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Translational *in vitro* research: integrating 3D drug discovery and development processes into the drug development pipeline

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As we witness steady progress towards the development of robust, scalable, and reproducible 3D tissue models for preclinical drug testing, there is a need for systematic physiological and pharmacological validation and benchmarking. Ongoing and future studies should generate evidence as to whether 3D tissue models are more predictive, help reduce the risk of failure rate, and can be used for decision making in the drug discovery and development pipeline. Here, we discuss the importance of harmonizing the validation of these models based on throughput capacity and physiological complexity as a requirement to establish their true translational capacity. We also outline our strategy for a novel 3D-tailored holistic drug discovery concept rather than piecemeal integration of 3D models into the current process.

Introduction

The failure rate from investigational new drug (IND) submission to drug approval has remained stubbornly close to 90% over the past decades [1]. This high failure rate has led the drug industry to a difficult situation of very low return on investment, and has pushed towards the pursuit of breakthrough innovative solutions to identify new clinically relevant targets and improve the clinical predictability of the drug discovery and development process [2]. For more than 50 years, the pharmaceutical and biotechnology industries have been incorporating major technological advances at the genetic, proteomic, and phenotypic levels to discover new disease targets and develop the next generation of disease interventions. However, despite the many impressive biotechnological advances, there have not been any major advances in the average success rates for the identification of new drug targets and the development of new drug entities. More recently, disruptive therapeutic modalities, such as gene therapy, cell-based therapies, and tissue regeneration, have emerged as the new therapies of the future. Regardless of the therapeutic modality to be developed, the need for disease-relevant *in vitro* models that are predictive with respect to efficacy and toxicity in humans will remain critical [3,4]. The hope is that these models will be at least as predictive as, if not more than, the current animal models, thus at least reducing the amount of animal testing in the shorter term, and hopefully eventually eliminating it. Increasing the throughput of testing clinical candidates with these alternative advanced cell models will also make choosing the best therapeutic entity for clinical development more efficient and cost-effective.

3D tissue models of different degrees of complexity have been developed for decades now and have been postulated to become the new dimension in biology, changing the way in which *in vitro* research is being done [5]. However, the integration of these advanced *in vitro*

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GLOSSARY

3D model validation tissue- and/or organ- and disease-specific processes to assess how closely the in vitro model reflects in vivo biology and disease progression.
3D^3 process a drug discovery and development process based on synergistically integrated 3D models for the efficacy, safety, and pharmacokinetic assessment of drugs.
Application describes the kind and/or type of biological information that is generated using a respective tissue, organ, or disease model.
Microphysiological system connects at least two different mammalian tissues or organs with a fluidic bridge. The whole system operates under flow conditions.
Physiological assay incorporates aspects that are driven by translational characteristics into drug testing in animals or patients, such as dosing concentrations and schedule, treatment time, endpoints, and the tissue model.
Physiologically disease-relevant assays comprises a tissue disease model that reflects an in vivo-like disease progression applying disease-relevant endpoints.
Translational capacity/value describes how closely a cellular in vitro model reflects in vivo biology with respect to: (i) morphology and cell composition; (ii) physiology; (iii) pharmacology; and (iv) tissue-specific pathways (Fig. 2 in the main text). Preferably, the validation aspects should be computed in such a way to generate quantifiable classification numbers to define how close a tissue, organ, or disease model reflects the in vivo physiology (quantitative-equivalency analysis).

3D tissue models into the drug discovery and development pipeline has taken longer than anticipated despite the fact that it is agreed upon within the research community that they replicate more closely the biology of a tissue or organ *in vivo* [6–8]. Only over the past 10 years has the use of 3D tissue models that capture native-like tissue physiology gained significant momentum in drug development, especially in toxicity testing. This is in large measure because of the steady progress towards the development of many robust, scalable, and reproducible 3D tissue models. The expectations are high that, by using these 3D tissue models as more physiologically disease-relevant assays (see Glossary), the development of drugs will become more efficient and, thus, the process will accelerate and the overall current high failure rates will reduce [9]. How realistic is it that the use of 3D tissue models will significantly improve the success rate of drug development? One would think that the closer the physiology of the 3D tissue models is to the *in vivo* tissues, the more predictive they should be and, therefore, their use should reduce the gap between *in vitro* therapeutic responses in human cellular models and *in vivo* patient responses. The question remains: how do we demonstrate whether 3D tissue models are more predictive of therapeutic responses in the clinic compared with monolayer cells in 2D models? This is not a trivial question to answer and it will need a multipronged approach to resolve. This will include, but not be limited to, establishing benchmarking libraries of compounds, together with longer-term integration of such models in screening strategies along with traditional 2D-based development methods and animal testing, which will eventually provide enough data for a retrospective analysis of predictive rates.

So far, the development of 3D tissue models has been done mostly in academic settings, working primarily on single tissues and organs in isolation, and with relatively low sample throughput, which has slowed their integration into the drug discovery and development process. Liver 3D tissue models are perhaps more integrated into a drug development pipeline because there is a well-established set of compounds for benchmarking and validation of these models for liver toxicity studies [10]. In general, this is not the case for efficacy studies, where there are no standard 'best-fit' models that address different biological questions and/or endpoints about the efficacy of various therapeutics regimens. Many technologies are maturing to the point that are converging into practical use to develop physiologically relevant 3D tissue models that reflect *in vivo*-like tissue architecture, biology, and pharmacological responses. These technologies include the use of patient-derived cells, both induced pluripotent stem cell (iPSC)-derived cells and primary cells, methods to produce 3D tissue models in a more reproducible and scalable manner, and methods that allow *in situ* quantitation of biomolecules [11]. Many of these technologies are being industrialized and becoming commercially available, including: (i) spheroid and organoid microtissue models produced in microwell plates that are compatible with automation and screening infrastructure and allow testing of thousands of drugs in efficacy and safety screens [12]; (ii) biofabricated tissues and 'organ-on-a-chip' technologies with lower screening throughput but that attempt to reflect more closely organ architecture and function, and are used for hit and lead validation [13,14]; and (iii) microphysiological systems for human-based systemic validation (such as body-on-a-chip or human-on-a-chip) [15,16] (Fig. 1). With these technologies at our disposal, the scientific community is well equipped with the tools necessary to work on a completely novel drug discovery and development paradigm based on 3D tissue models.

However, despite these technological advancements, the research community has yet to agree on a set of standards (e.g., reference compound sets that target major signaling pathways in disease biology, dosing regimens, and phenotypic and/or physiological endpoints) to use in efficacy and toxicity benchmarking. As an example, one such initiative to benchmark the predictability of *in vitro* assays, the Comprehensive in-vitro Proarrhythmia Assay (CIPA) program established a set of *in vitro* assays and compounds with known cardiac liabilities to facilitate the adoption of a new paradigm for assessment of clinical potential of torsade de pointes (TdP) that is not measured exclusively by potency of hERG block and not at all by QT prolongation. (http://cipaproject.org/). Establishment of similar focused sets of benchmarking compounds and endpoints for specific models will go a long way in establishing the predictive power of 3D models.

Developing translatable 3D tissue models

As we discussed above, a significant challenge is to determine the 'translational capacity' of 3D tissue model systems. The 'translational capacity' of a 3D tissue model depends on how we define the *in vivo* 'reality' and how it is measured quantitatively. Quantitative equivalency analysis (of the 3D *in vitro* models and *in vivo* data) can be performed on the morphological, phenotypic, and functional and/or physiological behavior of the *in vitro* 3D tissues and organoids, in an *ex vivo* assay with biopsied tissue from an organ, as a function of drug exposure over time. One obvious technology to quantify these properties is to use high-content imaging of 3D tissue constructs. Even with advanced instrumentation, there are many limitations to this technology, as pointed out by Carragher *et al.* in an elegant SWOT analysis for the application of high-content imaging of 3D tissues [17]. This paper clearly highlights the need to harmonize quantification and data-analysis standards to

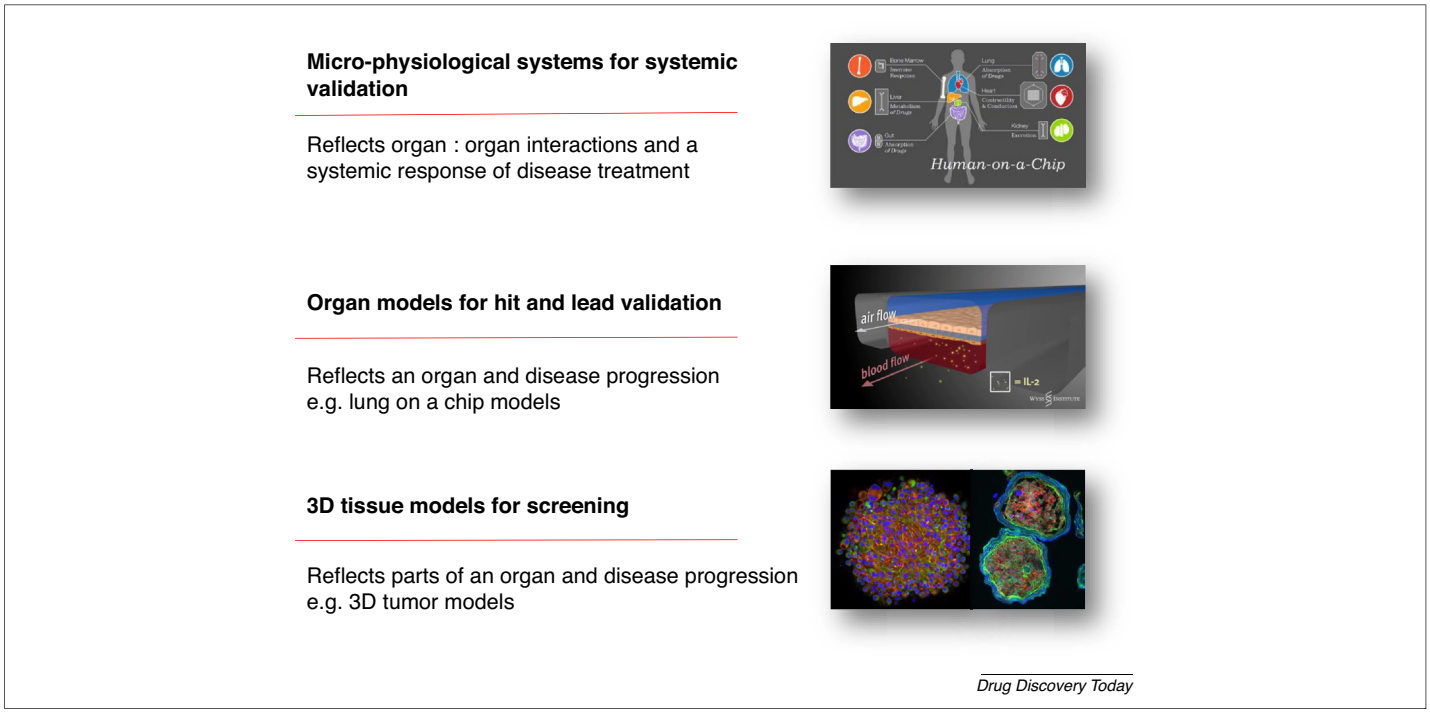

FIGURE 1

Harmonization of advanced 3D tissue models according to their use and scalability within the drug discovery and development pipeline. This is a mandatory step in their integration and the use of their outcomes in decision making points along the pipeline, because the various models differ significantly in their complexity and compound testing throughput. Spheroid or microtissue models (e.g. tumor spheroids or microskin) have a higher throughput capacity than tissue on-a-chip models. By contrast, organ-on-a-chip technologies operate under flow conditions that allow one to address additional endpoints, such as distribution, pharmacokinetic, and pharmacodynamic parameters. Therefore, we need to tailor validation schemes according to the biological question being asked. The human-on-a-chip and the lung-on-a-chip images are courtesies from the Wyss Institute at Harvard University, Massachusetts. The fluorescent image of a tumor spheroid was kindly provided by Dr. Molly Boutin (NIH/NCATS).

develop quantitative equivalency analysis concepts. The SWOT analysis correctly pointed morphological heterogeneity and size and shape-based variability of 3D constructs as a weakness. A major source of this variability is cell sourcing. For example, the same cell types (cell lines or primary cells) obtained from different vendors or laboratories differ widely in their behavior in both 2D and 3D constructs. Harmonized quality control standards for sourcing cells and their storage and stability are crucial aspects of validating 3D tissue models to establish translational capacity.

Indeed, both pharmacokinetic and pharmacodynamics (PK/PD) studies with these models can be applied for direct comparison to the *in vivo* situation to get a better sense of the translational capacity of the *in vitro* model. For example, a longitudinal study of the effect of chemotherapeutic agents on the proliferation and morphology of 3D tumor models would add a temporal component, 'time', as the fourth dimension, which could help to better replicate pharmacological effects observed in tumors *in vivo*, including onset and duration of efficacy and resistance. For example, human 3D liver tissue models can be used for metabolic studies (and reported toxicity studies) to explore exposure, residence times, and target engagement properties of compounds under investigation [9,18]. Similar studies can be designed with other 3D tissue models *in vitro*. Although these individual tissue models might provide the relevant stability and metabolic status of compounds, integrated microphysiological systems (such as body-on-a-chip) are needed for more complex information on drug clearance and PK/PD effects of parent drugs and metabolites, using microfluidics, perfusion, and barrier penetration models (e.g., of the blood–brain barrier) [18,19].

Although there are many examples showing how these 3D tissue models are as, or more, predictive of toxicity and/or efficacy with respect to the effects of small-molecule compounds in animal models and humans than the current state-of-the-art 2D cellular models [6,7,10,12,17–22], there is still an unmet need to demonstrate that a process that takes more than 10 years to reach a clinical outcome can benefit from 3D tissue technologies. We believe that improving *in vitro* biology with higher translational capacity will lead to higher efficiency in drug discovery, but it takes probably more than just adapting single 3D tissue models. Changing only minor pieces of the process will probably only incrementally improve the process, which is already good, but will not have the significant impact that one would expect. Instead, we need to work on 3D drug discovery and development processes ($3D^3$) to fully explore the potential of *in vitro* models with higher translational capacity.

Harmonizing *in vitro* 3D model validation

To implement a new $3D^3$ paradigm, there is a need to harmonize the validation of the models to allow comparison with respect to biology, tissue- and/or disease-specific physiology, and pharmacological response (Fig. 2). This is essential to better understand the models as well as to assess and compare the translational value of different *in vitro* models targeting the same tissue or organ in the context of disease progression. How to validate a model that spans research, therapeutics discovery, and development and regulatory approval is still a topic of intense discussion within the academic and industry communities, and, in most cases, the conclusion is: 'it depends on the application,

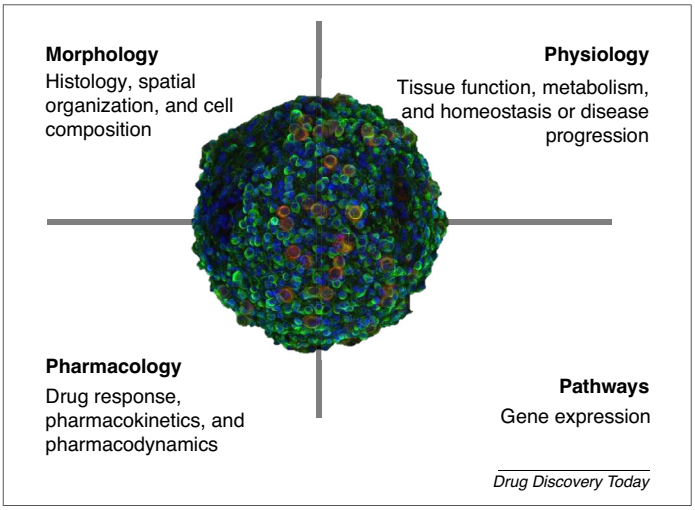

FIGURE 2

In vitro model characterization. *In vitro* models can be categorized into four classes. (i) Morphology: organs and tissues have a clear structure to function relationship. Therefore, advanced models can be assessed according to their spatial organization and cell distribution (morphology), which indicates the extent to which they reflect the native organ or tissue and how stable the model is over the envisioned assay time. (ii) Gene expression: gene expression displays whether major genetic drivers for a specific tissue function are on set and whether they change over time in the same way as their *in vivo* counterparts after environmental changes. Environmental changes can be either disease specific or biochemical changes, such as glucose concentration. (iii) Tissue and/or organ function: every tissue has a specific function that can be measured either by released proteins (e.g., albumin for liver or kidney) and specific metabolism (e.g., glucose metabolism in pancreatic islets). These functions can usually be measured and are a hard parameter to measure the extent to which the *in vitro* model reflects native tissue function. (iv) Pharmacological response: from most *in vivo* tissues and organs, we have a set of compounds at our disposal for which we know the pattern of metabolism in the body at the metabolite and kinetic level. This is a major evaluation parameter to the use of *in vitro* models in drug discovery and development. Integrating these four validation classes can lead to quantifiable and comparable measures for calculating the translational capacity and predictive power of 3D tissue models. Picture courtesy of Dr. Olivier Frey, Bio Engineering Laboratory, Department Biosystems Science and Engineering of ETH Zurich, Basel, Switzerland.

meaning what is the biological question to be addressed by the 3D tissue/disease model is being used'. The question that remains is: 'What assay(s) is (are) the best fit to study a particular biological question and what should the respective, representative 3D model be?' We believe that, to the extent to which an *in vitro* model reflects correctly *in vivo* biology, its translational capacity does not depend on the application. The pertinent, application-dependent information that we extract from each model opens the opportunity to further tailor the coevolution of a battery of different assays and/or models to answer complementary biological questions with respect to a particular disease phenotype. Recognizing this need for more complex information from physiologically relevant disease models, the high-throughput screening community is moving in the direction of multiplexing assay readouts to maximize informational output.

A key issue is how and who is defining validation procedures and how to implement them in the research arena. The manifold model formats in combination with the various analytical possibilities make it difficult to define a standard procedure for all models. As an example, published literature on various liver models on the market shows that it is not possible to do a straightforward comparison because of different biomarkers used to assess polarity and maturity of hepatocytes, exposed to different toxic and nontoxic reference compounds in different concentrations and incubation times; in addition, the number of compounds tested on these systems vary from only a handful to more than a 100. Even looking at something simple as albumin secretion, it becomes difficult to compare the systems because, in many cases, the values are provided in different units, which need to be normalized against each other, both in healthy or diseased liver. However, in assessing metabolic activity, the data acquired are more standardized because of clear regulatory guidelines set forth in the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) Guidance documents (2012) on how to assess CYP activity, which have been adapted for 3D *in vitro* liver models. In general, one can postulate that a diversity of compounds profiled in 3D tissue models would provide more reliable pharmacological responses in *in vitro* models with the same genetic alterations as the patients. However, the scalability of the model determines how many compounds are useful and should be considered for guiding validation schemes. We propose defining reference sets of compounds in combination with the respective endpoints tailored towards the tissue and disease models as the first relatively easy step towards harmonizing *in vitro* model validation. It is probably unfeasible to exactly define pharmacological profiling in association with specific biochemical or imaging-based endpoints, because some models will not be compatible with the respective assays. Therefore, we need tissue- and disease-specific guidelines that provide the researcher directions on, for example, biomarkers, functional endpoints, and compound selection (Fig. 2), but leave it to the scientist to decide which assay fits best to the model to gain the respective information. The quality of the validation is determined not only by the number of tissue and/or disease characteristics covered, but also by the quality of the data captured. Ideally, the validation data should be available and searchable in an open-access database.

Validating *in vitro* models does not yet belong to category of standard procedures if new tissue-engineering technologies are being developed. However, in the interest of the scientific community, publicly funded research projects in this area should help move the field in a direction that ensures a validation process. Again, good examples of such a consortium is the CIPA initiative to guide the validation of cardiac *in vitro* models or the cross-model validation initiative of the TOXRISK-21 project (www.eu-toxrisk.eu/).

The translational capacity of *in vitro* 3D tissue models

Determining the translational capacity of a particular *in vitro* 3D tissue model is a crucial aspect to ensure that data obtained are usable for decision making during the drug discovery and development process. The same *in vitro* model can be either used as an efficacy or toxicity model. For example, a 3D liver model comprising hepatocytes, Kupffer cells, and stellate cells can either be used for liver toxicity assessments or as a disease model to test an-

tifibrotic drugs, provided that appropriate insult gives a disease-relevant phenotype. The core model reflecting liver architecture and cellular composition is essentially the same [21,22]. Therefore, we should uncouple the validation of a 3D tissue model from the applications for which the model can be used. Only if these two aspects are uncoupled can the validation be harmonized. This harmonization would include characterizing the basic biological composition (how close does the *in vitro* model reflect tissue and/or organ architecture and cell composition), phenotyping (functional and/or physiological basis and on a transcriptomic or proteomic level) and pharmacological response (can major tissue and/or organ pathways be modulated with small molecules). The quantitative characterization of these endpoints should allow the compilation of information on the disease physiology captured by each, 'fit for purpose' model and the activity of perturbagens during hit and lead validation to synergistically determine their value in clinical predictability. Translating *in vitro* data to *in vivo* reference data will be similarly challenging because the data sets might be different and, most of the time, the *in vivo* and clinical data might not be easily available in publicly accessible databases (quantitative-equivalency analysis). Therefore, aligning the *in vitro* assays with *in vivo* responses and clinical endpoints along with the use of in-depth bioinformatics and artificial intelligence algorithms will be essential to gain an in-depth view of how close an *in vitro* tissue and/or organ model reflects the reality of disease progression and pathological response. Finally, to benefit the research community and enable them to build more predictive models, all the validation data generated as a result of these harmonization studies and the natural history and patient data should be compiled into a searchable and public accessible data base to truly demonstrate clinical validity for translatable 3D models.

Concluding remarks

3D tissue models for efficacy and safety testing have the inherent potential to not only improve our understanding of biology, but, more importantly, to also fundamentally affect the process of development of therapeutics, regardless of their modality. However, harmonized validation of advanced *in vitro* models throughout the discovery and development process is required to unlock the full potential of these promising technologies and to design knowledge-driven novel concepts for drug discovery and development.

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