A POSSIBLE ALTERNATIVE TO ANIMAL TESTING: THE ORGAN-ON-A-CHIP

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Introduction

Perspective

New innovations are popping up everywhere, from daily life to science. In this article, we aim to shed some light on an innovation aimed at reducing the usage of animals while not compromising the goal of making research to human disease more translational: the so-called organ-on-a-chip models. Here, we will explain what organ-on-a-chip models are, the advantages and disadvantages of using them, their current applications and their future possibilities.

Background

For the past centuries, men improved and replaced many old techniques. Horse and carriage were replaced by the car, the bow and arrow were replaced by the revolver and the cassette tapes were replaced by the CD. The world of medicine does not differ from this cycle of replacement and innovation, thinking of the imaging techniques, immunotherapy, prostheses created with computer-aided design and so on. Despite all the great innovations, however, we are still confined to one very old part of medicine and science: testing on animals. Even though animal testing is being regulated more and more, we are still dependent on it. The conditions in which animal testing is allowed have been evolving over the years. For example, there are strict guidelines and it is forbidden to test cosmetics on animals. In the Netherlands, research institutions need a permit for every single project from the central committee of animal research (CCD) [1]. The permit is only given when the usefulness and necessity of trials sufficiently outweigh animal suffering, and when there are no alternatives available, like research on tissues. Because of these strict guidelines, researchers have to be very specific about what they want to investigate, which feels like a burden by most researchers. These strict conditions do not take away our dependence on animal research. Most of the animals are used for applied and translational research, legislation required toxicity and safety tests, and fundamental scientific research. In 2015 the Netherlands used over 500.000 animals for animal testing [2]. But in line with the cycle of replacement and innovation, could this number not be different? The goals are to reduce, refine and hopefully replace animal studies, also known as the 3Rs [3]. But how can something as complex as an animal be replaced? Organ-on-a-chip models might be the answer for that!

What is an organ-on-a-chip?

An organ-on-a-chip is a 3D cell culture device reproducing a microenvironment that mimics the activity, mechanics and physiological responses of an organ or organ system from the body. Simple organ-on-a-chip devices consist of a single compartment, using a single cell type cultured directly on a channel's surface. These cells can be exposed to fluid shear stress, which is physical stress acting on the luminal surface of cells in the direction of the fluid flow, thus creating a frictional force, comparable to the *in vivo* situation in tissues. More complex devices contain multiple compartments divided by (semi)permeable membranes to allow for transcellular transport. In order to study cell-cell interactions, different cell types can be used in the different compartments of the device. Creating a chip that mimics tissue environments is thus achieved by structural architecture, mechanical forces and co-culture with multiple cell types [4].

A multitude of different organ-on-a-chip devices resembling single organs exist. Some examples of these are the liver-on-a-chip, skin-on-a-chip and kidney-on-a-chip. The latter is an *in vitro* recreation of the microenvironment of the renal tube. For kidney cells, the 3D structure is essential for its function, because it creates the barrier between the blood and pre-urine. When this matrix is disturbed, the kidney no longer retains its function. A kidney-on-a-chip can be comprised of two layers of silicon. This creates two channels, which are then separated by a porous membrane coated with extracellular matrix components. Proximal tubule cells are most often cultured in 3D channels (Figure 1). This site in the kidney is of special interest because it is the primary site of drug clearance and reabsorption. Lastly, a physiological level of flow is applied to create fluid shear stress, which seems to be especially important for the right level of transporter expression, such as Na/K-ATPase and aquaporin-1. Consequently, this gives the epithelial cells more height and a polarity that more closely resembles the situation *in vivo* [5].

Current limitations

Though organ-on-a-chip systems seem promising, there are still some limitations to overcome before implementation can take place.

The biggest limitation of many organ-on-a-chip models is the fact that no primary cells, which represent the *in vivo* situation best, are used in experiments using the devices. Primary cells are difficult to culture and generally have a short life-span, making them less convenient for longitudinal usage [6]. However, using primary cells also has the advantage of being more translational.

Another limiting factor regarding organ-on-a-chip models is that they need to be reproducible in order for the results to be valuable and useful. Therefore, the system has to be validated with established assays with approved read-outs before broad implementation. Reproducibility of these models, both technically as well as biologically, is still a challenge due to the fact that there is no standardisation for important properties such as flow and pressure fluctuations. Furthermore, the sensitivity and specificity of the 3D model have to be compared to existing *in vitro*, animal and clinical data to make sure that the organ-on-a-chip models have the same responses as the organs in our bodies [7]. It thus can be said that the organ-on-a-chip models still need improving, but as these models were only coined a few years ago, they already came a long way.

Current applicability of the organ-on-a-chip

Not only are many researchers trying to perfect their organ-on-a-chip models, but they are also performing experiments to unravel mechanisms of disease and pharmacotoxicology with them.

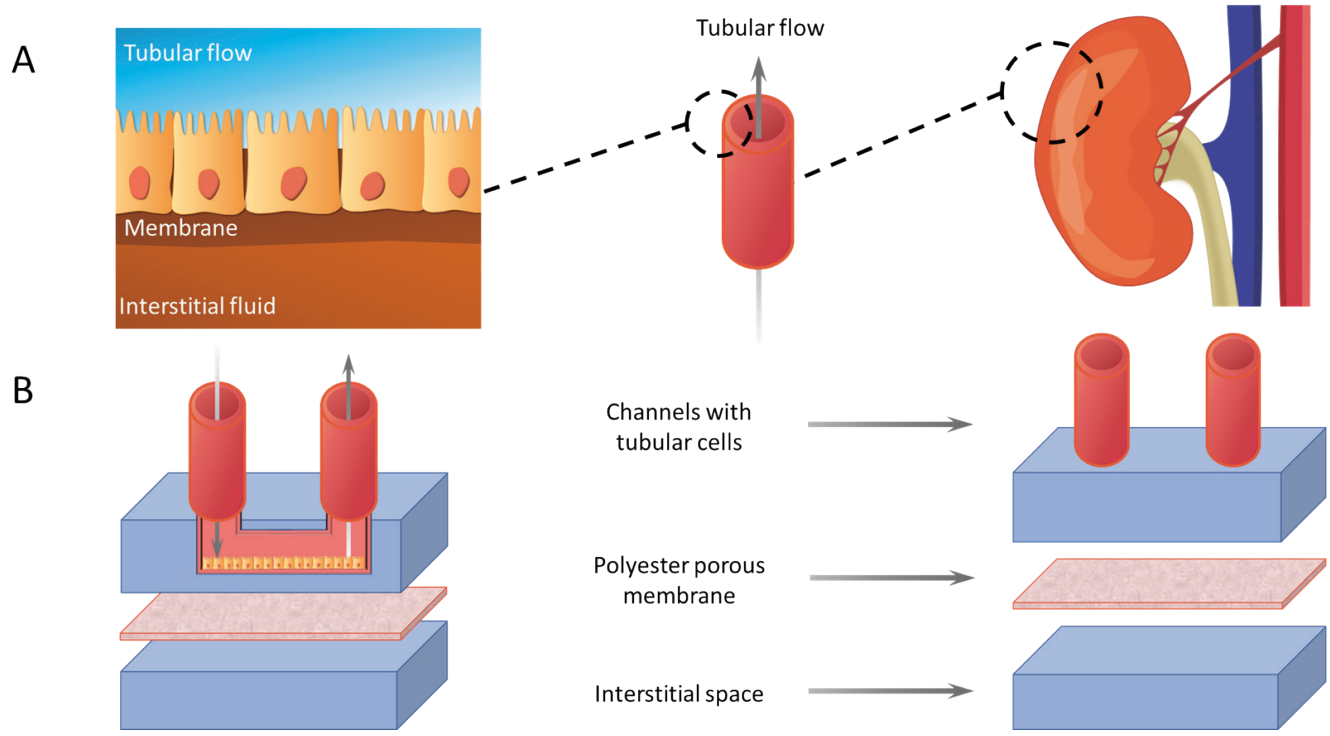

Figure 1: *The kidney-on-a-chip. The microfluidic device consists of two polymeric channels, resembling the proximal tubule and interstitial space, separated by a porous membrane coated with extracellular matrix components. Cells are cultured on top of the membrane, in the presence of a physiological level of flow. (b) Device assembly: The upper layer, polyester porous membrane, and lower layer are bonded together through surface plasma treatment.*

Wang et al. aimed to elucidate the mechanism underlying kidney damage in relation to exposure to high glucose levels, as seen in diabetic nephropathy, using a kidney-on-a-chip [8]. The first sign of diabetic nephropathy is commonly proteinuria. In proteinuria, patients lose albumin and other proteins through the kidney due to damage to the glomerular filtration barrier. Wang et al. used a kidney-on-a-chip that mimics this glomerular filtration barrier containing glomerular endothelial cells, basement membrane and podocytes. The artificial glomerulus was exposed to high concentrations of glucose in the blood compartment to mimic the pathological responses as seen in patients with diabetes mellitus. They found an increased barrier permeability to albumin caused by the high concentrations of glucose, which shows that the artificial glomerulus shows a similar response to high glucose levels as seen in patients. These results reveal that hyperglycemia plays a crucial role in the development of increased barrier permeability to albumin and thereby glomerular dysfunction leading to proteinuria. Moreover, the kidney-on-a-chip mimics diabetic nephropathy that has not been possible by cell-based and animal models, which makes it a useful platform for studying the mechanism of diabetic nephropathy and developing an effective therapy in glomerular diseases.

Kim et al. used the kidney-on-a-chip model to investigate which pharmacokinetic profile of a drug would result in the least nephrotoxicity [9]. Kidneys, together with the liver, are the most important organs for the metabolization and elimination of drugs. Research dedicated to determining the fate of substances in the body, called pharmacokinetics, thus often focuses on the kidneys and liver. However, being part of the pharmacokinetics of potentially very toxic drugs comes with a price for these organs: the kidney and the liver can be severely damaged. In most cases, it is necessary to use animal models to examine this so-called nephrotoxicity and hepatotoxicity, but animals and humans are not the same. The metabolism at cell-level is different, which makes animal studies less reliable. With the rise of organ-on-a-chip models, new opportunities arise. Kim et al. use a microfluidic kidney model containing epithelial cells to examine the nephrotoxicity of the antibiotic gentamicin. Gentamicin is mainly metabolized by the kidneys and is known for its nephrotoxicity and neurotoxicity [10]. The epithelial cells of the microfluidic kidney model were exposed to gentamicin using two different pharmacokinetic profiles: bolus injection and continuous infusion. The researchers concluded that gentamicin bolus injection causes less nephrotoxicity in their model compared to a continuous infusion regimen. For the use of organ-on-a-chip models, it means that the organ-on-a-chip is suitable because it seems to have the same reactions as the organ would have in the body. These studies show that even though the organ-on-a-chip models are still in development, they could already have an important role in refining toxicology studies in animal models.

Multi-organ systems

Diseases and toxicity seldom restrict themselves to a single organ but tend to disrupt homeostasis across multiple organ systems. With an animal model, this could be assessed because it is a multi-organ system by itself. Therefore, to simulate homeostasis disruption *in vitro*, a more challenging system with two or more organ chips is needed. With such a system one could study multi-organ physiological functions and pathophysiology in human cell lines directly [12].

Bauer et al. have given an example of a pathophysiological study using multiple organ-on-a-chip models. They developed a two-organ-chip model to study the interaction between human liver- and pancreatic islet cells. Type 2 diabetes mellitus (T2DM), with multi-organ morbidity, surely seems to be a good candidate for a multi-organ chip model. An analysis of rodent models mimicking human T2DM reveals significant interspecies differences at every level of glucose regulation. This seems to explain why animal studies have poor translations to understand and improve glucose metabolism in humans [12]. Bauer et al. interconnected liver spheroids, which are packages of cells with many features of the human hepatocyte and human pancreatic islet microtissue. In this chip-based model, glucose tolerance tests (GTT) showed a functioning

feedback loop for insulin and glucose. Moreover, when they performed the second and third GTT, they noticed that the islet microtissue had a reduced ability to release insulin. This indicates that prolonged hyperglycemia impairs islet function and therefore the multi-organ chip model mimics basic T2DM pathophysiology. Still, the model in this study is far from representing what happens *in vivo*, because the model only consists out of two organs. Additional organs could be incorporated such as kidneys, a gut, the heart or fat tissue, to further unravel the disease progression of T2DM. Also, a chip-based pathophysiological model could potentially provide a helpful tool to identify new drug targets [13].

Human-on-a-chip and other future ambitions

Thus far we reviewed the potential of an organ-on-a-chip and even a multi-organ system to help resolve problems of animal models. But what about a body-on-a-chip? Could such a system actually reflect *in vivo* parameters of the human body accurately?

A body-on-a-chip would consist of linked together human organ-on-a-chip models (Figure 2). This model would basically resemble a human, scaled down roughly 100,000 times. However, when the human body is scaled down to a micro-device model, imbalance seems inevitable [13]. Organ models would need to have the same relative volume as they have in the human body. In addition, a cell culture medium would be required that mimics blood and with the correct blood flow rate, because both factors influence diffusion across the endothelial membrane. However, there is lots of variety in the culture media used for differently established organ-on-a-chip models, because they use different cell lines. Therefore, making a body-on-a-chip is not as easy as just connecting existing organ-on-a-chip models. Regarding this compatibility issue, tissue engineers are still looking for the "Swiss Army knife" among cell sources [11]. Another consideration is the bioavailability of drugs, which is a phenomenon of drug disposition in the gut and liver or skin whereby the concentration of a drug is reduced before it reaches the systemic circulation [11,13]. Several companies, such as TissUse in Germany, are already trying to perfect this new technique. It is hard to tell when the human-on-a-chip will be common practice in laboratories, but experts estimate this to happen within the next 20-30 years.

There are numerous future ambitions for both human-on-a-chip as well as organ-specific chips. Preclinical testing of pharmacodynamics and kinetics, as well as body toxicology, would be exiting applications for the organ-on-a-chip models. Animals have a different genetic background and therefore often translate poorly to the human clinic [11]. Still, researchers are obligated to test their new medicines on at least two species of rodents and two bigger mammalian species. Altered legislation and involvement of regulatory agencies would be needed to implement the organ-on-a-chip models more in preclinical testing. This could eventually help bridge the gap between preclinical predictions based on animals and outcomes in clinical trials [13]. Furthermore, if newer stem cell technology is integrated into organ-on-a-chip models, personalised models

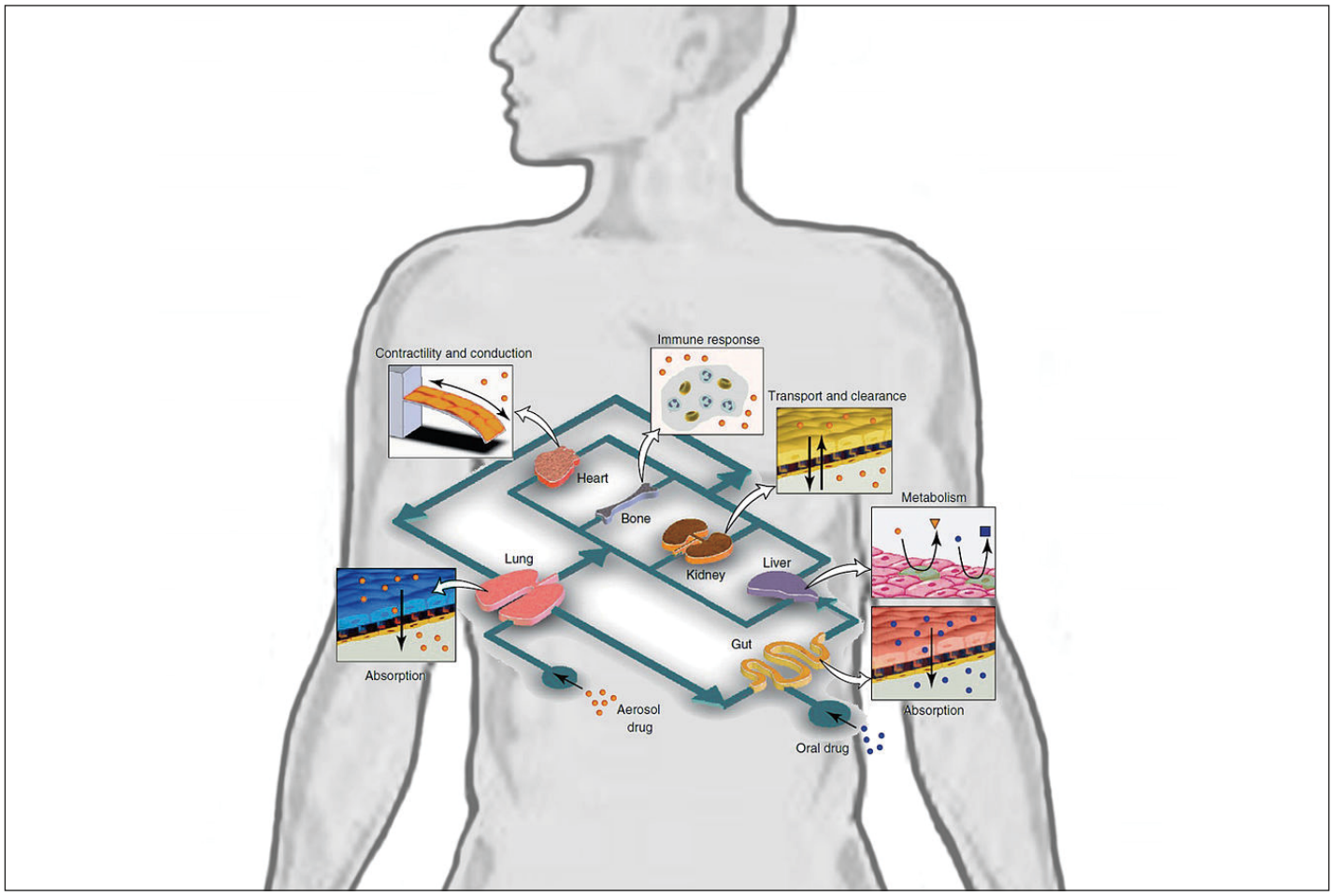

Figure 2: *Human-on-a-chip. Designing a whole body biomimetic device will potentially correct one of the most significant limitations of organ-on-a-chip models: the isolation of organs. Image from https://commons.wikimedia.org/wiki/File:Conceptual_Schematic_of_a_Human-on-a-Chip.jpg, user Timothy Ruban, reuse under CC BY-SA 3.0 licence.*

could make patient-specific predictions concerning toxicology and drug efficacy [13]. Moreover, organ-on-a-chip models could be used in research where actual clinical trials are hard to carry out (e.g. in paediatric diseases and rare diseases).

Conclusion

Organ-on-a-chip models have great potential in their various forms. Kidneys-on-a-chip already prove useful in pharmacokinetics. Multi-organ systems and the body-on-a-chip are likely to let us learn more about (patho)physiology and could allow for preclinical testing of new drugs. Hopefully, organ-on-a-chip models will eventually be able to replace animal experiments or reduce them to an absolute minimum, without the need for compromises; both ethical and scientific. In the meantime, the organ-on-a-chip can reduce the number of animals needed for research, by playing a role in studies like toxicology, with the great advantage of the genetic resemblance of the human.

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References

1. Vergunningen - Centraal commissie dierproeven https://www.centralecommissiedierproeven.nl/onderwerpen/themas/vergunningen (accessed 26 Jan 2018).
2. Aantal dierproeven - Proefdiervrij. Proefdiervrij. 2018.https://proefdiervrij.nl/aantal-dierproeven/ (accessed 14 Jan 2018).
3. Radboudumc. Jaardocument 2016. (2016).
4. Nieskens, T.T.G. & Wilmer, M.J. Kidney-on-a-chip technology for renal proximal tubule tissue reconstruction. European Journal of Pharmacology 790, 46-56 (2016).
5. Jang, K.J., et al. Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment. Integrative biology : quantitative biosciences from nano to macro 5, 1119-1129 (2013).
6. Nieskens, T.T. & Wilmer, M.J. Kidney-on-a-chip technology for renal proximal tubule tissue reconstruction. Eur J Pharmacol 790, 46-56 (2016).
7. Paoli, R. & Samitier, J. Mimicking the Kidney: A Key Role in Organ-on-Chip Development. Micromachines 7, 126 (2016).
8. Wang, L., et al. A disease model of diabetic nephropathy in a glomerulus-on-a-chip microdevice. Lab on a chip 17, 1749-1760 (2017).
9. Kim, S., et al. Pharmacokinetic profile that reduces nephrotoxicity of gentamicin in a perfused kidney-on-a-chip. Biofabrication 8, 015021 (2016).
10. Gentamicine. Farmacotherapeutischkompas.nl (2018). at <https://www.farmacotherapeutischkompas.nl/bladeren/preparaatteksten/g/gentamicine__parenteraal_> (accessed 14 Jan 2018).
11. Dehne, E.-M., Hasenberg, T. & Marx, U. The ascendance of microphysiological systems to solve the drug testing dilemma. Future Science OA 3, FSO185 (2017).
12. Bauer, S., et al. Functional coupling of human pancreatic islets and liver spheroids on-a-chip: Towards a novel human ex vivo type 2 diabetes model. Scientific Reports 7, 14620 (2017).
13. Esch, E.W., Bahinski, A. & Huh, D. Organs-on-chips at the frontiers of drug discovery. Nature reviews. Drug discovery 14, 248-260 (2015).

EXAM QUESTIONS

As RAMS aims to enlighten both students and professionals, we would like to present you two exam questions. Find out if you can remember what you have learned during the bachelor!

We challenge you!

Question 1

A patient with an acute asthma attack is treated with intravenously administered prednisolone, in addition to inhalers. Prednisolone acts on the glucocorticoid receptor, a so-called nuclear receptor. When is the effect of prednisolone likely to occur?

A. Within seconds.
B. Within minutes.
C. Within hours.

(Topic: Farmatoxocology, Module Q6 Movement and Flow 2017)

Question 2

Gastric acid secretion can be inhibited by negative feedback from the intestine. Which of the following hormones from the duodenum inhibits the release of gastrin from the pyloric antrum?

A. Acetylcholine.
B. Bombesin.
C. Histamine.
D. Secretin.

(Topic: Digestion, Module Q6 Movement and Flow 2017)

The answers to these questions can be found on page 16 in this journal.