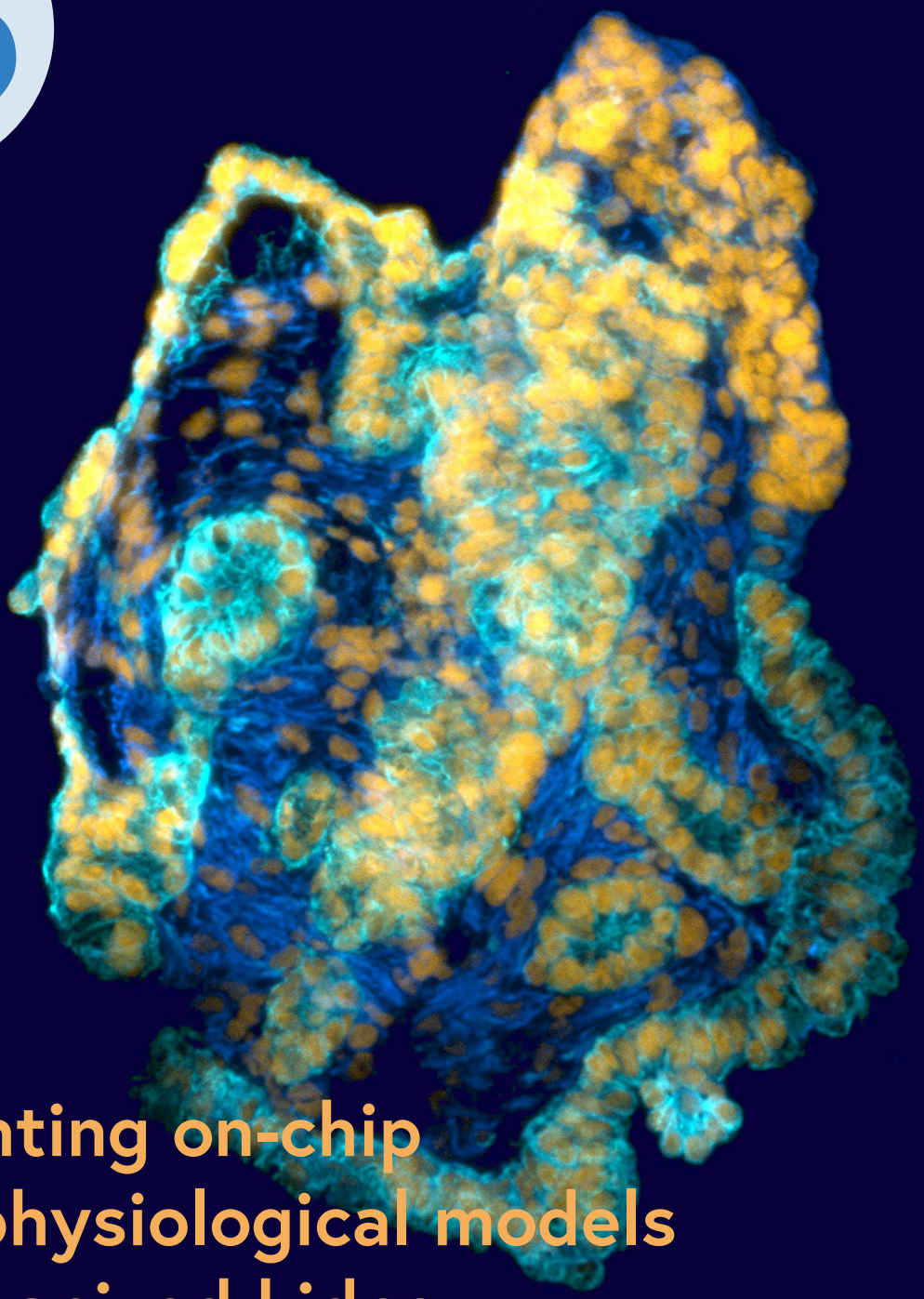
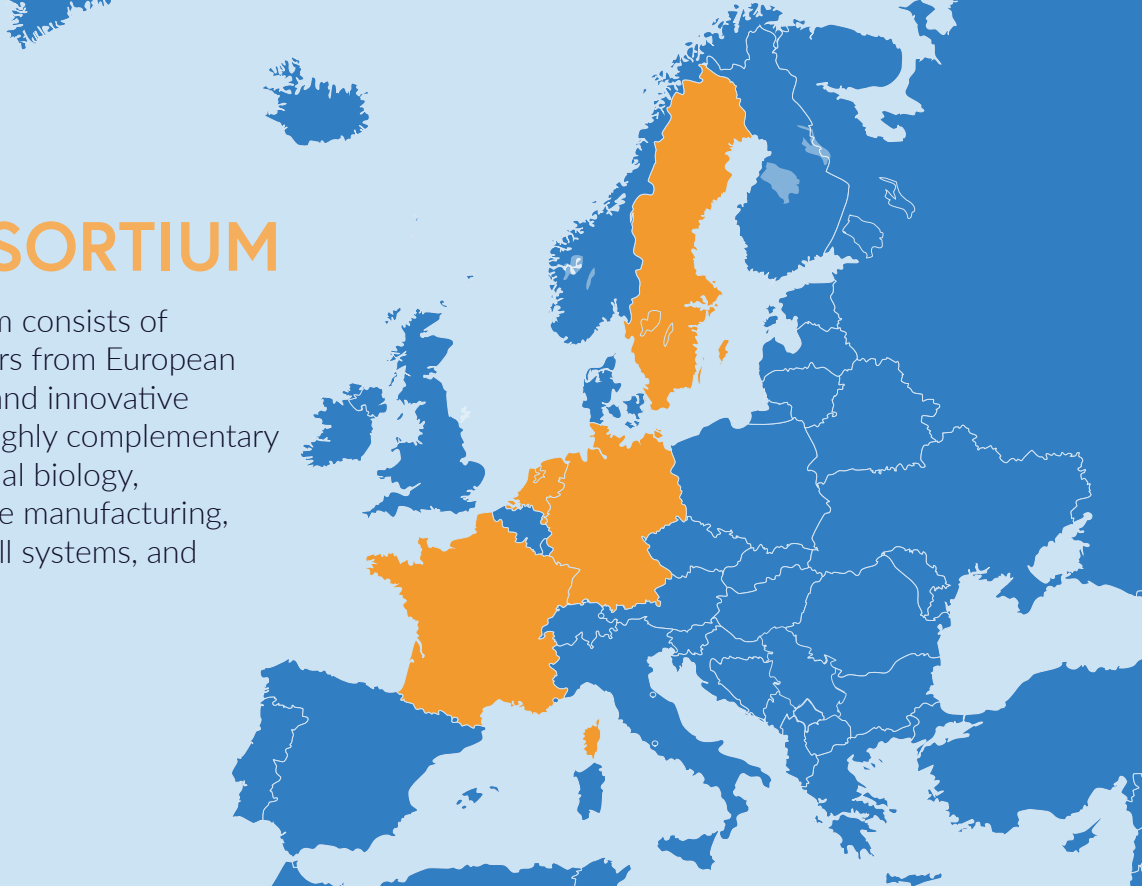


Bioprinting on-chip microphysiological models of humanized kidney tubulointerstitium

BIRDIE Stakeholder report 2023

THE CONSORTIUM

The BIRDIE consortium consists of experienced researchers from European academic institutions and innovative SMEs. The team has highly complementary expertise in translational biology, bioprinting and additive manufacturing, microfluidics, single-cell systems, and organ-on-chip models.



Maastricht University

Maastricht University (MU) is the youngest and most international university in the Netherlands. Ranked 7 in QS "Top 50 under 50", the university has more than 16.500 students, 4.000 staff and 55.000 alumni, with over 70% foreign PhD students from more than 100 student nationalities. MU is part of the Maastricht health campus and the constant interaction with hospital physicians ensures the proper environment for the development of clinically and physiological relevant models.

Nantes Université

Nantes Université (NU) has approximately 38,000 students enrolled each year, one doctoral college with eight post-graduate schools and 3,200 research staff working in 75 accredited labs. NU has research agreements with industry and shares its discoveries with the society at large. As a multidisciplinary university, programs in most fields of knowledge and academic paths are offered.

TISSUSE Emulating Human Biology

TissUse is a Berlin, Germany-based, biotech company who has developed a unique "Multi-Organ-Chip" platform to accelerate the development of pharmaceutical, chemical, cosmetic, and personalized medical products. Therewith, TissUse's Multi-Organ-Chips provide preclinical insight on a systemic level using human tissue and enable the direct prediction of effects of substances and their metabolism on near real-life models.

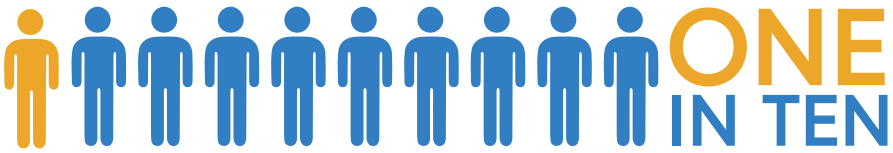
fluicell®

Fluicell AB is a Sweden-based, publicly traded, biotech company with a commercialized product portfolio for biomedical research. The company is a world leader and pioneer in open-volume microfluidics for the life sciences and holds a strong IP and patent position with five different patent families in the estate. By zooming into the level of individual cells, the company redefines the approach to cell biology with its unique microfluidic technologies.

THE BURDEN OF CHRONIC KIDNEY DISEASE

Chronic Kidney Disease (CKD) is predicted to become the 5th leading cause of death worldwide*. Among the risk factors for the development of CKD, drugs, and in general nephrotoxicants, and viral infection (BK Virus) play a major role. Moreover, therapeutic options offered to patients are still limited to renal replacement therapies such as dialysis or, in extreme cases, transplantation.

*C. P. Kovesdy, Epidemiology of chronic kidney disease: an update 2022. *KI Supplements*. **14**, 7-11 (2022).



843.6 Million affected globally (2017)*

Building a foundation for future therapies through state of the art biomedical technology

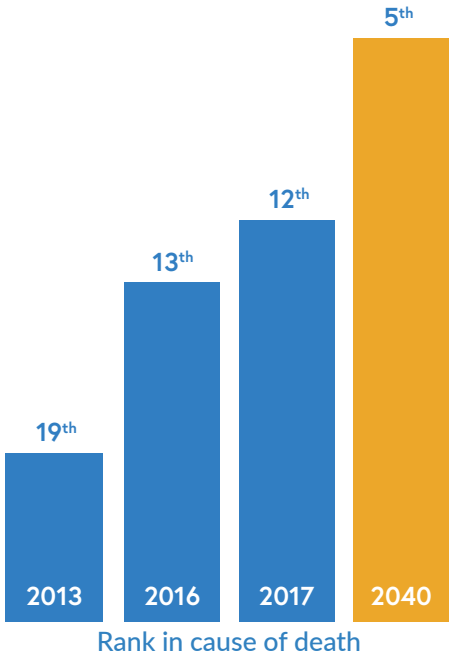
The BIRDIE project was formulated with an emphasis of bringing broad societal impact from the development of state-of-the-art biomedical technology. Our vision is that the humanized kidney *in vitro* models developed within the BIRDIE project will allow further understanding of kidney disease while supporting future therapies for patients. Furthermore, the aimed *in vitro* models will be essential to test new therapies administered to patients (e.g. during drug development) or ultimately being able to generate patient-specific *in vitro* models (derived for iPSCs generated from patient cells) allowing personalized medicine approaches.

Microphysiological Model
Leader: Nantes Université

Organ-on-Chip
Leader: TissUse GmbH

iPSC-based models
Leader: Maastricht University

Bioprinting
Leader: Fluicell AB



Rank in cause of death

+41.5 % Death rate increase 1990-2017*

THE BIRDIE APPROACH

Microphysiological model

During BIRDIE project we are looking to set up long-term microphysiological models of human renal proximal cells cultured under physiological and disease states. The optimized model will be challenged with both BK virus infection and nephrotoxic drugs, and transcriptomics will be used as a guidance system to constantly assess the proximity of our models compared to healthy or diseased native renal sorted cells and tissues.

Organ-on-Chip

Organ-on-a-chip systems enable a co-culture of physiologically relevant tissue models in a closed microfluidic circuit emulating the blood perfusion. During the BIRDIE project a novel chip enabling a dual perfusion of a kidney model by overlapping a blood and a urinary microfluidic circuit will be developed by TissUse GmbH. This chip will harbor the 3D bioprinted models developed within the other work packages.

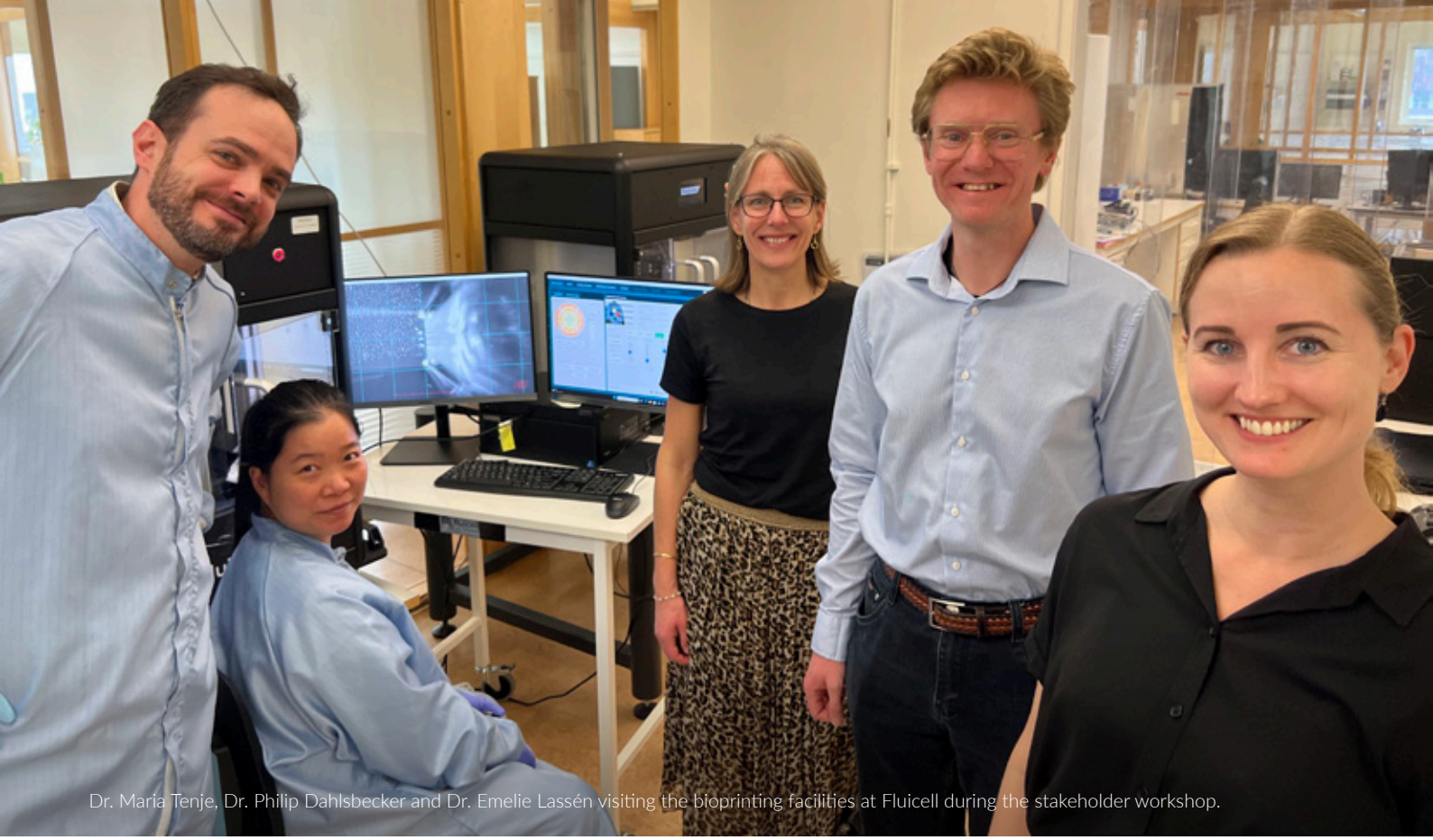
IPSC-based models

Induced pluripotent stem cells (iPSCs) allow the generation of renal progenitors and organoids relevant for kidney *in vitro* models. IPSCs-derived organoids will be generated and combined with bioprinting and microfluidics for nephrotoxicity and viral infection screenings.

Bioprinting

The combination of multiple bioprinting techniques is of paramount importance to achieve a broader range of complexity and mimicry in kidney models. Within BIRDIE we will use multiple bioprinting techniques to produce macro-size features and Fluicell's unique single cell bioprinting to fine-tune cellular compositions within the kidney tubulointerstitium space.

THE BIRDIE IMPACT



Dr. Maria Tenje, Dr. Philip Dahlsbecker and Dr. Emelie Lassén visiting the bioprinting facilities at Fluicell during the stakeholder workshop.

STAKEHOLDER MEETING

Non-animal models for research and drug development

Late-stage failures is a major driver behind increasing drug development costs. *In vitro* research models that combine human cells with accurate modeling of organ and multi-organ physiology has the potential to offer predictive power at an early stage of development.

On October 18th, 2023, the BIRDIE consortium gathered experts from the pharmaceutical industry, academia and regulatory authorities to discuss opportunities and challenges associated with implementing new non-animal research models in biomedical research and drug development. A goal behind the workshop was to stimulate dialogue between stakeholders that represent different aspects of the research sector to identify what is required from new *in vitro* models, in which areas the chance for successful implementation is highest and what the pitfalls are along the way.

The workshop also covered different aspects on advanced *in vitro* kidney models, both from a developer and from an end-user perspective.

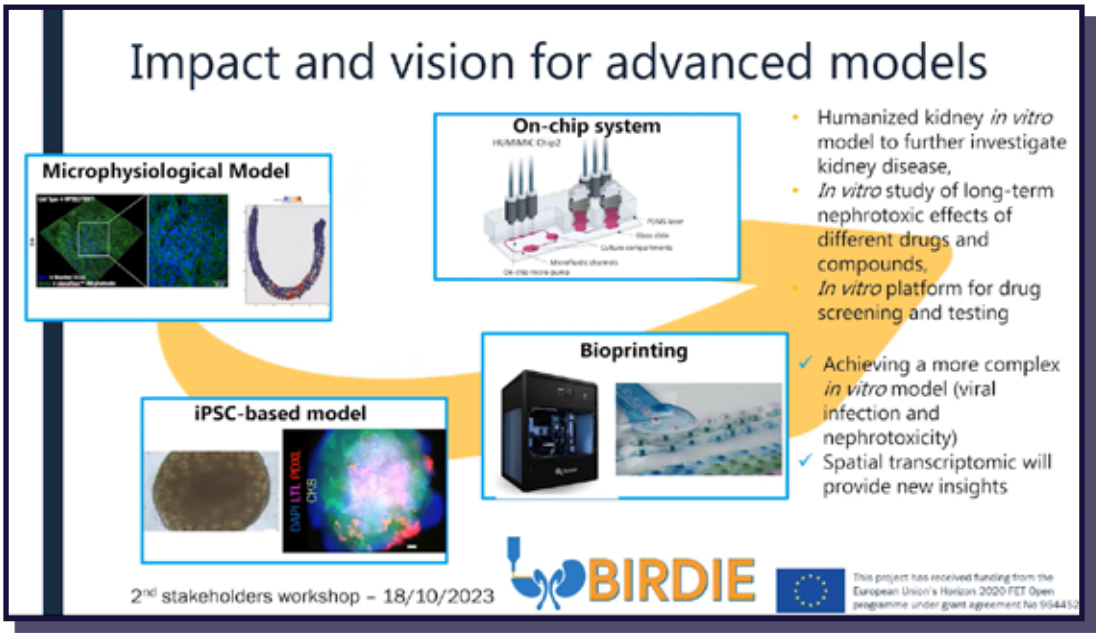
The invited stakeholders were Prof. Maria Tenje (Uppsala University), Dr. Silvia Mihăilă (Utrecht University), Dr. Camilla Svensson (Swedish Medical Products Agency / EMA 3R Working Group), Dr. Emelie Lassén (AstraZeneca), and Philip Dahlsbecker (AstraZeneca).

The workshop opened by consortium leader Dr. Carlos Mota introducing the BIRDIE project and it aims to achieve. During the workshop, each of the invited speakers gave an individual presentation, followed by questions from the other participants and from the audience. The workshop concluded with a panel discussion, featuring all of the participants.

On the following pages, we provide brief summaries of all the talks and of the panel discussion. Here, we provide some of the key take away points from the workshop:

- It is There is unlikely that to be a one single microphysiological in vitro model will be the solution that fits all research purposes, especially for something as complex as the kidney. Instead, new technologies need to be developed in conversation with end-user to address their specific needs.
- Irrespective of model design, a key challenge is creating the right balance between model simplicity that promotes ease of use and workflow integration, and model complexity that enables accurate biological mimicry.
- The BIRDIE approach covers many of the key aspects to achieving an accurate *in vitro* kidney biomimicry by creating a model that bridges microscopic cell-level tissue modeling with macroscopic biomechanical modeling.
- Integration of non-animal models in regulatory testing is currently challenging due to lack of “universal” guidelines, and lack of inter laboratory comparisons and standardization.
- The easiest point of entry for new advanced *in vitro* technologies is likely during drug discovery to replace simpler cell based models by providing datadata that are more accurate earlier during drug development.

WATCH THE RECORDED WORKSHOP →



Bioprinting on-chip microphysiological models of humanized kidney tubulointerstitium

PRESENTED BY: DR. CARLOS MOTA, MAASTRICHT UNIVERSITY / BIRDIE

BIRDIE consortium leader Dr. Carlos Mota gave the first talk of the workshop. In his talk, Carlos focused on the motivation behind the BIRDIE project, the rationale behind its approach and the goals that the project has set out to achieve. This summary provides an overview of some of the key points during the talk.

Kidney disease is a growing health concern and is rapidly becoming one of the leading causes of death worldwide. The BIRDIE project aims at creating humanized kidney *in vitro* models that can allow further understanding of kidney disease, enable development of new treatments and create opportunities for personalized medicine approaches.

The first scientific work package is focused on microphysiological models and involves using primary tissue from patients and comparing with existing models being used nowadays.

One of the key focus areas of this part of the project is to study BK virus infection, an important cause for kidney disease. To correlate *in vitro* what is happening in the patient, the project uses spatial transcriptomics to identify the specific location where an infection occurs in the tissue.

In parallel, the consortium is establishing iPSC-protocols to generate kidney organoids as well as endothelial and epithelial cells. To capture the complexity of the kidney, the goal of the project is to first combine the iPSC-derived cells with high precision bioprinting. In the final work package, the bioprinted constructs are integrated in an organ-on-chip device to enable long term studies of nephrotoxicity and viral infection.

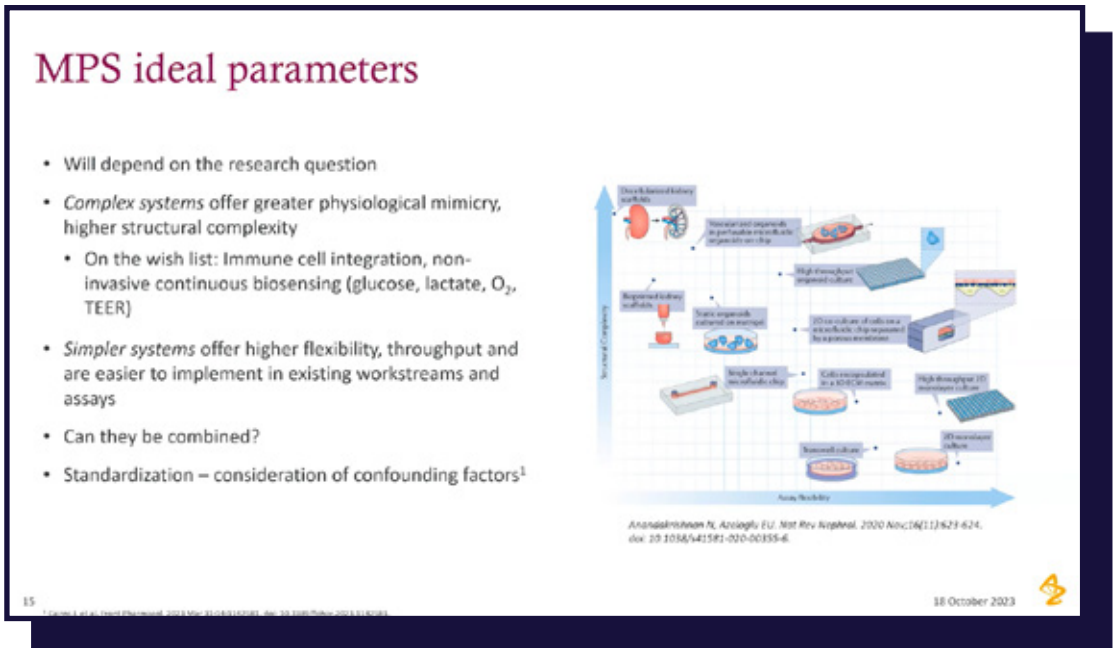
The primary bioprinting technology used in the project enables selective deposition of cells with high precision. The goal is to use this technology to replicate the cellular arrangement found in the tubular interstitium and to combine this with tubules that are bioprinted on a chip model.

To create a fitting chip model for the project, the consortium is redesigning the chip technology from TissUse, one of the project partners. The chip is a closed circuit with actuating pumps that enables continuous flow across multiple circuits.

The intended chip design contains one circuit for urine and one for blood. The goal is then to integrate the bioprinted constructs in the chip. At the current stage of the project, there is a lot of engineering work happening to optimize all the individual components that will be part of the final design.

A main challenge for BIRDIE is the size and scale that the project is aiming for. Even though the bioprinting technology allows high precision cell deposition, integrating the multiple components into a chip and mimicking the complexity of the tubular interstitium in a very confined space remains an important challenge for the project to solve.

If these challenges can be resolved, the BIRDIE project will result in a humanized *in vitro* model that can allow investigation of kidney disease and long-term toxicity, drug testing and personalized screening, and that can constitute a more accurate model for viral infection, providing new insights into disease progression and development of antivirals on the long term.



Modeling the proximal tubule for drug target validation

PRESENTED BY: DR. EMELIE LASSÉN & DR. PHILIP DALSBECKER, ASTRAZENECA

Dr. Emelie Lassén is Associate Principal Scientist at AstraZeneca in the renal bioscience team and Dr. Philip Dalsbecker is a postdoctoral fellow at AstraZenca civilian safety Science under Clinical Pharmacology and Safety sciences. The focus of their talk was to present how AstraZeneca is using renal *in vitro* models and microphysiological systems (MPS) in their research and to give insight into what is needed and desired from new technologies for them to be incorporated in their research toolbox.

The core of AstraZeneca's development strategy is what they label as 5R: right target, right tissue, right safety, right patients, and the right commercial potential. Within renal bioscience, the primary concern is the two Rs right target and right tissue, but right safety also comes into consideration.

In all these three Rs, using human material has the potential to improve the link within the studied target and the disease and provide a translational advantage. Many diseases, such as diabetic kidney disease, are difficult to model in rodents and the strategy for identifying new drug targets has changed to be more focused on human target validation. In the search for the right tissue, it is also interesting to be able to perform pharmacokinetics, pharmacodynamics, and similar kinds of assays.

MPS come into play for *in vitro* target validation. The choice of more advanced system is motivated by better physiological mimicry and higher structural complexity, including parameters such as vascular organization and immune cell integration.

The increased complexity and biological relevance offered by MPS comes at the expense of screening capacity. At the same time, having a simpler, more high-throughput model risks introducing pitfalls from having a too reductionist approach.

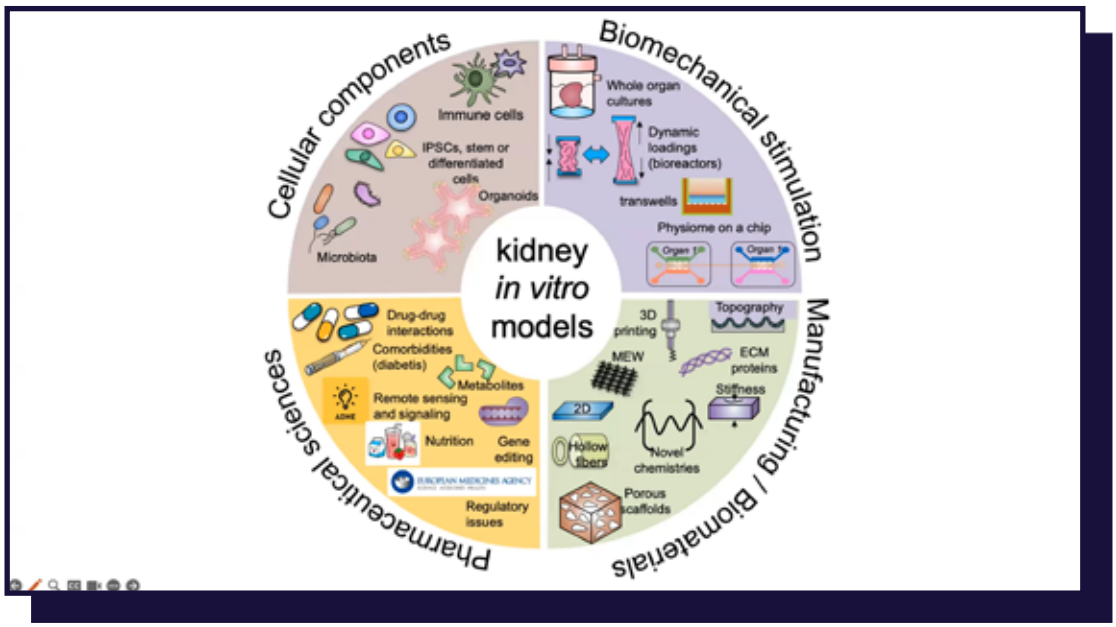
In proximal tubule modeling, one thing that the researchers at AstraZeneca are interested in recreating is cell polarization, something that is lost in 2D models but that can be retain in more advanced systems.

Moreover, having the ability for non-invasive biosensing to for instance monitor parameters such as glucose, lactate, oxygen and transepithelial electrical resistance in real time is also a point of interest. There is also an interest from AstraZeneca and other comparable actors to be able to model intracellular transport, reabsorption and excretion in increasingly physiologically relevant systems for use in mode of action, efficacy and safety studies.

Other qualities that are desirable from a user perspective have to do with assay compatibility and having flexible readout capabilities such as morphological changes, immunofluorescence staining or uptake of fluorescent ligands. This must be achieved while also meeting standardization and robustness requirements that takes things such as inter-operator variability into account.

To summarize, the ideal system from the pharmaceutical industry's point of view combines the flexibility and ease of integration offered by simpler system with the high level of mimicry of the more complex systems. So far, none of the tested MPS devices has succeeded in achieving this goal.

The value for BIRDIE: For the BIRDIE project, this means that there is a need to develop new devices that have a wide applicability and that can be tailored to the end-users' needs. Proximal tubule models need to model transcellular transport, reabsorption and excretion in a physiologically relevant setting. Designing systems with possible readout modalities in mind is also important.



INs and OUTs of the proximal tubule

PRESENTED BY: DR. SILVIA MIHĂILĂ, UTRECHT UNIVERSITY

Dr. Silvia Mihăilă is an assistant professor of *in vitro* models of disease at the Department of Pharmacology of Utrecht University. Her work aims to develop humanized *in vitro* models to replicate pathological complications associated with kidney injury failure in the pursuit of unraveling mechanisms of disease and potential therapeutic targets. The focus of her talk was to provide insight into the kidney disease research conducted at her division and the diverse ways they model kidney function.

The main driver behind Silvia Mihăilă's research is to look at processes in the kidney and to try to reproduce those in the lab to gain mechanistic understanding and to find potential therapeutic methods.

One of the group's research interests is protein-bound uremic toxins (PBUTs). These toxins bind to albumin and cannot be removed by conventional dialysis. They accumulate in the plasma of CKD patients and are associated with a wide range of comorbidities. To improve dialysis, it is desirable to find a way to separate toxins from albumin.

To achieve this Silvia and her team looked at how this happens in the kidney. There, proximal tubule cells that have active transport properties remove the compounds. To recreate this process, they use a proximal tubule epithelial cell line, modified so that it expresses the transporter protein OAT1 that handles the removal of the toxic compounds.

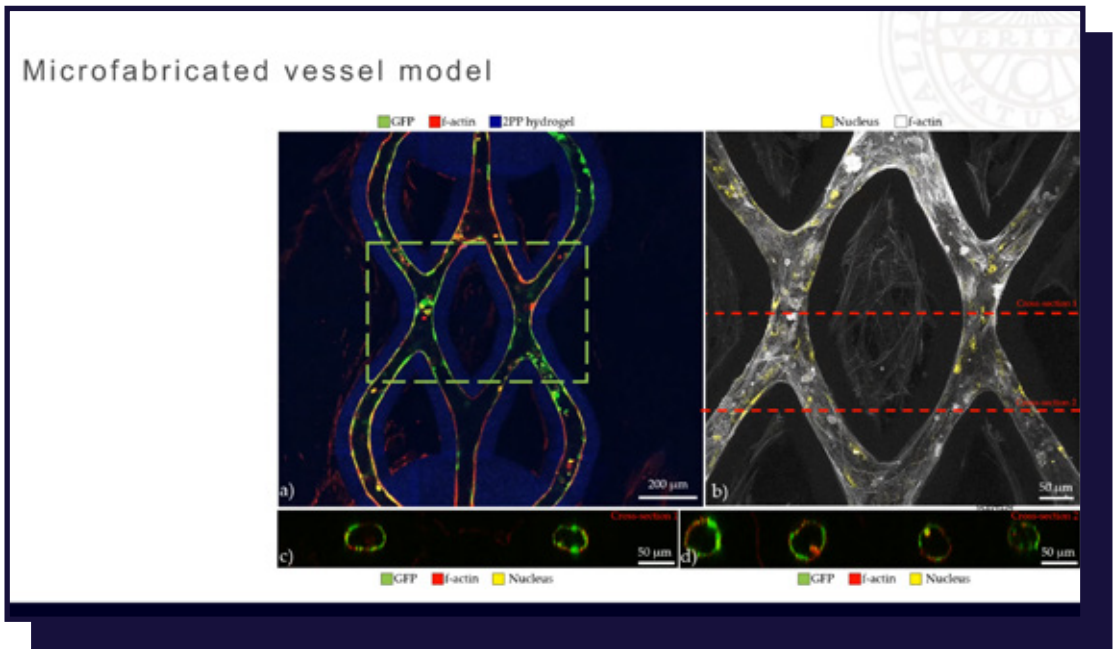
With this cell line and the proximal tubule cells, the researchers now had a model of kidney function, making it possible for them to start answering the question of why do kidneys fail?

One thing that they wanted to replicate was renal ischemia reperfusion, something that is normally studied in animals. In their study, they managed to mimic the injury conditions *in vitro*, and also used treatment with MSCs to investigate if they could rescue that damages cells, indicating a potential treatment.

Another area that they wanted to investigate was the link between medication and kidney damage. It is common for people with kidney disease to require a high amount of medication to treat comorbidities related to their kidney disease. These medications can be handled by OAT1, preventing the transporter from fulfilling its function to remove toxins. To study this, Silvia and co-workers treated their cell line with drug compounds showing an effect on transporter activity.

The cell line, and the knowledged from it, is now being integrated in a bioartificial kidney system, where the cells are being coated on a hollow fiber. The system is being designed to replicate the process in the kidney where PBUTs are being separated from albumin, returning to the original motivation behind the study of the transporter function. This project not only involves the modified cell line, but also studies of biomaterial fabrication to active the correct filtering properties and material-cell interaction.

The value for BIRDIE: A take-home message for the BIRDIE project is the opportunity offered by tailoring cell lines that mimic specific function in the kidney and the value of creating strong dialogue with kidney disease scientist to create further uses for the system being developed in the project.



Microfabricated hydrogel-based barrier models

PRESENTED BY: DR. MARIA TENJE, UPPSALA UNIVERSITY

Dr. Maria Tenje is Professor at Uppsala University and leads the ENBLA research group, specializing in biomedical and life science applications based on advanced micro and nanofabrication methods and microfluidics. In her talk, Maria Tenje zooms in on the design and fabrication of biomaterials and how that creates opportunities for new types of devices for research and drug development.

At the core of this research, the main research question being investigated, is the question: How can we tune the properties of biomaterials to make new devices possible?

One example of such a design approach is tuning hydrogel properties to steer cell adhesion to specific geometries, therapy providing the ability to control positioning and orientation of cells by varying the dimensions of the geometries that can influence cells adhesion.

A key focus area for Maria Tenje and her group is looking at models for biological barriers. Currently used barrier models are generally 2D, present a static environment and use synthetic materials.

One approach is to encapsulate cells in a barrier environment in 3D, using e.g. collagen. This creates a system based on bio-derived material that can function as a barrier.

The challenge was to integrate the collagen barrier into a microfluidic chip to create a system that is not static. Here, being able to have sufficient mechanical support for the collagen barrier presented a challenge.

The solution was to merge the barrier construct with a supporting mesh structure that could be integrated in a transwell system, creating a free-hanging barrier.

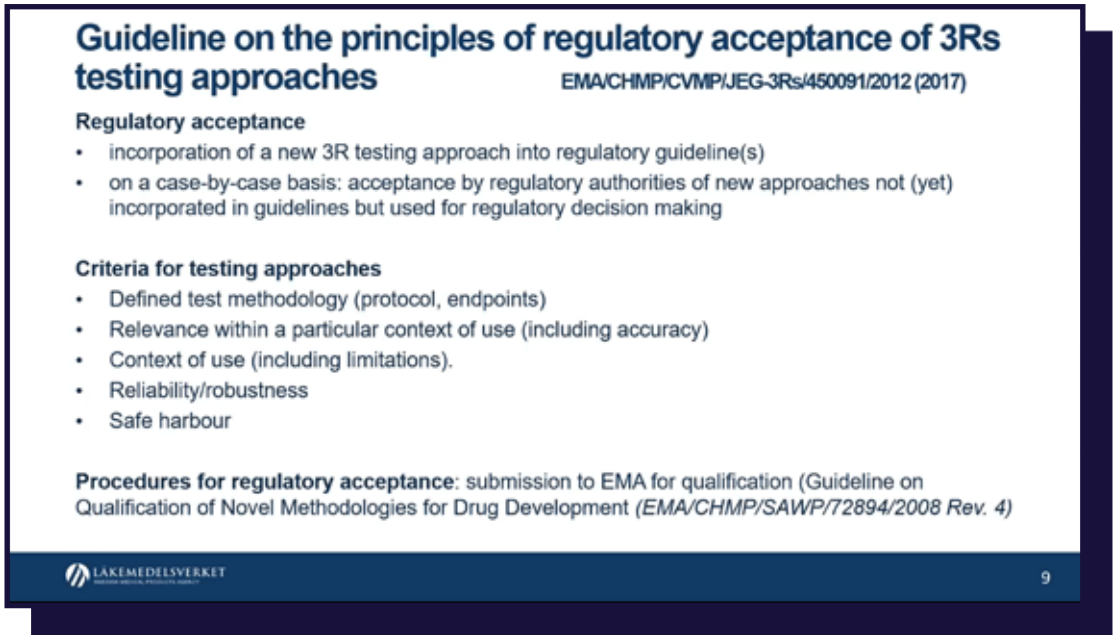
The design also provided the possibility of combining multiple stacks, creating a multi-layered barrier system.

Another technique being used in Maria Tenje's group is two-photon polymerization. This technique is being used to create *in vitro* models, which has the benefit of enabling free-form fabrication, but which require presence of photo-activated initiators.

One way this technique can be used is to ablate materials to create perfusable channels that can be seeded with cells. This method was used in the lab to create a microfluidic vessel model.

This vessel model is constructed as an on-chip system consisting of both GelMA and fibrin gels. The vessel backbone is first created without cells and then the cells can be added. This approach lets the co-cultures be placed in close proximity of each other enabling cell communication, with a barrier that is down to 40 µm thick.

The value for BIRDIE: Barriers play an important role in many organs and tissues, and this is also the case for kidneys. Maria Tenje's work can provide inspiration for how to address design and engineering challenges related to replicating the biological barriers in the kidney in an *in vitro* system.



3Rs in drug development, the regulatory perspective

PRESENTED BY: DR. CAMILLA SVENSSON, SWEDISH MEDICAL PRODUCTS AGENCY / EMA

Dr. Camilla Svensson is Scientific Director in Pharmacology/Toxicology at the Swedish Medical Products Agency and member of the EMA 3R Working Party. Her talk focused on ongoing work among regulatory authorities within 3R and their perspective on new *in vitro* methods.

The goal of medical products agencies across Europe is to ensure the quality, safety and efficacy of medicinal products to safeguard public health, as well as animal health. Another important role is also to support innovation.

Currently 10.6 million animals are used in research and testing. Approximately 17 % of those are for regulatory use. Alternative cell- and in silico-based methods are available, in particular in early drug development. Alternative methods can also be used for better mechanistic interpretation of *in vivo* data.

Regulatory testing is made in a very broad sense because it is not possible to know what the effects of a substance will be and what safety issues will emerge. It is therefore necessary to ensure that the substances has an acceptable safety profile before going into humans.

Regulatory testing needs to provide information on the uptake and distribution of the substance, and to understand if there are metabolites that could be a safety concern. The safety data is supposed to answer what exposure is safe, and what are the potential risks, both of the short term and long-term use. The data should also answer if the effects are reversible and possible to monitor in clinical trial.

Because of the complexity of the knowledge that the regulatory testing needs to provide, the approach from regulatory authorities in this area is mainly to focus on the two Rs Reduce and Refine.

Where new alternative methods have the highest potential for use is within efficacy and dose testing. While this data is needed to go into clinical trial, there is no guidance on exactly what type of requirements that need to be met. Since there are many different diseases and disease models, it is up to the developer to justify the choice of methods used to support efficacy and to show that the methods have acceptable standardization.

Currently, regulatory agencies support 3R by considering alternative methods in guideline revisions and when providing scientific advice. Support for development of new *in vitro* methods is primarily handled on a European level by EMA.

In their **regulatory science strategy document**, EMA specifically points out enforcement of 3R as a priority and has formed a specific group to engage with stakeholders and to review guidelines.

The value for BIRDIE: From the talk provided by Camilla, it is clear that the highest chance of implementation for non-animal research models is within basic research and early-stage drug development. This is also the area with largest use of laboratory animals.

Although broad of *in vitro* models in safety testing is challenging in the near future, alternative methods have a potential use in preclinical efficacy testing. However, additional work needs to be done to achieve sufficient method standardization.

THE CONSORTIUM LEADERS

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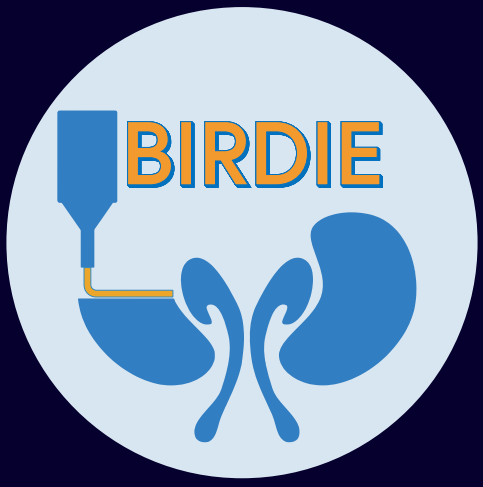

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