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Organ-on-a-chip for assessing environmental toxicants

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Man-made xenobiotics, whose potential toxicological effects are not fully understood, are oversaturating the already-contaminated environment. Due to the rate of toxicant accumulation, unmanaged disposal, and unknown adverse effects to the environment and the human population, there is a crucial need to screen for environmental toxicants. Animal models and *in vitro* models are ineffective models in predicting *in vivo* responses due to inter-species difference and/or lack of physiologically-relevant 3D tissue environment. Such conventional screening assays possess limitations that prevent dynamic understanding of toxicants and their metabolites produced in the human body. Organ-on-a-chip systems can recapitulate *in vivo* like environment and subsequently *in vivo* like responses generating a realistic mock-up of human organs of interest, which can potentially provide human physiology-relevant models for studying environmental toxicology. Feasibility, tunability, and low-maintenance features of organ-on-chips can also make possible to construct an interconnected network of multiple-organs-on-chip toward a realistic human-on-a-chip system. Such interconnected organ-on-a-chip network can be efficiently utilized for toxicological studies by enabling the study of metabolism, collective response, and fate of toxicants through its journey in the human body. Further advancements can address the challenges of this technology, which potentiates high predictive power for environmental toxicology studies.

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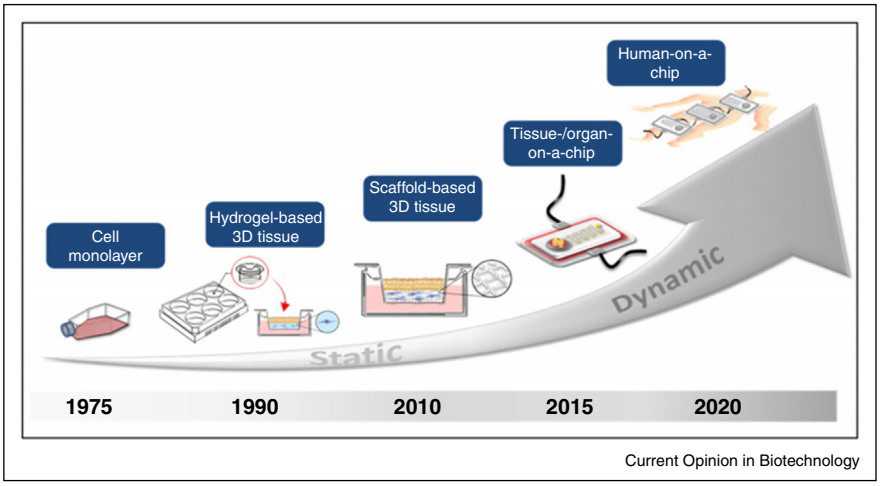
Introduction

With the momentous advancement of technologies, introduction of man-made toxic xenobiotics, or toxicants, are accumulating in the environment that are poorly understood and/or not yet identified. The United States Centers for Disease Control and Prevention (CDC) reported over 80 000 chemicals used in 2012, which 2000 chemicals are manufactured or imported into the U.S. in amounts of at least one million pounds per year, commonly referred to as high production volume (HPV) chemicals [1]. Due to the rate of toxicant accumulation, unmanaged disposal, and the unknown toxicological effects to the environment, there is a crucial need to quickly and efficiently evaluate the potential adverse health effects upon inevitable integration into the human body. Unfortunately, most of the previous research has concerned with identifying human exposure to HPV chemicals rather than addressing the need to understand toxicological effects in human physiology-relevant models.

One of the most well-known conventional screening methods is Toxicity Forecaster or ToxCast in short, which is a high throughput screening (HTS) based method employed by the U.S. Environmental Protection Agency (EPA). ToxCast prioritizes HPV chemicals in *in vitro* models, of which over 1800 chemicals have been at least partially analyzed, whose data is then compared to the results of animal studies. This method, however, remains time-consuming, costly, and still relatively low-throughput [2•,3]. *In vitro* models are limited in high predictive power due to significant shortcoming in the use of *in vitro* 2D models, which are incomparable to the complex, *in vivo* 3D microenvironment detailed in human physiology. The 3D microenvironment exhibits a well-organized architecture possessing intimate cell–cell interactions and cell-extracellular matrix (ECM) network that is essential for recapitulating the human physiology. In addition, toxicity studies from animal models may inaccurately portray toxicological effects in the human body due to obvious inter-species differences [2•,3].

As illustrated in Figure 1, recent innovations in microfluidic technologies have produced organ-on-a-chip (OOC) platforms, which integrate advanced 3D tissue engineered constructs with microfluidic networks to minimize the shortcomings of *in vitro* 2D models [2•,4•]. Such cohesive platform enables important physiological cues, such as the vasculature and interstitial fluid flow, which improves mimicry of the *in vivo* physiological conditions for studying stem cell differentiation, metastasis, and so on. In addition, inter-species differences can be eliminated through the use of human cells. Furthermore, OOC researchers have begun to investigate interconnecting multiple OOC systems into a network (Figure 1), in order

Figure 1



Evolution from *in vitro* models to multi-OOC systems.
Figure reproduced from Planz et al., with permission from Elsevier [70].

to emulate inter-organ relationships and ultimately objectify human-body-like microphysiological systems [4•]. While OOC systems have primarily been utilized for stem cell, cancer, and drug testing, they can also be used toward environmental toxicology studies. In this mini-review, conventional environmental toxicology screening will first be summarized for select HPV toxicants. OOC technologies will then be discussed in regard to its potential for assessing environmental toxicants, in addition to what challenges must be addressed to produce a better alternative to *in vitro* 2D models and animal models.

Conventional environmental toxicology screening

Conventional HTS relies on 2D cultured cells to evaluate the cytotoxicity to drugs or toxicants, whose responses differ from those obtained *in vivo* due to the lack of physical and humoral interactions provided by the ECM, cell–cell interactions, and other molecular components of the native organ [5]. Indeed animal models do reproduce organ complexity more accurately, but deduction of toxicological responses may be ambiguous due to inter-species differences and thus remain irrelevant to human physiological responses. Also, the time consumption, costs, and ethical concerns of animal testing disfavors its use in toxicological research [5,6].

Microfluidic HTS systems (typically considered a precursor to OOC systems), where cells are cultured in microfluidic channels, do incorporate flow components in a miniaturized manner (leading to low fluid consumption, assay miniaturization, and parallel processing) [7–10]. Yet, they cannot assess detailed information regarding the effects of generated metabolites, bioaccumulation, cell–ECM interactions, and processing via organs as it travels throughout the human body.

On the other hand, precision-cut organ models, where thin tissue slices are used rather than 2D cultured cells, demonstrate the sheer advantage of direct interspecies comparison with respect to metabolic capacity and sensitivity for toxicants [6], and therefore has been identified as useful models for toxicological assessment [5,11,12]. However, obtained tissue slices are largely constrained by the limited viability for toxicological testing, which inhibits long-term toxicity studies [5].

Known HPV toxicants

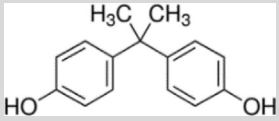
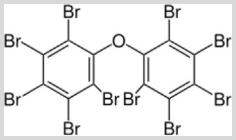
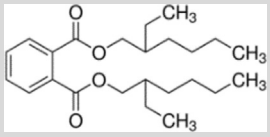
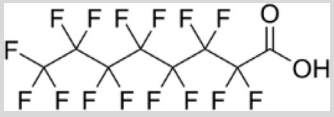
Select known HPV toxicants of interest (especially prioritized by the CDC) are listed here: environmental phenols, polybrominated diphenyl ethers (PBDE), phthalates, and perfluorinated chemicals (PFCs) (Table 1).

Many environmental phenols, notably bisphenol A (BPA), serve as endocrine-disrupting chemicals (EDCs), which mimic or antagonize endogenous hormones due to similarities in their chemical structures [13,14]. Although the use of BPA has strictly been limited, BPA is ubiquitously prevalent in manufacturing plastics and frequently leaches into water sources, resulting in bioconcentration in the environment [14–18]. Alarmingly, BPA can induce endocrine-disrupting health effects at modest concentrations of nanograms per liter [19–22].

PBDEs, of which the most common form is decabromodiphenyl ether (DECA), are utilized as flame retardants in commercial products with well-documented varying effects in numerous animal organisms [23–25]. Human susceptibility to PBDEs through inhalation, dermal

Table 1

Summary of select environmental HPV toxicants, shown together with how their adverse health concerns have been addressed with *in vitro*, animal, and human physiological response models. The most common health concerns of interest were those altering reproductive systems, altering developmental systems, inducing cancer, altering neurological systems, and inducing acute toxicity responses. Although there are statistical studies determining potential associations between existing adverse effects and human exposure of toxicants, those were not considered as human physiological response models.

Group	Representative chemical and its structure	Half-life	*In vitro* or animal model available to address adverse health concerns?	Human physiological response model available to address adverse health concerns?
Environmental phenols	BPA 	4–5 hours [78]	Reproductive: Y Developmental: Y Cancer: Y Neurological: Y Acute: Y All from Ref. [78]	Reproductive: Little Developmental: N Cancer: N Neurological: N Acute: N All from Ref. [78]
PBDE	DECA 	Average 15 days. Longer half-lives with decreasing bromides (37–91 days) [79]	Reproductive: Y [27] Developmental: Y [27] Cancer: Y [80] Neurological: Y [27] Acute: Y [18]	Reproductive: Y [29,81] Developmental: N Cancer: N Neurological: Y [29,82] Acute: N
Phthalates	DEHP 	12 hours [83]	Reproductive: Y [84] Developmental: Y [35,85] Cancer: Y [36] Neurological: N Acute: Y [36]	Reproductive: N Developmental: N Cancer: N Neurological: N Acute: N
PFCs	PFOA 	3.5 years [37,38]	Reproductive: Y [86] Developmental: Y [86,87] Cancer: Y [86] Neurological: Y [88] Acute: Y [89]	Reproductive: N Developmental: N Cancer: N Neurological: N Acute: N

absorption, and ingestion is substantially high due to their lack of chemical binding to products [26]. Several limitations from previously conducted *in vitro* models include inaccurate use of PBDE dose-dependent concentrations [27] and evaluation of culture medium rather than quantifying PBDE accumulation in cells [28–30], which ultimately challenges translation of *in vitro* to *in vivo* results.

Like PBDEs, phthalates are industrial chemicals that also exhibit leaching behavior [31]. The most common phthalate toxicant is diethylhexyl phthalate (DEHP), which contaminates inhaled indoor air [32–34]. Majority of *in vitro* studies support phthalates as xenoestrogens [35], but remain unverified for *in vivo* studies. The plethora of *in vitro* toxicological studies have specifically yielded species-specific results that are not human physiology-relevant [36].

PFCs are used for protective coating of products, notably heat-resistant non-stick coatings. With limited water solubility and low volatility, PFCs, such as perfluorooctanoic acid (PFOA), bioaccumulate in the environment and in the body [37,38]. This slow elimination time challenges the determination of how lifestyle, diet, and other exposure-related factors influence physiological responses, and ultimately understanding the kinetics of the toxicant metabolism and subsequent removal from the body.

OOC technologies

OOCs are microfluidic-based systems with advanced 3D tissue engineered constructs and cultured human cells to replicate a human organ of interest [2•]. Microfluidic channel networks are designed and fabricated to mimic the organ structure (*e.g.*, liver sinusoid, nephron in a kidney, *etc.*). The channel surfaces are usually modified with the layers mimicking the ECM, allowing the human cells to adhere, spread, and proliferate within the channels (thus requiring tissue engineering technologies). Once OOCs are constructed, fluid flow is applied to generate mechanical forces that recapitulate the *in vivo* microenvironment experienced by cells [2•,39]. Specifically, organ-specific fluid flow enables gradient formations of molecular components and maintenance of cell–cell interactions [39,40•,41], which are vital to emulating

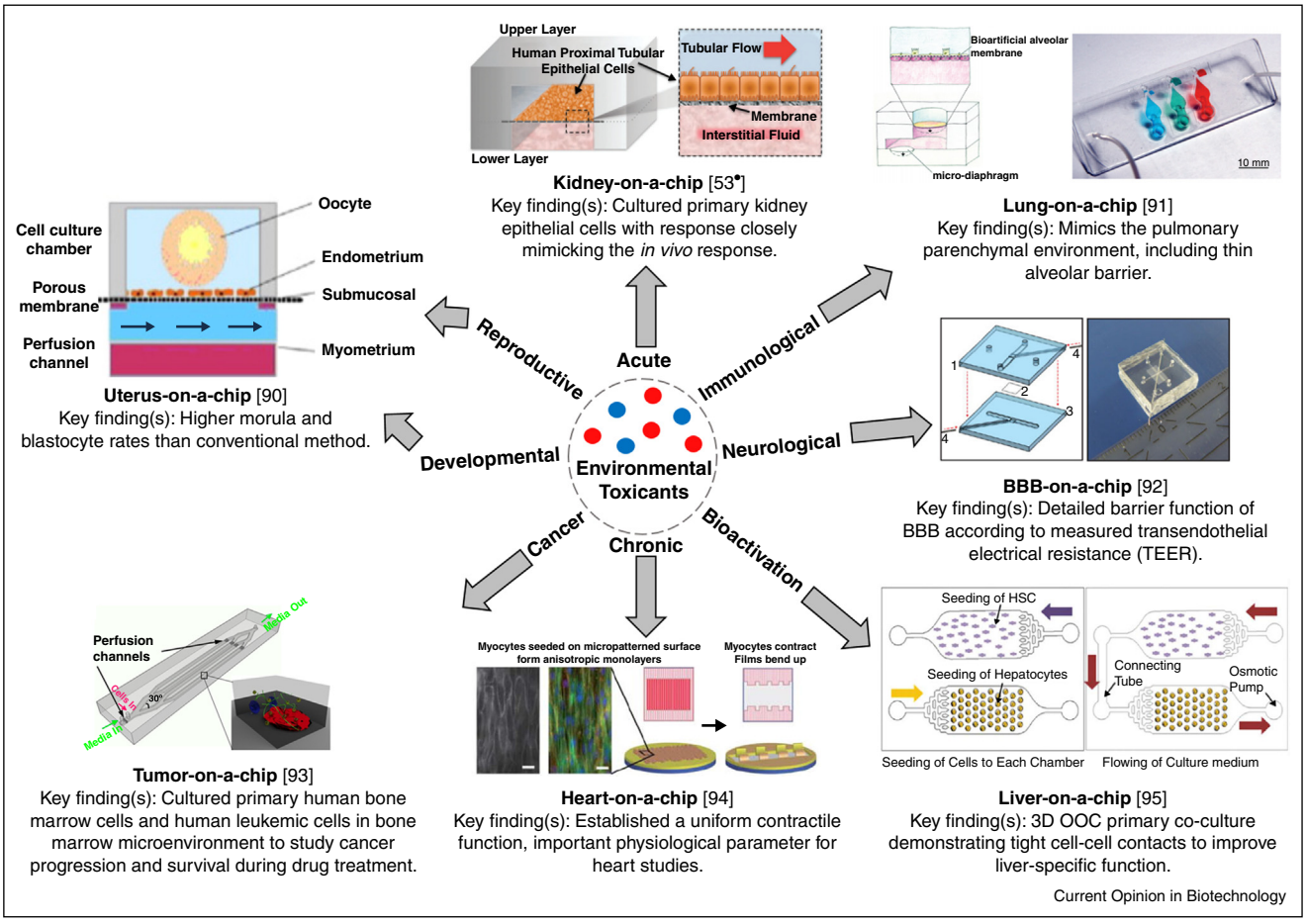
human physiological responses. Previous research has demonstrated that incorporated ECM networks yielded *in vivo*-like behaviors such as apical-basal polarization [42], lumen formation [43], increased differentiation [44], and appropriate protein expression [45]. Finally, OOCs are significantly low-cost, possesses tunable properties, mass-producible, with low reagent consumption and waste production compared to the conventional 2D assays [46,47]. Figure 2 provides a summary of available OOC technologies that may be used to address significant health concerns of toxicants, although most of them are not designed to study them.

While many different OOC systems have already been demonstrated for various applications, we are particularly interested in kidney, liver, and lung OOC systems (referred to as kidney-on-a-chip, liver-on-a-chip, and lung-on-a-chip) due to their important roles in bioactivation, filtration, and susceptibility to the environmental toxicant exposures.

Kidney clears endogenous waste and exogenous toxicants from the body, and is highly susceptible to xenobiotic and metabolite-induced nephrotoxicity [46]. Unknown consumption of food disinfectants may induce oxidative stress to the human kidney [48–50]. Various existing literature demonstrates a strong promise of kidney-on-a-chip devices for studying drug-induced toxicity and drug interaction studies through recreating renal tubule microenvironment within microfluidic channels [51,52,53•,54] with appropriate transport functions [54], crucial for investigating filtration capabilities. Some fundamental requirements essential to reproducing efficient kidney-on-chips include the biocompatibility of chip materials, fabrication with non-cell-adhesive materials [55], and the control of fluid shear stress to facilitate tight monolayer formation.

Liver is responsible for drug bioactivation, drug clearance, and production of reactive metabolites that can interact with other downstream organs [56–58]. During these

Figure 2



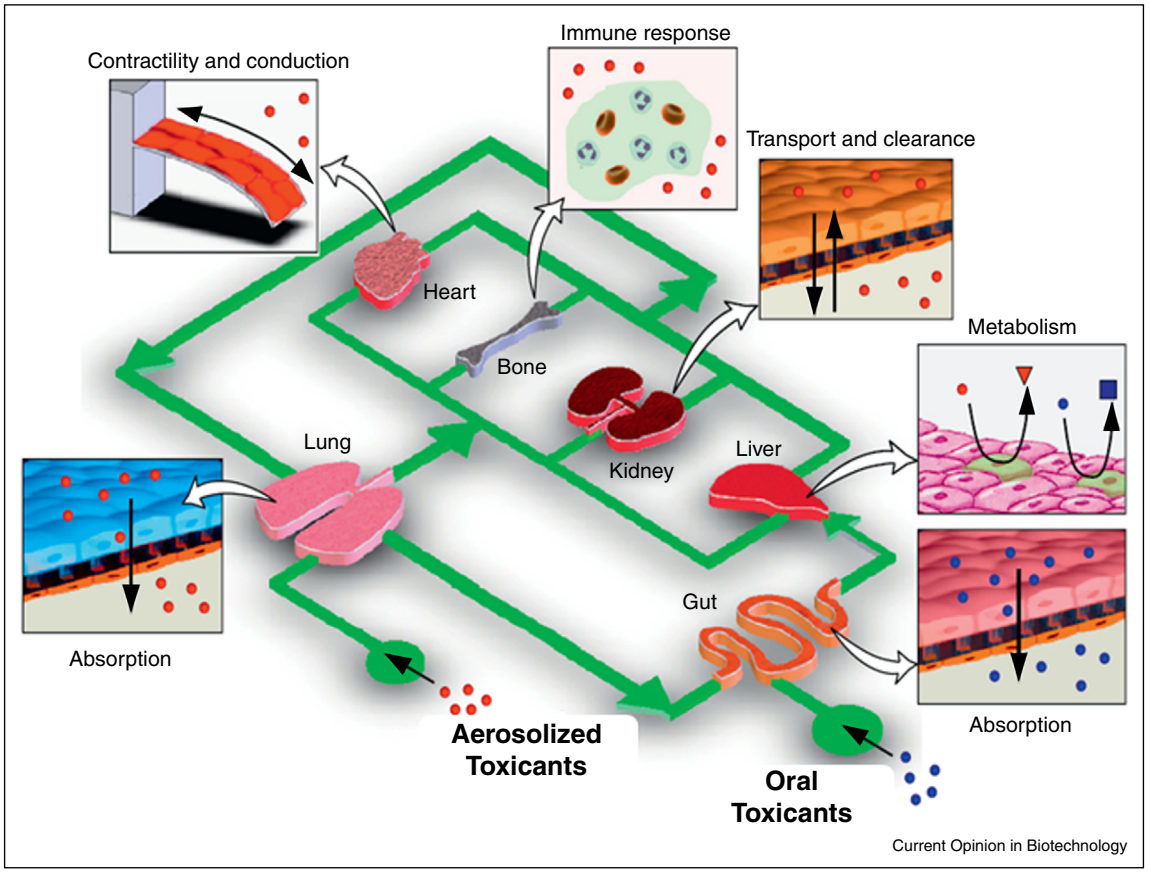
Summary of existing OOC technologies that may be used to address adverse health effects of environmental toxicants. Figure of uterus-on-a-chip was adapted from Ref. [90] with permission from Elsevier. Figure of lung-on-a-chip was adapted from Ref. [91]. Tumor-on-a-chip figure was reproduced from Ref. [93]. Figure of heart-on-a-chip [94], kidney-on-a-chip [53•], BBB-on-a-chip [92], and liver-on-a-chip [95] were reproduced with permission from the Royal Society of Chemistry.

processes, liver is also susceptible to drug-induced injury. Multiple biomimetic liver-on-a-chip platforms have been established for drug toxicity testing [59–62]. Liver-on-a-chip is essential in multiple-OOC systems because of its high concentration of biotransformation enzymes that may bioactivate xenobiotics [63•]. A metabolically active liver model must be integrated into *in vitro* models in toxicology studies in order to determine toxicological effects of metabolites and serve as an ideal representation of human physiology [64], which is generally considered very challenging in 2D models. One such important interplay among organs is that of the hepatic and renal systems. According to human physiological processes and anatomical placements, the liver nearly receives all of the xenobiotic-containing blood perfused by the intestinal system (gut), whose bioactivated metabolites may compromise the renal system during subsequent hepatic first pass effect during circulation [63•]. A good alternative is the interconnected, multiple OOC systems (*e.g.*, liver-on-a-chip and kidney-on-a-chip connected in a series), which is discussed later.

The respiratory tract is a significant entry port of the human body due to the thin mucosal barrier with adsorptive surface area of the alveoli, which enables rapid access to the bloodstream [2•]. Lung-on-chips engineered with appropriate alveolar-capillary interface and vacuum strain to mimic physiological breathing can be used to study aerosolized toxicants. The challenge resides with the reconstruction of a reliable alveolar-capillary barrier without a complex culturing process [65].

Since OOCs are easy to construct, small-scale, and flexible in changing their designs, they can be made into not only high-throughput systems but also interconnected, multi-OOC systems. Such multi-OOC systems can better simulate the overall physiological responses of human body, especially for toxicants. These multi-OOC systems can be built into a human-on-a-chip system, which will greatly improve *in vivo* physiological responses due to the better relationship modeling and correct anatomical placement (Figure 3).

Figure 3



Schematics of interconnected multi-OOCs, with microfluidic circulatory system to ultimately yield an anticipated human-on-a-chip. As toxicants enter the integrated model via inhalation through the lungs, or ingested through the gut, an intimate study of toxicant bioactivation, metabolism, transport, and fate can be observed. Such interconnected system model is crucial for understanding potential *in vivo* responses to the toxicants from various organs. Figure was adapted by Huh et al. with permission from Elsevier [76••].

Use of OOCs toward environmental toxicology

Governmental funding programs are currently expanding worldwide on developing innovative drug screening tools, in particular, *in vitro* cell-based or tissue-based models reproducing human physiology [66]. OOCs are obviously superior platforms over those *in vitro* models, and better predict the *in vivo*-like responses. While OOCs have substantially been used for drug screening, several drug toxicity studies are currently emerging, which may be adapted to environmental toxicology assessment. For example, Homan *et al.* recapitulated human kidney's dose-dependent responses to a nephrotoxin on a 3D bioprinted proximal tubules on chip [67]. In addition, primitive OOC systems that utilize cell spheroids still serve as adequate alternatives to 2D *in vitro* models and are being tested for drug toxicity assessment. Ziolkowska *et al.* presented a microfluidic chip with carcinoma cell spheroids to investigate the efficacy and toxicity of an anticancer drug [68]. Wei *et al.* presented a similar concept using primary hepatocyte spheroids on innovative fibers, which achieved excellent prediction of *in vivo* drug clearance rate [69]. Although these preliminary studies have been demonstrated for drug assessment, they may be translated into environmental toxicology.

Many researchers acknowledge that the future direction must entail influence of multi-organ crosstalk and ultimately reconstitute the human-on-a-chip that is capable of ideally replacing animal studies [70]. This multi-OOC feature is a very important aspect for assessing environmental toxicants, to fully and correctly emulate the inter-organ and systemic responses from human body. Toxicity studies with multi-OOC do exist, albeit for drug screening but not for environmental toxicology: Maschmeyer *et al.* connected and maintained four OOCs to profile drug metabolism and processing among organ cross-talking networks for 28 days [71••]. Oleaga *et al.* demonstrated a 14-day sustained multi-OOC model with functional cardiac, muscle, neuronal and liver activities [72••]. More significantly, they evaluated 5 drugs with known adverse health effects, whose results were in general agreement with available human and animal data [72••]. Such productive step toward human-on-a-chip may render an ideal tool for assessing environmental toxicants.

However, the number of such multi-OOC toxicology studies remain minimal, and those small number of studies continue to focus primarily on drugs, not environmental toxicants. With the development of physiologically relevant OOC models, toxicant screening may become possible with reduced cost, time, and labor. Not to mention, further questions may be answered that current technologies have not been able to address with such multi-OOC models — for example, the effects of environmental toxicants on the development of allergies [15].

Nonetheless, there will be several challenges that need to be considered with advancing OOC technologies. The chip materials must be made tissue-compatible and optimized to closely capture the complexity of native tissues [73]. Use of primary cell culture is ideal, while they have a limited lifespan, undergo rapid phenotypic alterations, and show large variability over different batches of isolation [2•]. Because of these limitations, well-established, immortalized cell lines are most commonly used in OOC studies, while their immortalized trait often presents false tissue-specific functions [74••,75]. Additionally, current analytical methods for OOCs (mostly fluorescence microscopy-based) remain tedious, disruptive, time-consuming, and lack real-time *in situ* analysis capability [53•,73,76••]. Non-invasive monitoring tools for *in situ* OOC analysis has been previously demonstrated [47], again still quite small in number, which may facilitate the assay analysis with low costs.

Conclusion

There is an overwhelming burden of assessing numerous HPV toxicants present in the environment. *In vitro* models and animal models are inadequate for understanding the *in vivo* toxicological responses. In addition, they are severely limited in detecting additive or synergistic interactions of environmental toxicants occurring within the human body [77]. With the recent advances of OOC technologies that better recapitulate human physiology, adverse health effects of toxicants and assessment of multiple exposure of various toxicants can be evaluated. There are scarcely any conclusive studies of human responses to toxicants available with OOC technologies. In fact, the majority of OOC literature have been focused on preclinical studies of pharmaceutical drugs, but not on environmental toxicology. In this sense, we strongly suggest that OOC technologies should be employed for identifying and understanding environmental toxicants, which will significantly benefit the general public toward complete understanding on numerous environmental toxicants.

Conflict of interest

There is no conflict of interest relating to this article.

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