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In vitro immunity: an overview of immunocompetent organ-on-chip models

Andrew I. Morrison [ID][1,2], Mirthe J. Sjoerds [ID][1],
Leander A. Vonk [ID][1], Susan Gibbs [ID][1,2,3]
and Jasper J. Koning [ID][1,2]*

[1]Molecular Cell Biology and Immunology, Amsterdam UMC Location Vrije Universiteit Amsterdam,
Amsterdam, Netherlands, [2]Amsterdam Institute for Infection and Immunity, Inflammatory Diseases,
Amsterdam, Netherlands, [3]Department of Oral Cell Biology, Academic Centre for Dentistry
Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit, Amsterdam, Netherlands

Impressive advances have been made to replicate human physiology *in vitro* over the last few years due to the growth of the organ-on-chip (OoC) field in both industrial and academic settings. OoCs are a type of microphysiological system (MPS) that imitates functional and dynamic aspects of native human organ biology on a microfluidic device. Organoids and organotypic models, ranging in their complexity from simple single-cell to complex multi-cell type constructs, are being incorporated into OoC microfluidic devices to better mimic human physiology. OoC technology has now progressed to the stage at which it has received official recognition by the Food and Drug Administration (FDA) for use as an alternative to standard procedures in drug development, such as animal studies and traditional *in vitro* assays. However, an area that is still lagging behind is the incorporation of the immune system, which is a critical element required to investigate human health and disease. In this review, we summarise the progress made to integrate human immunology into various OoC systems, specifically focusing on models related to organ barriers and lymphoid organs. These models utilise microfluidic devices that are either commercially available or custom-made. This review explores the difference between the use of innate and adaptive immune cells and their role for modelling organ-specific diseases in OoCs. Immunocompetent multi-OoC models are also highlighted and the extent to which they recapitulate systemic physiology is discussed. Together, the aim of this review is to describe the current state of immune-OoCs, the limitations and the future perspectives needed to improve the field.

Abbreviations: 3D, Three-dimensional; BBB, Blood-brain barrier; DC, Dendritic cell; EC, Endothelial cell; ECM, Extracellular matrix; FDA, Food and Drug Administration; FRC, Fibroblastic reticular cells; HLA, Human leukocyte antigen; HUVEC, Human umbilical vein endothelial cells; IL, Interleukin; LC, Langerhans cell; LPS, Lipopolysaccharide; MPS, Microphysiological systems; OoC, Organ-on-chip; NK, Natural Killer; PBMC, Peripheral blood mononuclear cells; PDMS, Polydimethylsiloxane; RhS, Reconstructed human skin; TCR, T cell receptor; TNFα, Tumour necrosis factor alpha.

1 Introduction

Disease is a major burden on society; economically, socially, physically and psychologically. Worldwide, over 7.6 million people die annually from transferable diseases, like influenza and COVID-19, and over 40 million people from non-transferable diseases, such as cancer and cardiovascular diseases (1). Over the past decades, progress at healthcare organisations and pharmaceutical industries has advanced to such a level that many diseases can be prevented, controlled or even cured. Significant improvements have been made, with key driving factors including vaccination programs, innovative research on disease pathophysiology, discovery of new drug targets and advancements in toxicity screenings (2, 3).

Given the complexity of human physiology and ethical considerations, many human diseases have been investigated using animal models or *in vitro* cultures of human cells (4). Animal studies have traditionally been the gold standard in the drug development process preceding clinical trials. While they have been recognised as a necessity for evaluating drug metabolism, toxicity and efficacy, they do have several drawbacks. These include poor translatability to humans, low reproducibility rates, high costs and a time-consuming nature (5). Together, this results in around 90% of drug trials that are pre-screened in animals failing in humans due to differences with drug efficacy and toxicity effects (6). In addition to *in vivo* models, conventional *in vitro* assays have been widely used for predictive drug testing. These make use of human cells derived from either fresh human tissues/organs, or immortalised cell lines cultured under static conditions (7). However, such cultures generally lack the intricate three-dimensional (3D) multicellular organisation of a human organ, including vascularisation, which is complex to recreate in a static model. As such, this has led to the birth of MPS; a more realistic human physiological microenvironment represented in an *in vitro* setting.

1.1 Microphysiological systems and organ-on-chip platforms

MPS is a hypernym for *in vitro* models capable of replicating features of human physiology on a micro-scale that is biologically suitable for their intended function (8). OoCs are a type of MPS platform in a microfluidic device that can control and allow the imitation of native tissue/organ functions such as dynamic, organisational and physiological responses. An OoC microfluidic device can act as a small scale bioreactor to maintain fresh human biopsies or reconstructed organotypic tissues/organoids for extended periods of time (9). These microfluidic devices can enable additional mechanical parameters like flow rate, stretch and pressure, which are traditionally lacking in static two-dimensional (2D) and 3D cultures (10). Such parameters allow constant supply of oxygen and nutrients to the organ models, as well as removal of toxic metabolites, while also facilitating cell migration and multi-organ crosstalk. The design of the microfluidic device varies based on the requirements for culturing single or multiple organ types within the device and the biological questions

that need addressing (11, 12). Examples of such microfluidic OoC platforms can be seen in Figure 1. Typically, microfluidic devices are made from cell culture-compatible materials, namely polydimethylsiloxane (PDMS), and feature micro-channels for media flow and culture compartments that can be filled with cells, ECM-like gels, organotypic models or biopsies (13). OoCs have been made in academic bioengineering laboratories and are also commercially available from industrial companies. Both sectors have generated promising results in terms of modelling true human representative organ functions-on-chip. This includes toxicity screens performed during drug development and disease mechanisms that can be further understood to a deeper level than what is currently possible in animal models (14). The more accurate portrayal of human physiology *in vitro* has led to official acknowledgement by the United States (US) FDA, who has authorised the "use of certain alternatives to animal testing" that includes OoC models to investigate the safety and effectiveness of a drug (15, 16).

1.2 The necessity to incorporate the immune system

Although encouraging advancements have been made in OoC innovation, the inclusion of the immune system is still significantly lacking and is crucial for these models to reflect more optimally human physiology and disease (17–20). The human immune system has a major underlying role in the pathophysiology of almost every disease, whether that be cancer (21), metabolic disease (22), infection (23) or autoimmunity (24). The process by which the human body reacts to external or internal threat is called inflammation, and this can be an acute reaction, where unwanted pathogens, wound debris or toxins are swiftly removed, or chronic where the response persists for weeks or even years (25). Inflammation in itself is an umbrella term for a cascade of events that result in the recruitment and activation of immune cells via release of pro- or anti-inflammatory mediators, such as lipids, cytokines and enzymes (26). The landscape of these inflammatory mediators varies throughout different organs, resulting in organ-specific immune responses.

Immune cells develop within primary lymphoid organs and can then migrate through complicated blood and lymphatic vascular networks to secondary lymphoid organs and tissues. Lymphoid organs systemically co-operate with innate and adaptive immune cells, who can be migratory or tissue-resident (27). Innate immunity is the first line of defence and includes cells such as dendritic cells (DC), macrophages, monocytes, neutrophils and mast cells from myeloid origin, and nature killer (NK) cells. These innate cells sense danger via pattern recognition receptors (PRRs) (28) and offer a quick immune response upon pathogenic challenge, such as phagocytosis and secretion of inflammatory cytokines. In contrast, T and B cells are the main subsets in adaptive immunity, which exhibit memory capabilities and are specific in their immune response. T cells become activated after the T cell receptor (TCR) recognises its cognate antigen in the lymph nodes via presentation by DCs using human leukocyte antigen class II
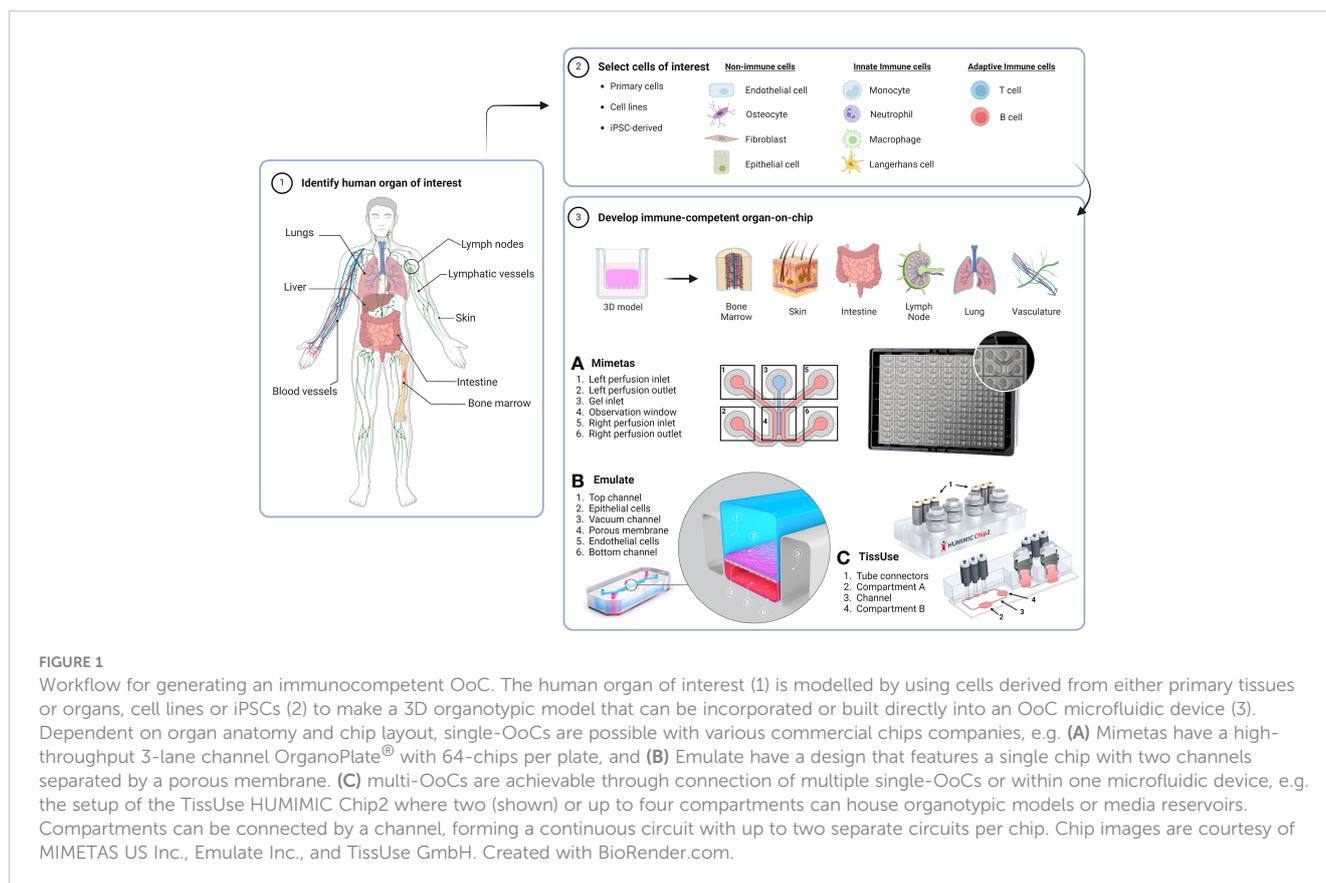
FIGURE 1

Workflow for generating an immunocompetent OoC. The human organ of interest (1) is modelled by using cells derived from either primary tissues or organs, cell lines or iPSCs (2) to make a 3D organotypic model that can be incorporated or built directly into an OoC microfluidic device (3). Dependent on organ anatomy and chip layout, single-OoCs are possible with various commercial chips companies, e.g. **(A)** Mimetas have a high-throughput 3-lane channel OrganoPlate® with 64-chips per plate, and **(B)** Emulate have a design that features a single chip with two channels separated by a porous membrane. **(C)** multi-OoCs are achievable through connection of multiple single-OoCs or within one microfluidic device, e.g. the setup of the TissUse HUMIMIC Chip2 where two (shown) or up to four compartments can house organotypic models or media reservoirs. Compartments can be connected by a channel, forming a continuous circuit with up to two separate circuits per chip. Chip images are courtesy of MIMETAS US Inc., Emulate Inc., and TissUse GmbH. Created with BioRender.com.

(HLA) molecules. These molecules are composed of different subtypes, termed HLA-DR, -DP, -DQ. T cell activation also depends on co-stimulatory molecules, such as CD27 from T cells bound to CD80/CD86 on antigen presenting cells (APCs). Once T cells are activated, they can either stimulate other lymphocytes to respond to potential threat or directly eliminate the target through the release of cytotoxic proteins, like granzymes. B cells also become activated after stimulation from T-helper cells or via antigen recognition directly, and can produce antibodies to neutralise the pathogen and/or facilitate opsonisation; a specific type of directed phagocytosis.

Until now, innate immunity has been predominantly simulated in OoC platforms. Unlike adaptive immune cells, innate immune cells do not rely on HLA molecules for their activation. Complexity greatly increases when adaptive immune cells are to be used due to their associated HLA-molecules, which may result in immune cell activation when other HLA-mismatched cell types are also present in the platform. This undesirable effect, mirrors the phenomenon known as graft *versus* host disease (GVHD) which leads to organ rejection by the adaptive immune system (29). In order to avoid cytotoxicity initiated by HLA-mismatch in OoC when investigating adaptive immune responses, all cell types should be derived from the same individual or at least be HLA-matched, introducing a major limitation of cell sourcing for current models. While efforts have been made by researchers to integrate immune cells into OoCs (30), development of human immunocompetent-organ models is needed to help us further understand how immune cells interact with organs during health and disease. This is particularly important for understanding how drugs can influence these interactions (e.g. localised immunotherapies for treating autoimmune disorders or cancer).

Therefore, the aim of this review is to provide a comprehensive overview describing the extent in which the human immune system, specifically innate and adaptive immune cells, has been incorporated into both single- (Table 1) and multi-OoC (Table 2) models, and to discuss their current limitations and future perspectives. The focus is mainly on the body barriers (lung, skin, intestine and liver) and lymphoid organs.

2 Organ barriers

Tissue barriers (e.g. lung, skin and intestine) play a vital role in maintaining systemic homeostasis by protecting internal organs from direct environmental assault, such as pathogens. In this way, the tissue barriers preserve organ functions and provide a robust defence against immunological challenges. Additionally, although the human liver is not directly connected and exposed to the external environment, it is an immune-rich tissue that acts as a checkpoint for the clearance of foreign intestine-derived antigens before they can enter the systemic blood stream (105). Therefore, characteristics of single organ barriers-on-chip with an immune component, including the liver, are summarised in Table 1 and are further described in the following text below.

TABLE 1 Overview of immunocompetent single-organ-on-chip models.

Organ	Simulated feature(s)	Cell types	Microfluidic device and readouts
Lung	Inflammation (31–34)	IC: (P) PBMCs (31, 32, 35), macrophages (36, 37), neutrophils (33, 38, 39), T cells (40, 41)	MD: commercial OoC (Emulate) (32, 34, 36–43) and in-house (31, 33, 35), unidirectional open systems
	Cell crosstalk (37, 38, 40–42)		
	SARS-CoV-2 (35–37, 39, 43)	SC: (P) alveolar epithelial cells (32, 34–38, 40–42), microvascular ECs (31, 34, 36, 37, 39–42), lung fibroblasts (33), HUVECs (31, 33), airway epithelial cells (33, 39)	R: IC migration and adhesion, cytokine secretion, gene and protein expression, barrier permeability, metabolomics and proteomics
Skin	Barrier function (44)	IC: (P) PBMCs (45), macrophages (46), T cells (47), neutrophils (47)	MD: in-house (44–49), dynamic open/closed systems
	Wound healing (46)	(CL) U937-monocytes (44), HL-60 cells (48)	
	Inflammation (47–49)	SC: (P) keratinocytes (44, 49), fibroblasts (46, 48, 49), HUVECs (46), ECs (45) (CL) HaCaT cells (46–48)	R: barrier function (microscopy, electrical resistance and fluorescent tracers), inflammation (cytokine secretion), viability, cell-cell interactions, IC phenotype and migration
	Adipose (45)		
Intestine	Host immune-microbiome interactions (50–53)	IC: (P) PBMCs (52), monocyte/macrophages (54, 55), moDCs (56), neutrophils (57)	MD: in-house (53, 54, 56, 59) and commercial OoC (Emulate (43, 50, 51), ChipShop (52), and Mimetas (55, 57, 58, 60)), unidirectional/bidirectional/dynamic open/closed systems
	Barrier function (57)	(CL) THP-1 cells (57–59), MUTZ-3-DC precursors (58), U937 cells	
	IC migration (56)	SC: (P) HUVECs (54, 59), microvascular ECs (41, 52), organoids/iPSC (43, 55, 56, 59, 60)	
	Inflammation (41, 54, 55, 58–60)	(CL) epithelial cells (Caco-2), HT-29-MTX-12 cells (58),	R: barrier function (permeability), cell viability, bacterial activity, gene and protein expression, cytokine secretion, transcriptomics
Liver	Inflammation (61–66)	IC: (P) PBMC-isolated macrophages (61), Kupffer cells SC: (P) Hepatocytes, HUVECs (61)	MD: in-house (64), commercial OoC (CNbio (65, 66), Emulate (63), Mimetas (62), ChipShop (61)), unidirectional/ bidirectional/dynamic open/closed systems
	IC infiltration (61)	(CL) stellate cells LX-2 (61, 63), HepaRG (66)	R: IC migration, cell-cell interactions, gene and protein expression, cytokine secretion, metabolomics, cell viability
Bone Marrow	Haematopoiesis and niche formation (67–69)	IC: (P) HSPCs, BMNCs (70), CD34+ progenitors (70, 71) (CL) SUP-B15 (72)	MD: in-house (67, 68, 71, 72) and commercial (TissUse (69) and Emulate (70)) OoC, dynamic/unidirectional closed/ open systems
	Bone marrow and cancer cell interactions (71, 72)	SC: (P) BMSCs, MSCs, HUVECs (71), osteoblasts (71)	R: cell survival and phenotype, cell-cell/ matrix interactions, gene and protein expression, cytokine secretion, oxygen consumption
	Shwachman–Diamond syndrome (70)		
Lymph Node	IC interaction and trafficking (73–75)	IC: (P) PBMCs (73, 76–78), DCs (75), moDCs (77), T cells (75),	MD: in-house (73–76, 79, 80) and commercial (Emulate (78), TissUse (77)) OoC, unidirectional/dynamic open/closed systems
	Cellular organisation (78, 79)	(CL) MUTZ-3 cells (73), THP-1 cells (79, 80), Jurkat cells (74, 79)	
	Antigen-antibody	SC: (CL) fibroblasts (75)	R: DC maturation and migration, T cell activation, cell adhesion, antigen-specific antibody secretion, cytokine production and permeability

(Continued)

Organ	Simulated feature(s)	Cell types	Microfluidic device and readouts
	responses (76, 77)		
	Cancer metastasis (80)		
Spleen	Blood filtration (81, 82)	IC: (CL) THP-1 cells (83) Other: RBCs	MD: in-house (81–83) R: cell viability, morphology, metabolomics, mechanical parameters, microscopy
	Sickle-cell disease (83)		
Vasculature	Blood and lymph vessel-IC interaction (84, 85)	IC: (P) PBMCs (86), T cells (84, 85, 87–89), neutrophils (90–92), moDCs (93), (CL) THP-1 cells (87)	MD: in-house (86–89, 91–93) and commercial [Mimetas (84, 85, 90)] OoC, dynamic/bidirectional open systems
	IC migration and infiltration (88–90, 93)	SC: (P) HUVECs (84, 87, 89, 90), (CL) HMEC-1 ECs (85)	R: DC maturation and migration, T cell activation, Cell adhesion, antigen-specific antibody secretion, cytokine production and permeability
	Cancer metastasis (87, 91, 92, 94–97)	Cancer cells: (CL) melanoma A375 cells (85), breast cancer cells (87)	

P, primary; CL, cell line; IC, immune cell; SC, stromal cells; MD, microfluidic device; R, readout.

2.1 Lung

The first OoC model to be established, which represented a more complex human micro-physiology, compared to what could be achieved in static models, was a model of the lung (10). The airways are prone to infection and inflammation and therefore have numerous mechanisms for protection such as; mucus surfactant for trapping foreign particles, epithelial cell barriers and resident immune cell populations, where the most prevalent are the alveolar macrophages (106).

The lung-on-chip mirrors human physiology by replicating essential tissue characteristics, including dynamic airway movements, surfactant release, the alveolar-capillary interface, airway inflammation and incorporation of blood vessels (42). Such chip models contain an air-liquid interface (ALI) and are generally constructed in two-channel microfluidic devices. These channels can house primary lung alveolar epithelial cells and pulmonary microvascular endothelial cells (ECs) on either side to simulate an epithelial barrier, separated by a porous membrane. A standard configuration of a lung-on-chip is illustrated in Figure 1, which shows a design associated with the Emulate lung-on-chip system.

Incorporation of immune cells into existing lung-on-chips has been used mainly for disease models, namely that of the lung's response to SARS-CoV-2 from the COVID-19 global pandemic, but also inflammatory diseases like asthma (38). The most common approach to introduce immunity involves the use of peripheral blood mononuclear cells (PBMCs) obtained from buffy coats, which consist predominantly of T cells and different proportions of B cells, NK cells, monocytes and DCs. PBMCs can be administered into the vascular endothelial lined channels of the chip, simulating the circulation of immune cells from blood. In the context of a lung-on-

chip exposure to SARS-CoV-2, PBMC-derived macrophages were found to contribute towards a SARS-CoV-2 induced interferon β (IFNβ) inflammatory response. The use of an inhibitor targeting the type 1 IFN intracellular pathway demonstrated the capability to alleviate the inflammation-on-chip, bringing the IFNβ levels down to those observed in an uninfected chip (36). Additionally, macrophages were recorded to have the ability to phagocytose SARS-CoV-2 damaged ECs (35). Furthermore, a severe immune overreaction, known as a cytokine storm, was modelled when SARS-CoV-2 infected patient samples were tested on a lung-on-chip, where the high cytokine levels were suppressed after monoclonal antibody treatment (37). In addition, clinically relevant SARS-CoV-2 treatments demonstrated drug efficacy by reducing viral load and inflammation on a lung-on-chip when PBMCs were present, indicating a benefit for the use of immune cells (43).

While most disease-related inflammatory lung-on-chip models focus on SARS-CoV-2, other viral infections have also been studied. PBMCs in a lung-on-chip model of rare acute respiratory distress syndrome (ARDS) displayed extravasation from one chip channel to another that was dependent on the presence of an endothelial barrier, ECM density/stiffness, and the flow profile (31). Bidirectional flow delayed the extravasation of immune cells compared to unidirectional flow, highlighting the importance of organ-specific dynamic flow conditions. Another study focusing on influenza virus-induced endothelial inflammation found that the number of PBMCs adhering to the lung-on-chip's endothelium was 100 times higher compared to uninfected chips (32), demonstrating their capability to react to pathogenic challenges.

In addition to PBMCs, single immune cell populations have also been brought into lung-on-chips. For instance, neutrophils, a major component of the lung's innate immune system (42), have

TABLE 2 Overview of all immunocompetent multi-organ-on-chip models.

Organ	Simulated feature(s)	Cell types	Microfluidic device and readouts
Gut/ Skin (98)	Gut inflammation and lipid uptake on skin	IC: (P) macrophages SC: (P) dermal fibroblasts, keratinocytes (CL) Caco-2 cells	MD: in-house R: cell viability, metabolite production, cytokine/ chemokine secretion, permeability and immunofluorescence
Liver/ Gut (99, 100)	Inflammation mediated modulation of drug disposition.	IC: (P) Kupffer cells, DCs, T cells (100) SC: (P) hepatocytes (CL) Caco-2 cells and HT29-MTX (99)	MD: in-house R: cytokine/ chemokine secretion and gene expression
Skin/ Gingiva (101)	Nickel induced inflammation	IC: (CL) LCs SC: (P) keratinocytes, fibroblasts	MD: commercial HUMIMIC multi-OoC (TissUse), dynamic system
Lung/ BBB (102)	Lung cancer metastasis to the brain	IC: (CL) THP-1 monocytes SC: (CL) lung cancer cells, fibroblast, ECs, epithelial cells, astrocytes, ECs	MD: in-house R: barrier integrity and permeability, immunofluorescence and cytokine/ chemokine secretion and gene expression
Lung/ Liver/ Heart (103)	Drug toxicity screening	IC: (P) Kupffer cells SC: (P) hepatic stellate cells, hepatocytes, iPSC-derived cardiomyocytes, cardiac fibroblasts, ECs, stromal mesenchymal cells, bronchial epithelial cells	MD: in-house R: barrier function (microscopy, electrical resistance and fluorescent tracers), inflammation (cytokine secretion), viability, metabolite secretion
Gut/ Liver/ Kidney/ Bone Marrow (104)	First pass metabolism, PK and toxicity.	IC: (P) CD34+ progenitor cells	MD: in-house R: viability and cell tracking by immunofluorescence

P, primary; CL, cell line; IC, immune cell; SC, stromal cell; MD, microfluidic device; R, readout.

displayed migratory chemotactic properties across the EC barrier to epithelial cells upon Influenza A exposure in lung-on-chips (39), as well as in fibrotic lung-chips (33). Next to this, T cells are the predominant adaptive immune cell present in the airways and consist of mainly tissue resident memory T cells. When applied to lung-on-chips in a pool of PBMCs, activated T cells also had a migratory capacity towards epithelial cells upon inflammation using different viruses (39). Since T cells have a prominent role in recognising infected cells or cancer cells, effort has been made to increase their killing capability via multiple mechanisms, including the generation of bi-specific antibodies (40). The safety efficacy of a bi-specific antibody coupling CD3$^+$ T cells to tumour antigens has been evaluated using an alveolus-on-chip, highlighting the practical use of such a model in the toxicology field (41). T cells also have a

pathophysiological role in asthma, and while T cells have not yet been used in asthma-on-chip, interleukin (IL)-13 was used to represent a T-helper cell type 2 suited microenvironment in a microfluidic device that replicated clinical data in terms of mucociliary clearance and increased mucus secretion (34).

In summary, immunocompetent lung-on-chips have rapidly evolved, heightened by the COVID-19 pandemic, and have started to characterise the role of immune cells in viral and bacterial infections and their effect on epithelial and ECs. There is still a need for further representation of the innate and adaptive immune system in these chips, particularly for more inflammatory diseases such as respiratory allergies to elucidate the role of allergen-related immune cells i.e., mast cells, and for understanding drug mechanisms.

2.2 Skin

The skin is another protective barrier against external pathogens, chemicals and physical stimuli. It consists of two main layers; the epidermis and the dermis. The epidermis is composed of highly specialised keratinocytes, melanocytes and immune cells, such as Langerhans cells (LCs). The dermis is a fibroblast-populated ECM compartment containing the vasculature and immune cells such as dermal DCs, T cells and macrophages (107). These skin immune cells exist in either resident or migratory populations, where upon tissue damage the APCs (LCs and DCs) become activated and migrate towards the skin-draining lymph nodes through the lymphatic vasculature.

A major characteristic of *in vitro* human organotypic skin models is their exposure to the air from the epidermis side, known as the ALI, which promotes spontaneous epidermal differentiation and stratification. Nutrients are supplied via culture medium in contact with the basal layer of the epidermis in reconstructed human epidermal (RhE) models or via the dermis side in full-thickness reconstructed human skin (RhS) models. RhS are typically bi-layered structures with keratinocytes seeded on top of a fibroblast-populated collagen-based 3D matrix (108). This design offers several advantages over *ex vivo* skin biopsies, e.g. prolonged culture duration with defined cell types present and can be readily used for safety/risk assessment, wound healing, drug delivery and allergen induced inflammation/disease. However, immunocompetent skin-on-chip models are still in their infancy and relatively simple in terms of their cellular setup.

One of the first reported immunocompetent skin-on-chip models was developed using epidermal keratinocytes, cultured together with the U937 monocyte-like cell line under dynamic flow. The model showed improved keratinocyte tight junction formation and general long term cell survival (44). Such immunocompetent skin-on-chips have evolved further by the addition of dermal fibroblasts with PBMC-derived T cells (47) or human umbilical vein endothelial cells (HUVECs) with macrophages (46) to simulate a vascular channel. These immune cell additions facilitate the study of tissue infiltration, cytokine production and dynamic cell-cell interactions that more closely resemble normal native skin processes. In addition, vascularised

skin-on-chip models have demonstrated neutrophil migration from endothelium to dermis upon ultraviolet (UV) radiation exposure (48), further show-casing the ability to recapitulate dermatology-based phenomenon. Increased complexity of skin-on-chip has been described to include neopapillae into the dermis hydrogel, which are precursors of the hair follicle (109), but this has yet to include immune cells.

The pathophysiology of numerous skin related-diseases show immune cell involvement. For example, the addition of an adipocyte layer to RhS model has displayed an essential role in stabilising the metabolic properties of the skin (110), and as such, obesity has been mimicked with immune cell incorporation into a white adipose tissue (WAT)-on-chip (45). Adipocytes and ECs were isolated from skin biopsies and co-cultured in a chip with the same patient derived PBMCs, namely CD14$^+$ monocytes and T cells. The addition of these immune cells to the model could recapitulate endocrine and immunomodulatory WAT functions. For more inflammatory and allergy-associated skin diseases-on-chip, incorporation of immune cells has yet to be achieved. However, potential does exist to address these disease mechanisms. For example, atopic dermatitis (AD) was modelled on chip using the disease-relevant cytokines IL-4 and IL-13. This resulted in tissue dehydration, keratin exfoliation and suppression of barrier-related genes (49).

In summary, skin-on-chip models have been comprehensively characterised and demonstrate robust properties in comparison to native human skin, albeit still lacking key features such as adipocyte layers, glands, nerves and growth of hair follicles. However, their immune-compatibility is still in its initial phases, primarily relying on the use of innate immune cells.

2.3 Intestine (Gut)

Like the skin and airways, the intestine (gut) is a barrier organ that is constantly in contact with external stimuli, harbouring a microbe dense microenvironment to fine tune a balance between tissue homeostasis and pathogenic infection. Hence, this is why the gut houses an extensive population of resident immune cells in the body. These immune cells can be found in an area of connective tissue called the lamina propria that house a plethora of e.g. macrophages, T cells and DCs (111, 112). The intestinal architecture contains villi, which are small projections of epithelium extending into the lumen to increase the surface area for nutrient uptake. The majority of gut-on-chips have overlapping attributes with lung-on-chips, such as inclusion of vacuum chambers to replicate peristaltic movement and chip-channels separated with a semi-porous membranes or ECM-rich hydrogel to enable culture of human intestine (or colon) epithelial cells and ECs (Figure 1).

Immune cells of myeloid origin have generally been used in multiple gut-on-chip models. Similar to skin-on-chip models, the first immunocompetent gut-on-chip model introduced the monocyte cell line U937, perfused through a two-channel chip containing the epithelial cell line CaCo-2. After lipopolysaccharide

(LPS) or tumour necrosis factor alpha (TNFα) exposure to trigger inflammation, the epithelium increased barrier permeability and induced immune activation (44). To date, gut-on-chip models have become more complex by the addition of HUVECs for the endothelial compartment, and gut commensal microbiome components such as probiotic bacterial strains as detailed below. Gut-on-chip co-cultures with microbial species have included either complete PBMCs or PBMC-isolated macrophages/DCs perfused through endothelial channels to study several different parameters, such as microbe-dependent tissue inflammation, damage and cell differentiation (50–52). Such addition of immune cells in these experiments have displayed protective properties of the endothelium, as the ECs were normally subjected to inflammation-associated tissue damage from the microbial species when immune cells were not present.

The major chronic inflammatory disease associated with the gut is inflammatory bowel disease (IBD), encompassing conditions like ulcerative colitis and Crohn's disease. This condition poses significant challenges for individuals, which is why gut-on-chip models are an appealing choice for IBD disease modelling and testing drug efficacy. When epithelial barriers are damaged, it leads to leaky gut, allowing pathogens to enter the bloodstream. In the context of gut-on-chips, IBD has been recapitulated through combinations of inflammatory cytokines, *E. coli* or LPS, and has involved the use of monocyte-derived DCs, macrophages, and PBMCs (53, 54, 56). More specifically, after cytokine exposure, pro-inflammatory M1 macrophage differentiation occurred via crosstalk with epithelial cells (55) and the monocyte cell lines THP-1 and MUTZ-3 were able to provoke synergistic inflammation through increase in IL-8 secretion in a chip (58). The theme of gut inflammation has extended to the use of neutrophils in an LPS-induced gut-on-chip, which mimics epithelial damage by neutrophil invasion and inflammatory crosstalk between resident and circulating immune cells (57).

Interestingly, there has been limited incorporation of adaptive immune cells in gut-on-chip models. One study has explored the safety and efficacy of T cell bi-specific antibodies targeting tumour antigens, which was conducted in parallel with an alveolus-on-chip model, as previously mentioned (40, 41). Nonetheless, for an incapacitating disease like IBD, the addition of immune cells, such as macrophages, and inflammatory mediators to the intestine-on-chips recapitulates a physiologically-relevant disease setting. This is particularly relevant since IBD patients have a higher abundance of macrophages and inflamed epithelium compared to healthy individuals (113, 114). Notably, models of the human intestine have a well distinguished organoid profile, whether derived from patient samples or induced pluripotent stem cell (iPSCs). Such organoids are now being cultured in gut-on-chip models that have an immune-like environment, such as iPSC-derived gut-like tubules that secrete IL-6 and IL-8 under an inflammatory stimulus (60). Likewise, a vascularised colon organoid demonstrated monocyte adherence to ECs, which then transmigrated towards the epithelium to undergo macrophage differentiation (59).

To summarise these immune gut-on-chip studies, it becomes apparent that they have predominantly featured innate immune cells over adaptive immune cells. This is reasonable given their

prominent involvement in gut-related diseases to a certain extent. However, it is worth noting that the gut contains a substantial population of T cells, which play a foundational role in other gut disorders, like Crohn's and celiac disease. Our intestine is influential to general health, so it would be pivotal to include adaptive immunity into future gut-on-chip models.

2.4 Liver

As stated in the introduction to the organ barriers, the liver is included in this review. The liver directly receives intestine draining-blood from the portal vein and thereby can act as a barrier to systemic infection. Since it is crucial for filtering blood and metabolising drugs and toxins which have bypassed the lymph node and spleen, this makes it an ideal organ for on-chip immunotoxicity testing. The liver is an immune privileged organ, with specialised resident macrophages known as Kupffer cells (115).

Liver-on-chip models normally use primary human hepatocytes, but the inclusion of immune cells at the single-OoC level are still lacking and are in a relative infant state. Such liver-on-chips can be used to study hepatocyte differentiation and culture stability e.g. LPS-induced inflammation with PBMC-derived macrophages resulted in macrophage polarisation to a M2 phenotype and demonstrated their adhesive properties and infiltration into hepatic cell channels on the chip (61). Inclusion of Kupffer cells allow for a more physiological immune model, where their use has been validated in a liver-chip as a hepatotoxic screening platform based on metabolic readouts (62, 63). These Kupffer cells have also been administered to study liver-related diseases on chip, such as advanced stages of non-alcoholic fatty liver disease (NAFLD), where exposure to an overload of long-chain free fatty acids induced pro-inflammatory biomarkers that could then be attenuated with drug application, indicating a proof-of-concept for hepatotoxicity testing of drugs (64). Likewise, in a liver-on-chip model of hepatitis, the Kupffer cells responded to LPS and hepatitis B virus (HBV) infection by secreting pro-inflammatory molecules associated with the disease (65). Additionally, glucocorticoids were assessed on liver-on-chips containing Kupffer cells to evaluate their anti-inflammatory properties (66).

Henceforth, it is clear that so far only innate immunity is partially represented in liver-on-chip models with the use of Kupffer cells. Integration of other innate immune cells to represent liver functioning is still required for future studies.

3 Lymphoid vasculature and organs

Lymphoid organs are a crucial component of the human immune system and distributed throughout the body to regulate and support immune responses. These specialised organs are integral in the production, maturation and activation of various immune cells. This all begins in the primary lymphoid organs and proceeds to the secondary lymphoid organs, which are strategically located to drain interstitial fluid from tissue. These secondary lymphoid organs play a fundamental role in immune surveillance, tissue specific immunity and memory responses.

The trafficking of immune cells between organs and tissues takes place in blood and lymphatic vessels, which serve as a key structural element for systemic immunology e.g. to guide immune cells from the tissue into secondary lymphoid organs and to direct them to the sites of inflammation. Since it is evident that vessel-on-chip has almost unlimited potential for its integration into the OoC field amongst numerous scientific disciplines (116, 117), this review focusses only on literature which includes vessel-on-chip to study immune cell migration. As such, features of lymphoid organs- and immunocompetent vasculature-on-chip are summarised together in Table 1 and discussed in detail below.

3.1 Primary lymphoid organs

In comparison to other organ models, lymphoid organ-on-chip models are distinctive for their abundance of immune cells since they play a primary role in regulating our immune system. The bone marrow and thymus are essential constituents of our immune system. Innate immune cells arise in the bone marrow and mature in tissue. Adaptive immune cells stem from the bone marrow, functionally mature in the thymus and differentiate in the lymph nodes. In this review, we focus on bone marrow-on-chip models for immune cells, as immunocompetent thymus-on-chips are yet to be developed.

The bone marrow is a complex organ that consists of several unique niches with differing microenvironments of ECM structures to perform several functions, namely erythropoiesis, myelopoiesis and lymphopoiesis. The first bone marrow-on-chip model was created by implanting a PDMS device into the bone marrow of mice, loaded with bone marrow-stimulating growth factors. The device was then explanted after 8 weeks of growth and maintained for up to seven days *ex vivo*. The model, albeit using mice in this study, was shown to accurately mimic physiological niches in the bone marrow and was later used in drug toxicity tests (67). Progress without using animal material for bone marrow niches are now widely modelled on chip. Numerous studies have recreated the endosteal niche, located at the surface of the bone for hematopoietic stem and progenitor cell (HSPC) differentiation, on chip to highlight the importance of mesenchymal stromal cell adhesion and cytokine secretion for CD34$^+$ HSPCs maintenance and haematopoiesis (68, 70, 71). Currently, one bone marrow-on-chip study has demonstrated the formation of macrophage colonies from HSPCs (69) and, to date, the generation of further immune cell subsets are yet to be recapitulated.

One of the most studied diseases on bone marrow-chips is bone marrow cancer. As the bone marrow is closely situated to the blood supply, cancers that develop in the bone marrow can be prone to metastasis. This proximity provides a route for cancer cells to enter the bloodstream, facilitating their travel to distant organs and the subsequent formation of secondary tumours. This is particularly perilous, as the spread of cancer cells can impact the normal functioning of various organs. Cancer cells have been included in bone marrow-on-chips to study tumour migration, indicating preferential metastasis to different niches (71). Moreover, anti-leukaemia drugs have been screened in bone marrow-on-chips.

Here, the 3D microenvironment was deemed to protect the cancer cells from drug-induced apoptosis compared to 2D cultures (72), highlighting the advantages of chip models for the study of drug efficacy. In addition to bone marrow cancer-on-chip, the genetic disease Shwachman–Diamond syndrome (SDS) was emulated on chip, where mechanisms of the disease pathophysiology were revealed to indicate association with neutrophil maturation impairment (70).

The modelling of bone marrow-on-chip is a complex task since the bone marrow features an intricate organisation of different compartments, each with their own functions. Future improvements could be made by demonstrating haematopoiesis for multiple immune cell subsets and/or the inclusion of lymphoid progenitors. Further development of these models is crucial in our understanding of the bone marrow microenvironment.

3.2 Secondary lymphoid organs

Secondary lymphoid organs are strategically located throughout the body and are inter-connected by a network of lymphatic vessels that transport lymph-fluid drained from the peripheral tissues. These organs include lymph nodes, tonsils, spleen and peyer's patches. Their key feature is a complex multicellular environment that is organised into special niches by lymph node stromal cells (LNSCs), and this is where the adaptive immune response is orchestrated (118). Due to the involvement of secondary lymphoid organs in the pathophysiology of inflammatory diseases and tumour metastasis, recreating an organotypic lymph node environment that encompasses every biological process is challenging.

While there is a lot of complexity to immunological processes within lymph nodes, the majority of models have used PBMC-derived moDCs, T cells and B cells to investigate immune cell clustering. This research has included dynamic perfusion with adaptive immune readouts such as plasma cell differentiation, antigen-specific antibody formation and cytokine productions (76–78). Furthermore, imitation of immune cell chemotaxis across lymph nodes has extended to include DC maturation and migration in the direction of flow to T cell compartments (73, 79). DC migration could be standardised using the commercially available drug hydroxychloroquine, which induced reactive oxygen species in T cells on the chip (74). Cell-cell interactions have also been explored by investigating adhesion molecules in a subcapsular sinus model (80), although the extent of this cellular characterisation remains somewhat limited.

The spleen is another secondary lymphoid organ which, unlike the lymph node, filters blood. Initial efforts have been made to recapitulate core spleen-functions, such as blood filtration, from perfusion of ex-vivo spleen tissue (81) and spleen-on-chip devices using red blood cells (RBCs) (82). Intriguingly, macrophages were used in a spleen-on-chip model of sickle cell disease that revealed differences in their phagocytic capabilities between sickled red RBCs and non-sickled RBCs, under hypoxia (83). In spite of the fact that robust spleen features have been well-characterised with immune cells-on-chip, the technical developments to advance spleen-on-chip models are still at an early stage. For example, further studies to recreate immune cell behaviour with spleen-on-chip are required to more accurately portray splenic tissue biology, as well as using such models to better investigate infectious diseases, like visceral leishmaniasis (119).

The challenges of modelling secondary lymphoid organs, the lymph node in particular, lies in their complexity. A vital feature across all lymph node-on-chip models to date is their lack of stromal cells, in contrast to other tissue/organ models-on-chip, which all contained a stromal component, as detailed above. Currently, there is one chip model has included fibroblast reticular cells (FRCs) from a cell line source (75), which showcased DC and T cell migration towards this FRC compartment. There is clearly an unmet need for incorporation of these FRCs, given their role in not only shaping immune responses within the lymph node (120), but also their importance for immune cell survival and functioning in a 3D environment (121). Considering the abundance of lymph nodes in the human body and their central role in continuous filtration of interstitial fluid containing toxins, metabolites, and immune cells from large organ barriers (such as skin), lymph nodes are an ideal candidate for incorporation into multi-OoCs. Combined with other organ models, this can allow the recreation of a systemic immune response *in vitro*, as depicted in the schematic of Figure 2.

3.3 Vasculature

Blood and lymphatic vessels play an important role in immunological processes by trafficking immune cells between organs, tissue and the lymphatic system. Blood vessels allow for the transport and circulation of a plethora of lymphocytes from primary lymphoid organs into secondary lymphoid organs via high endothelial venules, as well as multiple other organs and tissue types. Lymphatic vessels drain interstitial fluid, which contains waste metabolites, pathogens and activated APCs, from all tissue to secondary lymphoid organs through afferent lymphatic vessels, for filtration and to initiate adaptive immunity. Once primed in secondary lymphoid organs, immune cells leave through efferent lymphatic vessels, and re-join the peripheral circulation. Therefore, without vasculature, it is not possible for a systemic immune response to occur. Vascularisation of organ models within microfluidic devices has become somewhat of a hot topic in the OoC field. This includes the integration of ECs under a single organotypic model and/or the seeding of ECs in vessel-like compartments entering or leaving the organ model. These models can provide insight into significant parameters such as blood and lymphatic vessel permeability of endothelial walls, shear stress, vessel formation and inflammatory responses, such as cytokine production. In addition, immune cell migration and cancer metastasis can also be modelled. Typically, the configurations of vessels-on-chip involves a primary microfluidic channel that can be populated with ECs, allowing immune cells to be administered through this channel. ECs can form lumen-like structures, which are often surrounded by a matrix containing stimulants or even tumour cells.

As such, vascular inflammation-on-chip with immune cell migration revealed that cytokine-stimulated PBMCs could change EC barrier properties, such as affecting EC morphology and upregulation of certain adhesion molecules (84). Similarly, T cells were characterised on their response to chemotactic gradients and shown to interact with ECs through transmigration into ECM hydrogels (85–87). The versatility of T cells was explored in other vascularised OoCs to highlight their functionality against tumour cells (88, 89).

Another immune cell type regularly integrated into vessels-on-chip is the neutrophil. It has been shown that ECM components can dictate their migration capacities (90). Neutrophils were shown to exacerbate tumour cell metastasis in an ovarian cancer-on-chip device, indicating their unique role in cancer progression, which may have been overlooked in standard static cultures (91). A similar result was also observed with LPS-stimulated neutrophils that disrupted EC barriers and enhanced tumour cell extravasation (92). Likewise, APC characteristics could be recapitulated with vessels-on-chip, where DCs exhibited their antigen capturing and presenting ability along a chemotactic gradient (93) and macrophages exhibited phagocytic capacities (94). M1 macrophages were also seen significantly inhibited tumour-induced angiogenesis on chip (95). These cellular functions even extended to NK cells, which underwent trans-endothelial migration and killed tumour cells on chip (96). Further NK cell killing properties were also displayed through trans-endothelial migration into breast cancer spheroids-on-chip (97).

In summary, there are numerous prospects to explore immune cell migration through vessels-on-chip. While it is already at a promising stage, this could be better accomplished by defining the use of blood ECs (BECs) or lymphatic ECs (LECs) for showing specific immune processes. As such, vessels-on-chip possesses unlimited potential for integration into pre-existing organ models and multi-OoC devices, aiming to truthfully replicate a systemic immune response *in vitro* (Figure 2).

4 Multi-organ-on-chip

The systemic physiological nature of the human immune response ensures a coordinated, total-body defence against a variety of health threats, like infections, cancer and toxic substances. This is why multi-OoCs have been developed. The arrangement of multi-OoCs can either be one microfluidic device that contains multiple interconnected organ models via channels and chambers, or multiple separate single-OoC microfluidic devices that are externally connected through tubing. The goal of such an integrated system, even without immune cells, offers a beneficial predictive value for drug safety and toxicity testing at a more systemic physiological level (123). From an immunology perspective *in vitro*, the potential of multi-OoCs are ideal for replicating dynamic immune cell migration between human organs to initiate tissue-specific immune responses. A comprehensive summary of all multi-OoC attributes with immune cells is displayed in Table 2, visualised in Figures 2, 3, and elaborated upon below.

At a two-organ immunocompetent multi-OoC level, crosstalk between the gut and liver during LPS-induced inflammation was
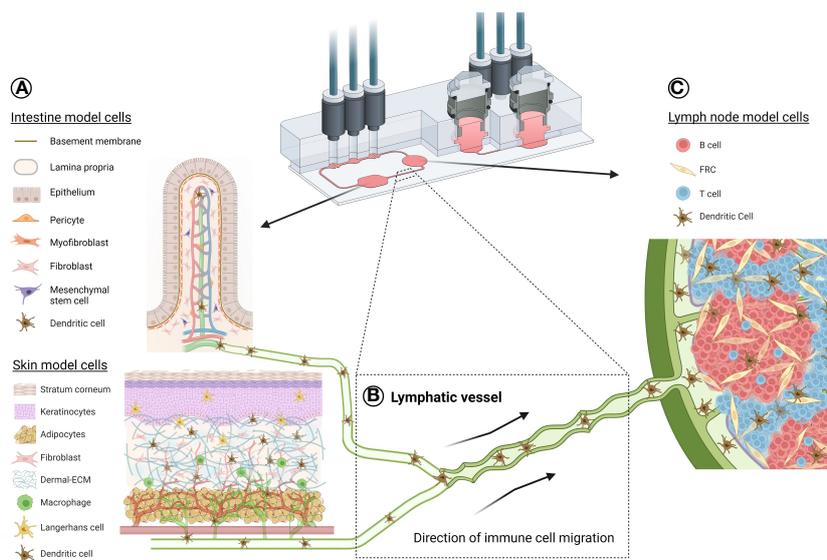
FIGURE 2
Schematic illustration of a potential organ-draining lymph node-on-chip. Exemplar use of a TissUse multi-OoC device to demonstrate immune cell migration between an organotypic skin or intestine model **(A)** through lymphatic vasculature **(B)** to an organotypic lymph node model **(C)**. This is representative of a standard immune response, where skin Langerhans cells and skin/intestine dendritic cells can become activated in the epidermis or dermis/lamina propria, respectively, due to either allergen/bacterial exposure, injury or disease. APC migrate into the lymphatic vessels for their journey to the lymph node for antigen presentation to the adaptive immune cells. Such a response can be possible using multi-OoCs, as well as other types of organ-crosstalk models. Chip image is courtesy of TissUse GmbH. Image of intestine model is credited to and adapted from (122). Created with BioRender.com.

FIGURE 3
Schematic overview of a human-on-chip design from all possible immunocompetent multi-OoCs. Immune cells (innate and adaptive) are colour-coded to show the combinations to which they are currently used in various biological processes and applications. Connecting and expanding such multi-OoCs would create the ability to recapitulate human physiology in an *in vitro* setting, albeit on a more simplified level. Image is courtesy of TissUse GmbH. Created with BioRender.com.

recreated by administering DCs to the gut compartment and Kupffer cells to the liver model. Here, the upregulation of immune pathway genes and pro-inflammatory cytokines was detected when the immune cells were present in each organ compartment (99). In a separate gut-liver axis study, the addition of circulating T cells enhanced IBD-like conditions on the multi-OoC, enhancing its practical use for a disease-based model (100). In another study, the skin was separately combined in two multi-OoCs devices, such as connection to gingiva (101) and the gut (98). The skin-gingiva organotypic model on chip mimicked a clinical case study where topical allergen exposure to the gingiva resulted in activation of a skin immune response, as illustrated by LC migration in the skin model thus representing a systemic inflammatory response. The gut-skin multi-OoC assessed the downstream effect of fatty acid absorption by the gut model on skin inflammation, where macrophages increased nitric oxide uptake, associated with a pro-inflammatory response. Next to this, a lung-blood brain barrier (BBB) multi-OoC was developed containing monocytes to study the epigenetics of non-small cell lung cancer. The outcome was a consistent data profile between mice and patient studies, indicating an accurate proof-of-principle set up that does not require animal models (102).

A singular immunocompetent three-organ multi-OoCs has been developed, where lung-liver-heart organs were connected through external tubing linking microreactors. Using liver Kupffer cells, cardiotoxicity was to be mediated by pro-inflammatory cytokine secretion after inducing toxicity to the lung model with bleomycin, a chemotherapy antibiotic (103).

Four-organ multi-OoCs have demonstrated crosstalk among organs in both lung-brain-bone-liver and gut-liver-kidney-bone marrow multi-OoCs. The lung-brain-bone-liver multi-OoC showcased immune involvement, where monocytes seeded in the lung tissue differentiated to M2 macrophages after tumour cell introduction. This provoked transmigration of cancer cells into other tissues and inflicted damage on the astrocytes (brain tissue), osteocytes (bone tissue) and hepatocyte (liver tissue) organ compartments (124). The gut-liver-kidney-bone marrow multi-OoC used CD34$^+$ progenitor immune cells to represent the bone marrow and study metabolomics with pharmacodynamic and pharmacokinetic parameters. However, such readouts were not immunology-related (104).

In summary, immunocompetent multi-OoCs are still in their initial stages of development, predominantly featuring innate over adaptive immune cells. Multi-OoCs represent the ultimate frontier in replicating systemic immunological processes in an *in vitro* environment. It is evident that progress is being made in the immunotoxicity field, but the path ahead is long and numerous challenges still need to be overcome.

5 Conclusion and perspectives

OoCs are crucial for advances in studying immunity *in vitro*. However, scientists are challenged by the most optimal chip design, fabrication and implementation to address biological questions. Human immunology is complicated on a physiological level and this review has aimed to summarise the extent of which the immune system has been recapitulated into OoC models.

In general, the innate branch of the immune system has most frequently been included into single- and multi-OoC compared to adaptive immune cells. From a logical perspective, these innate cells are the first line of defence against external pathogens, disease and inflammation, and therefore offer an unspecific and diligent response that may be easier to model. Such immune cells are readily available through PBMC-isolation from blood donors or as cell lines, allowing practical universal benefits and a somewhat unlimited source of cells. However, as we learn more about tissue microenvironments, the importance of tissue-specific immune cells becomes apparent and must be considered. This for example includes the liver Kupffer cells or skin LCs, which each exhibit specific environmental profiles that dictate their tissue functioning.

While adaptive immune cells have been employed sparingly in non-lymphoid OoCs, these cells present a genuine assessment of functional specificity due to their long-lasting and memory-like properties representing the adaptive immune response. Once successfully integrated and thoroughly evaluated, this development will mark a significant step towards systemic immunology *in vitro*, further complementing areas such as disease modelling, drug testing and personalised medicine. As OoCs become more and more advanced and complicated, this will allow the opportunity to use tissue-specific cells that can either be obtained from biopsies or more appealingly; iPSCs. The strength of using iPSC-derived immune cells above primary cells or cell lines will be that they will enable donor-matched autologous cell types to be integrated into OoCs, thus bypassing the allogenic responses in a donor HLA-mismatch, a vital hurdle for immune modelling. However, the current limitations with iPSCs include the incomplete maturation status of the differentiated cells, unknown ability to skew towards tissue specificity and, especially in the immunology field, their unknown ability to generate diverse repertoires of T and B cells. The origin of these cell types can naturally raise complex questions concerning whether an individual's sex, age or lifestyle choice might influence their functionality in an OoC, especially considering how our immune system alters over time under the influence of epigenetic factors [125].

As mentioned above, OoCs still lack the inclusion of all organ-specific cells. This is where the organoid field can act as a promising tool for creating models of the immune system for organ-on-chip. Such examples include kidney organoid-on-chip [126] and BBB-on-chip [127], which both feature T cells that demonstrate tumour eradication and adhesion, extravasation, and migration under inflammatory conditions. Additionally, a primary NK cell model has illustrated dynamic extravasation into a physically separated tumour cell niche on a microfluidic device [128], indicating that immune cell migration can be recapitulated without using an actual organ model on the chip. For immune organs, human tonsil organoids have been established to show early promise for modelling vaccine efficacy [129] and a lymphoma microenvironment [130]. However, these tonsil models do not feature or acknowledge stromal cells, which are integral for lymphoid organ functioning as alluded to earlier. In future studies, we anticipate a greater shift towards making these organoid models into more immunology based-on-chip.

The gold standard consideration is to what extent one must delicately balance the complexity and simplicity of the biological and technical OoC design. For example, a liver-on-chip was able to detect close to 90% of drug-induced liver toxicity in patients, a result that went completely under the radar in an animal model [131] [132]. While this liver-on-chip did not include immune cells, it is just one initial example of the potential promise that immunocompetent OoCs will have in superiority over animal experiments. Likewise, the very first lung-on-chip, developed by Emulate, mimicked lung pathology to a level that was never observed before in an *in vitro* setting [42]. Not to mention, the technical design of such microfluidic devices must be considered for adding an immune element. The 3D environment of tissue models, whether that be built around biological or synthetic scaffolds, need to mimic the correct ECM of the native organ [133], and should be bio-compatible and spatially suitable for bringing into a microfluidic compartment [134]. Care must also be taken in regards to the properties of such biomaterials on immune cell sensitivity. For example, hydrogel components like fibrinogen can modulate immune cell behaviour in either a suppressive or supportive manner [135, 136]. Other factors such as the mechanical stimuli within the microfluidic device, such as shear stress, flow rate etc., and the type of experimental readouts, such as in-line or end-point sampling, must all be acknowledged when studying human immology.

How far this work can progress into human immunology-on-chip, rather than multi-OoCs, is somewhat of a paradoxical outstanding question that only time will tell if it will be a tangible possibility or whether it is even needed. The recent approval by the US FDA for OoCs to be used in pre-clinical testing [15] has complemented the progress of this field which works towards efficient standardisation and safe robust use of such chips, as well as incorporating the immune system. This has already seen a benefit from a drug repurposing perspective, as a human lung-on-chip accelerated the discovery of a novel class of RNA-based therapeutics, where a pathological role of receptor found on lung alveolar cells was identified in viral infections [32]. Such an avenue is also highly appealing for big pharma, as it has been reported that OoCs could reduce up to 26% of the costs for each drug that is approved [137], therefore applicable companies with the appropriate resources can invest to scale up OoC research, and in turn save finances, accelerate drug development and deliver the promise of personalised medicine in the near future [138, 139].

In conclusion, improvements of immunocompetent single-OoC and multi-OoC models are critical for their utilisation in both the fundamental research and drug development field. The closer these models come to accurately represent physiological systemic processes, the more widely available and applicable they will

become. In this way, they will play a pivotal role in the need to study human diseases in more physiologically relevant models.

Author contributions

AM: Conceptualization, Writing – original draft. MS: Writing – original draft. LV: Writing – original draft. SG: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. JK: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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