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Trends in Biotechnology

Opinion

3D human tissue models and microphysiological systems for HIV and related comorbidities

3D Human Tissue Models for HIV Working Group[1,*,@]

Three-dimensional (3D) human tissue models/microphysiological systems (e.g., organs-on-chips, organoids, and tissue explants) model HIV and related comorbidities and have potential to address critical questions, including characterization of viral reservoirs, insufficient innate and adaptive immune responses, biomarker discovery and evaluation, medical complexity with comorbidities (e.g., tuberculosis and SARS-CoV-2), and protection and transmission during pregnancy and birth. Composed of multiple primary or stem cell-derived cell types organized in a dedicated 3D space, these systems hold unique promise for better reproducing human physiology, advancing therapeutic development, and bridging the human–animal model translational gap. Here, we discuss the promises and achievements with 3D human tissue models in HIV and comorbidity research, along with remaining barriers with respect to cell biology, virology, immunology, and regulatory issues.

Highlights

3D human tissue models/microphysiological systems (3D/MPS) can represent human physiology. Linking organ models can improve biological fidelity and predictive capability.

3D/MPS may be infected with HIV, enabling studies of pathogenesis, host–virus interactions, inflammation, neuropathogenesis, and HIV reservoirs.

Integrating immune cells or lymphoid tissues into 3D/MPS facilitates study of HIV immune responses, leukocyte depletion, immune ontogeny, immunotherapies, and vaccines.

Application of 3D/MPS to therapeutic development facilitates sophisticated preclinical pharmacology, *in silico* modeling, predictions of human response and safety, and evaluation of potency of biologics.

Tissue sources and/or modifications permit study of unique conditions, including TB co-infection, substance use, and special populations (e.g., medically fragile, pregnant or lactating people, and children).

The rise of 3D human tissue models

Synergy between biomedical research, tissue engineering, and microfluidics has generated advanced 3D human-derived tissue-culture models. These models are also known as **microphysiological systems (3D/MPS)** (see Glossary) and include **tissue-chips** or organs-on-chips, **organoids**, tissue explants, and cells grown in 3D extracellular matrix[i]. Each model possesses distinct features that enable them to be more physiologically relevant. Organ-chips, with cells cultured in microfluidic devices, incorporate physical characteristics such as muscle compression, oxygenation, and fluid flow [1]. 3D culture conditions emulate complex cell–cell interactions and biophysical characteristics such as stiffness and density of surrounding tissue structures [1]. Organoids form by *in vitro* self-assembly of single-cell suspensions of mature or precursor cells and can mimic *in vivo* differentiation, development, and ontogeny [2].

A recent development in the use of *in vitro* models is the FDA Modernization Act 2.0[ii]. Signed into law in December 2022, this statute allows certain alternatives to animal testing when evaluating a drug or biological product. While the statute does not eliminate animal testing, it clarifies that various nonclinical test methods such as 3D/MPS, as well as computational models, can be used prior to and during the clinical phase of the investigation of the safety and effectiveness of a drug or biological product. The National Institutes of Health (NIH) has been exploring how complementary, non-animal approaches may advance biomedical research, and the NIH Advisory Committee to the Director formed a Working Group on Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research, tasked with further evaluation of the potential applications and limitations of 3D/MPS[iii]. These models offer unique advantages for studying **human immunodeficiency virus (HIV)** and other human-tropic pathogens, such as *Mycobacterium tuberculosis* (*Mtb*), that reside in complex pathological structures (e.g., granulomas).

[1]The collaboration members, names only, can be found in the special section 'Collaborators' near the end of the paper. For a full set containing the names, ORCIDs, and affiliations, see the Supplemental information online.

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To explore the possibilities for adoption of advanced tissue models, members of the NIH and academic communities organized a workshop entitled '3D human tissue models for HIV and related comorbidities: how can organoids and microphysiological systems advance the field?' held on 20–21 September 2022 (Box 1). Here we summarize the proceedings from this workshop, including the technological relevance, opportunities, and challenges for 3D/MPS to synergize with the science of HIV and related comorbidities.

Technologies relevant to HIV

Efforts to develop and refine 3D tissue and organ printing platforms have progressed over the last decade [1], presenting opportunities for research on HIV and related comorbidities (Figure 1). Models with human cells and tissues are particularly important: HIV is adapted to humans and its growth is regulated by numerous interactions with the host cell machinery that are not found in cells from other species. By using consistent cell sources, 3D/MPS can improve reproducibility over existing models. Conversely, using heterogenous cell sources, 3D/MPS can capture a large spectrum of human diversity, including demographic, geographic, and genetic variation [3].

Effective models for HIV, **tuberculosis (TB)**, and other comorbidities must account for the various disease stages and biological contexts of infection. 3D/MPS offer improved physiological relevance in controlled, tunable conditions, permitting the addition of infectious agents or co-infecting pathogens at defined concentrations. Gene editing preliminarily enables previously intractable mechanistic studies in human tissues [4,5]. By varying cell sources, researchers may examine the influence of genetics or other factors on the activity of a drug or biological agent [6]. Developmental models (e.g., placental and fetal 3D/MPS) [7–9] could model HIV disease and drug development for pregnant, lactating, and pediatric populations.

3D/MPS of diverse tissue types can be applied to HIV research (Figure 2), including models established for use in non-HIV viral pathogens, non-infectious diseases, and drug development (Table 1, Key table). Frequently, 3D/MPS recapitulate features of disease or pharmacokinetics that animal and 2D cell models fail to capture. To date, research with cerebral organoids has uncovered contributions of HIV latency and reactivation in microglia to HIV-associated neurocognitive disorder (HAND) [10–12]; tonsil explants have delineated spatial and cellular features with respect to lymphoid tissue and germinal centers in chronic HIV [13]; and 3D models incorporating human peripheral blood mononuclear cells (PBMCs) and microfluidics generated TB-like structures reflecting the cellular composition of granulomas [14,15]. Organ-chips have enormous potential to advance HIV and *Mtb* prevention and treatment through application to drug discovery, toxicity, and studies of vulnerable populations and coexisting conditions. HIV-related applications are promising.

New opportunities

HIV pathogenesis

3D tissue-culture models can facilitate the study of many aspects of HIV pathogenesis, including latency, reactivation, and chronic inflammation, especially in difficult-to-access tissues such as the brain [10]. The great remaining challenge in HIV research is how to eradicate or inactivate the virus in patients. Potent antiretroviral therapy (ART) effectively controls the virus but does not eliminate HIV from infected cells; the virus rebounds if treatment is stopped [16]. Despite effective virally suppressive ART, people living with HIV often experience chronic immune activation and persistent inflammation that may drive comorbidities [17,18], including the prevalence of HAND, which still occurs in up to 50% of people with HIV [19].

3D/MPS have already proven useful in elucidating HIV pathogenesis. *Ex vivo* human tissue cultures maintain the cellular architecture, complexity, and interactions needed to study viral pathogenesis

Glossary

HIV reservoir: in an HIV infection, a small number of immune system cells in the body are infected with HIV but are not actively producing new virus. HIV can hide inside these cells for years, forming a latent reservoir. However, at any time, the latent virus can become active again and start making more virus[viii].

Human immunodeficiency virus (HIV): the virus that causes acquired immunodeficiency syndrome (AIDS).

Inducible pluripotent stem cell (iPSC): differentiated cells that have been reprogrammed back into a state in which they have the ability to differentiate into all of the cells of the adult body[ix].

Microphysiological system (3D/MPS): uses microscale cell culture platform for *in vitro* modeling of functional features of a specific tissue or organ of human or animal origin by exposing cells to a microenvironment that mimics the physiological aspects important for their function or pathophysiological condition[i].

Organoid: a 3D, mini-organ-like structure made by growing self-organizing cells in the laboratory. Organoids contain many types of cells and closely mimic the structure, organization, and some of the functions of the human tissues and organs[x].

Physiologically based pharmacokinetic (PBPK): a drug development tool that mathematically integrates physiological, physiochemical, and drug-dependent preclinical and clinical information to predict an investigational drug's absorption, distribution, metabolism, excretion, and pharmacokinetics[xi].

Tissue-chip: a tissue-chip or organ-on-chip is a subset class of microphysiological systems and consists of a miniaturized physiological environment engineered to yield and/or analyze functional tissue units capable of modeling specified/targeted organ-level responses[i].

Tuberculosis (TB): the disease caused by *Mycobacterium tuberculosis*.

Box 1. 3D Human Tissue Models for HIV and Related Comorbidities Workshop

Introduction
Co-chairs Melanie Ott, MD, PhD; Gladstone Institute of Virology, University of California, San Francisco Lishomwa Ndhlovu, MD, PhD; Weill Cornell Medicine *Opening remarks* Carl Dieffenbach, PhD; Division of AIDS, NIAID, NIH
Keynote: Molecular basis for HIV latency and reactivation in microglial cells Jonathan Karn, PhD; Case Western Reserve University
Technology of 3D human tissue models
Microphysiological systems for drug development: opportunities and regulatory considerations David Strauss, MD, PhD; Center for Drug Evaluation and Research, FDA
Human pluripotent stem cell-derived organoids to model infectious diseases Shuibing Chen, PhD; Weill Cornell Medicine
Regenerative medicine strategies for body-on-a-chip Anthony Atala, MD, FACS; Wake Forest Institute of Regenerative Medicine, Wake Forest University
HIV pathogenesis and disease modeling
Complex synthetic and organotypic cell cultures models to study HIV-1 pathogenesis Oliver T. Fackler, PhD; University Hospital Heidelberg
Viral pathogenesis in human tissue ex vivo Leonid Margolis, PhD; NICHD, NIH
Single cell transcriptomic profiling of a novel 3D neuron-astrocyte coculture model of tauopathy Christine Cheng, PhD; University of California, San Diego
Comorbidity biology including tuberculosis
Translational tissue culture models of HIV & TB Larry S. Schlesinger, MD; Texas Biomedical Research Institute
Lung organoids: a love nest for co-infections Carolina Garcia de Alba Rivas, MD, PhD; Boston Children's Hospital, Harvard Medical School
Modeling tissue-resident immunity in organoids Calvin Kuo, MD, PhD; Stanford University
Therapeutics
Microphysiological systems for assessing the efficacy of regenerative medicine cellular products Kyung Sung, PhD; Center for Biologics Evaluation and Research, FDA
Renal transporter function and chronic kidney disease: utility of a dual-channel microphysiological system Catherine Yeung, PharmD, PhD, MPH; University of Washington School of Pharmacy
Integration of a 3D-hepatocyte spheroid assay into the GSK hepatotoxicity strategy for small molecules Melanie Sakatis; GlaxoSmithKline
Organs-on-chip platform with human tissue niches linked by vascular flow Gordana Vunjak-Novakovic, PhD; Columbia University
Maternal and pediatric
Towards a placenta MPS for evaluating polyclonal IgG antivirals during pregnancy Evi Struble, PhD; Center for Biologics Evaluation and Research, FDA
Using SOSRS to model neural tube defects in vitro Andrew Tidball, PhD; University of Michigan
Exploring the impact of HIV on germinal center reservoirs using lymphoid tissue explant and organoid models J. Zachary Porterfield, MD, PhD; University of Kentucky, University of KwaZulu-Natal, Africa Health Research Institute
Vaccinology
Modeling autoimmunity in immune organoids Mark Davis, PhD; Institute of Immunity, Transplantation, Infection, Stanford University School of Medicine
Spatially organized models of the lymph node using ex vivo *tissue and organs-on-chip* Rebecca R. Pompano, PhD; University of Virginia

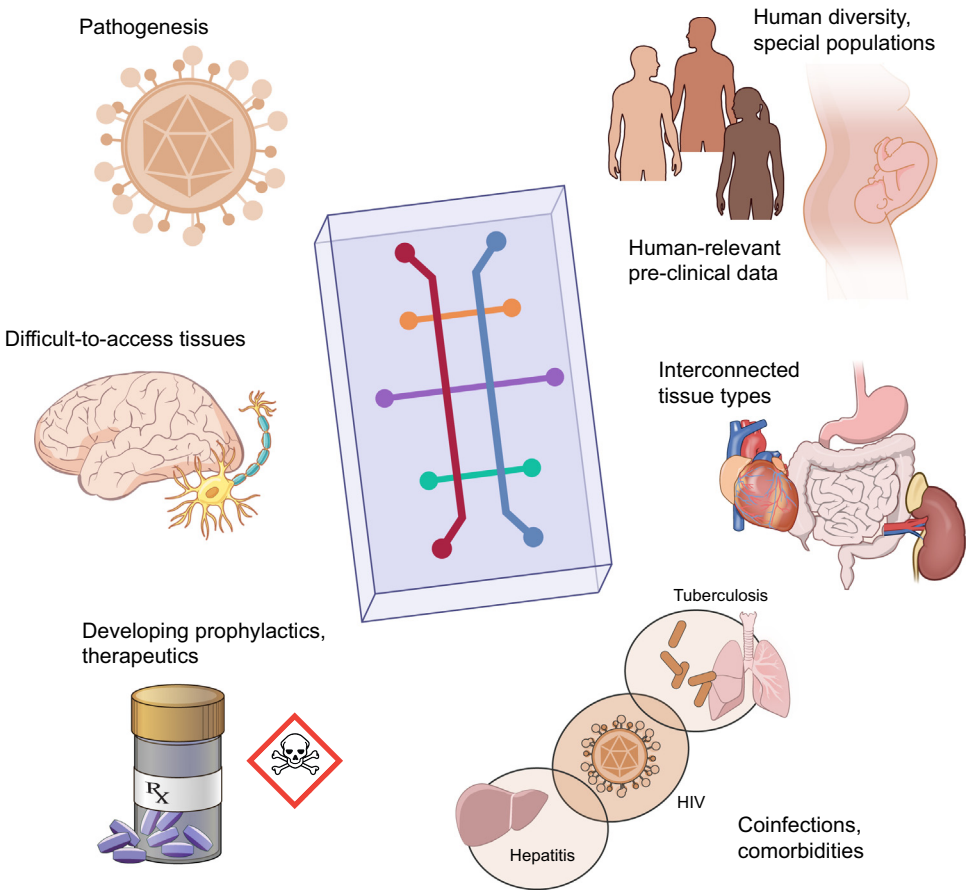

Figure 1. Advantages of 3D human tissue models/microphysiological systems (3D/MPS) with opportunities to advance HIV and related comorbidity science. (A) Pathogenesis. HIV infects and forms reservoirs in tissues, in addition to the blood/peripheral blood mononuclear cells. 3D/MPS allow study of disease pathogenesis and immune interactions at the tissue level. (B) Human-relevant data. HIV is human-tropic and interindividual variability may affect disease progression and drug disposition. 3D/MPS allow for study of human-relevant research on the disease, therapeutics, cure strategies, and interindividual or subpopulation variability. (C) Interconnected tissue types. HIV and its comorbidities are multisystem diseases interacting with the immune system. Interconnected tissue types allow for better biological fidelity to study HIV disease, its complications and comorbidities, and clinical pharmacology. (D) Co-infections and comorbidities. HIV leads to an immunodeficiency and inflammation, rendering the person living with HIV susceptible to co-infections (e.g., *Mycobacterium tuberculosis*) and comorbidities (e.g., neurocognitive, liver, and kidney dysfunction). 3D/MPS facilitate investigation of the varying and interconnected tissue types involved in these co-infections and comorbidities. (E) Developing prophylactics and therapeutics. 3D/MPS have demonstrated use in therapeutic and prophylactic development, in some cases with better predictive accuracy than traditional preclinical models. (F) Difficult-to-access tissues. Difficult-to-access tissues (e.g., the human brain) can be infected by HIV and form reservoirs, but they cannot be easily sampled and studied. The ability to generate, for example, via inducible pluripotent stem cells, and experiment with these tissues allows human-relevant investigations that would otherwise be challenging. Abbreviation: HIV, human immunodeficiency virus.

[20]. Human tonsil explants from children with HIV demonstrated severe and persistent depletion of circulating innate lymphoid cells despite ART. Remaining innate lymphoid cells upregulated genes associated with activation and metabolic perturbation [21]. Additional models are still needed.

Although lymphoid tissue contains much of the **HIV reservoir**, HIV also infects the brain. Infected T cells and monocytes enter the brain after blood–brain barrier damage, leading to infection of

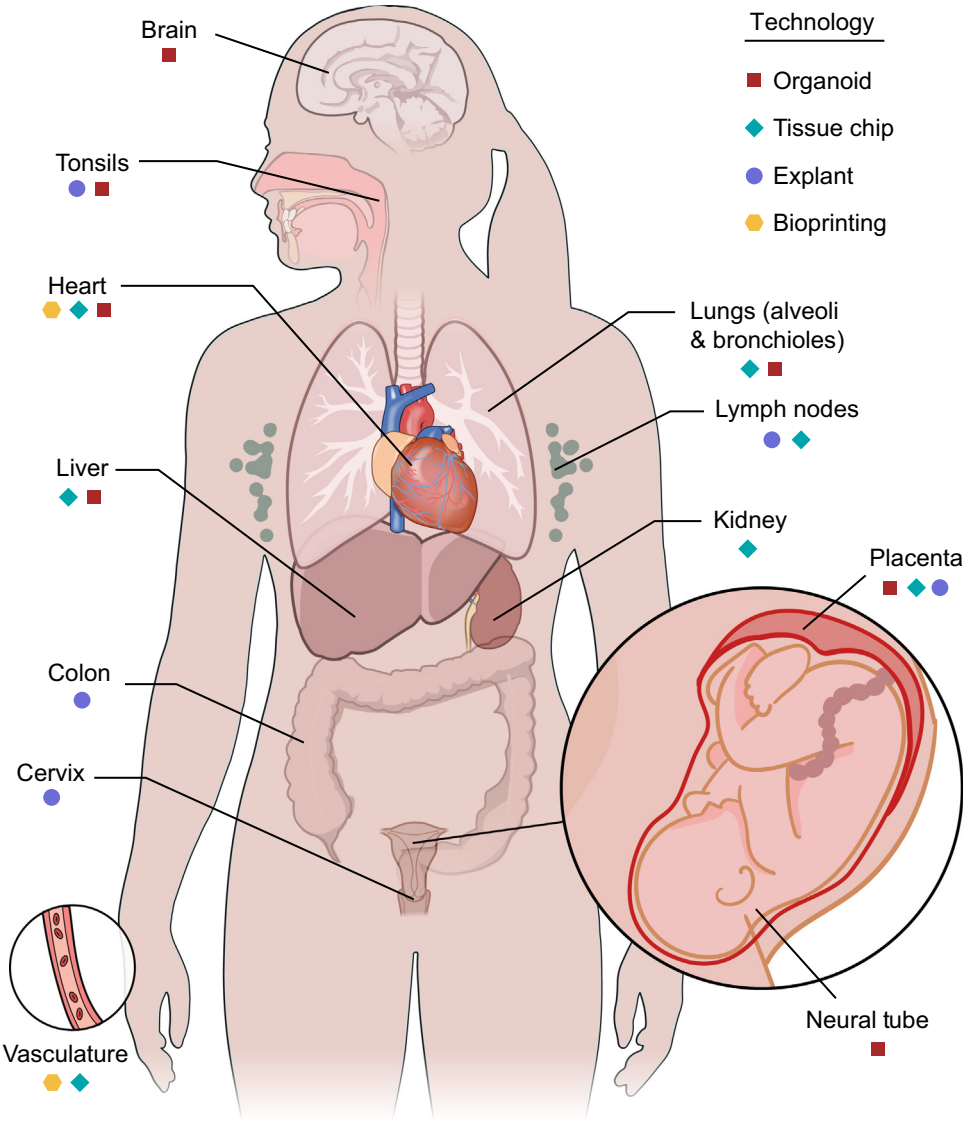

Figure 2. The 3D human tissue models/microphysiological systems (3D/MPS) with potential application to scientific questions about HIV and related comorbidities. Certain tissues or organs have relevance to specific areas of HIV and comorbidity research: (A) Sites of HIV infection, pathology, and systems immunity may be modeled using the cervix, colon, tonsil, lymph node, and brain. The cervix/vagina and rectum/colon may be applied to study HIV horizontal transmission, early immune pathogenesis, and agents for prevention. The lymphoid tissues and brain are sites of HIV reservoirs and may be used to study HIV pathogenesis, inflammatory complications, and cure strategies. (B) Sites of HIV complications and comorbidities include the lungs, heart, liver, kidneys, and colon. The lungs are the primary site of *Mycobacterium tuberculosis* infection and disease. HIV and its treatments may lead to complications of the heart, liver, and kidneys. (C) Sites of relevance to HIV therapeutics and clinical pharmacology include the liver, kidneys, blood, brain, and lymphoid tissues. (D) The placenta, neural tube, and brain are applicable to the study of pregnant and pediatric populations, including maternal–fetal pathogenesis, vertical HIV transmission, and prevention. (E) Cellular therapies are a focus for potential HIV cure strategies. The vasculature model provides an example of a potency bioassay that may be used to assess cellular product quality, although not specific to HIV. The symbols depict various types of 3D/MPS technology (i.e., organoids, tissue-chips, human tissue explants, or bioprinting) that may be applicable to HIV-related investigations. Abbreviations: 3D/MPS, 3D human tissue models/microphysiological systems; HIV, human immunodeficiency virus.

Key table

Table 1. Potential applications of non-HIV-specific 3D human tissue models/microphysiological systems to HIV and related comorbidity science[a]

Non-HIV viral infections				
Pathogen	Model	Non-HIV application	Applications to HIV or TB	Refs
Influenza	Human tonsil organoid reassembled from disaggregated surgically discarded tonsil tissue	• Interrogation of the immune response to inactivated and live-attenuated influenza vaccine • Functional germinal centers, with dark and light zone architecture, formed after *in vitro* stimulation with influenza antigens	• Immunogenicity of HIV vaccine candidates • Host immune responses to HIV infection • Screening of adjuvants and antigen/adjuvant combinations	[40,42]
SARS-CoV-2	ACE-2-expressing alveolar lung organoid generated from adult type 2 alveolar lung cells	• Recapitulation of host responses and after *in vitro* infection with SARS-CoV-2 by upregulation of type I and type III interferon responses, loss of surfactant proteins	• Host–pathogen interactions in *Mtb* infection • Drug target discovery	[91]
SARS-CoV-2	Mini-tissues with human pluripotent stem cell-derived cardiomyocytes cocultured with human pluripotent stem cell-derived macrophages	• 1280 FDA-approved drugs were screened for ability to prevent formation of reactive oxygen species and cardiomyocyte apoptosis in SARS-CoV-2 infected mini-tissues • Ranolazine prevented myocardial damage by blocking reactive oxygen species, and tofacitinib prevented damage by inhibiting the JAK/STAT pathway	• Identifying drug candidates for prevention and/or therapy • Therapeutics for comorbidities	[92]
Zika virus	iPSC array derived from 77 individual donors and iPSC-derived cerebral organoids from selected donors	• GWAS identified seven single nucleotide polymorphisms in a *cis*-regulatory element of the gene *NDUFA4* associated with permissiveness to Zika virus infection *in vitro* • Experiments with these iPSC indicated that loss of *NDUFA4* results in mitochondrial stress and increased type I interferon signaling	• Identification of genetic or epigenetic determinants of HIV silencing and control of inflammation • Host–pathogen interactions • Identification of host susceptibility factors	[6,22]
Zika virus and cytomegalovirus	Human-based placental barrier organ-chip using multicompartment channels with a placental barrier (fibroblasts, syncytiotrophoblasts, HUVECs) connected by a channel to a fetal compartment (undifferentiated HSC neurospheres)	• Modeling vertical transmission of Zika virus • Testing transplacental transfer of immunoglobulin G for protection of the fetus from infection	• Modeling vertical transmission of HIV • Exploring viral, antiretroviral, immune, and inflammatory mechanisms of pathology observed in HIV-exposed infants with and without HIV	[7,63,93,94]
Non-infectious diseases				
Disease	Model	Non-HIV application	Applications to HIV or TB	Refs
Alzheimer's disease	iPSC-derived neuron-astrocyte assembloids containing human tau oligomers	• Single cell transcriptomics uncovered changes in heat shock protein (HSP) chaperone systems in disease states • Treatment with HSP90 inhibitor PU-H71 reduced misfolded tau and neuroinflammatory transcripts	• HIV neuropathogenesis • HIV latency and reactivation in brain • Effects of antiretroviral therapy and substance use (e.g., opioids)	[10,11,25,26]
Autoimmunity	Human tonsil organoid self-assembled *in vitro* from disaggregated tonsil tissue	• Preliminarily, *in vitro* gene editing using CRISPR/Cas9 to inactivate the canonical regulatory T cell transcription factor, Foxp3, resulted in production of autoreactive antibodies to dsDNA, the histone core, other proteins	• Mechanistic studies of preventative or therapeutic interventions for HIV by inactivation of target genes • Studies of HIV infection in primary lymphoid tissue	[4,95]

Drug development				
Use	Model	Non-HIV application	Applications to HIV or TB	Refs
Pharmacokinetics/ drug disposition	Dual channel vascularized proximal renal tubule model with proximal tubule epithelial cells grown in the vascular endothelial channel and HUVECs grown in the tubular epithelial channel	• Renal tissue-chips, combined with physiologically based pharmacokinetic modeling, predicted drug disposition of morphine and its metabolite morphine-6-glucuronide. • Varying glomerular filtration rate simulates different stages of chronic kidney disease	• Pharmacokinetics of anti-HIV preventative or therapeutic drugs in healthy individuals, people who use substances, and/or people with coexisting organ impairment • Improved understanding of drug- and disease-specific factors affecting drug elimination and risks of toxicities • Relevance of genetic variants altering drug disposition to support efforts in individualized medicine	[46–48]
Pharmacodynamics/ drug response	Patient-derived samples of target tissues for construction of 3D organoids	• Patient-derived colon cancer samples self-arranged into 3D organoid structures, demonstrated to yield reproducible assay results, and then evaluated in dose-response assays with 16 different compounds • An integrated bone and heart organ-chip platform for Ewing's sarcoma better represented linsitinib's observed Phase 2 clinical efficacy and cardiotoxicity as compared with traditional preclinical models	• Relationship between target tissue (e.g., lymphoid) penetration and effects on HIV replication and reservoirs • Protection against HIV acquisition in rectal and vaginal models • Improved characterization of individualized drug responses	[45,96,97]
Drug–drug interactions	3D human liver and tumor microtissues loaded into compartments with microfluidic channels	• Co-incubation of the anticancer prodrugs, cyclophosphamide and ifosfamide, with ritonavir revealed reduced hepatic metabolism of anticancer agents and reduced antitumor activity	• Screen existing and novel anti-HIV and TB medications with other concomitant medications • Provide mechanistic insights into complex interactions with safety and/or efficacy concerns	[52]
Placental drug transfer	*Ex vivo* cotyledon models isolated from placental tissue and placed inside a perfusion chamber; maternal and fetal blood circulation established through cannulation of intervillous space and chorionic artery	• Sildenafil transfer assessed using placentas from uncomplicated and early onset pre-eclampsia • Administered to the maternal circulation with collection of maternal and fetal samples at timed intervals to determine ratios of fetal to maternal ratios • No differences identified in extent of transfer between placenta types	• Assessing *in utero* exposures to antiretroviral and TB compounds by the developing fetus • Testing transplacental transfer of broadly neutralizing antibodies for prevention of vertical transmission of HIV • Relationships between drug exposures and pregnancy and/or infant outcomes	[8,64,65,98]
Teratogenesis	Self-organizing single rosette cortical organoid for evaluating neural tube defects	• Treatment with valproic acid recapitulated neural tube defects seen in humans and mice • The non-teratogenic metabolite valnoctamide did not • Knockout of SHROOM3 suggested a shared mechanism as for valproic acid	• Screening candidate HIV and TB compounds for teratogenic risk of neural tube defects • Evaluating drug-induced and genetic mechanisms for neural tube defects	[9]
Drug safety: cardiotoxicity	Multi-organ-chip systems connect iPSC-derived tissues by microfluidic flow and enable communication between tissues	• Screened 28 drugs linked to low, intermediate, and high torsades de pointes risk to assess drug-induced arrhythmia-like events and prolongation of repolarization	• Screening existing or candidate HIV and TB drugs for cardiotoxicity alone or in combination	[53,60,99]

Table 1. (continued)

Drug development				
Use	Model	Non-HIV application	Applications to HIV or TB	Refs
		• Successfully screened a panel of drugs recalled by the FDA versus nontoxic compounds		
Drug safety: hepatotoxicity	Liver-chips with two channels and a porous membrane, with primary human hepatocytes are grown on one side of the membrane and human liver sinusoidal endothelial cells, Kupffer cells, and stellate cells grown on the other.	• 27 Blinded compounds with known hepatotoxicity or nontoxicity were applied to 870 commercially available human liver-chips, which predicted drug-induced liver injury with 87% sensitivity and 100% specificity	• Screening existing or candidate HIV and TB treatment or prevention drugs for hepatotoxicity • Improving the understanding of underlying mechanisms behind organ toxicity	[53,54, 61,100]
Quality assurance	Blood vessel-chip enhanced throughput bioassay to measure bioactivity of mesenchymal stomal cells for stimulating vasculogenesis	• Reproducibly assessed mesenchymal stomal cells' vasculogenic bioactivity across laboratories and operators • These experiments identified a correlation between vasculogenic potential and baseline expression of several genes involved in vasculogenesis	• Quality assurance, quality control of cellular products for HIV cure strategies	[59]

[a]Abbreviations: FDA, United States Food and Drug Administration; GWAS, genome-wide associational study; HIV, human immunodeficiency virus; HSC, hematopoietic stem cells; HUVECs, human umbilical vein endothelial cells; iPSC, inducible pluripotent stem cells; *Mtb, Mycobacterium tuberculosis*; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TB, tuberculosis.

microglia and perivascular macrophages. Left untreated, this infection results in acute neurotoxicity; dysfunctional regulatory signals among neurons, microglia, and astrocytes; and increased neuronal death, microglial reactivation, and virus production [10,11].

Research using both immortalized human microglial cells and **inducible pluripotent stem cell (iPSC)**-derived human microglia indicates that signals from neurons can promote HIV latency [11]. HIV silencing in the brain is mediated by the nuclear receptor related-1 protein (Nurr1), which recruits epigenetic factors to counteract the effects of inflammation, cell cycle, and metabolism on promoting viral replication [22]. These observations are now being extended to cerebral organoid systems. An exciting advance for the technology is to combine neural progenitor cells and hematopoietic progenitor cells to permit the co-differentiation of neurons, astrocytes, and microglia [23]. These organoids show superior microglial migration and distribution than can be achieved when mature microglia are added to mature organoids. In preliminary results using a modification of these organoid systems, microglia in the organoids become latently infected by HIV but reactivate, as shown by the expression of HIV Tat and spliced mRNA after exposure of the organoids to TNF-α and other inflammatory signals (J. Karn, personal communication). This phenomenon is likely to mimic episodic reactivation of the virus in HAND, which correlates strongly with systemic and central nervous system inflammation [10,24]. Brain organoids from neurodegenerative conditions may additionally illuminate HIV infection and progression to neurocognitive dysfunction [25,26].

Unlike 2D culture systems, 3D organoid systems recapitulate parameters that determine HIV replication, immune recognition, tissue $CD4^+$ T cell depletion, and the relationship between cell motility and virus spread [27]. HIV-infected PBMCs embedded in 3D-collagen matrix can be imaged to study infection kinetics as well as migration speeds and encounter frequencies between infected and bystander cells [27–29]. This tissue-like system incorporates environmental signals and collagen density, which are important for HIV spread occurring almost exclusively via cell-to-cell transmission under these conditions [29]. In this 3D matrix compared with a 2D

suspension, immature dendritic cells adopt an elongated morphology with increased deformability, and these biophysical adaptations are associated with increased productive infection mediated, at least in part, by elevated DC-SIGN surface expression [30].

Organoids hold great promise for studying complex intracellular signaling networks and transcriptional changes that control HIV latency and viral reactivation in architecturally complex and inaccessible tissues. Further, organoids may facilitate HIV cure studies, including gene editing approaches, immunotherapeutics, and novel 'shock-and-kill' or 'block and lock' strategies to eliminate the viral reservoir or suppress HIV replication.

Immune responses

Despite promising results in non-human primate (NHP) models [31], no vaccine regimen has replicated highly effective prevention of HIV acquisition in humans. HIV's species tropism has been an obstacle to translating vaccine candidates from preclinical models to clinical use. The common approach of infecting NHPs with a simian-human immunodeficiency virus (SHIV) hybrid has limitations, such as the SHIV human Env protein's reduced affinity for rhesus CD4 [32]. 3D/MPS using only human cells can avoid some limitations of SHIV and NHP models, since the virus-susceptible cells express human CD4 and the relevant HIV coreceptors and proteins required for virus production.

In addition to the human-centric nature of HIV immunology, vaccine development often relies on analysis of PBMCs, which are abundant and easily accessible. However, PBMC analysis does not capture the highly ordered and necessary interactions between T and B cells occurring in the lymph node germinal centers during an adaptive immune response, nor does it capture the unique functions of tissue-resident lymphocytes [13,33]. With 3D micropatterning and microfluidics, lymphoid chips can mimic the complex architecture of lymph node germinal centers and enable studies of germinal center T and B cells [34,35]. 3D tissue models can replicate the chemokine gradients necessary for generating lymphoid tissue architecture and organizing cell interactions [35,36]. Given the central role of lymphoid tissues in HIV infection and vaccine responses, integration of lymphoid models with other tissues [37] has the potential to illuminate mechanisms of protection by vaccine-induced adaptive lymphocytes and their migration to and surveillance of non-lymphoid tissues.

Beyond vaccines, analyses of human lymphoid tissues and *in vitro* tissue models have revealed novel biology about HIV infection and the associated adaptive immune response. Germinal centers, which are found in lymphoid tissues and contain abundant $CD4^+$ T cells, are a major latent reservoir for HIV [38]. Human tonsil explants have been used to examine the effects of HIV on adaptive immunity [13]. HIV-1 *nef* expression in $CD4^+$ T cells interfered with T and B cell interactions to block antibody production in a mouse model. Infection of human tonsil explants confirmed this finding; infection with wild-type HIV prevented antibody responses to tetanus toxoid, while infection with *nef*-deleted HIV permitted antibody responses to the same antigen [39]. Single cell analyses of tonsil explants from people with HIV uncovered differences between blood $CD8^+$ and lymph node resident $CD8^+$ T cells. Tonsil $CD8^+$ T cells were primarily located outside germinal centers in HIV-negative and -positive donors on ART, but viremic donors showed some $CD8^+$ infiltration into the germinal center. Gene expression analysis of the tonsil-derived $CD8^+$ T cells from the HIV-positive donors also showed increased signatures of chronic T cell activation and resident memory profiles but lower cytolytic gene signatures compared with blood $CD8^+$ T cells [13]. Such differences demonstrate the need to study the HIV response in the context of organized tissues.

Measuring immune responses in human mucosal and lymphoid tissue models can increase throughput while improving and complementing the predictive value of existing models, thereby

optimizing selection of vaccine products for efficacy testing in large-scale trials. *Ex vivo* lymphoid models can also enable the discovery of correlates and mechanisms of protection while retaining the diversity of human immunity. Protective efficacy will likely require multiple effector responses and models for mucosal tissues will be critical to effective vaccine development [40]. These human *in vitro* systems permit interrogation of T cell responses with new degrees of depth and accuracy [13,41] and could be applied to screen novel and existing vaccine adjuvants [42,43]. 3D/MPS will aid the development of highly useful, predictive tools for immunology and vaccinology.

Therapeutics

The HIV field has several ongoing efforts to develop and optimize new and existing therapeutics for use in HIV treatment, prevention, and cure. 3D/MPS hold promise for preclinical evaluation of novel therapeutics and to bridge gaps across currently utilized therapies, with potential applications spanning the ability to evaluate mechanisms of action, drug pharmacokinetics and pharmacodynamics, *in silico* modeling, toxicology, and integrated multisystem analysis of physiological processes and drug impacts.

Human tissue explants have been used to evaluate viral pathogenesis, antiviral mechanisms of action and activity, cytotoxicity, HIV-triggered tissue immune deficiencies, and viral interactions within tissues [20]. Early studies of acyclovir were complicated by cell-culture systems that were not adequately infected by HIV. Later studies using tonsil explants confirmed that acyclovir exhibits anti-HIV activity only in tissues that carry endogenous human herpesviruses, which provide the necessary phosphorylation step to enable drug activity [44]. Recently, rectal and vaginal tissues from recipients of the anti-HIV broadly neutralizing antibody (bNAb) VRC01 showed protection against *ex vivo* HIV-1 challenge [45].

Kidney and liver organ-chips can model drug metabolism and elimination and refine **physiologically based pharmacokinetic (PBPK)** models. A vascularized human renal proximal tubule tissue-chip was able to predict renal clearance of a substrate for renal uptake transporters in the presence and absence of an inhibitor [46]. Integrating the model's data into PBPK modeling improved prediction of renal clearance, drug and metabolite disposition, and resulted in better alignment with observed plasma concentration-time profiles of both drugs and metabolites in healthy and chronic kidney disease patients [47,48]. Similar work has been applied to the antiretroviral tenofovir, where modeling renal clearance in persons with kidney disease was optimized after incorporating the effect of uremic toxin interactions with renal transporters [49]. These approaches, if incorporating drug absorption, may improve pharmacokinetic modeling of novel long-acting antiretrovirals, where prolonged dosing intervals require optimizing precision [50]. Liver organ-chips are reproducible and suitable for metabolism studies, including quantification of CYP450 and phase II enzyme activity, which are commonly involved in the metabolism of antiretroviral and TB medications [51]. These assessments are a critical component in drug development and set the stage for identifying potentially concerning drug–drug interactions and understanding complex interactions among multiple enzyme and transporter pathways [52].

Although not yet applied frequently to HIV therapeutics, 3D/MPS could screen antiretrovirals and TB therapeutics for difficult-to-identify toxicities, such as arrhythmias and drug-induced liver injury (DILI). The body-on-chip *Ex Vivo* Console of Human Organoids platform identified potentially fatal arrhythmias not previously identified in animal studies or human clinical trials [53]. Screening for DILI is critical yet complex. A pharmaceutical industrial setting has integrated a 3D-primary human hepatocyte (PHH) spheroid assay into its screening strategy of new and existing compounds for drug-induced hepatotoxicity potential [54,55]. The 3D-PHH spheroid assay more readily identifies direct cytotoxicity and has higher overall sensitivity than 2D HepG2 systems

[54]. However, when applying such liver systems to assessments for hepatoxicity in the HIV therapeutic area, the *in vitro* data generated must be considered together with other disease-specific factors, such as potential concomitant medications with additive hepatotoxic effects, altered immune status potentially increasing risk of immune-mediated idiosyncratic hepatotoxicity, and populations that may exhibit differential hepatotoxicity risks such as pediatrics. We speculate that future development of such *in vitro* tools could allow evaluation of immune mechanisms for DILI risk, applications to biological agents, and in combination with computational methods, predict hepatotoxicity risks in complex populations.

Patient-specific cell sources used in 3D/MPS can advance drug efficacy and safety predictions under personalized medicine approaches [56], while disease-specific modifications can facilitate evaluation of complex clinical conditions. Renal organ-chips can model chronic kidney disease and altered drug clearance [46]. Liver-specific models may be tailored to HIV disease states, applied for high-throughput screening, and potentially used to evaluate alternative complications, such as liver fibrosis, along with therapeutic interventions. 3D/MPS could also identify biomarkers for early detection or monitoring of comorbid conditions.

Major advances in HIV cure have been driven by biological and cellular therapies, especially hematopoietic stem cell (HSC) transplantation [57,58]. 3D/MPS may contribute to quality assurance for cellular products, which have been hampered by functional heterogeneity that complicates demonstration of clinical efficacy and manufacturing quality control. Recently, a human blood vessel-chip potency bioassay reproducibly assessed cellular product vasculogenic bioactivity across laboratories and operators [59]. Further development and qualification of such 3D/MPS-based bioactivity assays could ensure quality if scalable cellular or gene therapy approaches to HIV cure are developed.

Body-on-chip technologies provide opportunities for the integrated analysis of multiple organs, providing a more accurate representation of physiological processes and toxicology. These platforms feature human tissue niches linked by microfluidics to mimic vascular flow. The InterOrgan-chip contains human iPSC-derived heart, bone, liver, and skin tissues that maintained the phenotype and function of each tissue type over 4 weeks of culture and accurately replicated induction of protein and miRNA markers of tissue-specific drug toxicity [60]. Multi-organ models may be further applied to study disease states, such as multi-organ hypoxia, tissue repair over a prolonged time, and complex toxicity, including detection of toxic events often missed in the traditional drug development pipeline [53,61].

Application of organ-chips to drug development has great potential to bridge the animal–human gap in preclinical compound evaluation, particularly when no appropriate animal model exists (e.g., placentas, where substantial differences have been observed between species) [31]. Organ-chips can provide data on preclinical human toxicology, deconvolute unexplained interindividual variability in drug exposures and responses, and evaluate unexpected postmarketing findings. Disease modeling requires greater biological fidelity, but with sufficient data on predictive ability, organ-chips may screen products under development for effects on disease progression. Ultimately, the biological and pharmacological relevance of 3D/MPS will depend upon the context of use, but potential near-term applications include bridging between *in vitro* and *in vivo* systems to better understand drug pharmacokinetic–pharmacodynamic relationships.

Maternal–infant studies

Vertical HIV transmission is the major route of pediatric HIV acquisition, with 130 000 new pediatric infections annually [62]. Placental 3D/MPS have been used to model vertical transmission

of non-HIV viral infections, teratogenicity, and transplacental transfer of antiretrovirals and immunoglobulin G (IgG) [7,8,63–65], thereby bridging important gaps in maternal–infant health. Animal models are poorly predictive of transplacental transfer in humans. Placental organ-chips could improve the understanding of drug exposure *in utero* and its potential associations with pregnancy and infant outcomes, protection against HIV transmission, and toxicities to the developing fetus. *Ex vivo* human cotyledon models have been used to estimate placental antiretroviral transfer [64]. Although some model systems demonstrated good agreement with cord blood–maternal plasma ratios in humans, others have been discrepant, such as bictegravir being predicted to have low-level transfer at ~7% [65] compared with ~140% in preliminary human measurements[iv]. Thus, models must be validated against human data and refined to identify potential mechanisms explaining discrepancies between model systems and humans.

With the Antibody Mediated Protection trials establishing proof-of-concept for anti-HIV bNAb prevention of horizontal transmission of neutralization-sensitive HIV viruses [66], bNAbs are an intervention-of-interest for preventing vertical HIV transmission, with recent phase I/II studies and cost-effectiveness modeling [67,68]. Maternal HIV-1 envelope-specific antibodies, particularly polyclonal, broadly multispecific bNAb activity, bNAb activity targeting uncommon epitopes, or pre-existing neonatal antibody-dependent cytotoxicity, may be associated with reduced vertical HIV-1 transmission [69,70]. Promising models to assess transplacental transport of antibody-based therapeutics include placental trophoblast monoculture and coculture [8]. Questions remain about dosing, potency, and specificity of antiviral IgG therapies in pregnancy and escape mechanisms that might be addressed with further development of placental organ-chips.

In 2018, pharmacoepidemiological surveillance detected a signal of increased neural tube defects linked to *in utero* dolutegravir exposure in pregnant people [71,72], although subsequent data indicated that the risk was minimal [73,74]. This unconfirmed signal resulted in slowing dolutegravir rollout [75] with potential consequences of increased maternal deaths and vertical HIV transmission [76]. We surmise that application of a neural tube organoid [9] could have ruled out the suspected teratogenicity of dolutegravir within weeks, as opposed to the years required for larger pharmacovigilance follow-up studies [73,74].

By using patient-derived tissues, one can foresee the ability to elucidate neural tube defect etiologies, particularly genetic or drug-related [9]. Given the demonstrated capability of using these neural tube organoids to identify a mechanism of neural tube teratogenicity by inhibition of histone acetylases leading to reduced apical constriction [9], these mechanistic insights have implications for histone deacetylase inhibitors under development as first-generation HIV latency reversal agents in HIV cure strategies.

Linking placental 3D/MPS with models evaluating teratogenicity may provide a better understanding of placental drug transfer, permit safety evaluation of intrauterine exposures, generate fundamental data to inform pregnancy PBPK models, and determine the teratogenic potential of genetic, chemical, or therapeutic etiologies. Combined models would allow for studies on infectious or antiviral effects on placentas (e.g., cytotoxicity, metabolic function, transcytosis, and proinflammatory signaling molecules) and the fetal compartment (e.g., infectious pathology, cytokine production, and toxicity). Given ethical considerations for research in pregnant populations[v], 3D/MPS enable a unique, safer opportunity for testing and developing therapeutics for use in pregnancy and pediatrics.

TB

TB remains one of the deadliest infectious diseases globally. TB is the leading global cause of death for people living with HIV, who have an 18-fold greater risk of developing TB than persons

without HIV[vi]. The TB field has largely relied on small-animal models, specifically murine, to study the basic biology of TB pathogenesis and the immune response. Murine models have provided great insights into the acute stage of TB disease but are not able to produce granulomas comparable with those observed in humans [77]. Through use of ultra-low dose *Mtb* infection models, the development of murine strains, including C3HeB/FJ mice that develop necrotic granulomas, and the Collaborative Cross mice that broaden the heterogenous immune induced against *Mtb*, attempts have been made to improve the murine model. It still does not fully recapitulate the entire scope of human TB disease. Furthermore, mice require immune system-based humanization to study TB/HIV co-infection. Currently, NHPs represent the best model for human TB disease, but they are expensive and limited in supply [31].

Human-based *in vitro* and *ex vivo* models are urgently needed to study TB. With *in vitro* models, the TB field has primarily relied on 2D cellular monoculture infection models, primarily of macrophages. In recent years, researchers developed 3D human models of specific aspects of TB pathogenesis, including immune cell cocultures in extracellular matrices (e.g., collagen) that seek to recapitulate the immunological complexity of granulomas, a pathology associated with chronic disease [14]. One such model was shown to be predictive of human TB and identified sphingosine kinase 1 as a potential target for host-directed therapy [15]. In another study, immune cells from individuals with latent TB formed granulomas faster than naïve individuals.

More recently, organoids and lung-on-chip systems [78,79] have been developed. These models are well-placed to understand the role of lung physiology, including epithelial cells and fibroblasts in TB, which are likely to be important in early and paucibacillary infection [80–82]. Preliminarily, a lung organoid has recently been developed using a platform previously utilized in the development of tumor organoids that incorporates a larger cohort of resident immune cells [41] and could conceivably allow for the study of the complex host immune response induced by *Mtb* in the lungs beyond the innate immune stage. Recent applications of these models for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) research (Table 1) highlight new opportunities for TB research [41,83]. Continued improvement of these and other models would provide greater flexibility in kinetic studies and much-needed human preclinical data to support and prioritize therapeutic and vaccine candidates to be advanced into clinical development.

Regulatory

The use of 3D/MPS in regulatory science may improve selection of more effective, less toxic agents with less animal testing. 3D/MPS facilitate high-throughput screening when most animal models do not and they can be used when an optimal animal model for an infectious disease is unknown or resource-limited, which are particular advantages for pandemic preparedness [31]. They can also complement non-specific safety studies and product evaluation for rare diseases. *In vitro* data generated using tissue models may overcome the challenges of small sample sizes, de-risk nonclinical findings (e.g., identifying therapeutics with reduced risk of hepatotoxicity), and alert to and/or reduce need for clinical trials evaluating drug–drug interactions. 3D/MPS may characterize therapeutic activity and measure potency, including tissue-specific activity, provide organ-specific pharmacokinetic modeling, and assess specific safety concerns. Models may be used for candidate selection, benchmarked against existing technology, and qualified as fit-for-purpose [51,59,84]. The FDA budget for 2023 includes new funding for implementation of an agency-wide New Alternative Methods program that will establish cohesive and comprehensive strategies to advance the development, qualification, and implementation of new alternative methods for regulatory use[vii].

Challenges

While noteworthy progress has been made in developing human 3D/MPS, important challenges remain. Translation of new technologies will require many steps to reach needed standards of safety, efficacy, and quality. Technical and biological hurdles will limit the full replacement of animal models in the foreseeable future, but efforts to improve and optimize 3D/MPS are ongoing.

First, the numbers and lifespans of cells in culture must be improved. For example, co-developing cerebral organoids from neural progenitor cells and microglia from HSCs improves microglial migration and distribution in the organoid system, but improved brain organoid models for HIV neuropathogenesis will require increasing the number of fully differentiated microglia and the number of dopaminergic neurons [85]. *Ex vivo* lifespans of 3D human models depend on tissue type and condition and currently limit application [17]. Studies of chronic exposure to therapeutics or toxins, antibody maturation, aging, and long-term sequelae require extending tissue lifespans from weeks to months. One key will be enhancing vascularization and perfusion methods [79]. Certain cerebral organoids, for example, develop necrotic cores over time because of insufficient oxygen and blood flow [86]. Cell egress and death limit tonsil organoid survival to 3–4 weeks, limiting their effectiveness for studying *de novo* immune responses [42]. Air–liquid-interface culture extends cerebral organoid lifespan from weeks to months and is a promising approach to human airway epithelial cultures for study of infectious diseases [87,88].

Second, integrating host adaptive immunity remains an obstacle because of alloreactivity. Coculture with relevant cell types or inclusion of immune system tissue models (e.g., bone marrow, lymph nodes) would markedly influence the biological fidelity and utility of models for studies of HIV and TB. Researchers have adopted two strategies to address alloreactivity: obtaining all tissues from the same donor or omitting lymphocytes from the models altogether. More versatile approaches to generate organoids containing tissue-resident lymphocytes and body-on-a-chip containing circulating lymphocytes will expand the utility of tissue models for studying HIV and TB.

Third, as we explore new human tissue models for HIV research, we must develop methods to benchmark data from human models against previous data from animal models, including animal-derived tissue models [89]. Tissue models should be built as standardized, user-friendly platforms, but models must be fit-for-purpose to address specific research questions [84]. Finally, the large volumes of data generated by these models will require integration and interpretation. We must work to integrate knowledge and maximize research impact.

Fourth, human tissue models still do not allow examination of complex multistage TB and HIV infections in their entirety. We can study airway or alveolar *Mtb* infections individually with organoids, but no model encompasses both anatomical locations [78–81,83]. Nevertheless, these organoid systems may be suitable for modeling co-infections and drug responses. Future models with more sophisticated host immunity should aim to capture the transition from acute to chronic *Mtb* infection and the development of viral reservoirs after an acute HIV infection.

Fifth, another challenge in developing models of TB/HIV co-infections is knowing exactly what a model needs to effectively represent human infection. TB *in vitro* models do not fully recapitulate observations from granulomas in NHPs and human tissue. Yet, the important question is whether these *in vitro* models, while not perfect, are sufficient to model potential therapeutic candidates, antimicrobial efficacy, or pathogenesis. TB and HIV models should be designed and used with specific goals in mind (i.e., testing therapeutics, identifying biomarkers,

evaluating host-directed agents, modeling disease transmission or progression, and testing vaccine candidates).

Sixth, current 3D/MPS, although promising, are not yet sufficient for comprehensive safety evaluation. For example, the 3D-PHH model constitutes a single component of an integrated hepatotoxicity screening pipeline [54]. Because multiple, complex mechanisms underlie DILI, various mechanisms must be evaluated with multiple complementary assays to determine hepatotoxicity potential [90]. Despite the generally greater sensitivity of 3D-PHH, the 2D system identified a few known hepatotoxins that the 3D system did not [54,55]. Replacing 2D assays with 3D systems in integrated hepatotoxicity screening pipelines will require further optimization.

Finally, broader uptake in HIV research will need stimulus. For all their caveats, established animal and 2D *in vitro* models have known advantages, limitations, and an existing body of data. For example, the murine model is the established preclinical model for initial TB antibiotic development because it is a reliable indicator of clinical benefit [77]. 3D/MPS must be more reliable in selecting therapeutics for clinical development and we need a better understanding of their limitations. We must also consider how these 3D/MPS will be utilized by the general research community. Is the goal of the community to equip every lab for the use of these models, to establish cores at research institutes, or to develop fee-for-service infrastructure? The increasing complexity of 3D/MPS models, particularly body-on-chip platforms, requires specialized knowledge. Research labs will need to know their niche in the system to adopt 3D/MPS. To guarantee success of these new platforms for the study of HIV, the scientific community must increase cross-disciplinary efforts while continuing to engage regulatory entities. Increasing uptake requires including modelers in discussions with researchers in pathogenesis, therapeutics, and vaccines for HIV and related comorbidities.

Concluding remarks

Unique opportunities are presented by 3D/MPS to leverage advances in systems biology, stem cell technology, tissue engineering, and computational methods to understand HIV pathogenesis, inform on HIV reservoirs for cure potential, evaluate immunity and vaccine responses, predict therapeutic efficacy and safety, elucidate comorbidity biology, and model special populations. Collaborations between 3D/MPS modelers and HIV investigators provide an opportunity for potentiating applications of these novel technologies to critical questions in HIV research. Although 3D/MPS is early in development, these models may address progress-limiting questions (see Outstanding questions).

Future directions may include addressing multiple clades of HIV, repurposing existing and down-selecting new therapeutics for HIV, evaluating coexisting conditions (e.g., substance use and dependency), assessing differences by sex, extending research to subpopulations across the age spectrum, and accelerating HIV vaccine development. Advances in 3D/MPS technology have positioned the scientific community to overcome many challenges in HIV and comorbidity research.

Collaborators

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Outstanding questions

What are the limits of 3D/MPS? Which technological improvements are most pressing for application to HIV research?

How can 3D/MPS model horizontal and vertical transmission dynamics? How well will 3D/MPS predict response and safety of preventative agents?

How can 3D/MPS enable studies of pathogenesis in difficult-to-access tissues? What existing models can be adapted?

How can 3D/MPS model the latent HIV reservoir and factors affecting reservoir establishment and maintenance? How applicable will 3D/MPS be for modeling combination cure interventions?

How can adaptive immunity be incorporated into 3D/MPS? Can 3D/MPS overcome the challenges of alloreactivity? How can 3D/MPS evaluate vaccine responses?

How can we most effectively leverage 3D/MPS to study interactions in multi-organ system diseases and what parameters must be considered?

What are the most appropriate models for HIV-related comorbidities and co-infections, such as TB? What are the technical and biological considerations when studying these conditions?

How well can 3D/MPS be adapted to special populations? Will 3D/MPS sufficiently recapitulate ontogeny within their tissue lifespans?

How can 3D/MPS be leveraged for development of therapeutics and biological agents for use in pregnant, pediatric, and other special populations? What are the most appropriate models for preclinical safety and efficacy in these populations?

How do data from existing 3D/MPS compare with existing human data? How can these models be compared with preclinical animal models? Where can 3D/MPS most effectively bridge the translational gap from animals to humans?

What standards should be employed for cell sources and engineered platforms to allow cross-validation,

reproducibility, qualification, scale-up, and manufacturing?

What is the best approach for dissemination and adoption of 3D/MPS in scientific communities investigating HIV and related comorbidities?

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Declaration of interests

D.E.Y. was previously an unpaid technical advisor for the non-profits Cover the Globe and Maipelo Trust. K.M.B. has received consulting fees from ViiV Healthcare. S.C. is the cofounder of OncoBeat, LLC, and a consultant of Vesalius Therapeutics. A.R.M. is a cofounder and has an equity interest in TISMOO, a company dedicated to genetic analysis and human brain organogenesis focusing on therapeutic applications customized for the autism spectrum disorders and other neurological disorders origin genetics. The terms of this arrangement have been reviewed and approved by the University of California, San Diego, in accordance with its conflict-of-interest policies. M.Z.S. is an employee of GlaxoSmithKline Research and Development, United Kingdom. L.E.W. is a co-inventor on a US patent describing systems and methods to model adaptive immune responses, assigned to Stanford University. The remaining authors declare no competing interests.

Supplemental information

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Resources

[i]www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda

[ii]www.congress.gov/bill/117th-congress/senate-bill/5002/text

[iii]https://acd.od.nih.gov/working-groups/novel-alternatives.html

[iv]https://programme.ias2023.org/Abstract/Abstract/?abstractid=681

[v]www.nichd.nih.gov/sites/default/files/2018-09/PRGLAC_Report.pdf

[vi]www.usaid.gov/global-health/health-areas/tuberculosis/tbhiv

[vii]www.fda.gov/media/157192/download

[viii]https://hivinfo.nih.gov/understanding-hiv/fact-sheets/what-latent-hiv-reservoir

[ix]https://stemcells.nih.gov/info/basics/stc-basics

[x]www.cancer.gov/publications/dictionaries/cancer-terms/def/organoid

[xi]www.fda.gov/drugs/news-events-human-drugs/development-best-practices-physiologically-based-pharmacokinetic-modeling-support-clinical

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