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Human organ-on-a-chip technology as a catalyst for drug discovery☆

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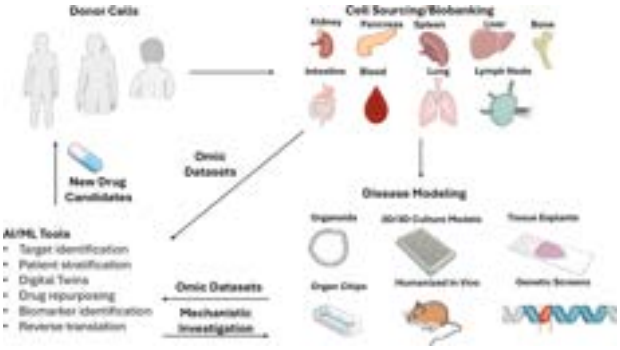
HIGHLIGHTS

- The pharmaceutical industry faces a critical attrition crisis, with ~90% of drug candidates failing in clinical trials largely due to the inability of conventional cell cultures and animal models to accurately predict human responses.
- Organ Chips enable testing of therapeutics using clinically relevant pharmacokinetic profiles and administration routes, supporting more predictive preclinical assessment of drug efficacy, toxicity, and mechanisms of resistance across a broad range of organ systems and disease states.
- Integration of Organ Chips with high-throughput screening, functional genomic approaches, and artificial intelligence represents an emerging shift that enables rapid target identification, drug repurposing, and therapeutic discovery, while potentially reducing clinical failure rates.

GRAPHICAL ABSTRACT



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ABSTRACT

The pharmaceutical industry currently faces a critical attrition crisis, with approximately 90% of drug candidates failing during clinical translation. A major contributor to this high failure rate is the inability of preclinical models, namely conventional cell cultures and animal studies, to accurately predict human responses. In recent years, human Organ-on-a-Chip (Organ Chip) microfluidic culture technology has emerged as an alternative and potentially transformative approach to overcome these limitations. Unlike static cell cultures or organoids, Organ Chips recapitulate organ-level pathophysiology by incorporating tissue–tissue interfaces, dynamic fluid flow, mechanical cues, and immune cells, enabling a higher level of physiological mimicry as well as the testing of therapeutic responses using clinically relevant drug pharmacokinetic profiles. In this article, we focus on how human Organ Chip technology has begun to be used to facilitate drug development, its advantages and

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disadvantages relative to traditional preclinical models, and its recent integration with artificial intelligence (AI) and high throughput screens, which have the potential to accelerate discovery while ameliorating translation rates.

1. Introduction

Despite an explosion in the number of registered clinical trials, the rate of new drug approvals has stagnated. Approximately 90% of candidates that enter clinical trials fail to receive approval, with lack of efficacy accounting for roughly 50% of these failures, and unexpected safety issues accounting for approximately 25%[1,2]. This efficiency crisis begs a fundamental question: why do so many therapies that appear promising in preclinical studies fail in human patients? The answer largely lies in the predictive limitations of currently accepted preclinical models.

Advancement of therapies from pre-clinical to clinical investigation typically relies on *in vitro* cell cultures and *in vivo* animal studies as required by the FDA. Commonly Used *in vitro* cell cultures permit investigation of molecular mechanisms underlying disease pathology and response to therapies, but they are often optimized for cell growth rather than accurate physiologic mimicry[3,4]. They typically lack complex extracellular structures and stimuli (e.g., tissue-tissue interfaces, immune cells, dynamic fluid flow, mechanical forces) that define human physiology and disease states within living organs. Conversely, animal models provide systemic complexity but are frequently confounded by interspecies differences in genetics, anatomy, immunology, and metabolism, sometimes leading to catastrophic errors in predicting human toxicity and efficacy[4].

More recently, advances have been made in the development of microphysiological systems (MPS) that better replicate the form and function of living human tissues and organs[5]. MPS include static three-dimensional (3D) engineered culture systems and organoids grown within 3D extracellular matrix (ECM) gels, as well as microfluidic organ-on-a-chip (Organ Chip) devices that culture living cells and tissues under dynamic fluid flow [5,6]. Experimental preclinical models created with tissue engineering approaches that mimic the microarchitecture, cell-cell interactions, and ECM of native tissues include Transwell™ cultures, micropatterned tissue constructs, scaffold-directed tissue constructs, and 3D bioprinted tissues. Organoids are self-organizing cultures of adult stem cells or induced pluripotent stem cells (iPSCs) grown within a 3D ECM gel, such as Matrigel, which mimic the structure and function of living tissues or parts of organs [7]. Organ Chips are microfluidic culture devices lined with living human cells from multiple tissues that are positioned to recreate organ-specific tissue-tissue interfaces and exposed to dynamic fluid flow as well as physiologically relevant mechanical cues [8].

Engineered MPS and Organ Chips can be constructed using human cells, either primary adult cells isolated from mature tissues obtained either at surgery, biopsy, or autopsy (many now often sold by commercial suppliers); iPSCs that are created by reprogramming adult cells [9] (e.g., fibroblasts, immune cells) genetically and/or chemically to become embryonic-like stem cells; or adult stem cells grown and expanded as organoids within ECM gels, which may be subsequently dissociated and cultured in Organ Chip systems.

Compared to other *in vitro* models, Organ Chips are able to reconstitute organ-level behaviors and multi-organ level responses (when multiple chips are linked fluidically) and thus, are the superior choice for drug development and regulatory safety assessments. They are the only preclinical human model that enables drugs to be tested using clinically relevant administration routes (e.g., oral versus intravenous) and which can simulate pharmacokinetics (PK) and pharmacodynamics *in vitro* [10]. In this article, we briefly review how Organ Chips are currently being utilized to accelerate the development of therapeutics and drug delivery systems. We highlight the convergence of Organ Chips with high-throughput screening, and artificial intelligence (AI) as an emerging paradigm shift that enables rapid drug repurposing as well as the discovery of novel therapeutic targets. We further discuss the potential of these human-relevant models to replace animal testing in regulatory assessments, thereby shortening drug development timelines and improving clinical success rates.

2. Microfluidic human organ chip technology

The first microfluidic Organ Chip model that reconstituted human organ-level structure and function was a Lung Alveolus Chip that contained two parallel microfluidic channels separated by a porous ECM-coated membrane with human lung alveolar epithelial cells on one side and human pulmonary microvascular endothelium on the other, thereby recapitulating the alveolar-capillary interface in vitro [11]. These channels were surrounded on either side by two full-height hollow chambers and the body of the device was composed of a flexible, optically clear, silicone polymer (poly-dimethyl siloxane). Thus, by applying cyclic vacuum to these side chambers, the central walls of the chamber and attached porous membrane with the adherent tissue-tissue interface could be rhythmically stretched and relaxed to mimic physiological breathing motions (**Fig. 1A**). This same design has been used with different organ-specific cells to develop Organ Chip models of healthy and diseased lung alveolus and airway, small and large intestine, kidney tubule and glomerulus, liver, skin, vagina, cervix, brain, bone marrow, and lymph node, among others [12–33].

The Organ Chip field encompasses a plethora of different device materials and designs, tissue types, diversity of cell types represented, and physiological cues present in each model (**Fig. 1B-J**). For example, other microfluidic Organ Chip designs that utilize Transwell-like open type chambers, which integrate a lower flow chamber with or without a lining endothelium through which culture medium is perfused, have been used to create models of a similar broad range of different organ types [8,34,35] as well as to create multi-Organ Chip models [36–39]. Another Organ Chip design incorporates a wide microchannel filled with a central column of ECM gel along its length, creating two hollow microchannels with flow on either side that can be lined with different tissue types (e.g. lung epithelium and endothelium) [275,276]**.** This creates a tissue-tissue interface with an ECM through which cells can migrate; however, the thickness of the gel can potentially complicate drug distribution studies. Microvascular endothelial cells also can be cultured within thicker ECM gels within microfluidic channels under conditions that promote formation of functional capillary networks [40]; these devices are particularly useful for cancer studies [41].

In their most complex configuration, multiple different types of Organ Chips (e.g., lung, liver, kidney, intestine, etc.) can be fluidically linked to create human "body-on-a-chip" systems that can recapitulate interactions between multiple different organs, simulating multiple layers of human physiology and organ-specific cues [28,38,42]. However, the physiological complexity of multi-Organ Chip models comes at the expense of ease-of-use as multiple different cell types must be cultured simultaneously and synchronized so that each Organ Chip is at the correct level of differentiation when it is to be linked with the others. The number of samples and analyses that must be carried out also can expand dramatically and so this often requires a large research team resulting in prohibitively expensive studies. Thus, the complexity of a multi-Organ Chip model must be balanced by the technical demands of its usage [43], and most multi-Organ Chip studies at present link a limited number of different models (e.g., liver and intestine; cervix and vagina) [21,27,44]. Future designs may need to be tailored to balance

the operational needs of a specific stage of the drug discovery pipeline against system complexity, and these types of experiments could be facilitated by use of robotics and AI.

3. Organ Chips as preclinical tools for drug development

The drug development process is time-consuming, costly, and unreliable [45]. Owing to their versatility, Organ Chips can be integrated into multiple stages of this process. The most obvious and often pursued application for human Organ Chips is for preclinical assessment of drug toxicities as a potential replacement for animal testing [277,278]. But human Organ Chips also can be used to the assess efficacy and safety profiles of drugs when administered using clinically relevant dosing regimens, elucidate underlying mechanisms of action and toxicity, model evolution of drug resistance, identify potential new therapeutic targets by elucidating novel disease mechanisms, and rapidly repurpose existing drugs for new applications [8,279–281]. In addition, Organ Chips are beginning to be used to better understand and predict patient-specific responses to therapies to develop personalized therapeutic regimens. Through these applications, Organ Chips represent the potential for a faster, less costly, and more patient-centered drug development process in the future. A few examples are summarized below.

3a. Toxicity assessment

While the first publication describing the human Lung Chip did not explore responses to drugs, it was shown to be an effective tool to measure toxicities, in this case lung inflammation, induced by exposure to silica nanoparticles that were simulants of airborne smog particulates [11]. Nanoparticle exposure resulted in increased production of reactive oxygen species (ROS), enhanced expression of ICAM-1 on the surface membrane of the pulmonary microvascular endothelium, and induced recruitment of circulating neutrophils that were perfused through the vascular channel. Interestingly, the inflammatory responses induced by exposure to nanoparticles in the Lung Chip were augmented when the lung tissues were exposed to physiological breathing motions, and hence they would have been missed using conventional static culture models.

The same human Lung Chip was also used to reproduce drug toxicity-induced pulmonary edema observed in human cancer patients treated with interleukin-2 (IL-2) at similar doses and over the same time frame [46]. Again, mechanical forces associated with physiological breathing motions were found play a crucial role in the development of a toxicity response, in this case increased vascular leakage that leads to pulmonary

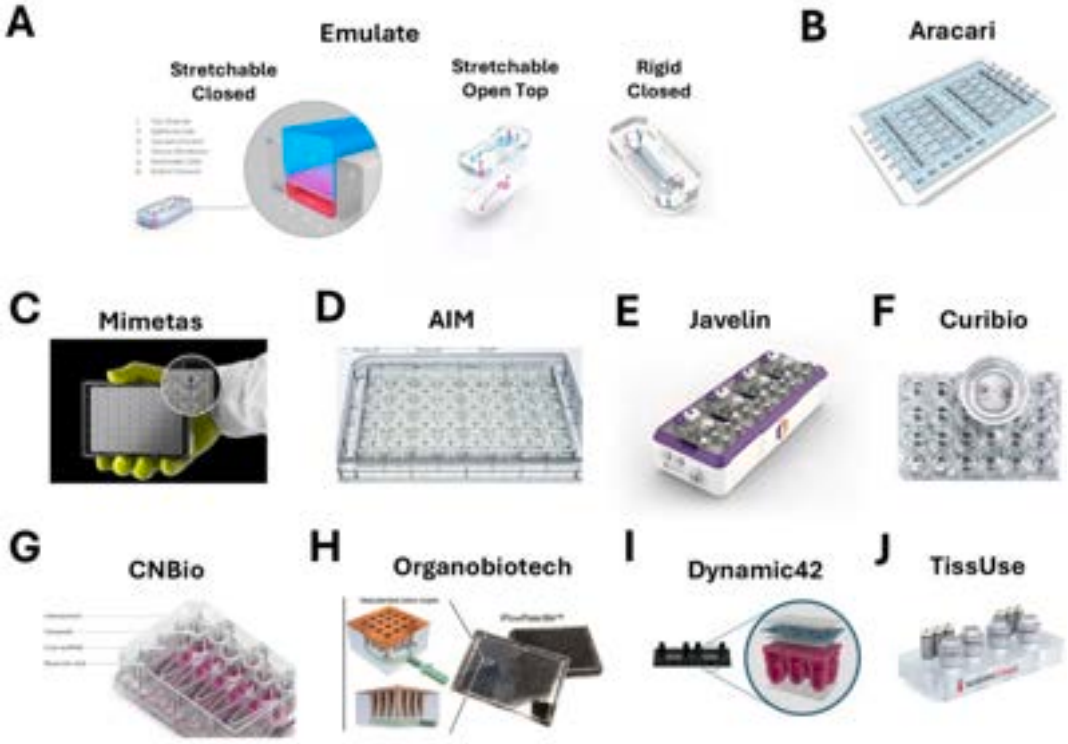

Figure 1. Examples of commercially available microfluidic Organ Chip systems. A) Emulate Organ Chips are optically clear and contain two parallel channels separated by a flexible porous membrane with two full height chambers on either side through which vacuum can be applied to mechanically stretch and relax the membrane and adherent cells. Different tissues can be cultured on the top and bottom of a shared ECM-coated membrane to recreate tissue-tissue interfaces or channels can be filled with an ECM gel containing one or more types of cells. extracellular matrix containing human immune cells. Variable configurations are now available such as open-top chips with a large top chamber that can contain a thicker ECM gel or tissue mass; chips made from flexible (PDMS) or rigid plastic that exhibit lower adsorption of small lipophilic molecules are also available. The company has sold a 12 chip Zoe instrument for automated culture of their Organ Chips, but they now offer a higher throughput 96 chip instrument (AVA) with integrated incubator and fluorescence microscope. B) Aracari multiplexed microfluidic platform features two endothelialized channels flanking a central diamond-shaped chamber filled with ECM gel. This configuration supports capillary sprouting and the formation of 3D microvascular networks, while enabling co-culture with embedded cells embedded within the ECM, such as cancer cells. C) Mimetas markets a highly multiplexed array of 3 parallel microfluidic channels. The middle channel is filled with and ECM gel that restrained from the parallel flow channels with a phase guide and cells are cultured on either side of the gel. D) Multiplexed AIM biotech device that is similar to Mimetas but instead of phase guides, the middle ECM channel is separated by triangle shaped pillars. E) Javelin Organ Chip has a flat cell culture surface over which medium is circulated and liked to an oxygenation chamber. F) Curbio Organ Chips enables stretching of connective tissues containing by using 2 pillars that replicates muscular motions. G) CNBio Organ Chip uses Transwell culture inserts but also enables circulating flow from apical through basal chambers. H) The multiplexed Organobiotech chip contains ECM patterned as intestinal crypts above a vascular channel. I) The Dynamic42 Organ Chip has 3 stacked channels: the middle channel contains ECM and other channels are used for culturing epithelial or mesenchymal cells. J) TissUse's Humimic multi-chamber Organ Chip system contains multiple mini-bioreactor chambers positioned on a flat plate, which can be cultured individually or fluidically coupled through a shared underlying fluidic channel.

edema. This unusual toxicity response also would not have been detected in static culture models, and thus, the ability of human Organ Chips to replicate physiologically relevant mechanical deformations is a critical advantage of this preclinical model.

Poor prediction of drug-induced kidney injury (DIKI) prior to clinical trials is a key risk of drug development[47]. Building on the success of human Lung Chips, a human Kidney Proximal Tubule Chip was similarly found to be able to detect renal cisplatin toxicity and Pgp efflux transporter activity that more closely mimicked human responses observed in vivo than previous results obtained with animals or cultured cells [18]. Subsequent studies have used Kidney Proximal Tubule or Glomerulus Chips to further investigate nephrotoxicities induced by treatment with cisplatin [17,48,49] as well as multiple other drugs, including adriamycin [20], cyclosporin [48–50], polymyxin antibiotics [51,52], digoxin [53], amphotericin B [54], doxorubicin [52], sunitinib [52], and tenofovir [49]). Adriamycin, for example, was shown to induce albuminuria and podocyte injuries in a Kidney Glomerulus Chip that were similar to toxicities induced in human patients by this cancer drug [20]. Other studies investigated toxicity to kidney chips of orally administered drugs by administering physiologically relevant doses of compounds to the epithelial side of Intestine Chips which were fluidically linked to Kidney chips by their vascular channels [44,50,55]. Together, these findings establish vascularized Kidney Chips as a promising platform for human-relevant nephrotoxicity assessment, addressing the long-standing challenge of predicting drug-induced kidney injury before entering costly and risky clinical trials.

The great potential translational value of Organ Chip technology was further demonstrated by showing that human Organ Chips lined with human endothelium and perfused with human whole blood can detect vascular thrombosis induced by a therapeutic anti-CD40L monoclonal antibody (Hu5c8)[56]. This is important because clinical development of Hu5c8, which was being developed for treatment of autoimmune disorders (e.g., lupus), was ended abruptly due to thrombotic toxicities detected in human clinical trials. These toxicities had not been seen in preclinical animal models, including studies with non-human primates (NHPs)[57].

A human Bone Marrow Chip that supports differentiation and maturation of multiple blood lineages over a month in vitro was able to replicate bone marrow injury, including myelo-erythroid toxicity after exposures to chemotherapeutic drugs and ionizing radiation, as well as marrow recovery after drug-induced myelosuppression [32]. Using this approach, it was possible to recreate known regimen-specific toxicities to 5-fluorouracil (5-FU) and to a candidate cancer drug (AZD2811) on-chip that were seen in human patients in a previous clinical trial but difficult to replicate in animals. Importantly, both these results were made possible by perfusing the drug through the endothelium-lined vascular channels of these two-channel Organ Chips so that they recreated clinically relevant PK of the drug, which in the case of AZD2811 was based previously quantified changes in drug levels in plasma of patients in a past Phase I clinical trial. This is a unique advantage of microfluidic Organ Chips over organoids and other static culture models. Other studies in Bone Marrow Chips have evaluated the toxicity of doxorubicin [58], transferrin receptor-targeting IgG1 antibody [59], T cell bispecific antibodies [59,60], CAR-T cell therapy [61], ionizing radiation exposure [62], and bacterial lung infection [62]. These studies confirmed hematopoietic lineage-specific toxicities observed in Bone Marrow Chips matched with toxicities observed in patients.

Rat, dog, and human Liver Chips containing four different primary liver cell types (hepatocytes, sinusoidal endothelium, Kupffer cells, and stellates) oriented in their relevant positions also have been created and used to study species-specific drug toxicity responses [63]. When a drug compound (fialuridine) known to cause fatal liver toxicity in humans, but not in animals, was perfused through these chips, hepatotoxicity was observed in the human chips but not in the animal chips, thus recreating the clinical observations. This study and numerous follow-up studies have shown that human Liver Chips can replicate various types of liver toxicities in response to drug treatments, including hepatocellular injury [64–67], steatosis [68–71], cholestasis [63,72], and fibrosis [73–76].

In a more recent landmark study, human Liver Chips created with the same four different primary liver cell types obtained from three different human donors and challenged with 27 known hepatotoxic and non-toxic drugs demonstrated an ability to detect small molecule drug-induced liver injury (DILI) with 87% sensitivity and 100% specificity, vastly outperforming standard animal models[77]. This human Liver Chip model was the first microphysiological system to enter the FDA's Innovative Science and Technology Approaches for New Drugs (ISTAND) Program that is designed to qualify new drug development tools for regulatory use*[78]*, and it is now in the final stages of evaluation. A microfluidic multi-organ "Body-on-Chips' model containing heart, liver, bone and skin tissue niches fluidically coupled by recirculating vascular flow was also used to model drug toxicities induced by the anticancer drug doxorubicin[79]. This study recapitulated the known organ-specific toxicities of this drug and allowed for the identification of early miRNA biomarkers of cardiotoxicity.

3b. Therapeutic efficacy evaluation

Human Organ Chips have also been shown to be excellent in vitro models for evaluating efficacy of a broad range of therapeutics in human-relevant disease models [46,80–132]. For example, studies using the Lung Chip model of pulmonary edema induced by IL-2 led to identification of potential new therapeutics for pulmonary edema, including angiopoietin-1 (Ang-1) and a transient receptor potential vanilloid 4 (TRPV4) ion channel inhibitor (GSK2193874) that later entered human clinical trials [46].

Another Lung Alveolus Chip that was perfused with human whole blood, which recapitulates *in vivo* pulmonary thrombosis responses, including clot formation and platelet-endothelial dynamics, was used to assess the efficacy of antithrombotic drugs[80]. When the protease activated receptor-1 (PAR-1) inhibitor, parmodulin-2, was perfused through these Lung Chips treated with lipopolysaccharide endotoxin (LPS) that promotes clot formation and disruption of the tissue barrier, it was found to significantly decrease platelet binding to the endothelium, thrombi formation, and pulmonary vascular leakage. These results were confirmed when similar studies were carried out in a mouse LPS lung injury model. The original Lung Alveolus Chip was also modified to model the small airway of the lung by lining the chip with a primary human mucociliary bronchiolar epithelium and an underlying microvascular endothelium[81]. Asthma was modeled in these chips by exposing the epithelium to IL-13, which replicated the goblet cell hyperplasia, cytokine hypersecretion and decreased ciliary function of asthmatics.

Other Lung Airway Chips that were lined with epithelial cells from patients with chronic obstructive pulmonary disease (COPD) displayed selective cytokine hypersecretion, increased neutrophil recruitment, and clinical exacerbation by exposure to simulants of viral and bacterial infections, as observed clinically in COPD patients[81]. Studies with the Asthma Lung Chip model revealed that a JAK inhibitor drug (tofacitinib) could suppress goblet cell hyperplasia, decrease secretion of G-CSF and GM-CSF, and restore normal cilia beating frequency, whereas treatment with dexamethasone was ineffective, providing results consistent with clinical studies in human patients. Similarly, when the COPD Lung Chip was stimulated with poly I:C to mimic viral infection and neutrophils were perfused through the vascular channel, a glucocorticoid drug (budesonide) that generally lacks activity in many COPD patients was also found to be ineffective at suppressing immune cell recruitment. In contrast, another drug (BRD4 inhibitor) that was shown to suppress lung inflammation in a mouse model significantly reduced immune cell recruitment on-chip. Importantly, when these same drugs were tested on similarly differentiated airway epithelium co-cultured with an underlying endothelium in static Transwell cultures, the effect of BRD4 inhibition was significantly reduced. This study revealed that the drug appears to act by preferentially inhibiting early neutrophil adhesion and

rolling responses via suppression of expression of endothelial adhesion molecules (e.g., E-selectin, VCAM-1, ICAM-1). These responses would have been missed in a conventional static culture.

This work was followed by several studies that used Lung Chips to develop in vitro models of disease that can be used to assess drug efficacy [82–86]. These models, which involved induction of injuries by various materials (e.g., formaldehyde [84], cigarette smoke [50], electronic cigarette vapor [51], nanoplastics [53]) as well as infectious agents, including microbial toxins [52,54], bacterial pathogens (e.g., *Mycobacterium tuberculosis* [87], *Haemophilus influenzae*[88]) and viruses (e.g., influenza, SARS-CoV-2, HCoV-NL63 viruses[89,90]), can serve as excellent models for assessing efficacy of drugs that target these conditions. For example, use of a Lung Chip model of respiratory infection by Influenza A, SARS-CoV-2, HCoV-NL63 confirmed that treatment with oseltamivir effectively reduced viral replication during Influenza A virus infection while camostat mesylate only showed weak inhibition of SARS-CoV-2 infection [89]. These results aligned with the weak effects on all-cause mortality and negative PCR results later observed clinically in COVID-19 patients with camostat mesylate [91]. Lung Chips also have been used to analyze responses to various anticancer therapies, including cisplatin[92,93], etoposide[92,94], gefitinib[82,95], MK-571 (an inhibitor of MRP-1)[94,95].

Having developed Organ Chip models of both lung alveolus and airway, it was possible to develop human Orthotopic Cancer Chip models. This was done by culturing non-small cell lung cancer (NSCLC) cells that have an activating mutation and a second acquired EGFR point mutation within Lung Alveolus Chips versus Lung Airway Chips and measure the effects of tyrosine kinase inhibitor drugs[96]. Comparison of tumor growth in these two different organ microenvironments revealed that the tumor cells proliferated much faster in the alveolus chips, which replicated their growth patterns seen in vivo. Interestingly, this study also revealed that the NSCLC cells are significantly more sensitive to the inhibitory effects of a third generation compared to a first-generation tyrosine kinase inhibitor (rociletinib versus erlotinib, respectively) as expected based on the double mutation. However, the NSCLCs were almost completely resistant to the inhibitory effects of rociletinib when growing in Lung Chips exposed to physiological breathing motions, which were also found to suppress overall tumor growth and invasion in the absence of drug. Using the Lung Chip model, the breathing effects on drug action were shown to be mediated through effects on epidermal growth factor receptor (EGFR) and MET protein kinase.

Subsequent work has confirmed that exposure to physiological breathing motions is crucial for accurately modeling disease phenotypes and hence, for assessing drug responses in human lung in a more clinically relevant manner [97–100]. For example, physiological breathing motions were shown to be necessary for the development of pulmonary fibrosis on-chip [97,101–104] and this model was used to assess effects of drug treatments, including the FDA-approved drugs, nintedanib and pirfenidone [101–103,105,106]. In one study that embedded flexible micropillars in a Lung Chip model of pulmonary fibrosis, two candidate small molecule drugs, BMS-986020 and KD025 were compared against nintedanib and pirfenidone [107]. This chip design enabled *in situ* sensing of contractile force produced by pulmonary fibroblasts and overall tissue stiffness. In parallel, the authors performed immunofluorescence-based expression of α-smooth muscle actin (SMA) and type I collagen to evaluate compound efficacies. They showed that both of their candidate drug compounds were similarly effective at reducing TGFβ-induced pro-collagen expression and tissue stiffness, and they were more effective at reducing α-SMA expression and contractile force generation than nintedanib but not pirfenidone [107]. These findings aligned with results of a previous study in which pirfenidone was found to be superior to nintedanib at attenuating the combined effect of TGFβ and physiological breathing motions on fibroblast contractile force, whereas the two were comparable in the absence of mechanical deformations [101].

Bone Marrow Chips have also been used to assess drug effects in models of marrow malignancies [108,109] and metastatic progression [110–112]. The effect of cross talk between osteosarcoma cells and immune cells within the bone marrow niche was studied in Bone Marrow Chips containing patient-derived osteosarcoma cells [105]. This study revealed increased CXCR4-CXCL12 interactions with tumor in Marrow Chips treated with bone marrow stromal cell-derived extracellular vesicles (EVs)[112]. The study also showed that this pathway could be inhibited using a small molecule inhibitor of CXCR4 (plerixafor) and that tumor cell death was further increased by co-administration of doxorubicin.

Drug efficacy also has been evaluated in Organ Chip models of the human small and large intestine [16,113–124]. Live imaging of a human Colon Chip that supports accumulation of a mucus with a bilayer structure and thickness similar to that observed in vivo revealed that addition of prostaglandin E2 (PGE2), which is increased during inflammation, causes rapid mucus volume expansion mediated by an increase in hydration state of the mucus[16]. Pretreatment of the chip with a drug (bumetanide) that inhibits the Na-K-Cl co-transporter 1 prevented this PGE2-induced increase in mucus thickness on-chip without altering total mucin content. During COVID-19, human Intestine Chips lined by primary duodenal organoid-derived epithelium and an intestinal microvascular endothelium were used to study infection with the coronavirus NL63 that uses the ACE2 Receptor for cellular entry much like SARS-CoV-2, and to evaluate the effects of antiviral drugs [113]. Interestingly, the organoid-derived intestinal epithelium was found to express ACE2 at higher levels when grown under flow and with peristalsis motions on-chip than when cultured statically as organoids in 3D ECM gels. This study showed that the approved protease inhibitor drug, nafamostat, could inhibit viral entry and reduce both viral load and cytokine secretion, while the direct acting antiviral drug, remdesivir, was not effective and that it was toxic to the endothelium. The same model of intestinal infection was also used to test the effects of other drugs that had been proposed for potential repurposing against SARS-CoV-2. Oral drugs that had been shown to inhibit infection by SARS-CoV-2 and other viruses in vitro, including toremifene, nelfinavir, clofazimine, and fenofibrate, were also tested in this model. Toremifene reduced NL63 viral load like nafamostat, while the other antiviral drugs were inactive at the doses tested.

Human Intestine Chips also have been used to model acute radiation injury and evaluate responses to radiation countermeasure drugs [12,114,115]. Exposure of Organ Chips lined by human intestinal epithelial cells and endothelial cells to γ-radiation resulted in ROS production, apoptosis, and increased DNA strand breaks as well as villus blunting, disruption of tight junctions, and intestinal barrier compromise. However, all these injury responses were prevented by pretreating the chips with dimethyloxaloylglycine[114]. Other Intestine Chips have been used to evaluate the therapeutic effects of small molecule drugs [116–119], antibody-based therapeutics [60,120], and probiotics[15,121–123]. In one example, Intestine Chips infected with *E. coli* bacteria that display increased barrier permeability and inflammatory cytokine release respond well to treatment with treatment with antibiotics, such as ceftazidime, amikacin, or penicillin-streptomycin, as well as probiotic treatment with *L. rhamnosus* GG [121]. In another study, *Bifidobacterium bifidum*, a known commensal bacterium, also was shown to protect against TNFα-induced increases in intestinal permeability [15]. These results with probiotic formulations suggest that microfluidic Organ Chips that enable co-culture of human cells for multiple days in direct contact with living microbiome across a physiological mucus layer[26,29,124] offer a novel preclinical tool to evaluate the potential the therapeutic efficacy of live biotherapeutic products (LBPs) that are composed of living bacteria or bacterial consortia.

Similarly, human Vagina and Cervix Chips have been developed that permit co-culture with either healthy (optimal) vaginal microbiome dominated by a consortium of *Lactobacillus crispatus* bacteria or a

consortium containing *Garderenella vaginalis* and other dysbiotic bacteria, which induces a phenotype similar to that observed in women with bacterial vaginosis[26,29]. The Vagina Chip model is being used to evaluate the disease reversing effects of treatments with LBPs containing multiple *L. crispatus* strains. It also has been used to test the therapeutic efficacy of an endolysin (BNT331) that specifically targets and lyses *G. vaginalis bacteria, alone and in combination with a L. crispatus* consortium[125].

More recently, a human Endocervix Chip created with patient-derived endocervical epithelium interfaced with matched stromal fibroblasts and exposed to hormones to mimic the third trimester of pregnancy was shown to support cervical mucus plug formation in vitro [126]. Cervical insufficiency that has been associated with pre-term labor can be modeled on this chip by including a dysbiotic vaginal microbiome on-chip, which promotes ECM dissolution in the stroma, increases pro-inflammatory cytokines, and promotes cervical ripening and mucus plug breakdown, similar to that seen in patients experiencing preterm labor. Importantly, use of this Organ Chip model led to the discovery that the inflammatory cytokine IL-1 act directly on the cervix to promote collagen breakdown in the stroma and administration of an approved therapeutic IL-1 receptor antagonist (anankinra) fully protected against development of cervical insufficiency in this model. Interestingly, perfusion of a drug that was previously approved for prevention of preterm labor but then deemed ineffective by the FDA (hydroxyprogesterone caproate) also was found to be ineffective in this model. In a separate study, a human Fallopian Tube Chip lined by patient-derived fallopian tube epithelium and stromal cells was shown to be useful as a preclinical tool to assess the effects of non-hormonal contraceptives by treating chips that are impregnated with living human sperm[127].

In addition, human Lymphoid Organ Chips have been developed to assess the efficacy of therapeutic vaccines and adjuvants [128,130,131,269–271]. For example, by culturing primary human B and T cells with antibody presenting cells in an ECM gel within one channel of a two-channel Organ Chip and perfusing medium through the parallel channel, it was possible to promote self-assembly of lymphoid-like structures[128]. Importantly, these chips respond to vaccination with commercial influenza Fluzone vaccine by organizing germinal center-like structures, forming plasma cells, generating high affinity IgG directed against the hemagluttinin A antigen in the vaccine, and secreting cytokines that appear similar to those detected in patients receiving a similar vaccine. The additive effects of adjuvants were also detected in this model as well as development of cellular and humoral immune responses to a DNA origami scaffold-based vaccine when conjugated with antigenic peptides or proteins[129]. More recently, this Lymphoid Organ Chip was combined with a muscle cell culture chamber to create a model of intramuscular vaccination, which was used to replicate vaccination by self-replicating mRNA vaccines and commercial Moderna and Pfizer mRNA COVID-19 vaccines as well as to demonstrate vaccination responses to a naive antigen (Rabies virus) and provide evidence of a somatic hypermutation response on-chip [130]. A modified version of this Lymphoid Organ Chip also was shown to mimic a vaccine boost in response to perfusion with the SARS-CoV-2 spike protein by inducing spike-specific memory B cells, plasmablast differentiation, and secretion of spike-specific antibodies[131]. In this study, responses to Wuhan monovalent and Wuhan/Omicron bivalent mRNA vaccine boosts were compared and found to show similar induction of Omicron neutralizing antibodies, consistent with immune imprinting that has been reported in vivo.

Finally, multi-organ human Body-on-Chips systems have been used for drug testing as well [28,38,42,132]. One study fluidically coupled microfluidic devices lined by HepG2/C3A liver cells, MEG-01 megakaryoblasts (to represent bone marrow), MES-SA uterine cancer cells, and a multidrug-resistant variant of these cancers cells (MES-SA/DX-5) to test the efficacy of different drug combinations against toward multidrug resistance[132]. The findings suggest that combining the anti-cancer drug doxorubicin with multi-drug resistance modulators (cyclosporine and nicardipine) will be more efficacious than use of any one of these agents alone. Importantly, while the drug combination killed the drug-resistant tumor cells, it had tolerable effects on normal tissues.

3c. Evolution of drug resistance

One of the critical challenges in therapeutic development is the emergence of drug resistance. Human Organ Chips provide a model to simulate this process [133–147]. For example, human Lung Airway Chips were used to mimic human-to-human transmission of influenza viral infections by sequentially passing mucus droplets from the airspace of one infected chip to the air channel of another while being treated with suboptimal doses of the antiviral drugs amantadine or oseltamivir [133]. This study demonstrated the spontaneous evolution of influenza virus through both mutation and gene reassortment, resulting in the spontaneous emergence of clinically prevalent resistance mutations, and strains that were resistant to both drugs. On the other hand, viral resistance was not observed when the chips were treated with nafamostat, an inhibitor targeting host serine proteases. This human preclinical model may enable the preemptive design of new and more effective antiviral drugs and vaccines that are less likely to lead to resistance, which may be extremely valuable for confronting viral pandemics in the future.

As described above, a different type of resistance to drug treatment was observed in human Lung Cancer Chips that were treated with rociletinib when the chips were cultured in the presence or absence of breathing motions. That study revealed that breathing motions suppressed growth inhibition by this TKI drug, thus demonstrating that physiological mechanical forces can play a critical role in how cancers resist drug treatments. A subsequent study showed that co-culture with cancer associated fibroblasts (CAFs) could promote resistance to osimertib, another TKI targeting EGFR[134]. Similar results were obtained with other anti-cancer therapies, including Toposar[94,135,136], Gefitinib[137–139], paclitaxel[138], and gemcitabine[36,138,140]. In addition to cancer drug resistance, several studies have investigated resistance mechanisms to a variety of pathogens, including influenza [141–143], *Staphylococcus aureus*[141,144,145], *Enterococcus faecium* [146], *Candida Albicans* [147], and Pseudorabies Virus[148].

3d. Prediction of drug pharmacokinetics

The human Body-on-Chips approach also has been used in combination with computational PK/PD modeling to quantitatively predict human drug PK parameters (e.g., C_{max}, $t_{1/2}$) for an oral drug (nicotine administered into the lumen of an Intestine Chip) and an intravenous therapeutic (cisplatin introduced into the vascular flow path) using only data generated from 3 different fluidically linked Organ Chips (intestine, liver, and kidney or liver, kidney, and bone marrow, respectively)[55]. In this study, fluids were transferred from the outflow of one chip to the inlet of another using a robotic liquid handler rather than tubing to minimize dead space and permit mass spectrometric analysis of drug and metabolite levels within every compartment of the multi-organ model. An "arterio-venous reservoir" also was incorporated into the flow path to facilitate medium mixing and permit fluid sampling so that it is not linked to effluent of any one Organ Chip and more similar to a blood sample removed from a peripheral vein in a patient.

Multiple other multi-Organ Chip studies also have leveraged fluidic linking and dynamic fluid flow to successfully validated several drugs (e.g., midazolam, diclofenac, hydrocortisone) against known human PK parameters in vitro [149,150]. Key PK-relevant parameters that have been modeled in vitro using this approach oral bioavailability, intestinal permeability, hepatic clearance, and tissue-specific distribution and metabolism, among others.

4. Drug discovery enabled by organ chips

There are multiple examples where analyzing fundamental disease mechanisms using Organ Chips have led to identification of potential

new therapeutics. However, a persistent challenge in translating Organ Chip technology into mainstream drug discovery pipelines has been throughput. While individual chips offer unparalleled physiological fidelity, their complexity of use has historically limited scalability relative to conventional 2D cell cultures and organoid-based platforms. Recent work has begun to address this limitation performing 'omics' on Organ Chips [12,151–153], and there are now many different types of commercial Organ Chip platforms, some of which offer higher-throughput chip configurations capable of supporting compound screens (**Fig. 1**) [31,60,154,155]. However, most recently genetic screens [115,151,156] and AI analysis [89,115,157,158] have been combined with Organ Chips for rapid target validation in a human-relevant context (**Fig. 2**), as will be described below.

4a. Therapeutic advances based on new mechanistic insights from Organ Chips

Early Lung Chip studies that revealed the important role of breathing motions and activation of the mechanosensitive TRPV4 ion channel for development of pulmonary edema led to advancement of a TRPV4 inhibitor into human clinical trials[46]. This was followed by demonstration that administration an adeno-associated virus (AAV) vector delivery system encoding the high homology domain of the TRPV4-associated transmembrane protein CD98 can protect against development of pulmonary vascular leakage [159]. Indeed, one of the major advantages of microfluidic Organ Chips that contain tissue-tissue interfaces and experience dynamic fluid flow compared to organoids or static cultures is this ability to assess the efficacy of therapeutics when they delivered in clinically relevant drug delivery vehicles. For example, in another study focused on creating a pan-viral treatment strategy, polymeric nanoparticles were used to deliver CRISPR RNA (crRNA) antiviral therapeutics targeting highly conserved regions of the viral genome in a Lung Alveolus Chip model of influenza A virus infection [160]. These crRNAs effectively inhibited replication of both H1N1 and H3N2 influenza viruses, while producing minimal off-target effects, as confirmed by transcriptomic analysis. More recently, a lipid nanoparticle delivery system modified to target preferentially to lung was shown to induce dose-dependent inhibition of tumor cell growth when loaded with a immunostimulatory duplex RNA that potently induces production of protective Type I and III interferons and perfused through the endothelium-lined vascular channel of a human Lung Cancer Chip[161].

Analysis of the mechanochemical mechanism by which breathing motions suppress viral infection in a human Lung Alveolus Chip also led to repurposing of a drug as a therapy to prevent cytokine storms in COVID-19 patients[152]. In this study, mechanical forces associated with breathing motions were found to activate innate immunity (e.g., protective Type I interferons). Transcriptional analysis related that this response was mediated by activation of TRPV4 and by signaling via the receptor for advanced glycation end products (RAGE). These findings suggested that TRPV4 and RAGE may serve as new therapeutic targets for patients infected with influenza virus. Indeed, administration of the RAGE inhibitor azeliragon, which was previously tested in human Phase III clinical trials for Alzheimer's Disease, resulted in near complete suppression of cytokine induction, while inhibition of TRPV4 suppressed both inflammation and viral burden in infected Lung Chips with breathing motions. Data from this manuscript were included in a pre-investigational new drug (IND) application to the FDA by Cantex Pharmaceuticals (the pharmaceutical company that manufactures the drug) and the compound moved into human clinical trials for COVID-19. Results obtained testing a therapeutic monoclonal antibody[161] that blocks complement activation in a Neuromuscular Junction Chip model of demyelinating neuropathies that includes motor neurons linked to Schwann cells[162] also were included in an IND application submitted by Dianthus Therapeutics, Inc., which enabled a Phase II clinical trial for treatment of generalized myasthenia gravis.

More recently, a modified version of the Lung Alveolus Chip model of influenza infection was used to model macrophage-exacerbated lung injury and how mechanical overdistension associated with ventilator use drives bacterial superinfection[153]. By incorporating macrophages

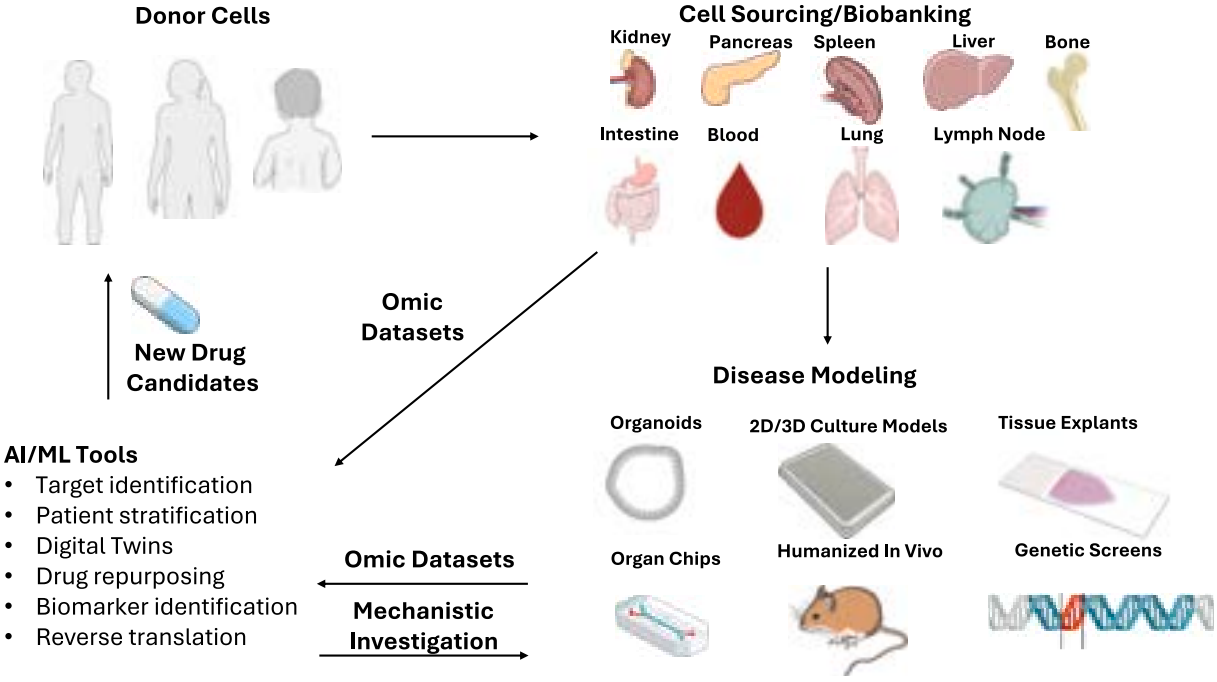

Figure 2. Schematic illustrating how human Organ Chip platforms can integrate with and enhance traditional drug discovery workflows. Early-stage target identification and validation are supported by genomic tools (e.g., CRISPR-based screening) and high-throughput in vitro assays. Human Organ Chips bridge this translational gap by enabling physiologically relevant modeling of human tissue- and organ-level physiology in the presence of dynamic flue flow and mechanical cues, supporting more predictive assessment of drug efficacy, toxicity, and pharmacokinetics. Integration with AI facilitates analysis of complex datasets from humans, animals, or Organ Chips accelerates drug repurposing and target discovery. Together, these approaches position Organ Chips as a central platform linking high-throughput screening to clinically relevant human biology, with the potential to reduce reliance on animal models and improve clinical success rates.

into the air channel of the chip and applying abnormally high levels of mechanical strain, the presence of macrophages were shown to drive key hallmarks of severe influenza, including activated cell death programs. In addition, pathological levels of mechanical distension suppressed innate immunity and enhanced secondary *Pseudomonas aeroginosa* infection. Use of computational gene network analysis led to the identification that SIRT1 suppression underlies both responses, and experimental administration of SIRT1 activator drugs confirmed this mechanism.

During COVID-19, use of human Lung Airway Chips infected with pseudotyped SARS-CoV-2 virus led to the identification of the antimalarial drug amodiaquine as a potential therapeutic for this disease whereas hydroxychloroquine and chloroquine were not active in this model[90]. These results support the translation of amodiaquine into clinical trials for COVID-19 at multiple sites in Africa. Studies using the same Airway Chip model infected with influenza virus revealed that co-administration of the protease inhibitor drug nafamostat with the lead antiviral drug oseltamivir (also known as Tamiflu) doubled the time that oseltamivir displayed effective viral inhibition from two to four days, which could have significant value in the clinic.

Patients with COPD are known to experience exacerbations of the disease when infected with influenza virus [272]. Human Lung Chips lined by epithelium from COPD patients replicated this exacerbation when infected with influenza virus *in vitro* as they exhibited higher viral titers, greater barrier disruption, and more intense inflammation than similar chips lined by healthy epithelium[143]. Transcriptional studies revealed upregulation of genes encoding serine proteases that are known to facilitate viral entry and downregulate interferon-related genes responsible for antiviral immune responses. Importantly, treatment of the virus-infected COPD chips with the protease inhibitor drug, nafamostat, prevented development of disease phenotypes.

Organ Chip models of the female reproductive tract also have been useful for gaining insight into complex diseases that resulted in identification of potential new therapeutic approaches [26,125–127]. For example, use of the human Vagina Chip model of bacterial vaginosis[26] revealed that administration of a probiotic *L. crispatus* consortium synergizes with an endolysin therapeutic candidate, BNT331, that targets the dysbiotic *G. vaginalis* bacteria that play a central role in this disease [125]. Administration of *L. crispatus* alone failed to engraft or displace the *G. vaginalis* bacteria, but it suppressed inflammation while BNT331 only killed the dysbiotic bacteria. However, simultaneous administration of both treatments resulted in higher engraftment of *L. crispatus* on-chip, inhibition of *G. vaginalis*, and a reduction in inflammation. As described above, use of a human Endocervix Chip also led to the identification of IL-1 as a major mediator of changes in the cervix that lead to cervical insufficiency associated with preterm labor. In that study, the FDA approved IL-1 receptor antagonist, anakinra, was shown to prevent these effects, thus raising the possibility that it may offer a new therapy for this application[126].

Use of human Intestine Chip models of nutritional deficiency-induced enteric dysfunction lined by cells from young women also revealed that an antimalarial combination of antibiotics (sulfadoxine-pyrimethamine) can reverse multiple intestinal absorptive abnormalities and suppress inflammation that can be seen in these patients[163]. This is an important finding because this antimalarial therapy has been surprisingly found to increase the birth weight of infants in pregnant women in sub-Saharan Africa, independently of malarial infection, in a past study [273,274]. Thus, these chip results support the possibility that sulfadoxine-pyrimethamine could be used to improve maternal absorption and thereby promote healthier fetal growth.

There are also examples where use of human Organ Chips led to insights that raised the possibility of new therapeutic strategies even though specific drugs were not identified [164,165,282]. For example, using a Liver Chip, two naturally occurring polyphenols (quercetin and hydroxytyrosol) were found to inhibit development of free fatty acid-induced hepatocellular steatosis based on their antioxidant and lipid-lowering properties. These two compounds, or drugs based on their structures, potentially could be useful for preventing or treating nonalcoholic fatty liver disease[164]. By studying a Heart Chip created with Barth Syndrome patient-derived iPSCs to model this disease, a mitochondrial-targeted ROS scavenger compound (MitoTEMPO) was found to partially reverse the contractile defects in human cardiac tissue, again offering a new drug development path to explore[165].

4b. Synergy with high throughput screens

To more directly integrate Organ Chips with the drug discovery pipeline, several groups have altered Organ Chip designs to support higher-throughput testing (**Fig. 1**)[19,38,69,89,154,166–169]. One early example involved development of a scaled-down horizontal microchannel design that enables approximately 350 microchannels to be interrogated in parallel[154]. This platform was used to screen a library of 159 compounds for hepatotoxicity, demonstrating the feasibility of organ-level toxicity screening at a scale not previously achievable with conventional Organ Chip formats[19]. More recently, this platform was used to perform a 1537 kinase inhibitor screen to identify potential angiogenesis therapeutics [170]. Although not used directly for drug development, in another study an array of micropumps compatible with an array of Organ Chips was aligned with optical oxygen sensors to assess oxygen consumption rates in high-throughput [171]. Human renal proximal tubule cells were seeded in these devices, enabling high-throughput real-time measurement of oxygen consumption in response to compound treatments. While designed in a 96-well format, the scale of these designs is still somewhat prohibitive as compared to 2D and organoid-based platforms. To be broadly applicable to pharmaceutical development, reproducibility and scalability concerns must be addressed[172]. Much work has focused on scalability and reproducibility of device fabrication or bioprinting[173–177], and recent work has begun to address reproducibility of results obtained in various ways [178,267,268]. One example is through development of a fully-automated end-to-end process, dubbed “Screening Station No. 2,” using a large-scale SCALE12-MR rocking incubator and automated image analysis[178]. This platform was demonstrated to be capable of identifying both a positive angiogenic effect of treatment with known vascular growth factors (VEGF, S1P, PMA), as well as a negative, dose-dependent effect of Sunitinib treatment.

Rather than using Organ Chips to screen for new drugs directly, an alternative approach involves using Organ Chips to validate compounds identified using high throughput screens in conventional 2D cultures [90]. During the COVID-19 pandemic, one study tested FDA-approved anti-viral compounds that inhibited other coronaviruses in early studies in cultured Huh-7 cells treated with SARS-CoV-2 pseudoviruses to assess their potential ability to inhibit cell entry. Notably, Organ Chips successfully filtered out false-positive hits obtained in the Huh-7 cell studies, including chloroquine and hydroxychloroquine[83], which were later also confirmed to be inactive in non-human primates as well as humans, as described above. In contrast, it predicted amodiaquine to be active against viral entry. This result was confirmed in studies with infectious SAR-CoV-2 in a hamster model, which helped move the drug to human clinical trials.

An alternative approach involves integrating Organ Chips with functional genetic screens. For example, a screen using greater than 200 small interfering RNAs (siRNAs) was first carried in cultured human lung cells to identify gene targets that protect these cells against infection with influenza virus [179]. Two siRNAs were identified that protected the cultured cells as well as human Lung Airway and Alveolus Chips against viral infection by inducing large increases in secretion of Type I and III Interferons. Interestingly, when control studies were carried out with other siRNAs directed against the same gene targets they were inactive. This led to the serendipitous discovery of a new class of potent immunostimulatory, short, duplex RNAs that produce broad-spectrum inhibition of infections by many different respiratory viruses (e.g., SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-NL63, influenza H1N1 and H3N2) virus in cell lines, human Lung Chips and a mouse SARS-

CoV-2 infection model[179]. In addition to testing the efficacy of potential gene therapies, Organ Chips have been used to test the effectiveness of proposed cell therapies, particularly CAR-T cells[180–183]. One study used a vascularized, perfused, breast-cancer-on-a-chip model to assess transmigration of perfused immune CAR-T cells, infiltration into the tumor, and cytokine release for up to 8 days[182]. Notably, patient-specific responses to CAR-T cell therapy due to differences in ROR1 expression were conserved in this chip system.

The versatility of the CRISPR/Cas9 system provides another exciting approach because it enables genetic perturbation across the human genome with a single experimental toolkit[184,185]. In a recent study, kinome-scale CRISPR screening was performed on human endothelial cells from different tissues of origin (intestinal, lung, umbilical vein) to identify potential therapeutic targets for development of radiation countermeasures that protect against injuries induced byacute γ-radiation exposure[156]. This study identified CLK2 inhibition as a potential way to protect against radiation damage, and pharmacological inhibition of CLK2 using the drug TG003 protected endothelial cells against radiation injury in human Intestine and Lung Chip models even when added after radiation exposure. Notably, the same treatment did not protect Caco-2 intestinal epithelial cells against radiation damage, and hence this target would likely not have been identified if the screens had been performed in immortalized cell lines. A recent study demonstrated proof-of-concept for CRISPR screens in engineered systems, although an Organ Chip was not utilized. In this study, functional genomic screens were carried out directly within a perfused, tissue engineered construct containing MBA-MB-231 cancer cells co-cultured with primary stromal cells and endothelial cells [186]. Notably, the authors showed that patient-specific results were attainable via this approach.

4c. AI-enabled drug repurposing

Multiple types of computational algorithms have been developed for target identification [187–191], predicting efficacy [192–195], and drug design [196–202]; however, the integration of these tools with Organ Chips is only a recent development [203–207]. One of the most successful integrations to date of Organ Chips with AI has been through automated analysis of high-content imaging [203,208]. More recent applications of AI in drug discovery have demonstrated significant impact on drug identification and repurposing and it is being increasingly used to analyze the complex datasets generated by in vitro models as well as clinical datasets. During the COVID-19 pandemic, AI-enabled dynamic molecular modeling was utilized to discover compounds which target a conserved region of the SARS-CoV-2 spike protein which mediates membrane fusion[209]. The study revealed bemcentinib, an orally-available, FDA-approved AXL kinase inhibitor as capable of binding to this site and inhibiting viral entry, independent of its known activity. The study then used AI-enabled chemical structure selection to predict novel bemcentib analogs that should exhibit antiviral activity, without exhibiting AXL kinase inhibitory activity. Chemical synthesis and testing of these compounds confirmed that they did not inhibit AXL kinase, yet the effectively prevented viral entry in a human Lung Chip infected with multiple types of pseudotyped SARS-CoV-2 viral strains and suppressed SARs-CoV-2 infection in an animal model [196]. A related study used this AI-enabled molecular dynamics simulation approach predicted that self-assembly of flavonoids, such as quercitrin, should slow molecular activities, which was predicted to increase cell viability under stress[210]. The study also experimentally confirmed that pretreatment with quercitrin protects against cellular toxicity induced by exposure to with γ-irradiation.

The Network Model for Causality-Aware Discovery (NemoCAD) algorithm represents a different AI-enabled drug repurposing approach. This algorithm compares disease-associated transcriptomic signatures with drug-induced signatures from the LINCS database that contains many FDA-approved drugs, and then prioritizes potential drug candidates for repurposing by comparing results in context of gene-gene and gene-drug networks using Bayesian analysis with machine learning [142,143]. Use of NemoCAD to compare transcriptomic profiles of a MeCP2 knockout *Xenopus laevis* tadpole model of Rett syndrome versus healthy controls led to the identification of the FDA-approved drug vorinostat as a potent effective therapy. Importantly, this drug was shown to improve central nervous system abnormalities as well as gastrointestinal, respiratory, and inflammatory phenotypes observed in patients with this disease in both MeCP2-null tadpole and mouse models [143]. Based on these results, vorinostat recently entered human clinical trials for treatment of Rett syndrome. NemoCAD has also been used to repurpose the drug donepezil as a therapy that can slow metabolism induce a torpor-like state, which was found to be useful for increasing viability of whole organs, which may be useful for surgical transplantation [211].

More recently, NemoCAD was applied to repurpose drugs for treatment of acute radiation injury by comparing transcriptomic data from the epithelial and endothelial compartments of human Lung Alveolus Chips exposed to γ-radiation versus controls [12]. HMOX1 emerged as one of the top ten putative targets, and treatment with lovastatin and prednisolone attenuated radiation-induced lung injury. A similar approach was carried out with human Intestine Chips lined by ileal epithelial cells and intestinal microvascular endothelial cells exposed to radiation. Use of NemoCAD led to the identification of the FDA-approved antifungal drug, miconazole, as a potential radiation countermeasure and this prediction was rapidly validated in the human Intestine Chip model[115].

Beyond drug repurposing and target identification, AI has been integrated with microfluidic platforms[212–216] and Organ Chips [207,217–220] as a tool for predictive analytics and data analysis. In an early iteration, the sizes of microdroplets produced by water-in-oil emulsions was predicted using a simple neural network with 10 neurons and droplet length as a single output parameter[213]. A subsequent study used a recurrent neural network (RNN) to predict the magnitude and location of contact pressure in soft sensors, the prediction of which is often confounded by the non-linearity of responses by these devices [214]. In addition to prediction of physical parameters relevant to Organ Chip cultures, AI has been used as a tool for experimental data analysis and for predicting biological outcomes [217–220]. In one example, a pre-trained convolutional neural network (CNN) was shown to be capable of accurately tracking and characterizing single cells in time-lapse images derived from Organ Chip co-cultures of HER2+ BT474 breast cancer cells, fibroblasts, endothelial cells, and PBMC[221]. Notably, the effect of treatment with the immunotherapy drug trastuzumab, an antibody therapy directed against the HER2 receptor which promoted interactions between PBMC and HER2+ breast cancer cells, was accurately characterized by the CNN.

In a separate study, a deep learning-based neural network was successfully able to predict the differentiation of hematopoietic stem and progenitor cells solely by assessing changes in cell morphology and motion in a microfluidic chamber[218]. Impressively, this network was able to predict the differentiation trajectories of individual cells prior to the appearance of conventional molecular markers of hematopoietic differentiation. A myriad of generative and non-generative deep learning-based tools have also been developed for the integrated analysis of multi-omic data[110,222–231], which have been reviewed extensively elsewhere[231–233]. While not developed using Organ Chips, the BEHAV3D system integrates high-content imaging data capturing functional interactions between tumor-derived organoids and T cells with single-cell transcriptomic data from the same cells[234]. This system was able to identify 27 genes with no previously known T cell function whose expression could predict the likelihood of cells engaging in killing behavior, highlighting its potential for analysis of more complex systems, such as cancer-on-a-chip models[235].

5. Advantages and disadvantages of organ chips versus other preclinical models

Organ Chips incorporate physiological cues and interactions

between different cell types that are highly valuable; however, this limits their throughput compared to traditional 2D cultures, which at present can be scaled to millions of data points[236–239]. In contrast, common Organ Chip designs are limited to tens to hundreds of devices due to individual seeding requirements. Additionally, cutting-edge biotechnologies are often tested in cancer cell lines and mice, resulting in limited translation to native human tissues[240–242]. In contrast, Organ Chips can model physiological dosing regimens in human cells as well as heterogeneous cell-cell interactions, enabling earlier identification of human-relevant efficacy and toxicities[11,32,243,244]. Thus, in comparison to 2D cell cultures, Organ Chips represent a superior platform for predicting drug actions in humans, but as of yet they cannot reach the scale of throughput of traditional cultures.

In comparison to organoids, Organ Chips reconstitute tissue-specific architectures, enabling more relevant interactions among relevant cell and tissue types, but this adds greater model complexity. Nevertheless, the importance of this structural complexity is clear. For example, the formation of a mucus layer with in vivo-like thickness and bilayer structure in human Colon Chips requires dynamic fluid flow, even when the chips are lined by organoid-derived epithelium that do not exhibit these features in static culture [245]. Transcriptomic profiles from Intestine Chips lined with human duodenal organoid-derived epithelial cells also more closely mimic freshly isolated duodenal tissue than static organoid cultures [100,209]. Similar results have been obtained by other groups studying gastrointestinal organoid-based Organ Chip models[246–248]. Furthermore, organoids possess substantial variability in formation, size, morphology, and cell types present due to factors such as lack of supporting cell types, an uncontrolled culture environment, an undefined animal-derived 3D culture matrix, and the inherent stochasticity of *in vitro* cell fate decisions[43]. Organ Chips address several of these concerns by controlling the geometric arrangement of cells, providing a defined chemical environment, as well as providing biophysical cues for development[8]. Organoids also rely on embedding in Matrigel or similar 3D ECM gels, such that high-throughput screens using this system are technically constrained in ways that conventional 2D cultures are not [249]. Nonetheless, they have recently been shown to be capable of being integrated into high-throughput discovery platforms, enabling hundreds to thousands of compounds to be screened simultaneously [250–253]. Thus, for the purposes of high-throughput drug discovery, organoids represent a valuable intermediate between traditional 2D cultures and Organ Chips.

In contrast to animal models, Organ Chips are higher-throughput and represent interactions between human cells, with key disadvantages being issues with cell sourcing, donor-to-donor variability, and inability to model complex phenotypes such as cognitive functioning[254]. Furthermore, versatile genetic toolkits have been developed for animal models beyond what have been developed for human Organ Chips, and genetic modification of primary cells is notoriously difficult. In addition, issues with cell sourcing adds to technical challenges involved in genetic modification of primary cells. Thus, future work will need to focus on developing improved genetic tools for use in Organ Chip models.

The bigger concern for animal models, despite functioning as the gatekeeper for human trials over the past fifty years, is their poor predictive accuracy as more than 70% of drugs that pass safely through animal testing fail in the clinic[1,77]. Despite this, well-defined, in-bred, animal models enable studies to be rapidly reproduced with similar results, whereas Organ Chips lined by cells from different human donors may exhibit greater variability. However, this is also a strength of human Organ Chips as this variability mimics the natural variation observed in human clinical studies. In addition, results from Organ Chips have shown the potential to improve predictions of human drug responses compared to animal models[77], therefore, their inclusion as additional gatekeepers of clinical development merits serious consideration[254].

6. Limitations and future research recommendations

While significant advances have been made in the Organ Chip field, several important limitations remain to be addressed. Challenges related to cell sourcing, standardization, and scalability have been extensively reviewed elsewhere[8,255]. Although Organ Chip models are gaining acceptance for safety assessment and toxicology studies, the integration of stromal and immune cell populations remains limited in many platforms, which may reduce their ability to accurately predict complex disease responses. With only a few notable exceptions, most current models do not incorporate both circulating and tissue-resident immune cells or complex human organ-specific microbiome, despite their critical roles in disease pathogenesis and therapeutic response. For example, a recent inflammatory bowel disease (IBD) Organ Chip study demonstrated that IBD-associated fibroblasts can regulate epithelial barrier dysfunction and immune cell infiltration[256]. However, IBD is a highly complex immune-mediated disease involving dynamic interactions among epithelial, stromal, immune, microbial, and metabolic components[257–263]. Consequently, modeling additional disease features such as microbiome dysbiosis, tissue-resident immune populations, fibrosis and stricture formation, and mesenteric adipose tissue expansion within a single Organ Chip system remains challenging. Multi-organ and interconnected Organ Chip platforms have been successfully established by several groups[8,10,37,162], and future efforts could leverage these approaches to model interactions among distinct intestinal compartments, including the mucosa, muscularis propria, and mesenteric adipose tissue. Such systems may provide a more comprehensive representation of human intestinal physiology and pathology, particularly in complex diseases such as IBD.

AI and ML algorithms clearly have significant potential in drug discovery, but several limitations remain. First, the integration of AI with Organ Chip technology is still in its early stages, and relatively few studies have demonstrated its full capabilities. AI models are highly dependent on the quality and quantity of experimental data, meaning inaccurate or incomplete datasets can lead to unreliable predictions [264,265]. Many AI-based drug discovery approaches also require validation in human-relevant models such as Organ Chips before their predictions can be trusted. Finally, despite promising results in drug repurposing and target identification, AI-generated findings still require extensive experimental and clinical validation before they can be translated into approved therapies[266].

7. Conclusion

Human Organ Chips provide insight into molecular mechanisms in physiologically relevant human organ-relevant contexts, incorporating tissue-tissue interfaces, dynamic fluid flow, mechanical forces, and immune cell interactions. These features distinguish Organ Chips from conventional static cell cultures, organoids, and animal models, and they underpin the ability of this technology to reveal new disease mechanisms, identify novel therapeutic targets, and enable preclinical evaluation of drug safety and efficacy in a human-relevant context. Human Organ Chips have the capacity to generate clinically actionable insights that other preclinical models cannot, including prediction of safety risks which escaped animal testing (e.g. thrombosis induced by anti-CD40L antibody Hu5c8, fatal hepatotoxicity by fialuridine) and identification of new therapeutic candidates (e.g. TRPV4 inhibitors for pulmonary edema).

Still, challenges to widespread adoption remain. Cell sourcing, device standardization, and scalability continue to be active areas of development, and the incorporation of stromal and immune cell populations into Organ Chip models, while advancing, remains incomplete in many platforms. The complexity of multi-organ systems, while enabling more physiologically comprehensive modeling of drug absorption, distribution, metabolism, and excretion, also introduces substantial technical demands that limit their accessibility and throughput.

Furthermore, while the human Liver Chip has made landmark progress as the first microphysiological system to enter the FDA's ISTAND program for regulatory qualification, broader regulatory adoption of Organ Chips as accepted tools for safety assessment will require continued generation of cross-platform validation datasets and standardized reporting frameworks. The extensive testing required for the Liver Chip in the ISTAND program suggests that future acceptance will be time-consuming and costly, as well as being dependent on existing clinical datasets such as those published by the IQ consortium[78]. Therefore, regulatory acceptance will likely proceed slowly despite the promise of these systems.

The convergence of Organ Chip technology with high-throughput screening and artificial intelligence, while still in its early stages, offers a compelling path toward addressing the throughput limitations that have historically constrained the role of these systems in mainstream drug discovery pipelines. As described in this review, scaled Organ Chip platforms have already demonstrated the feasibility of compound library screening at organ-level physiological fidelity, and functional genomic approaches including siRNA and CRISPR-based screens have been successfully integrated with Organ Chip validation to identify and confirm new therapeutic targets in human-relevant tissue and organ contexts. AI-enabled approaches, including NemoCAD-based transcriptomic drug repurposing, automated high-content image analysis, and deep learning-based prediction of cellular behavior, have further expanded the analytical capabilities of these platforms, enabling insights that would not have been attainable through traditional experimental approaches alone. Nonetheless, the dependence of these AI tools on high-quality, well-annotated experimental datasets, and the need for extensive experimental and clinical validation of AI-generated predictions, underscores the importance of continued investment in generating robust, reproducible Organ Chip datasets that can serve as reliable training and validation resources.

The full utility of Organ Chips will require integration across the drug discovery pipeline, pairing high-throughput formats and genomic screens for early target identification with multi-organ systems for pharmacokinetic modeling and toxicity assessment, incorporating patient-derived iPSCs and organoid-derived cells, and pairing experimental data generation with AI-driven analytics to accelerate target identification, compound prioritization, and mechanistic understanding. As regulatory agencies, including the FDA, increasingly recognize the limitations of animal models and express openness to alternative approaches through initiatives such as the FDA Modernization Act 2.0, the scientific and regulatory landscape is becoming increasingly favorable to broader adoption of Organ Chip technology. By providing a human-relevant preclinical model that can recapitulate the complexity of living human tissues and organs at a level not previously achievable in vitro, Organ Chips hold promise to improve the speed, cost-efficiency, and clinical success rate of bringing new therapies to patients, while simultaneously reducing the reliance on animal testing that has long defined preclinical drug development.

Declaration of competing interest

D.E.I. is a founder, member of the board, and chair of the scientific advisory board of Emulate Inc.; he also holds multiple patents covering Organ Chip technology.

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Data availability

No data was used for the research described in the article.

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