (healthy) human looked like inside. The Greek physician Galenus (second century AC) wanted to know the underlying cause of what he had observed by external examination of his patients. Morgagni, Da Vinci and later Vesalius, Servetus and Harvey – physicians, anatomists and scientists during the Renaissance (1400-1600) – were also driven by scientific questions such as how the blood circulation and kidneys function.

We can still learn a lot from *post mortem* research. Moreover, our research techniques have become so refined that we can learn much about causes of death and lethal diseases by means of autopsy. Unfortunately, the number of autopsies performed in recent decades has gradually decreased. There are several reasons for this. One important reason is the misconception that we think we know why a patient has died because of modern medical research during life. However, at least 30% of autopsies result in important unexpected findings.

Is the sick human the ideal test person?

A disadvantage of *post mortem* research is, of course, that we investigate end stages of disease processes when life has ended. For many scientific questions, we prefer to study living organisms. Animal experiments are essential in this regard: experiments with worms, flies, fish, mice, rats, rabbits and monkeys provide essential building blocks for our knowledge about humans because they allow us to change things experimentally: if we take away this gene or add that protein we can see how the bodies of these species react. And that may be sufficiently similar to the reaction in humans that we obtain new clues on how the human body works. The fact that even experiments with "primitive" animals such as worms, flies and fish provide important data for humans is thanks to the considerable similarities (and sometimes also informative differences) in structure and function in biology: these species all have heads and tails, left and right and many different organs in common with humans. However, extrapolating findings from animal experiments to humans remains the ultimate challenge and it is essential to provide evidence for the assumptions made.

Young healthy men

Is the healthy individual the ideal test person? This also depends on the research question. Healthy test persons who participate in scientific research – if they are paid or not –are not always representative of the population as a whole or patients in particular. When a new drug is administered to humans for the first time (phase I) in clinical trials, these are often young healthy men, even if the drug is ultimately intended for old people. This can lead to incorrect conclusions on the benefit or adverse effects on the intended patient group.

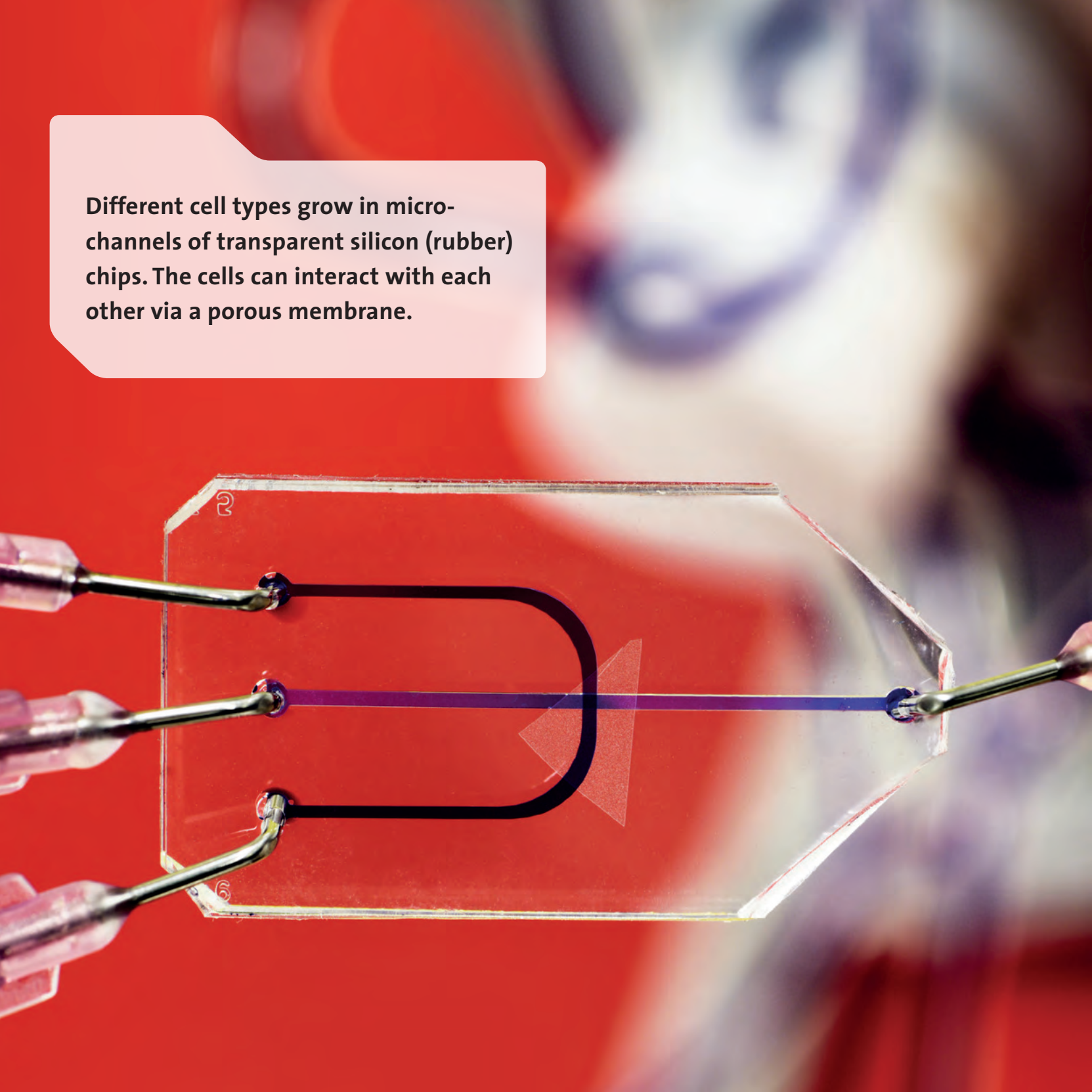
Nevertheless, healthy test individuals are very important. Examples are the testing of endotoxins in volunteers, which is important for research on blood poisoning (sepsis), and of the human malaria model, which is vital for research on malaria vaccines.

When healthy test persons are exposed to interventions, like a drug, a toxin such as an endotoxin, malaria parasites or a new vaccine, the risks for the test person need to be carefully weighed against the potential outcome for knowledge and the beneficial effects for humanity. Fortunately, and actually for the last 50 years, this kind of research requires examination and approval by independent ethics committees. This is also the case for drug and other research in patients.

The sick human

Modern imaging techniques in medicine, such as the CT scan, MRI scan, spectroscopy, ultrasound and even the more basic research on DNA, RNA, proteins and metabolites, offer the possibility to gain many insights from both healthy test individuals and patients.

For me, as a physician, the sick human is the biggest source of inspiration and challenge and thus, despite the limitations, the ideal test person.



Different cell types grow in micro-channels of transparent silicon (rubber) chips. The cells can interact with each other via a porous membrane.

2 What are Organs-on-Chips?

A human body is not simply a layer of cells in a Petri dish that we normally grow in the laboratory. A body is a three-dimensional mixture of soft and hard material that is flexible and dynamic, with blood and lymph vessels that carry fluids and cells under flow through it. The challenge for Organ-on-Chip developers is to capture the specific functions of organs and body parts in a microenvironment (the chip) in which a three-dimensional synthetic tissue can behave as in the body. In order to mimic this, many different scientific disciplines need to work closely together. An important question is: how do you obtain the right cells? How do you enable the tissue in the chip to breathe or contract? And how do you know what happens at the microscale?

How does the body work, and how do you mimic it?

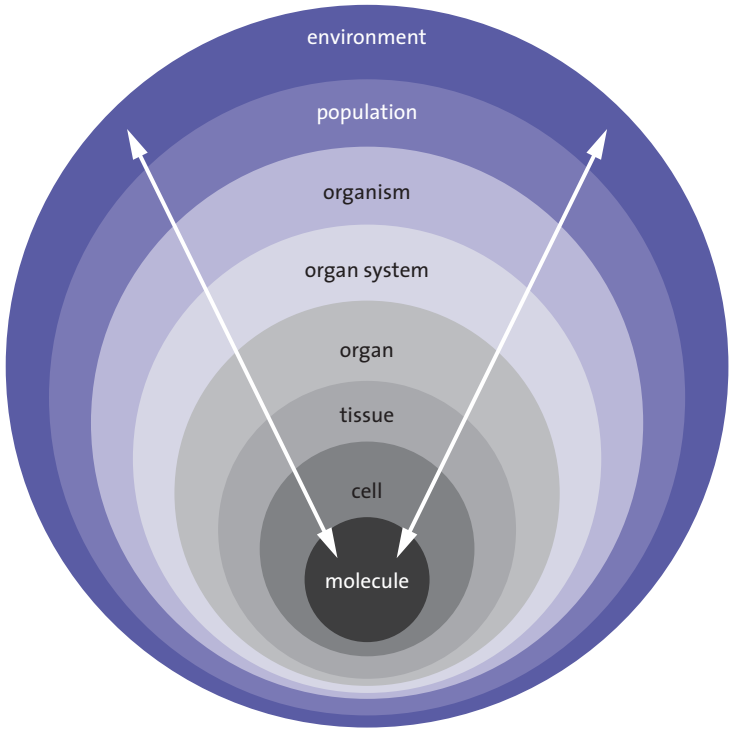
DR. BEREND VAN MEER

The most advanced computer is not that complex as our body. Everything we do, walking, eating, working and sleeping, requires collaboration of about 10 to 100 trillion (that is more than a million times a million, or a thousand times a billion) cells in our body. These cells are not all the same; they have different functions. For example, there are muscle cells, lung cells or blood cells. In order to collaborate effectively, the cells have organized themselves into tissues, for example muscle bundles, lung alveoli or blood vessels. The combination of several tissues forms an organ, such as the heart, lungs or vascular system.

Nutrition and environment

In order to mimic parts of the human body in the laboratory, the body's own cells can be used and the same conditions as in the body can be re-created. Cells are therefore cultured at 37°C at the same humidity and pH value as in the human body. The cells are usually cultured in a plastic Petri dish in *medium*, a liquid that contains a cocktail of nutrients necessary for cells to grow and function. All cells need medium but the composition of the nutrients differs per cell type.

In addition, some cells or tissues also need other



Cells in an organ live in an extremely complex micro- and macro-environment.

specific conditions. Those conditions are referred to as the microenvironment, which often means the biomechanical aspects of the real tissue. For example, the heart continually pumps blood, a blood vessel is used to "feeling" blood flow inside it, and the cells in the lungs experience a constant elongation and stretch due to breathing. If blood vessel cells are cultured in a Petri dish, they form a flat cell layer and the medium is static. Research has shown that these cells function less well than when cultured in small hollow tubes (microchannels) through which the medium flows.

Mutual communication

Even if you could perfectly succeed in mimicking tissues and their microenvironment in a laboratory, you would still not have achieved the complexity of the body. That is because our organs communicate with each other via nerves, hormones or other substances that are transported through the blood circulation. Some organs are more important than others in this interaction. Certain organs control other organs (like the brain or the pituitary gland) or they convert substances in our body into other substances (for example, the liver), whereas other organs mainly let themselves be controlled (for example, muscles).

Healthy and sick tissue

Even if the interaction of organs is under control, that is still not enough. For proper testing of the effects of drugs, diseased, not just healthy tissue needs to be mimicked. By administering a potential drug to such "disease models" it will become possible to establish whether the drug can benefit the condition or even cure the disease.

Diseases have different causes (that include genetic, viral or bacterial origins) and can occur at different levels: some diseases destroy body cells, whereas others obstruct the blood circulation. Therefore, to mimic these diseases, other "parts" are also needed in the model, for example relevant cells with the genetic abnormality (see: Where do the cells come from?) or a virus (see: Models for viral infections) or bacteria (present in the normal microbiome of the gut, lung, and skin for example).

In addition to these aspects of the model, it is important to realise that (except for identical twins) no single human is the same. That not only applies to personalities or fingerprints but also to the rest of the body. For everyone, everything works slightly different and therefore it is almost impossible to develop a single model for all people. Genetic background, age, diet and living environment make people different and this, in turn, causes big differences in how diseases progress and the effectivity of drugs. That is often the underlying reason why one patient is cured by a specific

The first Organ-on-Chip: the lung

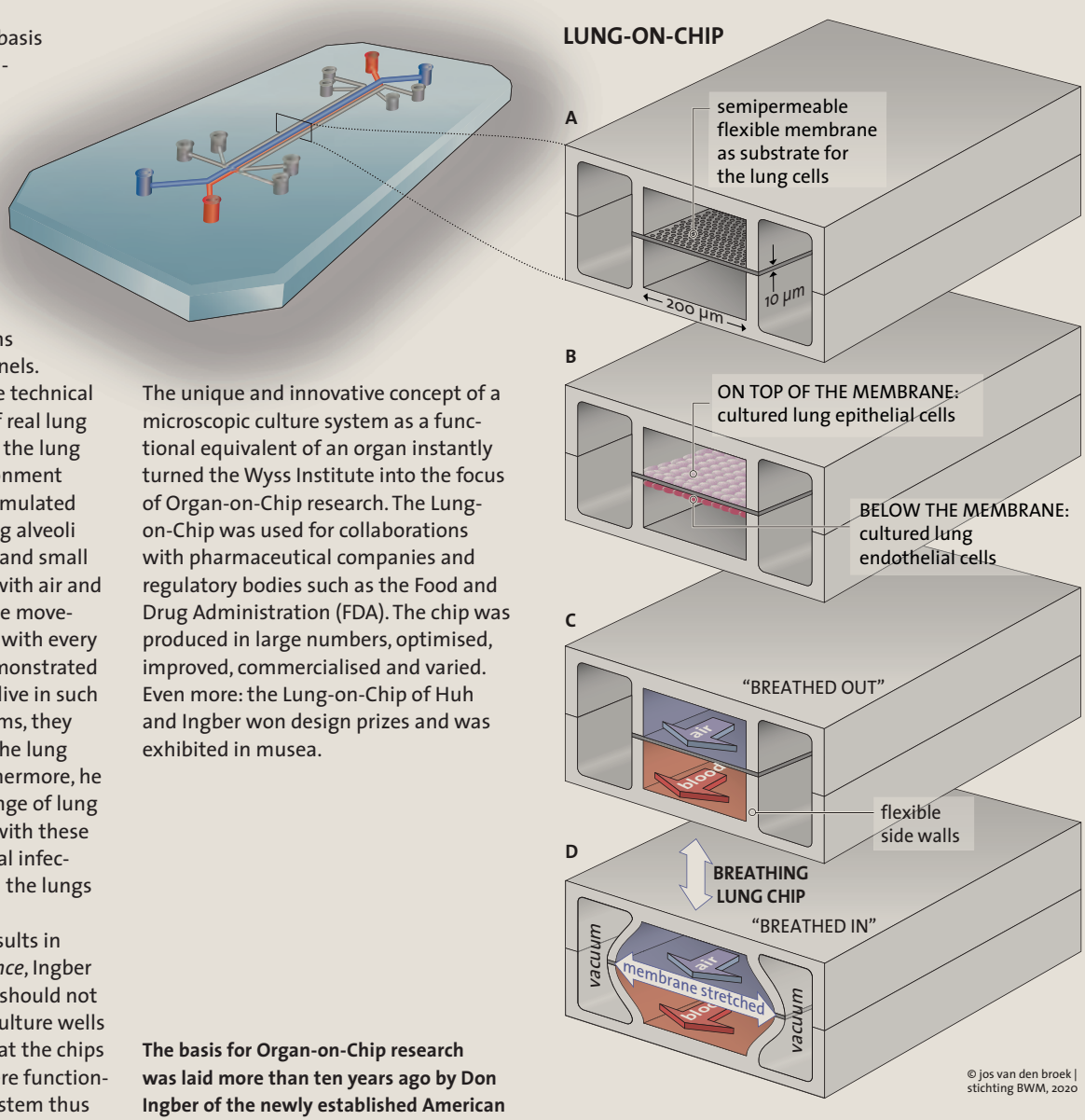
DR. ANDRIES VAN DER MEER

More than a decade ago, the basis was laid for current Organ-on-Chip research at the newly established American Wyss Institute for Biologically Inspired Engineering of Harvard University (wyss.harvard.edu). Researcher Dongeun Huh in the group of Prof. Don Ingber cultured different cell types in "chips" – small culture systems with microscopic fluidic channels. Huh's research focused on the technical simulation of the structure of real lung alveoli. In an attempt to offer the lung tissues in his model an environment as realistic as possible, Huh simulated many different aspects of lung alveoli in his chips: thin membranes and small channels which he provided with air and blood flow. He even copied the movement of the lungs associated with every breath. In his research, he demonstrated that if lung tissues are kept alive in such technical micro-culture systems, they could mimic the function of the lung alveoli in a realistic way. Furthermore, he demonstrated that a wide range of lung diseases could be simulated with these culture systems, from bacterial infections to fluid accumulation in the lungs (oedema).

In the publication of these results in the top scientific journal *Science*, Ingber emphasised that Huh's chips should not simply be seen as advanced culture wells for lung cells. He indicated that the chips and the incorporated cells were functionally "merged" and that the system thus mimicked the function of an entire organ: the Lung-on-Chip was a fact.

The unique and innovative concept of a microscopic culture system as a functional equivalent of an organ instantly turned the Wyss Institute into the focus of Organ-on-Chip research. The Lung-on-Chip was used for collaborations with pharmaceutical companies and regulatory bodies such as the Food and Drug Administration (FDA). The chip was produced in large numbers, optimised, improved, commercialised and varied. Even more: the Lung-on-Chip of Huh and Ingber won design prizes and was exhibited in musea.

The basis for Organ-on-Chip research was laid more than ten years ago by Don Ingber of the newly established American Wyss Institute for Biologically Inspired Engineering with the Lung-on-Chip model.



treatment, whereas the other patient remains sick. The challenge is to be able to predict the effects and solve the problem.

What is needed?

Mimicking the complete body in the laboratory is very complex and difficult to realise. For this reason, it is important to think carefully about *why* something needs to be mimicked. In other words: what is the research question? If we want to test a new heart drug, then the stomach might not be relevant, but it is appropriate to study the interaction with the brain because the brain controls the heart. While the best test system for our highly complex body is in the proces of development, many more simple aspects can already be studied along the way.

The ideal test system for a person would be a system of different (relevant) samples of sick and/or healthy 3D organ tissue with the correct microenvironment, linked to each other by blood vessels and nerves. Ideally, tissues, environmental factors and nutrients should also be specific for the person concerned. As mentioned earlier, systems like this are a sharp contrast with the hard, plastic Petri dishes that are used for most laboratory research. Organs-on-Chips could be the game-changer to simulate the complexity of the body in the best possible way.

An Organ-on-Chip refers to a population of tissue cells in a "smart" micro-environment (chip)

What is an Organ-on-Chip?

DR. BEREND VAN MEER

Organs-on-Chips are small samples of – mostly human – organ tissue cultured on a smart substrate. This substrate has been designed in such a way that the cells of that tissue behave as naturally as possible and can simulate different functions of the human body. The major difference with normal cell culture in a Petri dish is that, because of the smart substrate, researchers can offer the right microenvironment to the cells so that "they feel at home". The more the cells behave as in the human body, the better they will predict what happens in the real human body if it becomes sick or if a drug is administered. Globally, an Organ-on-Chip consists of cells (organ) and a microenvironment (chip).

The cells

Different cell types are needed, depending on the organ model. For a Lung-on-Chip model, lung cells are needed of course but often an organ is not composed of just a single cell type; it contains various other cell types referred to collectively as connective tissue. In lung alveoli, epithelial cells are present on the side where the air flows. These cells are very different from the type of tissue that is present in the lung alveoli on the side of the blood flow through the blood vessels. A single organ may consist of dozens of cell types. Usually, research does not need all cell types. If researchers want to mimic a disease where only blood vessel cells of the lung play a role, then it will usually not be necessary to include epithelial cells in the model.

The next question is which cells are suitable to put in the chips (see also: Where do the cells come from?). Organ specific cells can often be produced from pluripotent or adult stem cells but primary tissues can also be used, such as tissue sections from patient biopsies, or cell lines from organ

specific tumours that keep dividing continuously. Each of these different cell types has advantages and disadvantages, such as availability, genetic background and any patient-specific abnormalities. The cells can be genetically modified, for example by allowing them to produce a fluorescent "label", so that it is easier to monitor their behaviour in the chip.

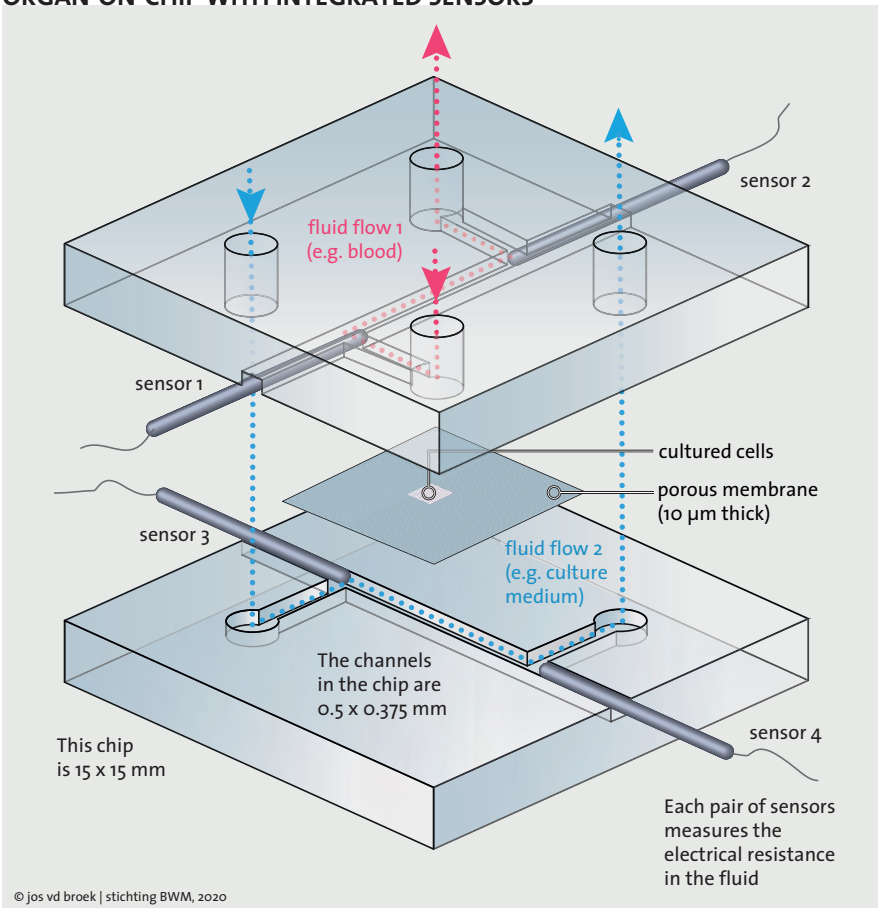
The microenvironment

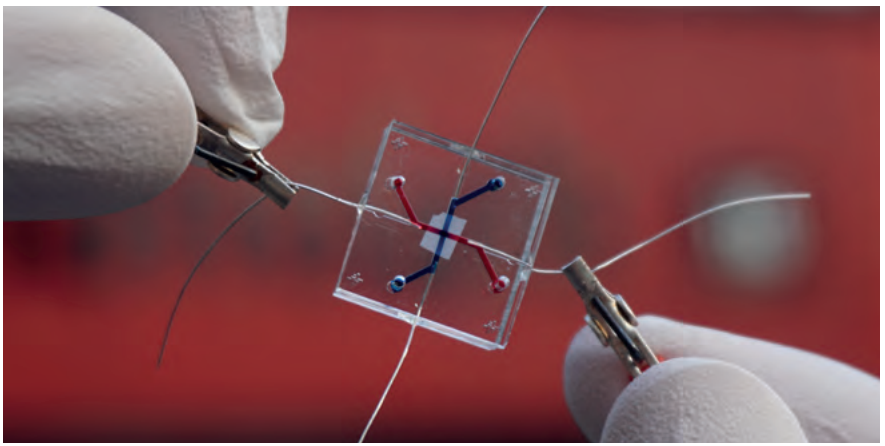
The chip must be designed such that it can mimic the natural environment of the cells. Imagine that a Blood Vessel-on-Chip is needed. A Petri dish, with the blood vessel cells in a flat layer is clearly not an option since, although a network of vessel like structures is often formed, it is not a round tube as a normal blood vessel (it has no "lumen"). So, in this case, a chip or 3D culture environment is required. In the chip, which already has the format of a round tube, the blood vessel cells can grow on the wall and the diameter of the tube can be controlled to mimic everything from an artery and vein to a tiny capillary. The geometry of an organ is an important aspect for the designers. For studies of two different cell types, as in the Blood-Brain Barrier-on-Chip (which mimics the blood vessel "barrier" protecting the brain from invading bacteria or immune cells), porous membranes are a useful substrate for culturing the cells.

However, there is another major difference between the Petri dish and the real body. A Petri dish as we have said before is hard, inflexible and lacks movement, whereas the body consists of soft, flexible tissues that are continuously moving. For this reason, the chips are often made of a soft material such as silicone rubber. Rubber can mimic the flexibility and elasticity of the body very well. If the chip is developed in the right way, the cells can even be exposed to mechanical elongation and stretch (see: The first Organ-on-Chip: the lung).

Finally, there is another very important aspect,

ORGAN-ON-CHIP WITH INTEGRATED SENSORS





A mixture of disciplines

DR. ANIKA NAGELKERKE

Usually you do not ask a plumber to install electricity or an electrician to paint the walls and window frames. However, bringing together the expertise and knowledge of different professionals, results in a house in top condition. The same holds for the development of Organs-on-Chips. For this purpose, a technologist is not asked to culture the cells, or a cell biologist to design the chips. Everyone has their own expertise but many different disciplines must closely work together to mimic processes in the human body, even more than for building a house.

For organ-on- chip research it is important to facilitate collaboration between different disciplines.

Mimicking a human body

The human body is an incredibly complex system in which dozens of organs and tissues each carry out their own task and at the same time collaborate in a very intelligent way. Asking researchers from different disciplines to mimic the human body would probably result in a wide range of different ideas. A doctor sees a complete system of different organs, a biologist thinks about structures with functional cells, and a chemist sees a variety of molecules and chemical reactions. A physicist will present a wide range of physical processes: from diffusion to conduction of electrical signals. The engineer will design an ingenious system of tubes and filters driven by a pump system and a central computer. All these insights are necessary to develop realistic Organs-on-Chips.

Collaboration is essential

The challenge is to bring the different disciplines together. In practice, this is not simple. Each discipline speaks its own language. Furthermore, researchers have more and more become experts in a specific field. By setting up special working groups in the field of Organs-on-Chips, where the different scientific worlds come together and ideas and results are being shared with enthusiasm, the first step is taken towards understanding each other and collaborating. And then suddenly one plus one becomes three and the collaboration has concrete added value. Especially now that chip systems are becoming increasingly complex and can mimic the human body even better. In other words, development of Organs-on-Chips definitely requires a multidisciplinary approach.

that is relevant throughout the body, but not in a Petri dish, namely fluid flow. While the "surrogate blood" (medium) in a Petri dish is static, in the body the blood is flowing continuously. It is not surprising that blood vessel cells behave more naturally when they are exposed to a flowing medium. Micropumps are needed to pump the fluid through the microchannels.

Cell layers or cell clumps

Organoids differ considerably from Organs-on-Chips. Organoids are three-dimensional cell cultures, usually a clump of cells "swirling" around in a culture medium, which, in general, completely organise themselves and differentiate without external control. The resulting structures resemble a small biopsy from a real organ or tissue. They have great value as "stand alone " systems for drug and disease research, particularly of cancer. However, they do not usually contain blood vessels or blood flow.

Cell cultures in Organs-on-Chips by contrast are controlled by the microenvironment and are exposed to fluid flow. These cell cultures thus do not need to be three-dimensional. They usually attach to the chip material so that they do not flow away when the liquid is pumped through. Organoids have been used in Organ-on-Chip systems, but the fluid flow does not enter the relatively thick three-dimensional cell culture, unless there is a (synthetic) vascular system, or when the three-dimensional structures are opened up.

Smart chips

Chips are often made by micromanufacturing techniques. This is necessary to produce the small geometric shapes that are about the same size as the cells (about 10-100 micrometres) or vessels. Similar techniques are used to produce chips for smartphones and computers. That makes it relatively simple to make these chips "smart", incorporate sensors and link them to readout methods. For example, in heart tissue models, electrodes can be incorporated in the chip, which can measure the electrical activity of the cells, resembling an electrocardiogram. One of those electrodes can also be used the other way around to transmit an electrical signal that initiates the contraction of the heart muscle cells. This is similar to a pacemaker.

Organ-organ interaction

Via the blood vessel channels, several Organs-on-Chips can be interconnected to examine the interaction between organs. For example, the influence of certain nutrients on the gut can be determined and the resulting indirect effect on the brain. Or the occurrence of side products can be examined, that might be harmful for the heart and are the result of breakdown of the drug in the liver. The interaction of the different organs is often crucial for determining the safety of a substance or drug.

Consequently, there is no single Organ-on-Chip model but as many models as one can imagine. There is also no single model for heart muscle tissue either. Some researchers are interested in the contraction of the heart after the administration of a drug, whereas others want to know how the drug influences the electrical conduction in the heart. That requires a different tissue size and other cell types. In this way, each time the cells, chips, sensors and flow and movement are precisely tuned to the required organ tissue and research question, and that largely increases the number of options.

There are as many Organ-on-Chip models as there are organs (and more)

Where do the cells come from?

PROF. CHRISTINE MUMMERY AND DR. VALERIA ORLOVA

Pluripotent stem cells can develop into all cells in every organ of the body

THE HUMAN body consists of 10-100 trillion cells with about 200 different cell types in 78 organs. Amazingly, all these cells are derived from one single cell: the zygote, an egg cell which is fertilised by the sperm. The formation of the zygote is followed by a rapid cell division and specialisation in different cell types. This is called differentiation and the structure formed is called the *blastocyst*. The blastocyst consists of about 100 cells, which can divide into several hundred cells in just a few hours. The blastocyst consists of *an inner cell mass* (ICM) which becomes the embryo itself and an outer layer of cells, called trophoblast, which develops into the placenta. The ICM can be removed from the blastocyst, and the cells can be stimulated in a Petri dish to divide. This is how human embryonic stem cells are obtained. There is usually an excess of human blastocysts due to the considerable number of egg cells that must be fertilised for successful *in vitro* fertilization treatment (IVF). Human embryonic stem cells (hESCs) have the same development potential as the ICM and can form every cell type of the human body.

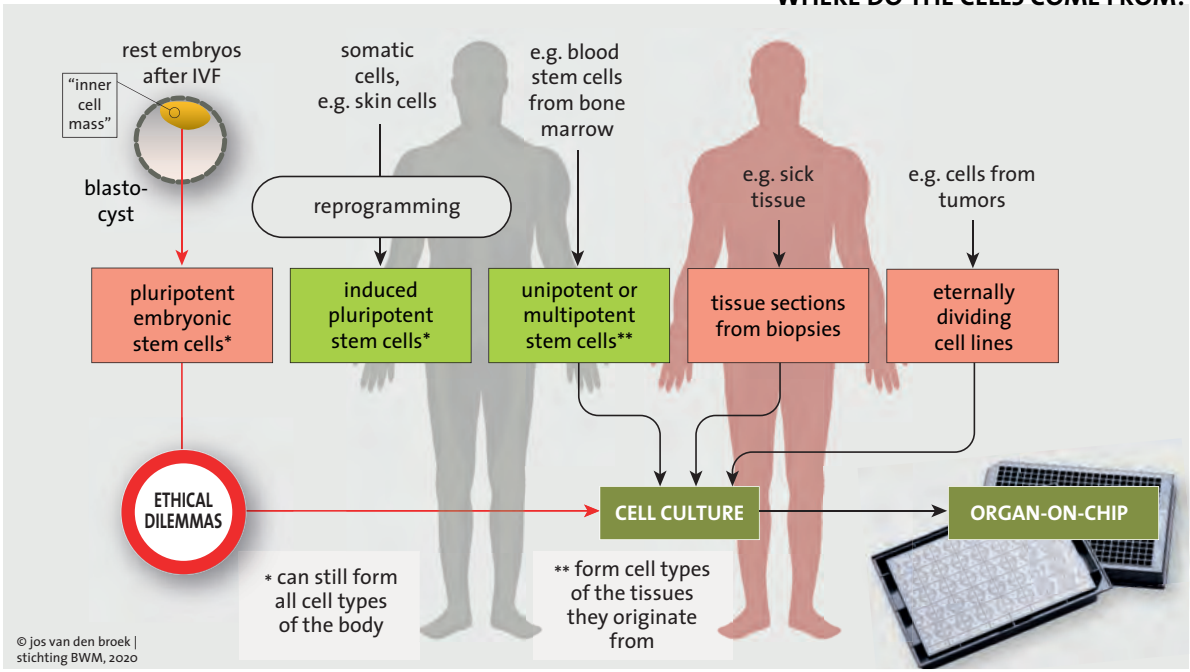
Reprogramming cells

Due to their origin, ethical objections are associated with the use of hESCs, and in almost all countries their use is strictly regulated. Some people consider the destruction of embryos to be comparable to destroying a living individual. A scientific breakthrough, which provided an alternative to avoid this ethical issue, was the discovery that cells which are similar to hESCs can be obtained via a molecular "trick" – genetic reprogramming – in which ordinary body cells are converted into human induced pluripotent stem cells (hiPSCs). Researchers can now produce hiPSCs from almost every type of adult cell, including skin-, blood- or hair cells and even cells present in the urine. Just like hESCs, hiPSCs can also be used to produce many different cell types that are present in various organs of the body, and that would otherwise be very difficult to obtain, such as cells from the heart, brain, lung, liver, gut, and also blood vessels.

The major advantage of hiPSCs is that these can be made from the body cells of each separate individual, both sick and healthy people. hiPSCs can be kept in the laboratory for an unlimited time. They can be genetically modified to produce different cell-specific "markers" such as fluorescent proteins, as a result of which the behaviour and identity of the cells can be followed and controlled (see: How do you know what happens in a chip?). Also, specific genetic mutations can be inserted or repaired, such as replacing a single DNA building block (nucleotide) but also larger pieces of the DNA (chromosomal translocations). Consequently, it is possible to produce genetically equivalent pairs of hiPSCs, with and without the mutation that causes the disease, as a result of which correct comparison between the diseased and healthy cells is possible. hESCs and hiPSCs are jointly referred to as pluripotent stem cells or hPSCs because they can develop into all cells from all organs in the body.

Adult stem cells

Besides hPSCs, there are also other stem cells, which are present in the adult body. Unlike hiPSCs – which can still become all cell types of the body – adult stem cells are undifferentiated precursor cells that can only become one or several specific cell types. They are unipotent or multipotent. These cells are present throughout the entire life of an adult and facilitate the growth of organs (during childhood) and the repair of these. The best-known adult stem cells are blood stem cells (haemopoietic stem cells). These cells are present in the bone marrow and throughout the entire life produce



There are several possible sources of cells for Organs-on-Chips: primary cells directly from donated tissue, tumor cell lines or stem cells. The stem cells include human embryonic stem cells from an early (discarded) IVF embryo, induced pluripotent stem cells derived by reprogramming any cell of the body, and uni/multipotent stem cells from adult organs. The pluripotent stem cells can develop into all cells of the body, uni/multipotent adult stem cells into one or more cell types of the tissues from which they were collected. In addition, immortal cell lines and intact pieces of tissue (tissue sections) from biopsies can be used in the chips. Which cells or tissues are best depends on the particular research question.

all types of blood cells (red and white blood cells, and immune cells). Many organs that are continuously renewed or are highly sensitive for damage contain multipotent stem cells, such as the gut, the skin, the hair roots, the liver, the bone and skeletal muscles. However, some organs, such as the heart, do not have adult stem cells. Therefore, for the heart, hiPSCs or hESCs are the only way to culture organ-specific cells in the laboratory. In recent years, extensive research has been done not only to develop improved methods for the differentiation of hESCs and hiPSCs into organ-specific cell types, but also to develop new methods for the so-called "direct reprogramming" of adult cells or hPSCs into organ-specific cells.

Becoming mature

The most important question is how similar the cells, derived from hPSCs, are to the original cells in the human body. Many of the cells that are currently derived from hPSCs are very immature, and they are more similar to cells from a foetus. Whether it is possible to allow these cells to become more adult (mature) in the laboratory and whether they can be used to simulate age-related disorders is a question that needs to be answered in the coming years.

What are suitable materials for the chips?

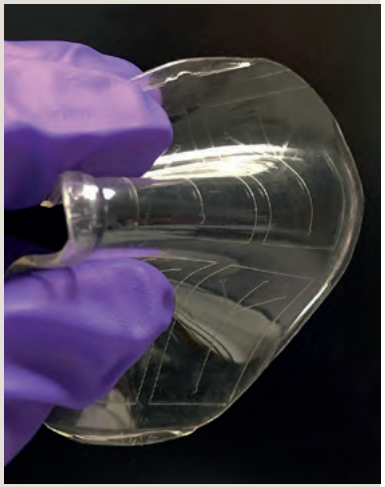
PROF. JAAP DEN TOONDER, DR. MASSIMO MASTRANGELI AND PROF. ROMAN TRUCKENMÜLLER

Materials used for the chips need to meet many requirements. First of all, they should be "cell-friendly", or biocompatible and should not have a negative effect on the cells and tissues. They should also be flexibile (mechanically movable), optical transparent (for microscopy) and manufacturable (amenable to production in a factory).

Rubber chips
Rubber polydimethylsiloxane (PDMS) is the mostly used material for research and development of Organs-on-Chips. This material meets many of the requirements above. It is biocompatible and permeable for oxygen so that it is very cell-friendly. It is also optically transparent for microscopy, very flexible so that it can be actuated mechanically, and it can be produced with high precision in all shapes via a simple moulding process. For this reason, PDMS is a preferred material, in particular in the research phase of chip development, for quickly designing and testing Organ-on-Chip prototypes. However, PDMS also has significant disadvantages, as a result of which it is less suitable for industrial production. First of all, the material absorbs small molecules, including potential drugs, in a non-specific way that is difficult to predict. This is a problem for its use in drug development since it is very difficult to determine exactly what concentration of a drug cells will be exposed to. Secondly, the PDMS manufacturing process is difficult to scale up because it is time-consuming, so that cost-effective production of chips cannot be realised.

Plastic chips
Research on alternative materials is increasing. Plastics like polystyrene and polycarbonate are suitable for cell culture and they are optically transparent. Furthermore, these materials can be produced on a large scale by industrial processes such as injection moulding. However, these materials are less flexible than PDMS, so that also alternatives such as polyurethane and hydrogel are being investigated. Although these are flexible, they also have disadvantages, including limited transparency (polyurethane), and manufacturability (hydrogel).

Silicium chips
Silicon is a traditional material used in the electronics industry for the fabrication of microchips in combination with oxides, nitrides and metals; silicon chips can be produced on a large scale and with high precision. However, silicon is not optically transparent and is very rigid, and these are major disadvantages for Organ-on-Chip applications. The future probably lies in the combination of different materials in hybrid chips – for example integration of soft, rubber-like materials in silicon chips or in the stiffer plastic chips. These possibilities are currently being investigated by an increasing number of Organ-on-Chip scientists and technologists.



The ideal chip material is transparent, flexible and biocompatible and also easy to produce in large quantities.

How do you make a chip that mimics a body?

PROF. JAAP DEN TOONDER, DR. MASSIMO MASTRANGELI AND PROF. ROBERT PASSIER

Fluid flow in microchannels of Organ-on-Chip devices provides cells with nutrients and removes waste

Laboratory-on-Chip

Microfluidic systems were originally developed to scale down complex biochemical processes that take place in laboratories and to integrate these on a chip of a few centimetres in size. In these "Lab-on-Chip" devices, blood samples for medical diagnostics can be analysed. Their advantage is that a medical test can be performed with just a few drops of blood, a minimum amount of reagents and in much shorter time than in standard laboratory tests. In this way diagnostic testing becomes faster, less expensive and often more precise.

A microfluidic chip contains small reaction chambers in which biochemical reactions take place. The chambers are connected by microchannels in which a fluid can be transported in a controlled way to accurately manage this process.

Culture Chambers-on-Chip

Microfluidic chips also form the basis for Organs-on-Chips. The chambers are advanced culture compartments, often separated by a porous membrane, in which three-dimensional tissues, often consisting of several cell types, can be cultured. The microchannels provide the cells with the necessary nutrients and remove waste. But they do much more. The microchannels in the chip that can be precisely engineered, make it possible to provide the mini organs with the correct (bio)chemical environment, as in the body. Oxygen and carbon dioxide levels and the acidity (O_2, CO_2 and pH) can be precisely set, but also gradients that occur in our body can be simulated.

Manufacturing chips

Microfluidic chips are frequently made from a high-grade polymer, such as polydimethylsiloxane (PDMS), a type of rubber (see box: What are suitable materials for chips?). This soft, silicone material is transparent, permeable to oxygen and flexible and easy to process. The PDMS monomer is poured into a precisely machined mould and left to harden. After removal of the mould, small channels are visible in the PDMS layer and half of the chip is ready. In many chips, a porous membrane is applied on top of the channels, on which the cells later can be grown. By connecting two of these PDMS halves the final chip is made. This process is called soft lithography and is suitable for the fast and inexpensive design and production of chips for small-scale tests, mostly for use in university laboratories.

Another technology for the production of chips for mini organs is silicon wafer technology. This technology is also used for chips in smartphones or computers. The production takes place using equipment in a cleanroom, a special working environment, which is designed to exclude contamination of products by the presence of dust particles. With this conventional and standardised micromanufacturing process, large-scale production of robust and reproducible chips is possible.

Force and stiffness

Endothelial cells (blood vessel cells) are grown on the walls of the microchannels to mimic blood vessels. Endothelial cells, just like many cells, are sensitive to mechanical stimuli. Blood exerts force on the blood vessel wall as it flows through. This force determines how the endothelial cells organise, line up in the direction of the flow and behave. By very precise control of the fluid flow in the microchannels, circulation of the blood can be realistically simulated in the chip.

Another important environmental factor that determines the behaviour of cells is the stiffness of the environment. In our body, different organs have a different stiffness. Think for example of strong and solid bones or flexible, elastic skin. Through the right choice and the design of materials in the chips, it is possible to recapitulate this mechanical microenvironment and control the behaviour of cells.

Electrical stimuli and actuation

Active stimuli are also important for the function of certain tissues, such as muscle cells or heart cells that need electrical stimuli. Thanks to microfabrication technology, it is possible to integrate electrodes in microfluidic chips that can deliver electrical stimuli to cells in the culture chamber, just as in the body. This is important, for example, for the development of a Heart-on-Chip. In the heart, specialised heart muscle cells (pacemaker cells) produce electrical impulses that activate other heart muscle cells. As a result of this, the heart can pump blood to other organs in a coordinated way. In the chip, the pacemaker function is realised by an electrode that produces electrical signals at the frequency of the heartbeat, so that neighbouring heart muscle cells synchronously contract.

By making use of flexible materials, tissues can be actuated, or forces can be exerted on the cells. Breathing by the lungs can be mimicked in a Lung-on-Chip. For this purpose, the lung cells can be cultured on a flexible membrane, which can be repeatedly inflated with a pump system. The actuation that is realised in this way, simulates that of

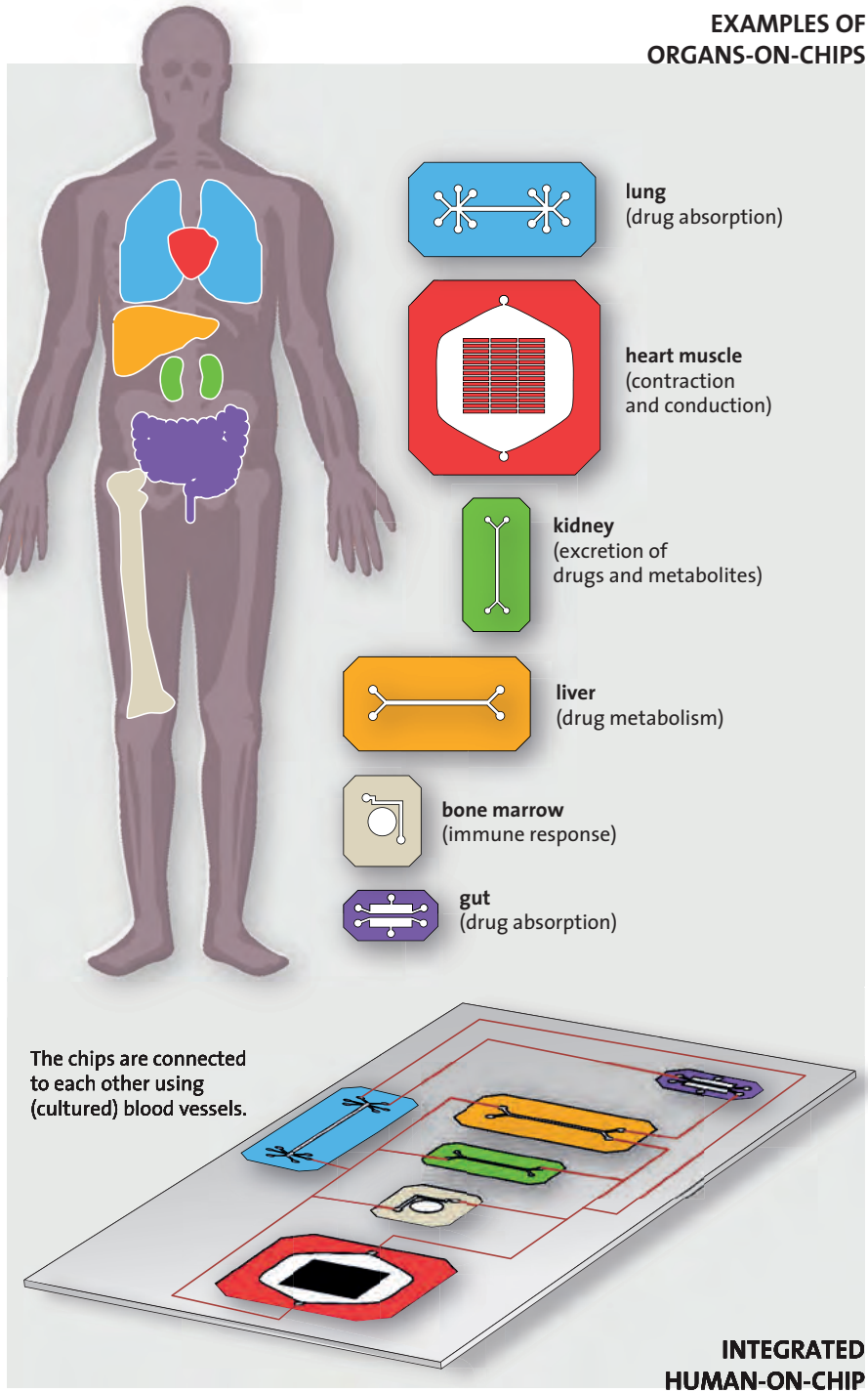
The Microfab Lab at the TU in Eindhoven is an advanced microfabrication facility, including a cleanroom, for the development of microsystems for Organs-on-Chips and related research. The yellow light protects the materials used from damage by UV radiation, present in daylight.

the lung alveoli during breathing in and out (see also: The first Organ-on-Chip: the lung).

Connecting culture chambers

Finally, it is technically possible to develop very complex chips, which consist of several interconnected mini organ models, each in their own specific microenvironment. The microchannels with blood vessels connect the compartments with mini organs and enable the exchange of proteins, cells and other substances, and thus the interaction between the organs, just as in our body. The ultimate vision of some is developing a "Body-on-Chip".

Together, all these technologies ensure that Organs-on-Chips provide much more realistic models than the current simple cell cultures in a Petri dish. The more precisely the microenvironment of organs in the body can be recapitulated in the chip, the more reliable Organ-on-Chip models will be for a wide range of applications, including a better understanding of the function of healthy and diseased organs, or the testing of drugs.



Self-organisation or control?

PROF. MARCEL KARPERIEN AND PROF. JOS MALDA

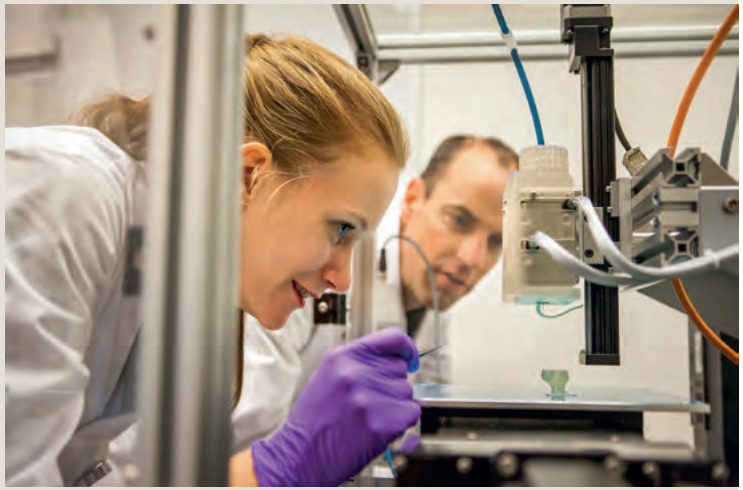
The organs in our body consist of a large number of different cell types that are organised in a hierarchal structure, that is typical for each tissue. Examples are different tissue-specific cell types, endothelial cells, nerve cells and lymph vessel cells to name but a few. These structures have developed through millions of years of evolution and they have been "selected" for specific functions of tissues. For research on the function (physiology) of a healthy or diseased organ system in an Organ-on-Chip, it is important to simulate this hierarchal tissue structure as precisely as possible. But how do you do this?

Spontaneous organisation

Broadly speaking, two different approaches are possible to mimic the natural structure, either by self-organisation or by control. During embryonic development, the (stem) cells that together form a new organ system, spontaneously organise themselves in the right hierarchy. We can simulate this process in a chip by directly combining tissue-specific stem cells with endothelial and nerve cells. Under the right culture conditions, the cells spontaneously organise into the typical tissue architecture and small blood vessel structures are formed. The disadvantage of this process is that it takes a long time and that there is no control over the format of structures that are finally formed. This means that each Organ-on-Chip is slightly different. The same holds for the test results; the response of the mini organs to a certain test stimulus will differ more strongly. That can be a disadvantage if you want to compare conditions in a large number of mini organs.

Bioprinting

Bioprinting can be used to accelerate this process of organisation. It is a technique that allows living cells to be printed layer-by-layer in a three-dimensional structure without losing their function or activity. Using this technique, the formation of the hierarchical tissue structure can therefore be controlled by reproducing the natural organisation at the cellular level. The building blocks in bioprinting are different types of "bio-ink", which are often a combination of living cells with a hydrogel. These hydrogels have the characteristics of the tissue-specific extracellular matrix, a kind of three-dimensional support structure, that is necessary for replicating a mini organ. Subsequently, the printed cells in turn have to form a functional unit. By controlling the tissue-forming process via bioprinting, we are able to develop complex mini organs in a much faster and more reproducible way.



Bioprinting of living cells to form a three-dimensional mini organ in the laboratory of UMC Utrecht.

How do you know what happens in a chip?

PROF. ERIK DANEN AND PROF. LOES SEGERINK

THE VALUE of every model system, and thus also of Organ-on-Chip models, is determined by the information you can extract from it. How do you know what happens in an Organ-on-Chip? What can you measure? And how does that information help you to understand how the mini organ develops, how it functions and how it responds to drugs or harmful substances, for example?

Gene expression patterns of cells reveal how similar mini organs are to the real organ

Gene expression and metabolites

Just like for a real organ, the function of tissue in an Organ-on-Chip is determined by the individual cells and their collaboration. Researchers can study whether the cells in the chip develop into a functional mini organ by looking at the gene expression pattern. That means that they map which genes in the cells are "on" and which are "off". That pattern is different for each organ and therefore provides information whether the Organ-on-Chip resembles the organ to be mimicked.

Nowadays, it is relatively easy to determine a gene expression pattern. The sequencing of the human genome at the beginning of the 21st century, was followed by a technological revolution, which led to what is called *next-generation sequencing* technology. This technology can be used to quickly determine, in a cost-effective way, the "on" or "off" position for all genes at once by measuring the quantity of RNA per gene. When a gene is switched on and is being expressed, its DNA is read and translated into RNA. In turn, the RNA is translated into a protein.

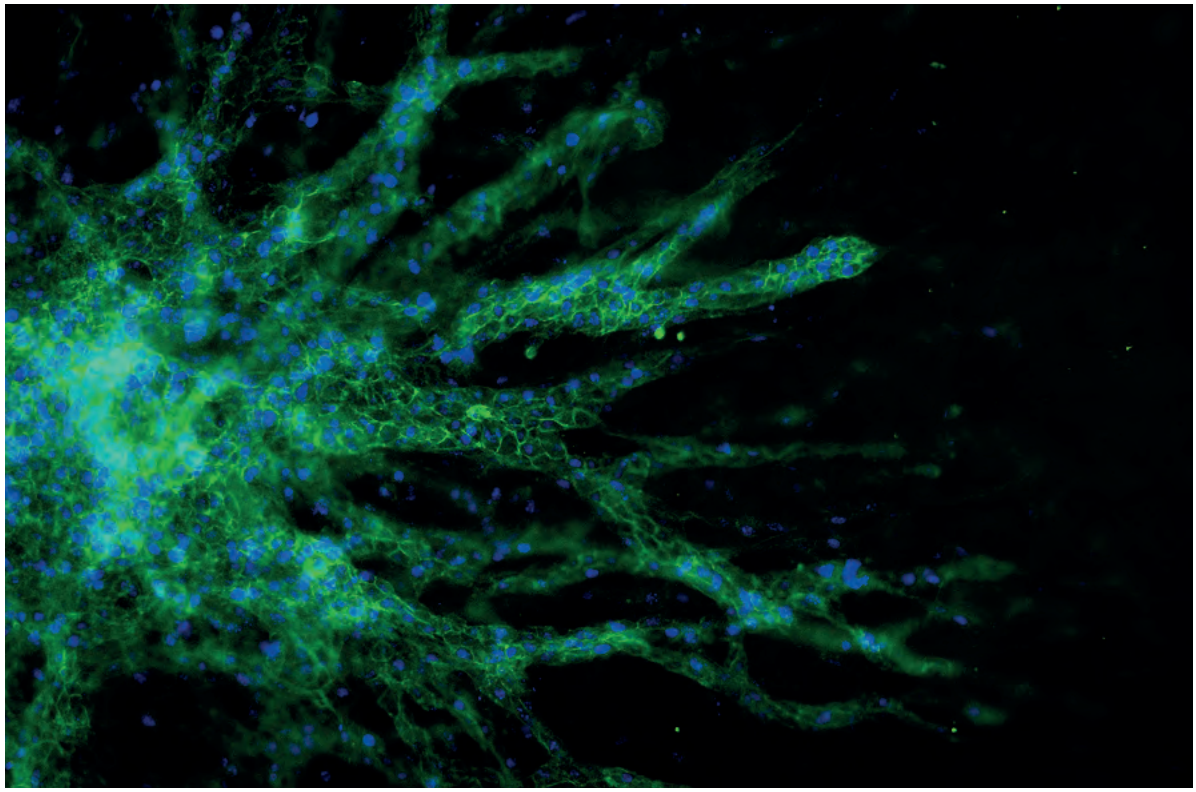
The gene expression pattern can also be used to study how an organ responds to changes, for example when it is exposed to a drug or harmful environmental factors. Repeated isolation of RNA does require sufficient cell material, which is not present in large quantities in most Organ-on-Chip models, because they are miniaturized. Fortunately, however, the sensitivity of the technology has greatly increased in recent years, so that very little RNA is needed to determine a gene expression pattern. Other molecules in the cell can also be analysed now from very small amounts of material. *Metabolomics* is a promising technology in this respect. Based on small molecules (metabolites) this technology can determine the chemical fingerprint left by cellular processes. Besides gene expression, this provides a unique picture of the tissue activity under normal or stressful conditions.

Tissue biopsies

Gene expression pattern and metabolite analyses are only possible if the Organ-on-Chip model is accessible for taking a biopsy. A biopsy can be a piece of cell tissue, like tissue of a patient, but also a small sample of liquid that circulates in the chip, similar to a blood sample from the patient. The advantage of such a fluid sample is that it can easily be taken several times without damaging the mini organ. Cells that have detached from the tissue and RNA molecules and metabolites that have leaked from the cells can be isolated from such a fluid biopsy. Also "signalling molecules" such as cytokines and hormones can be detected, which are produced by the mini organs in response to changes in the environment.

Barrier function and sensors

The barrier function of an organ is often also measured in an Organ-on-Chip. This function indicates how easily certain substances from the environment or blood can end up in the organ. An example is the blood-brain barrier. In a healthy state, this barrier ensures that harmful substances from the



Fluorescent reporter genes can be used to monitor the growth of cells and the expression of certain genes in the cell.

blood cannot simply enter the brain and cause damage. A permeability test can be performed to determine the barrier function of the Organ-on-Chip. This is done by placing a certain substance with a recognisable label, often a fluorescent molecule, on one side of the tissue layer, and by examining how much of this substance ends up on the other side after a certain period.

Another possibility is to integrate sensors in the Organ-on-Chip, for example by placing electrodes on both sides of the barrier. The resistance, that is being measured indicates how "tight" the barrier is, which is determined by the junction of cells in a cell layer. This method can also be used to measure the development of 3D structures, such as the formation of villi in gut epithelium.

Even more sensors can be incorporated in an Organ-on-Chip. These include sensors that monitor the pH, oxygen tension, temperature and fluid flow. All these data enable precise monitoring of the behaviour of the mini organs during the experiment.

Microscopy and fluorescent proteins

These different technologies make it possible to study the behaviour of the organ at the *tissue level*. An Organ-on-Chip contains different types of cells, just like a real organ. Analyzing these cells individually requires a different approach, namely microscopy. By fixing the tissue in the organ model and by treating it with specific stains, the tissue structure and the presence of important proteins in the cells can be visualized under the microscope. This allows mapping of certain characteristics of

the different cell types in the organ model. The disadvantage is that this analysis can only be done once, because fixation and staining are irreversible.

In recent decades, huge steps have been made in the development of advanced confocal laser microscopy that allows real-time studies of individual cells in the tissue. These microscopes are now controlled by robots and can rapidly create large numbers of detailed images. For microscopy the Organ-on-Chip model needs to be accessible. There are specific requirements regarding the choice of material for light transmission, so that only transparent chips are suitable (see box: What are suitable materials for the chips?).

By combining confocal laser microscopy with cells, containing so-called *reporter genes* in their genome, researchers can even more precisely examine cell processes. Reporter genes consist of the gene of study, linked to a gene for a fluorescent protein. As a result, the cells produce a fluorescent variant of the protein of interest. In this way, the production and even the location of the protein in the cell can be studied at the individual cell level in the Organ-on-Chip. This method can be used for proteins that are characteristic for a specific cell type activity, or proteins that are involved in a cellular response to stress.

By combining all these methods, it is possible to study biological processes with an unprecedented resolution, such as how tissues and cells in organs function but also the course of disease processes or the response to harmful substances. Questions such as "Which cells in a tissue are responsive to a therapy?" and "Which genes are on or off in different cell types in healthy or diseased tissue?" can then be answered using an Organ-on-Chip model.

Organs-on-Chips, what is possible? And for now, what not?

What can be mimicked by Organs-on-Chips, now or in the near future?	What cannot be mimicked by Organs-on-Chips yet?
– The function of many healthy individual organs, such as heart, lung, liver, gut, skin, kidney, bone, muscles and blood vessels. Example: for Heart-on-Chip it has been shown that all important internal mechanisms, responsible for contraction in the heart, are present and function as in the human body.	– development of embryos – reproductive organs – effect of ageing – immune system responses – the intact brain
– The loss of function of organs due to disease or damage suffered Example: with Lung-on-Chip it has been shown that a viral infection can be simulated and that the infection proceeds differently in a lung that does not "breathe" than in a lung with periodic stretching (to simulate breathing).	– damage to cognitive brain functions, such as memory – the onset of cancer and metastases – effect of radiation
– The interaction of organs with each other. Organs-on-Chips that are at this level of development: lung, heart, liver, gut, skin, muscles and blood vessels. Example: in a study where a Gut-on-Chip, Liver-on-Chip and Kidney-on-Chip were connected, it was possible to simulate how fast nicotine (for example from chewing gum) can be taken up in the body via the gut and subsequently secreted via the kidneys.	– spread of drugs through the entire body – scar formation in the heart after a heart attack – recovery of the brain after a stroke

What is still needed?

Very precise knowledge is still needed about many of the small steps in different biological processes, such as embryonic development or tissue aging and how this can be influenced. In some cases, it is not yet possible to produce the right cells in the laboratory. Today only a few simple cells of the immune system can be produced, although this plays a very important role in this sort of complex biology. Many leading scientific studies aim at obtaining this knowledge, also for the application in Organ-on-Chip models.

What could be the impact on health

NORA FRANZEN, MSC AND PROF. MAARTEN IJZERMAN

Imagine: cancer patients who receive a treatment that is tailored to their genetic profile, a food industry that checks ingredients regarding their allergic potential, or public health institutes that can better monitor harmful substances in drinking water. This might become possible thanks to new Organ-on-Chip technology.

While this technology is still in an early phase of development, there is certainly enthusiasm about possible areas of application. Among the most promising are personalising medical treatments and the development of new drugs in the pharmaceutical industry.

Healthcare costs

The research agency IMS (Institute for Healthcare Informatics) in the United States estimated he global spending on medicines to reach 1.26 trillion euros in 2020. That is an increase of 35% over the past five years. In this market, there is a considerable need for more efficiency in drug development to reduce the costs of innovative treatments, and secure access for more people. Technological innovations that enable this are currently one of the major challenges in healthcare at the moment.

The pharmaceutical industry, universities and regulatory bodies are continuously seeking to improve drugs and make them available as quickly as possible. Although the pharmaceutical industry does not publish the actual costs of drug development, it is estimated that these vary from 552 to 2520 million euros per new drug.

Besides the direct costs for research laboratories, there are two other big cost drivers: the long development time of a drug that takes five to ten years due to time-consuming and costly clinical trials, and the limited success rate of an effective and safe drug because potential drugs drop out throughout all phases of research. This explains the great societal interest in increasing the efficiency of the drug development process. This could be realised by reducing the research and development (R&D) costs or by increasing the success rate (of new drugs).

Predictive and applicable?

Why is this important for the discussion on the impact of Organs-on-Chips? In a study that was published in the journal *Drug Discovery Today* in 2019, a quantitative estimate was made of the possible impact based on an analysis of direct costs, development time and success rate. The researchers estimated that the use of Organs-on-Chips could result in a cost saving of 10-26% per new drug. This would mainly be due to the decrease in direct costs and an increase in the success rate of hits in the preclinical phase.

The same study revealed that there are still a lot of questions about the application and technological barriers. For example, it is questioned whether Organs-on-Chips can increase the efficiency of clinical trials with patient groups. Or whether a better understanding of the disease mechanism can lead to savings in the direct costs of clinical trials through more targeted patient recruitment. However, some people feel that Organs-on-Chips can contribute to the discovery of characteristics (biomarkers) for certain diseases, as a result of

care and economy?

which a given treatment can be better tailored to a patient who will benefit from it. However, the extent to which Organs-on-Chips are predictive and applicable for the physiological processes in the human body is yet to be seen.

Big challenges with respect to automation, standardisation and ease of use remain, which are crucial for the implementation of the technology in an industrial setting. Technical needs and adoption speed are different for more large-scale therapeutic applications than for the identification of disease mechanisms by universities, and will determine the impact of the technology on research and development.

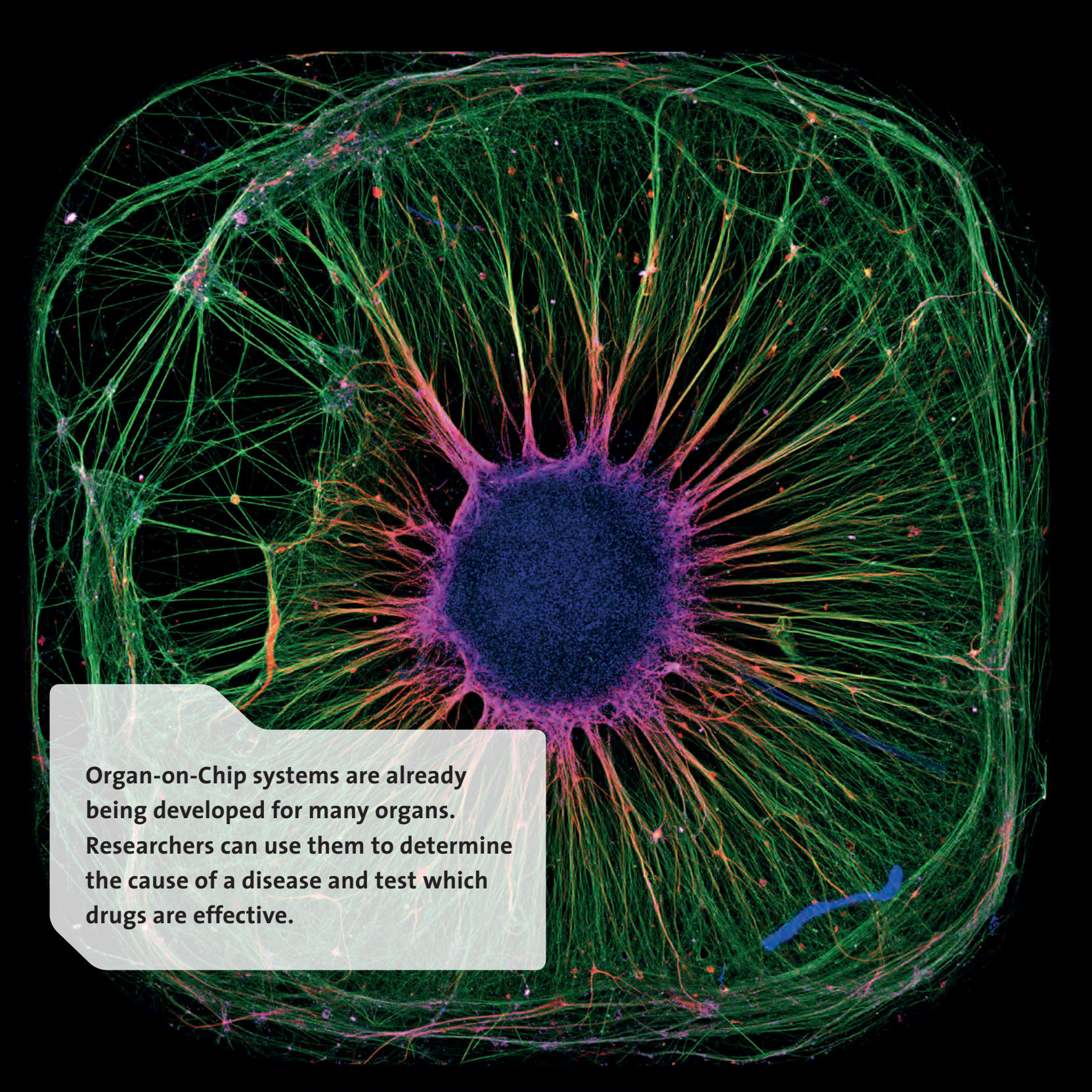
Transformation

Since the development of Organs-on-Chips is rapidly advancing, and it forms a serious alternative for animal models in drug development, new rules are needed for the actual application. At present, the strict regulations that the pharmaceutical industry encounters, still form a barrier for using the technology. The question remains how the use of the current *in vitro* systems and animal models can be changed. If Organs-on-Chips can only add value to the existing R&D procedures, then their impact will remain limited. However, if they can replace conventional protocols, this could trigger a transformation.

For the time being, regulators emphasize that the industry needs to provide data that gives insight into the reliability of Organs-on-Chips for determining the effectivity and safety of drugs. Researchers must be aware of this so that regulatory changes can keep pace with technological maturation.

Cost savings, faster availability and better effectivity of drugs for the patient. Is that possible?

The question is who drives the technological development and who benefits from it. Is that industry or the government or the citizen/patient, or a combination of these parties? In this regard, a dialogue is necessary about the impact of public investments in R&D and about cost savings for society through lowering of drug prices.



Organ-on-Chip systems are already being developed for many organs. Researchers can use them to determine the cause of a disease and test which drugs are effective.

3

What can Organs-on-Chips be used for?

The most important application domains for Organs-on-Chips are the development of drugs and treatments, toxicological tests and unravelling disease mechanisms. Many different Organ-on-Chip models are being developed. One model is almost being applied, whereas with another model the first experiments are just starting. Models are also becoming more complex due to the integration of the immune system and the microbiome, which are important components of the microenvironment for a Gut-on-Chip. Even if cells and tissues on chips can very well recapitulate the human organs, it is important to realise that they still remain models, and thus an approximation of reality.

From infancy to maturity

PROF. CHRISTINE MUMMERY AND
DR. JANNY VAN DEN EIJNDEN-VAN RAAIJ

Although the first Organ-on-Chip model was described some ten years ago, Organ-on-Chip technology as a whole remains in its infancy. Organ-on-Chip models have not yet been widely adopted by the pharmaceutical industry, nor have they become accepted yet by the regulatory bodies for drug testing. The recent use of human-induced pluripotent stem cells (hiPSC) has however now set the stage for major steps forward: Organs-on-Chips are poised to contribute to a wide number of biomedical applications.

Brain-on-Chip: you can see the axons (green), synapses (pink), cell nuclei (blue) and nerve cells with dendrites (red).

Toxicity and disease models

There are already several examples of Organs-on-Chips, among which a handful of hiPSC-derived models, that showcase the potential of the technology. These include toxicity models enabling drug safety tests for kidney in a **Kidney-on-Chip** and for the heart in **Heart-on-Chip**. The prediction of toxicity of nanoparticles is well advanced in **Lung-on-Chip models**.

Disease models have also been developed, and now include a model for thrombosis in **Blood Vessels-on-Chip**. A constriction in the microflu-

idic channel of the chip mimics an atherosclerotic plaque and blood clots form immediately behind the constriction. For another disease, called Hereditary Haemorrhagic Telangiectasia (HHT), a **3D Blood Vessel-on-Chip model** has been developed. People with HHT suffer from weak blood vessels which can result in severe haemorrhages. With the blood vessel model work is ongoing to identify existing drugs that could stabilise weak vessels.

Heart-on-Chip models can reflect essential processes of heart cells, such as force of contraction, calcium waves and the electrical action potential that jointly determine the heart activity. The models can also contain heart cells of patients with heart failure. These models are currently being used to screen for drugs to treat the disease. The results will be used to select drugs for clinical trials.

Models for lung infection and asthma are now ready for implementation in the drug discovery process. **Neurons and Glia Cells-on-Chip**, including cells from patients with (rare) genetic forms of neural disease, like amyotrophic lateral sclerosis (ALS), are being used to elucidate the pathogenesis of the disease. These models also form the basis for screening drug candidates. This is important because no treatments are available and no appropriate animal models that truly mimic this disease.

Another model is the **Blood-Brain Barrier model**. This model was used to identify the antibiotic minocycline, widely used for treating acne, which could possibly protect against the development of schizophrenia, particularly in families with a predisposition.

Cancer-on-Chip models with integrated 3D-organized blood vessels are being used to study the mechanism of metastasis and screen for treatments that inhibit this process.

Gut-on-Chip models are now "coming of age". Folded intestinal epithelium, the cell layer that covers the intestinal wall, can be made from either human adult stem cell organoids or from pluripotent stem cells. They can be flattened to a layer (2D) in order to grow them in Organ-on-Chip formats. Intestinal fluid flow is simulated on the surface of the epithelium, and the microbiome of the gut and cells of the immune system can be integrated. In this way it will be possible to see how a complex of the gut, microbiome and immune system interacts to affect human health and cause disease.

Multi-organ chips

While basic aspects of various organs have now been modelled, and also combined to form multi-organ chips, the biggest challenge is the inclusion of the vasculature, innervation and immune cells in the models. The ultimate goal is to maintain the internal environment of the organs in these models. This is called homeostasis and is important for proper functioning of organs in the body.

A remaining challenge is precise measurement of the pharmacokinetics and pharmacodynamics in multi-organ systems. Pharmacokinetics indicates what the body does with a drug, how the drug is taken up, converted and distributed in the different organs of the body. Pharmacodynamics shows where a drug interferes (biochemically), how it interacts with the organs, and which processes or substances disrupt the effect. The expectation is that, as the field develops, this will be explored in more depth.

Adoption and acceptance

The showcases described here are expected to encourage adoption of Organ-on-Chip technology by industry and acceptance by regulatory bodies. This will move the discovery of new, effective drugs forwards. The "early adopters" in the pharmaceutical industry have indicated that they already use Organ-on-Chip models for internal decision-making in the drug development cycle: is a drug promising or not for further development?

Application of Organ-on-Chip models in the pharmaceutical industry.

Organ-on-Chip model	Used for				
	identification, qualification and selection of drugs	discovery of new drugs	preclinical safety	pharmacokinetics and pharmacodynamics	Drug efficacy
Blood Vessel Vasculature- on-Chip					
Bone Marrow-on-Chip					
Gut-on-Chip					
Lung-on-Chip					
Liver-on-Chip					
Eye-on-Chip					
Kidney-on-Chip					
Liver-Pancreas-on-Chip					
Liver-Thyroid-on-Chip					
Skin-Tumour-on-Chip					

Source: ALTEX doi:14573/altex.2001241

However, most tests with Organ-on-Chip models are currently done outside the regular pharma portfolio. This research occurs in a pre-competitive setting: the research phase prior to competition between different industries. Users, developers and regulators have a common interest to work together on the implementation of Organ-on-Chip technology for a wide range of applications.

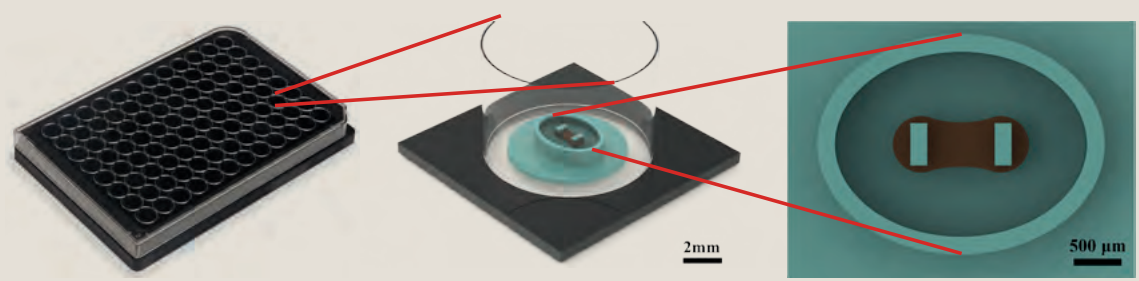
In sum, the most important areas of application of Organ-on-Chip models are toxicity tests, drug development (drug efficacy and drug repurposing) and unravelling disease mechanisms. Personalisation of the models will enable tailored therapies perhaps for individuals or more likely for specific groups, by the assessment of drug responses based on differences in sex, age and ethnicity.

How do you grow a Mini Heart-on-Chip?

LAURA WINDT, MSC AND MILICA DOSTANIC, MSC

A mini heart can consist of about 16,000 cells

Various cell types are needed to develop a miniature model of the human heart on a chip. The cells can be derived from a patient for research on heart disease, or healthy cells can be used for a better understanding of the functioning of the heart. The cells used do not originate from the patient's heart, but from urine, blood or skin. The cells are isolated and turned into stem cells by reprogramming them in the laboratory. By adding specific substances, the stem cells can develop into the cells needed for a mini heart: heart muscle cells, connective tissue cells and blood vessel cells. The cells are mixed in a ratio, which is equal to that in the human heart: 70% heart muscle cells, 15% connective tissue cells and 15% blood vessel cells. In total, this mini heart consists of about 16,000 cells.
Besides the cells, a chip is required that acts as the living environment of the cells. The chip is just a few millimetres in size and is produced with high precision in a cleanroom to avoid contamination by particles. This specially controlled room is necessary for production at the microscale. The chip, with pillars in the middle for growing the mini heart, is made of soft, elastic and cell-friendly material (PDMS).
Collagen gel is added to the cell mixture to transport the cells to the chip and keep them together. Because of its very small size, only 1 microlitre of the gel-cell mixture is needed to "fill" the chip. On the chip, the cells aggregate, organise themselves and form tissue-like structures. The length of the mini heart is then about 1 millimetre; smaller than a pinhead. The gel-cell mixture reorganizes, resulting in the formation of the mini heart as a bundle around the elastic pillars.
By using this material, the cells feel as if they are in the heart itself and behave accordingly. As soon as the tissue is formed around the pillars, it starts to contract spontaneously, just like a real heart. To keep the mini heart alive for several weeks to perform experiments, the cells are continuously provided with oxygen and fresh nutrients, as by the blood in the human body.
By integrating sensors and electrodes in the chip, the heart activity can be measured, and the effects of drugs. In this way certain therapies, like chemotherapy, can be tested for harmful side effects in the heart.



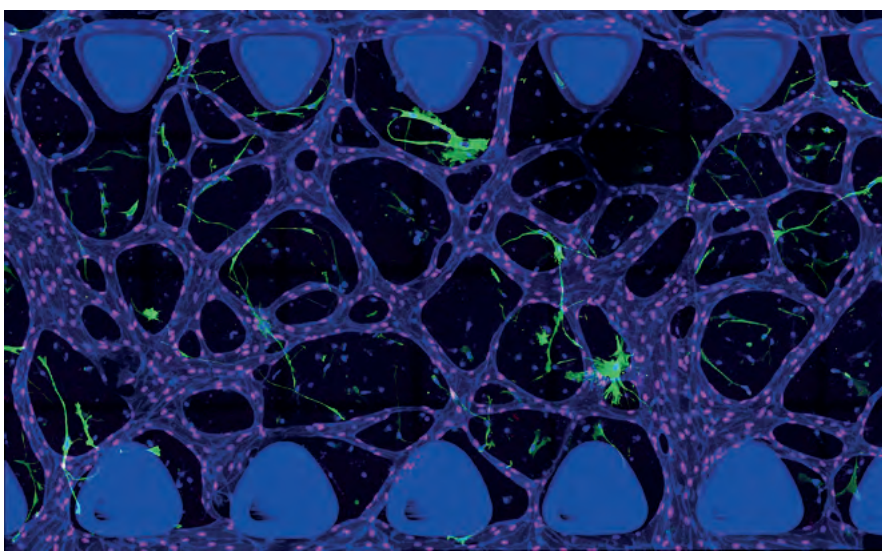
Schematic 3D model of a heart tissue platform, including a chip. At the bottom of the plate with 96 wells (96-well plate) is a plastic layer of PDMS (blue). The heart tissue (brown) has grown around the two pillars that are surrounded by an oval-shaped wall. Based on research from Leiden University Medical Center and TU Delft.

Blood-Vessels-on-Chip

PROF. CHRISTINE MUMMERY AND
DR. VALERIA ORLOVA

Blood vessels form a 60,000 kilometre long network of interconnected channels in our body. This network consists of a large aorta and arteries that carry oxygen-rich blood away from the heart. These arteries branch into smaller arteries which in turn branch into an extensive network of capillaries to efficiently provide the cells in the various organs with oxygen and nutrients. The oxygen-poor blood is subsequently turned back to the heart via a network of capillaries and veins. The diameter of the blood vessels varies from 5 to 10 micrometres in the capillaries to 2 to 3 centimetres in the aorta. The inner lining of the blood vessels consists of endothelial cells. The outside of small capillaries is covered with pericytes, and that of the larger blood vessels with smooth muscle cells. These outer cells stabilise a blood vessel and regulate its size.

A poor functioning of the blood vessels can lead to many different diseases, involving many organs, such as the heart, brain, lungs and gut, that can be affected. In addition, gene mutations that cause abnormalities in endothelial cells, pericytes and smooth muscle cells are linked to a range of vascular diseases. For this reason therapies, that can improve the health of blood vessels, can help prevent or treat organ diseases.



Blood vessel cells from a patient with Heritable Haemorrhagic Telangiectasia (HHT) on a Blood Vessel-on-Chip. Endothelial cells of the blood vessel (blue) with cell nuclei (pink) and pericytes (green). HHT patients have abnormalities in the blood vessels, making them very weak and easily bleed.

Replicating blood vessels

Today, human-induced pluripotent stem cells (hiPSCs) can be made from each individual and used to differentiate them into almost all cell types of the body (see: Where do the cells come from?). These hiPSCs are the basis for culturing the cell types needed for the development of blood vessels in the microfluidic chip. In the microfluidic channels of the chip, endothelial cells are first introduced, followed by pericytes or smooth muscle cells to form the blood vessel.

Researchers from Leiden have been able to simulate blood vessels of different diameters; small blood vessels of 10 to 20 micrometres in diameter to large blood vessels and veins of 300 to 500 micrometres in diameter. Using highly accurate mini-pumps, the blood vessels can be provided with artificial blood, and the fluid flow in the vessels can be mimicked. These chips can be placed under a microscope to monitor how the cells develop and move. The cells adhere to each other and form branches with a cavity in the network, just like real blood vessels in the body. This is very important, because the behaviour gives insight into the (mal)functioning of the blood vessels, and which factors can influence this. Using these blood vessel models, researchers can study different vascular diseases and try to find new treatment strategies.

Nosebleeds

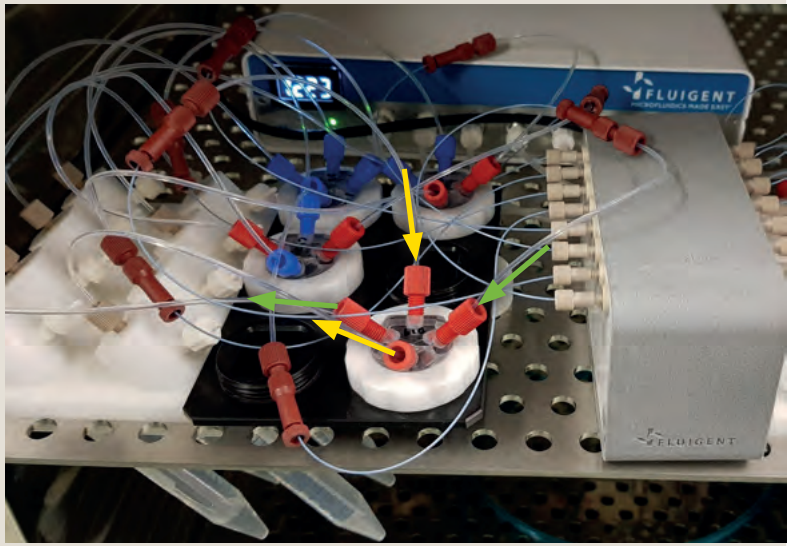
Heritable Haemorrhagic Telangiectasia (HHT) is one of the vascular diseases that is being studied

Breast Cancer-on-Chip

DR. DIK VAN GENT AND PROF. JOHN MARTENS

The mobility of tumour cells can be investigated in Organ-on-Chip models

Just imagine if every breast cancer patient knows exactly whether there is a chance of metastasis? And which therapy is most effective in case metastases are already present? Today therapies are still carried out that either prove unnecessary or have no effect on the tumour. This referred to as overtreatment. The patient experiences side effects, but does not benefit at all from the therapy. This gives the patient unnecessary suffering and the healthcare system unnecessary costs. Researchers have already been addressing these important questions for many years. It sounds simpler than it is. Determining whether a breast tumour has the capacity to metastasize or whether this is sensitive to a therapy first requires culturing of a piece of that tumour in the laboratory. The culture conditions for tumour growth in the laboratory should closely resemble those in the body. To achieve this, researchers from Erasmus Medical Centre in Rotterdam started to culture small pieces of breast tumour on a chip, that can further grow under precisely controlled conditions, with continuous supply of nutrients and removal of waste products. They managed to culture these pieces of tumour tissue for several weeks, just as in the human body. The next step is to closely mimic different treatments on a chip, including hormonal therapy, chemotherapy and combination therapy, so that the models can predict how the tumour in the patient will respond. The current chip model also allows studies of the motility of tumour cells, which might indicate whether they can metastasize.



A setup to culture tumour slices. The culture medium flows from the tubes on the right via the chip (black plate in the middle that contains the tumour tissue) to the tubes on the left.

More complex chips are required to determine whether tumour cells can pass through the body and accumulate in other organs. The chip with the piece of breast tumour tissue must then be connected, via cultured blood vessels, to a chip with healthy organs. This type of research lies in the future but is already underway. It is expected that in the end, test and measurement models will become available for investigating in parallel both the potential of the tumour to metastasize and the sensitivity of the tumour for all available treatments This enables the physician and researcher to determine in advance whether treatment is needed at all, and if so, which therapy is the best option for the patient.

at the Leiden University Medical Centre, together with the St. Antonius Hospital in Nieuwegein, using a Blood Vessel-on-Chip, made from patient cells. HHT patients suffer from heavy and recurring nosebleeds that can occur several times a week and last for minutes, with on average half a litre of blood loss each time. The only successful treatment that reduces the severity of the nosebleeds is to replace the nasal mucosa (the mucosa in the nose cavity) by a piece of skin as a graft. Because the therapeutic possibilities are very limited, there is a lot of interest in developing an *in vitro* model for this disease that can be used to select new drugs that can restore the blood vessels in HHT patients. For this purpose, first hiPSCs are made from these patients, that are subsequently differentiated into vascular cells to develop an HHT-Blood Vessel-on-Chip model. The blood vessels on the chip are unstable and sensitive for "bleedings", just like in the patient. The HHT Blood Vessel-on-Chip model is now being used to look for better drugs to reduce nosebleeds in HHT patients.

This approach can also be used for other diseases that are being caused by a defective blood vessel function. For example, the addition of heart muscle cells or nerve cells to the Blood Vessel-on-Chip model could help discover the underlying mechanisms for diseases such as heart failure and vascular dementia and to find better therapies.

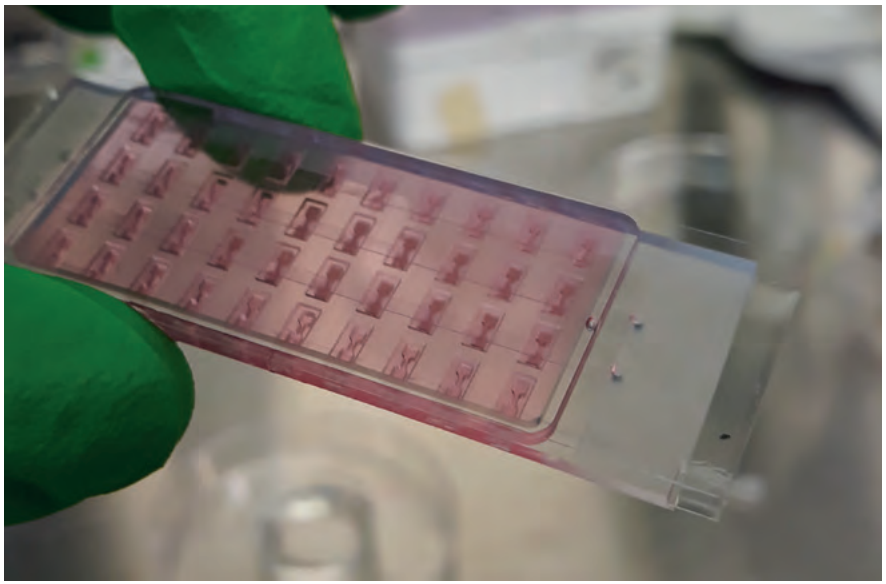
Heart-on-Chip

PROF. ROBERT PASSIER AND PROF. JOLANDA VAN DER VELDEN

THE HUMAN heart at rest beats on average about 60 to 70 times per minute and during major exercise up to 200 times per minute, while pumping 25 to 45 litres of blood around the body. This performance can be delivered due to a precisely regulated process. First pacemaker cells produce electrical signals, which are spread via conduction cells to heart muscle cells according to a fixed pattern. This results in the coordinated contraction of the heart muscle cells in the atria and ventricles of the heart. It is hardly surprising that disruptions of the generation of electrical impulses, conduction of the signal, or contraction of heart muscle cells can be life-threatening.

Heart failure

Cardiovascular disease is a collective term for heart and vessel disorders. These can vary from a disease



myHeart-on-Chip: an hiPSC-based Heart-on-Chip to study heart-blood vessel interactions and blood flow patterns. Each well contains a mini heart.

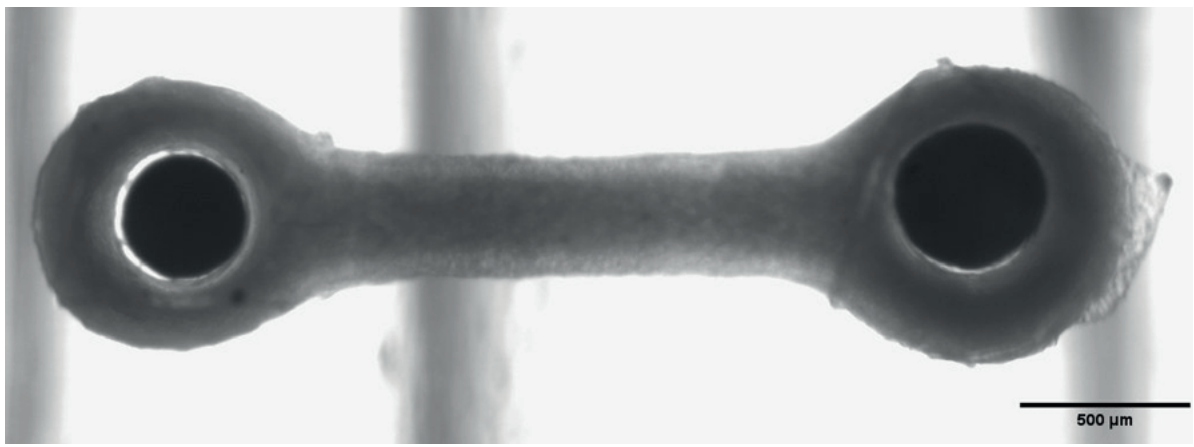
with just a single gene and cell affected, to a multifactorial disease, resulting ultimately in heart failure, for example a heart attack due to obstruction in the vessels. Risk factors do not always contribute equally to the pathology. Heart failure not only means less contraction of the heart muscle cells but also poor relaxation of these cells, resulting in a heart that is less filled with blood.

The complexity and the different types of cardiovascular diseases make it very challenging to treat these disorders and cure them. Although we have learned a lot from existing disease models (mostly laboratory animals) about the possible mechanisms responsible for the onset and progression of heart diseases, extrapolation to humans has proven to be very difficult. The functioning of the heart and the disease process in animals can differ significantly from humans (for example, a mouse heart beats about 7 to 8 times faster than a human heart). For this reason, new treatments for heart patients are often non-existent. Besides laboratory animals, the use of heart muscle tissue from patients – obtained during open-heart surgery – for both molecular and functional studies (such as the contraction and relaxation of heart muscle cells), can give insight into disease mechanisms in humans. However, the heart tissue can only be obtained at one specific moment in the disease process, and a proper control group is usually missing.

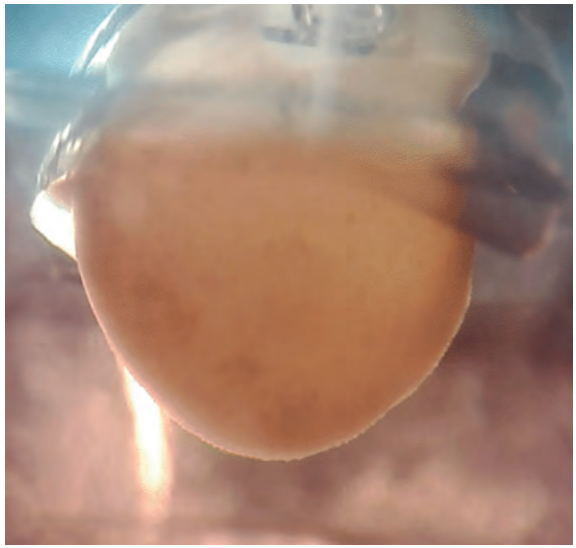
There is a clear need for other test systems that mimic the human heart better to study the course of cardiac disease. Researchers from Leiden University Medical Centre, Amsterdam University Medical Centre and the University of Twente are collaborating on developing such new models.

Mini muscles and mini hearts

To study diseases that manifest as reduced pumping function of the heart, human heart cells would ideally be organized as 3D heart tissues which resemble the adult heart tissue. By culturing the 3D heart tissue as a mini muscle bundle, around elastic micro-pillars (see: How do you grow a Heart-on-Chip?), the extent that these pillars bend reflects the force of contraction of the heart tissue; this can be captured on video and subsequently calculated into an absolute measure of force. This measurement is very informative for severity of disease pathology and the effects of drugs. However, there is no direct relationship with the pumping function of the heart. It has recently proven possible to resolve this issue and create a beating 3D mini heart, consisting of a single heart chamber. This



Top view of 3D heart tissue made from human stem cells, that has formed around two elastic micro-pillars (top side has black colour). Due to the force of contraction of the heart tissue, the two micro-pillars are bent towards each other. Recording of this movement by a camera enables measurement of the force.



Mini heart with a real pumping heart chamber.

enables measurement of the volume of fluid squeezed out of the chamber with each contraction via the microfluidic channels in the chip: a mimic of what is referred to as “ejection fraction” of the heart in clinical cardiology.

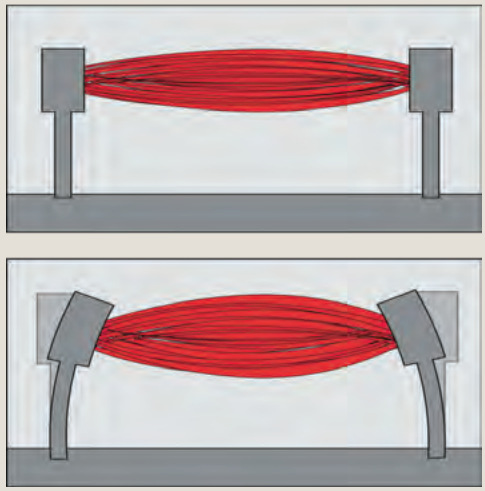
Even though these developments are promising, the actual added value of these complex 3D mini hearts still has to be proven. Do these models represent a realistic picture of heart diseases and does this result in better therapies for heart patients? Are these models superior to current animal models or a valuable addition for answering specific research questions? Is there a need for modifications to accurately recapitulate the function of the human heart and the onset of disease, for example multiple compartments, tissue organisation, valves, pressure, simulating other disorders (comorbidities) that play a role in the advancing pathological signs of cardiovascular diseases? To answer these questions, direct comparison with the current test models (both *in vitro* and *in vivo*) *and* the heart muscle tissue in humans is essential.

It is possible to create a beating 3D mini heart, consisting of a single heart chamber

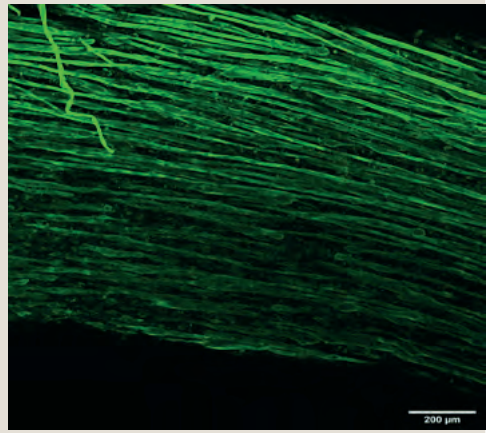
Muscle-on-Chip

DR. PIM PIJNAPPEL AND DR. JESSICA DE GREEF

Although more than 700 muscular diseases are known, therapies are only available for a few. An important reason for this is the paucity of models for human skeletal muscles. Researchers from Erasmus Medical Centre and Leiden University Medical Centre are working together on a three-dimensional model of human skeletal muscles to address this issue.



The Muscle-on-Chip model for skeletal muscles. The deformation of the pillars after muscle contraction is measured with a camera. In this way the force of the mini muscle can be calculated.



Parallel long muscle fibres in a human Muscle-on-Chip. The protein titin has been stained green using an antibody. This protein is part of the contractile unit of the mini muscle, the sarcomere. When several sarcomeres contract simultaneously, the result is contraction of the muscle.

Creating muscle cells

To create a muscle model, the muscle cells are made in the laboratory from induced pluripotent stem cells derived from a patient's skin cells. The muscle cells are then mixed with a liquid gel (containing the blood coagulation factor fibrinogen among other components) that is poured between two flexible pillars in the chip. Both the pillars and the chip are made of the cell-compatible polymer polydimethylsiloxane. After the addition of thrombin, an enzyme that converts fibrinogen into fibrin, a fibrin network is formed, resulting in hardening of the gel. The muscle cells continue to grow in this gel and ultimately form muscle fibres (multinucleate cells).

Forming muscle fibres

Because muscle cells can contract, they start to pull on the pillars during their growth. In this way, they organise themselves into parallel muscle fibres that together form a miniature muscle between the pillars. Dependent on the model, mini muscles can be several millimetres to half a centimetre in size. Using electrodes, the mini muscles can be electrically stimulated, so that they contract in a controlled way. Because the pillars are flexible, they will move during the contraction. By recording the shift of the pillars with a high-speed camera, the force of the muscle can be calculated. This is much like the approach used for heart muscle except that the kinetics of contraction are different: for skeletal muscle, it is slower. The organisation of the contractile units (the sarcomeres) in the muscle can be studied by staining of the proteins in the muscle cells. Furthermore, the quantity of muscle proteins can be measured by the use of antibodies or mass spectrometry. This provides information about the structure of the muscle and shows possible abnormalities, that may be involved in specific muscle diseases.

New therapies

With this muscle model, the course of a muscle disease can be studied and new therapies for individual patients can also be tested. The latter is an important theme, because it is known that there are big differences between individuals, that might result in very different responses to a certain therapy. The muscle model enables selection of therapies, which will benefit every individual patient.

Gut-on-Chip

DR. HANS BOUWMEESTER, DR. SEBO WITHOFF AND DR. EVITA VAN DE STEEG

A HEALTHY AND well-functioning gut (or intestine) is crucial for a healthy life. Malfunctioning of the gut not only results in less absorption of nutrients but negatively affects the immune system and other organs. The close relationship between the gut, the immune system, the brain and many other organs has only recently been recognized. Also, recent studies have shown that gut bacteria – or "microbiome" – play an important role in the functioning of the gut.

Although research on the gut has been ongoing for decades, little is yet known about the complex interactions between microbiome and other organs. This is mainly due to the use of relatively simple laboratory setups, containing just one cell type, to investigate the digestive process and the effect of gut bacteria on function or diseases of the gut. The development of microfluidic chips containing gut tissue – known as Gut-on-Chip – now enables studies of the interaction of the cells lining the gut with gut bacteria as they pass through the lumen, and with other organs. Such models are being developed by researchers from the University Medical Centre Groningen, TNO, the Hubrecht Institute and Wageningen University and Research.

Function and form

The most important function of the gut is the absorption of nutrients and the contribution to a good water balance. At the same time, the gut must ensure that pathogenic bacteria and other pathogens do not enter our body. Although these tasks seem difficult to combine, they can be performed because the first and last part of the gut look very different. Roughly speaking, the gut consists of three parts: the small intestine, the large intestine and the rectum, each with specific functions and anatomical and physiological characteristics. In order to study functioning of the gut in healthy and sick people, laboratory models for each part of the gut are required.

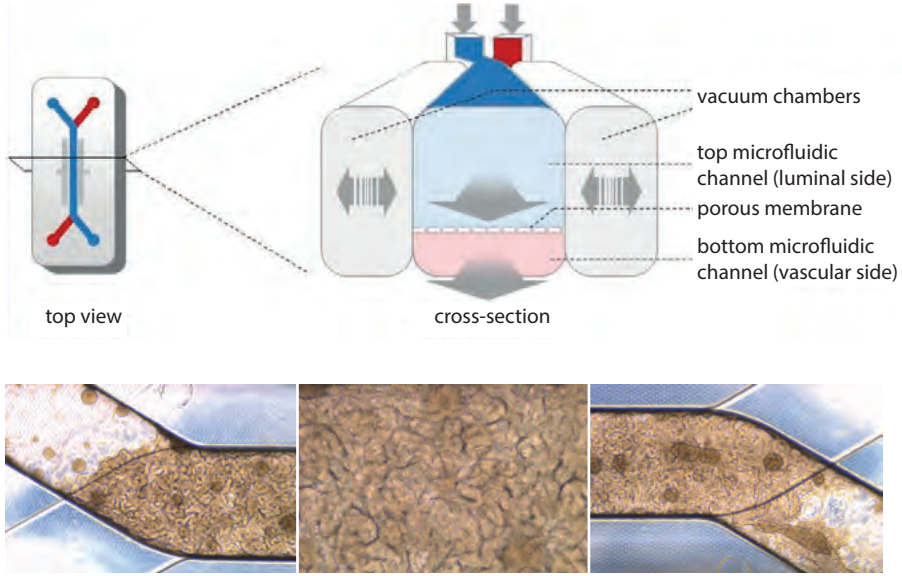
Small intestine

Most of the nutrients are absorbed in the small intestine. This is possible because the intestinal wall (intestinal epithelium) is highly folded in this part of the gut, resulting in a large surface to absorb nutrients. These intestinal folds are also called the crypt and villus structure. Only few gut bacteria are present in this part of the gut. Food is mainly digested by enzymes coming from the stomach wall and pancreas.

Five cell types are present in the gut epithelium: the enterocytes (cells for substance uptake), Goblet cells (produce a protective mucus layer), enteroendocrine cells (ensure the excretion of hormones for optimal control of gut function) and Paneth cells (important for immunity against foreign particles). These cells are continuously generated from the (adult) stem cells that renew the gut epithelium and are present deep in the crypts of the epithelial layer.

Methods have been developed that support culture of human gut cells from stem cells in the laboratory. Culturing of these cells on microfluidic chips generates the five cell types of the gut listed above. Although this forms a good basis for developing gut models, there is still another important step to go: creating peristaltic movement.

Our gut is continuously moving. Not only because of the continuous flow of partially digested food, but also because of the constant contraction (peristaltic movement) to transport the food. Gut cells are continuously stretching and relaxing and respond to the movement of substances through the lumen. This is mimicked



Schematic representation of a Gut-on-Chip (based on the design of Emulate Inc, Boston, MA, VS) and photographs of cultured mini guts in such a chip (UMCG). The top microfluidic channel simulates the gut channel and the bottom channel a blood vessel. The channels are separated by a porous membrane on which the epithelial cells can grow. The microfluidic channels are surrounded by vacuum chambers to simulate the peristaltic movement of the gut.

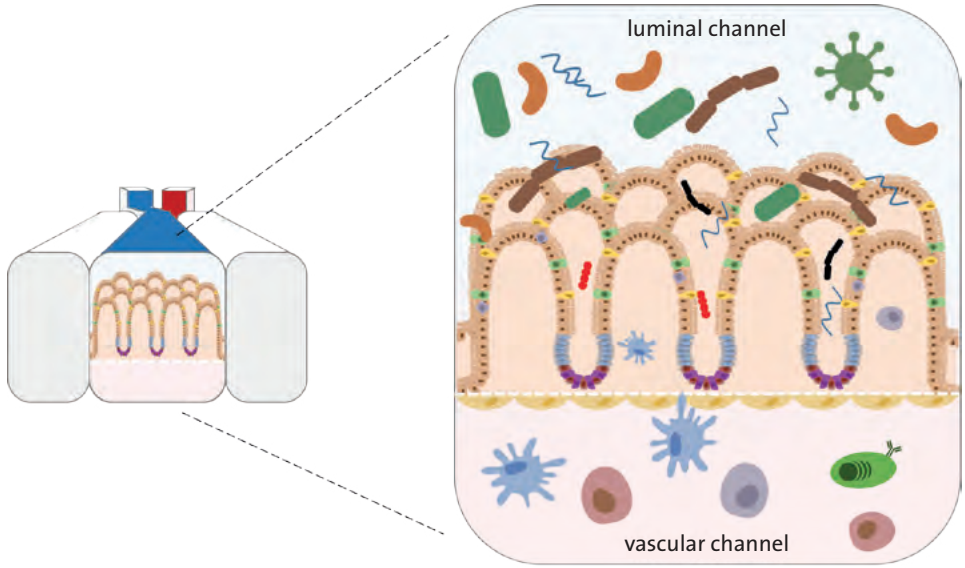
in Gut-on-Chip models by continuous fluid flow passing over the cells; in some models, the cells are even stretched, an even closer mimic of peristaltic movement. Interestingly, after seeding the gut cells on these chips, mini guts and – after several days – intestinal folds with crypts and villus structures develop. This 3D structure is not formed under static conditions.

Large intestine

The large intestine contains many gut bacteria (the gut microbiome). These bacteria break down any remaining food components and produce essential vitamins (vitamin K) and fatty acids. These by-products are not only the fuel of gut cells but have an important function for health throughout the entire body. In particular because of the important role of gut bacteria in the large intestine, it is much more difficult to develop adequate Gut-on-Chip models for the large intestine.

In the conventional laboratory model, bacteria often rapidly overgrow the gut epithelial cells, so that this model can only be used for a limited time. Continuous fluid flow in a Large Intestine-on-Chip model, prevents this overgrowth and enables bacteria to be kept in the system for up to two weeks. An important requirement of the ideal large intestine model is that no oxygen is present on the inside (lumen). Oxygen is essentially absent in the human large intestine and many gut bacteria even die in the presence of oxygen. However, the epithelial layer of the gut (blood side) does require oxygen. This is normally provided by the transport of oxygen via the blood but is simulated in Gut-on-Chip models that contain the different compartments (lumen and blood vessel side), separated by a porous membrane. Epithelial cells are cultured in the top compartment on the membrane and on top of this, bacteria can be added under oxygen-free (or oxygen-poor) conditions. The bottom compartment can be used for the supply of an oxygen-rich medium, and immune cells as required; these are also important for a healthy balance in the gut (homeostasis).

Nutrients and eventually immune cells can reach the gut (lumen) via the pores at the base of the epithelial layer.



Gut in health and sickness

Intestinal health involves more than the proper absorption of nutrients. There are many intestinal disorders, including various types of intestinal cancer, immune-mediated complex chronic diseases like chronic infection of the gut (inflammatory bowel disease, IBD; ulcerative colitis and Crohn's disease), irritable bowel syndrome (IBS) and celiac disease (gluten intolerance). These diseases can be mimicked in Gut-on-Chip models by populating the devices with stem cells isolated from patients (see also: Genetic Diversity). The interaction of gut cells with bacteria plays an important role in the uptake of nutrients and in these diseases. By making use of patient-specific gut bacteria, the role of the microbiome in sickness and health can also be investigated in Gut-on-Chip models.

The communication of the gut with other organs is also very important. Some chronic diseases appear to be caused partly by a disrupted microbiome in the gut (for example and somewhat surprisingly, schizophrenia and Alzheimer's disease). For studying such interactions it is essential that all cell types that play a role in gut biology (epithelium, microbiome, immune cells, endothelial cells, fibroblasts) are present on the chip, and that the gut can be linked to the brain (gut-brain axis), the liver (gut-liver axis; for metabolism) and the heart (gut-heart axis; short-chain fatty acids can cause heart problems).

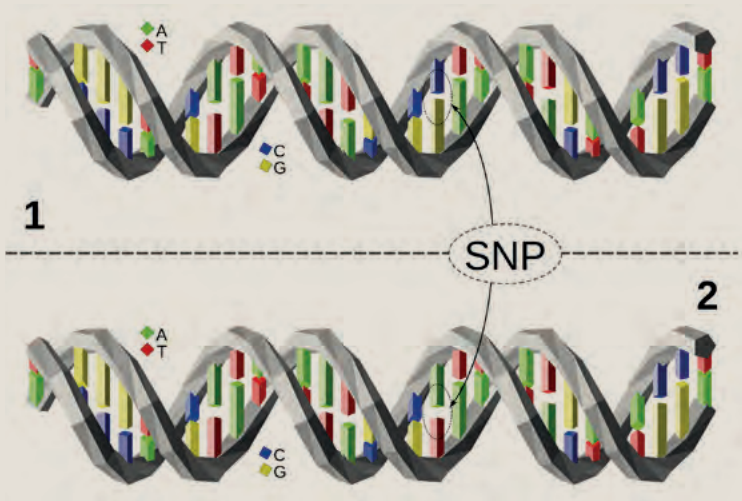
How this all works at the molecular and cellular level is now being investigated with new Gut-on-Chip models that can be connected to other Organ-on-Chip models.

Genetic diversity

DR. SEBO WITHOFF

SNPs can indicate sensitivity to drugs

Only 0.1% of the genetic information (the DNA) of an individual) differs from that of any other individual, who is not a family member. This difference seems small but as human DNA consists of about 3 billion "building blocks", this means that each individual has 3 million DNA differences compared to another individual. DNA only has four different building blocks: the nucleotides adenosine, cytidine, guanosine and thymidine, which are connected in a long chain.



The smallest difference that can be measured at the DNA level is a difference in one of the four building blocks of the DNA. This difference is called a Single Nucleotide Polymorphism (SNP).

Building blocks

The smallest difference that can be measured at the DNA level is that in one the four building blocks of the DNA. This difference is called a Single Nucleotide Polymorphism (SNP). These SNPs can occur throughout the DNA, also in or near genes that code for RNA (about 5% of the DNA, contains information for making proteins), resulting in a change of the function or the expression level of these gene products.
With the increase in major genetic studies and the establishment of population biobanks, which contain stored biomaterial and patient data, it has become clear that the genetic cause of complex diseases with a deregulated immune system – such as multiple sclerosis, rheumatism, diabetes and celiac disease (gluten intolerance) – largely lies in the presence of tens to hundreds of SNPs in the non-coding part of the DNA of the patients. These "non-coding" SNPs influence the expression level of genes (but not the nucleotide sequence of these genes which would disrupt the function).
Another important finding is that pharmacogenetics studies have shown that SNPs can determine the sensitivity to drugs. In the most extreme case, the presence of a certain SNP can even result in severe side effects.

Personalised models

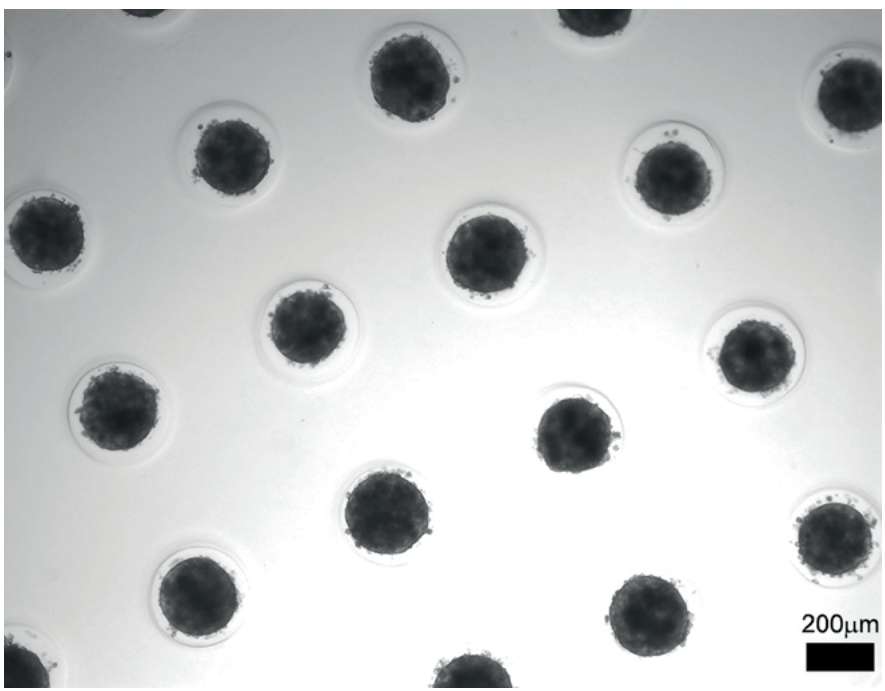
Due to the genetic diversity between individuals and between patients and healthy people it is important to develop personalised models for studies on diseases, but also on the specific drug responses per individual (personalised medicine). This can be realised by isolating tissue-specific stem cells from biopsies or by generating hiPSCs by reprogramming somatic cells of the body (see: Where do the cells come from?) – from both sick and healthy individuals – and by differentiating them into the tissue type of interest. Organ-on-Chip models made with these tissues are a promising platform to investigate why one individual does or does not develop a disease and which drug works for each individual patient.

Liver-on-Chip

DR. GEURT STOKMAN, DR. ROELAND HANEMAAIJER AND PROF. BOB VAN DE WATER

The liver plays an important role in the conversion of nutrients, drugs and harmful substances. The liver also stores certain nutrients. Most liver functions are realised by hepatocytes, the most frequently occurring cell type in the liver. In addition, stellate cells (involved in fat storage and fibrosis) and Kupffer cells (part of the immune system) play a role when liver functions are disrupted.

The liver is very susceptible to damage because of its important role in the conversion of many compounds. The formation or presence of toxic substances, or the accumulation of a drug or fat (steatosis) in hepatocytes can disturb the normal liver function, cause an inflammatory reaction (hepatitis) due to activation of Kupffer cells, and ultimately lead to cell death of hepatocytes and formation of scar tissue by stellate cells (fibrosis). Furthermore, various liver diseases can lead to a loss of liver function, resulting in insufficient processing of nutrients, drugs or harmful substances by the body.



Phase contrast microscope image of single liver spheroids with a diameter of 150-200 micrmetres, consisting of about 3000-5000 cells, during the culture procedure in wells on a plate.

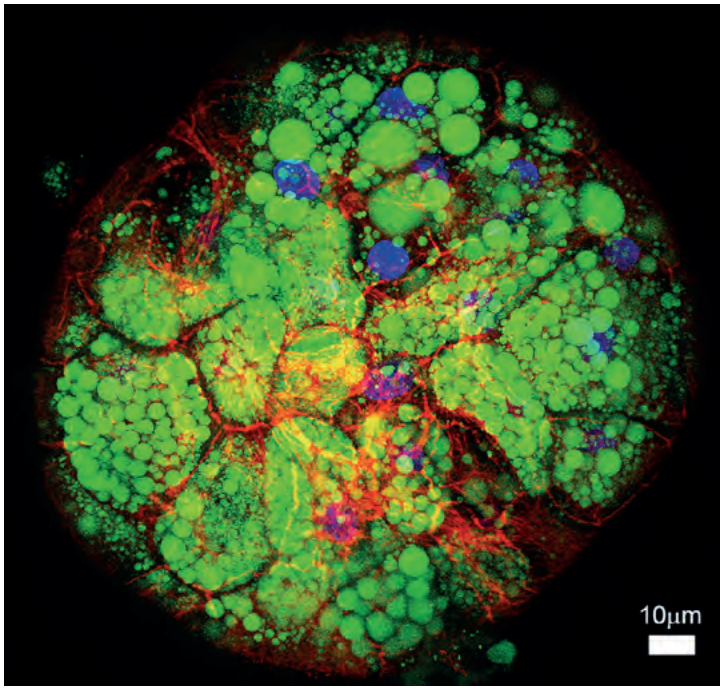
Because of the central role of the liver in health and disease, accurate measurement of its functions is important. Which substances are toxic to the liver and in what amounts? Which drugs are metabolized by the liver and how can a diseased liver be treated? Since this cannot be investigated in humans directly, cell and animal models are used for research.

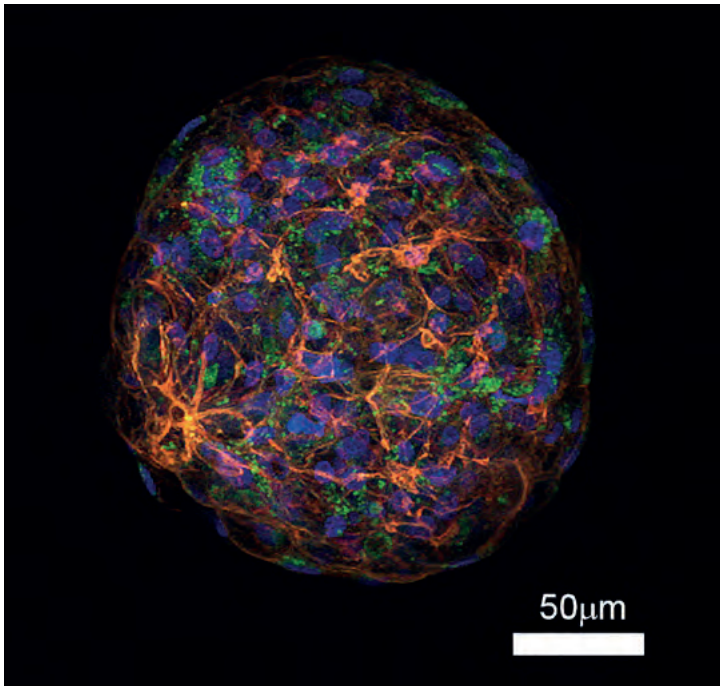
In current cell models, mostly one single cell type is studied or included. This limits the relevance of the model because a single cell type cannot reveal the complex interaction that occurs between the other cell types in the liver. This interaction does take place in animal models but there are differences between the metabolism of humans and animals; for example how hepatocytes deal with fats, toxic substances and drugs. These differences may mean that conclusions drawn using animal models may not translate to humans and thus be incorrect in predicting for example human risk. Ideally, a cell model is required that closely resembles the human liver.

Spheroids

Recapitulating the liver and its function from individually cultured cells is not easy. In a flat plastic Petri dish, hepatocytes and other cells rapidly lose their function. To preserve this as well as possible, liver cell culture conditions must be appropriate although this is difficult, because multiple cell types are needed to simulate processes in the liver and each cell type has its own culture requirements.

Researchers from TNO and Leiden University are working together on a solution. By culturing the





Confocal microscope images of liver spheroids with a diameter of about 150-200 micrometres. Left: The Kupffer cells (green), the actin cytoskeleton (red) and cell nuclei (blue) are coloured with fluorescent markers. Right: Steatosis in hepatocytes, visualized by staining the fat with a green fluorescent marker. The actin cytoskeleton (red) and cell nuclei (blue) have also been stained.

different human liver cell types into a small spherical clump of cells (spheroid) and passing fluid in the surrounding space, circulation of the liver is simulated and the spheroids remain well-fed and functional. The size of the spheroids (between 150 and 300 micrometres) can be determined quite easily by culturing them in cavities of the required dimensions. This allows the simultaneous formation of hundreds of individual spheroids. The hepatocytes, stellate cells and Kupffer cells used are derived from human donors. Hepatocytes can also be made from hiPSCs. The Kupffer cells are part of the immune system that mainly occurs in the liver and activates inflammatory responses during liver damage. By co-culturing these liver cell types, the complexity of a real liver can be recapitulated in a Liver-on-Chip model, including the metabolic processes that are characteristic for humans and relevant for research.

Fatty liver disease and liver fibrosis

TNO uses the liver spheroids for research on *non-alcoholic fatty liver disease* (NAFLD). The onset of NAFLD is strongly dependent on lifestyle: it is associated with a fat- and sugar-rich diet, insufficient exercise, high blood pressure and an increased BMI. The number of patients with NAFLD has rapidly increased in recent decades among both adults and children and now occurs in Europe in 25-30% of all people. Characteristic for NAFLD is steatosis, which can result in hepatitis, liver fibrosis and ultimately in a complete loss of liver function. There are no effective drugs yet, partly because of the lack of models for research that can accurately predict whether a potential drug will be effective in patients. Liver spheroids could change that.

Toxic substances

Leiden University is studying liver toxicity. The liver is the organ that can be most affected by

chemical substances, including drugs. For the entry of new chemical substances and drugs to the market, prediction of their toxicity for the liver is essential. In the past, laboratory animals often failed to predict liver toxicity of drugs in humans.

Liver damage can be the consequence of a toxic effect, caused by the drug in hepatocytes but it can also be due to an effect on the interaction between multiple cell types of the liver or of the immune system. These interactions are much more difficult to demonstrate. It is expected that by using multicellular liver spheroids, the safety of these substances can be predicted more accurately.

Brain-on-Chip

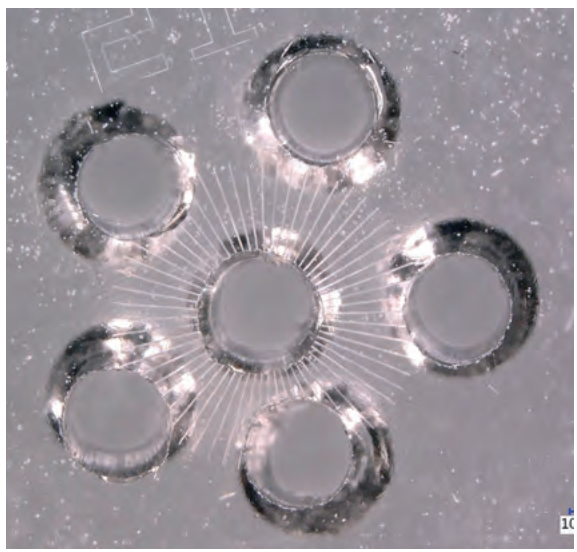
PROF. STEVEN KUSHNER AND DR. REGINA LUTTGE

Is it possible to replicate neural networks and human brain functions in the laboratory? And how can a Brain-on-Chip be used for the discovery of drugs for complex brain diseases such as Alzheimer's Disease, Parkinson's Disease and schizophrenia?

Brain diseases

Psychological and addictive disorders affect more than 1 billion people worldwide and about one-fifth of them live with that handicap for their entire life. For dementia alone, it is currently estimated that by 2060 almost 200 million people worldwide will suffer from dementia. By 2030, the healthcare costs for these people will already be more than 0.9 trillion euros worldwide.

Scientists and physicians still face numerous challenges in the development of more effective treatments for brain diseases. One of the most important limitations is the applicability (validity) of existing cell models to systematically investigate



This chip forms the basis for a new Parkinson Disease-on-Chip model. The model makes use of a radial chip with microtunnels to separate the different tissues from the nervous system. An organoid of the midbrain can be placed in the centre and the neural networks of the digestive system (which may be affected in Parkinson Disease patients) can be put in the reservoirs at the edge of the chip.

Research on brain cells cultured on a chip at Eindhoven University of Technology.

the cause of brain diseases, find targets for treatment and test possible drugs.

The brain is very well protected by a thick skull and a blood-brain barrier. It is also very difficult for brain scientists to do research on living brain tissue because the high quantity of neuronal cells has very specialised functions and no ability to recover. For this reason, access to brain tissue is very limited. Living brain tissue only shortly becomes available when somebody dies or when somebody must undergo surgery due to a brain disease (for example, cancer or epilepsy). This limits research on both healthy and diseased tissue, which is necessary to understand the processes in brain cells and the underlying molecular biology of brain diseases.

However, in recent years considerable progress has been made in unravelling the genetics of brain diseases. Thousands of genetic variants are now known of neurological development disorders that have far-reaching, lifelong consequences for children. But translating this knowledge into a better understanding of the underlying neurobiological mechanisms and possible therapies appears to be extremely difficult. This is due in particular to the lack of brain tissue for research in the early stages of diseases and the lack of reliable animal models.

Alzheimer-on-Chip

hiPSCs now offer a unique chance to tackle this problem. Today living human nerve cells derived from people with a known genetic risk (see box: Where do the cells come from?) are available for researchers.

Although the first studies of human nerve cells derived from hiPSCs were performed in a monolayer of cells in a Petri dish, the use of the 3D Brain-on-Chip model has considerable advantages. For example, for Alzheimer's disease it has already been demonstrated that a 3D Brain-on-Chip model can simulate more accurately the *in vivo* neuronal organisation, the biochemical changes and the pathological structures observed in the brain tissue of diseased patients.

Now that self-organising brain tissues can be cultured in the laboratory, brain scientists finally can investigate living human brain tissue at the cellular level. A historical, technical barrier has thus been overcome and makes it possible to combine cell biological and biomedical techniques. With Organ-on-Chip technology (based on fabrication techniques of the microelectronic chip technology and on the use of microfluidic fluid exchange) brain activity can now be followed real-time both at the cellular and molecular level. That is crucial in the search for cures for these diseases.

On average, it costs almost two billion dollars to develop a drug. For Alzheimer's disease, no drug has been found yet despite more than 30 years of research. This is partly because the current cell and animal models do not mimic the complexity of the human tissue and therefore have a limited applicability. Furthermore, animal models of brain diseases often have low evolutionary (cross-species)

Retina-on-Chip

DR. ANDRIES VAN DER MEER AND PROF. ANNEKE DEN HOLLANDER

One in five elderly people in the Netherlands has a form of age-related macular degeneration, a condition of the retina at the location of the yellow spot (macula). This results in partial blindness and increasingly far-reaching visual limitations in daily life. Such people have difficulties with driving, face recognition, reading and watching television.

Age-related macular degeneration is caused by a combination of genetic factors and lifestyle. Smoking results in a higher risk of macular degeneration, whereas a diet rich in omega-3 fatty acids and antioxidants (lutein, zeaxanthin) has a protective effect. The condition occurs slightly more frequently in women than men. Genetic studies have shown that the complement system, part of the immune system, plays an important role in the development of the disease.

In the case of macular degeneration, damage occurs to the light-sensitive cells of the retina, just behind the eyeball. Other tissues in the retina are also involved, including the retinal pigment epithelium and small blood vessels (capillaries). There is no effective therapy yet for macular degeneration. There is only a treatment based on injections in the eyeball with substances inhibiting vessel growth, that can slow down the most aggressive form.

Little is known about the exact course of macular degeneration. Which tissues in the retina are affected first? What role does the immune system play in the progress of the condition? How do genetic factors and sex hormones, such as oestrogen, play a role? What are the indications for an effective treatment? Laboratory models of the retina are essential for answering these questions. These models should not only capture a person's genetic components and other personal characteristics, but also the complex structure of this unique piece of tissue.

This is the view of somebody with macular degeneration.

Researchers from the University of Twente and Radboud University Medical Centre recently started to use Organ-on-Chip technology to replicate the human retina and study the course of macular degeneration. These Retina-on-Chip models simulate the structure of the retina, including the local capillaries with flowing blood (with Leiden University Medical Centre). The tissues in the model are derived from human stem cells, originating from individual patients, and therefore reflect a person's genetic material. With the incorporation of light-sensitive cells it looks like the Retina-on-Chip models are starting to "see" something.

validity which means that the results of studies with rodent models have a poor predictive value for the efficacy of drugs for human disorders of the central nervous system.

It is encouraging that scientists have already demonstrated that Brain-on-Chip models are very valuable for research on the effect of drugs. One example is unravelling the mechanism of neurotoxicity during a first in-human study regarding a hydrolase inhibitor that did not give any indications of toxicity in animal experiments (see: How a clinical test could go wrong). Or the example after the outbreak of the Zika virus, when an international series of studies with Brain-on-Chip could elucidate the cause of microcephaly in new-born children from women that were infected with the Zika virus. The cellular specificity and the molecular targets of this human virus could not have been identified using animal models.

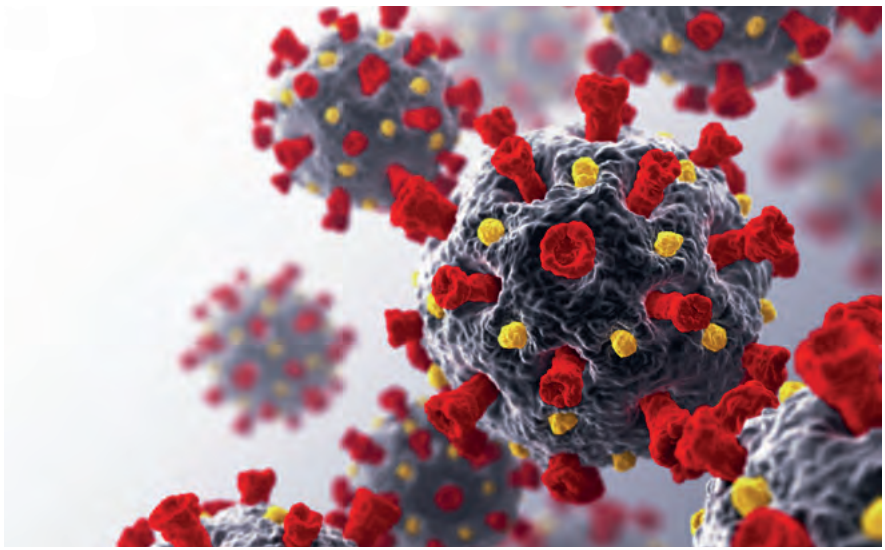
These examples illustrate the scientific and societal value of brain models based on real human material. They explain why there is a strong need for a next generation of advanced cellular models of the human brain to discover new drugs, and 3D Brain-on-Chip models are well qualified for that.

Models for viral infections

DR. KATJA WOLTHERS, DR. DASJA PAJKRT AND DR. BARRY ROCKX

After several people in China were treated for severe respiratory infection in December 2019, the cause was quickly discovered: the coronavirus SARS-CoV-2. Three months later, almost 3 million people had been infected worldwide and more than 200,000 of them died. Six months later, the numbers have doubled. The world economy was shut down, because in many countries, one lockdown after another was announced; a health crisis and an economic crisis in one.

Outbreaks of new viruses regularly occur, however rarely at such a scale. Many of the newly discovered viruses are transferred from animals to humans. The factors that subsequently determine whether a virus can infect humans, make them sick and spread further are very complex. Even for well-known viruses such as influenza, there are still many aspects that are unknown with respect to the spread of the virus and how people get sick.



Schematic representation of the coronavirus, the cause of Covid-19.

Cells, animals and organoids

Within virology, various experimental models are used to estimate the risk of viruses being transferred from animals to humans or from humans to humans. This knowledge is used to calculate the possible impact of new virus infections on public health.

To control virus infections, vaccines or antiviral drugs need to be developed. This requires living models in which an infection is induced. For this purpose, cell cultures or laboratory animals are used. In such models a vaccine or an antiviral drug can then be tested for its capacity to prevent or fight the virus infection. These models have limitations because they cannot always be translated to the situation in humans, or because such models do not work for certain viruses such as the frequently occurring intestinal pathogens rotavirus and norovirus.

With the availability of human organoids, viral infections of the gut, respiratory tract or brain now can be studied better. As a result, the use of human organoids for virology has increased tremendously.

Blood vessel and lung models

The use of Organ-on-Chip models for studying (new) viruses has been very limited so far, while the possibilities are unquestionably extensive. Researchers from Leiden have recently demonstrated that the leakage of blood vessels after infection with the Ebola virus can be simulated with a Blood Vessel-on-Chip model, and that the same model can subsequently be used to test new therapies for vessel leakage.

Other very promising results came from an American study by Don Ingber's group (Wyss Institute) with a Lung-on-Chip, that showed that the extent of the damage to the lung endothelium, and the immune response following infection by different flu viruses correspond to the severity of the disease in humans. The researchers also

demonstrated that this Lung-on-Chip is very well suited for screening of antiviral drugs, as during the outbreak of SARS-CoV-2.

The Organ-on-Chip models are also very suitable for measuring infection and immune responses. The first line of defence, the innate immune system, can already be measured very well in such models. However, the immune response of the human body to a virus is a complex interaction of infected cells in an organ or in blood with cells of the immune system. To simulate this interaction as well, cells of the immune system are now being incorporated in Organ-on-Chip models.

In this way, it is not only possible to gain more insight into the characteristics of (new) viruses, but also to test therapies in laboratory models that mimic the human body. As a consequence, new drugs could enter the market faster, with a reduction of the use of laboratory animals. At the same time, fewer test persons will be exposed to ineffective drugs with harmful side effects. Making use of cells from the patient, a personalised model could in principle also be developed for viral infections to test whether a certain drug is effective for that particular patient or has side effects.

With the development of biological models that simulate the complexity of an infected human, critical factors for disease, spread and treatment can be defined much faster and more objectively than with existing models. This is very promising in fighting viral infections and especially for rapid control of new virus outbreaks.

Lung-on-Chip can be used to screen for antiviral drugs

Regenerative Biomaterial Tests-on-Chip

PROF. CARLIJN BOUTEN AND DR. ANTHAL SMITS

Biomaterials are materials that are compatible for use with living organisms. They have been used in medical implants, prostheses and instruments for a very long time. Modern examples are the artificial hip made of titanium or stainless steel, contact lenses made of silicone hydrogels, nitinol stents, or (degradable) suture of nylon, silk or polyester.

The materials are typically present in the body for long periods – or they are in contact with the body – and should therefore be able to function in harmony with the body. On the one hand, the material needs to be "friendly" for the cells and tissues in the body; on the other hand, the cells and tissues should accept the material. Immune responses to foreign materials can result in chronic inflammation, thrombosis, encapsulation and fibrosis (proliferation of connective tissue) or even breakdown of the material, as a result of which prostheses fail or breakdown products are released. Such reactions can cause severe complications, such as leaking silicone breast implants or painful fibrosis as a consequence of synthetic pelvic floor implants.

Patient's own tissues and carrier materials

Biologists, material scientists, engineers and doctors are working on various solutions to improve the interaction between material and living tissue. These include chemical or physical adjustment of materials to make them more suitable for the human body. Materials of animal origin are also used, such as the heart valve prosthesis made of a tough membrane around the bovine heart, with all cells removed.

Furthermore, over the past two decades, *tissue engineers* even have developed living, autologous (the body's own) tissues in the laboratory: tissues made of cells and biomaterials, that can no longer be distinguished from the existing tissue after implantation, and that can adapt to changes in the body.

However, the most advanced solution consists of developing smart, degradable materials that stimulate the body to regenerate or replace damaged or missing tissues. For this form of regenerative medicine, a temporary carrier material – called a scaffold – is placed in the body as a prosthesis. Because the body recognises the scaffold as foreign material, it sends cells from the surrounding tissue and blood to the scaffold to clear this up and restore the tissue. Thanks to a smartly chosen chemical composition and physical structure of the scaffold, the response of the immune cells can be turned in the right direction: after controlled inflammation, the scaffold stimulates the cells to produce healthy tissue via a wound healing process while the scaffold itself is slowly broken down.

From animal to bioreactor to chip

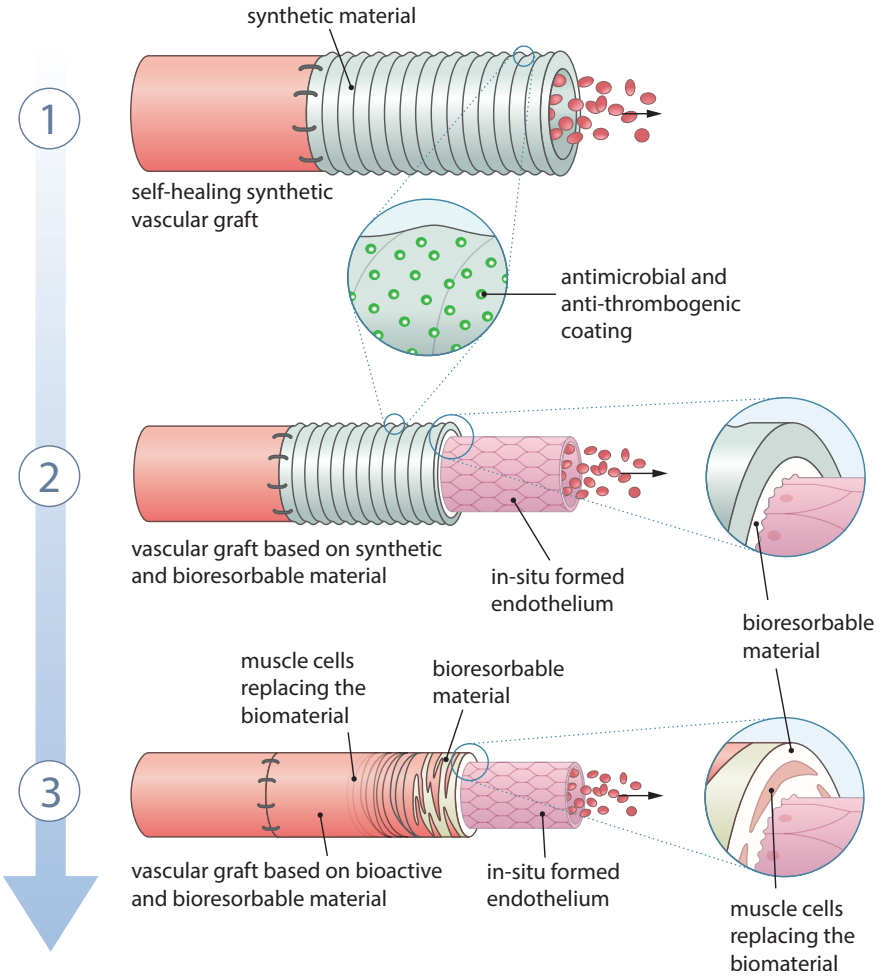
Just as for the testing of drugs, laboratory studies with isolated cells or tissues and animal models are conventionally used for the testing of biomaterials. Such studies mainly explore (under strict conditions) whether the biomaterials elicit an immune response. For these studies the animal model has the advantage of having an intact immune system.

More specific models are needed to predict more precisely, and with fewer animal experiments, how a biomaterial will behave in the body of a human or, even better, a sick patient. Human Tissue- and Organ-on-Chip models could provide a solution for this purpose as well. These models arose automatically so to speak when *tissue engineers* started to culture living tissues in the laboratory. Mostly, they used small pieces of tissue made of the body's own cells and a degradable scaffold. Bioreactors that mimicked the dynamic situation in the body were developed to produce functional tissue. An example is a bioreactor, including heart valve tissue undergoing rhythmic load application, which is comparable to the opening and closing of a real heart valve, for faster tissue formation and stronger valves.

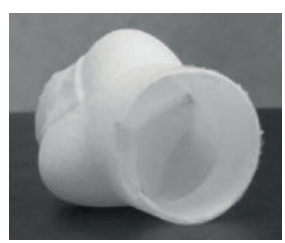
The culture in the bioreactors can include chemical or biological stimuli to simulate many different conditions. The bioreactors themselves are becoming increasingly compact for more efficient use of the cells and scaffolds and for better measurements, for example with microscopy. They are also produced on a larger scale in order to test many different culture conditions.

Regeneration-on-Chip

Nowadays, these systems are also used as test models for Regeneration (new tissue formation)-on-Chip. To monitor and understand the process of tissue formation in a regenerative scaffold in detail, 3D scaffolds are exposed in a controlled way to the same conditions as in the body: the forces on the scaffold, the presence of cells and an immune system, and blood flowing by. At the same time, tests can be performed that provide information about the function of the cells, the formation of new tissue, the stiffness and organisation of that tissue, the breakdown and breakdown products of the scaffold, and the response of immune cells. In summary: simulation and measurements are as specific as possible to predict what will take place in the human body. A major advantage of this approach is that the effects of changes in the scaffold material, such as the addition of a signalling substance that attracts certain cells, can be efficiently tested. Furthermore, certain aspects of the "recipient" (disease, sex, age, etc.) of the material can be simulated and their effect can be examined.



Scaffold for a vein (left) and a heart valve (right). The illustration shows how the scaffold of a vein transforms into living tissue.



An example: newly formed arteries

For the replacement of small arteries, a porous, degradable synthetic scaffold is being developed in the form of a small tube. Immediately after implantation this scaffold should be able to function as a blood vessel, and also continue to do so while the scaffold slowly breaks down and is replaced by new tissue.

To investigate this process, part of the tube is placed in a chip, in which the pressure of blood on the vessel wall and the rhythmic deformation of the wall are simulated. The system is equipped with a blood-substituting growth fluid with circulating immune cells, and vessel wall cells are seeded on the scaffold. This system is exposed to chemical or physical stimuli that simulate a disease, like diabetes, kidney failure or hypertension, which ultimately results in damaged blood vessels. This chip can be used to test which changes to the scaffold are needed to control the immune response and the formation of new blood vessel tissue. In this way, it will be possible to develop and test tailor-made vessel scaffolds for patients in the future.

Can a Brain-on-Chip feel anything?

DR. DOROTHEE HORSTKÖTTER

A special organ that can be mimicked as an organoid or on a chip, is the brain. However, the brain is fundamentally different from other organs such as the liver, kidneys or gut. That is because the brain is seen as the biological substrate for who we are as a person, of our self-understanding and actions and with that of the possibility to lead a meaningful life. This makes research on the functioning of the brain, but also interventions in the brain, very special. It is very important because brain disorders have a direct effect on a person's personal identity. At the same time, it is also highly problematic because research on and also with the brain can influence our ideas about what personality is.

Consciousness-on-Chip?

From an ethical perspective, the development of living brain models elicits important questions and a range of concerns. For example, culturing brain organoids and a Brain-on-Chip raise the question whether such models can develop their own consciousness or sensitivity. If that is the case, would they then deserve, at least in the long-term, the same rights and protection as humans or animals? Paradoxically enough, the development of consciousness could destroy an important ethical advantage. After all, the brain models are being developed with the idea that they might replace animal research and save sensitive beings from suffering. One could argue that the better the living brain models are, the more problematic it might become, from an ethical view, to do research on these models.

For the time being, consciousness in a Petri dish or on a chip is still far away. Nevertheless, it is important to take into account that these consequences could possibly arise in the future. At the same time, it is questionable whether a sensitive or conscious "brain being" in a Petri dish is a realistic perspective or rather a science fiction scenario. Because this implicitly assumes that consciousness and sensitivity can exist independently of a body and without any social interaction. And that conflicts with everything that is known about the importance of biopsychosocial interaction mechanisms for the development and functioning of every human being.

It should be admitted that living brain models already demonstrate a surprisingly high level of self-organisation and electrical activity. But this does not mean that there is a straight upward line between a single neuron without consciousness and a complex of possibly several tens of thousands or millions of neurons with something like a consciousness or self- understanding, or even a personal identity. That always requires a formative social environment, the acquisition of language and interaction with other people. This places the abovementioned ethical concerns in a more appropriate context. At the same time, it might force researchers to examine not just brain models of a patient with a psychiatric disorder, such as psychosis, depression or post-traumatic stress disorder, but also pay attention to the patient's personal circumstances. This broad view is more relevant for research with brain tissue than for studies with other organs.

And who is the owner?

Where is your personality located?

Informed consent and ownership

The development and use of a Brain-on-Chip raises other ethical questions. With the use of hiPSCs for the development of brain models, ethical questions concerning the use of embryonic stem cells no longer play a key role. However, relevant questions arise about consent that should be requested from people who provide cells for research, and about ownership of the stem cell lines. Donors of cells to a biobank are usually asked for consent in general terms and researchers keep research possibilities relatively open. Donors also give up ownership of their cells, which is then transferred to the research institution. This holds for the use of each mini organ.

The brain has a different cultural status because this organ has been assigned the fundamental role as the carrier of somebody's personal identity. For cell donors, this can be a reason to contribute to Organ-on-Chip research in general, but not to the development of living brain-like structures, in particular. In order to provide informed consent, donors should know what their body material will be used for, and research institutions should be as open and transparent as possible about the intended use. Donors should be given the option of conditional donation, so that they can for example exclude the use of their material for brain research, but permit other use.

Concern about the development of consciousness in the Petri dish does not have to criticize this type of research. However, ethical reflection remains essential. Ethics helps to clearly define the prerequisites: to determine how the autonomy of donors can be respected, how possible therapeutic advantages can be generated, and how these can subsequently be shared in a fair way among patients.

In the Netherlands, about 450,000 animal experiments are performed each year. Not only on mice and rats, but also on dogs, guinea pigs and cats.

4 Can Organs-on-Chips replace laboratory animals?

A potential indirect benefit of the development of Organs-on-Chips is that it might help to reduce the use of laboratory animals. In the Netherlands, a special acceleration programme has been initiated by the government: Transition Animal-free Innovation (TPI). Laboratory animals have already been banned for testing the safety of cosmetics. The Skin-on-Chip model is well advanced for tests of the effects of cosmetics. However, sometimes research cannot take place without laboratory animals.

Animals or no animals: how can we realise a more humane medicine?

PROF. DANIELA SALVATORI

Since time immemorial, animals have been used for science and research. For thousands of years, throughout the history of medicine, the anatomy and physiology of vertebrates have been studied. Physicians in ancient Greece dissected animals for a better understanding of the human body. Much of our knowledge about the function of cells, tissues and organs as well as disease mechanisms, has arisen from studies carried out on animals. All that knowledge has contributed to many medical applications, including the use of drugs, vaccines and surgical interventions.

Almost 90% of Nobel Prize research in medicine made use of animal experiments for the discoveries. The treatment of diabetes type I with insulin has first been established for dogs by Banting and Macleod (Nobel Prize 1921).

Animal models have helped to develop vaccines that have saved the lives of millions of people and animals. At the start of the 1920s, child mortality worldwide was considerably reduced after the introduction of a vaccine for diphtheria. In first instance this vaccine was tested on horses. Many new surgical interventions have been developed and tried out on pigs, because their organs are about the same size as those of humans.

Mouse models

Animals are similar, but not identical to humans. Rodents, in particular mice, are the mostly used mammals for experimental research. Mice are used because of their genome, which is 95% similar to the human genome. Furthermore, mice are small, easy to handle and they reproduce quickly.

Regardless of the similarities, mice do not always develop the same genetic diseases as humans. Researchers have to change the mouse genome first in order to cause a human disease in mice. Advanced gene technology is available that can change mouse genes such that the mice develop human diseases, the so-called mouse models for human diseases. Although many different mouse models are available to study human diseases, a successful treatment in a mouse does not guarantee the same result in a human. And that is not only due to the genetic differences between mice and humans.

No animal model can precisely recapitulate human disease: all are an approximation

Not all mice are the same

In laboratories, inbred strains of mice are mostly used. These mouse strains are produced by allowing "brothers and sisters" to mate for at least 20 successive generations, which results in a highly homogenous composition. The results are very much dependent on the specific inbred mouse strain that is used. A striking example was published in the journal *Science* in 2014. A research team stated that some mouse strains were completely resistant to the Ebola virus and that others died without specific symptoms, whereas mice from yet another strain developed fatal haemorrhagic fever. This example demonstrates how we should consider animal models: no single animal model is able to precisely recapitulate a certain human disease.

Furthermore, it is not possible to create all symptoms of a certain human disease in mice. In the case of age-related brain diseases such as Alzheimer or Parkinson, one must be careful in interpreting cognitive, emotional and (of course) language deficits that are characteristic for such diseases. For this reason, differences in development and function of the brain in rodents and humans need to be considered.

Legal obligation

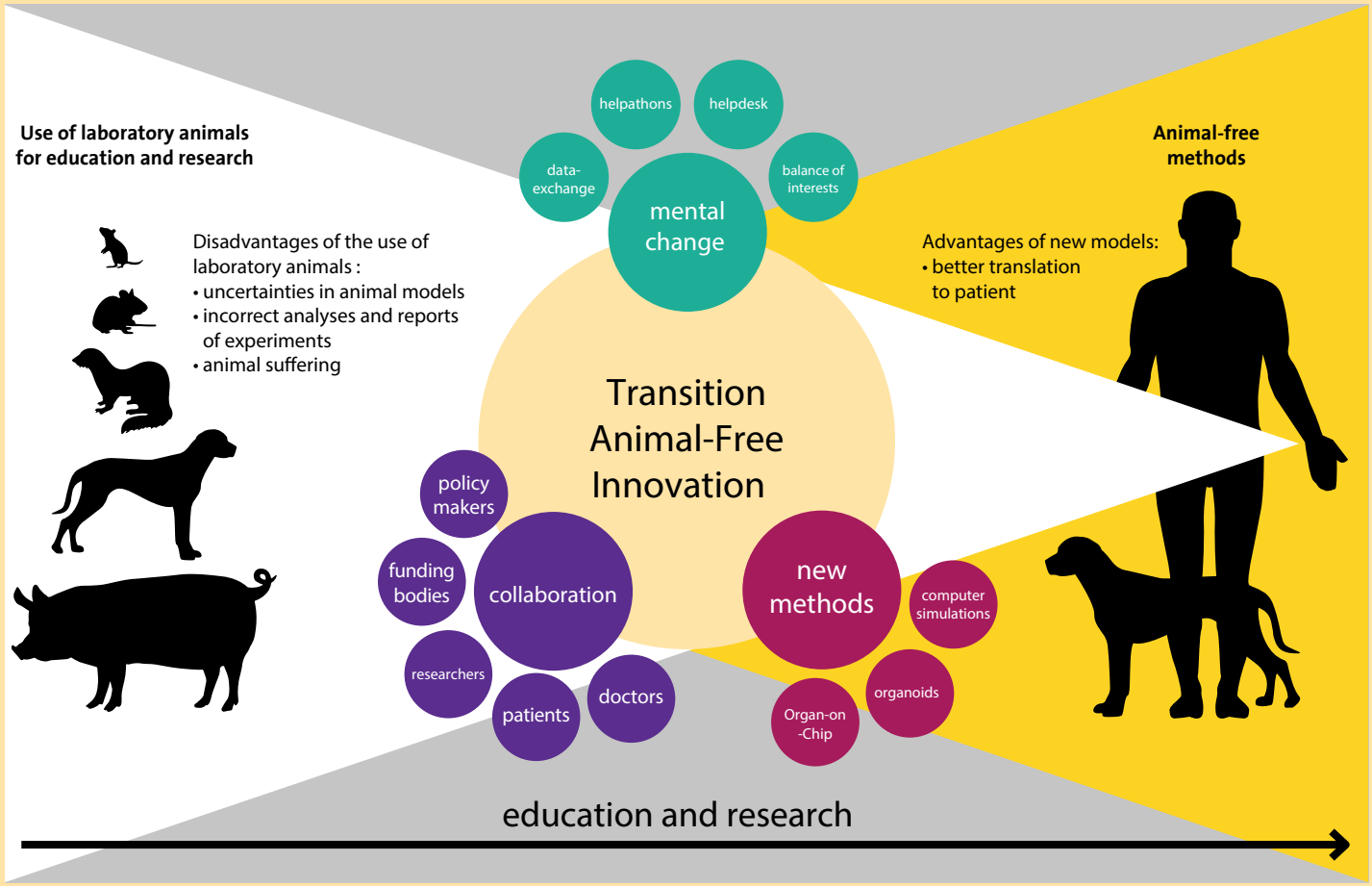
At present it is not permitted to directly test new drugs on humans because there is a legal requirement to carry out the tests on animals first (preclinical research). This procedure, which is mainly used by the pharmaceutical industry, requires on average 10 to 15 years per drug and is extremely expensive. Researchers in pharmaceutical companies decide on the basis of the results from animal experiments whether a possible drug will be effective and safe in humans. Despite all efforts, money, time and animal experiments, pharmaceutical companies are confronted with a serious problem: just one in ten drugs that have a positive effect on animals also works in humans. Only a fraction of the thousands of human diseases has an approved therapy, that causes in many cases also undesirable side effects. This is very worrying for all patients, who are hoping to be cured.

The causes of this unacceptable, worldwide failure have been analysed and have been attributed to errors in the study design, analysis and reporting of experiments. Although improvement is possible, this will not solve the differences that exist between humans and animal species. These differences make it impossible to directly translate preclinical results from animals to humans.

Accepting change

Much has improved over the past 50 years. The use of laboratory animals is ethically much debated and controversial. The Netherlands has the ambition to significantly reduce the use of laboratory animals in the coming years. The availability of

The route to animal-free education and research

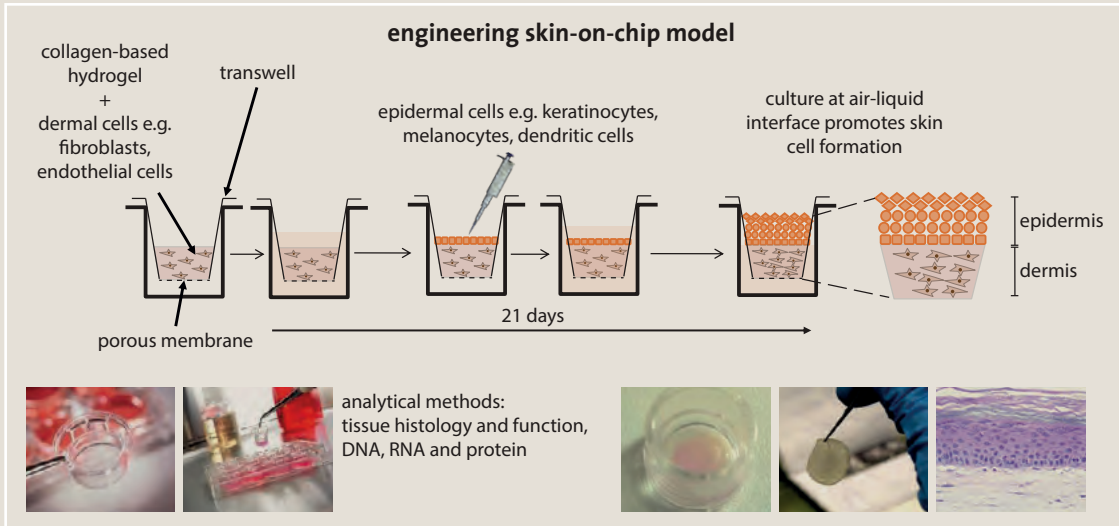


Entrepreneurs, researchers and civil society organisations are seeing a growing number of possibilities for research and testing without animals. A change in mindset, collaboration and the development of new methods are key aspects. Communication is a crucial factor: the exchange of data and the exchange of ideas, for example via the helpathons or helpdesks that answer questions and make contact with the experts. Helpathons are meetings to critically discuss a research proposal that involves laboratory animals, and suggest, where possible, replacement of the animal experiments by alternatives, or refer to knowledge that has already been generated in other research projects.

Skin-on-Chip

PROF. SUE GIBBS

During everyday life, our skin comes into contact with a wide range of products such as cosmetics, hair dye, perfumes and drugs as well as many household, industrial and agricultural products. Sometimes this contact is “desirable”, but often it is not and then nevertheless unavoidable.



In three weeks, a mini skin can be cultured and used for skin experiments. The cultured piece of human skin consists of the epidermis (keratinocytes, melanocytes and dendritic cells) on top of a dermis of hydrogel with collagen and connective tissue cells (fibroblasts). The different cell types are isolated from small skin biopsies. The cells are multiplied in culture and are used to produce large quantities of skin models. For example, a biopsy of 3 cm^2 of skin can produce a series of 50 skin models (each 4 cm^2 in size). Various methods of analysis are available to measure the effect of substances on the mini skin.

Absorption of substances

The skin protects our internal organs from substances in the environment. However, many of the substances applied to the skin are absorbed and subsequently activate the immune system before they enter the blood. Once in the blood, they can influence other organs.
For this reason, strict safety assessment of active substances and other ingredients in products and drugs, that are being applied to the skin, is essential to determine a safe concentration. This means that skin models are needed that can simulate the behaviour of human skin (the skin physiology), especially because Europe has banned the use of animals to test whether substances in cosmetics cause irritation, damage (2009) or allergies (2013).
Many tests have already been developed based on skin cells. Tests are available using a reconstruction of the human skin (epidermis), equipped or not with certain immune cells (dendritic cells). These tests are valuable to investigate which cosmetic ingredients cause irritation or allergies. Some of these tests have been officially validated by the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM).

More complex skin models

The challenge is to develop more complex skin models that include immune cells and that are connected with other organs via microfluidic channels. It will then be possible to determine whether substances applied to the skin can spread and cause harmful effects elsewhere in the body.
An example is to determine whether substances are harmful for the liver. The necessary Skin-on-Chip model could consist of a piece of skin, cultured in the laboratory and made up from epidermis, dermis and immune cells, which is connected with mini livers (organoids), via a system of microfluidic channels covered with blood vessel cells. Such models are currently being developed, and in the future will give more insight into the toxicity of substances, that are applied to the skin.

new, human-based methods such as Organ-on-Chip technology has increased enormously.

However, it is very difficult for young researchers to deviate from the standard procedures and opt for alternatives. Animal experiments have been an important part of research for a very long time. For an active search for alternatives, students should be made familiar with possible animal-free methods already at an early stage in their curriculum. In addition, an open dialogue with the government needs to be realised, together with initiatives that promote data sharing.

It is an emotional and difficult consideration but we must be willing to accept a change in mindset. Progress means investing in research methods and technologies that are relevant for humans. We have a moral obligation towards patients and animals to make medicine more humane.

Replacing animal experiments with animal-free innovations is challenging

The search for alternatives

DR. CYRILLE KRUL AND DR. ANNE KIENHUIS

For decades, the 3Rs have been a topic of research: refinement, reduction and replacement of animal experiments. On the one hand, because animal experiments are not always good predictors for humans. On the other, because of the request of the scientific field, society and politics to reduce the use of animal experiments. Many methods that could be alternatives have been developed in recent years. Examples are cells in a culture well, computer models, or more advanced 3D models, like Organs-on-Chips. Unfortunately, only a few of these alternatives have been developed and standardised to such extent that they can be incorporated in regulations.

Animal-free innovations

Replacing animal experiments with a completely new test that does not use laboratory animals, referred to as animal-free innovation, is not easy. It is not yet possible to study the effect of drugs and toxicity of chemical substances or nutrients without animal experiments. Proof is necessary for the safety of new drugs and substances, that will enter the market.

For the development of animal-free innovations, more knowledge is needed about human biology, the influence of drugs and substances on the human body, and the translation of that knowledge into relevant and reliable test methods. Collecting and exchanging knowledge and setting the right priorities requires investment in international collaboration between the various stakeholders from science, industry and government, and between different disciplines, such as technology, data analysis, biology, pharmacology and toxicology.

Organ-on-Chip is a promising, animal-free innovation, enabling the culture of (combinations of) human cells for long periods (longer than a month)

Better than the gold standard

DR. BEREND VAN MEER

A test model based on human heart cells sounds promising, but is it also indeed better than animal experiments or other available models? In order to answer that question a project was started in 2013 with a number of European research groups that all had developed their own test model with heart cells derived from human stem cells. Except for one group that used heart cells isolated from a rabbit heart. The rabbit heart model is the "gold standard" for testing possible side effects of new drugs on the heart. This means that all potential new drugs should be tested on these rabbit heart cells before they can be tested on humans.

A pharmaceutical company also participated in the project and provided 27 different drugs to the research groups to test on the heart muscle cell models. This was done 'blinded': researchers did not know which drug they were testing.

For each drug, the researchers noted – based on their test results – whether the heart of the patient would contract more strongly, less strongly or if nothing would happen. The effect of each of the 27 drugs on the human heart was already known from clinical practice, so that it could be easily checked whether the model predicted the effect correctly.

The research group from Leiden University Medical Centre (LUMC) developed a special microscope for very accurate measurement of small changes in the contraction of the heart muscle cells, the electrical activity and the calcium waves after the administration of a drug. The last two elements are vital for heart cells to contract properly.

The LUMC measurement system: a special microscope developed for very accurate measurements of small changes in the contraction of heart muscle cells, electrical activity and calcium waves after the administration of a drug.

The models with rabbit heart cells correctly predicted the effect in 67% of the cases. In general, this is comparable with the predictive value of animal experiments. The heart cells model derived from human stem cells in combination with the LUMC measurement method predicted the effect correctly in 78% of the cases, a signifcant increase and better than the gold standard – at least for the limited number of drugs that were tested. The other research groups that also used human heart muscle cells, but a different measurement method, scored between 56% and 67%.

The fact that the LUMC measurement method resulted in a better score than the measurements from some other groups with the same type of heart muscle cells reveals that it is not only important to have the right heart model, but also to combine this with the right measurement methods. And that is very well possible with Organs-on-Chips.

under conditions that resemble the physiology of humans. This allows the study of long-term effects of drugs or other substances but also any side effects. For several organs, including lung, skin, liver, kidney, heart and gut, Organ-on-Chip models are being developed. But where and how can they be used in research on the effect and toxicity of drugs and substances?

Toxicity

In the short term, the use of an Organ-on-Chip has the best chance of success in research on the efficacy and mode of action of a drug. An Organ-on-Chip model can be used to screen possible drugs. This can be done in a very early phase of the research, when many potential drugs are still available, but also in a later stage to gain a better understanding of how the promising drug works.

The use of Organ-on-Chip for research on the toxicity of drugs and chemical substances is much more complex, because it is only allowed to use methods that are included in international guidelines. However, the model systems are very promising for toxicity research because the cells can be exposed for a long period under physiological conditions. This might give insight into toxic effects that occur after a longer period of time. Organs-on-Chips can already be used in screening tests, to get an indication of possible harmful effects. However, an animal-free innovation must first be internationally accepted before it can be used to determine toxicity.

Validation tests

Validation is needed for inclusion of Organ-on-Chip technology in guidelines for toxicity tests.

Research on gut organoids at Utrecht University, one of the animal-free innovations that are being developed.

Animal-free asthma research

DEBBY WEIJERS, MSC

In Europe, more than 10 million animals are used each year for scientific purposes. Worldwide this number is more than 100 million but this is an estimate since a proper registration is lacking in many countries.
In the Netherlands, about half a million animal experiments are carried out each year. About 98% of these are done for the benefit of people. Examples are safety tests of substances, drug development or research on human diseases. For such experiments, researchers are continuously looking for a model that resembles a human as close as possible. For a long time (genetically-modified) animals were used, but in recent years science has developed rapidly. One of the latest innovations is the simulation of complex biological systems on a chip. By combining cell and chip technology it is possible to develop Organs-on-Chips, that have the potential to reduce the need for animal experiments.

Asthma-on-Chip
A good example of this development is the Asthma-on-Chip model. Asthma patients respond to a wide range of harmless stimuli, such as cold or pollen. A coughing and cramping reflex occurs, so that the muscles around the airways strongly contract and the asthma patient hardly can breathe. Until now, asthma research has only been done on animals, especially guinea pigs, because these animals can become short of breath, just like humans. However, asthma research in guinea pigs certainly does not provide all information needed to understand the disease. Researchers can see that the guinea pig becomes short of breath but they cannot investigate why that happens. This is because the anatomy of the nerves that reach the airways is different in guinea pigs than in humans. As long as the cause is not known, it is difficult to develop targeted therapies that can help asthma patients.
The University of Groningen has now developed an Asthma-on-Chip model that can help to better investigate what happens in the human body during asthma. In this way the researchers want to study the communication between nerve cells and lung muscle cells so that they can gain a better understanding of the cause of shortness of breath. Furthermore, the research is completely animal-free. Research that is better for humans and animals!

Instead of guinea pigs, plastic chips with human cells are now being used at the University of Groningen to study the mechanism of asthma development.

Organs-on-Chips provide the pieces of a jigsaw puzzle that can describe human biology. These pieces can be combined with a range of other relevant data, such as disease status, age, gender, genetic material and lifestyle of the donor. This can lead to a digital twin with predictive value for health and disease.

Investigating the validity of an Organ-on-Chip model will provide all stakeholders with detailed information about the relevance and reliability of that model for toxicity tests. Validation is a time-consuming and difficult process and on average can take 10 years per application. Another aspect that hampers validation is, that many different stakeholders are involved in this process, such as the developers of the model, the industry and the governmental bodies that evaluate the toxicity of drugs and substances.

For Organs-on-Chips various aspects are important in the validation process. First of all, they have to provide reliable predictive information about possible toxicity in humans. This requires that an Organ-on-Chip accurately resembles the specific mechanism in humans. This needs to be further specified in a standardised method. The method should be robust, which means that small differences in the conditions of the procedure do not influence the outcomes. Furthermore, these outcomes should be reproducible. In other words, an independent test performed by different laboratories with a single set of reference compounds should give the same results.

These aspects of validation apply to every animal-free innovation. During the development of an Organ-on-Chip, the complexity of the technology also plays a role. The principle is: develop a test that is *as simple as possible and as complex as necessary*. In this respect it is important to find a balance between a complex system with many cell types, which can accurately predict a specific mechanism of toxicity, and a system that is easy enough to use for obtaining reproducible results in different laboratories.

The virtual human

Organs-on-Chips yield pieces of the puzzle that can ultimately be used to describe human biology. Each piece leads to a set of data that can be combined with all sorts of other relevant data, including data about disease status, age, sex or genetic background, or even about lifestyle. With the help of artificial intelligence and *machine learning* this can result in advanced computer models with predictive power. Such a virtual approach (the virtual human) can make relevant predictions about the toxicity of drugs and chemical substances for each individual patient or healthy individual, based on his or her data. Although this is not yet possible in toxicology using the current approach (in which laboratory animals take a central position), developments related to this approach that uses the digital version of humans ("digital twin") to prevent harmful effects are fully underway.

Why laboratory animals are someti

PROF. GUUS SMIT

Can the mouse or another laboratory animal completely disappear from the laboratory? Probably not. Some organs are so complex that it is impossible to understand them by looking at cells in a culture well or even on a chip. A good example is the brain.

Complex network

The human brain consists of about 80 billion nerve cells surrounded by supporting glia. Recent research has demonstrated that at least 100 different types of nerve cells exist. These nerve cells all have unique connections and form large networks that are constructed during a development process of many years. That process already starts before birth and continues until at least the 20th year of life. Nerve cells continuously undergo changes due to interaction with each other and the processing of information from the living environment. The network of nerve cells can learn, make new connections or strengthen or weaken these connections. The brain receives information from sensors (such as the eyes and ears), integrates that information, and transfers a balanced signal to other organs, for example the muscles. So you "see" a car (you recognise it as a car), estimate how fast this will reach you and you decide that it is safer to jump aside. All of this occurs within one second. A complex system like the brain can only be studied if it is completely intact, for example in a living human or laboratory animal.

Brain development

Growing nerve cells in a culture well is not the problem. For decades, researchers have cultured nerve cells from rodents in the laboratory for research on the function of these cells. Thanks to the development of hiPSC technology, now also human nerve cells can be studied and animal experiments are therefore no longer required. However, these studies are all limited to the investigation of the "cell biology of the nerve cell". That yields interesting knowledge about the nerve cell and the interaction with a few cells around it. But these experiments are not sufficient to understand the complex communication between large numbers of very diverse nerve cells and their specific connections in the intact brain. This is not only due to their small number but mainly because nerve cells in culture lack the crucial development period of the brain, during which they develop by interacting with each other and by external information. Furthermore, with the current state of the technology it is not possible so far to recapitulate the immense diversity of brain cells in culture.

Brain diseases

Research on intact brain is not only important to understand how our brain develops and functions, but also how brain disorders can develop over time. Examples are depression or Alzheimer's disease, which are both the consequence of long-term processes by the interaction of the nerve cells with each other and with the environment. In Alzheimer's disease, different types of nerve cells and immune cells, while mutually interacting,

mes still needed

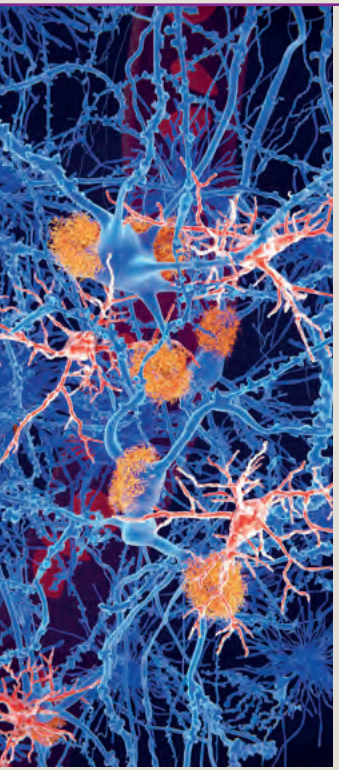


Illustration of nerve cells with amyloid plaques (orange) and microglia (red), the immune cells of the brain of somebody with Alzheimer's disease.

seem to play a very specific role in the disappearance of the memory. In the case of depression, the interaction of the individual with the environment determines the development of this psychopathology. For the best possible studies of the development of these complex brain disorders, intact brain is needed. Because this research in humans is difficult, and sometimes even impossible, laboratory animals, mostly mice or rats, are needed for experimental research. The additional use of human nerve cells in culture can be very useful in this regard, for example to understand what goes wrong inside the cell. However, in many cases the pathology is determined by lack of collaborating nerve calls in large networks, which cannot be easily mimicked in culture.

If research leads to a potential drug, the success of that therapy then still depends on tests in laboratory animals. It is difficult to know with certainty whether side effects, that only occur in a complex system, can be identified in simple culture environments. And that should be known prior to exposure of humans to the new therapy. In the end, humans are included in a small clinical study, that can be expanded to treatment of patients on a large scale in case of positive results.

Computer model

Would it be possible to mimic the brain and brain diseases in computer models or via artificial intelligence? That certainly helps to gain more insight, but also requires the combination with animal experiments. One of the most important aims of these models is to understand the functioning of the complex biological systems and the effects of external factors and substances on these systems. This is only possible by continuously interrelating the results from these two research areas. Modelling brain processes and the effects on those processes without biological confirmation of these neurophysiological processes in the brain will soon lead to a wrong interpretation of the reality.

Both computer models and brain cells in culture wells or on a chip form a useful addition to brain research. But in every brain study the ultimate question is: are we doing the "best research" using as few laboratory animals as possible or are we doing the "best possible research" without laboratory animals? A useful "addition", however, is not a "replacement" or "transition". Brain research takes a long time and is very expensive, but it is also needed to describe and understand the development and complexity of the biology of the brain as well as possible.

For brain research, and also other research that requires laboratory animals, it might be good to aim for the "best research" with as few laboratory animals as possible instead of the "best possible" research without laboratory animals.

A so-called “valley of death” must be crossed to bring an innovation from the lab to everyday practice. That usually requires collaboration between developers and end users, and everything in between, from an early phase onwards.

5

Challenges and barriers, what are the next steps?

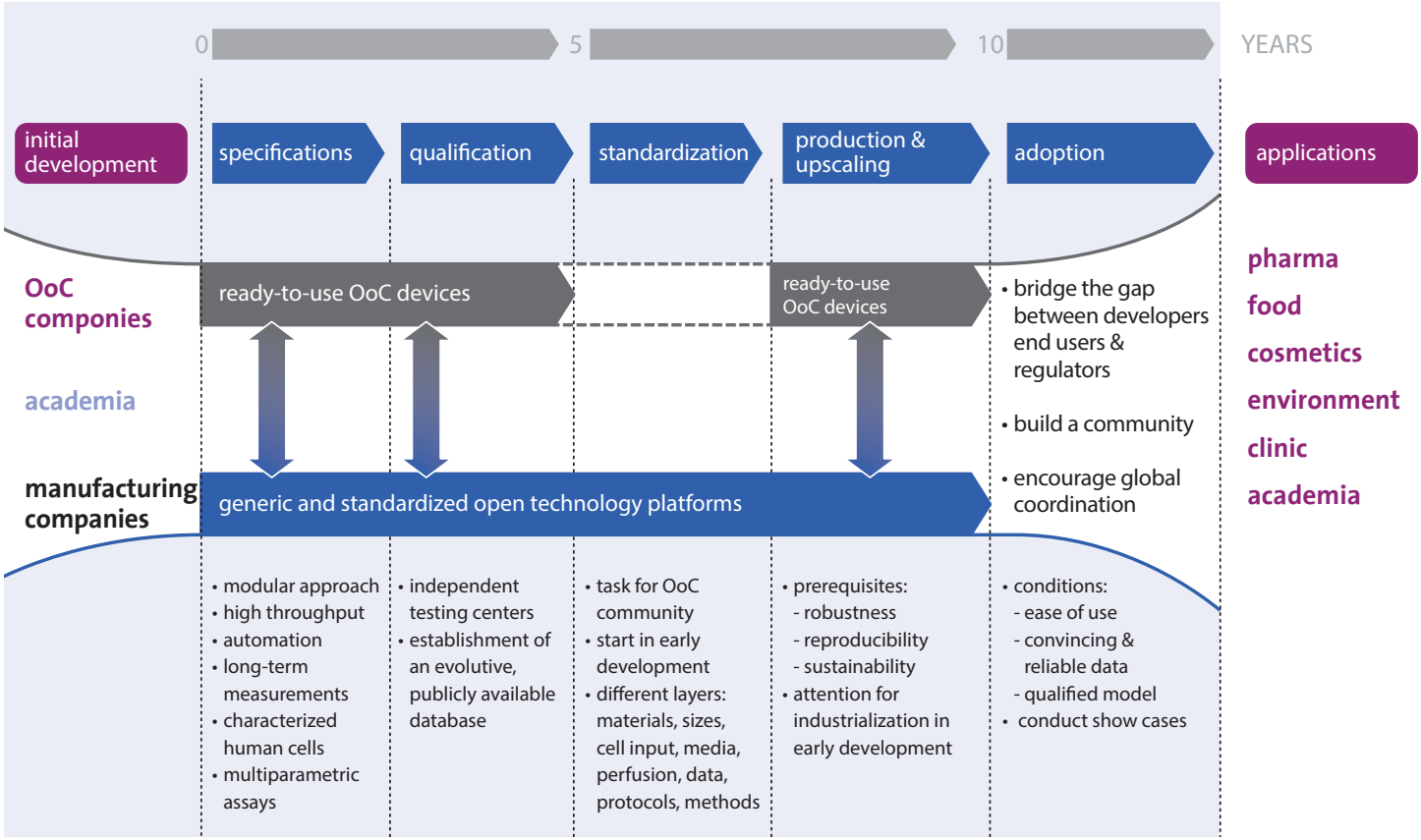
Much needs to be done before Organs-on-Chips can be and are used as test and research models in healthcare and by the pharmaceutical industry. Academics, industry and regulators in Europe have jointly developed a roadmap, indicating the steps required to implement these new models in practice. First of all, the models in different laboratories should yield the same results, and be suitable for standardisation and large-scale production.

European Roadmap

DR. JANNY VAN DEN EIJNDEN-VAN RAAIJ

What is the current state of the art with respect to Organs-on-Chips? What is needed to make these new test models useful and attractive for a wide range of end users? And what is the route to achieve this? In 2019, these issues were discussed by scientists, companies, regulators and end users from around the world. The meeting took place in the context of the two-year European project ORCHID (Organ-on-Chip In Development), which was coordinated by the Netherlands and ran until the end of 2019. Via literature studies, interviews and expert workshops, an inventory was made of the current state-of-the-art of the technology, of the users' needs and of the main challenges for the future.

The outcomes were outlined in a European roadmap. It is a guide which describes the steps and milestones that are required to implement Organ-on-Chip technology as an innovation in everyday practice. And to demonstrate that this technology offers solutions for societal challenges in healthcare. The most important application areas for Organ-on-Chip technology are currently personalised medicine, the efficacy of drugs, toxicity of substances and unravelling disease mechanisms. The models should be user-friendly, equipped with integrated sensors and pumps, connectable for the measurement of organ-organ interactions, and suitable for parallel tests. The three major challenges for the actual acceptance of this innovation are: qualification, standardisation and production and upscaling of Organ-on-Chip models.



European roadmap for Organs-on-Chips developed in the ORCHID project.

Qualification

A growing number of academics and companies are developing their own Organ-on-Chip models, resulting in a multitude of choices. But which models can now be used for which purpose? One of the recommendations from the ORCHID report was that the models should be independently qualified and characterised for a defined and specific purpose (*context of use*) (see Qualification, everywhere the same result). That independent qualification is extremely important for convincing end users about the reliability and reproducibility of a model.

Qualification requires a set of test compounds for which it is known what their effect is on organs in the human body and how this can be measured. This allows a model to be qualified for a certain purpose. The IQ MPS consortium, a non-profit organisation of 21 pharmaceutical and other biotechnology companies, has put together a list of such test compounds, so-called reference compounds. It is also important to ensure that the results of the independent qualification of Organs-on-Chips are made accessible for end users. An elegant way to achieve that is by storage of those data in a publicly accessible database.

Standardisation

For standardisation, Organ-on-Chip models should be reproducible, robust and connectable (interoperable). It involves not only the cells, but also the

chips. This implies that a Heart-on-Chip system of one company should fit perfectly with the Liver-on-Chip model from another company, and that they should be compatible with the laboratory equipment present. This would make researchers' lives much easier and could, for example, also accelerate studies on the toxic effect of drugs or their metabolites on the heart.

However, the reality is different: everyone is developing their own system and supporting equipment. Standardisation requires a broad European effort, which has already been initiated by the European Organ-on-Chip Society, EUROoCS (see: The international perspective). As demonstrated by the electronics industry (Electronic Components and Systems, ECS) standardisation is associated with accelerated innovation. Open technology platforms equipped with standard technology are already common practice in the electronics sector and form the basis for customized applications, that meet the needs of the (end) user (see: Standardisation and open technology platforms).

Qualification, standardisation, manufacturing, and upscaling are the biggest challenges

Production and upscaling

The number of chips and cells to be produced is dependent on the type of model and the extent of use. For some applications, such as screening hundreds of drugs by the pharmaceutical industry, systems with many chips are needed that can be connected in parallel. For academic research on disease mechanisms a single chip might suffice.

In order to eventually produce sufficient and affordable quantities, the design, dimension and structural materials of the chip should be taken into account, preferably from the start of the development. Furthermore, during the upscaling of the models, quality control of both technological and biological components is important. This requires clear guidelines to guarantee the robustness. Just like in the microelectronics industry, high-volume production will probably be associated with a reduction of the production costs and variability and will result in a high reproducibility of the chips. On the other hand, mass production of chips requires huge investments, which are not obvious as long as the systems are not qualified. That is why qualification, standardisation, production and upscaling go hand-in-hand.

Qualification, everywhere the same result

DR. BEREND VAN MEER, DR. JANNY VAN DEN EIJNDEN-VAN RAAIJ AND PROF. CHRISTINE MUMMERY

IF ORGANS-ON-CHIPS are so promising, why are they not yet being used in every pharmaceutical laboratory? And why are drugs not continuously being tested on these systems? There is, however, a big difference between development and use of this type of models in research groups, and application of such models in the pharmaceutical industry. For the industry, the models need to be robust and qualified, and it should be very clear what a model can do and cannot do. On the other hand, in a research group, new developments are paramount and less attention is paid to issues such as reproducibility and optimisation of protocols.

Ideally, the regulatory bodies, which assess whether the right experiments have been done to allow a drug to enter the market, should give approval for the use of the models. This approval should be based on the right information. But where should that information come from? Pharmaceutical companies usually do not invest in a model that might not be approved; they invest in drug development. The last thing they want is that a study with an alternative model yields something unexpected that is hard to interpret and could disturb the progress of drug development. Research groups on the other hand, do not invest in the application of models; they develop new models. Furthermore, both parties have a very clear interest so that their information about the model could be biased. Independent test centres that can qualify and validate existing and new models are thus needed to overcome this impasse.

A fit-for-purpose Organ-on-Chip model is one which has proven suitable for a specific purpose. In other words, it cannot be used for all types of tests but exclusively for a specific application. An Organ-on-Chip model is thus qualified for that specific application.

Establishing test centres

Qualification of newly developed tests is continuously taking place. It usually goes like this: a single central laboratory selects a new test model, which is subjected to a number of standard tests (can the model be used?) and subsequently to several model-specific tests (does the model mimic what it was supposed to mimic?). Afterwards, the same tests are performed in different laboratories to evaluate the reproducibility and check whether they yield the same results. If this is the case, showing a high reproducibility, this information can be shared with the regulatory bodies, that can approve the test models, and with the pharmaceutical industry, that can select which test model is suitable for which drug.

In Europe, test centres already exist for alternative test models for animal experiments (EURL ECVAM). They focus on less complex models, such as simple cell lines that are used for tests in large volumes. The way these models are tested, is a good example for the qualification of Organs-on-Chips. The Dutch Organ-on-Chip Consortium hDMT has already taken the initiative to realise test centres that specifically focus on Organs-on-Chips. With this, one of the most important steps described in the roadmap has been taken towards implementation of organ models in practice, enabling reliable drug testing and (partial) replacement of animal experiments.

Standard tests and model-specific tests

Although the organisation and approach of the test centres is clear, the crucial question remains which tests should be performed in order to prove that the organ model indeed simulates the situation in the human body. The standard tests and model-specific tests have different purposes: the standard tests should evaluate the basic requirements of the model, such as the suitability of the material to culture the cells, ease of use and the lifespan of the

Independent test centres are needed for qualification and validation of the models.

cell culture. The model-specific tests should clarify for which application the model can be used (fit for purpose), whether the model can predict the effect of a drug in the human body, and also what the conditions and limitations of the model are. The tests are very complex, because how could these aspects be determined? Most of the processes that occur in the body are unknown, and also the interaction between different cells, tissues or organs is not fully understood.

Reference drugs

Organs-on-Chips are too complex to be qualified based on our current knowledge of the human body. But it can also be the other way around. Organ models can be qualified using drugs with known effects in patients. So for models that mimic the contraction of heart muscle tissue, a list of drugs has been compiled that influence the heart in various ways. These drugs all use a different target of the heart muscle tissue: the drug nifedipine blocks certain calcium channels of heart cells and the drug omecamtiv mecarbil blocks proteins that are involved in the contraction of the heart muscle, while both drugs reduce the force of contraction. If the heart model does not respond to one of those drugs, it is possible that the heart model lacks the respective target, or that the target does not function properly in the model.

A set of different types of drugs for which the precise effect on the human body is known are called “reference drugs”. As different Organs-on-Chips simulate different parts of the human body, a list of reference drugs is currently being compiled for different organs and models. That allows models to be tested for their specific application and enables regulatory bodies to give approval for the use of a test model, based on the test results. It may seem paradoxical, but in this way, drugs can be used to test whether Organ-on-Chip models are suitable for drug testing.

Standardisation and open technology platforms

DR. MART GRAEF

Successful application of Organ-on-Chip technology in everyday practice requires a combination of the approach of the biomedical world with the working method of microelectronics. In medicine and the pharmaceutical industry, equipment and drugs are usually being developed for specific treatments, so that standardisation plays a minor role. However, this results in relatively high development costs, a long development time and in the end expensive healthcare.

Miniaturisation

On the other hand, in microelectronics standardisation forms the basis of success. For more than 50 years, standardisation has been the driving force behind the electronics industry, resulting in an increase of the number of components in a single chip from 10 in 1962 to the impressive number of 1 terabit (10^{12}) today. This development was made possible by "Moore's Law": the functionality of integrated circuits (ICs) increases exponentially over time, while the manufacturing costs decrease. In other words: integration of functions results in lower costs per function.

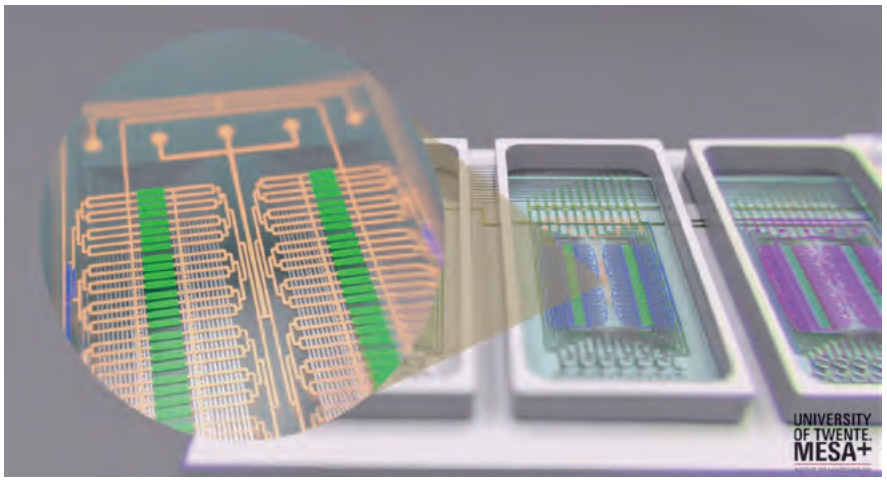
Open technology platforms

The increased complexity in microelectronics is not only a technological and scientific challenge, but also forms the core of its innovative strength. That requires far-reaching standardisation. The only way to manage the complexity is to divide technology into standardised process modules. In turn, these modules can be added as building blocks to various systems for a wide range of applications. A complete set of standardised process modules together forms an "open technology platform", that can be used by different developers to develop customized applications.

Through these open technology platforms, the microelectronics industry has been able to develop in a phenomenal way in recent decades. Would it be possible to use this approach, based on standardisation and multidisciplinary collaboration across the entire value chain, in the biomedical world? Such an idea could be named "Moore for Medical". It is not a goal in itself, but by making electronic components and systems available via open technology platforms in the biomedical domain, the enormous challenges in healthcare, with respect to both innovation and cost management, might be effectively tackled.

Technology platforms for Organs-on-Chips

Using standardised building blocks for Organs-on-Chips could enormously increase their applications. This is necessary, because every person, organ and research question is different and the ultimate models must be fit for purpose. That is only feasible if the development of these models is both flexible and affordable. Each chip must have a combination of cell-friendly microfluidic channels,



The University of Twente has developed a Translational Organ-on-Chip Platform (TOP) that fits different chip systems.

sensors to collect and process measurement data or images, as well as actuators, micro-electromechanical systems (MEMS), that can stimulate or trigger a micro organ. A technology platform for Organs-on-Chips is crucial for the integration and control of all these different components, and to meet the needs of different users.

Various technology platforms for Organs-on-Chips are available now. For example, the University of Twente has developed the *Translational Organ-on-Chip Platform*, which offers an open, standardised interface control for microfluidic chips from different developers. Another example is a platform based on a microfluidic channel embedded in a freestanding PDMS membrane, which has been developed at TU Delft.

The development of technology platforms for Organs-on-Chips is a huge challenge. This can only be realised by making effective use of the knowledge of many academic and industrial experts and other stakeholders who are part of the Organ-on-Chip ecosystem. The importance of interdisciplinary collaboration, both at the national and international level, is evident. A new European initiative in this context is the "Lighthouse Health.E" programme, which has been set up to facilitate the use of electronic components and systems in the medical domain. Several collaborative projects in this programme specifically focus on Organs-on-Chips and the development of technology platforms.

(Inter)national collaboration is crucial for the development of technology platforms

Upscaling, from model to factory

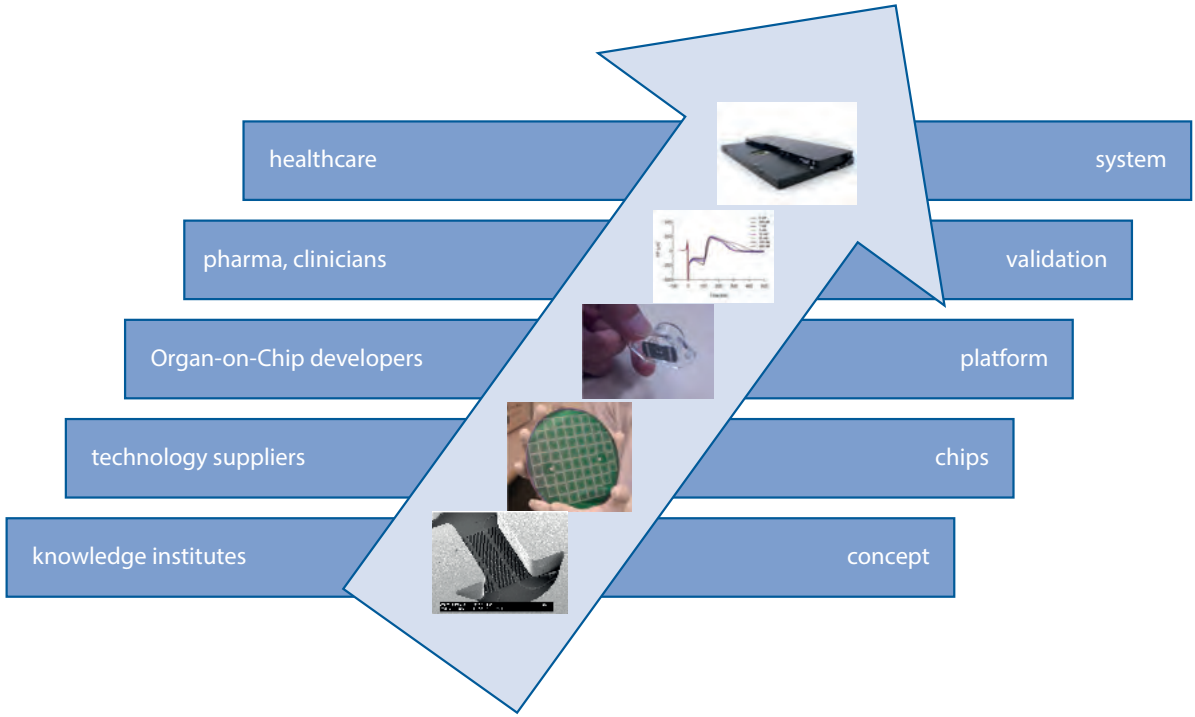
DR. MART GRAEF

The success of Organs-on-Chips will ultimately be measured by the extent to which they can be used for generating disease models, developing new (personalised) drugs and establishing the efficacy of drugs. That will only be possible if Organs-on-Chips can be produced and applied on a large scale, and the results obtained are reproducible and mutually comparable. That does not only require a high degree of standardisation, but also an industrial production of the necessary technology platforms and the associated instrumentation and measurement equipment.

The value chain

The problem of developing and marketing new products based on scientific research results is known as bridging the "valley of death", the imaginary gap between the university and the end user. In the academic phase often too little attention is paid to aspects such as reproducibility, manufacturability and cost price, so that a brilliant idea can still fail during the product development. This can be prevented by the involvement of all stakeholders in the value chain, already from the start of the innovation project.

The value chain of the Organ-on-Chip ecosystem includes knowledge institutions that focus on the development of mini organs and platforms, technology suppliers who take care of the manufacturing of these platforms, end users from (university) hospitals and companies, like the pharmaceutical industry and, finally, stakeholder organisations, such as patient organisations, health insurance companies, regulatory and governmental bodies. The insights and needs of all these parties are



The value chain for Organ-on-Chip technology. Knowledge institutions come up with an idea and design a first concept for an Organ-on-Chip. The components that are required, such as the microchip, electrodes, micropumps, sensors and cells, are made and delivered by different technology suppliers. These components are brought together in a plate that simulates the physiological microenvironment of the mini organ (a smart well plate). After qualification and validation this Organ-on-Chip platform can be used for the development and production of specific organ and disease models. The final result is a standardised system for the development of personalised drugs and other health applications.

essential for the development of optimal technology platforms that can bridge the gap between academic research and end users.

Use in practice is key

Because the platforms for Organ-on-Chip technologies should be tailored to different user options, the entire value chain needs to be involved in the development. For studies on disease mechanisms a single chip could be sufficient, but for research on the efficacy and toxicity of drugs there is a need for large-scale parallel analysis capabilities, so that many Organ-on-Chip models can be used simultaneously. For this purpose, *smart multi-well plates* are required, such as those that are being developed in the context of the European project *Moore4Medical*.

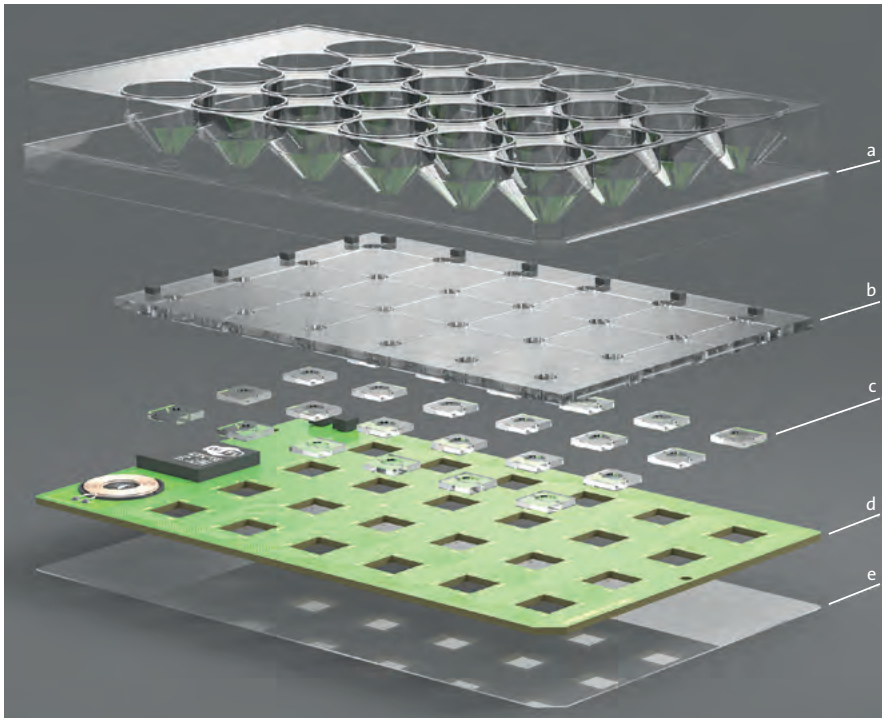
These platforms have a standard dimension and can be flexibly applied and customized for research on the functioning of organ tissues in models, such as a Pancreas-on-Chip, Heart-on-Chip or Skin-on-Chip. This requires separate measurement systems, each with their own micropumps, supply channels and sensors. The platforms can provide this. If the entire platform is only filled with Heart-

on-Chip systems next to each other, the integrated sensors can enable simultaneous measurement of the effect of a very large number of drugs to discover which drug inhibits the function of the heart (toxicity) and which drug does not. Direct involvement of end users during the design phase of the platform will be crucial for all these cases.

A business model

Organ-on-Chip technologies will only become commercially available for pharmaceutical industry or university hospitals if they are interesting enough for companies to invest in these. The current technology suppliers – the manufacturers of the Organ-on-Chip models – are often start-ups and spin-offs from universities and other knowledge institutes. Examples are Mimetas and Micronit (University of Twente), BI/OND (TU Delft) and Emulate (Wyss Institute, United States) for the chips, and Ncardia (Leiden University Medical Centre) for the cells.

From biomedical and technological perspective, developments in recent years have gone very quickly: from single cell cultures in Petri dishes to mini organs that can be studied in parallel in *smart well plates* with 12, 96, 384 or 1056 microwells. Nevertheless, this is only the start of the development. The concept of standardised technology platforms, which forms the basis of the business model of microelectronics, is also applicable in the new domain of Organ-on-Chip technology. As a result, this will imply that besides upscaling of the Organs-on-Chips, developed in research laboratories, to industrial applications, also established biomedical companies and start-ups will be attracted. These will in turn contribute to the availability of Organs-on-Chips for healthcare.



The smart multi-well plate that is being developed in the European project Moore4Medical. a) 6x4 hermetically sealed culture wells, b) plate with microfluidic channels and micropumps, c) chip modules for cell culturing d) printed circuit board with wifi, and e) transparent base plate.

The international perspective

DR. JANNY VAN DEN EIJNDEN-VAN RAAIJ
AND DR. MIEKE SCHUTTE

Collaboration is the key word to ensure that a technology like Organs-on-Chips is actually implemented in practice. Many parties are involved that complement and strengthen each other in the development, production and marketing of such models. Researchers come up with methods to enable cells to form mini organs and make designs for the housing (chips) for those organs. Companies contribute with their knowledge and expertise about production, upscaling and market. Regulatory bodies develop guidelines to use the Organ-on-Chip models in a safe and responsible manner. And ultimately, the models will be used in healthcare, but also for the testing of food, chemicals and cosmetics.

Organising national collaboration

By sharing and exchanging knowledge and ideas, more can be achieved than on your own. In the Netherlands, such collaboration is well organized by the institute hDMT as the driving force behind the development of Mini Organs-on-Chip. The acronym hDMT stands for *Institute for human Organ and Disease Model Technologies*, which is a non-profit consortium established by and for researchers in 2015. Within hDMT, public knowledge institutes and private organisations, such as companies and funds, work together. More than one thousand researchers, each with their own expertise, are involved and together provide added value for the consortium and the development of the technology. National and international companies contribute with their expertise about manufacturing, mass production and market perspectives. If patentable results emerge from the joint projects, the partners involved make agreements themselves about the intellectual property and the patent to be applied for.

Researchers from throughout the Netherlands regularly meet at the theme group meetings of hDMT. Here, new research results and ideas are discussed. There are now seven theme groups, each focused on a specific organ or disease. For example, researchers are developing models for blood vessels, gut and liver, heart, brain, eye, skeletal muscles and cancer, and combinations of these. This has led to a national ten-year research programme: NOCI (Netherlands Organ-on-Chip Initiative) funded by the NWO programme Gravitation, that aims to develop Organ-on-Chip models for the heart, brain and gut and study their interaction. One of the research topics is the question what happens in the brain if the microbiome (all microorganisms in the gut) is severely disturbed. Seven hDMT research groups are participating in this programme with many (young) enthusiastic researchers.

Across national borders

For the development of innovative Organ-on-Chip models it is not only important to collaborate across disciplines, but also across national borders. With the Dutch hDMT as example, various national Organ-on-Chip networks have now been formed in Europe, for example in the United Kingdom, the Scandinavian countries and Switzerland.

In the context of the European project ORCHID (see European Roadmap) co-coordinated by hDMT, the European Organ-on-Chip Society (EUROoCS) was established in 2018. Research groups and organisations from more than 20 European countries are already united in the EUROoCS-network. Furthermore, every scientist worldwide can become a member of EUROoCS. The aim of the society is to facilitate the development of Organ-on-Chip technology, by encouraging the exchange of knowledge and skills in this area, amongst

Some of the Dutch researchers participating in the Organ-on-Chip consortium hDMT during the annual consortium meeting (February 2020).

others via the annual conference. This conference, which is organised every year in a different country, brings researchers into contact with each other and with companies – from start-ups to large pharmaceutical companies. This strengthens existing relationships and initiates new collaborations, so that the international community keeps on growing.

In European context, also an International Training Network for Organs-on-Chips has been established. Within this network, a new generation of researchers is being educated and trained to develop innovative Organ-on-Chip models, and these researchers work together in international exchange programmes.

In addition, EUROoCS lobbies governments and funding agencies for new opportunities to support research on Organs-on-Chips, and to put the potential of this technology on the international agenda. Straightforward communication about the possibilities and limitations of the technology also makes citizens and society familiar with Organs-on-Chips.

Under the umbrella of EUROoCS, all these initiatives are contributing step-by-step towards a European Center of Excellence in the field of Organs-on-Chips. A strong foundation is thus being laid for the global development and the application of these promising models.

Are there legal barriers to use the m

DR. SONJA BEKEN

Before a drug can be tested on humans, regulations require that relevant data about the safety and efficacy of the drug have been collected according to established guidelines. These often require the use of laboratory animals.

3R approaches

Based on ethical considerations regarding animal welfare the use of animals should be limited as much as possible. The European Directive for the protection of laboratory animals (Directive 2010/63/EU) defines that the principle of the 3Rs (replacement, reduction and refinement) guides the use of test methods. These 3Rs are also consistently applied during the writing or revision of guidelines (recommendations) both at the European and global level. In recent years various 3R approaches have been included in guidelines. Unlike directives, guidelines are not legally binding and often are referred to as "soft law". Both regulatory bodies and applicants/holders of a trade licence such as pharmaceutical companies can deviate from a guideline, if scientifically substantiated.

In 2010, the European Medicines Agency (EMA) established a specific working group: the "Joint 3Rs *(Replacement, Reduction and Refinement)* Working Group". The task of this working group is to facilitate the implementation of the 3Rs in regulations for drugs and strengthen collaboration with other European groups. Since then, all relevant guidelines have been thoroughly revised, and the working group has made recommendations for further optimisation. This overview has been published on the EMA website and can be used by researchers as a guideline for the development of new test methods within critical areas of regulations.

At present, there are no specific guidelines that recommend the use of Organs-on-Chips to demonstrate the safety and efficacy of drugs. Research on these models is still in full progress and regulatory bodies are following these developments closely. In the beginning of October 2017, EMA organised a workshop with all parties involved. The aim was to gain insight into the state-of-the-art of this technology in Europe and to propose an action plan about how Organ-on-Chip models can become part of drug development.

Qualification studies

For the acceptance of each method within the scope of the 3R principle, including Organs-on-Chips, qualification studies are needed. These studies determine whether a method can be included in a guideline or can be used for taking decisions about the safety of drugs. These qualification studies aim to demonstrate the usability and reliability of a test method within a predefined context. In other words, it should be clear to what extent an Organ-on-Chip model can measure an endpoint for a predetermined series of reference compounds and provide the expected results. This endpoint is relevant for a specific purpose, within the applicable requirements for efficacy and safety of drugs *(context of use)*. It is important to detect in a very early stage of the development of a drug whether this substance can cause cardiac arrhythmias (=pur-

odels?

Recently, the head office of the European Medicines Agency (EMA) moved to the east side of the Amsterdam Zuidas. EMA left London due to the exit of the United Kingdom from the European Union.

pose). That could be realised by measuring the effect of that drug on the electrical conduction in Heart Muscle Cells-on-Chip (= endpoint). However, to be able to state with certainty that this measurement is correct, the model should be tested with compounds that are known to cause these cardiac arrhythmias (= reference compounds).

The qualification of new methods determines whether regulatory bodies, such as EMA, will accept the results obtained with these new methods. Qualification studies, performed for example by the end user, the model developer, or consortia, whether or not in collaboration with regulatory bodies, must follow specific procedures that are clearly described in EMA's guidelines.

International dialogue

The type of qualification study and the parameters and reference compounds to be investigated depend on the method or technology involved and the context of use. For Organs-on-Chips, there is a need for specific recommendations. This requires the communication between developers, end users and regulatory bodies at an early stage. The dialogue with regulatory bodies, such as EMA, ensures that they gain experience with the application of this new technology.

The development of Organs-on-Chips is taking place worldwide, and most guidelines about drug testing have an international character. International collaboration is therefore necessary. Communication between EMA and its American counterpart, the Food and Drug Administration (FDA), has already started. The experience currently being acquired by the FDA, through collaboration with commercial developers of Organs-on-Chips for the evaluation of this technology, is being closely monitored by Europe.

The road towards acceptance

Organs-on-Chips are a promising technology that can potentially be used for research on safety, biological availability and efficacy of drugs. There is still much uncertainty about the use of Organs-on-Chips models within the regulatory framework for drugs. This is due to a lack of experience and

independent qualification studies. More technical aspects, such as the broad diversity of chips per organ system, the range of cell types of human origin and uncertainty about the integration of different organ systems, are contributing to this.

The use of Organ-on-Chip models by the pharmaceutical industry could lead to a more accurate selection of possible drugs. Substances with a favourable safety profile and optimum efficacy could be identified in an early phase of drug development based on tests with Organs-on-Chips. The results generated can help to underpin the qualification of these methods and ultimately make it easier for regulatory bodies to accept them.

Guidelines are non-binding decisions that set the main contours of certain policy areas within the European Union. Although guidelines are not binding, they often form the framework for taking binding decisions. The EU provides guidelines in areas of common foreign and safety policy, employment policy, macroeconomic policy and, more practically, trans-European networks.

Epilogue: Your Body-on-Chip

PROF. CHRISTINE MUMMERY AND PROF. ALBERT VAN DEN BERG

Just imagine: several decades from now a patient with a heart complaint could go to the doctor, who would collect some blood to make hiPSCs and a few weeks later, a mini Heart-on-Chip. This could be used to test which drugs are suitable for the patient and which drugs might represent a risk. Or that for a patient with cancer, the tumour tissue is cultured on a chip and the doctor tests which chemotherapy is guaranteed to have an effect and which tumours are likely to metastasize so need careful monitoring. Actually, testing the effectivity of chemotherapy is already starting with tumour organoids.

Step-by-step

Such a future is possible, but big steps still need to be taken in the efficiency and speed with which Organs-on-Chips are used. The chips should become even smaller to reduce costs, and everything should preferably be done by robots. This is not only much more accurate, but a robot can work 24/7, and on thousands of chips at once. Only then there is a chance that personalised Organs-on-Chips will indeed become available for regular medicine. However, we are not that far yet. Knowledge about immunology and the microbiome has not been developed in such a way that we can modulate and change it in Organs-on-Chips. The immune system and microbiome differ between each individual, so it is difficult to predict how this could influence the results of drug tests, even if the cells in the chip are from the patient concerned.

For the time being, research proceeds by taking small steps, from a single cell type to several on a chip and from a single Organ-on-Chip to several interconnected Organs-on-Chips. Will this ever result in a whole Body-on-Chip? And regardless of the numerous challenges in technology, time and costs, is it actually worthwhile?

Connecting factor

One of the technical challenges is how the different Organs-on-Chips should be connected. Connecting the various microfluidic channels from one chip to another is not simple. Each cell and organ type needs its own type of "life fluid" with different nutrients, pH levels and energy sources, such as glucose, other sugars or fatty acids.

In our body, organs are connected by blood vessels with a total length of 60,000 kilometres. But not every blood vessel is the same. Some blood vessels transport oxygen-rich blood (arteries), whereas others transport oxygen-poor blood (veins). Sometimes the vessels are very thin with slowly moving blood (capillary blood vessels) and sometimes very broad and with a thick wall (for example, the aorta) with the innermost layer consisting of endothelial cells and the outermost layer of numerous layers of smooth muscle cells for strength. If the connections between different Organs-on-Chips really did resemble blood vessels, then it would be possible to use the same fluid for all organs.

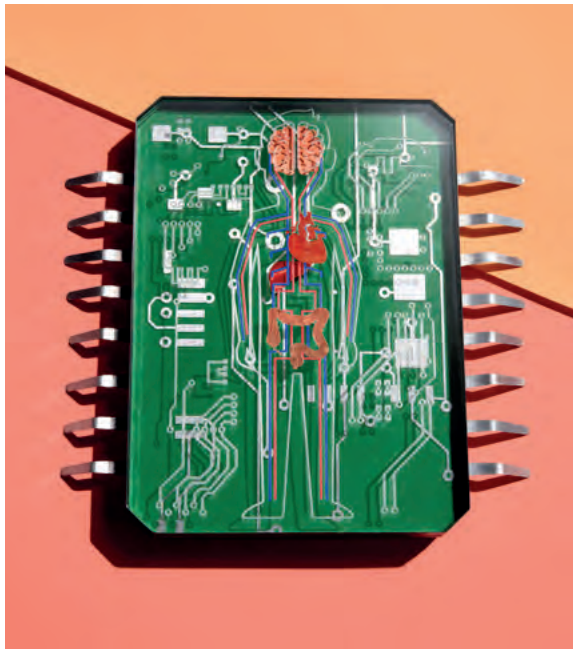
Body-on-Chip

Processes in the human body can be simulated by connecting Organs-on-Chips via the microfluidic channels. This will not only enable investigation of how the heart, liver or lung respond to drugs

but also whether the organs influence each other. For example, by testing whether the effect of a heart drug is different when the liver is connected to the heart, because the liver converts the heart drug into another form that either does not work, works less well or even has an opposite (toxic) effect.

Several laboratories in the world are already working on this. The team of Don Ingber, the American pioneer in the area of Organs-on-Chips, has connected eight different organ types on Chip – gut, liver, kidney, heart, lung, skin, blood-brain barrier and brain – with a "circulatory system" for a period of three weeks to monitor the interaction. Also, the influence of nicotine on human organs was simulated in a Multi Organ-on-Chip model.

The challenge is to develop a standard platform, a sort of print plate on which various components can be 'clicked' so that all necessary mini organs and vascular systems can be interconnected and the immune system and microbiome can also be included. The question is not whether this is possible, but when it will be reality.



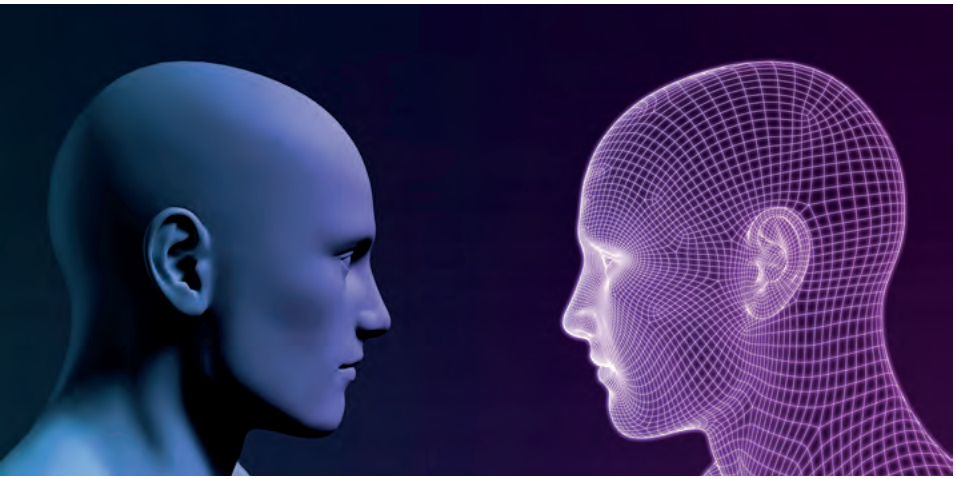
One day, a doctor will prescribe drugs based on how your Body-on-Chip responds to them.

Covid-19: role for Organ-on-Chip models?

At the end of 2019, it slowly became clear that people in China got sick by a very infectious and unusual virus. A disease that possibly started at a market where various exotic animals entered the food chain. Before the end of the first quarter of 2020, the entire world was affected by this "flu" pandemic, which is unparalleled in our times. The disease is called Covid-19.

Not everybody gets sick after infection by the virus, and some people only have mild symptoms, whereas others become severely ill. Besides causing fever, the virus appears to affect the lungs of a patient first, and an infected person often has a severe cough. Meanwhile, it has become clear that many other organs are affected, blood vessels become "leaky", the lungs fill with fluid and in some patients, blood clots develop throughout the body. The gut can also be affected, causing patients suffering from abdominal pains and diarrhoea. The liver also shows disease symptoms and the virus is rapidly multiplying in all these organs.

It is, therefore, extremely difficult to know how patients should be treated. Doctors prescribe known drugs such as various antivirals but these can have side effects on the heart, which are severe enough to cause a sudden heart failure. And they might not even fight the virus. Should doctors activate the patient's immune system or instead slowing it down to prevent a so-called "cytokine storm", a phenomenon that is similar to the consequences of blood poisoning (sepsis) and from which many people still die. The kidneys are often affected too: some patients need to have permanent kidney dialysis after they have been recovered from the viral infection.



Are digital twins the future solution for escalating healthcare costs?

What can Organ-on-Chip models offer? Earlier in this booklet, it was indicated that Organ-on-Chip models are already being developed for the lung, heart, liver, gut and blood vessels. All being organs that are affected in Covid-19 patients. These models, in combination with organoids of different organs, or with body cells derived from hiPSCs or adult stem cells, could and already do contribute to understanding and treating this new disease. Not only can the models provide insight into the mechanism of action of the virus in the body but they also help to determine what the effects and side effects of new or existing drugs are.

Digital twin

Another future perspective is that every patient or even every citizen will soon have an "Avatar", a virtual twin (Digital Twin) that not only contains medical data but also data about someone's general condition, lifestyle and social behaviour. Of course, privacy must be absolutely guaranteed, but such an Avatar could simulate the perfect doctor or general practitioner: The Avatar includes all X-ray photos, medication, consequences of accidents and causes of hospitalization, and could also contain the quantity and type of nutrients that have been taken, as well as sport activities and data from a wide range of smart wearable devices (fitbits).

In this development, data from Organs-on-Chips could be an important addition: how organs respond to certain external factors and drugs could be processed in the digital twin. Artificial intelligence could be used to provide the patient with tailored advice about his or her optimal therapy, and perhaps even better, dietary and behavioural recommendations to prevent diseases. Research has shown that 80% of health costs can be prevented, whereas only 3% of all health costs are spent on prevention.

In a nutshell, a Digital Twin, as long as it is provided with the correct information and perhaps data from Organ-on-Chip models, could represent the next "giant step for mankind" in healthcare efficiency and cost management. Organs-on-Chips could rescue the currently escalating costs of the healthcare system!

More information

Research on Organs-on-Chips

- Dutch research consortium: the Institute for human Organ and Disease Model Technologies: www.hdmt.technology
- Netherlands Organ-on-Chip Initiative, NOCI: noci-organ-on-chip.nl
- Organ-on-Chip Center Twente: www.utwente.nl/en/mesaplus/research/centres-of-expertise/oocct/
- European training network for Organs-on-Chips: www.eurooc.eu
- European Organ-on-Chip Society: www.euroocs.eu
- British Organ-on-a-Chip Technologies Network: www.organonachip.org.uk
- Organ-on-Chip in Development (ORCHID): www.H2020-orchid.eu

Videos

- *Het Klokhuis*, Season 30 episode Miniorgans (Children's TV programme in Dutch): www.npostart.nl/het-klokhuis/10-12-2019/VPWON_1260561
- Translational Organ-on-Chip Platform: www.youtube.com/watch?v=mbqdRpolSJw&feature=emb_logo
- Your heart on a chip? TEDx University of Amsterdam: www.youtube.com/watch?v=YRdGDpEYqbA
- A Body-on-Chip: www.youtube.com/watch?time_continue=22&v=nkkBu8GrExk&feature=emb_logo
- Bioprinting, how to 3D print human tissue: www.youtube.com/watch?v=uHbn7wLN_3k
- ICLON Gastles Proefdiervrije Innovatie & Proefdieren (Guest lecture Animal-Free Innovation & Laboratory Animals - in Dutch only): www.universiteitleiden.nl/gastlessen/cursussen/digitale-gastlessen/proefdiervrij
- Smart well plate for Organ-on-Chip: https://www.youtube.com/watch?time_continue=26&v=H5950GbMdyM&feature=emb_logo
- Animation about Lung-on-Chip, Gut-on-Chip: https://www.youtube.com/watch?v=0jf6Tor9WtA&feature=emb_logo
- Asthma research without laboratory animals (in Dutch Only): www.youtube.com/watch?time_continue=9&v=WOyTKxtwd8Q&feature=emb_title

Several research publications

- Impact of organ-on-a-chip technology on pharmaceutical R&D costs described in the box in Chapter 2, https://doi.org/10.1016/j.drudis.2019.06.003
- The Roadmap for Europe described in Chapter 5, Building blocks for a European Organ-on-Chip roadmap, https://doi.org/10.14573/altex.1905221

Laboratory animals and alternatives

- Royal Netherlands Academy of Arts and Sciences report Inventory: the importance of animal testing and possibilities for reduction of this in fundamental neuroscience research, 27 June 2019: https://www.knaw.nl/en/news/publications/inventarisatie-het-belang-van-dierproeven-en-mogelijkheden-tot-vermindering-daarvan-in-fundamenteel-neurowetenschappelijk-onderzoek?set_language=en (report in Dutch, summary available in English)
- TPI Utrecht University supports transition animal-free innovations: www.uu.nl/en/research/life-sciences/communities/tpi-utrecht
- Information about Dutch animal experiments can be found in 'Zo doende' (only in Dutch): https://bit.ly/39bgHl7
- Information about the worldwide use of laboratory animals: Facts and figures https://bit.ly/30nz1np Lush prize: https://bit.ly/2ZDFWcU
- Information about animal experiments in Europe: https://bit.ly/30lQKMo

Authors

- EURL ECVAM EU Reference Laboratory for alternatives to animal testing: https://ec.europa.eu/jrc/en/eurl/ecvam

Regulations

- Ethical use of animals in medicine testing: https://bit.ly/3he4v69
- Review and update of EMA guidelines to implement best practice with regard to 3Rs (replacement, reduction and refinement) in regulatory testing of medicinal products – report on actions taken: https://bit.ly/3fHhGfj
- Reflection paper providing an overview of the current regulatory testing requirement for medicinal products for human use and opportunities for implementation of the 3Rs: https://bit.ly/3fJhnAN
- First EMA workshop on non-animal approaches in support of medicinal product development: challenges and opportunities for use of micro-physiological systems: https://bit.ly/30rrUtQ (pdf)
- Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. For an overview see https://bit.ly/3fIZ26P (PDF)

Dr. Sonja Beken is coordinator of the non-clinical assessors at the Federal Agency for Drugs and Health Products (FAGG, Belgium) and member of the Safety Working Party of the European Medicines Agency (EMA).

Prof. Albert van den Berg is Professor of Sensor Systems for Biomedical and Environmental Applications at the University of Twente and (joint) director of the MESA + Institute for Nanotechnology.

Prof. Carlijn Bouten is Professor of Cell Matrix Interactions in Cardiovascular Regeneration in the research group Soft Tissue Engineering & Mechanobiology, Department of Biomedical Technology, Eindhoven University of Technology.

Dr. Hans Bouwmeester is Associate Professor of Toxicology and Advanced In Vitro models at Wageningen University & Research.

Dr. Janny van den Eijnden-van Raaij is Managing Director of hDMT.

Prof. Erik Danen is Professor of Cancer Drug Target Discovery at Leiden Academic Centre for Drug Research, Leiden University.

Milica Dostanic MSc is PhD Student in the Department of Microelectronics, TU Delft.

Nora Franzen MSc is PhD Student at the Netherlands Cancer Institute in Amsterdam.

Dr. Dik van Gent is Associate Professor of Genetics at the Department of Molecular Genetics, Erasmus MC in Rotterdam.

Prof. Sue Gibbs is Professor of Skin and Mucosa Regenerative Medicine at the Department of Molecular Cell Biology, Amsterdam University Medical Center and Academic Centre for Dentistry (ACTA) Amsterdam.

Dr. Astrid van de Graaf is Editor-in-Chief at De Ingenieur and Stichting Biowetenschappen en Maatschappij and freelance science journalist.

Dr. Mart Graef is Strategic Programme Manager at the Faculty of Electrical Engineering, Mathematics & Computer Science, TU Delft.

Dr. Jessica de Greef is Assistant Professor at the Department of Human Genetics, Leiden University Medical Center.

Dr. Roeland Hanemaaijer is Senior Researcher at TNO Metabolic Health Research in Leiden.

Prof. Anneke den Hollander is Professor of Molecular Ophthalmology at Radboudumc in Nijmegen.

Dr. Dorothee Horstkötter is Assistant Professor of Neuroethics and Practical Philosophy at the School for Mental Health and Neuroscience, Department of Health, Ethics and Society, Maastricht University.

Prof. Maarten IJzerman is Chair of Cancer Health Services Research of the University of Melbourne in Australia and part-time Professor in the Department of Health Technology and Services Research at the University of Twente.

Prof. Marcel Karperien is Professor of Developmental BioEngineering at the University of Twente.

Dr. Cyrille Krul is Professor of Innovative Testing in Life Sciences & Chemistry and director knowledge centre at Utrecht University of Applied Sciences.

Dr. Anne Kienhuis is Project Coordinator alternatives for animal experiments in toxicology at the National Institute for Public Health and the Environment (RIVM).

Prof. Steven Kushner is Professor of Neurobiological Psychiatry at the Department of Psychiatry, Erasmus MC in Rotterdam.

Dr. Regina Luttge is Associate Professor in the Microsystems section and Chair of Neuro-Nanoscale Engineering at Eindhoven University of Technology, Eindhoven University of Technology.

Dr. Anika Nagelkerke is Assistant Professor in the research unit Pharmaceutical Analysis at the Groningen Research Institute of Pharmacy, University of Groningen.

Prof. Jos Malda is Professor of Biofabrication in Translational Regenerative Medicine, Department of Orthopaedics of the University Medical Center Utrecht and at the Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University.

Prof. John Martens is Professor of Translational Cancer Genomics at the Department of Medical Oncology, Erasmus MC in Rotterdam.

Dr. Massimo Mastrangeli is Assistant Professor at the Department of Microelectronics, TU Delft.

Dr. Berend van Meer is researcher at the Department of Anatomy and Embryology of Leiden University Medical Center, Business Leader of the Organ-on-Chip Center Twente and responsible for the valorisation of Organ-on-Chip research at the MESA+ Institute of the University of Twente.

Prof. Jos van der Meer is Emeritus Professor and Former Head of the Department of Internal Medicine, Radboudumc in Nijmegen.

Dr. Andries van der Meer is Associate Professor at the Department of Applied Stem Cell Technology of the University of Twente.

Prof. Christine Mummery is Professor of Developmental Biology and Chair of the Department of Anatomy and Embryology of Leiden University Medical Center and chair of hDMT.

Dr. Valeria Orlova is an Assistant Professor at the Department of Anatomy and Embryology, Leiden University Medical Center.

Dr. Dasja Pajkrt is a Paediatric Infectious Disease Specialist at Amsterdam UMC, location AMC.

Prof. Robert Passier is Professor of Applied Stem Cell Technologies at University of Twente and Associate Professor of Molecular Genetics of Heart Development at Leiden University Medical Center.

Dr. Pim Pijnappel is Associate Professor at the Department of Paediatrics and head of the research group Molecular stem cell biology of Erasmus MC in Rotterdam.
Dr. Barry Rockx is Assistant Professor of Exotic Viruses at the Department of Viroscience, Erasmus MC Rotterdam.
Prof. Daniela Salvatori is Professor of Comparative Anatomy and Physiology, Faculty of Veterinary Medicine, Utrecht University.
Prof. Loes Segerink is Associate Professor of Biomedical and Environmental Sensor Systems at the Department of Biomedical and Environmental Sensor Systems (BIOS), University of Twente.
Dr. Mieke Schutte is Managing Director of hDMT.
Prof. Guus Smit is Professor of Molecular Neurobiology and head of the Department of Neurosciences at VU Amsterdam.
Dr. Anthal Smits is Assistant Professor in the Immuno-Regeneration group, Soft Tissue Engineering and Mechanobiology section, Department of Biomedical Technology, Eindhoven University of Technology.
Dr. Evita van de Steeg is Senior Researcher Human Cell Biology and Microbiology at TNO in Zeist.
Dr. Geurt Stokman is Project Leader and Researcher at TNO Metabolic Health Research in Leiden.
Prof. Jaap den Toonder is Professor of Microsystems at the Faculty of Mechanical Engineering, Eindhoven University of Technology.
Prof. Roman Truckenműller is Professor of Micro Tissue Engineering, Faculty of Health, Medicine and Life Sciences of Maastricht University.
Prof. Jolanda van der Velden is Head of the Department of Physiology and director of the Cardiovascular Institute of Amsterdam UMC.
Prof. Bob van de Water is Professor of Drug Safety at Leiden Academic Center for Drug Research of Leiden University.
Debby Weijers MSc is Director of Dutch Society for the Replacement of Animal Testing.
Laura Windt MSc is PhD Student at the Department of Anatomy and Embryology, Leiden University Medical Center.
Dr. Sebo Withoff is Associate Professor of Personalized Systems Biology of Complex Diseases at the Department of Genetics, University Medical Center Groningen.
Dr. Katja Wolthers is a Clinical Virologist at the Department of Medical Microbiology, Amsterdam UMC, location AMC.

Illustration credits

Cover: Dreamstime, 123RF

Elisabetta Citterio: p. 3 (left)
Loes Segerink: p. 3 (right)
iStockphoto: p. 5, 8, 16, 26, 60, 65, 74, 75, 83
Wyss Institute at Harvard University: p. 6
Jos van den Broek, Leiden: p. 10, 12, 25 b, 29, 33
Shutterstock: p. 13, 59, 91
Bob Daemmrich / Alamy Stock Photo / Imageselect: p. 15
123RF / Sittrop Grafisch Realisatie Bureau, Rotterdam: p. 17
Depositphotos: p. 19, 82
Device: Hossein Amirabadi and Jaap den Toonder (TU/e) / Photo: Bart van Overbeeke: p. 20
2019 Koninklijke Nederlandse Akademie van Wetenschappen (KNAW) (CC BY 3.0 NL) / Sittrop Grafisch Realisatie Bureau, Rotterdam: p. 22
Paul ter Braak / Marinke van der Helm; BIOS Lab on a Chip group, UTwente: 25 (bottom)
Darwin Microfluidics / www.darwin-microfluidics.com: p. 30
Tue / photo: Bart van Overbeeke: p. 32, 58
Jos Malda / UMC Utrecht: 34
Adapted with permission from Danen et al, Science Signaling Vol 7 Issue 312 ra15, 2014.: p. 36
123RF: p. 39, 95
Mark van der Kroeg, Femke de Vrij (laboratory of Steven A. Kushner, Erasmus MC, Rotterdam, The Netherlands): p. 40
Laura Windt / LUMC en Milica Dostanic / TU Delft: p. 44
Amy Cochrane / LUMC: p. 45
ErasmusMC: p. 46
Aisen De sa Vivas, University of Twente: p. 47
José Manuel Rivera Arbelaez en Marcelo Catarino Ribeiro, department AST, University of Twente: p. 48
Marcelo Catarino Ribeiro, department AST, University of Twente / River BioMedics: p. 49
Pablo Herrero-Hernandez / Erasmus MC: p. 50 (left)
Alessandro Iuliano / Erasmus MC: p. 50 (right)
Photos and Infographic: Renée Moerkens and Joram Mooiweer, The Intestine-Chip presented here is based on the design of Emulate Inc, Boston, MA, USA.: p. 52
Sebo Withoff / University of Groningen: p. 53
SNP model by David Eccles (Gringer) (CC BY 4.0): p. 54
TNO: p. 55, 56 (left, right)
Rahman Sabahi Kaviani, www.fet-proactive-connect.com: p. 57
Chemelot InSciTe: p. 63 (top)
Blood vessel scaffold and heart valve scaffold: Department of Biomedical Engineering, Eindhoven University of Technology: p. 63 (bottom left, bottom right)
Iris Sijboom, commissioned by the Dutch Society for the Replacement of Animal Testing: p. 66
Daniela Salvatori, Utrecht University: p. 69
Sue Gibbs / Amsterdam UMC: p. 70
Berend van Meer / LUMC: p. 72
University of Applied Sciences, Utrecht, photo Ivar Pel: p. 73
Science Photo Library / Alamy Stock Photo / Imageselect: p. 77
Getty Images: p. 78
based on: Building blocks for a European Organ-on-Chip roadmap, https://doi.org/10.14573/altex.1905221 / Sittrop Grafisch Realisatie Bureau, Rotterdam: p. 80
MESA+ Institute, University of Twente: p. 84
Moore4Medical, photo's TUDelft (bottom, mid), LUMC (top mid), Multi Channel Systems (top): p. 86
TUe: p. 87
Maartje Kuperus: p. 89
Adam Voorhes / Gallery Stock: p. 94

Thanks to

Netherlands Organ on Chip Initiative

HUMAN ORGAN AND DISEASE MODEL TECHNOLOGIES 2015 2020

EUROoCS
EUROPEAN ORGAN-ON-CHIP SOCIETY

And the financial support from NNGC.

This booklet is made possible by:

ZonMw

NWO

In this issue:

- Why are so many drugs failing?
- What is an Organ-on-Chip?
- What can you study with it?
- For which organs are chip models already being developed?
- Why are these models not in use yet?
- Could Organs-on-Chips replace animal models?

Editors:
Janny van den Eijnden-van Raaij
Astrid van de Graaf
Berend van Meer
Christine Mummery

With a preface from Christine Mummery and Albert van den Berg

Despite decades of research, there are still no drugs for many diseases. And the drugs that are available may only be effective in some patients or cause unwanted side-effects. This is partly due to insufficient knowledge on the causes of many diseases in humans and their underlying mechanisms, and partly because current research models, such as cell culture and laboratory animals, do not always predict disease effects and drug efficacy in humans.

Organ-on-Chip technology with human cells is a promising approach to address these problems. The models contain living human cells from specific organs that simulate the function, dynamics and structure of human organs in sickness and in health. Organ-on-Chip models could not only improve, but also accelerate the drug development process and reduce its costs. In addition, this technology contributes to reducing the use of laboratory animals.

In this booklet, cell biologists, geneticists, doctors, microbiologists, biotechnologists, engineers, analysts, ethicists, materials scientists, neurologists and research managers describe the challenges they face in developing these models and what is needed to implement them in practice.

bio wetenschap+ maatschappij

9 789493 232013