THE 3 DIMENSIONS OF ORGAN-ON-A-CHIP

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TNO innovation for life

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ABSTRACT

Even a brief glance at today's scientific literature and popular science magazines is enough to tell you that organ-on-a-chip technology is a bright new prospect. In most, if not all, expressions it not only enables the pharmaceutical industry to tackle the failure of new medicines in clinical trials but also provides an approach that will make animal testing redundant.

Organ-on-a-chip technology holds great promise, but what exactly is it? For one thing, it is more than just a single technology: it is about living cells coming together with supporting technologies to mimic aspects of human physiology and has applications not only for the pharmaceutical industry but also in the fields of diagnostics, food, cosmetics and chemical industry. Human biology, technology and applications – these are the three dimensions of organ-on-a-chip.

This paper will present TNO's perspective on organ-on-a-chip technology and provide some examples of the approach TNO is developing with its partners in order to create new organ-on-a-chip models that resemble specific organ processes and functionalities of human tissue. Our focus is on the liver, the gut and the lungs, a choice determined by our long track record of research related to these specific organ functions. Organ-on-a-chip models will teach us more about why some patients respond to a specific treatment while others do not. They can help in the stratification of patient populations and can contribute to a personalized therapeutic approach. While more closely mimicking physiological responses throughout the human body, organ-on-a-chip technology will also contribute to the reduction of animal testing. In other words, at TNO we seek to apply organ-on-a-chip technology in areas where there are concrete industrial and societal gains to be made.

INTRODUCTION

Preclinical and basic research into biological processes and the development of therapeutic strategies rely primarily on an extensive repertoire of methodologies and models that involve cell culture and animal models[1]. Although these approaches have provided extremely useful insights into physiological and pathophysiological processes in the human body, they are not suited for all relevant research and development questions. This is also demonstrated by the high percentage of compounds that fail during the clinical trial phase of drug development, mainly due to lack of efficacy and the occurrence of adverse effects [2]: ample proof that cell culture and animal models are often difficult to translate to the human situation and highlighting the need for a change in the conventional drug development strategy. This represents a move towards more accurate prediction of safe outcomes in patients, better insights into inter-individual variations, and animal free testing.

Over the past few years, the development of more physiologically relevant human-cell-based *in vitro* models has evolved[3,4]. These so-called organ-on-a-chip models are designed to outperform conventional cell-based models in mimicking tissue functions and architecture, for instance by using microfluidics to mimic blood flow and allow a continuous flow of nutrients and the excretion of breakdown products. With these models, it will be possible to study biological mechanisms more effectively and to more accurately predict pharmacological effects in humans. Organ-on-a-chip technology provides a promising approach to solving translational issues that are evident, not only in the pharmaceutical industry, but also in the nutritional, diagnostic, cosmetic, chemical and environmental industries[5]. The ultimate goal of organ-on-a-chip models is to mimic human physiology (and pathophysiology) within an *in vitro* system, ideally with simple readouts. Science as a whole, and drug development in particular, may greatly benefit from human functional organ-on-a-chip technologies, not only in terms of reliability of results but also in terms of lower costs and giving patients quicker access to new medicines.

While we are able to study physiological mechanisms in organ-on-a-chip systems and to predict pharmacological effects in humans, it is important to remember that a cell-based model cannot represent an entire organism. However, human stem-cell-based organ-on-a-chip systems do allow us to investigate the various critical biological pathways. By physically combining various organ-on-a-chip models (representing distinct organs) or by combining the results from various organ-on-a-chip models with computer-based *in vitro – in vivo* extrapolation, it may also be possible to more closely mimic whole human body responses. By providing a better selection of compounds to be tested in humans, these organ-on-a-chip models contribute to the reduction of animal testing in the development of therapies, for example.

BIOLOGY MEETS TECHNOLOGY – THE FIRST AND SECOND DIMENSIONS

Recent developments in multiple scientific fields, including stem cells, material sciences, micro engineering and microfluidics, have led to the integration of advanced humanized *in vitro* cell culture models. These organ-on-a-chip developments resemble the architectural tissue arrangements and biological complexity of living organs using human cells, including stem cells, cultured on microfluidic chips. This is a very promising new technology, as it potentially permits the study of diverse human biological processes that were impossible to study using conventional *in vitro* platforms and *in vivo* models. However, a number of obstacles exist with regard to the acceptance of these humanized *in vitro* models. These issues and examples of organ-on-a-chip models have been extensively reviewed in recent publications[6-10].

At an international level, organ-on-a-chip technology is attracting increasing attention from both technology-oriented parties and potential users of the technology, such as pharmaceutical companies. Although numerous models are being developed by universities and research institutes, only about 10 companies worldwide are commercially developing organ-on-a-chip systems for industrial purposes. To date, the models have been focusing on applications within acute toxicology, since short-term exposure and relatively simple endpoints are sufficient in these types of studies. The challenge is to take the models to the next level: organ-on-a-chip for human diseases, long-term exposure and patient-derived stem cells, providing an unique opportunity to discover personalized human drug targets, related to the underlying genetic background of the patient and geared towards the testing and selection of specifically designed medicines (Figure 1).

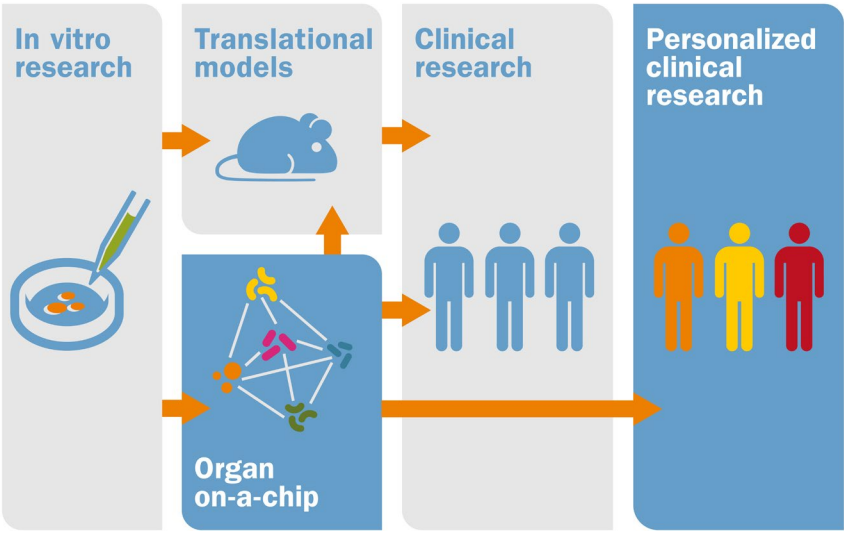

Figure 1. ***The potential of organ-on-a-chip****. The conventional drug development pathway involves preclinical* in vitro *research, preclinical* in vivo *research in animal models, followed by various phases of clinical trial studies in humans. Organ-on-a-chip technology has the potential to make animals studies redundant for certain specific research questions prior to clinical testing. By including patient-derived stem cells, the promise of stratification takes organ-on-a-chip technology to an entirely new level of personalized clinical research, which has a huge potential for disrupting the conventional drug development pipelines.*

ORGAN FUNCTION-ON-A-CHIP

The researchers and scientists at TNO have a broad background, ranging from biology to engineering and from psychology to economics. The combination of their knowledge and expertise, together with their collaborating partners in international industries, the health sector, research institutes, universities, government organizations and regulatory bodies, enables TNO to develop cutting-edge technologies and applications such as organ-on-a-chip.

The latest developments in the organ-on-a-chip models and technologies are very promising, but caution is advised. Some of the organ-on-a-chip models currently under development attempt to mimic entire organs or even humans-on-a-chip. While endeavouring to do so is a perfectly valid goal, it is important to ask what purpose this serves. A more relevant approach would be to mimic specific human organ functionalities and mechanisms in order to gain a clear understanding of physiological pathways and diseases, and how these can be affected by therapeutics or other compounds. This is why TNO prefers to call these technologies: organ **function**-on-a-chip.

TNO's approach is to identify specific biological processes relevant to the required application of the model and then to mimic these as optimally as possible in the organ function-on-a-chip system, using technologies such as scaffolds/biomaterials, fluidics, microfluidics and imaging. This approach not only provides focus, but also ensures the development of models with a clear application and relevant outcomes for decision making. By first focusing on the research application and developing our approach in line with user requirements with as much complexity as necessary, we can ensure proper utilization of resources and make realistic promises (Figure 2).

Instead of building a human on a chip, we combine computer modelling of the functional readouts from the various organ function-on-a-chip systems with knowledge gleaned from previous animal studies and clinical trials to offer unique perspectives on predicting full human body response.

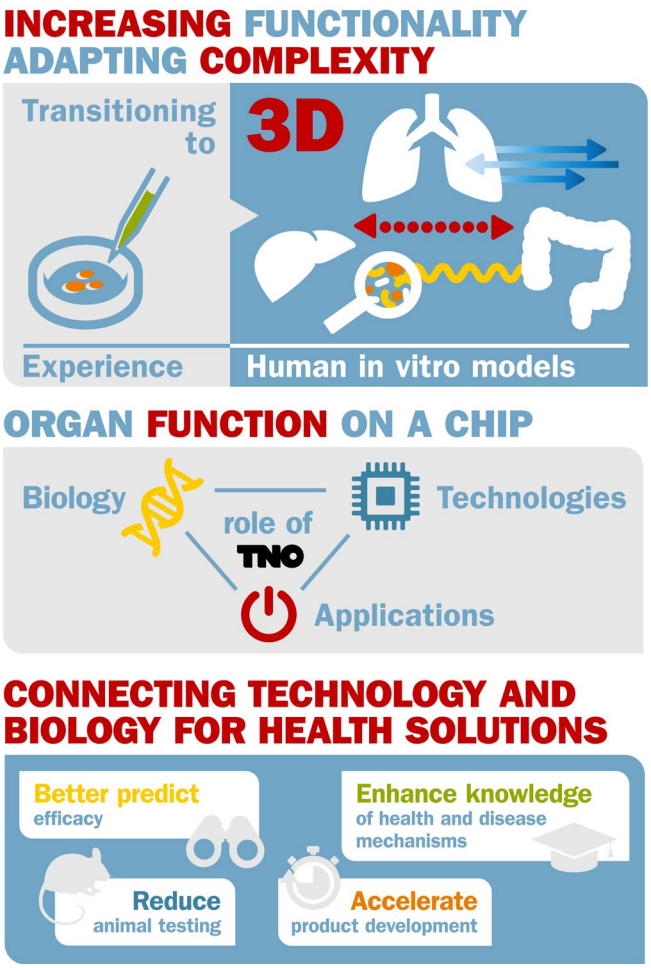

Figure 2. ***The role of TNO****: connecting innovative technologies with biology to create applications for health solutions.*

EXAMPLES OF TNO APPLICATIONS – THE THIRD DIMENSION

TNO's organ function-on-a-chip programme bridges technology and biology for health solutions. To achieve this, TNO collaborates with various partners who contribute their knowledge, expertise, materials and technologies to develop organ function-on-a-chip models. Would you like to find out more?
See TNO's organ function-on-a-chip website.

In its many years of preclinical and early clinical research, TNO has built an impressive track record in developing *in vitro* and *in vivo* models for toxicity, kinetics and efficacy, with a strong focus on specific organs: the liver, the gut and the lungs. TNO has also decided to focus on these organs for the development of its organ function-on-a-chip models.

LIVER FUNCTION-ON-A-CHIP

Obesity is associated with a spectrum of liver abnormalities, known as nonalcoholic fatty liver disease (NAFLD), characterized by an increase in triglyceride content (steatosis) and inflammation, leading to nonalcoholic steatohepatitis (NASH) and subsequent fibrosis that can ultimately lead to cirrhosis and

hepatocellular carcinoma. As a direct result of the obesity epidemic, NAFLD has become a major public health problem because of its high prevalence, potential progression to severe liver disease, and association with serious cardio-metabolic abnormalities.

Various animal models are available for the preclinical testing of new potential medicines for treating NASH. Some are more promising than others, but all have translational limitations. Many *in vitro* liver cell systems also show limited translational potential, due to the use of simplified single cell systems and induction methods. For liver culture systems, hepatocytes are the most frequently used cell types. A main disadvantage, especially in the application for liver disease testing, is dedifferentiation of hepatocytes in prolonged cell culture. Our initial results indicate that many of these disadvantages can be overcome by using human primary cells, a 3D-culturing system based on multi-spheroid scaffolds and disease-mimicking conditions. In the longer term, the use of cells (hepatocytes) from patient-derived stem cells (iPSCs) can open the door to a personalized cell system.

The goal is to combine innovations in the field of pluripotent stem cells (iPSC)and 3D-cell culture (scaffolds) with our extensive knowledge of liver disease pathways[11], resulting in a predictive *in vitro* NASH/fibrosis model. This advanced model will be defined by a co-culture of human hepatocytes and stellate cells on an innovative 3D-cell culture scaffold. The model will be applied to studying the effect of interventions on disease development, prevention and treatment and will open up possibilities for an *in vitro*-based precision medicine approach.

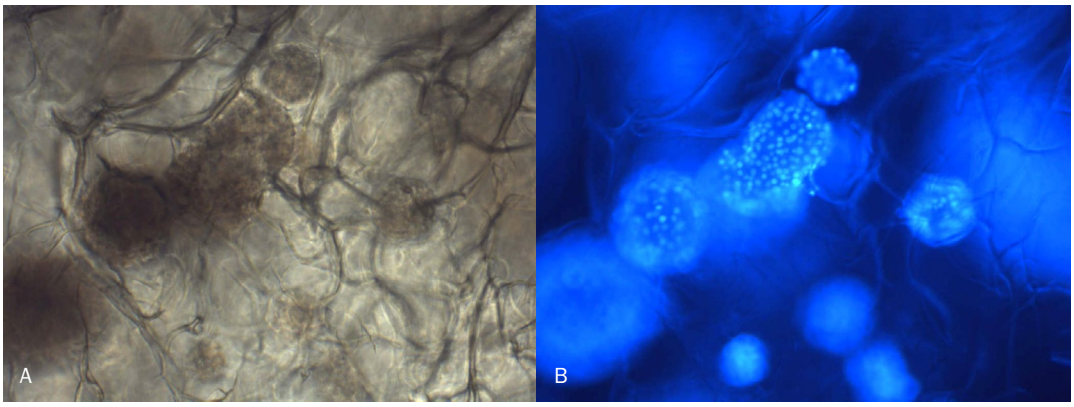

Figure 3. Spheroids of a co-culture of human hepatocytes and stellate cells in a Cellusponge scaffold. A: Bright field; B: DAPI stain.

GUT FUNCTION-ON-A-CHIP

The gastrointestinal tract plays a crucial role in providing a barrier between the luminal environment and the systemic circulation. This barrier is not only formed by epithelial cells that are closely linked by tight junctions, but also by an active mucus layer on top of the epithelial cells and by intraepithelial lymphocytes in the lamina propria underlying the epithelium, which actively extend sensory processes (dendrites) into the lumen. The challenge for the GI tract is to allow the efficient transport of beneficial nutrients and molecules (drugs) across the epithelium, while excluding harmful molecules and bacteria. Since the GI tract contains the largest reservoir of commensal bacteria in the human body, homeostasis in gut microbiota plays a crucial role in barrier functioning and in health and disease.

There are currently no *in vitro* models available to study these complex interactions. Cell lines, such as Caco-2 or HT-29 cell lines, only represent one cell type of the gut epithelium and are not well suited to investigating the various processes that determine oral bioavailability, such as intestinal metabolism or regional differences in absorption. Nor are they suited to studying the effect of food and pharma compounds on gut health, including host-microbe and immune responses. TNO, in collaboration with other research institutes and industry, therefore aims to develop a translational *in vitro* model of the human gut, representing the full range of epithelial cells in co-culture with human microbiota and immune cells[12]. For this purpose, stem cells will be isolated from crypts of human intestinal tissue, and cultured in a 3D context to form intestinal organoids. These 3D intestinal organoids exhibit a self-renewing capacity and express various important intestinal epithelial cell types, resembling the intestinal epithelium. In order to apply these intestinal organoids in the TNO gut-on-a-chip model to study intestinal absorption and gut barrier functions, they are cultured as a monolayer on permeable membranes. In order to closely mimic GI physiology, the model is subsequently combined with microfluidics (apical anaerobic and basolateral aerobic) and co-cultured with (pooled) human microbiota to study host-microbe interactions.

TNO believes that a range of gut-on-chip models are needed to study the different regions of the GI tract (duodenum, jejunum, ileum and colon) and for various diseased states (e.g. IBD, Crohn's disease), each with their specific physiological conditions and microbiota. Using intestinal organoids from distinct GI regions and/or from different patients is the first step towards achieving this. Importantly, using co-culture with personalized microbiota from individual patients (which can be derived from stool samples), this gut function-on-a-chip model can be applied to the study of individualized drug and food interventions in intestinal absorption and gut barrier functions.

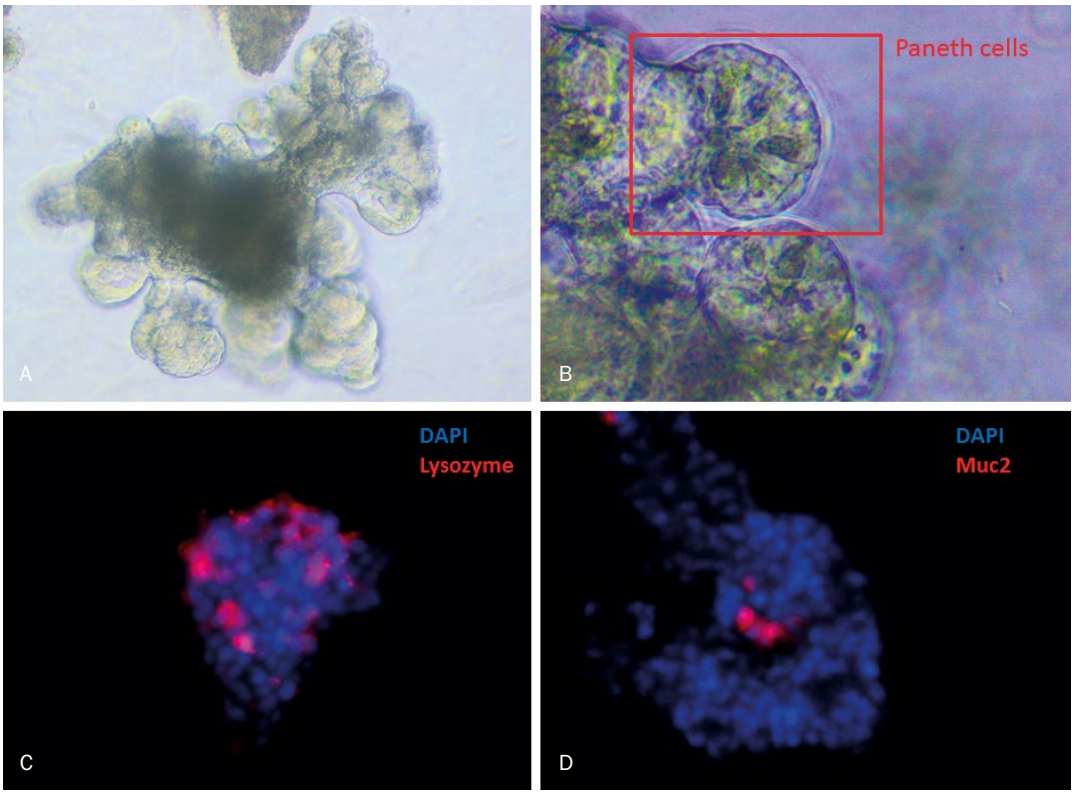

Figure 4. Characterization of intestinal organoids using light microscopy at 20× (A) and 40× (B) magnification. Red squares indicate the presence of Paneth cells filled with granules. Immunofluorescent staining for Paneth cell-specific marker lysozyme (C), and goblet-cell-specific marker Muc2 (D).

LUNG FUNCTION-ON-A-CHIP

The inhalation route is one of the main ways in which people are exposed to known and unknown compounds, such as complex environmental pollutants (traffic emissions, work place emissions, military exposures) and biological infectious agents. For the pharmaceutical industry, the inhalation route is important for administering drugs with a local effect (treatment of asthma, COPD, etc.) and there is a growing interest in using inhalation as an alternative route for administering drugs intended for systemic treatment.

Preventing respiratory diseases is critical for the general population and economic health. Given that many respiratory diseases are preventable to some degree (i.e. risk factors are related to human activities), it is vital to have procedures that enable accurate testing and the prediction of biological effects of airborne materials.

TNO believes that validated *in vitro* methods to assess the potentially adverse health effects and efficacy of aerosols are needed, as animal studies often fail to provide sufficient models for predicting human airway toxicity. These methods include:

1. improved cell models such as co-cultures of relevant primary human cells;
2. air-liquid interface exposure modules; and
3. aerosol generation technologies.

The epithelial cells that line the airways and alveoli are the first to be exposed to inhaled substances[13]. These cells are therefore the main focus of our cell culture models for inhalation toxicology and the testing of inhaled medications. Two-dimensional cell lines were initially used for this purpose but now a great step forward has been made with the development of more complex three-dimensional models[14], including an air-liquid interface in which air flows over the lung epithelial cells and fluid flows over the endothelial cells, and cells are stretched mechanically to mimic breathing.

As with the gut function-on-a-chip model, TNO aims to develop a translational lung function-on-a-chip model that makes use of human lung cells and stem cells derived from healthy or diseased persons. The inclusion of a human microbiome will make it possible to investigate how different microbiomes of various compositions can affect the epithelial layer's susceptibility to a range of compounds.

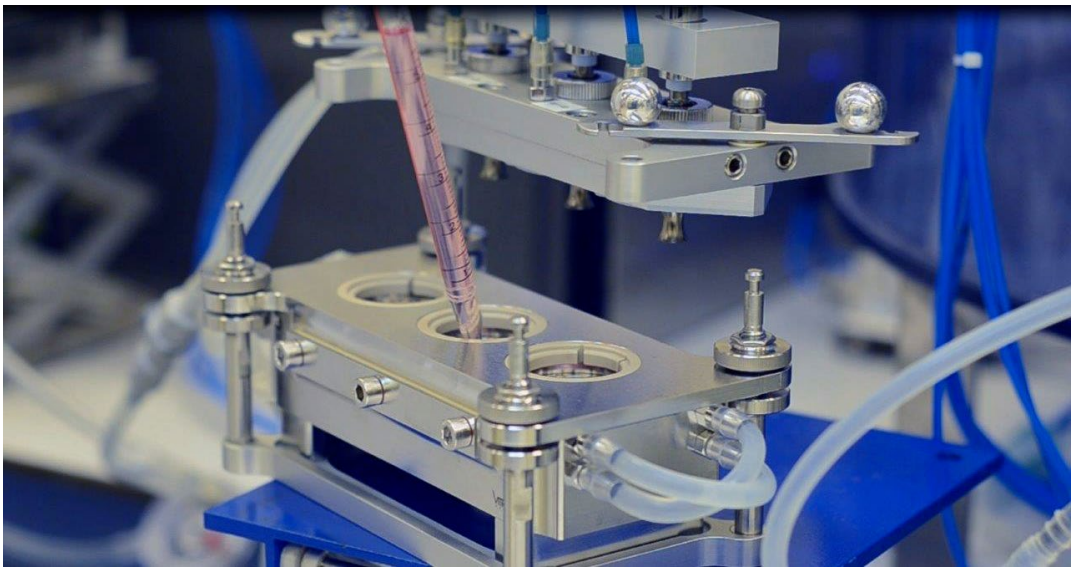

Figure 5. In vitro *inhalation model consisting of an air exposure module, equipped for three inserts containing 3D airway cell systems at the air-liquid interface. Courtesy of Triskelion B.V., Zeist.*

CONCLUSION

Organ-on-a-chip technology holds great promise and offers the prospect of a wide range of applications in a variety of industries. The most striking application is the pharmaceutical industry where there is a high demand for better predictive and translational models to study human physiological and pathophysiological processes related to toxicity, DMPK and the efficacy of new therapeutics. However, it is too early to conclude that organ-on-a-chip technologies will soon fulfil the promise of preventing late-phase clinical trial failures of new therapeutics or will succeed in making animal testing a thing of the past.

If this promising technological advance is to achieve its full potential, biologists and engineers must continue to collaborate on building models and on gaining a better understanding of what the results from these models have to say about the processes we can expect to find in humans. Bringing organ-on-a-chip models closer to practical applications also requires the involvement of the other stakeholders such as the end-users of these technologies, patient organizations, health insurers and regulatory bodies. TNO, as an internationally renowned independent research institute, has a crucial role to play in bringing these various stakeholders together. Their joint efforts can identify the fields where organ-on-a-chip technologies will be of added value, and the technological advances needed to predict how a new therapeutic will behave in humans with respect to efficacy, kinetics and/or toxicity.

As our biological/mechanistic understanding increases and better readouts are obtained, we foresee that organ-on-a-chip will find its place in fundamental and applied research, gradually ousting the existing experimental approaches, including laboratory animal research.

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