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Editorial overview: Disrupting the animal test with *in vitro* innovations

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Animal testing is the current gold standard for preclinical assessment of pharmaceuticals and regulatory toxicity testing of chemicals, but the low predictivity for effects in human patients has adversely affected the availability of much-needed disease treatments. At the same time, the increasing pollution of our planet with chemicals that are insufficiently regulated is threatening vulnerable ecosystems and is compromising the safety of our environment. If we want to achieve a sustainable, healthy environment, and sufficient availability of safe treatment options for our patients, it would be highly beneficial to develop human-relevant, better predictive test methods to assess pharmaceutical efficacy/safety and chemical toxicity.

The key to open the door to more predictive test models is human relevance; there simply are too many differences between the human patient and the laboratory animal to use the latter to accurately predict effects in the former. Of course, to replace something that falls short of expectations, we need to build something superior. A one-on-one replacement for animal models that can answer the big questions such as 'is this pharmaceutical/chemical safe?' at least as well as the animal model (or a combination of at least two species, as is common in drug testing) is not available; what we do have is a collection of advanced *in vitro* technologies and models with great potential that can be used to answer more specific questions. It should be emphasized here that is unlikely that the animal test will be replaced in the short term by a single *in vitro* model that can be used to answer all the relevant major questions, i.e. a 'human-on-a-chip.' It is much more likely that initially there will be models answering more specific questions, such as 'does this pharmaceutical/chemical adversely affect kidney function?' Validation of such models will be performed using a fit-for-purpose approach: the model should provide sensitive, robust, and predictive results for a certain purpose rather than for all possible purposes for which animals are currently used.

When we would have such models for the main organ toxicities observed in clinical studies (or after introduction of a medicine onto the market), such as the liver (most commonly responsible for drug withdrawal from the

market; e.g. the study reported by Patel et al. [1]), the kidney (nephrotoxicity is observed after treatment with chemotherapeutic agents or antibiotics; drug-induced nephrotoxicity accounts for around 18–27% of hospitalizations owing to acute kidney injury, [2]) and the heart (chemotherapy is a well-known cause, e.g. the study reported by Pai et al. [3]), this would be very useful for preclinical safety testing; of these, the liver is of particular interest because it is the primary site of metabolism, that is, most other target organs will be exposed to metabolites instead of the parent compound. If we want to study the absorption, distribution, metabolism, and excretion (ADME) of compounds and the related concept of drug metabolism and pharmacokinetics (DMPK), another relevant preclinical question for which animals are used integrating these single organ models in a multiorgan model where cells are grown into a barrier (e.g. intestine, kidney, liver) would be of great interest.

The development of innovative *in vitro* models has reached a stage where high-potential technologies have been developed that can be used to build advanced, more predictive *in vitro* systems. We have seen the arise of organoid technology, capable of growing mini-organs from human-derived cells with a three-dimensional structure and organ-like functionality that goes far beyond classical monolayer cell culture. Another major innovation is represented by organ-on-chip technology, with advanced microfluidic connections that can provide the ideal amount of shear stress for a certain cell type, resulting in superior differentiation and cellular functionality and a more realistic administration of a test compound of interest. State-of-the-art regenerative medicine has delivered advanced three-dimensional bioprinting technologies with which multiple cell types and matrix materials can be printed simultaneously in shapes similar to the structures that make up organs. Promising work has been performed bridging the gap between high-fidelity and high-throughput, which used to be mutually exclusive; multiwell plates with organ-on-chip technology are now available, which is an asset in drug target identification (a common application of *in vitro* methods in the pharmaceutical industry). Scientists have developed very small sensors with which functional and viability-related endpoints can be measured in real-time (viability, shear stress, barrier integrity, oxygen consumption, and so on), generating large amounts of temporal data without the need to harvest cells (i.e. killing them). Major steps have been made toward automation and robotization of cell culture methods, resulting in far higher throughput, better standardization, and lower risk of contamination. Another landmark innovation is the rise of artificial intelligence; in particular, machine learning will be very useful to, for instance, identify the combination of readouts and/or cellular models that is most predictive for a certain human endpoint. Meanwhile, adaptation of the systematic review methodology, that is, common in clinical science, to preclinical data (e.g. from *in vitro* models) can provide verifiable, transparent, and complete information on the predictivity of innovative *in vitro* models (e.g. studies reported by Leenaars et al. [4] and de Vries et al. [5]). Although time-consuming, this has clear advantages.

If we want to achieve the next level of *in vitro* innovation by building test systems that can replace animal testing to a significant degree, we will need extensive interdisciplinary collaborations to integrate such technologies, all of which require specific expertise. One can compare it to building a house, which is more than the sum of its components. Simply stacking bricks, isolation material, electrical cables, plumbing tubes, sheets of glass, doors, locks, and advanced equipment such as a heat pump and a mechanical ventilation unit together does not result in a house. To build a home that meets the high present-day demands in terms of energy efficiency, comfort, and practicality, experts with different specializations are essential. Their knowledge and skills need to be combined in a closely coordinated construction process; the plumber should not start his work when everyone else is ready, and the house is plastered, painted, and looks ready to be moved into.

We should try to achieve the same synergy for innovative *in vitro* models. Major collaborative efforts will be needed to get the most out of the extremely promising technologies that are already available. All these components need to be connected during a process in which each other's advantages and limitations are carefully considered in a mutual way; for instance, cell biologists, information technology experts, material scientists, physicists specialized in fluid dynamics and modeling, regenerative medicine experts need to enter each other's world to understand how synergy can be achieved. The input from regulatory experts early in development will be essential to facilitate the validation and implementation of such methods later in time.

In the current issue, overviews of the state of the-art of several *in vitro* innovations are presented, focusing on models for the main organs involved in pharmacokinetics: the intestine; the kidney; and the liver. Of course, these organs are also relevant for oral exposure to chemicals or food components and food additives/nutraceuticals. The presented *in vitro* models are also of value to assess toxicity in their specific organs; it is worth mentioning here that hepatotoxicity and nephrotoxicity are important and common reasons for drug attrition during pharmaceutical development. The first

set of reviews will present the state of the-art regarding liver models (Vinken), intestinal models (Donkers, Eslami Amirabadi, and van de Steeg), and kidney models (Vriend, Pye, and Brown). A systematic review is included that elegantly compared innovative and classic *in vitro* models for drug-induced kidney injury (Irvine, van Berlo, Shekhani, and Masereeuw). An innovative high-throughput method to assess nephrotoxicity is presented as well, showing a successful attempt at upscaling of an advanced microfluidic *in vitro* model (Vriend et al.). The challenging topic of *in vitro* models aimed at the assessment of absorption, distribution, metabolism, and excretion- drug metabolism and pharmacokinetics (ADME-DMPK) is tackled in a review of multi-organ-on-a-chip models (Van Berlo, van de Steeg, Eslami Amirabadi, and Masereeuw). Finally, an overview of the application of advanced *in vitro* models for personalized medicine, focusing on stem cells, organoids, and organ-on-chip approaches is included (Van Berlo et al.).

In vitro innovations such as those presented in the current issue are valuable assets for the development of better test strategies; we may never be able to predict what will happen to us and our world, but for safety and efficacy, we have the tools and the combined knowledge to construct something better. The building process may require an unprecedented level of collaboration between experts from different disciplines; building blocks must be developed and interconnected with mutual understanding of each other's possibilities and limitations.

Declaration of competing interest

The authors declare no conflict of interest.

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