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The convergence of high-tech emerging technologies into the next stage of organ-on-a-chips

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ABSTRACT

Recently, organ-on-a-chips (OoCs) have been proposed as highly innovative, truly predictive tools with limitless potential for organ function modelling, drug discovery and testing. By mimicking human key organ functions *in vitro*, they are proposed as models for studying physiological processes as well as disease-related mechanisms to elucidate pathological pathways and test the safety and efficacy of potential drug candidates, with unprecedented degree of physiological and clinical relevance. Despite the numerous efforts from biology and engineering, we expect that OoC will reach the next level by benefitting from high-tech technologies such as biofabrication, artificial intelligence (AI), robotics and automation.

The human body is a fascinating yet highly complex machine and fully understanding how humans function and the mechanisms behind human diseases is still a challenge. It is therefore undoubted that we need reliable models to dissect such machine to different levels of complexity, from the organ to the tissue, cellular and molecular scales. Yet, replicating just small parts of such "machine" has been proved to be a daunting task.

Animal models have represented an incredibly powerful tool for interpreting physiological and pathological hallmarks *in vivo* and driving drug discovery and development research. Over the years, researchers have become well aware of the discrepancy between animal and human studies, with the former often showing their limitations in predicting human responses to new drug compounds with high fidelity. For example, few years ago a potentially super-agonist antibody, TGN1214, was developed for chronic inflammatory diseases and cancer, but it caused a systemic inflammatory syndrome (cytokine storm) and multiple organ failure in the six healthy volunteers of phase I clinical tests, while preclinical animal (on non-human primates) studies had reported no adverse issues [1]. Yet, animal studies are still largely employed in pharmaceutical industry. *In vitro* studies, frequently complimentary to animal tests, have matured significantly in the last two decades, becoming considerably different from the conventional 2D cell culture in Petri dishes. Characteristic examples of this maturation path are spheroids and organoids, complex heterogeneous 3D self-assembling structures expressing a highly realistic biochemical and biomechanical microenvironment and showing great potential in cancer research and developmental biology [2]. Moreover, in cell biology large efforts have been devoted to the optimization of multi-lineage differentiation protocols for patient-derived induced pluripotent stem cells (iPSCs) and the creation of iPSC-derived organoids, establishing more realistic cell models for a plethora of different organs and diseases [3]. Nevertheless, these lack peculiar organ features, such as appropriate tissue-tissue interfaces, suitable mechanical properties of the organ-specific extracellular matrix and the possibility to control spatiotemporally the availability of oxygen, nutrients and molecules of interest. To overcome their limitations, technological advances in materials science and engineering have led to the synthesis of artificial extracellular matrices and the design of highly sophisticated bioreactors, emulating the cell microenvironment in an accurate and controlled way, thus leading to more physiological 3D models. Consequently, this allowed performing long-term experiments in precisely controlled environments. The further introduction of microfabrication techniques (optical and soft lithography) to biology on one hand, and the knowledge acquired in the field of cell biology and development in 3D on the other, have led to an inconceivable repertoire of new possibilities represented by organ-on-a-chips (OoCs), belonging to the microphysiological systems [4].

OoCs as *in vitro* models: the current paradigm

OoCs are miniaturized *in vitro* systems that can recapitulate the microenvironment and key functions of different organs with a high de-

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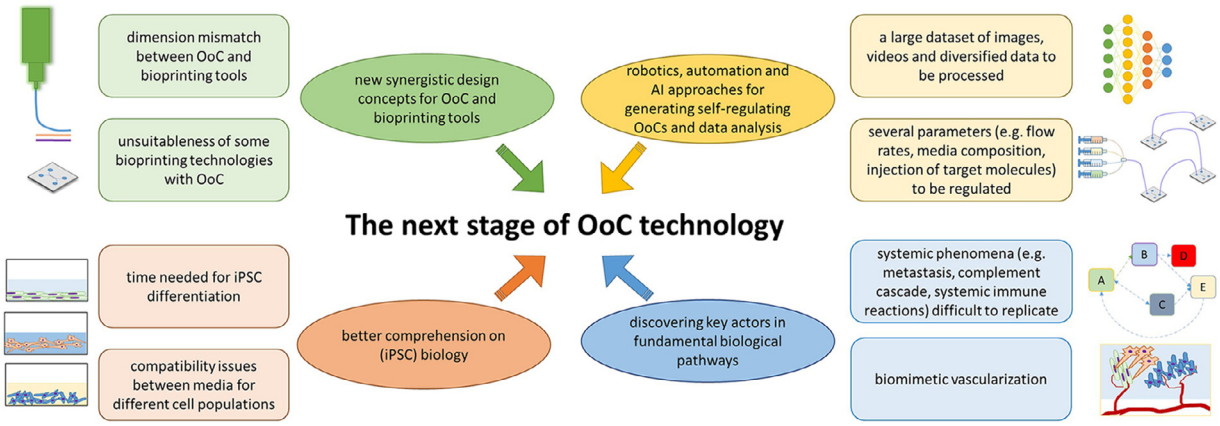

Fig. 1. Needs to be addressed towards the next stage of OoC technology.

gree of fidelity, facilitating the study of complex cell-cell interaction and regulation. They integrate relevant cell types, physical forces, (bio-)chemical concentration and gradients with unprecedented confidence, spatiotemporally recreating physical, biological and chemical features of the target microenvironment. Being a closer match to the *in vivo* counterpart in comparison to other systems, OoCs embody an extraordinary tool in pre-clinical research, potentially providing highly predictive data on drug efficacy and toxicity. Biomimetic OoCs emulating a plethora of functions from single or multi-organs, seldom displaying a vasculature-mimicking component, have been proposed towards a fully standardized, whole body-on-a-chip in the near future.

On the other hand, an increasing level of emulation of living organ architectures has been recently grasped by exploiting the latest achievements in the field of biofabrication, especially considering bioinks development and bioprinting technologies [5]. The fabrication of biomimetic 3D organ structures, displaying highly defined deposition and assembly of several materials and cell types, is more and more widespread, allowing accurate spatiotemporal control over cell–cell and cell–extracellular matrix interactions, but their combination in OoC is still in its infancy. Integrating such technologies rationally, exploiting their individual advantages in an orchestrated manner, would lead to *in vitro* models of organs and diseases with a high degree of fidelity to the real *in vivo* counterpart in terms of cell positioning, cell-cell and cell-matrix communication, taking into account physical and chemical properties of the microenvironment at the cell scale in an unprecedented manner. This is the current direction of biomedical research for obtaining human organ and disease models that are highly predictive of human responses.

Bringing the OoC technology to the next-level

Over the coming years, different technologies (i.e., microfluidics, biofabrication, patient-derived iPSCs, AI) will need to be developed synergistically towards their successful integration into a single OoC unit, meeting several technical and biological needs (Fig. 1). Once their integration is proved to be smooth and successful, an enormously rich number of readouts will need to be collected and analyzed to help clinicians and pharmaceutical companies taking fundamental go/no-go decisions in therapy and drug development, respectively. Minimal changes of cell behavior and functions, cell-cell interactions, tissue/organ microenvironment remodeling will be monitored over an extended period of time (i.e. from weeks to months) in OoC-based *in vitro* investigations, by recording a large dataset of images, videos and diversified data. These will be potentially analyzed by deep learning architectures, similarly to what has been conceived for histomorphometric evaluation of body tissues [6], e.g. drawing the relationship between images acquired from different tissues in OoCs and disease-specific outcomes.

Considering the intrinsic nature of OoCs, where multiple cell types are included, there is an increasing demand for highly precise high-throughput systems. In this direction, machine learning algorithms and, in general, AI would represent powerful tools for monitoring and interrogating these systems, by cracking the interactions within and between such complex networks. Ideally, data from an OoC could be analyzed real-time and used to train an AI-based control system that eventually will finely control the entire OoC and adjust several parameters, creating a self-regulated OoC. In the effort of developing parallelized OoCs for robust drug testing studies, we witnessed some examples of semi- or fully-automated OoCs, equipped with multiple biosensing capabilities and robotic liquid handling. Zhang et al. successfully integrated physical, biochemical, and optical sensing miniaturized tools in a fluidics-routing modular breadboard, handling a two-organoid system in a continuous, dynamic, and automated manner for drug testing [7]. Novak et al. brilliantly developed a sophisticated platform comprising eight different, vascularized OoCs, coupled with liquid-handling robotics and monitored by an integrated mobile microscope, allowing continuous, automated fluid control and cell culturing for three weeks [8].

Unsolved criticisms

The OoC technology will be increasingly adopted in public and private sectors transforming the biomedical research, once some critical issues will be faced. Reproducibility of OoCs at different end-user sites is often under debate, as particularly complex OoCs need highly-trained personnel and high-tech equipment to be reproduced and utilized. Sharing OoCs between labs brings also to biological questions, e.g.: should cells be seeded before or after shipment? Can they be frozen within the OoCs? Which is the shelf-life of cell seeded OoCs? Secondly, OoCs are often the result of lab-scale activities, where large-scale manufacturing technologies are neglected, making difficult a rapid translation to industry of a specific design or process. For example, OoCs fabricated by polydimethylsiloxane, the most popular OoC material, are difficult to be manufactured at a scale that will allow for broad use. On the other hand, it can adsorb small organic molecules interfering with their study when tested as drug candidates. New materials and standardized mass production-ready fabrication routes should be established. Third, from a systemic point of view, the vascular, lymphatic, neural, and immune systems should be better represented, especially in multi-OoCs, as they have been less considered so far [9, 10]. In addition, a consensus should be reached regarding body-on-a-chips: how should we scale the different organs? By their volume, weight, importance for the target application or something else? In this context, a standardized common medium composition should also be defined, able to connect several organ constructs without adversely impacting their functions. Finally, OoCs need to improve their user-friendliness, allowing to collect sufficient samples over

time from different districts for temporal drug quantification, either as medium or tissue portions.

Conclusions

As described here, OoCs have numerous advantages over conventional *in vitro* models and pre-clinical tools, but limitations must be considered. OoCs cannot emulate many complex full-organism phenomena, such as cognitive functions, organism behavior and systemic immune responses. Furthermore, the validation of OoCs is still an open debate across academia, industry and regulatory authorities, as there are no consolidated standards for such technology. Nevertheless, OoCs will continue to evolve and, if the full integration with latest advancements in biofabrication, AI, robotics and automation, and iPSC-based technologies is reached, will further expand their potential towards the establishment of pre-clinical models in a more physiological and clinically relevant way than many animal studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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