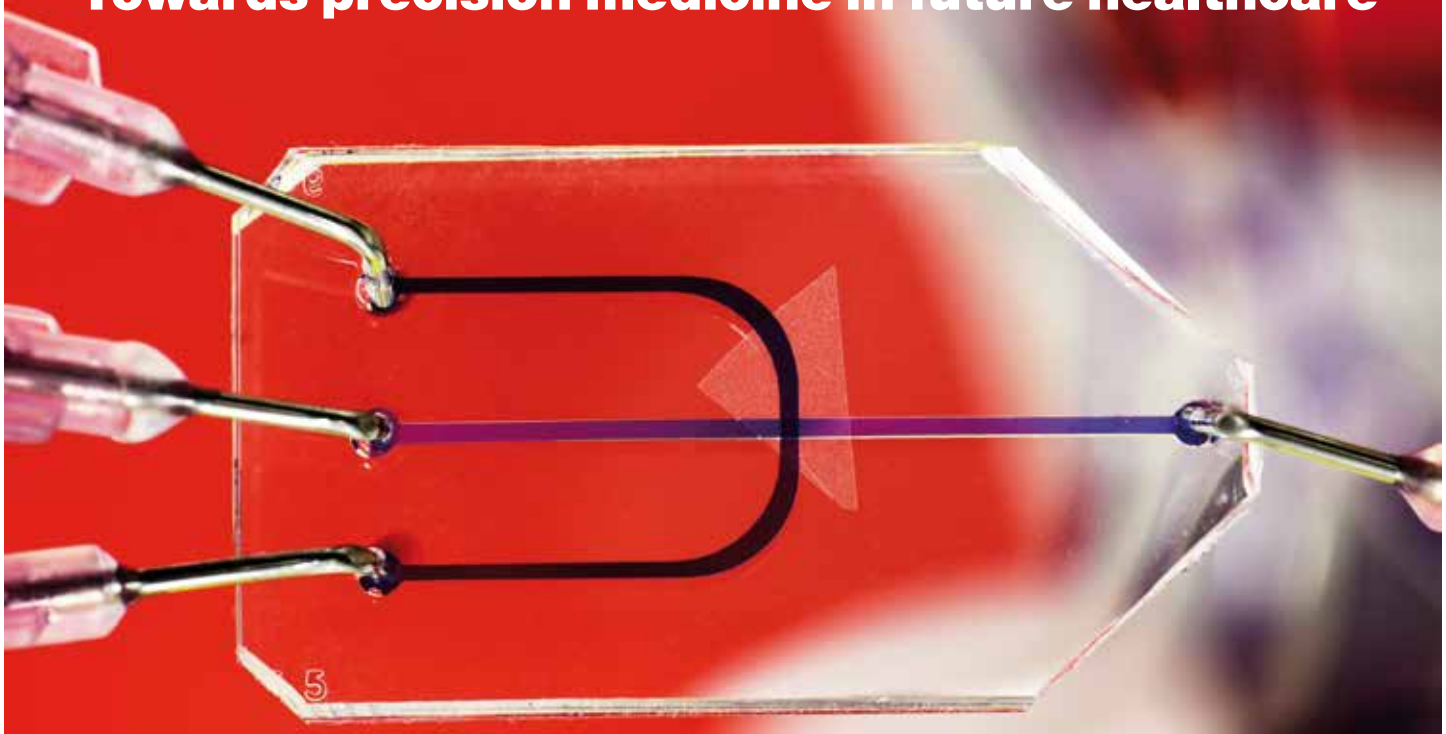
Towards precision medicine in future healthcare



WHITE PAPER

Organ-on-chip Technology and hDMT

hDMT
HUMAN ORGAN AND DISEASE MODEL TECHNOLOGIES

Cover: *Transparent organ-on-chip device made of poly-dimethylsiloxane (PDMS). Two crossing microchannels (blue and black) for liquid transport are separated by a porous membrane (opaque triangle) on both sides of which cells can be cultured.*
Device: *Hossein Amirabadi and Jaap den Toonder (TU/e)*
Photo: *Bart van Overbeeke*

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Executive Summary

ENGLISH

The goal: more reliable testing of new drugs

A major problem in developing new medicines is the limited availability of human model systems for preclinical research on disease target identification, drug efficacy and toxicity. This is still a major contributor to late (and expensive) drug failures in clinical trials. Laboratory animals or cells in standard tissue cultures, even if they are human, often do not respond to medication in the way cells in intact human organs in the body do. Organ-on-chip technology based on human cells may present solutions to this challenge of creating near-human test systems.

The technology: chips and cells

Organs-on-chips are devices with one or more biocompatible microfluidic chambers (known as 'chips') containing multiple cell types in 3D culture; the living cells interact much as they might do in tissue as either miniature organs or miniature tumors. The chip design allows the cell cultures they contain to be continuously perfused and mechanically or electrically manipulated. This mimics normal organ physiology or can be used to induce disease pathology at the organ and tissue level. It is even possible to link chips containing different organ and tissue types; these might become especially valuable when one tissue (like the liver) processes a compound to a metabolite that causes an effect for example on kidney, brain or heart. This is particularly relevant for drug toxicity screening.

The chips: high-tech micro-environments

The microfluidic chambers allow cell growth and maintenance under controlled, sterile conditions. The microfluidic flow can supply nutrients, drugs, immune cells, bacteria or viruses to the cell cultures inside the chips as necessary for the question to be addressed. Synthetic blood vessels can also be incorporated into the chip with real or synthetic blood flowing through. The chips allow manipulation of the physical (micro)environment of cells. For example by varying the stiffness of the cellular environment (as in bone), varying pressure or stress (as in muscle or flowing blood), or causing vacuum-driven pressure oscillation (as in lung tissue or beating heart muscle). These mechanical cues are of crucial importance in the recreation of the mini-organs. The chips allow molecular and functional monitoring, as well as real-time microscopic imaging of living cells.

The cells: stem cells

Stem cells can be used as the source from which to grow the miniature organs. These can be stem cells derived from healthy individuals or from patients. The resulting miniature organs are models that have the genetic characteristics of that particular individual. These could even be referred to as 'patients-on-chips'. These chips allow personalized analysis of drug responses and could thus facilitate identification of the most effective drug for an individual patient, before starting treatment. This would save time, money and unnecessary discomfort.

The application: disease models

Currently organs-on-chips are being developed for cancer, cardiovascular diseases, neurological and cognitive (brain) diseases, (auto)immune diseases, skin and a variety of others with complex genetic origins or with ethnic background impacting disease severity.

The research: a paradigm shift

It is expected that organ-on-chip models could result in a paradigm shift for biomedical research and the pharmaceutical industry, leading to new ways to identify effective drugs and improving the quality of medical care for many prevalent and severe diseases. They could provide near-human test systems for (pre)clinical trials with high relevance for individual patients. This would facilitate the development of novel treatment modalities, and allow assessment of the impact of inflammation and the immune system on treatments and disease. Organs-on-chips will make personalized medicine possible.

The societal effect: economic impact

Organs-on-chips could significantly reduce the costs of healthcare, because they would allow drug development to become better, safer, faster and cheaper. Presently the development of a new drug from start-to-market takes about 12 years, and costs minimally 1 billion euro per drug. This is due to inefficient drug development pipelines, with too many drugs failing late in development. Organs-on-chips will speed this up, thereby reducing costs. Drug repurposing – using a drug to treat a condition for which it was not originally developed – is considered an area most likely to benefit from these near-human models of organs-on-chips. Organs-on-chips support the 3Rs guiding principles of reduction, refinement and replacement of animal experiments.

The makers: institute for human Organ and Disease Model Technologies (hDMT)

Developing organs-on-chips means integrating different state of the art technologies. This requires creation of truly interdisciplinary research teams. The institute for human Organ and Disease Model Technologies (hDMT) is a consortium of internationally renowned scientists with backgrounds in biology, physics, chemistry, pharmacology, medicine and engineering. hDMT researchers work at academic research institutions, university medical centers and pharmaceutical companies in the Netherlands. In this ‘laboratory without walls’ multidisciplinary teams share their knowledge, expertise and facilities to deliver the human disease models of tomorrow.

For innovative disease models, hDMT currently prioritizes four themes: vessels-on-chip, heart-on-chip, cancer-on-chip and brain-on-chip.

hDMT is a precompetitive, non-profit technological R&D institute. hDMT will valorize organ-on-chip models developed through its biomedical and biophysical technologies to pharma companies and biotech, both in the Netherlands and internationally. hDMT also actively seeks collaboration with other researchers in the field of human organ and disease modeling worldwide, thus promoting open knowledge exchange and collaborations at the frontiers of organ-on-chip technology.

For more information see www.hDMT.technology

Management Samenvatting

NEDERLANDS

Het doel: betrouwbaarder testen van nieuwe medicijnen

Een belangrijk probleem bij het ontwikkelen van nieuwe medicijnen is de beperkte beschikbaarheid van menselijke modelsystemen voor preklinisch onderzoek naar aangrijpingspunten voor het behandelen van ziekten, de effectiviteit van het medicijn en de toxiciteit. Dit is er voor een groot deel voor verantwoordelijk dat het klinisch onderzoek naar een medicijn in een laat stadium afgeblazen moet worden, met alle kosten van dien. Laboratoriumdieren of cellen in standaard weefselkweken reageren (zelfs wanneer menselijke cellen gebruikt worden) vaak niet op dezelfde manier op medicijnen als cellen in intacte organen in het menselijk lichaam. Orgaan-op-een-chip technologie op basis van menselijke cellen zou oplossingen kunnen bieden voor deze uitdaging om testsystemen te ontwikkelen die het menselijk lichaam zo goed mogelijk benaderen.

De technologie: chips en cellen

Organen-op-chips zijn apparaatjes met één of meer biocompatibele microfluïdische kamers (zogeheten 'chips') die verschillende celtypes in een 3D-celkweek bevatten; tussen de levende cellen (in de vorm van miniatuurorganen of miniatuurtumoren) vindt een interactie plaats die overeenkomt met die in weefsels. Door het ontwerp van de chip kan voortdurend perfusie in de celkweek plaats vinden, wat een normale orgaanfysiologie nabootst. Ook kan de chip mechanisch of elektronisch gemanipuleerd worden en zo gebruikt worden om ziekteprocessen op orgaan- of weefselniveau te veroorzaken. Het is zelfs mogelijk om chips met verschillende organen en weefseltypes aan elkaar te koppelen. Dat zou belangrijk kunnen zijn als een weefsel (bijvoorbeeld de lever) een bestanddeel omzet in een metaboliet dat gevolgen heeft voor bijvoorbeeld de nieren, de hersenen of het hart. Dat is bijzonder relevant voor het screenen op toxiciteit van medicijnen.

De chips: hoogtechnologische micro-omgevingen

De microfluïdische kamers kunnen cellen onder gecontroleerde en steriele omstandigheden laten groeien en in stand houden. De microfluïdische stroom kan voedingsstoffen, medicijnen, immuuncellen, bacteriën of virussen naar de celculturen binnen in de chip vervoeren, afhankelijk van wat er nodig is voor de onderzoeksvraag. Ook kunnen synthetische bloedvaten in de chip verwerkt worden waar echt of synthetisch bloed doorheen stroomt. Op de chips kan de fysieke (micro)omgeving van de cellen gemanipuleerd worden. Bijvoorbeeld door het variëren van de stijfheid van de celomgeving (zoals in bot), variëren van druk of belasting (zoals in spieren of stromend bloed), of het teweegbrengen van vacuümgestuurde drukoscillatie (zoals in longweefsel of een kloppende hartspier). Deze mechanische prikkels zijn uitermate belangrijk bij het maken van miniatuurorganen. Het is mogelijk de processen op de chips moleculair en functioneel te volgen en real-time microscopische beelden van levende cellen te maken.

De cellen: stamcellen

Stamcellen kunnen gebruikt worden als basis om de miniatuurorganen uit te laten groeien. Dit kunnen stamcellen zijn die afkomstig zijn van gezonde personen of van patiënten. De miniatuurorganen die hieruit ontstaan zijn modellen met de genetische eigenschappen van de persoon in kwestie. Dit zou je zelfs 'patiënten-op-een-chip' kunnen noemen. Met deze chips kan een gepersonaliseerde analyse van de reactie op medicijnen gemaakt worden, waarmee voor de aanvang van de behandeling bepaald zou kunnen worden wat het meest effectieve medicijn is voor een individuele patiënt. Dit zou tijd en geld besparen en onnodig ongemak voorkomen.

De toepassing: ziektemodellen

Momenteel worden organen-op-chips ontwikkeld voor kanker, cardiovasculaire aandoeningen, neurologische en cognitieve (hersen)ziekten, (auto)immuunziekten, huidziekten en een scala aan andere aandoeningen met een complexe genetische oorsprong of met een etnische achtergrond die de ernst van de ziekte beïnvloedt.

Het onderzoek: een paradigmaverschuiving

De verwachting is dat orgaan-op-een-chip modellen een paradigmaverschuiving kunnen veroorzaken voor biomedisch onderzoek en de farmaceutische industrie en kunnen leiden tot nieuwe manieren om effectieve medicijnen te vinden en de kwaliteit van de medische zorg te vergroten bij veel voorkomende en ernstige ziekten. Ze kunnen testsystemen voor (pre)klinisch onderzoek opleveren die het menselijk lichaam zo goed mogelijk benaderen, met een grote relevantie voor individuele patiënten. Dit zou de ontwikkeling van nieuwe behandelmodaliteiten faciliteren en het mogelijk maken om de invloed van ontstekingen en het immuunsysteem op behandelingen en ziekten te onderzoeken. Door organen-op-chips wordt gepersonaliseerde geneeskunde mogelijk.

Het maatschappelijk effect: economische gevolgen

Organen-op-chips zouden de kosten van gezondheidszorg flink omlaag kunnen brengen, omdat de ontwikkeling van medicijnen daardoor beter, veiliger, sneller en goedkoper zou worden. Momenteel duurt de ontwikkeling van een nieuw medicijn, vanaf de start totdat het op de markt komt, ongeveer twaalf jaar en de kosten bedragen minimaal 1 miljard euro per medicijn. Dit komt doordat het ontwikkeltraject niet efficiënt verloopt en teveel medicijnen laat in de ontwikkeling toch niet bruikbaar blijken. Organen-op-chips zullen dit proces versnellen, waardoor de kosten teruggebracht worden. Herbestemmen van geneesmiddelen – het gebruik van een medicijn om een aandoening te behandelen waarvoor het oorspronkelijk niet ontwikkeld was – is een gebied dat waarschijnlijk het meest baat heeft bij deze modellen. Organen-op-chips ondersteunen het 3V-beleid voor dierproeven: vermindering, verfijning en vervanging.

De makers: instituut voor human Organ and Disease Model Technologies (hDMT)

Het ontwikkelen van organen-op-chips brengt het integreren van enkele van de nieuwste technologieën met zich mee. Hiervoor moeten brede interdisciplinaire onderzoeksteams opgezet worden. Het instituut voor human Organ and Disease Model Technologies (hDMT) is een consortium van internationaal erkende wetenschappers met een achtergrond in de biologie, natuurkunde, chemie, farmacologie, geneeskunde of techniek. hDMT-onderzoekers werken bij wetenschappelijke onderzoeksinstellingen, universitair medische centra en farmaceutische bedrijven in Nederland. In dit ‘laboratorium zonder muren’ delen multidisciplinaire teams hun kennis, expertise en faciliteiten om de humane ziektemodellen van morgen te ontwikkelen.
Voor innovatieve ziektemodellen heeft hDMT de volgende vier thema’s als prioriteit gesteld: vaten-op-een-chip, hart-op-een-chip, kanker-op-een-chip en brein-op-een-chip.
hDMT is een pre-competitief, non-profit technologisch instituut voor onderzoek en ontwikkeling. hDMT gaat orgaan-op-een-chip modellen die uit de ontwikkelde biomedische en biofysische technologieën voortkomen valoriseren bij farmaceutische en biotechnologische bedrijven, zowel binnen Nederland als internationaal. hDMT zoekt ook actief mondiale samenwerking met andere onderzoekers op het gebied van het modelleren van menselijke organen of ziekten en stimuleert zo de open uitwisseling van kennis en samenwerking aan de grenzen van de orgaan-op-een-chip-technologie.

Meer informatie kunt u vinden op www.hDMT.technology

Terminology

Adult stem cells	Undifferentiated cells, present among differentiated cells of tissues and organs.
Cardiomyocytes	Heart muscle cells.
Cell differentiation	Process by which (stem) cells specialize to perform specific functions. There are around 250 different differentiated cell types in the human body.
Endothelial cells	Cells that line the interior surface of blood vessels.
Extracellular matrix	Collection of extracellular proteins secreted by cells, providing structural and biochemical support to tissue.
Gene mutation	Permanent alteration in the DNA sequence of a gene.
hDMT	Institute for human Organ and Disease Model Technologies, founded in 2015 in the Netherlands. For more information: www.hdmt.technology
Immune system	System that protects the body against disease and infection; consists of immune cells, lymph nodes and lymph vessels.
In vitro	Outside the living body and in an artificial environment. For example, an in vitro experiment occurs in a laboratory vessel or other controlled experimental environment rather than within a living organism.
In vivo	In the living organism. For example, an in vivo experiment is done within a living organism or natural setting (person, animal or plant).
iPS cells	Induced pluripotent stem cells. iPS cells are stem cells derived (induced) by reprogramming fully differentiated cells, such as skin or blood cells, to become pluripotent. iPS cells have been generated from skin biopsies, blood or kidney cells in urine from living adults and children as well as rodents and primates.
Microenvironment	Immediate environment of cells, at the micrometer scale and smaller.
Microfluidics	Technology of manipulating and controlling fluids, usually in the range of microliters and smaller.
Organoid	3D cultures of adult stem cells that mimic organ (sub)structures. Sometimes also referred to as mini-organs.
Organ-on-chip	Microfluidics device (chip), typically made from silicone or glass, on which 3D structures of multiple cell types are grown (organ), under controlled laboratory conditions. The cell structures do not resemble complete organs but recapitulate the minimal functional unit of the organ in question.
PDMS	Polydimethylsiloxane, a flexible and transparent silicone material of which most chip devices are being made.
Stem cells	Cells with the ability to self-renew and to differentiate into specialized cell types of tissues. The fertilized egg is referred to as totipotent because it generates all cells of the body and tissues supporting fetal development like the placenta. Pluripotent stem cells form all cells of the body – including specialized cells such as skin, muscle or immune cells - as well as germ cells. Multipotent stem cells are found in adult tissue and form only those cell types of their tissue of origin.
Toxicity	The ability to poison or damage cells or a whole organism.
Vascular system	The network of vessels in the body, including arteries, veins and capillaries, that carry circulating blood.

1. The promise of organ-on-chip technology

Many current organ and disease models do not adequately capture the salient features of human diseases or organ function. This is a significant cause of late (and expensive) drug failure in clinical trials being faced today by pharmaceutical industry. The current preclinical models most widely used are either standard (monotypic) cell cultures or laboratory animals (zebrafish, mice and rats or dogs); these lack the complexity of human tissue and organs, and differ from humans in both anatomy and physiology. Animal models also raise ethical issues and the costs of animal experiments have a major impact on the budget required to bring a new drug to market. Pharmaceutical companies are thus in need of alternative, more representative and cheaper models in their search for new drugs and drug targets that have high human relevance.

Organ-on-chip technology is one solution to a better representation of the in vivo architecture and function of human organs and tissues. Organs-on-chips are living 3-dimensional cell structures, that are cultured on 'chips' – microfluidic devices with continuously perfused micrometer-sized chambers – that are designed to model physiological functions of tissues and organs. The chips allow cell growth and maintenance under controlled conditions. The microfluidic flow can supply nutrients and be used to deliver drugs, as well as expose target tissue mimics to immune cells, bacteria or viruses. The chips allow real-time microscopic monitoring, and manipulation of the physical and biochemical (micro)environment of cells. Physical cues may include varying the stiffness or hardness of the cellular environment as for example might be relevant for cells in bone versus muscle or brain, and mechanical cues like (time-dependent) pressure or stress, as for example might be relevant in stretched muscle or flowing blood. These parameters may be of great importance for normal or revealing diseased cell behavior. Organ-on-chip models can even represent 'breathing' lung tissue or beating heart muscle if subject to vacuum-driven pressure oscillation and the chips can incorporate synthetic blood vessels with real blood flowing through.

Conditions that would greatly benefit new models include cancer, cardiovascular diseases, neurological and cognitive diseases, (auto)immune diseases and a variety of diseases with complex genetic origins or an ethnic component impacting disease severity. By using stem cells derived from healthy individuals or patients, as appropriate, the genetic characteristics of that particular individual are captured in the model, allowing personalized analysis of drug responses and disease phenotypes. These 'patients-on-chips' could facilitate identification of the most effective drugs for a disease in those patients, saving time, money and unnecessary discomfort. It may also become possible to predict likelihood of disease onset in individuals with hereditary disease, presenting opportunities for lifestyle adjustment or drug prescription to delay the rate of health decline. Human organ-on-chip disease models thus will enhance fundamental understanding of complex diseases, help identify drug targets and could improve the outcome of drugs in clinical trials.

Beyond their potential use in drug research by pharma, organ-on-chip models could also be useful for many other applications, including the discovery of biomarkers for companion diagnostics, new treatment modalities for radiotherapy or hyperthermia, determining the effects of environmental contaminants, and testing food additives and cosmetics for safety. New methods for safety assessment of non-medicinal products are of increasing importance because Europe completely banned the use of animals in testing cosmetics and their ingredients in 2013. The promise that organs-on-chips more closely reflect human biology and simultaneously support the 3R's principle of reduction, refinement and replacement of animal experiments, makes them of significant interest in clinical as well as non-clinical applications.

It is important to note that the ultimate goal of organ-on-chip technology is not to build complete living organs but, rather, to create minimal functional (complex) tissue units that recapitulate human functionalities at the organ level. Organs-on-chips are not for use in the human body, but serve as laboratory models for the different applications. Organs-on-chips allow reliable *in vitro* analyses and their design is as simple as possible, yet as complex as necessary to address each specific question. A major challenge in further developing organ-on-chip technology lies in the integration of state of the art biological, physical, chemical, medical and engineering technologies – and thus requires creation of truly interdisciplinary research teams. This is the objective of hDMT, the Dutch Institute for human Organ and Disease Model Technologies: to establish multidisciplinary teams that can deliver the human organ and disease models of tomorrow.

2. State of the art technologies

Organ-on-chip technologies have undergone rapid development during the past decade, most recently enabled by breakthroughs in human stem cell technologies on the one hand which allow the replacement of primary tissue cells with renewable cell sources, and major advances in engineering biocompatible microfluidic devices on the other. Integration with advanced molecular biology, chemistry and increasing knowledge from medical sciences is now resulting in the next generation of organ-on-chip models.

A. Human adult stem cell technology

iPS cells and organoids

Human induced pluripotent stem (iPS) cells can relatively easily be generated from many different cells of the body, such as cells from skin, blood or urine. These cells can be kept in culture for long periods without losing their capacity to differentiate to many cell types of the body. They can be derived from healthy individuals of any age or ethnic background but also from patients with any disease of interest, on the genetic background associated with the disease. This is of particular use when the disease is caused by multiple genetic mutations or the gene mutations causing the disease are unknown. The differentiated cells have in many cases been shown to recapitulate the disease symptoms of the patient, most frequently when a disease has been autonomous to one cell type. For example, a mutation causing heart disease is evident in heart cells from patient-derived iPS cells, or brain disease is evident in neurons from patient iPS cells. Increasingly though, multiple cell types appear to be necessary for disease symptoms to become evident in the patient iPS cells. For example, endothelial cells from patients with an inherited vascular disease may appear normal in culture, but only in the additional presence of smooth muscle cells does their failure to interact properly with these cells become evident. Organ-on-chip can capture these complexities.

iPS cells have a shortcoming at present though that they are somewhat immature and differ from adult tissue cells. Adult stem cells derived directly from adult tissue biopsies do not have this limitation and for some purposes are more immediately useful as disease models. When cultured as aggregates these cells form complex structures called organoids. From intestinal tissue biopsies they are often referred to as ‘mini-guts’ and have already been demonstrated to exhibit disease phenotypes, for example abnormal chloride ion channel function in Crohn’s Disease. The Crohn’s organoids fail to swell under hypotonic conditions and effective drugs have already been identified using this simple swelling assay. Adult stem cells are however, limited in the range of cell types that they can form and they do not form the stromal or endothelial cells from the patient of interest. The two stem cell types thus yield complementary disease information and models.

Stem cell banks

There are several ongoing initiatives to generate large collections (libraries or banks) of iPS cell lines, both in Europe (Wellcome Trust, UK, StemBancc) and in the USA (NIH, New York Stem Cell Foundation and California Institute of Regenerative Medicine). Similarly, adult stem cell banks

to culture organoids are being generated in the Netherlands (Hubrecht Organoid Technology). These cell banks are currently generating cell lines for a variety of (genetic) diseases, from patients of different genetic backgrounds as well as from healthy individuals; these cell lines are expected to become widely available in the near future. Privacy issues and the use of cell lines for commercial purposes are already being considered upfront in the initial informed consent from tissue donors and are therefore not regarded as major problems. Disease-specific stem cell banks will provide unprecedented opportunities to re-create diseases *in vitro*, especially when a spectrum of different mutations is involved in the disease.

Through these human stem cell banks it will likely become possible to perform (pre)clinical trials-on-chips. Differentiated cells from hundreds of different patients, including either their diseased tissues or their healthy counterparts derived after genetic repair of the mutation, and healthy or defective immune cells could be collected on a single chip, allowing high throughput screening of new drug compounds or drugs that have been approved for another disease (i.e. drug repurposing or off-label use of drugs). (Pre)clinical trials-on-chips will greatly facilitate the development of novel treatment modalities, including immunotherapy, accelerating their entry into the market and lowering costs. It will eventually be only a small step towards personalized medicine, through prediction of drug efficacy for individual patients based on their 'personal' stem cell lines: the 'patient-on-chip'.

Stem cell differentiation protocols

In the last decade, major progress has been made in the development of reliable and reproducible protocols to differentiate human pluripotent stem cells into cells that are committed for particular organ or tissue functions. Standardized reagents and protocols are now (commercially) available not only for culturing the undifferentiated pluripotent stem cells but increasingly also for inducing the specialized differentiated cells from the stem cells. Cell differentiation is often initiated by adding one or more biochemical factors, sometimes sequentially, for specific time periods, and with spatial cues in 3D cultures. Additional cyclic stretch and/or electric stimulation appear to be necessary for full maturation of electrically active cells, like cardiomyocytes or skeletal muscle cells. Similarly, stiffness of the substrate on which the cells grow can play an important role in the differentiation of cells in specific directions. Likewise, adult stem cell culture as organoids is now becoming widely available, again using defined commercially reagents. The scaled production of these differentiated cell types is now also proving feasible. It is transforming these well-defined cells into appropriate assay formats that is the next challenge.

Molecular biology

Current molecular biology protocols allow the introduction of a single gene mutation in (stem) cells, by exchanging the mutation with the wild-type DNA sequence through homologous recombination. Homologous recombination thus allows generation of a series of mutated cell lines from the same starting (stem) cells, differing only in the introduced mutations. This technology is of particular interest for studying diseases that are caused by one or only few well-known gene mutations. Likewise, it is possible to repair a gene defect by replacing the mutated DNA sequence by the wild-type variant. Direct comparison of repaired and mutated cells treated with a drug of interest makes it possible to determine the extent to which the drug ameliorates disease symptoms.

It is also possible to modify a cell line by inserting a fluorescent 'reporter' gene into its genome, linked to a particular gene of interest. This approach can be used to monitor whether a signal transduction pathway is turned on or off in response to drug compounds, for example.

B. Microfluidic chip technology

Microfluidics and microfabrication

The chips on which cells are cultured allow extensive variation in design. Important aspects of chip design are biocompatibility, microfluidic conditions and options for physical manipulation, as well as chip production methodology and cost – including options for higher throughput devices. Polydimethylsiloxane (PDMS) is a silicone that is most commonly used to fabricate microfluidic chips, because of its biocompatibility, flexibility, optical transparency and its relatively easy fabrication by casting. A disadvantage of PDMS is its absorption of small hydrophobic molecules and, therefore, similar but absorption-resistant materials have been identified and are being further developed.

Stiffness and topography of the materials is also important for creating the appropriate microenvironment for the cell cultures. Coating the chips with extracellular matrix proteins, like fibronectin, collagen or Matrigel, is known to be important for mimicking physiological cell behavior. In this respect, membranes from natural extracellular matrix proteins are expected to have added value over PDMS membranes for some applications. In addition, by taking advantage of advances in 3D printing and 3D biofabrication, it will become possible to manufacture organs-on-chips that have a more well-defined 3D architecture and that more closely mimic the *in vivo* tissue organization.

The challenges for microfluidics integration into the chip are multifold. Flows of liquid or air through the microfluidic channels, for example with nutrients, drugs, immune cells, bacteria or viruses, need to be carefully controlled by passive or active pumping technology or microactuators. Also ease of seeding cells at specific locations, accessibility of cells for microscopic imaging and other analyses and, of course, sterility of the culture chambers and microfluidics connections throughout long term culturing are all very important to produce a functional device.

Monitoring and analysis

The architecture of the 3D cell structures on the chips is an important indicator for the viability of the cells, their response to external stimuli or agents, or how adequately they recapitulate physiological *in vivo* conditions. Preferably, architecture and functionality is monitored by real-time microscopic imaging but histopathological analysis on microscopic slides is also an accepted standard for characterization. Immunofluorescent labeling can be used to detect cell membrane proteins or exogenous extra cellular matrix proteins. Moreover, novel microscopic technologies have been developed that enable visualization of living cells and the 3D structure in a culture system like organ-on-chip. Confocal laser scanning microscopy is usually used for fluorescent imaging, although dual multi-photon fluorescent microscopy is better suited for 3D fluorescent imaging. Developments to improve the resolution and focus depth of 3D imaging technologies are continuously ongoing, aiming at analysing cellular processes at single molecule level. Reporter genes are used for lineage tracking studies or for identifying changes in cell phenotype.

Different bionanosensor technologies have already been developed to monitor specific micro-incubator conditions, including oxygen and CO2 concentrations, pH and fluid flow rate in the chip. One of the key features of organ-on-chip technology is that micro-incubator settings specific for one or more cell types allow accurate recapitulation of physiological conditions. It is this feature that enables maintenance of the cell cultures over a longer time frame than is possible under conventional culture conditions.

Major advances have been made in various molecular analysis technologies, such as PCR, DNA and RNA sequencing, mRNA expression profiling, proteomics, metabolomics and multiplex protein analysis. These analyses are currently performed on tissue slices or small samples of only a limited number of cells, or even at single cell level. This allows very accurate and detailed characterization of cells and monitoring of intracellular processes in response to drug compounds. Model-specific assays have also been developed, for example for heart-on-chips, to assess cardiomyocyte function based on mRNA expression data. The challenge will be to link these analysis tools to organs-on-chips in order to collect biomedically relevant data.

Computational modeling

A key aspect of organ-on-chip technology development deals with the integration and interpretation of the data from chips. Organs-on-chips yield a unique type of biomedical data that is based on the on-chip integration of multicellular and multifactorial aspects of tissue physiology and disease. This type of high-level (patho-)physiological information can normally only be obtained by animal testing.

The key challenge in analyzing the data from organs-on-chips is to link their physiological data to clinically relevant, low-level, high-throughput data, like molecular analysis of mRNA expression, proteomics, metabolomics, lifestyle, genetic risk factors. In order to accomplish this integration of different types of data, systems biology based on computational modeling is the most important approach.

By using computational models to analyze the results from organs-on-chips and to find the relations between the various factors involved in generating the final, clinically relevant endpoints, the data from organs-on-chips can be put to good use, for example by translating it into more simple biomarker profiles or by making predictions about disease progression.

Systems biology is an active field of research and the integration of different types of data can be accomplished by e.g. frequentist statistical modeling or by probabilistic Bayesian principles.

3. Available organ-on-chip models

A wide variety of organ-on-chip models are presently available for different areas of application. Apart from the examples below, other models are currently being developed for a number of diseases, such as cancer, cardiovascular diseases and those of other internal organs, cognitive disorders, cystic fibrosis, skin and hair.

A. PDMS-based models

PDMS-based microfluidic organ-on-chip models have been developed by Ingber and colleagues at the Wyss Institute of Harvard University. Their PDMS chip consists of two microfluidic compartments separated by a flexible porous PDMS membrane. Cyclic vacuum suction of two hollow side chambers allows mechanical stretching of the membrane, at specified strains and frequencies. Different cell types are grown on each side of the membrane, recreating critical tissue-tissue interfaces that are present in most organs. The Wyss lung-on-chip and gut-on-chip models have been instrumental in demonstrating the crucial importance of recreation of mechanical cues in the mini-organs. Only by mechanical stretching they were able to adequately recapitulate human organ functions and disease conditions.

Lung-on-chip

Lung-on-chip mimics the alveolus: the smallest functional unit of the lung. Therefore, an alveolar epithelial cell layer and a vascular endothelial cell layer are grown on either side of the membrane. By allowing air to flow over the epithelial cell layer and culture medium over the endothelial cell layer, the complex lung function was mimicked. An inflammatory response was simulated by adding bacteria to the air flow, while macrophages were added to the culture medium. Interestingly, the inflammatory response was only adequately simulated upon cyclic stretching of the flexible membrane between the two cell layers, inducing the macrophages to cross the membrane through the pores into the air flow and then digest the bacteria.

Gut-on-chip

The Wyss researchers also applied their PDMS chip to mimic the intestine. Human colon cancer cell line Caco-2 and a smooth muscle cell line were grown on either side of the membrane. Cyclic stretching, now simulating intestinal peristaltic contractions, was combined with a trickling flow through the luminal channel lined with Caco-2 cells. Interestingly, the cyclic stretching resulted in differentiation of the Caco-2 cells into villus-like structures resembling those seen in the human intestine, whereas this was not observed in the absence of stretching. Stretching also supported culturing gut microbes in equilibrium with the human cells – again a phenomenon not observed in the absence of stretching.

B. Cytostretch models

Dekker and colleagues at Delft University of Technology, in collaboration with Philips Research, have developed a stretchable chip specifically for electrically active cells, like neurons, cardiomyocytes and other muscle cells. Their Cytostretch chip consists of a thin, stretchable PDMS membrane with micro-patterned grooves on its surface and stretchable electrodes embedded in the membrane. Cells grown on the chips align themselves along the grooves. The electrodes pace the cells through electrical stimulation at a controlled frequency or record their electrical activity during cell stretching.

- **Heart-on-chip**
  The Cytostretch chip has been used to develop a heart-on-chip model. Human cardiomyocytes orderly align on the patterned membrane, and the embedded electrodes induce stretching at the frequency of the beating human heart. Variation in the beating rates allows detection of exercise-induced arrhythmias precipitated by drug compounds – a rather common failure of clinical trials for a wide variety of drugs.

C. Compartmentalized models

Van den Berg and colleagues at the University of Twente developed a microfluidic chip in which cells are cultured in PDMS microchannels of varying inner diameter. Upon injection of a mixture of endothelial cells, smooth muscle cells and collagen, the cells self-organize into a tubular structure that resembles vessels. Controlled microfluidic flows then allow analysis of cellular responses to drug compounds.

- **Vessels-on-chip**
  Vessels-on-chips mimic vascular tissues in a variety of human organs. Combining endothelial cells, pericytes (the precursors of vascular smooth muscle cells) and collagen into a microfluidic channel induced a vessel-like structure that followed the contours of the channel. Thrombus formation (atherosclerosis) due to arterial vessel stenosis was simulated by culturing human endothelial cells in a microfluidic channel with a local narrowing. Subsequent fluid flow revealed activation of the endothelial cells after the channel narrowing, with release of von Willebrand Factor and initiation of local coagulation through adherence of blood platelets.

D. Multi organ-on-chip models

The research group of Marx at the Technical University of Berlin was the first to explore the possibilities of multi organ-on-chip models. Their multi organ-on-chip was specifically designed for long term culture and maintenance of a number of different mini-organs or organoids on a single chip. The organoids are perfused using microfluidic flows that connect the different organoid chambers, thus mimicking *in vivo* circulation. Seeding the microfluidic channels with vascular endothelial cells is currently being explored as a means of recapitulating vascularization in the organoids. It is anticipated that such multi organ-on-chip models are especially valuable for drug toxicity screening.

4. Impact of organ-on-chip technology

A. Economic impact

Human organ and disease models-on-chips will initiate a paradigm shift in the medical and pharmaceutical communities. Organs-on-chips make personalized medicine possible, allowing drug development to become faster and cheaper, and – most important – the treatment modalities will be better and safer.

The problems that pharma face in drug development are three fold:

- Pre-clinical drug testing in animals does not always result in reliable prediction of drug efficacy or treatment outcome. Hence too many ineffective drugs reach phase I clinical trials.
- Many drugs cause severe side effects that only become evident at late stages of drug development, during clinical trials or once they are on the market, most notably heart, kidney, liver and brain toxicities.
- Even when effective drugs are developed, they may only give positive treatment outcomes in a subpopulation of all patients due to personal variability.

Together, these issues result in inefficient drug development pipelines where too many drugs fail late in development. The reality is that the development of a new drug from start to market currently takes about 12 years, and costs minimally 1 billion euro per drug.

Organ-on-chip models can solve at least some of pharma's problems. This is evident in the increasing interest of pharma in sponsoring or co-financing academic collaborations, and active participation in congresses and symposia on organ-on-chip and stem cell technologies, aside from initiating their own research programs. Clinical trials on chips and 'patients on chips' can provide more accurate assessment of efficacy as well as toxicity, reducing the need for animal testing or the numbers of animals required. The feasibility of this approach has recently been shown for the anti-epileptic drug Retigabine that was directly approved for clinical trials for ALS patients – without additional intermediate animal testing – based on its effectiveness in iPS cell-derived neurons from patients with an inherited form of this neurological disease.
The required financial investment in research and development of the human organ and disease models are real but not insurmountable. Even when the generation of iPS cell lines and their differentiated derivatives for all patients is taken into account, the costs will be much less than those currently invested by the pharmaceutical industry. The financial impact for pharma is potentially substantial and it is expected that organ-on-chip technology will soon enter their drug development pipelines.

B. Societal impact

As average life expectancy increases in developed societies, so does the prevalence of severe and often chronic diseases. There are no adequate treatments or cures for many cancers, cardiovascular diseases, neurological and cognitive diseases, (auto)immune diseases, or

certain hereditary diseases. Developing adequate treatment modalities or preventing diseases altogether requires an understanding of the underlying mechanisms that cause the diseases. Only then can precision drugs be developed that specifically target the biological aberrations rather than the disease symptoms, holding the promise of more effective treatment modalities. Even then, individual variability that results in almost equally variable treatment outcomes, including unanticipated drug toxicities needs to be taken into account.

Many of these issues can be addressed using human organ and disease models-on-chips. Organs-on-chips allow effective screening for drug efficacy and toxicity, specific for each individual. Because of such personalized medicine, there will likely be fewer treatment failures and fewer troublesome side effects that typically cause discomfort, anxiety, or worse. Governmental agencies should be encouraged to stimulate research more actively as development of organ-on-chip technology is still in an early phase of research, typically not covered by large pharma. The long term reward would be a significant reduction of the costs for healthcare but in the short term animal use could be reduced, new biotech/spin out companies could be established and valorization/licensing could provide a major stimulus to multidisciplinary academic research. But the ultimate reward will be the improved quality of medical care for many prevalent and severe diseases.

The ethical issues involved in animal experiments are recognized in the 3R principle of replacement, reduction and refinement of animal testing that governs animal use in many countries. In Europe the use of animals has been completely banned in testing cosmetics and their ingredients. Organ-on-chip technology can also provide solutions here: with a personal chip that includes a wide variety (or all) cell types, any clinical or non-clinical compound can be tested for any person – including environmental contaminants, food additives and cosmetics. The societal impact of organ-on-chip technology is the improved quality of life for many of us.

5. hDMT: focus and priorities

The institute for human Organ and Disease Model Technologies (hDMT) is a consortium of internationally renowned scientists with backgrounds in biology, physics, chemistry, pharmacology, medicine and engineering. Our researchers share their knowledge, expertise and facilities to develop organ-on-chip models, by integrating state of the art human stem cell technologies and top-level engineering with a wide variety of other expertise. hDMT researchers work at academic research institutions, university medical centers and pharmaceutical companies in the Netherlands, so hDMT is effectively 'a laboratory without walls'.
Although hDMT was only established in early 2015, it has already organized highly interdisciplinary research teams. hDMT's research projects fall within two research lines that reinforce and complement each other: development of innovative human organ and disease models, and development of organ-on-chip technology platforms. Priorities for specific organ and disease models meet the needs of pharma, clinicians as well as society and an active dialogue is encouraged to achieve this central aim. Four themes are currently prioritized: vessels-on-chip, heart-on-chip, cancer-on-chip and brain-on-chip. Other models will be added in the near future, including lifestyle models (skin and hair) and environmental models (nutrition and infection).
hDMT is a precompetitive, non-profit technological R&D institute. hDMT will valorize the developed organ-on-chip models through technology and pharma companies, both in the Netherlands and internationally. hDMT also actively seeks collaboration with other researchers in the field of human organ and disease modeling worldwide, thus promoting open knowledge exchange and collaborations at the frontiers of organ-on-chip technology.

A. Vessels-on-chip

Cardiovascular disease is one of the biggest health challenges in Western society and is caused by thrombosis in atherosclerotic arteries. The cause, progression and acute manifestation of cardiovascular disease is complex and multifactorial, with multiple aspects about lifestyle, genetics, vessel wall biology, blood-based biology, physical blood flow and immunology that integrate to cause the clinically relevant endpoint. Because of their integrative nature, organs-on-chips are ideal for modeling such a complex multifactorial process. Proof-of-concept results have already demonstrated that organs-on-chips can be used to study physical and biochemical mechanisms involved in thrombosis around atherosclerotic plaques. Organs-on-chips could become major tools in finding disease mechanisms and pharmaceutical drug targets in the multifactorial context of cardiovascular disease.
Diabetes is another disease of major importance. Diabetes is a chronic disease that is associated with serious vascular complications, especially in the kidneys and eyes. Renal failure is the leading cause of death in diabetics and diabetes is the major cause of non-age associated blindness. Since little is known about the underlying mechanisms of nephropathy and retinopathy, and even less about possible treatments, there is an urgent need for diabetic vasculitis models. Recapitulation on chips of the highly specialized glomerulus and retinal vessels will provide insight into the causes and development of these pathologies, ultimately leading to better therapies.

Understanding neurovascular dysfunction and the mechanisms that regulate the blood-brain barrier is essential for prevention of neurological and neurodegenerative diseases and for identification of potential drug targets. An adequate blood-brain barrier model could also provide new insights into how to deliver drugs to the brain across this protective shield – a vital requirement for treating diseases like brain cancer or epilepsy.
Vessels-on-chip models will also be developed for cancer, since tumors require infiltrating blood vessels to develop and metastasize. Combining human vessels with cancer cells on chips might provide a model for drug delivery by promoting formation of blood vessels in a tumor or, alternatively, blocking it to prevent nourishing the tumor.

B. Heart-on-chip

Heart disease is the most prevalent cause of death in Western society. Most current treatments only slow down the progression of the disease, creating a great need for the development of novel treatment modalities. Doing so successfully requires more insight into the molecular and genetic nature of the underlying diseases, for which adequate heart disease models are essential. Heart-on-chip is essentially the only available option, since animal and cell models so far have failed to effectively capture human heart disease. Heart-on-chip based on iPS cells offer unprecedented opportunities for studying hereditary heart diseases, since the genome of a patient is captured in the derivative iPS cell lines. Heart-on-chip allows analysis of (abnormal) electrical and metabolic activity, and of patient and/or mutation specific responses to drug compounds. More complex heart-on-chip models are needed when the dysfunction results from a complex interaction of different cell types, but this seems technically all possible.
Another, perhaps even more urgent application of heart-on-chip is drug toxicity testing. Unexpected cardiotoxicity is a major reason why new drugs are withdrawn from the market. Cardiotoxicity tests with heart-on-chip models that are based on iPS cells from patients will result in more accurate toxicity assessment of new drugs, through matching of patients with a particular genetic background with specific drugs (i.e., companion diagnostics). It is even conceivable that drugs that have been withdrawn from the market because of their adverse side effects are going to be reintroduced for a particular population.

C. Cancer-on-chip

Cancer is still a leading cause of death in Western society, despite large investments in cancer research. Cancer-on-chip models are urgently needed because animal models and conventional human cell line models do not adequately reflect human cancer *in vivo* and they have limited predictive value for drug responses. In addition, pharmaceutical companies are undergoing a transition towards precision medicine, implicating that drugs are directed against the specific biological defects underlying the tumor. Precision medicine requires the patient's tumor cells and thus iPS cell lines or organoids from adult stem cells. It is important to recapitulate the tumor's microenvironment and to incorporate the human immune system, such that the resulting immune-competent cancer-on-chip model allows for studying cancer growth and metastasis, drug target discovery, testing of drug compounds, and companion diagnostics. The challenge lies in developing such a complex cancer-on-chip model.

D. Brain-on-chip

Neurological diseases have a high incidence and yet adequate treatments are usually lacking. In the elderly, various forms of dementia are prevalent, either as isolated disease like Alzheimer's disease, or within the context of other diseases like Parkinson, depression, or vascular disease. Inherited developmental disorders, with serious cognitive problems, and various forms of severe autism are often already problematic in childhood.
In contrast to many other tissues, samples from brain tissue for (histopathological) research or primary cell cultures are difficult to obtain. In addition, most neurological and psychiatric diseases are distinctly human and therefore difficult to investigate using animal models. Obviously there is an urgent need for human brain-on-chip models. Protocols are being developed to differentiate various neuronal cells from iPS cells, including cortical neuronal cells and astrocytes. Brain-on-chip models with multiple neuronal cells will provide an opportunity to correlate *in vitro* brain cell function with (defects in) cognitive processes as well as responses to drug compounds. And since at least a subset of neurological diseases is caused by specific gene mutations or structural genomic aberrations, generating brain-on-chips based on iPS cells is opening also new avenues to study those cognitive disorders.

E. Organ-on-chip technology platforms

Ongoing technological development is required for evolution towards the next generation organ-on-chip models. Future organs-on-chips should be improved regarding controlled cell differentiation, 3D culture devices, readout technologies, and complex data collection and analysis, among others. A major aim of the technology platforms is to address the scalability of device components to high throughput, and to enable reproducible manufacturability. Current hDMT technology platforms are:

- *Stem cell production and differentiation* for high quality and reproducible production of pluripotent and multipotent stem cells and differentiation in a wide variety of cell types.
- *Device development and manufacturing* for engineering new modules for chip devices, like new materials, microfabrication of biocompatible membranes, microfluidics, sensors and actuators.
- *Extracellular matrix components* for development of defined substrates and scaffolds with various physical characteristics, like variations in stiffness and topology.
- *3D biofabrication* for the precise patterning and positioning of biological entities, including living cells, structural biological materials, biochemicals and signaling molecules.
- *Imaging and readouts* for the development of specific analysis technologies, like enabling real time 3D monitoring of cellular or molecular reactions.
- *Computational models* for the development of complex data modeling and high throughput analysis software.

6. Key publications

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Please visit www.hdmt.technology for links to the publications

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Back cover: *Striated heart muscle cells derived from human stem cells. The blue structures are nuclei. The red and green structures show parts of the contractile apparatus. These cells are used in a microchip for maturation studies by mechanical stretching.*
Photo: *Berend van Meer (LUMC)*