SLAS Meet Up Suisse Romande 2022

"Mind the Gap" Between Pre-clinical and Clinical Assessment

The Need for More Physiologically Relevant Models

Authors: Leïla Munaretto(SUN bioscience), Maria Clapés Cabrer (SUN bioscience).
Contributions from: Anna Goralczyk (Philip Morris International), Christian Parker (Novartis), Julio Martin (SLAS).

The development of new therapeutics is a long and costly process. From the early drug discovery phase to clinical trials, potential drug candidates are thoroughly tested and optimized. Starting with thousands of compounds during the drug discovery phase, only a few are selected to enter the preclinical phase. At this stage, drug candidates are thoroughly tested both *in vitro* and *in vivo*, employing a variety of models. This stage is crucial to evaluate the efficacy and toxicity of a given compound and assess its safety before it is tested in human subject. Very few of these drug candidates progress from preclinical to clinical settings, where an alarming 90% would then fail during the different phases of the clinical trials[1]. The entire procedure is lengthy and costly, taking up to 10-15 years before a single drug is finally approved by regulatory agencies and the estimated cost per newly developed compound hovering between $1-2 billion[1]. Reducing the attrition rate for potential drugs appears therefore crucial to decrease cost and time associated with drug development.

In this context, the standard *in vivo* animal models used in preclinical testing often face criticism for their relevance to human biology. Translating findings from animal studies to humans is far from straightforward, and this likely accounts for why fewer than 10% of drug candidates succeed in traversing all phases of clinical trials. Beyond scientific considerations, animal experimentation also triggers strong ethical concerns in our modern society. It's noteworthy that some industries, like cosmetics, have already entirely banned animal testing, reflecting a societal shift towards more humane research methods.

Thus, to decrease the drug attrition rate and to reduce the use of animal experimentation, the development of more complex test systems has become increasingly imperative. These include multicellular models, three-dimensional cultures, and organ-on-a-chip systems, generally known as complex *in vitro* models (CIVMs). The increased complexity aims at better emulating the functionality of human tissues and organs, while enabling the evaluation of more physiologically relevant endpoints. CIVMs hold substantial potential for a better translation from preclinical to clinical settings in the drug development pipeline. However, these new models have brought forth several challenges concerning their ultimate application in the industry. Among the most significant hurdles is building confidence in these new *in vitro* models for their widespread acceptance and use. Scientists must be confident that a given model accurately mirrors the *in vivo* situation and can satisfactorily address their research question. This necessitates comparing *in vitro* data with relevant clinical data, which are often not readily available. Therefore, while CIVMs hold immense promise, they also present unique challenges that the scientific community needs to address in the pursuit of more humane and relevant drug development methodologies.

The SLAS (Society for Laboratory Automation and Screening) meet-up Suisse Romande 2022, held on September 6th, 2022, and hosted by SUN bioscience in Lausanne, meant to address key questions around newly developed complex *in vitro* models. The event gathered excellent speakers: Nikolce Gjorevski (Organoid Engineering group Lead

at Roche Institute for Translational Bioengineering), Nathalie Brandenberg (co-CEO of SUN bioscience), Els Adriaens (consultant in statistical data analysis and CEO of Adriaens Consulting bvba) and Annie Moisan (HOPE Program Director at Wellcome Leap). The debate, chaired by Jan Lichtenberg (CEO of InSphero, SLAS Board Member), was centered around the use of CIVMs to reduce the gap between pre-clinical and clinical assessment. The panelists addressed the recent advances in CIVMs from a technical and biological perspective, challenges for their final application in industry and contextualized their use from a European regulatory perspective, both in the pre-clinical and clinical setting. The following sections summarize the key-takeaways of the event.

What is complexity?

When talking about CIVMs, one must start by defining what is a complex model and where does the complexity arise. For all speakers, a complex model represents an *in vitro* model that shows superior translational results compared to a cell line. Complexity can be built from various key characteristics of the model with the aim to better recapitulate the *in vivo* situation. For scientist Nikolce Gjorevski, multicellularity, 3D architecture and functionality appear to be the three fundamental characteristics of any living tissue that should be at least partially represented in new CIVMs.

One can therefore enhance the complexity of a model by introducing multiple cell types in a co-culture system or by shifting from 2D culture to 3D culture. Here, it's crucial to acknowledge that the shift from 2D to 3D cultures was not a recent development. In fact, one of the earliest successful examples of 3D culture, though in animal cells, was performed by Landry et al. in 1985, where they cultivated rat liver cells in a spheroidal aggregate culture[2]. For human cells, the first example was provided by Görlach et al. in 1993, demonstrating the capabilities of 3D cultures in human hepatoma cells[3].

However, a significant milestone in the advancement of 3D cultures was achieved by Sato *et al.* in 2009 when they reported that mouse intestinal stem cells, in the right 3D culture conditions, can self-organize and generate a structure with a crypt-villus architecture that recapitulates the cell types of the native tissue[4]. Two years later, they also provided a protocol to culture human intestinal organoids from adult tissue[5].

Since then, the work with organoids has expanded rapidly, with a multitude of models being established. However, the lack of standardization and the extensive need for human handling in these culture systems has been a hindrance to their routine use on an industrial scale. In response to this challenge, a considerable amount of work has been devoted to developing organoid models that enable more standardized and automatable workflows.

The development of Gri3D®, a product of SUN bioscience, falls precisely in line with this. As Nathalie Brandenberg explained, this platform enables high-throughput organoid cultures in a standardized and automatable manner. As an example, thousands of patient-derived colorectal cancer organoids were successfully grown and screened for anticancer drug candidates using Gri3D®. High-content image-based phenotypic analyses were then applied to reveal insights into the mechanisms of action of the drugs[6].

Alternatively, the introduction of functionality, as in organ-on-chips devices, is also another way to increase the complexity of a given model. By combining microfluidic

engineering and cell culture, this technology recapitulates flows or mechanical stresses found within the organ, resulting in a more physiological tissue *in vitro*. Annie Moisan and Nikolce Gjorevski are both experienced researchers who pioneered the use of organ-on-chip technologies for drug efficacy and safety profiling. Examples of their work include lung and gut-on-chip models, that have been used to assess the on-target, off-tumor, toxicity of immunotherapies[7], study auto-immune pathologies[8] or assess the epithelial barrier integrity of perfused intestinal tubes in real time[9]. These "organ-on-a-chip" devices appear as potential complex models for drug screening and toxicology studies and provide a low-cost alternative to animal and clinical studies.

The double-edge sword: complexity vs confidence

Increasing the complexity of an *in vitro* model allows to better recapitulate the physiological state of a given tissue. However, the speakers agree that CIVMs are not necessarily better than simpler ones, as complexity comes with more variability and cost. Indeed, with more complex models come often more intricate readouts and even more challenging data analysis. In this double-edged sword situation, the best choice goes for the simplest model sufficient to answer the scientific question with enough confidence of its biological relevance.

Building confidence in CIVMs is a significant challenge and requires careful benchmarking to ensure the model truly mimics the physiological function of the tissue *in vivo*. Nikolce and Nathalie do so by comparing *in vitro* data from the CIVM with relevant *in vivo* data and making sure that the research question can be answered confidently with the CIVM. For instance, they would check the expression of the drug target before testing the drug in the model. However, Annie Moisan notes that in the case of human models, *in vivo* data are obtained via clinical studies and are often not widely available. A good strategy to increase the certainty, as well as speed up development, would be to jointly run *in vitro* studies with *in vivo* studies to generate comparable data.

According to statistician Els Adriaens, larger sample sizes significantly bolster confidence in the results. Nevertheless, she emphasizes that the results are limited to the population tested, taking into account variability between gender, ethnicity, and age. Hence, it's essential to always consider the population under study. Additionally, given that most of the available data currently originate from animal studies, *in vitro* animal data should initially be compared to *in vivo* animal data. Subsequently, human *in vitro* data should be compared with human *in vivo* outcomes, i.e., clinical data.

In summary, while the development of CIVMs relies heavily on animal studies and these models have become instrumental tools in reducing the use of animal experimentation, they haven't fully replaced it yet. However, they are certainly propelling us towards a future where more humane and relevant research models can be the standard.

A new era: inclusion of the CIVMs in human drug regulation processes

From a regulatory perspective, the use of human CIVMs in industry and drug development is not a widespread practice yet. The majority of regulatory authorities heavily depend on data derived from animal studies for the approval and regulation of

human drugs. Nonetheless, the imperative to reduce drug attrition rates while minimizing animal experimentation has encouraged these authorities to regard CIVMs as a viable source of data.

A significant step forward in this direction is the recent acceptance of the FDA Modernization Act 2.0 in the USA. The revised section 550 of the Federal Food, Drug, and Cosmetic Act (FDCA) now permits human biology-based test methods — not solely those performed on animal models — to be considered for human drug approval. This category includes cell-based assays, microphysiological systems, and bioprinted models. The inclusion of CIVMs in the regulatory processes for human drugs marks a pivotal progression towards the development of alternatives to animal testing. This could potentially usher in an era of more efficient, economical, and ethical drug development.

However, for researchers, the regulation and validation of CIVMs themselves still pose considerable challenges and require extensive time. The files submitted to regulatory affairs are currently not public, meaning that researchers lack access to previously rejected submissions and the reasons behind these rejections. Moreover, CIVMs used within pharmaceutical companies are often tested only for internal validation, without the results being publicly shared. This situation underscores the importance of associations that promote data transparency and sharing among members, thereby fostering confidence in alternative methods.

Conclusion

The potential of CIVMs in the field of drug discovery and development cannot be understated. These advanced models offer an opportunity to "mind the gap" between pre-clinical and clinical assessment, offering a platform that more accurately mimics human biology and significantly reduces our reliance on animal testing.

Despite the inherent challenges such as cost, variability, and validation, there's a notable shift in the regulatory landscape that is supportive of CIVMs, as demonstrated by the recent FDA Modernization Act 2.0.

However, widespread acceptance and use of CIVMs require ongoing collaboration, data transparency, and knowledge sharing within the scientific community. It is through such collective efforts that we can overcome current limitations and move closer to fully realizing the benefits of these models in drug development.

In closing, the road towards incorporating complex *in vitro* models into drug testing may be long and challenging, but the promise they hold for improving our approaches to drug development is compelling. As the scientific community continues to innovate and collaborate, the future of CIVMs in drug discovery and development is indeed promising.

References

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