ADVANCED HEALTHCARE MATERIALS
www.advhealthmat.de

Biofabrication Directions in Recapitulating the Immune System-on-a-Chip

*Robine Janssen, Laura Benito-Zarza, Pim Cleijpool, Marta G. Valverde, Silvia M Mihăilă, Shanna Bastiaan-Net, Johan Garssen, Linette E. M. Willemsen, and Rosalinde Masereeuw**

Ever since the implementation of microfluidics in the biomedical field, in vitro models have experienced unprecedented progress that has led to a new generation of highly complex miniaturized cell culture platforms, known as Organs-on-a-Chip (OoC). These devices aim to emulate biologically relevant environments, encompassing perfusion and other mechanical and/or biochemical stimuli, to recapitulate key physiological events. While OoCs excel in simulating diverse organ functions, the integration of the immune organs and immune cells, though recent and challenging, is pivotal for a more comprehensive representation of human physiology. This comprehensive review covers the state of the art in the intricate landscape of immune OoC models, shedding light on the pivotal role of biofabrication technologies in bridging the gap between conceptual design and physiological relevance. The multifaceted aspects of immune cell behavior, crosstalk, and immune responses that are aimed to be replicated within microfluidic environments, emphasizing the need for precise biomimicry are explored. Furthermore, the latest breakthroughs and challenges of biofabrication technologies in immune OoC platforms are described, guiding researchers toward a deeper understanding of immune physiology and the development of more accurate and human predictive models for a.o., immune-related disorders, immune development, immune programming, and immune regulation.

1. Introduction

The immune system is a complex network comprising a broad panel of immune cells, well-organized immune tissues, and a variety of secreted immune mediators. It orchestrates the protection of the host's body against different kinds of danger signals by recognizing and responding to foreign/non-self-substances, pathogens, and malignant cells while safeguarding against auto-reactivity toward healthy endogenous cells and tissues (i.e., self-tolerance).[1] Traditional two-dimensional (2D) in vitro models and more complex 2D and three-dimensional (3D) co-culture models that combine several cell types, a.o., are used for drug testing, screening, and discovery, but also for studying underlying mechanisms. However, these fail to recapitulate the total complex structures and organization of mucosal immune tissues and systemic immune organs and their pivotal interconnection. To capture the intricate physiological interactions more faithfully, advanced human in vitro

R. Janssen, L. Benito-Zarza, P. Cleijpool, M. G. Valverde, S. M Mihăilă, J. Garssen, L. E. M. Willemsen, R. Masereeuw
Department of Pharmaceutical Sciences
Pharmacology
Utrecht University
Utrecht 3584 CG, The Netherlands
E-mail: r.masereeuw@uu.nl

S. Bastiaan-Net
Wageningen Food & Biobased Research
Wageningen University & Research
Wageningen 6708 WG, The Netherlands

J. Garssen
Danone Global Research & Innovation Center
Danone Nutricia Research B.V.
Utrecht 3584 CT, The Netherlands

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adhm.202304569

DOI: 10.1002/adhm.202304569

models are under development. Recently emerging platforms that aim at replicating complex and interconnected organs are the Organ-on-a-Chip (OoC) systems.[2] OoCs are miniaturized cell culture platforms that recapitulate key physiological events by providing cells with a biomimetic microenvironment, incorporating compartments, flow, 3D cues, biochemical gradients, and mechanical micro-stimuli.[2,3]

OoCs present a series of advantages over more traditional culture platforms, featuring inter-organ connection, controlled flow, and minimal reagent consumption. Particularly beneficial for studying immune responses in physiological and disease states, such as cancer, infectious disease, autoimmune disease, or (food) allergies, OoCs enable real-time measurements of crucial parameters such as immune cell migration, infiltration, and cellular crosstalk/activation. In addition, OoCs might overcome challenges encountered in animal models by providing the flexibility to easily manipulate individual parameters. This not only reduced the reliance on animals for biomedical and toxicological research but also enhances translatability, as OoC models replicate at least in part human in vivo physiology.[4] Nevertheless, current OoC models present a series of limitations, from exhaustive manufacturing processes to limited diversity of fabrication materials and lack of 3D complex architectures.[5] Other biofabrication technologies, such as 3D bioprinting, might be key in addressing these shortcomings.

This review addresses recent developments, covering peer-reviewed published findings between 2018 and 2023, in OoC models replicating human immune organs or studying solely immune cell characteristics (e.g., migration, adherence, and infiltration). Additionally, the integration of several biofabrication technologies in tissue engineering to enhance the recapitulation of the complexity of the immune system within in vitro platforms was explored.

2. Key Players in the Immune System

The immune organs are divided into primary (i.e., bone marrow (BM) and thymus) and secondary (e.g., lymph nodes (LNs) and spleen) tissues, all characterized by highly organized and specialized micro-structures to harbor immune cells' functions. A simplified overview of the immune organs and immune cells is shown in **Figure 1** and is described in further detail in the next paragraphs. Due to the scope of this review, the immune system is limitedly presented and the reader is referred to other review articles for a more detailed description.[1,6]

2.1. Immune Organs

The BM is a semi-solid tissue found in the cancellous portions of the bones and features distinct niches, including the endosteal region, subendosteal region, central region, and perisinuoisal region.[7] Hematopoietic stem cells in the BM generate various immune cells, with B cells maturing locally and T cells maturing in the thymus.[7] The thymus, providing a complex organized microenvironment with cortical and medullary compartments, fosters T cell development for a diverse T cell receptor repertoire.[8]

Secondary immune organs consist of LNs, spleen, tissue-specific tonsils, adenoids, mucosal-associated lymphoid tissues,[9] such as the gut-associated lymphoid tissue (including Peyer's patches[9,10]), nasal-associated lymphoid tissue, and bronchus-associated lymphoid tissue.[9–11] An important infrastructural component of these secondary immune organs is fibroblastic reticular cells. These cells form a conduit system made from extracellular matrix (ECM) microfibers. In addition, they are important to stimulate the activation and differentiation of lymphocytes.[12] Besides these primary and secondary immune organs, tertiary immune organs exist. These originate outside the lymphatic structures and consist of an accumulation of antigen-presenting cells and lymphocytes at places where permanent inflammation is present. Due to this review's scope, tertiary immune organs will not be discussed in depth. However, various review articles can be consulted to obtain a more detailed overview of these tertiary immune organs,[13] as well as for secondary immune organs.[12,14]

This review mainly addresses the LNs and the spleen. The LNs are important centers containing naïve T and B cells which are instructed by incoming antigen-presenting cells (APCs) to drive adaptive immune responses. Innate dendritic cells (DC) traffic from the tissue via the lymph to the local LNs to present their captured antigens to a.o. naïve T cells. The LNs are characterized by a highly specialized microstructure with well-defined domains. These domains ensure compartmentalization for B and T cells, and interaction with corresponding APCs, antigens, and inflammatory mediators, initiating adaptive immune responses. Domains that are found in LNs are, for example, the follicles including B cells and the paracortex, as well as the interfollicular cortex both include T cells. Furthermore, the LNs consist of a reticular fiber meshwork, providing the LN with a porous structure.[15] The spleen, apart from abnormal cell clearance and hematopoiesis, performs essential immunological blood surveillance. Both structure and function are closely intertwined in this organ, having two clearly differentiated regions: the white pulp surrounded by the red pulp. Most innate immune cells (monocytes, neutrophils, and DCs) reside in the red pulp, while lymphocytes populate the white pulp. While antigen-loaded APCs travel to the white pulp to initiate an adaptive immune response, effector T cells and plasma blasts are also able to migrate to the red pulp.[16]

2.2. Immune Cells

The immune system includes two special defense responses. The first response is the innate immune system (e.g., DCs, macrophages, monocytes, natural killer (NK) cells, and granulocytes), which acts quickly (within hours) against any sign of danger and is non-specific. The second response is the adaptive immune system (e.g., B cells and T cells), which acts more slowly (days to weeks) and is target-specific, leading to the development of the classical immune cell memory and which can lead to a rapid effector response at the next antigen challenge.[17]

Pluripotent hematopoietic stem cells originate from BM where they differentiate into two different lineages: common lymphoid progenitors and myeloid stem cells. The common lymphoid progenitors further differentiate into B, T, NK, and NK-T cells. Upon subsequent antigen exposure and B cell activation, plasma cells will develop, producing antigen-specific antibodies. Myeloid

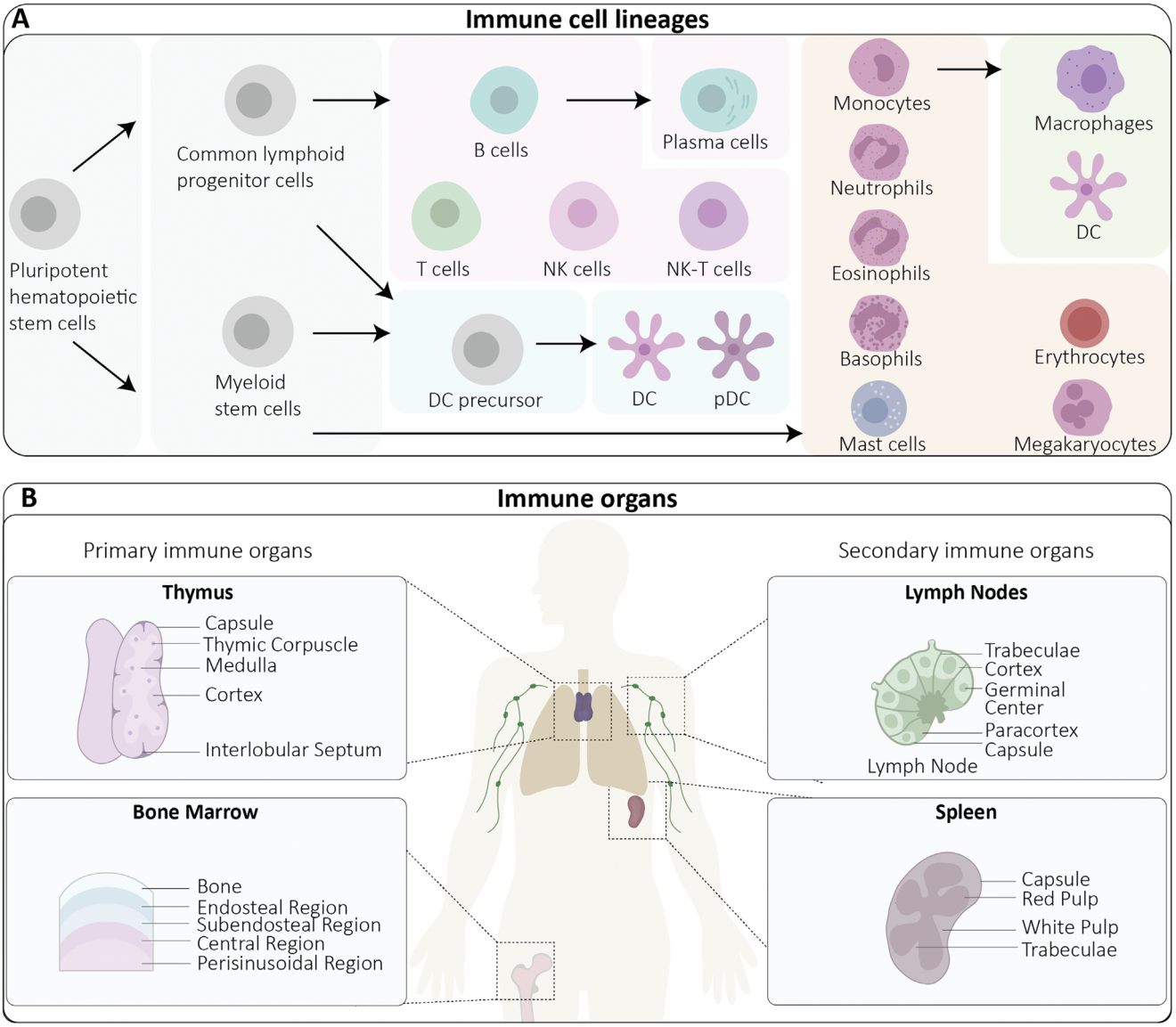

Figure 1. Schematic and simplified illustration of immune cells library and the compartmentalized immune organs. Immune cells originate from bone marrow (BM) A) in which pluripotent hematopoietic stem cells differentiate to common lymphoid progenitor cells and myeloid cells. From the first lineage, B cells, T cells, Natural Killer (NK) cells, and NK-T cells develop. B cells can differentiate further into plasma cells. The second lineage will finally develop monocytes, neutrophils, eosinophils, basophils, mast cells, and is involved in hematopoiesis (formation of erythrocytes and megakaryocytes and erythrocytes). Monocytes give rise to both macrophages and classical dendritic cells (DCs). Both lineages can develop DC precursors, which subsequently differentiate into either classical DCs or plasmacytoid DCs.[1] The lymphatic system consists of a set of different immune organs B), which can be classified as primary immune organs (i.e., BM and thymus) and secondary immune organs (e.g., lymph nodes (LNs) and spleen). Each individual immune organ is characterized by a complex organization and compartmentalization of its function. For the focus of this review, only the BM,[7] thymus,[8] LN,[15] and spleen[16] are displayed. Lymph vessels and LNs are in vivo not limited to the location as depicted in the figure but can be found throughout the body. Some cells illustrated were reproduced (adapted) under the terms of the Creative Commons Attribution (CC BY 4.0) license.[73] Copyright 2023, Elsevier Science & Technology Journals.

stem cells give rise to innate immune cells, including monocytes, neutrophils, eosinophils, basophils, and mast cells, but also hematopoiesis comprising of the formation of erythrocytes and thrombocyte forming megakaryocytes (Figure 1). Monocytes will subsequently differentiate into macrophages and classical dendritic cells in peripheral compartments. Furthermore, a common DC precursor derived from both lineages can finally differentiate into classical and plasmacytoid DCs.[1]

Besides the systems described, immune organs (e.g., LN) as well as non-immune organs (e.g., liver, skin, and gut), also include tissue-resident immune cells derived from myeloid and/or lymphoid progenitors. These immune cells' phenotype and function are specialized to the organ where they reside.[18] LNs for example include follicular DCs[19] as well as follicular T cells,[20] which both play important roles in B cell activation and antibody development. Other examples are epidermal Langerhans cells in

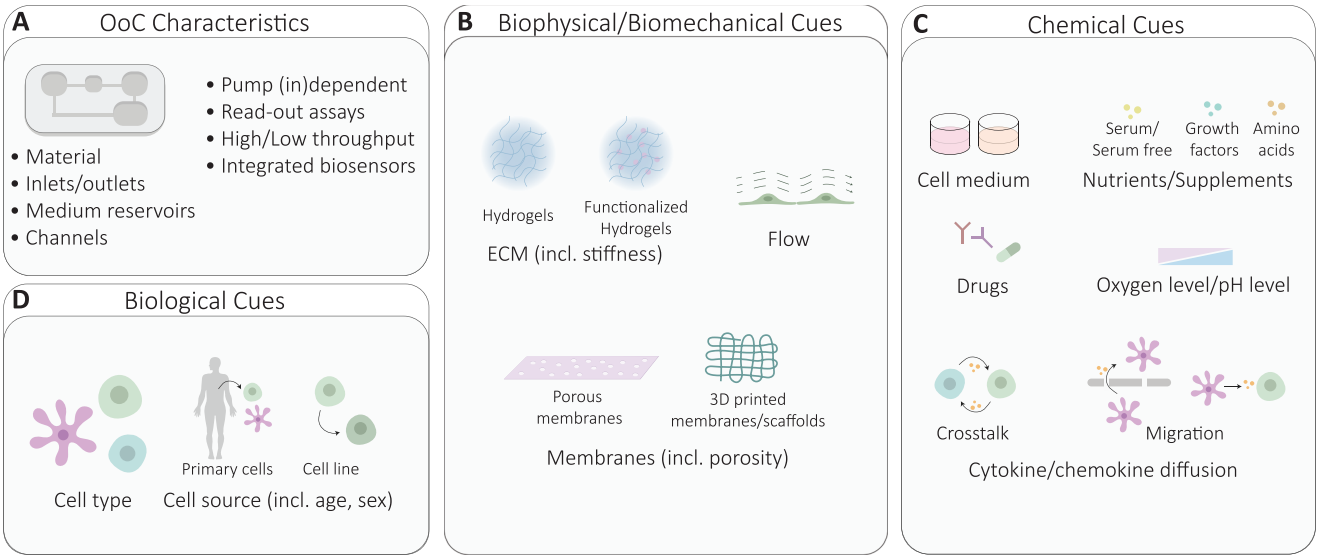

Figure 2. The need for environmental cues inclusion in current immunocompetent OoC technology. During the development of A) Organ-on-a-chips (OoCs) including immune organs and/or immune cells, several environmental cues need to be considered.[2,23,28,31] B) Biophysical/biomechanical cues consist of (functionalized) extracellular matrix (ECM) characteristics, fluid flow, pore sizes of membranes, and 3D printed membranes or scaffolds, among others. C) Chemical cues include the type of cell medium and its nutrients, drugs that can affect cells, oxygen, and pH levels, and chemotaxis or cell–cell-crosstalk by cytokine/chemokine secretion. D) Biological cues include cell type and cell source, like primary cells and cell lines.

the skin,[21] Kuppfer cells in the liver, microglia in the central nervous system, and pulmonary macrophages.[18] In addition, non-immune organs can contain tissue-resident T cells, for example in the gut, where tissue-resident memory T cells enable rapid immune responses.[18,22]

3. Environmental Factors Facilitating Immune Cell Function in Advanced in Vitro Models

To develop immunocompetent OoCs, a thorough understanding of environmental factors, illustrated in **Figure 2**, influencing immune cell characteristics is imperative. These factors, categorized as chemical, biological, and physical cues, play important roles in shaping the behavior and functionality of immune cells within the OoCs.[2,23] They critically determine the efficacy of these (advanced) in vitro models in faithfully recapitulating specific tasks, such as immunocompetence against tumor cells, viruses, or bacterial infections.

3.1. Chemical and Biological Cues

Chemical cues, including cell culture medium, growth factors, nutrient supplementation, oxygen concentrations and pH, drug release/exposure, and cytokine/chemokine diffusion, influence processes such as cell migration, differentiation, and apoptosis.[23] For example, it has been shown that the macrophage cytoskeleton can be rearranged upon stress hormone exposure, resulting in decreased macrophage deformability and increased chemotactic and phagocytic capability.[24] Chemokines can be encapsulated in hydrogels to sustain a gradient to attract immature DCs,[25] macrophages, and neutrophils,[26] facilitating directed immune cell migration. Growth factors, glycosaminoglycans, and/or proteoglycans can also be embedded in hydrogels to influence immune cell proliferation, activation, and differentiation.[27] Biological factors include the type of cells, cell source, disease state, and demographic variables, such as sex or age.[23,28] Specifically, age and sex differences are known to influence the immune cell composition in the body (e.g., number of NK and plasma cells), as well as immune function (e.g., T and B cell activity).[29] For this reason, it is crucial to take these biological differences into account when developing immune system models. For example, primary cells can be preferred over cell lines, as they more closely mimic native physiology.[30]

OoC systems can be superior in vitro platforms by accounting for the complexity derived from biological and chemical cues as described above. Many OoC platforms present separated channels and chambers, which enables the compartmentalization of cells and materials. This, in turn, facilitates the precise delivery of specific media and chemical factors to different cell types with spatiotemporal resolution.

3.2. Physical Cues, 3D Complexity and ECM Relevance

Physical cues encompass, a.o., parameters like stiffness and porosity of the ECM, as well as the induction of flow and the associated shear stress, impacting cell migration, phenotype, and function.[23,31] Flow can induce for example T cell activation and proliferation.[32] Cells growing on a flat surface or kept in suspension lack these flow-induced stimuli, as well as an in vivo-like 3D environment, such as ECM's topography and architecture.[33] Immune cells exhibit distinct migration patterns differently (e.g., interstitially or along cellular networks) in diverse 3D environ-

ments (e.g., loose connective tissue or highly organized lymphoid organs)[34] and therefore, the choice of ECM for in vitro models is crucial.

3.2.1. ECM-Immune Cell Interactions

In the past years, the relevance of ECM in cell behavior has been emphasized.[35] When it comes to the immune system, various studies have pointed out the importance to understand specific ECM-cell interactions. For instance, higher stiffness in the ECM leads to a pro-inflammatory macrophage response, a mesenchymal migration mode, and hampered phagocytic capabilities, while lower stiffness leads to an anti-inflammatory response,[36] amoeboid migration, as well as high phagocytic capabilities.[36,37] ECM's collagen I composition and stiffness also determine the remodeling of the cytoskeleton of lymphoid cells, thereby affecting their motility to change toward a more exploratory phenotype[38] as well as affecting the retention of memory T cells in the tissues.[39] For T cells, an increase in stiffness of 3D hydrogels has been shown to stimulate T cell proliferation, migration, and activation.[40] However, dense ECMs can also restrict immune cell migration.[41] Other examples include collagen-dependent neutrophil recruitment toward inflamed tissue via CXC-chemokine receptors[42] and macrophage activation promoted by the ECM component versican.[43] Furthermore, B cell differentiation and survival are supported by laminin and argin in the spleen.[44] These examples exemplify further the importance of careful selection of the ECM, depending on cell type, tissue's diseased state,[45] as well as hydrogel composition and stiffness.[46]

3.2.2. Hydrogel Formulation to Model ECM In Vitro

Features like stiffness, topography, chemical properties, and dimensionality have been studied to have an effect on the immune response.[46] Therefore, special attention is being paid to these naturally present biomechanical cues that ECMs provide to cells in vivo, to try to replicate them in vitro (Figure 2).[31] Hydrogel formulations range from more standardized polymeric solutions, such as synthesizing hydrogels of proteins (e.g., Matrigel and collagen I),[47] polysaccharides (e.g., alginate and chitosan)[48] or synthetic biomaterials (e.g., polystyrene, polyethylene glycol, and polycaprolactone),[49] to study immune cell–cancer cell interactions, to cutting-edge conjugations, including polyacrylamide,[36] methacryllated gellan gum,[50] and decellularized ECM.[51] These conjugations are carefully scrutinized in terms of their ability to modulate inflammation, induce monocyte differentiation and macrophage polarization, and improve cancer immunotherapy.[46,52] To further mimic the tissue's ECM, hydrogels can be functionalized by for example RGD motifs, which represent arginine–glycine–aspartic acids, to promote (immune) cell adhesion.[46]

However, 3D hydrogel models alone are unable to fully mimic the complexity of the ECM, as well as cellular interactions and other important physiological characteristics, such as flow (e.g., shear stress), heterogeneity of cell types, and microarchitecture.[34] Therefore, increasing the physical, chemical, and biological complexity in OoC could be key toward successfully mimicking these immune system features in vitro.

4. Immune Organs-oC

Recreating immune organs, such as BM, thymus, LN, the lymphatic system, and the spleen in vitro remains a difficult challenge due to the intricate micro-organization and compartmentalization inherent in the native organs (Figure 1). For the scope of this review, we focused exclusively on models that included human immune cells (**Figure 3**). For detailed biological insights, we refer the readers to Table S1 (Supporting Information) and for the technical details to Table S2 (Supporting Information).

4.1. Primary Immune Organs-oC

4.1.1. Bone Marrow-oC

Initial BM models primarily targeted the hematopoietic function of this organ,[53] with recent advancements also expanding into the formation and maintenance of immune cell lineages and the inclusion of immune cells. Notably, these models offer a unique avenue for studying diseases like leukemia, where immune cell development becomes dysregulated.[3,54]

One model mimicked the in vivo-like topography of BM using a 3D ceramic scaffold co-cultured with human mesenchymal stromal cells and human multipotent hematopoietic stem and progenitor cells (HSPCs), including a circuit for recirculation of media. In this system, HSPCs remained in their native state and maintained differentiable capabilities, such as immune cell or erythrocyte formation. However, immune cell responses, such as adhesion and migration, were not evaluated.[55] Another BM-oC device was developed to recapitulate the endosteal BM niche. BM stromal cells and BM cells (including HSPCs) were seeded in the apical compartment, whereas endothelial cells were seeded in the basolateral compartment separated by a porous membrane. Although this model was based on murine cells, the study confirmed HSPCs multi-lineage differentiation ability.[56]

To evaluate interactions between healthy and malignant hematopoietic cell niches, a BM niche-oC was developed, including arterial, osteoblastic, sinusoidal, and mesenchymal compartments. Immune cell responses, such as adhesion and different homing capabilities of healthy HSPCs, lymphoma monocytes, and leukemia cells were evaluated.[57] Besides, a two-channeled BM-oC that included endothelial cells, hematopoietic progenitors, and BM stromal cells, evaluated hematopoiesis, BM injury as well as chemotherapeutic toxicity in neutrophilic and erythroid lineages.[58] To further study BM pathologies, a three-compartment chip mimicking the BM-niche was constructed to evaluate chemoresistance mechanisms for several anti-leukemia drugs. The device consisted of three integrated hydrogel compartments, representing the central sinus and the perivascular and endosteal niche, containing endothelial cells, mesenchymal stromal cells, osteoblasts, or B cell acute lymphoblastic leukemia cells. Co-targeting of both leukemic cells and BM niche-derived signals increased the elimination rate of leukemic cells.[59] More recently, a BM-oC was presented containing two separate fibrin

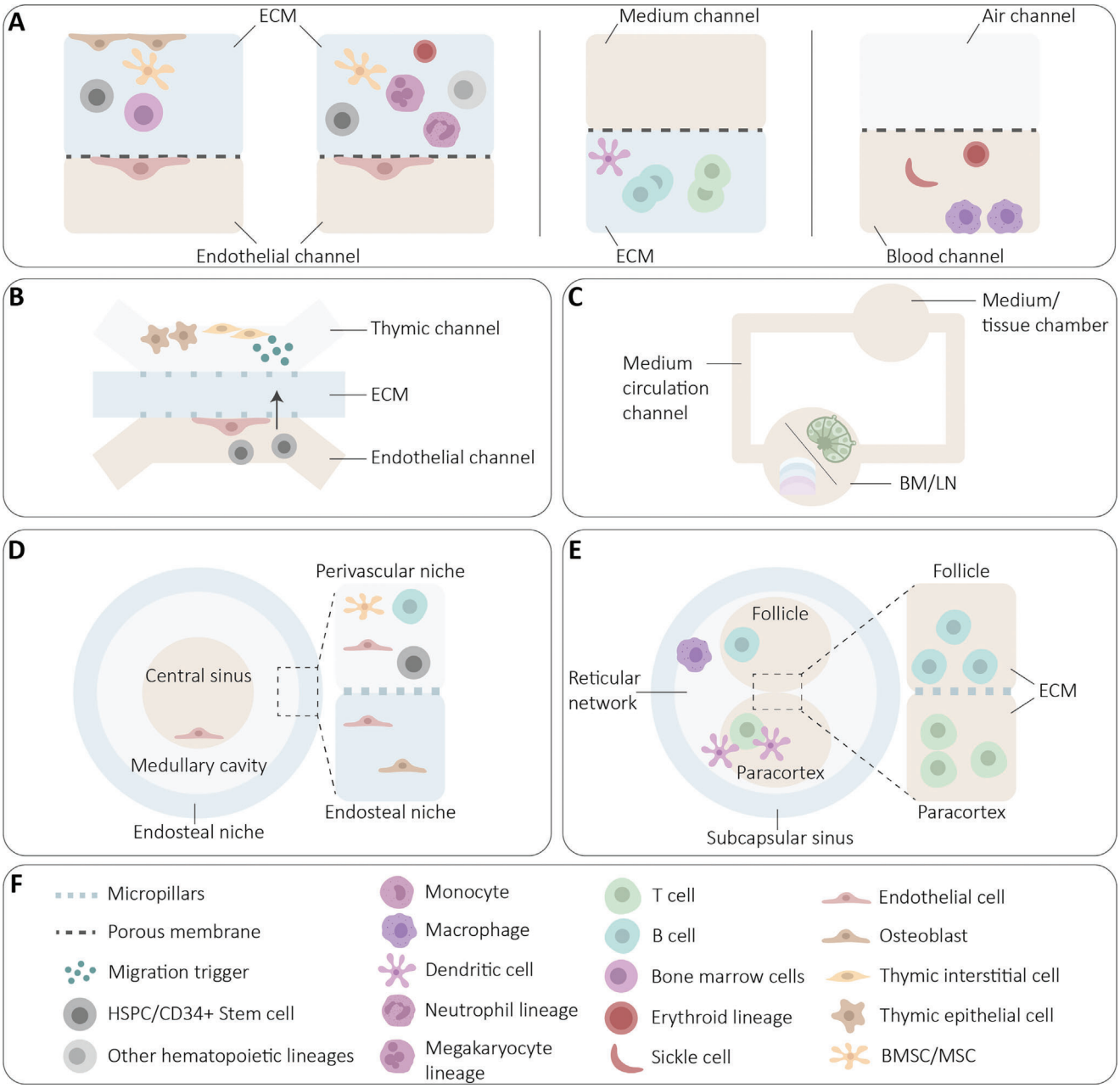

Figure 3. Schematic overview of the immune organ-oC models. A) Two-channeled immune organ-oC representing BM niches,[56,58] lymphoid follicle niches[67] and the spleen.[71] B) A three-channeled thymus-oC system including an endothelial channel, hematopoietic stem cell migration, and a thymic cell niche channel.[62] C) BM-oC[55] and (murine) LN-oC[63] systems using a circulatory channel system, connected to a culture and tissue chamber. Multi-compartment based immune organ-oC systems of D) BM[57,59,60] and E) LN niches,[65,66] or mimicking the subcapsular sinus microenvironment.[64] F) Illustrative legend to panels (A–E). ECM = Extracellular matrix, BM = Bone marrow, LN = Lymph node, HSPC = Human multipotent hematopoietic stem and progenitor cell, BMSC = Bone marrow stromal cell, MSC = Mesenchymal stromal cell.

gel-filled compartments for endothelial cells, BM stromal cells, osteoblasts, and HSPCs. The compartments mimicked the endosteal and perivascular niches and showed HSPCs maintenance and HSPCs differentiation into erythroid and myeloid lineages. Additionally, neutrophils were able to migrate out of the niches toward fluidic channels. Moreover, cancerous breast cells were introduced into the system, and these were able to migrate into the endosteal and perivascular niches of the BM.[60]

4.1.2. Thymus-oC

Despite its crucial role in self-tolerant T cell selection and/or maturation up until adolescence,[61] the thymus remains underrepresented in vitro models. The organized compartmentalization of the thymus could offer valuable insights into T cell development when recapitulated in OoC platforms. An early attempt of a thymus-oC device utilized a standard three contiguous-channels

design to evaluate the migration capabilities of hematopoietic stem cells. Positioned in an endothelial vessel (side channel), these cells migrated toward the interstitial and epithelial cells of the thymus (opposite channel). Even though immune cells were not yet included, the study laid the groundwork, shedding light on the significance of both thymic interstitial and epithelial cells to attract hematopoietic stem cells. This pioneering effort set the stage for the future development of immunocompetent thymus-oC devices.[62]

4.2. Secondary Immune Organs-oC

4.2.1. Lymph Node and Lymphatic Vessels-oC

Several studies have endeavored to recreate LNs in microfluidic systems. One approach included a dual-chamber microfluidic platform integrating ex vivo LN and tumor tissue, respectively. The chambers were connected in a closed-loop fashion to allow for indirect crosstalk between the tissues, in which the tumor tissue showed an immunosuppressive effect on LNs compared to healthy tissue. Although original tissue complexity was included, the model was still based on murine tissue.[63] From a more technical approach, lymphatic metastasis was studied by using a subcapsular sinus (SCS)-oC, mimicking the microenvironment of the SCS and entrance of metastatic cells. These models explored altered fluid flow profiles in quiescent and inflamed LNs, revealing that a change in the expression of adhesive ligands and the inclusion of co-perfusion of monocytes increased metastatic cell adhesion.[64] However, only the SCS niche of the LN was mimicked, missing other niches such as the reticular conduit, the follicle of B cells, and the paracortex of T cells. To capture the compartmentalized nature of the LN more faithfully, an LN-oC device with three hydrogel-encapsulating compartments was developed, representing the SCS, the reticular conduit, the follicle of B cells, and the paracortex of T cells. This LN-oC system replicated LN's in vivo compartmentalized structure and fluid flow regime, while it primarily focused on T cell expansion and co-localization of the incorporated DCs and T cells, lacking assessment on immune cell migration.[65] Subsequent miniaturization of this device, incorporating B and T cells, demonstrated its potential in drug screenings. For instance, the immunomodulatory drug hydroxychloroquine was found to negatively affect T cell motility, indicating the device's utility for studying immunomodulatory drug effects.[66] Furthermore, a simplified lymphoid follicle-like OoC consisted of collagen-embedded lymphocytes and a constant perfusion through a parallel channel (superfusion). This system enabled the formation of T and B cell-based ectopic lymphoid follicles and prevented lymphocyte autoactivation. Subsequent inclusion of DCs and seasonal influenza vaccine testing revealed antigen-specific IgG responses, highlighting the translatability of this relatively uncomplicated OoC device.[67]

In parallel studies, researchers have primarily focused on building lymphatic vessels, often excluding immune cells. Notably, one study utilized parallel channels to investigate the intricate crosstalk between breast cancer cells and lymphatic vessel endothelial cells.[68] Another innovative approach involved a 3-lane OrganoPlate (Mimetas BV), culturing mouse colon cancer organoids embedded in a collagen hydrogel adjacent to the lymphatic vessel. This tumor-lymphatic vessel co-culture model allowed the study of lymphangiogenesis induced by an altered, cancer-type-specific gene expression in lymphatic endothelial cells.[69]

4.2.2. Spleen-oC

Some advancements have been made in the past in developing spleen-oC devices,[70] however, one specific model has been developed recently, that is, between 2018–2023. This study leveraged a microfluidic platform and aimed at mimicking erythrocyte filtration capabilities of the spleen and macrophage phagocytosis of healthy and sickled erythrocytes. Results indicated that retention of sickled erythrocytes is faster under normal oxygen tension and becoming even faster in an oxygen-deprived condition compared to healthy erythrocytes.[71] Models lacking the implementation of splenic architecture and embedded immune cells underscore the critical need for establishing updated and relevant models that recapitulate the complex microarchitecture of this organ in humans which comprises of the body's largest secondary lymphoid organ.[16]

4.2.3. Other Secondary Lymphoid Tissues-oC

To date and to the best of our knowledge, no OoC devices have been specifically developed for mucosa-associated lymphoid tissues or tonsils. Nevertheless, current developments within the field of 3D organoids may offer viable solutions.[72] Notably, significant progress has been made in developing immunocompetent and inflammatory gut-oC systems,[73] capturing aspects of the immune response in the gut mucosal tissue. Nevertheless, these systems have yet to replicate complete Peyer's patches and/or mesenteric LN.

5. Immune Cells-oC

Accurate migration and homing responses of diverse immune cell types are crucial for the correct unfolding of the immune response. Various OoCs have successfully replicated these events by establishing chemotactic gradients. This section specifically focuses on OoC platforms that exclusively involve (human) immune cells (**Figure 4**). For in-depth biological details of studies discussed, we refer the readers to Table S1 (Supporting Information), and for technical details to Table S2 (Supporting Information).

5.1. Neutrophils-oC

Numerous studies in immune OoC systems have centered on neutrophils, particularly in response to chemotactic gradients generated by tissue cells during bacterial infections or exposure to chemotactic molecules. The LumeNEXT-Stacks (LENS) device, designed with stackable tube sections on top of a central chamber, facilitates the establishment of chemotactic gradients, enabling neutrophil transendothelial migration toward

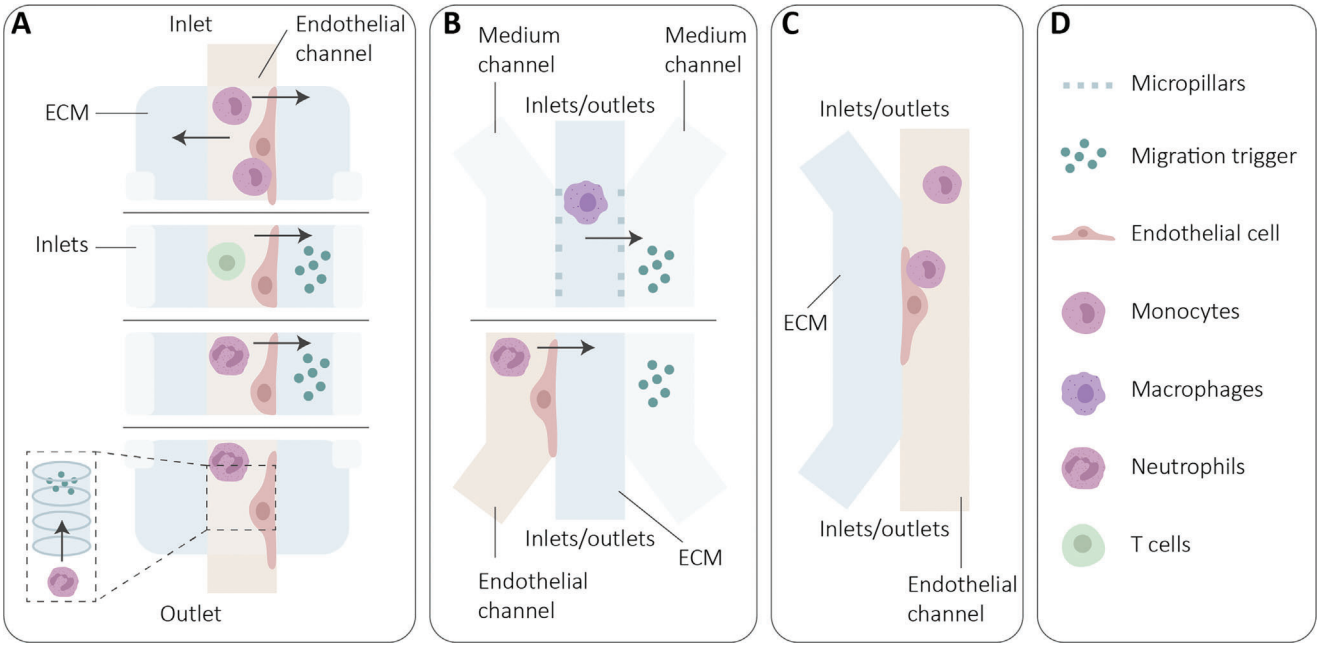

Figure 4. Schematic overview of immune cells-oC models. A) Platforms studying monocyte-endothelium adhesion and migration upon flow,[78] T cell migration[82] and neutrophil migration,[75,76,82] also including LENS technology.[74] B) Three-channeled immune-cell-oC systems based on macrophage[79] and neutrophil[77] migration. C) A two-channeled immune cell-oC studying monocyte-endothelium adhesion upon flow.[80] D) Illustrative legend to panels A-C. ECM = Extracellular matrix.

an interleukin-8 chemokine gradient.[74] Another study involving the LumeNEXT device, including human umbilical vein endothelial cells, assessed neutrophil migration and adhesion with cells from individuals lacking actin-related protein *ARPC1B*. Neutrophil migration and adhesion were observed to be reduced in comparison to healthy individuals.[75] Additionally, a study included induced pluripotent stem cell-derived endothelium in the LumeNEXT platform, which supported neutrophil migration and endothelium extravasation. Furthermore, pericytes were combined with the endothelium, mimicking the endothelial vessel more closely.[76] Furthermore, in a 3-lane OrganoPlate (Mimetas BV) neutrophil ECM-dependent migration performance was evaluated after exposure to inflammatory (TNF-α), chemoattractant (fMLP), and neutrophil proteases inhibitor. Neutrophil infiltration and migration capabilities increased in Geltrex-based gels compared to collagen I-based gel, whereas this effect was hampered by neutrophil protease inhibitors.[77]

5.2. Monocytes and Macrophages-oC

Different microfluidic devices have been proposed also for studying circulating monocyte and macrophage extravasation through the endothelial barrier for the purpose of mimicking cellular migration in response to shear stress, inflammatory insults, or infectious triggers. One of these devices utilized a hydrogel with a hollow channel containing an endothelial cell monolayer. Then, monocytes were introduced into the endothelial channel and their extravasation into the outer collagen gel was studied. Extravasation rate and not adhesion capabilities were the inhibiting factor in immune cell trafficking when exposed to flow.[78]

Further insights on macrophage mobilization upon exposure to different bacteria were studied by using a three-channeled chip including macrophages embedded in collagen hydrogels. The proof-of-concept experiments suggested macrophage migration, although not significant, toward fractions of pathogens and non-pathogens.[79] Furthermore, the 2-lane OrganoPlate device (Mimetas BV) was used to form a monolayer of endothelial cells in one channel perfused with monocytes, while having a collagen I hydrogel in the other channel. The platform showed that monocyte adhesion to endothelial cells is stimulated after the addition of inflammatory compounds.[80]

5.3. T cells-oC

The migratory behavior of T cells in the context of chronic obstructive pulmonary disease (COPD) has recently been studied in a 3-channeled microfluidic device, featuring docking micropillars for migration toward an established chemotactic gradient. Sputum from COPD patients was found to inhibit T cell migration, although the study focused on T cells within a simplified 2D microenvironment, leaving a more complex 3D microenvironment underrepresented.[81]

5.4. Multiple Immune Cells-oC

One recent study reported on neutrophils and T cells in separate LumeNEXT platforms, and compared extravasation and migration dynamics between neutrophils and T cells. The endothelial central channel was treated with TNF-α or was left untreated,

whereas the matrix included the chemoattractant C5a or was left untreated. Notably, upon TNF-α exposure, neutrophils exhibited significantly higher migration speed and distance than T cells. Interestingly, T cells migrated in a straight direction after TNF-α stimulation, while neutrophils followed more exploratory routes. The addition of the chemoattractant C5a temporarily negated the random migration of neutrophils, showcasing the dynamic interactions between different immune cell types in response to inflammatory stimuli.[82]

6. Other Relevant Immunocompetent OoC Systems

Besides solely focusing on immune organs or immune cells, some of the most recent OoC models of other organs, such as skin, gut, liver, and cartilage, incorporated immune elements in their platforms, enriching their complexity and the readouts possibilities.

6.1. Immunocompetent (Singular Organ) OoC Systems

Inflammatory immunocompetent OoC models, particularly for the gut and skin,[73] vary from simple to more intricate setups, combining intestinal epithelial cells with endothelial cells and studying their interaction with innate DCs and macrophages, thus modeling part of the intestinal mucosal immune compartment.[83] Furthermore, cancer OoC devices enable the study of immune cell-cancer cell interaction and the evaluation of different anti-cancer treatments.[84] Besides, immunocompetent OoCs were developed to assess monocyte-adipocytes interaction[85] and drug compound hepatoxicity.[86] In most cases, the choice of materials for emulating the ECM varied, exploring commercial options to study DC migration, such as Matrigel and VitroGelORGANOID 1–4, alongside collagen.[87]

6.2. Immunocompetent Multi-OoC Systems

Despite limitations in reproducing events across multiple organs, especially immunocompetent multi-OoC systems,[88] a few studies successfully incorporated immune components in these challenging multi-OoC devices. One of these is the multicompartment chip to model ulcerative colitis through the gut-liver-immune axis, facilitating interaction among colon organoids, macrophages, DCs, hepatocytes, and Kupffer cells, and antigen-presenting cell interaction with circulating regulatory and inflammatory Th17 T cells.[89] Furthermore, a multicompartment device for toxicological assays of systemic drugs was created, which interconnected hepatocytes, cardiomyocytes, skeletal muscle cells, and recirculating monocytes, including integrated biological microelectromechanical systems.[90] Another device consisted of four stacked layers representing the gastrointestinal barrier, the liver, BM, and the kidney, including physiological volumes in the microfluidic circulation. Although immune cells were not incorporated in this system, the presence of a BM compartment brings this system one step closer to including these cells in the future.[91]

7. Harnessing 3D (Bio)Printing for Improving Physiological Complexity of Immune OoC Systems

A recurrent trend seen in the aforementioned immune cells-oC devices is the simplicity of modeling immune reactions and studying only a sub-event of a full immune response cascade. Microfluidic devices mostly focus on chemotaxis or extravasation, overlooking immune cell cascades, recruitment of other immune cells, immune cell crosstalk, phagocytosis, or antigen presentation. Besides, as immune organs are complex tissues, current devices are not able to recapitulate the organ's true structure and function. Furthermore, the incorporation of migrating immune cells in the development of immune organ-oC devices is currently limited. The development, selection, and activation of immune cells in immune organs and the trafficking of immune cells between immune organs and other tissues are strongly intertwined processes. Therefore, the highly dynamic behavior of immune cells and the structures and organization of immune organs pose challenges that advanced in vitro models must address.

Cutting-edge biofabrication technologies, such as 3D bioprinting, might be key for addressing the limitations of current immune system models. The field of 3D bioprinting stems from additive manufacturing (AM) and specializes in the automated fabrication of 3D functional tissue replicates by combining living cells, bioactive biomolecules, and biomaterials as building blocks.[92] By using filament deposition (extrusion 3D bioprinting,[92] electrospinning,[93], melt electrowriting (MEW),[93]) light (stereolithography,[94] digital light processing,[94] volumetric (bio)printing[95]) or droplets generation (inkjet (bio)printing[92]), cells and materials can be deposited or encapsulated creating a hierarchically and spatially organized tissue (**Figure 5**). For readers who are unfamiliar with the fields of biofabrication and bioprinting, overviews of the printing modalities and approaches can be found in the literature and brief definitions are included in **Table 1**.

Some of the most attractive aspects that 3D printing has to offer with respect to OoC technologies include design versatility, accuracy, and the automation of fabrication processes. Reducing manual handling during device fabrication could decrease the variability between models while allowing for an increase in platform complexity. Moreover, the design and fabrication options are broadened, especially when it comes to replicating tissue-specific structures, such as vessels, membranes, and compartments. Bioprinting techniques present different size resolution capabilities, from 0.1 to 1000 µm.[96] As a general rule in the biofabrication field, size resolution is indirectly related to fabrication time. Thus, the broad resolution range within bioprinting also accounts for a wide variety of fabrication times, which increases the adaptability of techniques to different fabrication needs (e.g., from the creation of very detailed complex structures to the faster prototyping of simpler platforms). In addition, the diversity of techniques and their compatibility to work with several polymers simultaneously enables the conception of multi-material platforms, providing different types of environmental cues, from material gradients to topographic features. The potential of 3D printing technologies to generate microfluidic platforms in an integrated, single-step process has already been leveraged by many studies,[97] but is yet to be fully exploited for the immune system.

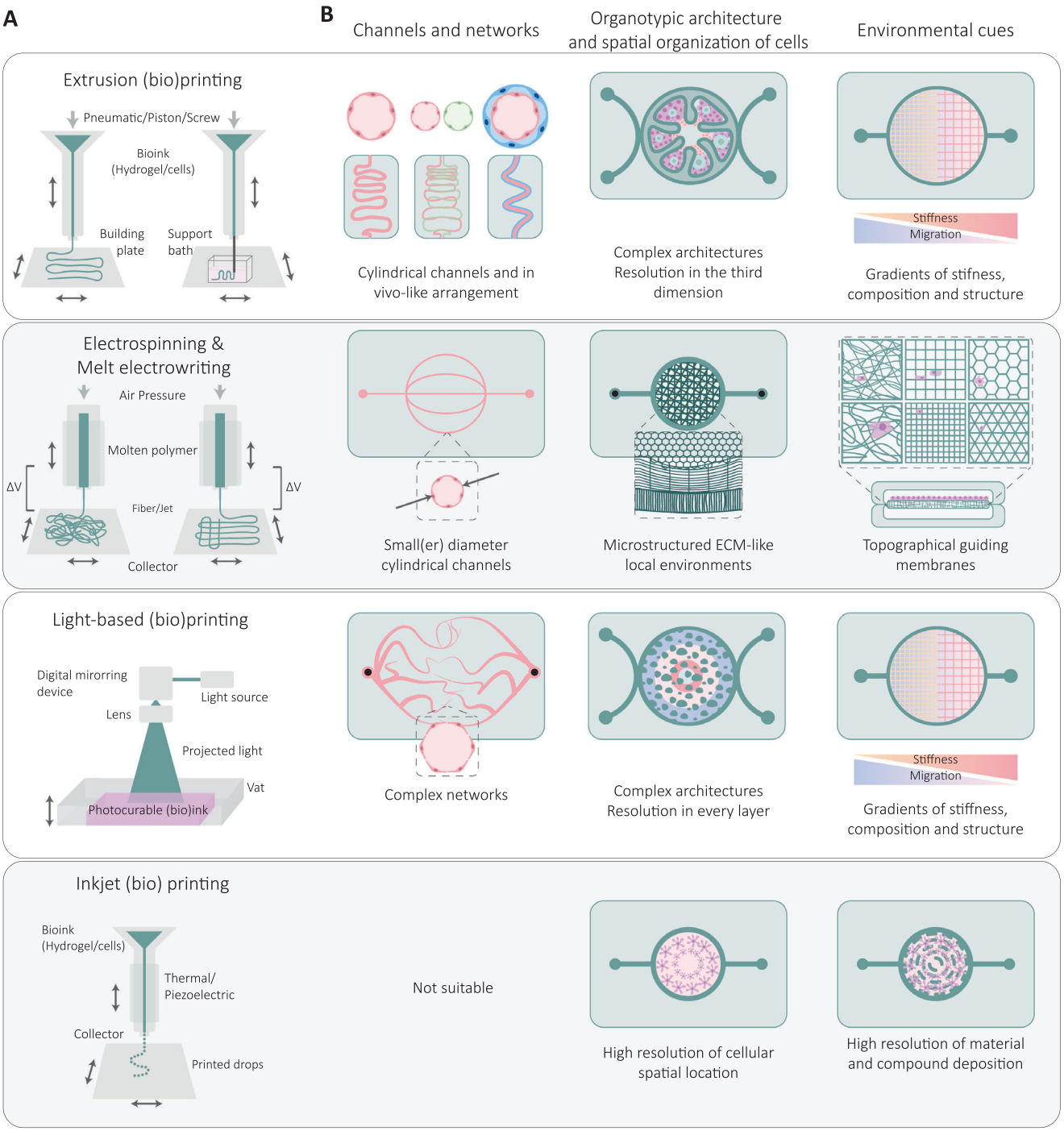

Figure 5. The use of biofabrication technologies to increase the physiological relevance of current and future immunocompetent OoC devices. A) Schematic on the working principles of the main 3D (bio)printing technologies with the potential to support the next generation of immune organs and immune cells in microfluidic systems. From top to bottom: extrusion (bio)printing,[92] electrospinning and melt electrowriting (MEW),[93] light-based (bio)printing[95] and inkjet (bio)printing.[92] B) Each technology can assist in the creation of channels with different degrees of complexity, branching, and diameters, replicate the organotypic architecture and spatial organization of the immune organs, and different environmental cues, such as stiffness or chemoattractive gradients, that can facilitate immune cell migration. ΔV = Voltage difference.

Table 1. Definitions of chip fabrication strategies, biofabrication, and bioprinting-related terms.

Technique	Main principle	Ref
Biofabrication	Automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as micro-tissues, or hybrid cell-material constructs, through bioprinting or bio assembly and subsequent tissue maturation processes.	[92]
Bioprinting	Field of biofabrication in which 3D printing methods are used for the fabrication of 3D scaffolds able to instruct or induce the cells to develop into a tissue mimetic or tissue analog structure through distinctive cell interaction, hierarchical induction of differentiation, or functional evolution of the manufactured scaffolds falls within bioprinting.	[92]
Digital light processing	Crosslinking of a photopolymerizable material layer-by-layer by the projection of a 2D image.	[94]
Electrospinning	Fiber production method in which a molten thread of polymer is extruded while subjected to an electric field.	[93]
Extrusion (bio)printing	Controlled deposition of material filaments being extruded from a nozzle, where the material is usually in a liquid or gel state.	[92]
Inkjet (bio)printing	Controlled deposition of semiliquid material in droplets.	[92]
Light-based (bio)printing	Creation of solid structures by exposing a photopolymerizable liquid resin to a pre-designed pattern of light.	[95]
Melt electrowriting	Variation of electrospinning in which fibers are deposited in a controlled and organized way.	[93]
Photolithography	(Micro)fabrication process in which a (UV) light source is used to transfer a geometrical pattern from a template mask to the substrate of interest rendering high resolution. An optical mask is irradiated with UV light to transfer the existing geometric pattern over the optical mask to a substrate via a light-sensitive chemical process.	[98]
Stereolithography	Crosslinking of a photopolymerizable material point-by-point with a ray of light.	[94]
Volumetric (bio)printing	Crosslinking of a photopolymerizable material in 3D bulk by projecting 2D images within a volume.	[95]

7.1. Channels and Networks

The integral role of vessels in the immune system, spanning from lymphatic capillaries and blood vessels, the blood innervation of the BM to leucocytes patrolling, underscores the significance of advanced channel design, aimed to replicate these structures. Traditional microfluidic fabrication techniques render rectangular channels and polygonal-shaped chambers, which fail to reproduce the natural shape of vessels in our bodies, which are intertwined, cylindrical conduits. Despite some 3D (bio)printing technologies not being able to compete in terms of resolution and surface finishing with standard photolithography,[98] they do allow for the creation of unrestrained structures, featuring cylindrical, coaxial, and branched structures.

Extrusion bioprinting, using sacrificial materials, facilitates the direct fabrication of hollow tubules within microfluidic platforms.[97,99] In short, sacrificial materials act as a template, removed post-printing.[100] This method extends to creating concentrical channels with coaxial nozzles. Two (or more) bioinks containing different cells and materials can be simultaneously extruded into hollow and coiled vessels-like structures.[101] Lymphocyte trafficking from and toward immune organs via the bloodstream and the lymphatic system, respectively, is crucial to unfold a correct immune response. Extrusion printing methods could be extended to print the larger blood vessels and collecting lymphatic vessels, starting from diameters of 50 µm[102] with a high(er) organization degree and architecturally relevant features. Yet, reduced diameters are needed for mimicking, for example, networks of 4–10 µm wide blood capillaries.[103]

Overcoming size limitations, MEW of sacrificial fibers can be used to create micro-vessels with branching structures, resulting in smaller lumens (≈65 µm) successfully lined with endothelial cells.[104] Hierarchical branching of vascular networks is replicated with stereolithography technologies, allowing the generation of perfused and vascularized microfluidic platforms.[105] The simplicity of fabrication, combining cells, biomaterials, geometries, and perfusion, is a notable advantage to be taken into account.

The choice of (bio)material(s) should be carefully considered as it could be a limiting aspect. Enabling single-cell migration of immune cells (e.g., extravasation, immune cell trafficking) is crucial because their movement and cell–cell interactions are necessary to successfully develop the immune response.[52]

7.2. Membranes

In OoC platforms, membranes are used to compartmentalize spaces, acting as a barrier while facilitating nutrients, drugs, and metabolites exchange and providing physical support for cellular adherence and growth.[106] Depending on factors like membrane thickness or pore size, cells can migrate across it, enabling essential cell crosstalk, colonization, organization, or co-localization of immune cells in immune organs. Other design parameters to be considered are undesired binding of ligands, surface roughness, wettability and hydrophilicity, permeability, microstructure, mechanical properties, and optical clarity for imaging.[107]

While the use of PDMS and other polymeric membranes is quite widespread, mostly for commercially available devices, their functionality is limited to the separation of channels.[108] Alternative 3D printing methods can further reproduce the in vivo-like microenvironment by incorporating topographical nano-, micro-, and macro- cues. Electrospun membranes have successfully been incorporated in on-chip models of the retinal epithelium and endothelium[109] or to generate an airway-endothelial model.[110] In addition, the versatility of this technique allows for the creation of 3D fibrous scaffolds, providing structural cues at the micro and macro levels.[106]

Another parameter of interest over which electrospinning and MEW provide control is pore size. Membranes with larger pores grant accessible compartments, so the patrolling and responding immune cells can easily migrate to their targets. MEW showcases interesting possibilities for assembling customized pores in membranes as they become not only physical barriers but also stir cell behavior.[111] This feature proves relevant in, for example, modeling certain types of cancer for which ECM's physical characteristics are essential to understand immune-tumor interactions.[112] Moreover, tissues in health and disease can have different ECM alignments, for example in cancer stroma. A tumor's ECM can be remodeled from a wavy and non-parallel collagen structure into parallel aligned and straightened collagen, which promotes for example metastasis.[113] To transit from normal isotropic collagen organization into aligned collagen 3D hydrogels of bioprinted (OoC) devices, collagen fibers can be bound to magnetic beads[114] or magnetic nanoparticles,[115] which will subsequently align upon exposure to a magnetic field. Furthermore, shear stress applied to cells is known to support, for example, endothelial[116] cells as well as ECM alignment.[117]

The highly porous MEW structures would allow for enhanced cellular organization, epithelial/endothelial cell–immune cell crosstalk and would enable better immune cell integration and migration into the tissue. Furthermore, this printing technology sheds light on the possibility to more closely mimic micro-scaled structures of specific immune organs, especially in combination with ECM remodeling techniques using magnetic fields and shear stress to further align the micro-architecture.

7.3. Organotypic Architecture and Spatial Organization of Cells

In recent years, special attention has been paid to the importance of micro and macro-architectures when replicating an organ in vitro.[118] Features like the 3D arrangement of elements and the degree of compartmentalization in a certain organ are tightly related to cell behavior, identity, and function.[2] Especially in complex organs like the BM, LNs, the thymus, or the spleen in which function is strongly characterized by the architecture. OoC devices are still far from being able to recapitulate the 3D complexity of these organs and a good part of in vivo spatial information is lost.

Inkjet and extrusion bioprinting can recapitulate native spatial information with high accuracy. The combination of both techniques enabled a multi-step approach for printing a layered skin model. From building de novo the microfluidic foundation with extruded polymers, to the layering of hypodermis, dermis, and epidermis containing their characteristic cell types and vasculature.[97] This highlights how the combination of different bioprinting techniques may support the complexity and maturation of organ models. However, the characterization of the living construct is limited to specific tissue markers and no functionality is proven.[97] The same approach could be used to precisely deposit tissue-resident immune cells, such as Langerhans cells in the epidermis or dendritic cells in the dermis,[119] in each printed layer to directly mimic the tissues' organization. Furthermore, this layer-by-layer construction holds the potential for building the different layers of immune organs, such as the multi-layered BM[7] or lobules in the thymus[8] and LNs,[15] and might support further the ease of immune cell migration and co-localization.

Bioprinting technologies also pose strong potential to create 3D structures given the freedom in design they provide. Of special interest is to generate perfusable and complex 3D structures in a single step. Volumetric (bio)printing increases manufacturing speed and feature resolution by sculpting constructs without the use of layers. For instance, volumetric (bio)printing has recently been applied to encapsulate liver organoids in a perfusable gyroidal structure,[120] opening new possibilities for the integration of fluidic forces in complex architectures. This is of high relevance when modeling afferent and efferent lymph vessels in immune organs. Furthermore, mucosal tissues such as the gut-associated lymphoid tissue, which contains a lumen surrounded by the intestinal mucosal barrier, including Peyer's patches right below the epithelium and draining LNs,[10,121] could be more closely reassembled including perfusion.

7.4. Environmental Cues

Particular emphasis has been drawn to the effect of the substrate's topography on cell behavior and function.[118,122] For instance, a series of micropatterns can be generated on synthetic hydrogels using a digital light processing printer, which serves as guidance for cells to attach and orient themselves.[123] Replicating as closely as possible the topographic features of the ECM, such as patterns, porosity, and stiffness, could help to stir and regulate cell behavior as proven extensively.[46,124]

Environmental cues for immune responses involved chemotactic events reproduced by the addition of the chemoattractant directly in the model or by the incorporation of cells producing them. However, chemokine diffusion and its concentration gradient strongly depend on ECM composition, stiffness, and porosity. Aiming at generating such in vivo-like gradients, natural thermogelling hydrogels were leveraged using an extrusion 3D printer, manufacturing gradients of hydrogel stiffness and cell composition in a predictable and tunable way.[125] Another study combined a microfluidic chaotic mixer with a digital light processing 3D printer to generate composable gradients exerting control over the cellular, chemical, and mechanical properties of the generated gradients.[126] Tunable hydrogel properties could possibly increase controllability over the speed of diffusion of chemokines as well as their diffusion distance, thereby providing cells with more in vivo-like gradients.

In conclusion, advanced 3D printing technologies offer diverse solutions for improving physiological complexity in immune OoCs. These technologies, with their versatile design capabilities, contribute to the creation of more biomimetic in vitro models, addressing the limitations of current immune system representations.

8. (Bio)Printing and its Application in Recapitulating the Immune System In Vitro

While the integration of biofabrication technologies with immune organs or cells is still in its infancy, certain strategies

applied have emerged, primarily focusing on electrospinning and MEW printing. For example, MEW-printed scaffolds, featuring different pore geometries, have been instrumental in studying the impact on immune cells. Notably, box-shaped geometries were found to enhance the spontaneous differentiation of human monocyte-derived macrophages into M2, with smaller pore sizes further supporting the M2 phenotype and macrophage elongation.[127] Recent studies using MEW-printed with a rhombus geometry of the pore demonstrated elevated secretion of anti-inflammatory mediators by human primary monocyte-derived macrophages.[128] Electrospun meshes also facilitate T cell expansion for T cell therapies, revealing enhanced cell proliferation with a smaller fiber spacing of 200 µm compared to larger pore sizes.[129] In another approach, electrospun skin meshes releasing the anti-inflammatory cytokine interleukin-10 showcased potential in scarless wound healing by suppressing inflammation and enhancing fibroblast performance.[130]

In the realm of (more recent) studies, a limited number have directly coupled bioprinting with OoC platforms for recapitulating targeted immune cell migration. One notable example employed different printing and fabrication techniques in a bottom-up approach to generate a vascularized ovarian tumor-on-a-chip model. The model allowed studying the role of neutrophils in tumor invasion by growing tumor spheroids on a 3D matrix, preserving their migratory behavior. The 3D-printed perfusable vessel enabled the native-like incorporation of neutrophils, granting them the freedom to migrate toward the tumor site in response to chemoattractant cues. This innovative combination of static 3D cultures with printed channels for perfusion demonstrated the importance of cellular dynamic interactions.[131] Another study employed a combination of extrusion and coaxial bioprinting to develop a perfusable tumor microenvironment with blood and lymphatic ducts. The design featured parallel vascular and lymphatic channels alongside an array of melanoma spheroids, allowing evaluation of melanoma cell invasion and intravasation evaluation toward the channels. From a technical point of view, sacrificial coaxial printing allows to lay the ducts, while extrusion printing of the organoids enables the precise deposition of the spheroids between the channels.[132] This unprecedented combination of extrusion printing with OoC technology, including immune cells, shows the potential to generate and mimic complex compartmentalized and immunocompetent in vivo-like tissues.

9. Future Outlook and Conclusions

The potential of OoCs, when compared to conventional 2D counterparts and static 3D cultures, particularly stands out in facilitating, for example, cell migration and tissue infiltration for effective cell-pathogen interactions. This positions OoC technology as an ideal candidate for mimicking specific aspects of the immune system. However, challenges persist in matching the heterogeneity of immune cells and the unique characteristics and functions of tissues. Primary and secondary immune organs demand compartmentalization and microstructural organization, while immune cells rely on vessels and biomimetic ECMs for migration. Current immune-on-a-chip (immune-oC) devices are diverse, and their complexity and translatability are limited. Some setups have focused on replicating neutrophil,[74–77,82] monocyte/macrophage[78–80] and T cell[81,82] responses in less complex chips, others have advanced more on the technological aspects including BM niches,[55,57,59,60] thymus[62] and LN structures.[64–66] The application of more advanced biofabrication approaches could cover these demands simultaneously. However, with respect to extensive validation, translation, and regulatory acceptance, 2D co-culture systems currently are the gold standard in screening and mechanistic immune response studies. Newly developed immune-oC models, including applications of biofabrication technologies, must pass these validation steps and in the future will help to complement and/or refine output derived from 2D co-culture systems. Parallel use of complementary 2D and 3D models will most likely be needed to bridge the translational gap in immunology.

One of the most relevant advantages of bioprinting is the diversification of materials and techniques to produce in vitro platforms, including biochemical and biomechanical cues for the cells. Special attention should be placed on the choice of materials used in the fabrication of complex OoC in terms of their immune safety and reactivity. The use of more biologically relevant materials would enable the modulation of the immune responses in vitro, priming certain responses and preventing undesired unspecific cell activation.[46,133] While natural polymers present improved cell affinity and biocompatibility, synthetic materials have more controllable physical-chemical features and may be favorable because these are less capable of inducing inflammatory responses.[134] Both types of polymers have successfully been processed to be printable, and include, for example, collagen, alginate, or even decellularized tissue-derived ECM, as well as polyethylene glycol, poly(lactic-*co*-glycolic acid), and polycaprolactone.[135] Furthermore, the importance of generating an OoC that includes a 3D environment becomes stronger when dealing with immune cells, given their high spatiotemporal specificity and migration characteristics. The potential of bioprinting to generate 3D structures is one of the main advantages of this technology, which could be leveraged to produce advanced 3D immune in vitro models of improved spatial complexity.

The integration of multi-organ OoC technology with bioprinting holds promise for providing new insights into the circulation and interaction of immune cells within the immune system and other organs. This combination allows for the recreation of natural-like compartments with a free-form design in a modular way, recapitulating immune events more faithfully. The ability of bioprinting technologies to generate 3D compartments in vitro platforms also enables the possibility to include higher-order biological structures, like spheroids and organoids, which are valuable tool to study higher complexity physiological processes. However, a balance between low experimental complexity and sophisticated physiological features to (re)produce relevant data in a realistic as well as a cost-effective manner should be considered. Besides, immune devices could benefit from the incorporation of high-resolution multi-sensors (e.g., electrochemical biosensors or gauge sensors), as many relevant immune events happen in a very short time span and in an exact localized manner.

An omnipresent limitation for immune system engineering is the source of cells. Most of the discussed immune-oC systems include immune cell- and immune organ specific immortalized cell lines and/or primary cells. The use of immortalized

cell lines, commercially available primary cells or primary immune cells derived from healthy donors, can be advantageous with regards to cell availability and time efficiency when developing and testing new immune models. However, some of the current immune-oC systems might lack correct representation and might not be identical to the patients' tissue or might not represent patients' variability.[30] Therefore, matching the donors of the human primary cells for the different immune cell lineages is strongly encouraged to increase the translatability toward human physiology. However, the most important limitations in using patient-derived primary cells are donor availability, ethical issues as well as patient variation. An alternative for future studies, although more time-consuming, could be the use of differentiating (pluripotent) stem cells into the various immune cells and immune organ-specific tissue cells to be applied in one model, to ensure donor compatibility.[136] However, it should be taken into account that variability in developed cell lineages might still arise, as laboratories may use different protocols. To further improve the physiological relevance of OoC platforms, immune models should cover tissue-resident immune cells, such as follicular DCs and T cells, or Langerhans cells and microglia, to more accurately replicate the immune responses within specific tissue. Besides, the evaluation of the immune cell responses is usually limited. Immune cell features, such as the ability for adhesion, migration, cell–cell interaction, cell recruitment, phagocytosis, type of immune response, and immune cell memory development, should be considered. In addition, device characteristics such as physiological fluid flow, ECM (specific porosity and stiffness), porous bio membranes and multiple compartments should be included in combination with organ-specific pH and oxygen levels, to increase the physiological relevance of the developed model. Especially, interconnected compartmentalization of the immune response or specific cellular niches inside immune organs will allow future studies to better incorporate the immune system in a more physiological manner.

Advancing toward the next generation of 3D in vitro models to study immune function requires further incorporation of immune components and structures such as LNs in the testing platforms. Up to date, the technological power offered by microfluidics has sufficed to produce perfusable and accessible-for-readouts single-organ 3D immune in vitro models. Nevertheless, challenges appear when trying to scale up these models to recapitulate events of higher complexity. A general pitfall in current immune-oC models is the high variability and low reproducibility between models associated to custom-made devices. Commercial microfluidic platforms are gaining increasing momentum, with more than a fifth of the reviewed articles making use of them. Furthermore, the recent implementation of AM technologies, such as 3D bioprinting, to the OoC field could be a promising strategy to palliate these shortcomings and to improve current immune-oC models. Future developments should prioritize immune-oC devices that mimic inter-organ migration of specific immune cell subsets to drive innovation in the field.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors would like to thank the knowledge partners in the consortium (Wageningen UR dep. Wageningen Food & Biobased Research, Erasmus MC dep. Allergology and clinical immunology, and Utrecht University div. of Pharmacology) for their valuable scientific input. This work was funded by the Dutch Ministry of Economic Affairs program TKI-AF under grant agreement LWV200123: PRedictive model For the sensitising capacity of novel nutritional pRoteins (PREFER study), and industrial partners Nutricia Research B.V., Cargill R&D Centre Europe, Arla Foods Ingredients Group P/S, Société des Produits Nestlé S.A., Phycom BV, The Seaweed Company B.V., and Mycorena AB and by Utrecht Institute for Pharmaceutical Sciences. The views expressed in this manuscript are those of the authors and do not necessarily reflect the position or policy of the collaborators.

Conflict of Interest

The authors declare no conflict of interest.

Author Contribution Statement

R.J., L.B.Z., and P.C. contributed equally to this work. R.J., L.B.Z., P.C., and M.G.V. wrote the first draft of the manuscript. S.M.M, S.B-N, J.G., L.E.M.W., and R.M. reviewed/edited the manuscript. All authors read and contributed to the final version of the manuscript.

Keywords

3D printing, biofabrication, immune cells, immune organs, immune responses, immune system, microfluidics, organ-on-a-chip

Received: December 21, 2023
Revised: March 19, 2024
Published online:

[1] D. D. Chaplin, *J. Allergy Clin. Immunol.* **2010**, *125*, S3.
[2] D. Huh, G. A. Hamilton, D. E. Ingber, *Trends Cell Biol.* **2011**, *21*, 745.
[3] a) D. Kim, M. A. Lokuta, A. Huttenlocher, D. J. Beebe, *Lab Chip* **2009**, *9*, 1797; b) G. Santos Rosalem, L. A. Gonzales Torres, E. B. de Las Casas, F. A. S. Mathias, J. C. Ruiz, M. G. R. Carvalho, *PLoS One* **2020**, *15*, e0243840.
[4] I. W. Mak, N. Evaniew, M. Ghert, *Am. J. Transl. Res.* **2014**, *6*, 114.
[5] K. Thakare, L. Jerpseth, Z. Pei, A. Elwany, F. Quek, H. Qin, *J. Manuf. Mater. Process.* **2021**, 5.
[6] J. S. Marshall, R. Warrington, W. Watson, H. L. Kim, *Allergy Asthma Clin. Immunol.* **2018**, *14*, 49.
[7] a) E. Cordeiro-Spinetti, R. S. Taichman, A. Balduino, *J. Cell. Biochem.* **2015**, *116*, 6; b) E. Zhao, H. Xu, L. Wang, I. Kryczek, K. Wu, Y. Hu, G. Wang, W. Zou, *Cell Mol. Immunol.* **2012**, *9*, 11.
[8] P. Thapa, D. L. Farber, *Thorac. Surg. Clin.* **2019**, *29*, 123.
[9] P. Brandtzaeg, H. Kiyono, R. Pabst, M. W. Russell, *Mucosal Immunol.* **2008**, *1*, 31.
[10] A. M. Mowat, *Nat. Rev. Immunol.* **2018**, *18*, 405.
[11] N. H. Ruddle, E. M. Akirav, *J. Immunol.* **2009**, *183*, 2205.
[12] S. E. Acton, L. Onder, M. Novkovic, V. G. Martinez, B. Ludewig, *Trends Immunol.* **2021**, *42*, 782.
[13] a) C. Gago da Graca, L. G. M. van Baarsen, R. E. Mebius, *J. Immunol.* **2021**, *206*, 273; b) Y. Sato, K. Silina, M. van den Broek, K. Hirahara, M. Yanagita, *Nat. Rev. Nephrol.* **2023**, *19*, 525.

[14] a) T. D. Randall, D. M. Carragher, J. Rangel-Moreno, *Annu. Rev. Immunol.* **2008**, *26*, 627; b) S. Arroz-Madeira, T. Bekkhus, M. H. Ulvmar, T. V. Petrova, *Circ. Res.* **2023**, *132*, 1203.
[15] C. L. Willard-Mack, *Toxicol. Pathol.* **2006**, *34*, 409.
[16] S. M. Lewis, A. Williams, S. C. Eisenbarth, *Sci. Immunol.* **2019**, 4.
[17] a) J. Parkin, B. Cohen, *Lancet* **2001**, *357*, 1777; b) L. Sun, X. Wang, J. Saredy, Z. Yuan, X. Yang, H. Wang, *Redox Biol.* **2020**, *37*, 101759.
[18] J. I. Gray, D. L. Farber, *Annu. Rev. Immunol.* **2022**, *40*, 195.
[19] J. Kranich, N. J. Krautler, *Front. Immunol.* **2016**, *7*, 225.
[20] S. Crotty, *Immunity* **2014**, *41*, 529.
[21] K. Clayton, A. F. Vallejo, J. Davies, S. Sirvent, M. E. Polak, *Front. Immunol.* **2017**, *8*, 1676.
[22] Y. Lyu, Y. Zhou, J. Shen, *Front. Immunol.* **2022**, *13*, 912393.
[23] a) M. Akhmanova, E. Osidak, S. Domogatsky, S. Rodin, A. Domogatskaya, *Stem Cells Int.* **2015**, *2015*, 167025; b) J. H. Hammel, J. M. Zatorski, S. R. Cook, R. R. Pompano, J. M. Munson, *Adv. Drug Delivery Rev.* **2022**, *182*, 114111.
[24] T. H. Kim, C. Ly, A. Christodoulides, C. J. Nowell, P. W. Gunning, E. K. Sloan, A. C. Rowat, *FASEB J.* **2019**, *33*, 3997.
[25] A. Singh, S. Suri, K. Roy, *Biomaterials* **2009**, *30*, 5187.
[26] E. Schiffmann, B. A. Corcoran, S. M. Wahl, *Proc. Natl. Acad. Sci. USA* **1975**, *72*, 1059.
[27] J. Taipale, J. Keski-Oja, *FASEB J.* **1997**, *11*, 51.
[28] S. L. Klein, K. L. Flanagan, *Nat. Rev. Immunol.* **2016**, *16*, 626.
[29] Z. Huang, B. Chen, X. Liu, H. Li, L. Xie, Y. Gao, R. Duan, Z. Li, J. Zhang, Y. Zheng, W. Su, *Proc. Natl. Acad. Sci. USA* **2021**, 118.
[30] G. Kaur, J. M. Dufour, *Spermatogenesis* **2012**, *2*, 1.
[31] a) M. Huse, *Nat. Rev. Immunol.* **2017**, *17*, 679; b) J. Kroll, M. J. A. Ruiz-Fernandez, M. B. Braun, J. Merrin, J. Renkawitz, *Curr. Protoc.* **2022**, *2*, e407; c) S. F. B. Mennens, M. Bolomini-Vittori, J. Weiden, B. Joosten, A. Cambi, K. van den Dries, *Sci. Rep.* **2017**, *7*, 17511; d) K. Wolf, M. Te Lindert, M. Krause, S. Alexander, J. Te Riet, A. L. Willis, R. M. Hoffman, C. G. Figdor, S. J. Weiss, P. Friedl, *J. Cell Biol.* **2013**, *201*, 1069.
[32] K. P. Meng, F. S. Majedi, T. J. Thauland, M. J. Butte, *J. Exp. Med.* **2020**, 217.
[33] S. Breslin, L. O'Driscoll, *Drug Discovery Today* **2013**, *18*, 240.
[34] a) P. Friedl, E. Sahai, S. Weiss, K. M. Yamada, *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 743; b) P. Friedl, B. Weigelin, *Nat. Immunol.* **2008**, *9*, 960.
[35] D. Manou, I. Caon, P. Bouris, I. E. Triantaphyllidou, C. Giaroni, A. Passi, N. K. Karamanos, D. Vigetti, A. D. Theocharis, *Methods Mol. Biol.* **2019**, *1952*, 1.
[36] R. Sridharan, B. Cavanagh, A. R. Cameron, D. J. Kelly, F. J. O'Brien, *Acta Biomater.* **2019**, *89*, 47.
[37] T. Okamoto, Y. Takagi, E. Kawamoto, E. J. Park, H. Usuda, K. Wada, M. Shimaoka, *Exp. Cell Res.* **2018**, *367*, 264.
[38] F. Puttur, L. Denney, L. G. Gregory, J. Vuononvirta, R. Oliver, L. J. Entwistle, S. A. Walker, M. B. Headley, E. J. McGhee, J. E. Pease, M. F. Krummel, L. M. Carlin, C. M. Lloyd, *Sci Immunol* **2019**, 4.
[39] S. J. Ray, S. N. Franki, R. H. Pierce, S. Dimitrova, V. Koteliansky, A. G. Sprague, P. C. Doherty, A. R. de Fougerolles, D. J. Topham, *Immunity* **2004**, *20*, 167.
[40] a) L. H. Lambert, G. K. Goebrecht, S. E. De Leo, R. S. O'Connor, S. Nunez-Cruz, T. D. Li, J. Yuan, M. C. Milone, L. C. Kam, *Nano Lett.* **2017**, *17*, 821; b) F. S. Majedi, M. M. Hasani-Sadrabadi, T. J. Thauland, S. Li, L. S. Bouchard, M. J. Butte, *Biomaterials* **2020**, *252*, 120058.
[41] a) H. Salmon, K. Franciszkiewicz, D. Damotte, M. C. Dieu-Nosjean, P. Validire, A. Trautmann, F. Mami-Chouaib, E. Donnadieu, *J. Clin. Invest.* **2012**, *122*, 899; b) J. Weiden, D. Voerman, Y. Dolen, R. K. Das, A. van Duffelen, R. Hammink, L. J. Eggermont, A. E. Rowan, J. Tel, C. G. Figdor, *Front. Immunol.* **2018**, *9*, 2798.
[42] A. Gaggar, P. L. Jackson, B. D. Noerager, P. J. O'Reilly, D. B. McQuaid, S. M. Rowe, J. P. Clancy, J. E. Blalock, *J. Immunol.* **2008**, *180*, 5662.
[43] S. Kim, H. Takahashi, W. W. Lin, P. Descargues, S. Grivennikov, Y. Kim, J. L. Luo, M. Karin, *Nature* **2009**, *457*, 102.
[44] J. Song, Z. Lokmic, T. Lämmermann, J. Rolf, C. Wu, X. Zhang, R. Hallmann, M. J. Hannocks, N. Horn, M. A. Ruegg, A. Sonnenberg, E. Georges-Labouesse, T. H. Winkler, J. F. Kearney, S. Cardell, L. Sorokin, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E2915.
[45] T. E. Sutherland, D. P. Dyer, J. E. Allen, *Science* **2023**, *379*, eabp8964.
[46] W. Bu, Y. Wu, A. M. Ghaemmaghami, H. Sun, A. Mata, *Regen Biomater* **2022**, *9*, rbac009.
[47] a) G. K. Chimal-Ramirez, N. A. Espinoza-Sanchez, D. Utrera-Barillas, L. Benitez-Bribiesca, J. R. Velazquez, L. A. Arriaga-Pizano, A. Monroy-Garcia, E. Reyes-Maldonado, M. L. Dominguez-Lopez, P. Pina-Sanchez, E. M. Fuentes-Panana, *Biomed Res. Int.* **2013**, *2013*, 279505; b) N. Linde, C. M. Gutschalk, C. Hoffmann, D. Yilmaz, M. M. Mueller, *PLoS One* **2012**, *7*, e40058; c) X. Q. Liu, R. Kiefl, C. Roskopf, F. Tian, R. M. Huber, *PLoS One* **2016**, *11*, e0156268; d) B. Weigelin, P. Friedl, *Biochem. Pharmacol.* **2010**, *80*, 2087.
[48] a) V. Phan-Lai, S. J. Florczyk, F. M. Kievit, K. Wang, E. Gad, M. L. Disis, M. Zhang, *Biomacromolecules* **2013**, *14*, 1330; b) D. Rama-Esendagli, G. Esendagli, G. Yilmaz, D. Guc, *Mol. Biol. Rep.* **2014**, *41*, 2885.
[49] M. N. Temples, I. M. Adjei, P. M. Nimocks, J. Djeu, B. Sharma, *ACS Biomater. Sci. Eng.* **2020**, *6*, 4179.
[50] Z. Xu, D.-G. Hwang, M. D. Bartlett, S. Jiang, K. M. Bratlie, *Biochem. Eng. J.* **2021**, 165.
[51] F. Taraballi, M. Sushnitha, C. Tsao, G. Bauza, C. Liverani, A. Shi, E. Tasciotti, *Adv. Healthcare Mater.* **2018**, *7*, e1800490.
[52] X. Li, Y. Shou, A. Tay, *Adv. Nanobiomed. Res.* **2021**, *1*, 2000073.
[53] a) M. R. Nelson, D. Ghoshal, J. C. Mejias, D. F. Rubio, E. Keith, K. Roy, *Biomaterials* **2021**, *270*, 120683; b) Y. S. Torisawa, T. Mammoto, E. Jiang, A. Jiang, A. Mammoto, A. L. Watters, A. Bahinski, D. E. Ingber, *Tissue Eng., Part C* **2016**, *22*, 509; c) Y. S. Torisawa, C. S. Spina, T. Mammoto, A. Mammoto, J. C. Weaver, T. Tat, J. J. Collins, D. E. Ingber, *Nat. Methods* **2014**, *11*, 663.
[54] C. Moreno, C. Munoz, M. J. Terol, J. A. Hernandez-Rivas, M. Villanueva, *J. Exp. Clin. Cancer Res.* **2021**, *40*, 321.
[55] S. Sieber, L. Wirth, N. Cavak, M. Koenigsmark, U. Marx, R. Lauster, M. Rosowski, *J. Tissue Eng. Regen. Med.* **2018**, *12*, 479.
[56] A. Sharipol, M. L. Lesch, C. A. Soto, B. J. Frisch, *Front. Bioeng. Biotechnol.* **2022**, *10*, 855777.
[57] J. Aleman, S. K. George, S. Herberg, M. Devarasetty, C. D. Porada, A. Skardal, G. Almeida-Porada, *Small* **2019**, *15*, e1902971.
[58] D. B. Chou, V. Frismantas, Y. Milton, R. David, P. Pop-Damkov, D. Ferguson, A. MacDonald, O. Vargel Bolukbasi, C. E. Joyce, L. S. Moreira Teixeira, A. Rech, A. Jiang, E. Calamari, S. Jalili-Firoozinezhad, B. A. Furlong, L. R. O'Sullivan, C. F. Ng, Y. Choe, S. Marquez, K. C. Myers, O. K. Weinberg, R. P. Hasserjian, R. Novak, O. Levy, R. Prantil-Baun, C. D. Novina, A. Shimamura, L. Ewart, D. E. Ingber, *Nat. Biomed. Eng.* **2020**, *4*, 394.
[59] C. Ma, M. T. Witkowski, J. Harris, I. Dolgalev, S. Sreeram, W. Qian, J. Tong, X. Chen, I. Aifantis, W. Chen, *Sci. Adv.* **2020**, 6.
[60] D. E. Glaser, M. B. Curtis, P. A. Sariano, Z. A. Rollins, B. S. Shergill, A. Anand, A. M. Deely, V. S. Shirure, L. Anderson, J. M. Lowen, N. R. Ng, K. Weilbaecher, D. C. Link, S. C. George, *Biomaterials* **2022**, *280*, 121245.
[61] J. Gordon, N. R. Manley, *Development* **2011**, *138*, 3865.
[62] S. A. Watson, Y. Javanmardi, L. Zanieri, S. Shahreza, R. Ragazzini, P. Bonfanti, E. Moeendarbary, *Bioeng. Transl. Med.* **2023**, *8*, e10454.
[63] S. Shim, M. C. Belanger, A. R. Harris, J. M. Munson, R. R. Pompano, *Lab Chip* **2019**, *19*, 1013.

[64] K. G. Birmingham, M. J. O'Melia, S. Bordy, D. R. Aguilar, B. El-Reyas, G. Lesinski, S. N. Thomas, *iScience* **2020**, *23*, 101751.
[65] A. Shanti, B. Samara, A. Abdullah, N. Hallfors, D. Accoto, J. Sapudom, A. Alatoom, J. Teo, S. Danti, C. Stefanini, *Pharmaceutics* **2020**, 12.
[66] N. Hallfors, A. Shanti, J. Sapudom, J. Teo, G. Petroianu, S. Lee, L. Planelles, C. Stefanini, *Bioengineering* **2021**, *8*.
[67] G. Goyal, P. Prabhala, G. Mahajan, B. Bausk, T. Gilboa, L. Xie, Y. Zhai, R. Lazarovits, A. Mansour, M. S. Kim, A. Patil, D. Curran, J. M. Long, S. Sharma, A. Junaid, L. Cohen, T. C. Ferrante, O. Levy, R. Prantil-Baun, D. R. Walt, D. E. Ingber, *Adv. Sci.* **2022**, *9*, e2103241.
[68] J. M. Ayuso, M. M. Gong, M. C. Skala, P. M. Harari, D. J. Beebe, *Adv. Healthcare Mater.* **2020**, *9*, e1900925.
[69] N. Frenkel, S. Poghosyan, C. R. Alarcón, S. B. García, K. Queiroz, L. van den Bent, J. Laoukili, I. B. Rinkes, P. Vulto, O. Kranenburg, J. Hagendoorn, *ACS Biomater. Sci. Eng.* **2021**, *7*, 3030.
[70] a) M. Antia, T. Herricks, P. K. Rathod, *PLoS Pathog.* **2007**, *3*, e99; b) Q. Guo, S. J. Reiling, P. Rohrbach, H. Ma, *Lab Chip* **2012**, *12*, 1143; c) L. G. Rigat-Brugarolas, A. Elizalde-Torrent, M. Bernabeu, M. De Niz, L. Martin-Jaular, C. Fernandez-Becerra, A. Homs-Corbera, J. Samitier, H. A. del Portillo, *Lab Chip* **2014**, *14*, 1715; d) J. P. Shelby, J. White, K. Ganesan, P. K. Rathod, D. T. Chiu, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14618.
[71] Y. Qiang, A. Sissoko, Z. L. Liu, T. Dong, F. Zheng, F. Kong, J. M. Higgins, G. E. Karniadakis, P. A. Buffet, S. Suresh, M. Dao, *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2217607120.
[72] A. L. Kenter, J. M. Richner, *Trends Immunol.* **2021**, *42*, 367.
[73] R. Janssen, J. W. M. de Kleer, B. Heming, S. Bastiaan-Net, J. Garssen, L. E. M. Willemsen, R. Masereeuw, *Trends Biotechnol.* **2023**, *42*, 119.
[74] P. H. McMinn, L. E. Hind, A. Huttenlocher, D. J. Beebe, *Lab Chip* **2019**, *19*, 3697.
[75] L. Kempers, E. G. G. Sprenkeler, A. C. I. van Steen, J. D. van Buul, T. W. Kuijpers, *Front. Immunol.* **2021**, *12*, 678030.
[76] P. N. Ingram, L. E. Hind, J. A. Jiminez-Torres, A. Huttenlocher, D. J. Beebe, *Adv. Healthcare Mater.* **2018**, 7.
[77] R. B. Riddle, K. Jennbacken, K. M. Hansson, M. T. Harper, *Sci. Rep.* **2022**, *12*, 6855.
[78] S. Perez-Rodriguez, S. A. Huang, C. Borau, J. M. Garcia-Aznar, W. J. Polacheck, *Biomicrofluidics* **2021**, *15*, 054102.
[79] S. Perez-Rodriguez, C. Borau, J. M. Garcia-Aznar, J. Gonzalo-Asensio, *BMC Microbiol.* **2022**, *22*, 211.
[80] C. Poussin, B. Kramer, H. L. Lanz, A. Van den Heuvel, A. Laurent, T. Olivier, M. Vermeer, D. Peric, K. Baumer, R. Dulize, E. Guedj, N. V. Ivanov, M. C. Peitsch, J. Hoeng, J. Joore, *ALTEX* **2020**, *37*, 47.
[81] X. Ren, J. Wu, D. Levin, S. Santos, R. L. de Faria, M. Zhang, F. Lin, *Ann. N. Y. Acad. Sci.* **2019**, *1445*, 52.
[82] A. C. I. van Steen, L. Kempers, R. Schoppmeyer, M. Blokker, D. J. Beebe, M. A. Nolte, J. D. van Buul, *J. Cell Sci.* **2021**, 134.
[83] M. Maurer, M. S. Gresnigt, A. Last, T. Wollny, F. Berlinghof, R. Pospich, Z. Cseresnyes, A. Medyukhina, K. Graf, M. Groger, M. Raasch, F. Siwczak, S. Nietzsche, I. D. Jacobsen, M. T. Figge, B. Hube, O. Huber, A. S. Mosig, *Biomaterials* **2019**, *220*, 119396.
[84] G. Trujillo-de Santiago, B. G. Flores-Garza, J. A. Tavares-Negrete, I. M. Lara-Mayorga, I. Gonzalez-Gamboa, Y. S. Zhang, A. Rojas-Martinez, R. Ortiz-Lopez, M. M. Alvarez, *Materials* **2019**, 12.
[85] Y. Liu, P. Kongsuphol, S. Y. Chiam, Q. X. Zhang, S. B. N. Gourikutty, S. Saha, S. K. Biswas, Q. Ramadan, *Lab Chip* **2019**, *19*, 241.
[86] K. M. Bircsak, R. DeBiasio, M. Miedel, A. Alsebahi, R. Reddinger, A. Saleh, T. Shun, L. A. Vernetti, A. Gough, *Toxicology* **2021**, *450*, 152667.
[87] M. D. Cherne, B. Sidar, T. A. Sebrell, H. S. Sanchez, K. Heaton, F. J. Kassama, M. M. Roe, A. B. Gentry, C. B. Chang, S. T. Walk, M. Jutila, J. N. Wilking, D. Bimczok, *Front. Pharmacol.* **2021**, *12*, 707891.
[88] N. Picollet-D'hahan, A. Zuchowska, I. Lemeunier, S. L.e Gac, *Trends Biotechnol.* **2021**, *39*, 788.
[89] M. Trapecar, C. Communal, J. Velazquez, C. A. Maass, Y. J. Huang, K. Schneider, C. W. Wright, V. Butty, G. Eng, O. Yilmaz, D. Trumper, L. G. Griffith, *Cell Syst.* **2020**, *10*, 223.
[90] T. Sasserath, J. W. Rumsey, C. W. McAleer, L. R. Bridges, C. J. Long, D. Elbrecht, F. Schuler, A. Roth, C. Bertinetti-LaPatki, M. L. Shuler, J. J. Hickman, *Adv. Sci.* **2020**, *7*, 2000323.
[91] L. Chen, Y. Yang, H. Ueno, M. B. Esch, *Microphysiol. Syst.* **2020**, *4*.
[92] L. Moroni, T. Boland, J. A. Burdick, C. De Maria, B. Derby, G. Forgacs, J. Groll, Q. Li, J. Malda, V. A. Mironov, C. Mota, M. Nakamura, W. Shu, S. Takeuchi, T. B. F. Woodfield, T. Xu, J. J. Yoo, G. Vozzi, *Trends Biotechnol.* **2018**, *36*, 384.
[93] N. T. Saidy, T. Shabab, O. Bas, D. M. Rojas-Gonzalez, M. Menne, T. Henry, D. W. Hutmacher, P. Mela, E. M. De-Juan-Pardo, *Front. Bioeng. Biotechnol.* **2020**, *8*, 793.
[94] H. H. Hwang, W. Zhu, G. Victorine, N. Lawrence, S. Chen, *Small Methods* **2018**, *2*, 1700277.
[95] R. Levato, O. Dudaryeva, C. E. Garciamendez-Mijares, B. E. Kirkpatrick, R. Rizzo, J. Schimelman, K. S. Anseth, S. Chen, M. Zenobi-Wong, Y. S. Zhang, *Nat. Rev. Methods Primers* **2023**, *3*, 47.
[96] T. Zandrini, S. Florczak, R. Levato, A. Ovsianikov, *Trends Biotechnol.* **2023**, *41*, 604.
[97] a) B. S. Kim, G. Gao, J. Y. Kim, D. W. Cho, *Adv. Healthcare Mater.* **2019**, *8*, e1801019; b) H. Lee, D. W. Cho, *Lab Chip* **2016**, *16*, 2618; c) N. Y. C. Lin, K. A. Homan, S. S. Robinson, D. B. Kolesky, N. Duarte, A. Moisan, J. A. Lewis, *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 5399.
[98] A. Mohanty, N. Swain, A. Ramadoss, in *Smart Supercapacitors*, Elsevier, **2023**, p. 559.
[99] S. Massa, M. A. Sakr, J. Seo, P. Bandaru, A. Arneri, S. Bersini, E. Zare-Eelanjegh, E. Jalilian, B. H. Cha, S. Antona, A. Enrico, Y. Gao, S. Hassan, J. P. Acevedo, M. R. Dokmeci, Y. S. Zhang, A. Khademhosseini, S. R. Shin, *Biomicrofluidics* **2017**, *11*, 044109.
[100] K. A. Homan, D. B. Kolesky, M. A. Skylar-Scott, J. Herrmann, H. Obuobi, A. Moisan, J. A. Lewis, *Sci. Rep.* **2016**, *6*, 34845.
[101] a) Q. Pi, S. Maharjan, X. Yan, X. Liu, B. Singh, A. M. van Genderen, F. Robledo-Padilla, R. Parra-Saldivar, N. Hu, W. Jia, C. Xu, J. Kang, S. Hassan, H. Cheng, X. Hou, A. Khademhosseini, Y. S. Zhang, *Adv. Mater.* **2018**, *30*, 1706913; b) A. M. van Genderen, M. G. Valverde, P. E. Capendale, M. V. Kersten, E. S. Garvi, C. C. L. Schuurmans, M. Ruelas, J. T. Soeiro, G. Tang, M. J. Janssen, J. Jansen, S. M. Mihaila, T. Vermonden, Y. S. Zhang, R. Masereeuw, *Biofabrication* **2022**, *14*; c) X. Zhou, M. Nowicki, H. Sun, S. Y. Hann, H. Cui, T. Esworthy, J. D. Lee, M. Plesniak, L. G. Zhang, *ACS Appl. Mater. Interfaces* **2020**, *12*, 45904.
[102] a) A. K. Miri, I. Mirzaee, S. Hassan, S. Mesbah Oskui, D. Nieto, A. Khademhosseini, Y. S. Zhang, *Lab Chip* **2019**, *19*, 2019; b) A. M. Platt, G. J. Randolph, *Adv. Immunol.* **2013**, *120*, 51.
[103] A. M. Taylor, B. Bordoni, in StatPearls, © 2023, StatPearls Publishing LLC., Treasure Island FL, **2023**.
[104] a) J. C. Kade, P. D. Dalton, *Adv. Healthcare Mater.* **2021**, *10*, e2001232; b) S. Wang, M. Sarwat, P. Wang, D. C. Surrao, D. G. Harkin, J. A. St John, E. C. L. Bolle, A. Forget, P. D. Dalton, T. R. Dargaville, *Macromol. Rapid Commun.* **2020**, *41*, e2000295.
[105] R. Zhang, N. B. Larsen, *Lab Chip* **2017**, *17*, 4273.
[106] K. Corral-Najera, G. Chauhan, S. O. Serna-Saldivar, S. O. Martinez-Chapa, M. M. Aeinehvand, *Microsyst. Nanoeng.* **2023**, *9*, 107.
[107] M. Rahimnejad, F. Rasouli, S. Jahangiri, S. Ahmadi, N. Rabiee, M. Ramezani Farani, O. Akhavan, M. Asadnia, Y. Fatahi, S. Hong, J. Lee, J. Lee, S. K. Hahn, *ACS Biomater. Sci. Eng.* **2022**, *8*, 5038.
[108] C. M. Leung, P. de Haan, K. Ronaldson-Bouchard, G.-A. Kim, J. Ko, H. S. Rho, Z. Chen, P. Habibovic, N. L. Jeon, S. Takayama, M. L. Shuler, G. Vunjak-Novakovic, O. Frey, E. Verpoorte, Y.-C. Toh, *Nat. Rev. Methods Primers* **2022**, *2*, 33.

[109] J. Chuchuy, J. Rogal, T. Ngo, K. Stadelmann, L. Antkowiak, K. Achberger, S. Liebau, K. Schenke-Layland, P. Loskill, *ACS Biomater. Sci. Eng.* **2021**, *7*, 3006.
[110] N. Higuita-Castro, M. T. Nelson, V. Shukla, P. A. Agudelo-Garcia, W. Zhang, S. M. Duarte-Sanmiguel, J. A. Englert, J. J. Lannutti, D. J. Hansford, S. N. Ghadiali, *Sci. Rep.* **2017**, *7*, 11623.
[111] a) A. M. van Genderen, K. Jansen, M. Kristen, J. van Duijn, Y. Li, C. C. L. Schuurmans, J. Malda, T. Vermonden, J. Jansen, R. Masereeuw, M. Castilho, *Front. Bioeng. Biotechnol.* **2020**, *8*, 617364; b) M. von Witzleben, T. Stoppe, T. Ahlfeld, A. Bernhardt, M. L. Polk, M. Bornitz, M. Neudert, M. Gelinsky, *Adv. Healthcare Mater.* **2021**, *10*, e2002089.
[112] T. I. Maulana, E. Kromidas, L. Wallstabe, M. Cipriano, M. Alb, C. Zaupa, M. Hudecek, B. Fogal, P. Loskill, *Adv. Drug Delivery Rev.* **2021**, *173*, 281.
[113] R. Malik, P. I. Lelkes, E. Cukierman, *Trends Biotechnol.* **2015**, *33*, 230.
[114] C. Guo, L. J. Kaufman, *Biomaterials* **2007**, *28*, 1105.
[115] A. L. Wright, L. Righelli, T. J. Broomhall, H. C. Lamont, A. J. El Haj, *Front. Bioeng. Biotechnol.* **2022**, *10*, 797437.
[116] R. Sinha, S. Le Gac, N. Verdonschot, A. van den Berg, B. Koopman, J. Rouwkema, *Sci. Rep.* **2016**, *6*, 29510.
[117] T. A. Russo, A. M. M. Banuth, H. B. Nader, J. L. Dreyfuss, *PLoS One* **2020**, *15*, e0241040.
[118] D. Baptista, L. Teixeira, C. van Blitterswijk, S. Giselbrecht, R. Truckenmuller, *Trends Biotechnol.* **2019**, *37*, 838.
[119] J. A. S. Quaresma, *Clin. Microbiol. Rev.* **2019**, *32*, e00034.
[120] P. N. Bernal, M. Bouwmeester, J. Madrid-Wolff, M. Falandt, S. Florczak, N. G. Rodriguez, Y. Li, G. Grossbacher, R. A. Samsom, M. van Wolferen, L. J. W. van der Laan, P. Delrot, D. Loterie, J. Malda, C. Moser, B. Spee, R. Levato, *Adv. Mater.* **2022**, *34*, 2110054.
[121] J. I. Park, S. W. Cho, J. H. Kang, T.-E. Park, *Tissue Eng. Regener. Med.* **2023**, *20*, 341.
[122] G. Sahin, E. Vrij, R. Grant, S. Giselbrecht, *Adv. Mater. Technol.* **2023**, *8*, 2300344.
[123] a) X. Qu, W. Zhu, S. Huang, J. Y. Li, S. Chien, K. Zhang, S. Chen, *Biomaterials* **2013**, *34*, 9812; b) A. P. Zhang, X. Qu, P. Soman, K. C. Hribar, J. W. Lee, S. Chen, S. He, *Adv. Mater.* **2012**, *24*, 4266.
[124] a) C. J. Bettinger, R. Langer, J. T. Borenstein, *Angew. Chem., Int. Ed.* **2009**, *48*, 5406; b) X. Cun, L. Hosta-Rigau, *Nanomaterials* **2020**, *10*, 2070.
[125] M. Kuzucu, G. Vera, M. Beaumont, S. Fischer, P. Wei, V. P. Shastri, A. Forget, *ACS Biomater. Sci. Eng.* **2021**, *7*, 2192.
[126] M. Wang, W. Li, L. S. Mille, T. Ching, Z. Luo, G. Tang, C. E. Garciamendez, A. Lesha, M. Hashimoto, Y. S. Zhang, *Adv. Mater.* **2022**, *34*, 2107038.
[127] T. Tylek, C. Blum, A. Hrynevich, K. Schlegelmilch, T. Schilling, P. D. Dalton, J. Groll, *Biofabrication* **2020**, *12*, 025007.
[128] C. Mondadori, A. Chandrakar, S. Lopa, P. Wieringa, G. Talo, S. Perego, G. Lombardi, A. Colombini, M. Moretti, L. Moroni, *Bioact Mater* **2023**, *21*, 209.
[129] B. Delalat, F. Harding, B. Gundsambuu, E. M. De-Juan-Pardo, F. M. Wunner, M. L. Wille, M. Jasieniak, K. A. L. Malatesta, H. J. Griesser, A. Simula, D. W. Hutmacher, N. H. Voelcker, S. C. Barry, *Biomaterials* **2017**, *140*, 58.
[130] L. Chen, L. Zhang, H. Zhang, X. Sun, D. Liu, J. Zhang, Y. Zhang, L. Cheng, H. A. Santos, W. Cui, *Bioact. Mater.* **2021**, *6*, 3218.
[131] V. Surendran, D. Rutledge, R. Colmon, A. Chandrasekaran, *Biofabrication* **2021**, *13*, 35029.
[132] W. W. Cho, M. Ahn, B. S. Kim, D. W. Cho, *Adv. Sci.* **2022**, *9*, e2202093.
[133] a) M. Kharaziha, A. Baidya, N. Annabi, *Adv. Mater.* **2021**, *33*, 2100176; b) H. Yang, L. Song, B. Sun, D. Chu, L. Yang, M. Li, H. Li, Y. Dai, Z. Yu, J. Guo, *Mater. Today Bio* **2021**, *12*, 100139.
[134] a) A. G. Niculescu, C. Chircov, A. C. Birca, A. M. Grumezescu, *Int. J. Mol. Sci.* **2021**, *22*; b) D. Salthouse, K. Novakovic, C. M. U. Hilkens, A. M. Ferreira, *Acta Biomater.* **2023**, *155*, 1.
[135] W. Aljohani, M. W. Ullah, X. Zhang, G. Yang, *Int. J. Biol. Macromol.* **2018**, *107*, 261.
[136] a) U. Ahmed, R. Ahmed, M. S. Masoud, M. Tariq, U. A. Ashfaq, R. Augustine, A. Hasan, *Biomed. Mater.* **2021**, *16*, 042003; b) W. Zakrzewski, M. Dobrzynski, M. Szymonowicz, Z. Rybak, *Stem Cell Res. Ther.* **2019**, *10*, 68.

Robine Janssen received her B.Sc. in Biomedical Sciences (2017) and M.Sc. in Molecular Mechanisms of Disease (2020) from the Radboud University, The Netherlands. Currently, she is a Ph.D. candidate in the group of Prof. Dr. Rosalinde Masereeuw, Dr. Linette Willemsen, and Prof. Dr. Johan Garssen at the Utrecht Institute of Pharmaceutical Sciences of the Faculty of Science of Utrecht University (NL), in collaboration with Dr. Shanna Bastiaan-Net, Wageningen University & Research (NL). Her research is focused on engineering the gut-immune-skin axis, in which she studies epithelial – immune cell crosstalk, interorgan crosstalk, as well as the development of an organ-on-a-chip device.

Linette Willemsen is an associate professor Immunopharmacology at the Utrecht Institute for Pharmaceutical Sciences of the Faculty of Science at Utrecht University (NL). Her research focuses on mucosal immunology and linking mechanisms by which pharma and/or nutrition can protect against chronic noncommunicable immune disorders such as allergies. For this purpose, her team develops in vitro co-culture models studying the crosstalk between structural (epithelial) and immune cells as exemplified by the recently developed sequential series of co-culture models mimicking the allergic sensitization cascade in mucosal tissues. The next step is to further develop this into organs-on-chip devices.

Rosalinde Masereeuw is a full professor of Experimental Pharmacology at Utrecht Institute for Pharmaceutical Sciences and vice-dean at the Faculty of Science of Utrecht University (NL). Her research is focused on understanding the pathways that can be pharmacologically triggered to enhance repair after organ injury and on developing novel regenerative therapies to replace organ function. For this, humanized in vitro systems are being applied that functionally mimic (patients) organs, which includes the use of innovative technologies for 3D tissue cultures such as microfluidics (organs-on-chip technology) and biofabrication.