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Resubmission of the Draft Manuscript

Computational Approaches for Modeling and Analysis of Human-on-Chip Systems for Drug Testing and Characterization

Submission to *Drug Discovery Today* as a “Guest Editorial” Article

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Cover Letter and Response to the Editor's Comments

October 31, 2016

To:

Dr. Stephen Carney, PhD

Editor, Drug Discovery Today

Dear Editor Carney,

Re:

Manuscript Title: "Computational Approaches for Modeling and Analysis of Human-on-Chip Systems for Drug Testing and Characterization"

Ms. Ref. No.: DRUDIS-D-16-00163

First, I apologize for the delay in the submission.

Thank you very much for reviewing and accepting the manuscript for publication as Guest Editorial article in Drug Discovery Today. I am herewith submitting the revised manuscript with minor editorial changes to facilitate better structure and flow of information. The references have also been updated to better articulate the proposed ideas. Also, attached in this resubmission is the photograph of the corresponding author per the journal requirements.

Look forward to receiving the Author's Proof of this manuscript.

Thanks and best regards,

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Toward novel preclinical platforms for predicting human-relevant responses to drugs

The development of new drugs intended for the treatment of human diseases is a time- and resource-intensive process. To satisfy regulatory requirements for drug approval, the generation of preclinical data on safety and efficacy (S&E) from *in vitro* experiments (using cell culture) and *in vivo* experiments (using animal models) is required before clinical trials, i.e., human testing, can be undertaken [1]. However, most drugs that exhibit promise in preclinical animal trials fail in clinical trials and therefore do not make it to market. This discrepancy can be attributed to the inherent differences that exist between cell-level biology and organ-level physiology [2-3] and the probable lack of reproducibility regarding the use of animal dose-response relationships for assessing human S&E [4]. To address these challenges, U.S. research agencies such as the National Institutes of Health (NIH), the Food and Drug Administration (FDA), the Defense Advanced Research Projects Agency (DARPA) and the Defense Threat Reduction Agency (DTRA) have organized workshops and sponsored multi-million dollar research programs to aid in the development of novel preclinical models for generating human-relevant S&E data for different drug compounds [5-6]. Such initiatives have inspired the idea of using human-on-chip systems (HoCs), an *in vitro*, preclinical, microfluidic-based technology, to rapidly and reliably predict human responses to drugs under development [5-7].

A single HoC system generally integrates several *in vitro* organ-on-chip (OoC) devices cultured with human-derived cells to create a physiologically consistent, holistic system for systematically quantifying and analyzing pharmacological responses to both known and new drug compounds. The ultimate goal of such systems is the creation of a preclinical platform for human extrapolation. The known compounds utilized for testing in HoC systems are typically FDA-approved drugs with well-characterized *in vitro* and *in vivo* behaviors, whereas the new drugs submitted to analysis in these systems are typically compounds under development. The idea is that by calibrating and validating HoC system data for known drugs, human responses to new compounds can be reliably predicted. While *in vitro* testing using multi-well plates is a common step in the drug discovery pipeline, the use of microfluidic HoC technology for drug testing and evaluation to predict human responses is a more recent endeavor [7-10]. Once developed and validated, there will likely be a “sweet spot” for HoC technology in the later stages of lead optimization during drug development.

Challenges faced in the development of HoC systems

Although the HoC concept appears promising, HoC system development poses several challenges related to individual OoC design and fabrication, cell functionalization and viability, organ-organ linking, drug delivery, blood surrogate selection, bio-analytics and, most importantly, the development of tools and methods for data interpretation and extrapolation. As OoC-based HoC technology has evolved, many research groups have deployed an iterative “build-and-try” approach in which the likelihood of producing physiologically inconsistent designs and *ad hoc* dosing protocols can affect drug development time and the reliability of drug testing. Therefore, when developing the constituent OoCs of a HoC system, it is imperative to define the basis of an intended design, i.e., the expected functionalities of the OoC must be clearly specified because it is not practical to replicate all *in vivo* organ-level functions *in vitro*. Generally, OoCs intended for pharmacological evaluation emulate the functionality of the

“elemental unit” of an organ. For example, gut-on-chip designs are primarily used to study drug absorption [8], whereas lung-on-chip designs are used to study drug transport across the alveolar air-liquid interface [9], and liver-on-chip designs are used to investigate drug metabolism and first-pass effects when linked with gut-on-chip designs [10]. Additionally, while one may expect human-like responses from HoCs using human-derived cells, at least for specific intensive quantities, such as normalized concentrations, rate constants and barrier transport properties, innate *in vitro* platform-specific attributes can lead to variances with the *in vivo* environment and may also convolute the *in vitro-in vivo* correlations. Some of these attributes include design aspects, fabrication constraints, media properties (e.g., protein content and additives), physiological linking (e.g., missing organs), cellular properties (e.g., source and expression) and process parameters (e.g., flow rates). In microfluidic chips, in addition to coupled convective and diffusive transport, significant drug loss into the polymeric package can also distort the interpretation of on-chip data.

To address some of the inherent differences between *in vitro* OoCs and their *in vivo* counterparts, we propose that advanced biologically and physiologically inspired computational modeling, discussed in greater detail below, can support and accelerate device design and development in addition to enabling the creation of physiologically consistent organ-linking protocols and rational pharmacotherapy experiments, as well as the robust extrapolation of *in vitro* on-chip outcomes for predicting human responses, which is key for the rapid and successful development of new drugs.

Computational approaches for modeling HoC systems and cross-platform scaling

Figure 1A conceptually illustrates the overall approach used to address the aforementioned differences between *in vitro* and *in vivo* environments via computational modeling, which entails the application of two sequential steps: *in-vivo-to-in-vitro replication* (IVIVR) and *in-vitro-to-in-vivo extrapolation* (IVIVE). Whereas IVIVR intends to emulate *in vivo* microenvironments in OoCs, IVIVE aims to extrapolate chip outcomes to predict human-relevant pharmacokinetic (PK), pharmacodynamic (PD) and toxicodynamic (TD) responses. The starting point for the implementation of these steps is the development of mathematical models of *in vitro* OoCs, based on *in vivo* physiology and pharmacology, described using first principles-based descriptions of media flow, drug transport across cellular barriers, and subcellular and metabolic losses. To ensure the models are effective for new drug testing, i.e., that they are able to predict the *in vivo* behavior of any drug, known or unknown, the governing equations of the models must rely on physicochemical drug properties that can be readily measured using standard assays. Moreover, the parametric nature of first-principle mathematical models should allow for the use of the same set of governing equations in both platforms. Therefore, *in vivo* physiologically-based pharmacokinetics (PBPK) and *in vitro* HoC models represent mathematical equivalents of a living human, and the sequential application of *replication* and *extrapolation* steps is thought to allow for the intelligent exchange of data between the two platforms. Such exchange should enable model-guided testing and evaluation of new drug candidates, first *in vitro* and then *in vivo*, as discussed below.

Replication: IVIVR is based on the hypothesis that cellular functions and behavior occurring *in vitro* will mimic those that occur *in vivo* when a microenvironment sufficiently replicates the

physiological environment, such that an OoC can be tuned to emulate key organ functions, both qualitatively and quantitatively, within the experimental scope by accounting for constraints in design, fabrication, cell-sourcing quality and culture conditions. To establish such equivalence, IVIVR aims to minimize differences between platforms by optimizing OoC design and process parameters using advanced biologically and physiologically inspired mathematical models with embedded optimization routines. Examples include the optimization of media flow rates to achieve ideal shear stress for cell viability and function, the optimization of confluence and transporter expression properties for proper barrier function, and the selection of appropriate polymeric materials to minimize anomalies, such as drug loss to the surrounding package. IVIVR can also be adopted for the rational design of drug delivery protocols to better characterize the PK/PD responses of OoCs (e.g., *in vitro* dosing reminiscent of human PK profiles) and facilitate better effluent sampling for robust measurements and comparison with *in vivo*. For example, by simulating the *in vivo* drug biodistribution patterns and PK responses for a specified administration route (e.g., intravenous infusion or oral administration) *a priori* using a computational *in vivo* human PBPK model, the model-predicted drug-concentration profiles could be used to set initial conditions *in vitro*, circumventing guesswork related to dosing protocols. Such predictions will be of great value for experiments using OoCs that are linked together in a physiological sequence. It should be noted that while IVIVR intends to minimize the differences between the two platforms, its ability to fully replicate the complexity and characteristics of a living human may be limited by the *in vitro* design constraints involved in HoC development. Some of these aspects include morphological properties, hormonal regulation, endogenous feedback loops and the physiological implications related to the fact that some organs are inevitably missing from *in vitro* HoC systems. Therefore, while replication can tune a HoC system so that it qualitatively emulates *in vivo* behavior *in vitro*, it is likely that the absolute quantitative outcomes of an *in vitro* HoC system will not perfectly superimpose with the corresponding *in vivo* measures in every instance. To address this bottleneck in quantitative emulation, model-guided extrapolation can be used to help derive the quantitative meaning of on-chip *in vitro* data in clinical scenarios, as discussed below.

Extrapolation: Following IVIVR-guided design of HoCs, robust algorithms must be applied to extrapolate on-chip data to humans, which can be accomplished through an IVIVE process. Scaling factors (SFs), which are ratios of intensive PK/PD quantities between *in vivo* and *in vitro* systems, are useful for this purpose. Intensive quantities allow for seamless exchange between platforms and can be used to acquire the local properties associated with a platform (e.g., a cellular-level vs. organ-level platform). Examples of such quantities include intrinsic clearance rates, barrier transport descriptors and mass transfer coefficients. Next, using a HoC, SFs should be estimated for a range of known drugs with well-characterized *in vivo* behavior and available clinical data. Once SFs are derived from a set of known drugs, SF maps can be established by plotting each SF against the specific drug properties for a given HoC configuration and design, as illustrated in Fig. 1B. We expect that the nature of this type of SF correlation will rely on the design characteristics of a given *in vitro* system, such as media type, cell properties, cell culture conditions and drug properties. Accordingly, the SF correlation for a given HoC design may be executed using a scalar value (unit or non-unit) or as a function of one or many drug properties. Therefore, a 1:1 match of the outcomes generated between two platforms (*in vivo* and *in vitro*) should not be used as a measure of the reliability and “functional closeness” of a given *in vitro* system; rather, the goal should be to obtain a consistent SF correlation, the reliability of which

can be built by establishing SFs based on multi-parametric formulations of physicochemical properties for a significant number of tool compounds over a broad range in a system with a pre-specified media and cellular configuration, as illustrated in Fig. 1B. We hypothesize that model-guided replication (IVIVR), as discussed above; can help ensure that consistent and reliable SF correlations are made. For a new tool compound, after estimating the intensive PK/PD quantities associated with a given HoC system, the corresponding *in vivo* values can be deduced using SF maps established from known compounds, and these scaled values can then be used as input data for a computational *in vivo* human PBPK model to predict PK/PD responses. Thus, in the process of IVIVE, the need for a computational human PBPK model is necessary to derive the *in vivo* meaning of on-chip data and to make clinical predictions. A related question that may arise is whether an intensive PK quantity estimated from a HoC system can be directly used as input in an *in vivo* PBPK model without extrapolation; we do not recommend such an approach because of the intrinsic differences that exist between the platforms and due to OoC design constraints that may limit quantitative replication. As described above, such residuals may skew HoC outcomes toward reliable human-relevant predictions, even for intensive quantities.

FIGURE 1:

Outlook

Although the development of HoC technology for new drug testing is a challenging and long-term endeavor, the use of coordinated experimental-computational modeling approaches can help mature this technology for the standardization of experimental methods and pharmacotherapy protocols to facilitate the generation of high-quality, reproducible drug data. Together with model-guided replication and extrapolation, HoC systems can evolve as a reliable preclinical drug-testing platform to predict human-relevant responses and accelerate drug development, creating a winning scenario for drug developers, regulatory bodies, and patients. Over the short term, we expect OoCs that are of immediate relevance to routine drug testing, such as liver-on-chip, gut-on-chip and heart-on-chip OoCs and their combinations, will be the first ones to mature and the lessons learned from the research and development of these systems will eventually facilitate the development of more holistic HoC variants.

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Disclaimer: The ideas, opinions and assertions in this article are those of the authors and do not necessarily reflect the official views or opinions of DARPA or DTRA.

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List of Figures

Figure 1: Overview of *in vitro* human-on-chip (HoC) technology, which enables the rapid and reliable prediction of human responses to different drugs and tool compounds. A HoC system generally integrates several *in vitro* organ-on-chip (OoC) devices that are cultured with human-derived cells to create a physiologically consistent, holistic system for systematically quantifying and analyzing pharmacological responses to both known and new (i.e., under development) drug compounds. (A) Computational design of a HoC system to emulate an *in vivo* microenvironment using OoCs via model-guided replication. The use of such a system accelerates device design and development, enables physiologically relevant organ linking and enables the rational design of pharmacotherapy experiments and model-guided cross-platform scaling to extrapolate chip outcomes to predict human responses. (B) The nature of scaling factor (SF) correlations between the *in vivo* and *in vitro* platforms and the application of SF maps to predict pharmacological responses of new compounds.

Model-Guided Design of Human-on-Chip System (HoC) and Cross Platform Scaling

(A)

IVIVR: In Vivo to In Vitro Replication

(recreate *in vivo* micro-environment in the organ-on-chip to emulate specific organ functionality)

IVIVE: In Vitro to In Vivo Extrapolation

(extrapolate *in vitro* chip outcomes such as PK/PD/Tox. quantities to predict human responses)

In Vivo Human PBPK-PD/TD Model

Physiology Pharmacokinetics (PK)
Pharmacodynamics & Toxicodynamics (PD/TD)

IVIVR

Step 1

Same System of Equations
(first principles-based)

In Vitro HoC PBPK-PD/TD

e.g., Five-organ linked HoC

IVIVE

Step 2

Scaling Factor (SF) = Ratio of PK/PD quantities *in vivo* to *in vitro*

(B)

(i) Determinants of SF

(ii) Instances of SF