Report workshop Organ on a chip for human organ and disease models, December 1 and 2, 2014.

Introduction

A workshop 'Organ on a chip' was held in the historical Trippenhuis in Amsterdam, seat of the Royal Netherlands Academy of Sciences (KNAW), on December 1st and 2nd 2014. This workshop was organized in the context of finalisation of the KNAW 'Over Grenzen' (Beyond Borders) project and the introduction of the new 'Organ on a chip' hDMT Institute in the Netherlands. Around 50 participants, including representatives of pharmaceutical companies, scientists and medical specialists joined the meeting, which was by invitation only. The program and list of participants is included.

The purpose of the workshop was to introduce the scientific research plans for the new Dutch Institute for human Organ and Disease Model Technologies (hDMT), planned to be founded in January 2015. Crucial for the success of hDMT is the dialogue with pharmaceutical industry to understand their needs for human healthy organ and disease models on which to base novel assays for improved drug development. The workshop was meant to set the stage for this dialogue: a combination of presentations of ongoing 'Organ on a chip' model development by (stem cell) biologists, experts in imaging, biochemists, physicists/ engineers, and presentations by industry to create a better understanding of the need to support their future product development. A summary of the topics discussed during the workshop is given below.

A new Dutch Institute (hDMT)

Background

Reinder Coehoorn (project leader of the 'Over Grenzen' grant, Philips and TU Eindhoven) indicated that the goal of the 'Over Grenzen' grant was to develop a route towards a leading position of the Netherlands in the emerging research field on Organ on a chip technologies. This was based on the world-wide strong position in stem cell research and microfluidics technologies and should result in the development of truly representative human organ and disease models. A Dutch technology consortium was formed and during the project this consortium unanimously expressed the wish to establish a new technological R&D institute: the Institute for human organ and Disease Models Technologies (hDMT).

Outline hDMT institute

Janny van den Eijnden-van Raaij (managing director hDMT together with Miriam Luizink) presented the outline of the hDMT institute, being a precompetitive non-profit technology foundation. The aim of hDMT is to develop and stimulate the use of in vitro human organ and disease model systems on a chip for multiple purposes by combining expertise and facilities from different disciplines and multiple organizations. This will primarily meet the need for representative human test model systems and as an additional goal also will reduce the use of animal experiments. hDMT is a public-private consortium of 8 founding partners, including the

University of Technology Delft, University of Twente, University of Technology Eindhoven, the Leiden University and University Medical Centre, the Hubrecht Institute, and two companies Genmab and Galapagos. As a central point of contact and a one-stop shop hDMT is envisaged as the gateway for pharmaceutical companies to 'Organ on a chip' technology in the Netherlands, supported by world class stem cell science. hDMT will consolidate expertise in the Netherlands and collaborate with experts from abroad in this area to develop complementary international programs that will lead to new insights in human disease, and to new treatments and diagnostic tests. This is especially relevant for those diseases that are uniquely human, that is diseases with a strong genetic background, diseases with a strong immune component and brain disease.
Knowledge-based Bayesian type computational modeling of the disease process or organ function to be mimicked will complement experimental findings to enable generation of a complete picture of the disease or organ.

Implementation of the model systems
The main implementation in the development process of novel therapies will be in drug testing in the latest preclinical stage before starting clinical trials, in the format of a "clinical trial on a chip". This means that for a specific organ or disease model a number of chips will be available, each including a patient-derived organ/disease model derived from iPSC isolated from a specific patient. In this way the real patient population is adequately represented in an in vitro form as "patients on a chip". This will be done in a low throughput format. Using such an approach a blood-based diagnostic test can simultaneously be developed to predict whether a specific patient is likely to respond to the drug. Importantly, while patients can only "be used" once in a clinical trial, these in vitro "patients" can in principle be generated as often as needed.

Research programs: Vessels, Heart and Cancer on a chip
Initial focus of the hDMT scientific program for disease models is on human stem cell-based cardiac, vascular and cancer models, while options for development of other human models, including those for neurological and auto-immune diseases are being explored; for healthy organ models for toxicity testing initial focus is on heart and blood-brain barrier. With respect to a lifestyle program, the focus will be on organ models for skin and hair and the microbiome (skin, intestines, oral cavity, vagina, lung) – this can all be extended to respective disease models. All hDMT research programs are interdisciplinary programs to which each partner contributes with a specific technology focus, embedded in ongoing research, clinical interest and expertise.

Vessels on a chip
Christine Mummery (coordinator hDMT research program 'Vessels on a chip', LUMC) and Albert van den Berg (UTwente) discussed the pioneering of the modeling of human vessels on microfluidic chips based on iPSC. The latter cells can be turned into endothelial cells and pericytes/smooth muscle cells that, under conditions of flow, organize themselves into three-

dimensional blood vessels closely resembling the vessels in the human body. By adding immune cells and/or other blood components (like platelets, cytokines, van Willebrand Factor) to the circulating fluid the effects of inflammation on vascular integrity or the process of thrombosis can be studied. The use of human iPSC offers opportunities for mimicking genetic vascular disorders in vitro and enables the microfabrication of patient-specific vessels on a chip, allowing preclinical testing of drugs for personalized medicine in the format of a "clinical trial on a chip", which includes a number of different patient-derived vessel models with different genetic backgrounds, representative for the "real" patient population.

Heart on a chip

Robert Passier (coordinator hDMT research program 'Heart on a chip', LUMC) and Ronald Dekker (Philips, TU Delft) described the modeling of the beating heart during various levels of exercise, on a microfluidic chip based on induced Pluripotent Stem cells. iPSC can be efficiently differentiated into different functional cardiac cell types, including beating cardiomyocytes. The cardiomyocytes have an immature phenotype, but are a powerful tool for safety pharmacology studies. For the latter purpose hDMT is developing a model mimicking the heart in action in order to test new drugs for possible side-effects that cause lethal arrhythmias under exercise conditions. This so-called Cytostretch device is capable of stretching heart muscle cells synchronized with contraction, while simultaneously measuring cellular electrical activity, for example after adding a drug. The use of human iPSC from cardiac patients in heart disease models enables studies of the response of individual patients to different drugs and development of a blood-based test to identify patients at risk for cardiotoxicity. Thus drugs that were classified as cardiotoxic may be rescued by the ability to administer the drug only to those patients for which the drug will not be cardiotoxic. hDMT will stepwise develop more complex heart on a chip disease models ultimately mimicking the complicated interaction of all cell types that make up the heart. Extension to multiple disease models is possible.

Cancer on a chip

Anja van de Stolpe (coordinator hDMT research program 'Cancer on a chip') and Kees Storm (TU Eindhoven) discussed the ways to mimic interactions between tumor cells and their biochemical and physical environment on a chip. hDMT is pioneering the modeling of tumors on microfluidic chips that are based on a unique culture method of primary cancer tissue (organoid culture of Hans Clevers), and will ultimately incorporate the human immune response. An immunocompetent cancer on a chip model will be developed by using a modular system linking a cancer on a chip to a lymph node on a chip and introducing blood inflammatory cells. Both the influence of the innate (inflammatory) and the adaptive immune system can be studied in this model. In addition the process of metastasis will be mimicked by enabling migration and intravasation of cancer cells into a surrogate blood vessel system in the cancer-chip and by developing a metastatic site on a chip, such as liver. Tumor tissue-specific (artificial) extracellular matrices and different types of porous membranes will be designed for the microfluidics chip system. As the models can incorporate the patient's genome they can be

used for 'clinical trial on a chip' testing and thereby pave the way to more efficient, personalized and therefore more effective treatments.

Pharma's interests: challenges and collaboration

Structural Genomics Consortium

Chas Bountra (SGC, Oxford) discussed the open access research policy of the Structural Genomics Consortium (SGC) (UK). SGC aims to accelerate the development of new medicines and to reduce duplication in research. The Structural Genomics Consortium (SGC) is an international not-for-profit public-private partnership of 10 global pharmaceutical companies and over 250 academic institutes. The SGC works on novel protein families and combines structural and medicinal chemistry expertise to produce molecular structures, new chemical probes and assays. All this output is used by the SGC and its collaborators to successfully explore new protein families with therapeutic potential. The SGC places all information, reagents and know-how into the public domain without restriction and in the absence of patents. Since animal models and cell lines are not sufficiently representative of human disease the SGC is now building human disease assays using cells/tissues from healthy individuals and patients. The objective is to use high quality chemical probes for novel human proteins and robust human functional assays to identify new targets for chronic inflammatory and neurodegenerative diseases.

National Centre for the Replacement Refinement & Reduction of Animals in Research

Sam Jackson (NC3Rs, London) introduced the activities of the NC3Rs, an independent scientific organization set up by the Government to support the UK science base through the application of the 3Rs. NC3Rs is dedicated to replacing, refining and reducing the use of animals in research and testing. The organization collaborates with scientists and organizations from across the life sciences sector, nationally and internationally, including universities, the pharmaceutical, chemical and consumer products industries, other research funders, and regulatory authorities. The NC3Rs receives its funding from the Government, charitable and commercial sectors. It supports the commitment of the scientific community to the 3Rs by funding research and early career development, supporting open innovation and the commercialization of 3Rs technologies, and stimulating changes in policy, regulations and practice. Funding possibilities for Organ on a chip are CRACK-IT (commercialization of new 3Rs technologies) and Innovate UK competition-based funding (development and application of non-animal technologies for drug development).

Pharma's interests: challenges and collaborations

Several representatives of pharmaceutical companies described the gaps in drug development and the expectations from the pharmaceutical industry on organ on a chip and incorporation of stem cell technology.

Ard Teisman (Janssen Pharmaceutical companies) indicates that, due to upcoming changes in the FDA guidelines, there is an urgent need to develop human in vitro model systems resembling functional mature cardiomyocytes in their endogenous environment in the body. These model systems will be used as an industry standard for cardiovascular safety pharmacology studies to obtain regulatory approval. There is a need for reliability in cardiomyocyte cell provision and for consistency between cell batches and test outcomes; for fast shipment of cells, for reproducible culture media and for better software platforms for faster analysis.

Tom Crabbe (UCB, Slough UK) also prefers the use of Organ on a chip technology since cell lines do not replicate the complexity of organs in the body. Organ on a chip model systems allow a better functional screening of preclinical stage drugs. Validation of the systems is very important and should be achieved by well-defined test molecules provided by pharma. The question is whether complex structures, like the glomerulus in the kidney, can be mimicked in the Organ on a chip models.

Gianni Dal Negro (GSK, Stevenage UK) expressed his concern about the lack of validated preclinical models that are good predictors of efficacy and toxicity of drugs in humans. There is a need for human 3D model systems of progressing complexity, consisting of different cell types in a controlled physiologically-relevant microenvironment. The cells should be cultured in a microfluidic system regulating transport of fluids and soluble factors, and should be linked with analytical devices that can probe the biochemical processes and enable longitudinal assessment. It is expected that Organs on a chip systems using iPSC can meet these needs. For GSK, organs of particular interest include but are not limited to the lung, gut, kidney and liver.

During the general discussion led by Richard Janssen (Galapagos) priority was given by the audience to development of the following Organ on a chip model systems: kidney, lung, heart, liver, vasculature, pancreas. There was scepticism about brain and gut on a chip because of the high complexity. It was recommended to focus also on other sectors than the drug development field, in particular the cosmetic industry but also the chemical sector and food industry, that are desperately waiting for reliable human test models.
In the case of development of more simple but high throughput models, pharmaceutical companies have different opinions about the question who should perform the compound screening. Some prefer to set up a screening assay and perform the screening in-house, others prefer handover to a CRO.
There is a clear need for increased collaboration to share the knowledge and combine the unique strengths between industry and academia. Pharmaceutical companies are willing to share the costs for development of a model system (see NC3Rs, CRACK IT). A suggestion from the audience was to start with a feasibility study as the first step, paid by multiple pharma companies and performed by hDMT. After publication of the results of this study the second step could then be contract development for commercial use, which occurs outside hDMT.

World-wide developments in 'organ on a chip'

Towards ' humans on a chip'

Uwe Marx (TissUse/TU Berlin) discussed the roadmap towards 'humans on a chip'. For the latter purpose different primary tissue cultures (organoids) will be connected on a multi-organ platform using a miniaturized circulatory network with an integrated micropump to provide pulsatile circulation of microliter-volume of medium to the tissues. Via the microfluidic channels nutrients and test substances as well as waste materials are transported in a similar way to blood. At TissUse a two-organ on a chip has already been designed consisting of 3D liver tissues and skin biopsies that appeared to be a potential new tool for systemic substance testing. In addition a four-organ-chip combining human intestine, liver, skin and kidney equivalents into a functional ADMET test assay has been established. The ultimate goal is to develop a 'human-on-a-chip' platform, in which ten organs-on-chips are interconnected and the vasculature and immune system are integrated. This system will predict human responses on drugs more accurately and will reduce animal use in research.

Advanced in vitro systems for modeling human disease

Andries van der Meer (Wyss, Boston) announced the launching of Emulate, a new company in the field of Organ on a chip technology. The company's mission is to commercialize its Organs-on-Chips bioemulation products, automated platform and software to enhance innovation and accelerate the development of pharmaceutical, chemical, cosmetic, and personalized medicine products. The company's founding team carried out their ground-breaking foundational research, engineering and technology translation at the Wyss Institute for Biologically Inspired Engineering at Harvard University. The latter institute developed various organ on a chip model systems, such as an alveolus on a chip for drug toxicity and drug efficacy studies. This microsystem reproduces breathing movements and the associated cyclic strain experienced by cells at the alveolar-capillary interface. In addition a small airway on a chip, a gut microbiome on a chip and a blood-brain-barrier on a chip have been developed. In the future iPSC with different genetic backgrounds will be used for population studies with organs on a chip.

Biorepositories in standardization and distribution of resources

Michael Sheldon (RUCDR, Piscataway USA) provided information about the RUCDR Stem Cell Laboratory. This laboratory houses a collection of postnatal-to-adult human control and patient-derived somatic cells and their reprogrammed derivatives, including 500 induced Pluripotent Stem Cell lines (iPSCs). As a fee for service the Stem Cell Laboratory derives iPSC by reprogramming of source cells, such as fibroblasts, cryopreserved blood cells, lymphocytes, lymphoblastoid cell lines and olfactory epithelium. Quality control of these iPSC occurs using a wide range of test markers, consistent with the FDA guidelines. Recently the different world-wide large-scale iPSC initiatives agreed international coordination and optimization of these resources. In addition the hiPSC initiatives were all willing to promote open exchange of hiPSC lines from their banks (Soares et al. (2014), Stem Cell Reports 3, 931-939).

Pharma's needs: 'Organ on a chip'
Representatives from several pharmaceutical companies discussed the possible use of Organ on a chip systems in preclinical studies.

Matt Sleemann (Medimmune, Cambridge UK) recommended Organ on chip model systems as a powerful tool for preclinical antibody studies. Medimmune is focused on using advances in immunology and other biological sciences to develop important new products that address significant medical needs in areas including respiratory, inflammation and autoimmunity; cardiovascular and metabolic disease; oncology; neuroscience; and infection. For the generation of therapeutic antibodies the Organ on chip approach maybe a powerful tool in selecting the potential therapeutic agents as well as helping predicting the patients who benefit most from them. Biologics, such as antibodies, typically have very high specificity and selectivity meaning that the mechanism of action of the drug can't be properly evaluated in 'classic' in vivo rodent models of disease. Consequently, more complex in vitro systems with human cells that replicate certain aspects of human physiology may provide a great platform to better understand the drugs mechanism of action. Coupling this technology with cells from patients with specific genetic polymorphisms or from specific diseased tissue could be extremely powerful in translating these observations into clinical research.

David Fischer (BioFocus, a Charles River company, Leiden, The Netherlands) indicated the importance of 3D organotypic assays in early drug discovery, ADME/tox drug property screening and chemical safety screening. Examples of these assays are glucose metabolism in vitro assays (human primary hepatocytes), co-culture anchorage independence assay (cancer cells and stromal cells), electrophysiological and complementary assays for cardiac risk assessments (utilizing iPSC-derived cardiomyocytes) and epithelial irritation and penetration assays (ocular, airway and skin explants). Currently most models are low to medium throughput. The challenge is to further develop these assays and increase the throughput, especially with more of these assays becoming regulatory requirements in both pharma and chemical industries.

Mario Monshouwer (Janssen Pharmaceutical companies) described the developments in necessary tools for pharmacokinetic and pharmacodynamic research in order to obtain mechanistic insight regarding drug disposition and safety of potential drug candiates before they enter into clinical development. These tools include analytical methods (including imaging), emerging platforms (hiPSC, 3D cell coculture, organ on a chip, humanized mice) and translation of safety/ADME markers (drug disposition markers). In the case of TAK-875 or fialuridine the availability of a hepatocyte-based system as a preclinical test system might have prevented drug-induced liver injury during late stage clinical phase.

New challenges

Skin on a chip

Human skin disease models require multi-organ models to identify and test novel therapeutics for diseases including cancer, psoriasis, adverse scar, vitiligo and atopic dermatitis. These models require cross-talk between skin and immune system, and should represent the 3D tissue complexity to mimic multi-cellular skin organ. Sue Gibbs (VUMC Amsterdam) presented the results thus far on the development of an immune competent skin on a chip. This model system consists of skin cells (keratinocytes, melanocytes and fibroblasts), immune cells and blood and lymph vessels. Skin equivalent organ culture containing Langerhans cells is grown on a porous membrane with endothelial cells. This membrane will ultimately enable controlled stretching of skin equivalent and trafficking of immune cells (Langerhans Cells, monocytes) through the endothelial layer. The skin equivalent is then transferred to the microfluidics device. In the future patient-derived samples (tumor, fibrosis) may be used for the skin on a chip enabling preclinical trials on a chip and response prediction (personalized medicine).

Gut on a chip

The number of food-related products containing silica nanoparticles increases. To understand the safety of such products the uptake of these nanoparticles following consumption needs to be assessed. Hans Bouwmeester (Wageningen University) demonstrated the use of a gut on a chip for studies of the toxicity of nanoparticles on gut epithelium. The epithelium consists of enterocytes, goblet cells and M-cells and particles can cross the epithelium dependent on the properties of the nanoparticle. The microfluidic chip contains human intestinal cells that are cultured on a porous membrane that can be exposed to nanoparticles. The device is coupled to analytical instrumentation that enables particle detection. Using this technology the gut on a chip model can also function as a test system for the quality of food in the future.

Brain on a chip

Steven Kushner (Erasmus MC, Rotterdam) indicated that the application of iPSC technology creates the unique opportunity to develop physiologically relevant models of human brain diseases using 3D neural cell cultures. Optimization of a neural differentiation protocol has yielded a standardized method for deriving a mature population of neurons and glia and which closely replicates their *in vivo* cellular proportions. Within a 3D environment, this *brain on a chip* application results in spontaneously active and synaptically connected neural networks, the functionality of which was confirmed by electrophysiology and real-time calcium imaging. Longitudinal metabolomic analysis of the extracellular medium has been demonstrated to offer the possibility of non-invasively assaying neuropathological processes. Patient-specific iPSC with site-specific genome editing therefore provides a solid platform for therapeutic discovery, as well as neurotoxicity and safety pharmacology studies including seizure risk.

Immune response on a chip

Anja van de Stolpe (Philips Eindhoven) emphasized the importance of incorporating the immune system in disease models on a chip since every disease is influenced by the immune response of the patient. The components of a human immunocompetent disease model on a chip include (1) cultured disease tissue, including tissue-specific dendritic/immune cells, (2) lymph node, (3) bone marrow/thymic tissue (dependent on model) and (4) a proper microfluidic device. This model, making use of human iPSC-derived immune cells, will mimic the interaction between the innate and the adaptive immune system and the interaction with the diseased tissue. The 3D architecture is very important for maturation and function of the immune cells in a lymph node or thymus. These models will enable development of novel therapies with associated companion diagnostic tests.

Conclusions

The workshop proceeded in a constructive and open atmosphere. The participants, including companies, were enthusiastic about the hDMT initiative and have expressed their willingness to cooperate with hDMT in the near future. The hDMT foundation will be established and hDMT will keep in contact with the participants for the start-up of concrete collaborative projects on Organ on a chip. These will result in representative model systems that are of benefit to the society and enable improved and safe development of novel treatments for many diseases.

Janny van den Eijnden-van Raaij
April 16th, 2015.