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Organ-on-chip models for infectious disease research

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Research on microbial pathogens has traditionally relied on animal and cell culture models to mimic infection processes in the host. Over recent years, developments in microfluidics and bioengineering have led to organ-on-chip (OoC) technologies. These microfluidic systems create conditions that are more physiologically relevant and can be considered humanized in vitro models. Here we review various OoC models and how they have been applied for infectious disease research. We outline the properties that make them valuable tools in microbiology, such as dynamic microenvironments, vascularization, near-physiological tissue constitutions and partial integration of functional immune cells, as well as their limitations. Finally, we discuss the prospects for OoCs and their potential role in future infectious disease research.

During microbial infections, interactions between the pathogen, host tissue, immune system and microbiota play important roles, making these processes complex to study[1]. Global healthcare systems are facing increasing numbers of opportunistic infections associated with rising antimicrobial resistance as well as emerging pathogens[2]. Therefore, an in-depth understanding of microbial infections is required to foster the development of new diagnostic tools and therapeutic approaches.

For decades, host–microbe interactions have been studied using animal models. Their physiology, metabolism and immune system made them indispensable for studying infectious diseases (Table 1). In vivo models remain crucial for fundamental and applied research, especially in preclinical studies to evaluate antimicrobial drug and vaccine candidates. However, owing to interspecies differences, animal models cannot always recapitulate human host and microbial phenotypes of infectious disease; which is a challenge for current drug discovery pipelines[3]. The use of animal models comes with practical limitations (such as the need for highly trained personnel and specialized facilities), ethical considerations and high costs. This has led to policy changes and introduction of the three Rs principle to replace, reduce and refine the use of animals in research[4]. Additionally, the limitations of animal-based research have been acknowledged by the US Food and Drug Administration (FDA) through implementation of the Modernization Act 2.0. This abolishes the requirement for all drugs to be tested in animal models[5].

Studying host–microbe interactions using in vitro cell lines can be advantageous over animal models owing to ease of use and greater control over experimental variables. These models have therefore become widely adopted in infection biology research. In vitro models can vary widely in formats and complexity (Table 1). Most commonly, monolayers of immortalized cell lines are grown in multi-well plates or trans-wells. Samples from healthy individuals and patients can be cultured as primary cells, stem cells or tissues ex vivo. Compared to immortalized cell lines, these cultures are more representative of physiological conditions.

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Table 1 | Advantages, limitations and applications of different models for infection biology

Model		Advantages	Limitations	Applications
'Traditional' in vitro models	2D cell culture in well plates	• High throughput • User friendly • Low cost • Well established • Controlled environment • Reductionistic model	• Usually lack 3D tissue structure • Limited cell–cell interactions • Oversimplification of human physiology • No compartmentalization • Common use of immortalized cell lines that do not accurately mimic tissue physiology • Static culture conditions • Prone to microbial overgrowth	• High-throughput screening • Study of single-cell interactions (for example, cell damage, host responses, pathogenicity mechanisms) • Compatible with (high-throughput or live-cell) microscopy
	Cell culture in trans-wells	• User friendly • Low cost • Controlled environment • Compartmentalization, that is, to create air/liquid interfaces, chemical gradients, polarization of cells, or specific manipulation of apical and basal tissue sides • Allows dissection of contact dependency	• Often lack 3D tissue structure with limited cell–cell interactions • Static culture conditions • Lower throughput than well plates	• Air/liquid interfaces • Study of interactions between two compartments • Study of contact independent cell–cell signalling • Study of immune-cell chemotaxis • Study of single-cell interactions • Some models facilitate 3D tissue structure (for example, skin) • Compatible with microscopy
Microphysiological systems	OoC	• Dynamic system, can recapitulate physiological forces and physical/chemical gradients in a controlled manner • Extended lifetime of cells through active perfusion of media • Perfusion removes waste and microbial overgrowth • Flexibility due to scalable biological complexity of cellular composition and arrangements • Multicellularity, compatible with cell differentiation in subtypes, and functionality • Compartmentalization allows specific manipulation of apical and basal tissue sides, as well as independent analysis of the compartments (for example, refs. 19,23–25,66,93) • Differentiation of 3D tissue architecture	• Low throughput • Can be more difficult and expensive to handle • Specific equipment requirements • Standardization in progress (Box 1) • Compounds can be absorbed by some OoC materials, for example, PDMS • Difficult to dissect interactions on a single-cell level, owing to higher complexity	• Modelling biochemical and cues • Work with highly proliferative microorganisms, stable microbiota • Circulation of peripheral immune cells • Modelling biophysical processes (for example, blood flow, peristalsis, urination) • Study diseases that lack suitable animal models (for example, refs. 91,92,94,95) or conditions that are lethal for the animal (for example, ref. 13)
	MOoC	• Can simulate systemic responses • Organ modules can be added, removed or exchanged	• Tissues often have different biological origins when established with immortalized cell lines • More difficult to set up and handle • Complexity increases with each organ addition • Balancing conditions for multiple organs is challenging (that is cell culture media and additives)	• Studying organ–organ interactions, or more than one organ simultaneously • Modelling organ-specific drug absorption, distribution, metabolism and toxicity
	Organoids	• Recapitulates 3D structure of tissues and multiple cell subpopulations • Cells recapitulate in vivo features, for example, receptor pool, biomarkers • Can simulate organ development and functionality • Recapitulates human variability • High throughput	• Limited experimental and analytical access • Challenging to adjust multiplicity of infection to organoids with different size and cell numbers • Standardization of organoid size and shape possible, but needs experience and specific equipment • Mostly static conditions • Donor variability	• High-throughput screening • Recapitulating different human genetic backgrounds • Usable in personalized medicine • Suitable for developmental studies
	Organoids-on-chip	• Combines benefits of OoC and organoids • Differentiation into organ-specific cell subtypes • Recapitulates human variability • More controlled environment than organoids	• More difficult to set up and maintain. • Higher costs than OoCs or organoids in other formats • Variability between organoid donors • Difficult to dissect interactions on a single cell level	• Vascularization of organoids possible • Easy infection of apical side of organoids • Usable in personalized medicine

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Table 1 (continued) | Advantages, limitations and applications of different models for infection biology

Model		Advantages	Limitations	Applications
'Traditional' ex vivo models	Human ex vivo blood	• Contains diverse types of functional primary immune cells • Same composition as in vivo blood • Recapitulates human variability • Easy to sample	• Donor variability • Difficult to standardize • Does not capture solid tissue contexts • Ethical considerations • Safety concerns regarding blood transmissible diseases • Obtaining blood requires trained personnel	• Dissect the role of different immune cells separately • Whole-blood infection assays • Usable in personalized medicine
	Human ex vivo tissue	• Donor specific • Preserves the tissue structure, multicellularity and functionality	• Challenging to recapitulate tissue-tissue interactions. • Ethical considerations • Difficult to dissect single-cell interactions • Limited availability (for example, biopsy material) • Often diseased tissues • Difficult to control for medication and comorbidities • Donor variability • Limited life span due to large number of necrotic cell material, viability is time sensitive	• Studying rare cell types in preserved structural environment • Studying disease-related alterations in tissue function and composition • Usable in personalized medicine
'Traditional' in vivo models	Non-mammalian in vivo models	• Complex, 3D and dynamic system, reproducing a whole organism • Includes functional immune system • Often compatible with high-throughput and intravital microscopy	• Large interspecies differences • Can be more difficult and expensive to handle and equipment may be required (for example, for zebrafish and *Drosophila* models) • Difficult to dissect single-cell interactions • Differences in microbiota composition	• Metabolism and toxicology • In vivo screenings • Developmental studies • Studying systemic disease features
	Mammalian in vivo models	• Complex and dynamic system • Come closest to recapitulating human physiology • Holistic view of interactions • Recapitulates fully functional immune and humoral system • Some models (for example, mice) are widely used with plenty of protocols and tools available	• Interspecies differences • Environment not highly controlled • Usually requires highly trained personnel and dedicated facilities • Difficult to dissect single-cell interactions • More difficult to perform microscopy than in other models • Ethical hurdles, especially when studying late-stage, life-threatening or chronic disease • Differences in microbiota composition	• Preclinical studies (pharmacology and efficacy) • Reproducing diseases at body level/systemic disease • Microbiome studies

More complex in vitro models have been developed to better mimic human tissues compared with traditional cell cultures. These models, termed microphysiological systems (MPS), can recreate physiological characteristics such as tissue function, 3D structure, cell morphology, biochemical environment and mechanical forces. MPS include widely used models such as organoids (in vitro grown miniaturized and simplified versions of an organ) and organ-on-chip (OoC) models (Table 1).

Here, we review features of OoC models and their value for infectious disease research. We discuss how these models have been applied in the context of microbial pathogenicity, host-pathogen interactions, and therapeutic approaches. We aim to introduce OoC models to infection biologists and highlight how they can provide useful insights into microbial pathogenesis, similar to animal models, while maintaining the control and flexibility of in vitro systems.

Characteristics of OoC models

OoC models come in diverse shapes and formats. The European Organ-on-Chip Society (EUROoCS) defines OoC models as "microfluidic devices, containing living engineered organ substructures in a controlled microenvironment, that recapitulate one or more aspects of the organ's dynamics, functionality, and (patho)physiology in vivo under real-time monitoring". The FDA defines them as "microphysiological systems that consist of a miniaturized physiological environment engineered to yield and/or analyse functional tissue units capable of modelling specified/targeted organ-level responses"[6].

Most OoC models are cell culture chambers approximately the size of a microscopy slide, and consist of one or more compartments that are separated by a membrane or hydrogel (Fig. 1a). They are made of optically transparent material to allow for microscopy and are integrated with microfluidic systems to perfuse medium through the culture compartments, or vacuum pumps to stretch and contract the chambers (Fig. 1b). By varying the types of cells seeded in the compartments of OoC models, different tissues can be modelled that are relevant to the specific research question (Fig. 1c). For example, a vasculature-like compartment can be included to study immune cell recruitment or pathogen dissemination. Although these features are also present in animal models and some in vitro models (Table 1), utilizing human cells in combination with microenvironment features like biomechanical forces and scaffolds in a single in vitro model makes

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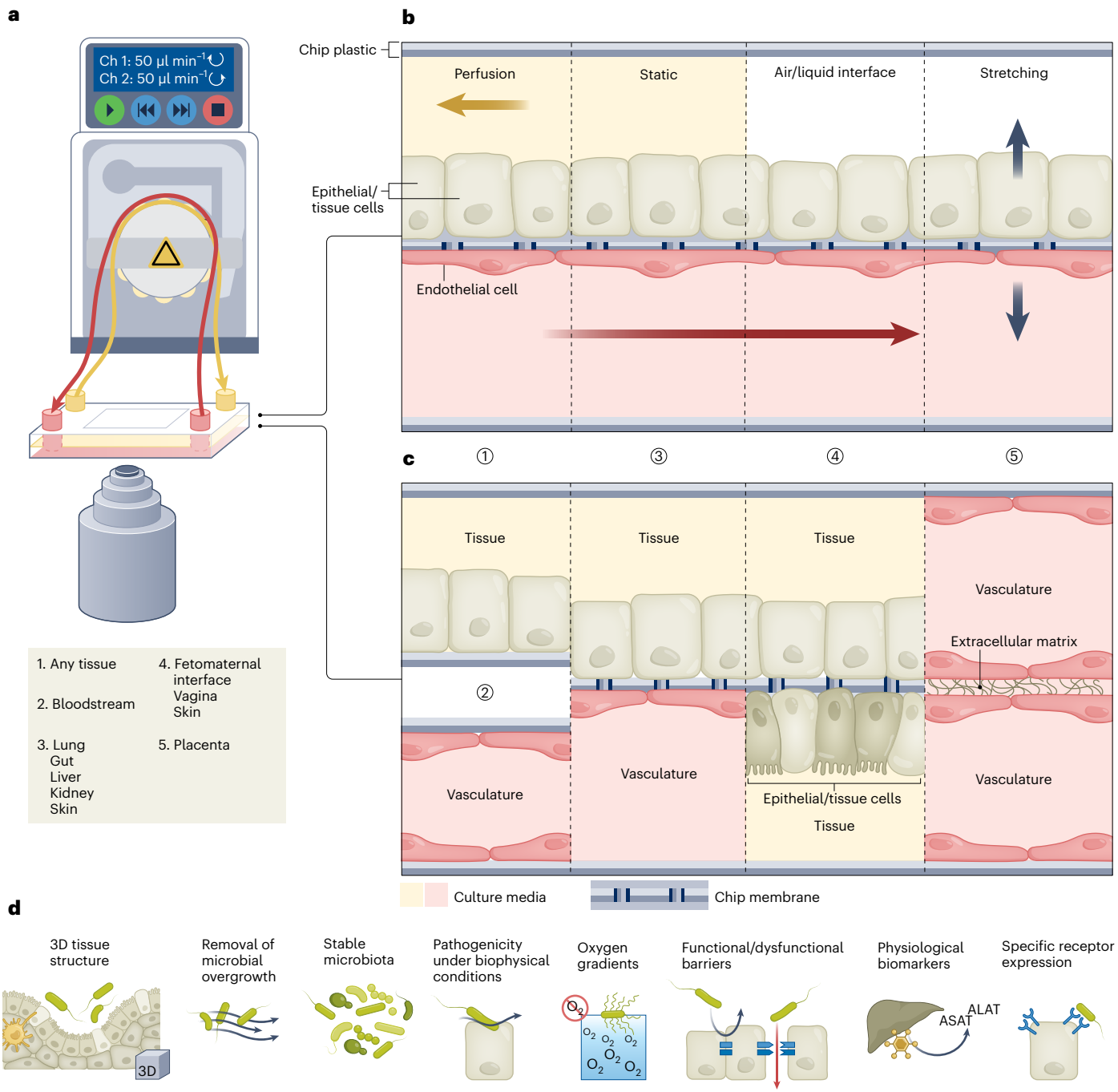

Fig. 1 | Common OoC models. a, Schematic representation of an OoC model with a microfluidic system. A microfluidic pump (top, not shown to scale) is connected to the chip through tubing, which perfuses media through compartments of the chip. This microfluidic configuration is compatible with microscopy techniques and supports imaging of cellular processes and interactions inside the OoC while maintaining controlled fluidic conditions. A microscope lens is shown below the chip. **b**, By varying experimental set-ups, OoC models can be configured to integrate relevant biophysical forces. Microfluidic flow can be applied to multiple compartments to simulate physiological flow in organs. Only one compartment may be subjected to flow while the other remains static. Supplying the tissue with essential nutrients through a different compartment can facilitate the establishment of an air/liquid interface in the other; simulating air exposure. Some models apply vacuum systems to stretch and relax the tissue. **c**, Diverse tissues can be modelled by arrangements of the cells grown in the different compartments of OoC models. Most common OoC models consist of: (1) a single tissue compartment; (2) blood-vessel-on-chip, where endothelial cells line the entire compartment; (3) vascularized tissue, with tissue cells on one side of a membrane and endothelial cells on the other side; (4) different cell/tissue types grown in each of the compartments; and (5) tissue co-culture with extracellular matrix separation. **d**, Features contributing to the physiological microenvironment that OoC models often combine into a single model for infectious disease research. Tissues in OoCs can acquire the 3D structure of specific niches. The microfluidic flow in OoC models can remove microbial overgrowth, help to establish stable microbial communities and allow the study of pathogenicity under biophysical conditions. Oxygen gradients can also be generated inside the OoCs by supplying specific compartments with oxygen while others are kept under anaerobic conditions. Cells within OoC models can acquire in vivo-like characteristics, allowing mimicking of functional and dysfunctional barriers, the study of specific biomarkers (for example, alanine transaminase (ALAT) or aspartate transaminase (ASAT) for liver function) and the interaction of a pathogen with specific receptors otherwise not expressed in vitro.

OoC devices attractive study systems for human infectious disease research (Fig. 1d).

In fact, OoC models can be important when appropriate animal models are missing. For example, the antibody TNT005 aimed to treat chronic inflammatory demyelinating polyneuropathy (a neurological autoimmune disease) was approved for human clinical trials after preclinical testing in OoC models, since an animal model for this disease is not available[7]. This could be an avenue for strict human pathogens or those that lack accessible or suitable animal models[8–11]. Advanced in vitro models using human cells are therefore a highly valuable research tool.

Potential of OoC models in infectious disease research

Several tissues relevant for studying infectious diseases have been replicated in OoC models (Figs. 1c and 2). Skin and mucosal tissues can be more accurately replicated, because they usually feature less structural and functional complexity. However, as biological complexity increases, it becomes more difficult to recreate full organ functions in vitro. To address this, researchers have started focusing on small functional units rather than an entire organ, for example, the neurovascular unit[12] versus the brain. OoC devices have been used to model infections caused by viruses, bacteria, fungi and parasites (Fig. 2). Organs such as the skin, intestine and lung are of major interest as they are often the first contact site during pathogen invasion. Organs frequently targeted by infections such as liver, brain and kidney can also be partially modelled with OoCs. OoC models have been particularly useful for studying pathogenic agents that leverage specific cell receptors (such as many viruses) and those that cross tissue barriers. This has led to well-developed models, which have been applied for both basic infection research and drug testing.

OoC models can be used to bridge specific gaps between traditional cell culture models and animal models, for example, by combining physiologically relevant features found in vivo with human cells in the controlled environment of in vitro models. Further, compatibility of OoC with microscopy allows to observe processes in higher spatiotemporal resolution than in vivo[13], which has been leveraged to monitor, characterize and quantify infections[14–17]. However, as for any model, their limitations must be understood before using them (Table 1). Animal models outperform OoCs when studying conditions or outcomes that affect multiple organs or adaptive immune responses, such as sepsis. Traditional cell culture or organoid systems are more easily applied for high-throughput approaches compared with OoCs. It is critical to identify research objectives where OoCs can be useful, especially as the required expertise and technical challenges can be comparable to animal work.

Modelling biophysical host properties in OoC models

Traditional cell culture cannot model mechanical forces within the human body, such as flow or peristaltic stimulation. As microfluidic devices, OoCs offer unique advantages as they can mimic biophysical properties of the host, by perfusing media or inducing tissue stretching and relaxing (Fig. 1b).

Mechanical forces can change the morphology or pathogenicity mechanisms of certain microorganisms[18]. Several studies in OoC models have reported changes in host–pathogen interactions when comparing static versus dynamic models[19,20]. Flow also altered responses of intestinal cells to enterohemorrhagic *Escherichia coli* enterotoxin[21]. Stretching and relaxing of the tissue in a bladder-on-chip model (mimicking filling and emptying the bladder) led to higher burdens of uropathogenic *E. coli* in comparison to static conditions[20]. Biophysical stresses, such as flow, can also force microorganisms to express pathogenicity mechanisms that are required in vivo, which might not be required in static models. For example, the fungus *Candida albicans* requires induction of filamentous morphology to facilitate adhesion to endothelial cells in a vasculature-on-chip model. Fungal adhesion to the host vasculature, a first step to disseminate to other organs, was regulated by the protein Hcg1, which was only expressed under dynamic (comparable to in vivo) but not static conditions[22].

The flow in OoCs also allows assessment of tissue–tissue interactions. For example, in a model of the fetomaternal interface various maternal and fetal cell layers were connected and subjected to perfusion[23]. This allowed the study of an epithelial infection by bacteria on the maternal cervix and fetal tissue (Fig. 3a). Immune responses, the role of exosomes from *Ureaplasma parvum*-infected cells[24] and the role of alarmins[25] were dissected, and biomarkers for preterm delivery were identified[25].

Many microorganisms replicate very fast, making the use of static models challenging. Microorganisms can quickly overgrow host cells, consume nutrients in the media and eventually kill host cells without inducing phenotypes that correspond to those observed in vivo. Typical solutions involve media that prevent cell damage, regular medium exchange, shorter experimental timeframes or use of antibiotics, which can bias results. By contrast, the continuous flow in OoC models can remove non-adherent microorganisms, preventing the detrimental effects of microbial overgrowth to the model. This allows both the pathogenesis mechanisms of rapidly proliferating microorganisms to be studied and to integrate stable commensal microbial communities. The latter is particularly important for studying microbiota as it can modulate the host immune system[26] and influence growth, as well as the virulence of many pathogens[27]. In line with this, several OoCs have demonstrated the beneficial effects of microbial communities on the host, such as preventing infections and reducing inflammation[28–32].

During infection, microbial pathogens interact with a variety of host cells and tissues, each with their specific properties (such as a specific pool of receptors, organotypic 3D structure, secreted factors or metabolism). For some microbial pathogens, strong phenotypic differences have been reported between in vivo and in vitro experiments[19]. In these cases, it is essential to mimic the tissue in a physiologically relevant way to reproduce the infection in vivo[33].

Immortalized cell lines are used in most in vitro models and often fail to recapitulate the phenotypes, functionality, cellular diversity and 3D structures of tissues in vivo. Although cell lines are used in many OoC models, the mechanical forces applied in these devices can induce their differentiation and lead to acquisition of morphological, structural and functional changes that closely resemble human tissues. For example, shear stress by microfluidic flow was shown to induce differentiation of Caco2 cells (intestinal tumour cell line) into absorptive (cells that absorb nutrients from the intestinal lumen), mucus-secretory (which produce the mucus layer that covers the intestine), enteroendocrine (which secrete hormones to regulate digestion and nutrient intake), and Paneth cells (which produce antimicrobial peptides and other molecules involved in immunity)[34,35]. Shear stress also contributed to a less permeable blood-brain-barrier in a neurovascular unit-on-chip composed of endothelial cells, pericytes, and neuronal cells[12]. In addition, peristaltic forces can induce tissue differentiation in the intestine[35] and the lung[36]. Thus, mechanical forces in OoCs can enable cell lines to be used in a more physiologically relevant way. For example, a microfluidic distal tubule-on-chip reproduced the barrier structure and reabsorption ability of the kidney better than traditional trans-well models. This enabled the effects of rabies virus infection on kidney cells to be characterized. Consistent with renal dysfunction observed in patients, the virus disrupted tight junctions in the kidney impairing electrolyte reabsorption[37].

Incorporating functional cells into physiologically relevant structures and a dynamic environment can be essential to study specific pathogenesis mechanisms. The intestinal pathogen *Shigella flexneri* can cause serious diarrhoea in humans even at a very low infection dose. However, in static in vitro models, high bacterial burdens are required to cause invasion and damage to enterocytes[19]. Using an intestine-on-chip

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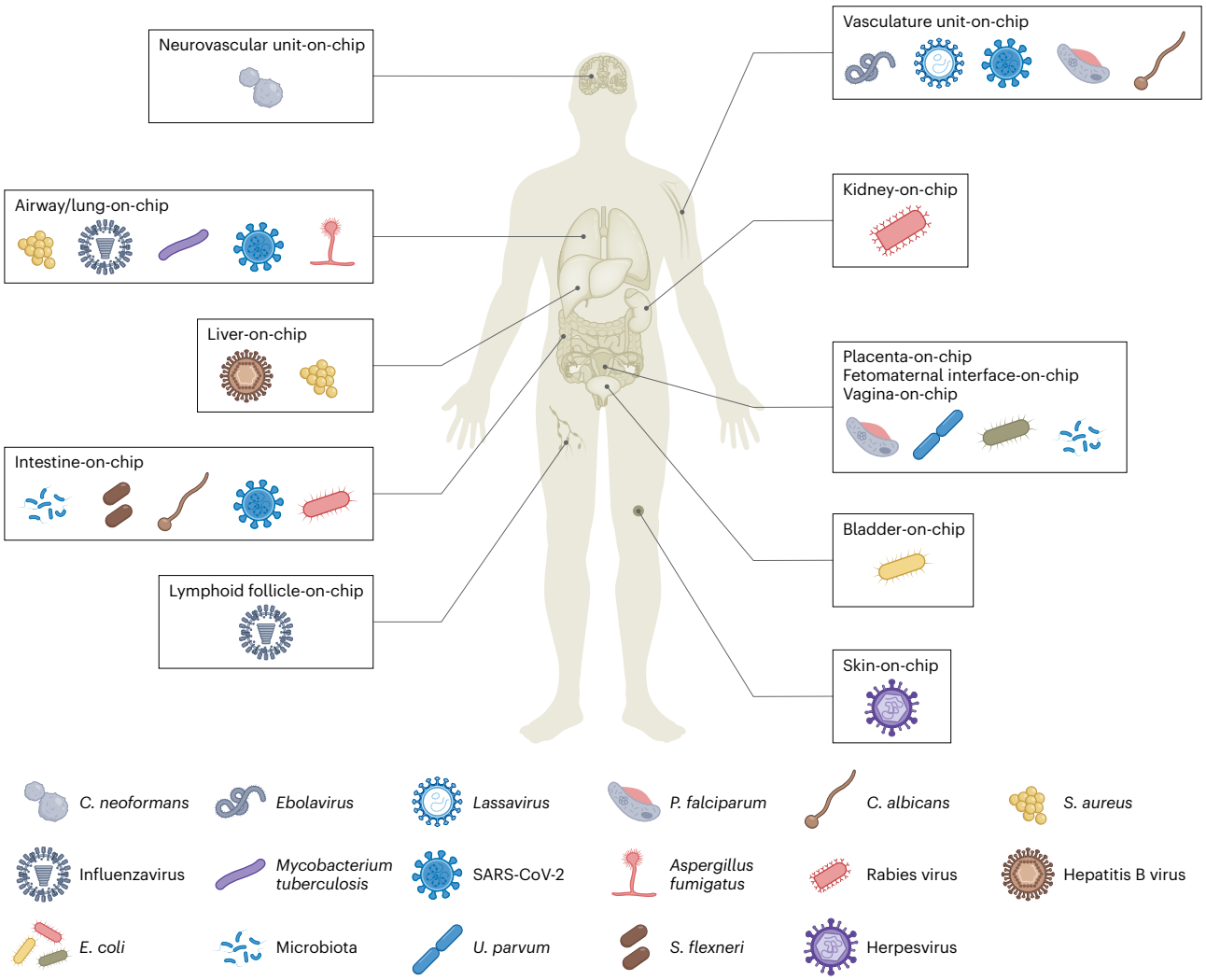

Fig. 2 | OoC models in infection biology. Overview of some of the OoC models that have been developed and used for infectious diseases research and the microorganisms that have been studied.

model featuring villi and crypts, peristaltic motion and shear force induced by flow, it was shown that all of these features were crucial to recapitulate the full pathogenicity of *S. flexneri*. In vivo-like adhesion, invasion, propagation within enterocytes, toxin secretion and host damage were observed from only a few hundred bacteria, an efficiency comparable to infections in humans[19]. Reproducing in vivo-like host–pathogen interactions is also crucial for testing drug. This is particularly important when the drug is targeted at preventing pathogen entry into host cells, intracellular replication, spreading to other tissues or reducing any detrimental inflammatory responses, as is the case for antiviral drugs[38]. For example, ACE2 (the receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the lung and gastrointestinal tract) was expressed in patient-derived intestinal epithelial cells cultured in an OoC model, whereas it was not expressed when those cells were grown in organoids or trans-wells[39]. In an airway-on-chip model, differentiated target cells showed similar characteristics compared to cells in human lungs, including expression of proteases involved in viral entry[40], and pathogenicity of several viruses, including influenza A and SARS-CoV-2, was recapitulated. This allowed identification of antiviral drugs and testing of therapeutic regimes, highlighting the power of OoC models as tools for drug discovery[39,40].

Studying chronic infections in primary hepatocytes is challenging, as they change into non-functional cells after a few days in culture. The constant flow within OoC devices supplies hepatocytes with fresh nutrients and oxygen, while removing waste products, thus enabling longer time periods for experiments. Incorporation of hepatocytes in a liver-on-chip model allowed functional cells to be maintained for 40 days so that chronic hepatitis B virus (HBV) infection could be studied in vitro. Hepatocytes in the chip showed increased susceptibility to HBV infection compared with other models that required high levels of infectious units compared with in vivo models. The liver-on-chip recapitulated observations made in patients regarding both viral and human biomarkers[41]. This provides evidence that OoC models can be used to assist the search for new immunotherapies and curative drugs, as well as in the development of personalized medicine approaches using both patient-derived liver cells and HBV isolates[41].

Vascularization in organ-on-chip models

A major advantage of applying flow in OoC devices is the integration of vasculature into tissue models (Fig. 1b,c). As a result, dissemination of pathogens through the bloodstream, recruitment of immune cells or distribution of drugs can be studied. Many OoCs used in the field of infection biology achieve vascularization by lining one of the compartments with endothelial cells. Although there are other approaches to include vascularization (for example, cancer research often requires a more complex vasculature network)[42], a single dedicated channel is easier to set up and manipulate, and is sufficient to offer unique insights into the infection process.

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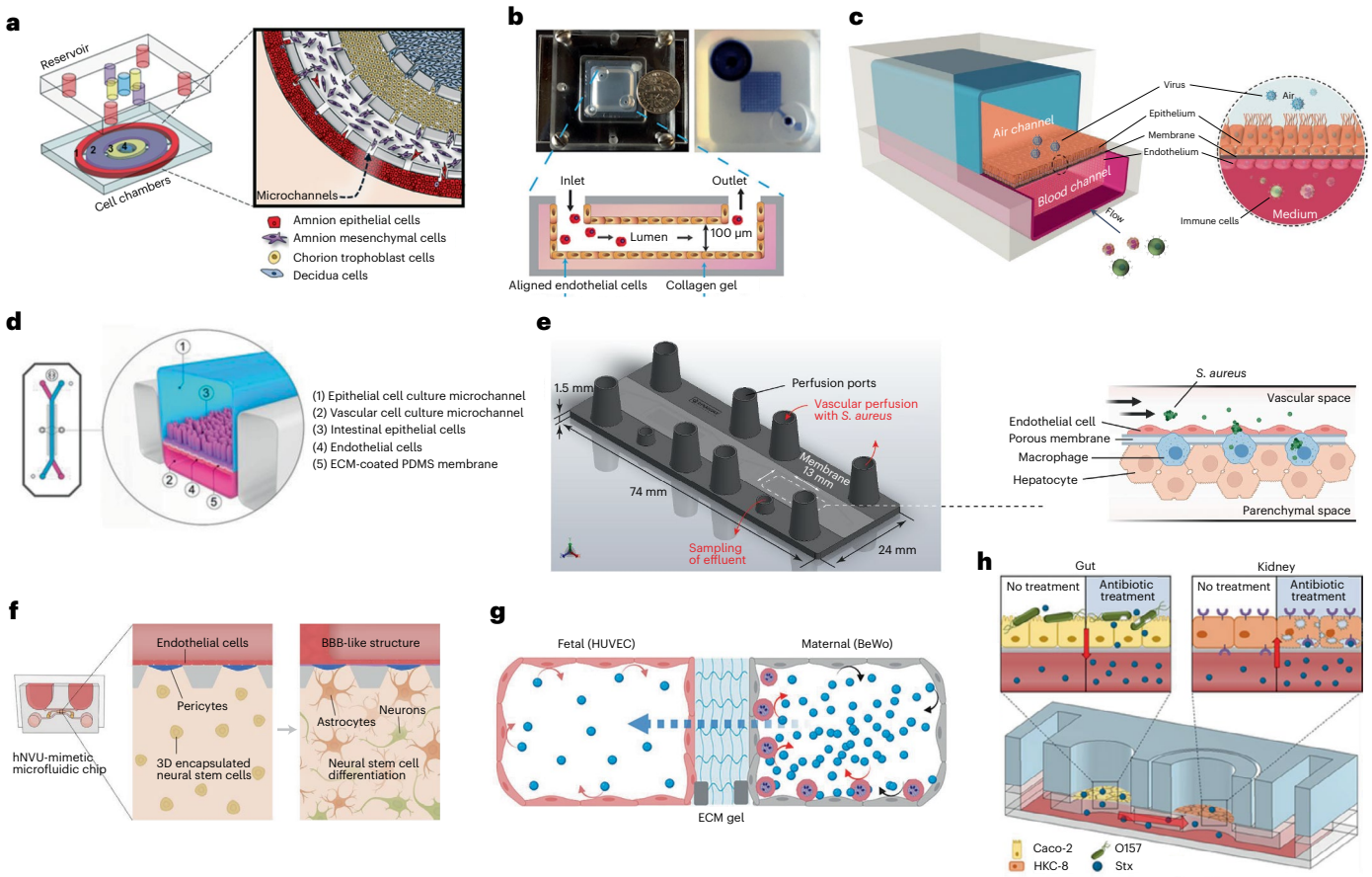

Fig. 3 | Exemplary OoC models used in infectious disease research. **a**, Schematic of a fetomaternal interface-on-chip, including different cell types that organize in layers interconnected by the ECM[25]. **b**, Three-dimensional fluidic microvessel device (left) and perfused with dye (right), and schematic cross-sectional diagram of the blood microvessel, consisting of endothelial cells lining the network of tubes in a collagen matrix[45]. *P. falciparum*-infected erythrocytes (red cells) can be perfused through the channel. **c**, Lung-on-chip on an Emulate chip with a vascular compartment, circulating immune cells and an air/liquid interface[40]. **d**, Intestine-on-chip in a PDMS chip from Emulate (left), which recapitulates both shear and peristaltic stresses (flow and stretching of the tissue), with a vascular compartment in the bottom channel[96]. **e**, Liver-on-chip on a Dynamic42 GmbH chip with tissue-resident macrophages and a vascular compartment in the top channel[15]. **f**, An hNVU including a membrane-free BBB. The barrier contains endothelial cells and pericytes, and separates the brain compartment, embedded in ECM, from the vascular compartment over this ECM[12]. **g**, Placenta-on-chip on a Mimetas chip, where an ECM hydrogel separates the fetal vascular compartment, lined with human umbilical cord endothelial cells (HUVECs), and the maternal compartment lined with trophoblasts (BeWo cell line). Bidirectional flow can be applied in both compartments[50]. **h**, MOoC consisting of gut and kidney compartments that maintain contact through the basal channel, mimicking the bloodstream[63]. Red arrows indicate the diffusion of molecules from the intestinal to the vascular compartment, and from there to the kidney compartment. Panels reproduced with permission from: **a**, ref. 25, Royal Society of Chemistry; **b**, ref. 45, under a Creative Commons licence CC BY 4.0; **c**, ref. 40, Springer Nature Limited; **d**, ref. 96, under a Creative Commons licence CC BY 4.0; **e**, ref. 15, under a Creative Commons licence CC BY 4.0; **f**, ref. 12, Springer Nature Limited; **g**, ref. 50, under a Creative Commons licence CC BY 4.0; **h**, ref. 63, under a Creative Commons licence CC BY 4.0.

Studying host–microbe interactions in the bloodstream

Blood-vessel-on-chip models (Fig. 3b) are used to study the interaction of blood-borne pathogens with the vasculature and those that compromise vasculature integrity (such as haemorrhagic viruses)[43,44]. By perfusing fluorescent albumin and combining it with time-lapse microscopy, vasculopathy induced by Ebola and Lassa viruses has been studied[43,44]. Ebola virus-like particles caused vascular leakage and activated the Rho–ROCK pathway, which is related to severe complications of the disease[43]. Further investigations into the Rho–ROCK pathway resulted in the identification of molecules that reverted vascular leakage at clinically relevant concentrations[43].

To study specific host–pathogen interactions, it can also be relevant to reproduce the 3D structure, biomarkers and physical properties of the microvasculature. Cerebral malaria occurs when *Plasmodium falciparum*-infected erythrocytes are sequestered in the brain microvasculature[45]. Using a perfused 3D brain microvessel, two proteins mediating malaria-infected erythrocyte adherence to the endothelium were identified[45].

Many pathogens can disseminate via the bloodstream to other organs, causing life-threatening systemic diseases[46,47]. This process can be modelled and molecular mechanisms studied using OoCs with a vascular compartment (Fig. 3c–e). For example, *C. albicans* can cause systemic candidiasis when it enters the bloodstream from the intestinal tract. Fungal growth, morphological changes, tissue damage and translocation (features of systemic candidiasis) were modelled in an intestine-on-chip with a vascular compartment. Pathogen invasion into the vasculature was quantified using this approach and further demonstrated that probiotic microorganisms can prevent systemic candidiasis[34].

Modelling physiological gradients and tissue barriers

Vascularized models can simulate physiologically relevant gradients, for example, from the blood across multiple cell layers. For drug development, this is an advantage compared with traditional cell culture models. The effective drug concentration is affected by the delivery route and host metabolism, which are processes that can be reproduced

in OoC models to some extent[48]. This was tested with antifungals in an invasive aspergillosis-on-chip model[16], and with antivirals in a herpesvirus infection model using a 3D vascularized skin device[49] where the drugs were applied through the vasculature.

Oxygen gradients between the blood and surrounding tissues are needed to recapitulate interactions of anaerobic or facultative anaerobic microorgsanisms with the host intestine. Hypoxic conditions in the colon were modelled by maintaining the OoC system in a hypoxic chamber while perfusing oxygen-saturated medium through the vascular compartment to enable oxygen supply to the tissue[28]. As a result, microbial communities similar to those detected in human faeces could be studied[28].

Individual biological barriers between organs or tissues are important when studying pathogen dissemination via the bloodstream or the recruitment of immune cells to infection sites. Accurate representation of different barrier-forming cells with intercellular junctions and structures that connect to the extracellular matrix (ECM) is important when studying their role in host defence against pathogens[12,34,43,50]. This includes the blood–brain barrier (BBB; Fig. 3f). A human neurovascular unit-on-chip (hNVU) with a surrogate BBB (to separate vascular and brain compartments) was used to demonstrate dissemination of *Cryptococcus neoformans*, a fungal pathogen that can cause meningitis, to the brain tissue[12]. This hNVU chip allowed direct visualization of host–pathogen interplay using microscopy. In another example, modelling the placenta showed that *P. falciparum*-infected erythrocytes from the mother can damage the fetus by interfering with glucose transport across the placental barrier[50]. This was demonstrated using a placenta-on-chip model where an ECM layer separated trophoblasts and endothelial cells (Fig. 3g).

Finally, vascularization is a key feature for integrating circulating immune cells in OoC models and a common approach in the development of multiorgans-on-chip (MOoC; Fig. 3h) as discussed in the following sections.

Incorporating functional immune cells into OoC models

Although the immune system is critical to clear infections, it can also play an important role during pathophysiology (such as in inflammatory bowel disease (IBD)[51] or vulvovaginal candidiasis[52]). To date, in vivo models are still needed to study the effects of a complete immune system. However, some functional aspects can be emulated in vitro with OoCs.

Circulating innate immune cells such as neutrophils can be perfused through vascular compartments in OoC devices to model chemotaxis, migration through the endothelial barrier, infiltration of tissues and interactions with the pathogen (Fig. 4a). The dynamic behaviour of neutrophils, including NET (neutrophil extracellular trap) formation and swarming, was shown using live-cell imaging while the immune cells infiltrated bladder tissue to clear uropathogenic *E. coli*[20]. Neutrophil recruitment and responses were also modelled during viral infections in lung- and skin-on-chip models[40,53]. Similarly, monocytes were shown to revert hepatic dysfunction after sepsis in a vascularized liver-on-chip. Monocytes perfused through the vascular compartment, attached to the endothelium, infiltrated the hepatic tissue section of the model, and improved liver cell function during acute inflammation, thus recapitulating these in vivo processes[54].

OoCs also represent a promising platform for studying mucosal immunology and the cross-talk between epithelial tissues and innate immune cells. For example, tissue-resident macrophages are critical for tissue homeostasis and play key roles in inflammation and pathogen defence by releasing cytokines and sensing those released by other cell types (Fig. 4a). For this reason, macrophages have been incorporated into various OoC models to study host–pathogen interactions[13,16,17,34,54] (Fig. 4a). Before introducing them into an OoC model, macrophages can be differentiated accordingly into a subpopulation of interest, such as alveolar macrophages patrolling the lung alveolar tissue[13,16] (Fig. 4a). However, cells can also be differentiated inside the device to model their natural development into various cell populations, as shown in an intestine-on-chip model. Here, monocytes differentiated into tissue-resident macrophages and lumen-sampling dendritic cells[34,55]. Responses to endotoxin varied between the vascular and intestinal compartment; endotoxemia (the presence of bacterial endotoxins or lipopolysaccharide in the bloodstream) was observed in the vascular compartment, while the endotoxin was 'tolerized' in the intestinal compartment[34,55] (Fig. 4b).

Thus, intestinal OoCs containing functional innate immune cells can help model essential aspects of intestinal barrier physiology and immunopathology linked to inflammatory diseases. Clinical hallmarks of IBD, such as villus injury and intestinal barrier disruption caused by proinflammatory cytokines like interferon-gamma[55], as well as responses to bacterial overgrowth in the intestinal compartment[30] were also mimicked in OoC models (Fig. 4b).

However, not all OoC models incorporate immune cells, and those that do often only incorporate innate immune cells, such as monocytes, macrophages and neutrophils. Models with a fully functional immune system are not yet available, and their development will be challenging. Since OoC models often involve cell lines with different origins, incorporating immune cells has largely been limited to including cells that do not induce allogeneic responses, that is, responses against foreign cells from another individual of the same species. Incorporation of cytotoxic CD8+ T cells or natural killer (NK) cells can lead to allogeneic immune reactions and destruction of the model. Nevertheless, peripheral blood mononuclear cells (PBMCs) containing these cells have been perfused to mimic inflammatory responses in a liver-on-chip model reproducing liver inflammation[54].

Although functional immune cells are incorporated in a variety of OoC models, lymphoid organs have also been successfully modelled[56–58]. In a lymphoid follicle-on-chip model, human B and T cells autonomously assembled under flow and recapitulated antibody responses after vaccination similar to human tonsils ex vivo[58]. Such models can be a major contribution to preclinical evaluation of vaccination strategies against infectious agents, especially considering that immune responses widely differ across mice models and humans[59]. The future combination of immunological tissues with other tissues in multiple OoC models will be an important next step in the direction of studying a functional immune system under in vitro conditions.

Modelling multiorgan infections with OoC

Microfluidic compartments of OoC models can be connected to integrate several different tissues into one platform. Such MOoC platforms allow studying defined research questions focusing on systemic infections, including the role of circulating immune cells or metabolite exchange between organs[60]. For example, gut–kidney or liver–kidney MOoCs could be useful to investigate systemic candidiasis, and skin–liver platforms may mimic *Staphylococcus aureus* infections. An existing example is the malaria-on-chip model. *P. falciparum* has a complex life cycle involving several human organs. This MOoC, containing endothelial, spleen, liver and circulating red blood cells, could maintain infection for eight days and reproduce various stages of *P. falciparum* infection, as well as show susceptibility and resistance to treatment. Therefore, it can serve as an important resource to study malaria pathogenicity mechanisms and test anti-malarial drugs[61].

MOoCs also allow the effects of infection on distant organs to be studied. Shiga toxins produced by certain *E. coli* strains can cause kidney damage even though infection occurs in the intestine[62]. A MOoC that interconnects gut and kidney compartments helped to dissect the response of each tissue to intestinal bacterial colonization and the effects of antibiotic treatment[63]. Host–pathogen interactions across different organs can also be studied using a MOoC. For example, a MOoC emulating the function of the central nervous system and the

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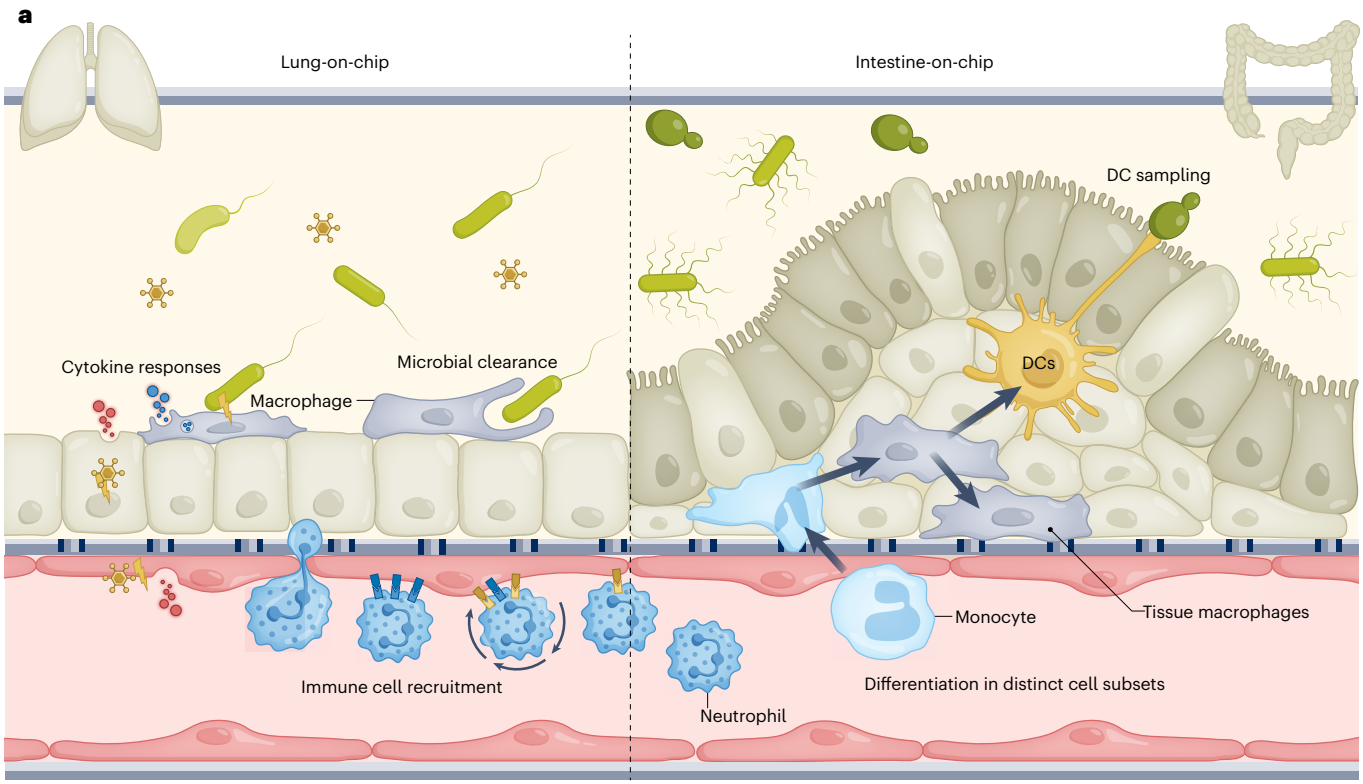

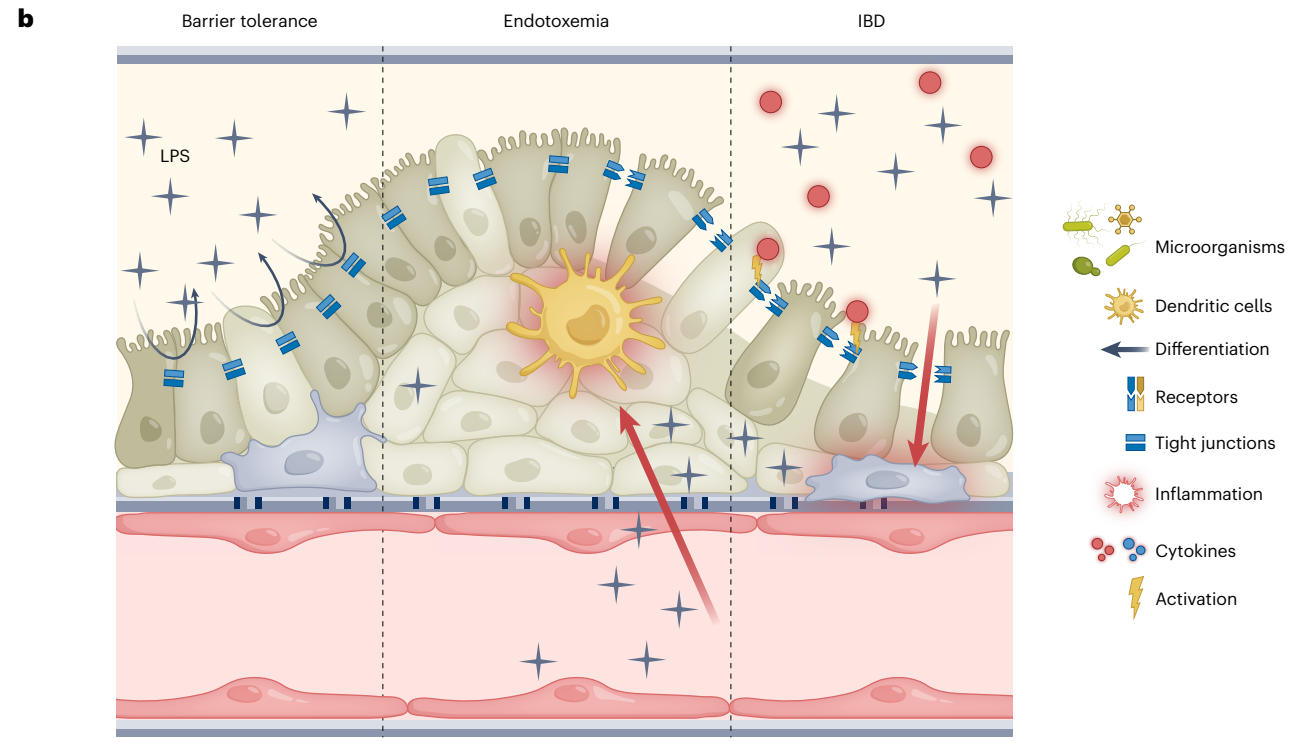

Fig. 4 | Integrating functional immune cells in OoC models. a, Specific functions of the innate immune system that have been modelled in OoC models. Overall inflammatory response of tissue, endothelium and incorporated immune cells can be assessed. Tissue resident macrophages, such as alveolar macrophages, contribute to microbial clearance and immune responses within the tissue microenvironment. Neutrophils and other circulating immune cells can be recruited from the vascular channel and extravasate into the tissue upon infection or inflammation. Monocytes can also differentiate into distinct cell subsets within the tissue, for example, dendritic cells (DCs) sampling the contents of the lumen or tissue resident macrophages. These aspects can be simultaneously recapitulated in an OoC model, allowing the introduction and study of more immune responses to the infection in a dynamic environment. **b**, A healthy epithelial barrier that integrates tissue resident immune cells in an intestine-on-chip is tight and when lipopolysaccharide (LPS) is applied to the epithelial lumen, it does not elicit inflammatory responses (left). When LPS is introduced through vascular compartment (mimicking endotoxemia) inflammatory responses from the tissue resident macrophages are elicited, leading to inflammation and barrier disruption (middle). During pathogenesis, like IBD, abnormal responses to stimuli in the intestine (such as the cytokine interferon-gamma or microbial overgrowth) can compromise the barrier and drive inflammatory responses (right).

liver was infected with *C. neoformans*[64] to determine the neurotropism of this pathogen.

MOoCs also have applications in drug development since they can reproduce key physiological parameters, such as absorption, distribution, metabolism, excretion and toxicity in vitro. In this context, liver models have been employed to reproduce the uptake of compounds and their catabolism by hepatic enzymes. For example, the increased nephrotoxicity of specific drugs after bioactivation was demonstrated using a MOoC with liver and kidney compartments[65]. Absorption can also be modelled by combining functional liver and intestinal compartments with a mixing chamber to recapitulate human plasma. Metabolite concentrations can be measured in the different compartments to obtain information about drug pharmacokinetics[66]. In the future, employing MOoCs for infection biology and drug testing will open up new avenues to study the effects and niche-specific efficiency of antimicrobial drugs on host–drug–pathogen interactions in detail.

Limitations of OoC systems

The relevance of OoC models for future research has been increasing in the last years, but they still have limitations. One major challenge is the need for standardization and validation, which is a prerequisite to incorporate these models into pre-clinical research. Many efforts are currently being directed towards standardizing OoC models (Box 1).

In addition, the materials used in many chips can pose a challenge in the drug discovery pipelines. Polydimethylsiloxane (PDMS) is a widely used material to fabricate chips, because of its flexibility, transparency, versatility and ease of use. Yet, PDMS absorbs hydrophobic molecules, and induces adsorption of proteins to its surface[67]. Other plastics used in tubes or reservoirs could have the same effect. This may alter the concentration profiles of molecules in media, negatively affecting results. In addition, artificial membranes or scaffolds that separate and structure cells limit diffusion and exchange of chemokines, metabolites and microorganisms between the tissues.

OoCs are generally lower throughput compared to other models, such as well plates or organoids. This makes them less appropriate to screen candidate drugs and limits their application to further stages of the pipeline where only a few lead compounds remain.

OoC models might not be suited for every research facility. They are more difficult to handle than traditional cell culture models, and require specific equipment and materials that can elevate the costs of the experiments[68]. Their higher complexity (such as different cell types, tissue structure, and mechanical forces) can also be a disadvantage when dissecting interactions at molecular or cell level, as more variables need to be taken into account.

Finally, OoC models cannot completely replace animal models, since these are still needed to assess systemic outcomes and holistic processes. Most diseases and treatments are not limited to a single cell-cell interaction, but involve multiple tissues and organs. OoCs can recapitulate several tissues and structures, but they do not fully recapitulate the high complexity of a human body[68].

Future perspectives

OoC models have been advancing through the development of innovative solutions, which enhance the mimicry of human tissues and organs, expand their applications and overcome some of their limitations[69]. As an emerging field, input from various areas of research, including bioengineering, biomaterials, and tissue engineering, is aiding this progression. OoC models are steadily improving and have become increasingly capable of answering complex research questions and allow investigation of the molecular mechanisms underlying infectious diseases.

To overcome the absorption of molecules on PDMS or other chip plastics, different materials, such as polystyrene or other thermoplastics, much more suited for biological applications, are being increasingly leveraged[67]. To further reduce unwanted surface effects, the chip materials can be modified with protein or extracellular matrix coatings to increase compatibility with biological material (biocompatibility).

Introduction of biocompatible substrates (such as hydrogels) can mimic the composition and function of the ECM and can be tailored to the need of specific models. Incorporation of organ-specific ECM components improves biocompatibility, physiological rigidity, and biochemical properties of cell substrates. This, for example, involves incorporating cell adhesion molecules, growth factors and signalling molecules that are released to promote tissue development[70] that is functionalization of substrates. Such substrates could be used to recreate conditions and cues required for sophisticated cell types such as

BOX 1

Standardization and validation of OoC models

To enable wider implementation of OoC technology in infectious diseases research, it is crucial to address issues concerning standardization and validation. To ensure that OoCs can be used in different laboratories and their results are reproducible, specific standards are needed for both the chips and the cell culture. In addition, guidelines to define and classify the models, as well as to measure and benchmark their performance are necessary[97].

The standardization of OoC technology is a priority for several government-sponsored programmes in the United States and Europe. The development of reproducible and robust OoC models is further supported by guidelines in related fields, such as the Good Cell Culture Practices and the Good In Vitro Method Practices. These guidelines are already applied for immortalized cell lines. However, it remains a challenge to reach similar guidelines for primary or stem cells. Accessories used with the chips, such as connectors, tubes, needles, or pumps, are already standardized[97] as they can be bought from commercial suppliers. However, they do need to be properly reported to ensure reproducibility.

Reliable OoC models that are relevant for infectious diseases research can only be obtained through validation. Internal validity, referring to the model's robustness and reproducibility, involves establishing clear benchmarks for chip fabrication, model assembly and monitoring batch-to-batch variability[97,98]. External validity refers to the model predictability, or ability to accurately translate its findings to real-world situations. OoCs can be validated based on clinical data from patients. An important tool in this regard is the Microphysiological Systems Database[99] that gathers published in vitro and in vivo data for chemical compounds. This database, together with other published resources, can be used for external validation of OoC models, since human responses to a specific chemical, in terms of specific biomarkers and ranges, can be compared with the data obtained using OoC models.

Some OoC companies already provide well-established commercial OoC platforms, ensuring high reproducibility of standardized assays. However, other platforms are still in the research and development phase with room for innovation, standardization, and adjustments for their use in infection biology research. In the next years we can expect more resources and effort towards the standardization and validation of OoCs from the community of producers, regulators and end users. Other products comparable to OoCs have also been recently standardized, such as the Tissue Engineered Medical Products, which help provide a reference and roadmap towards standardization of OoC models.

stem cells. Additionally, membrane-free models have been developed[71], for example using membranes or hydrogels that can be degraded with enzymes, hydrolysis, light, or mechanical stimulation[72–75] or hybrid solutions, such as fibre meshes and hydrogels[76].

Immortalized cell lines are commonly used to model tissues in OoCs. While this helps ensure standardization, it limits OoC applications in some cases. The tumour origin of these cells or the process of immortalization of cell lines usually affects their gene expression, morphology, and function, thus not always representing the in vivo situation. For example, the widely-used hepatic cell line HepG2 has a low expression of drug-metabolizing enzymes[77]. The skin cell line HaCaT shows major defects in the epithelial–stromal signalling, preventing it from forming a normal stratified epithelium[78]. To circumvent these drawbacks, OoCs can be built with primary cells and cells derived from organoids[39,55,79], combining the advantages of OoCs with organoids and primary cells (Table 1). Emerging alternatives for developing personalized models are adult stem cells and induced pluripotent stem cells. Since it is possible to grow and differentiate each cell type separately and then combine them inside the chip, OoCs may be built with customized cells. For example, patient-derived cells, stem cells from the same origin or specific cell lines. This enhances the physiological relevance of these systems for studying human infections and offers high reproducibility[80], as well as enables customization of the model and inclusion of more diverse cells. Stem-cell-based models could circumvent allogeneic immune reactions that would occur when integrating immune cells from different origins, enabling the study of T cells and NK cells in OoC. Additionally, the impact of specific genetic backgrounds (such as polymorphisms) on susceptibility to infection and risk of severe disease could be investigated using patient-derived stem cells in OoC models.

To expand their applications in drug discovery, higher throughput chip formats are being developed. Interesting examples are mini OoCs in 96-well plate format[43,44,50] or stackable chips[12]. Even though the model complexity and readouts that can be obtained using these models may not be as extensive, these assays enable higher throughput analysis of various conditions, which can aid drug discovery and testing. In addition, in the coming years we can expect diverse OoC formats in the market that will likely improve user-friendliness, reduce the need for specialized equipment, and decrease costs.

Incorporating sensors into OoCs can allow for continuous monitoring of specific parameters impacting infection, such as oxygen, pH, glucose, cytokines, and metabolites. This could offer unique insights in their dynamics during infection[81]. Trans-epithelial electrical resistance measurements in OoCs provide real-time assessment of barrier functions[82]. Combining OoC models with single-cell and spatial transcriptomics could also enable acquisition of high-resolution data on gene expression patterns and cell-to-cell variations, thereby enhancing our understanding of infection dynamics at the cellular level.

By improving the optical properties of OoC materials, such as glass bottom plates, these systems become more compatible with microscopic technologies, offering a comprehensive approach to studying human infections. Combined with advances in the optical microscopy field, this opens up possibilities to observe host-pathogen interactions with high spatiotemporal resolution. Live-cell imaging allows dynamic monitoring of the infection[83], whereas super-resolution optical microscopy approaches can visualize the organization of proteins with so far unprecedented resolution[84]. Different options for specific applications are available depending on the need for temporal resolution, long acquisition times, field-of-view, or three-dimensionality[85,86]. It remains to be seen whether alternative high-resolution imaging approaches such as expansion microscopy[87], which is based on dedicated sample preparation protocols, will be applicable for molecular-scale investigations in OoCs. Achieving high temporal resolution and long acquisition times is essential for dissecting dynamics of cells and proteins. However, it usually comes with the cost of reducing spatial resolution and field-of-view, especially in 3D samples such as OoCs[85,86]. For fluorescence microscopy, labelling needs to be optimized in general, but presents a special challenge in OoCs as labels need to penetrate several cell layers, calling for tailored protocols, for example, using smaller labels. Exchangeable tags, which reduce photobleaching after intense or prolonged imaging by exchanging the bleached tags with fresh ones after each round, can also be useful for live imaging or imaging in 3D samples, prone to photobleaching. Functional dyes, that can report on environmental parameters such as pH, ion concentrations, or membrane fluidity can provide several types of data in real-time[85,88,89]. Finally, advanced optics, such as special objective lenses or adaptive optics, could improve visualization into deeper cell layers in OoCs[90].

Conclusion

Various studies mentioned here showed that OoC models can help recapitulate human tissue compositions, architectures, functions and environments. Even though several assets are not exclusive for OoC models, they combine many advantages into one platform, allowing them to tackle complex research objectives in infection biology, while maintaining the flexibility and controllability of in vitro work.

Of note, OoCs are not superior to other models per se, but provide a different option to other in vitro systems and in vivo models. In fact, the use of different models to address specific parts of a research question is a common approach[17,24,25,91,92]. OoC models can be powerful tools when applied to the right research questions. They are particularly useful to model infectious diseases that involve a dynamic environment or the interconnection of several tissues.

OoC models can be designed and adapted in a flexible manner to suit scientific or industrial needs. The current market also offers OoCs with diverse degrees of complexity, versatility, user-friendliness, and price. However, the decision to use OoC models (if at all) and which type should be made on a case by case basis by taking the discussed points into account.

Taken together, (M)OoCs have gained momentum in the fields of infection biology, immunology and biomedicine. In the coming years, we can expect a progression in the development of different (M)OoCs formats and applications to model health and disease. Considering their increasingly diverse, complex and reliable readouts, they will offer new insights into infectious diseases and can be expected to aid the discovery of novel anti-infective compounds.

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Author contributions

R.A.-R. was responsible for conceptualization, literature research, writing and editing the manuscript, and preparing figures. A.S.M., M.T.F., C.E. and F.H.S. were responsible for writing sections of the manuscript and editing the manuscript. K.P. was responsible for editing the manuscript. B.H. was responsible for conceptualization, supervision and editing the manuscript. M.S.G. was responsible for conceptualization, supervision, writing sections of the manuscript, editing the manuscript, and preparing figures.

Competing interests

A.S.M. consults for and holds equity in Dynamic42 GmbH. R.A.-R., M.T.F., M.S.G. and B.H. are members of the Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute (Leibniz-HKI), which holds a cooperation agreement with Dynamic42 GmbH. The Leibniz-HKI is also member of the EU consortium FunHoMic, which maintains a consortium agreement and includes the company Mimetas. The other authors declare no competing interests.

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