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Modeling Immunity In Vitro: Slices, Chips, and Engineered Tissues

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Abstract

Modeling immunity in vitro has the potential to be a powerful tool for investigating fundamental biological questions, informing therapeutics and vaccines, and providing new insight into disease progression. There are two major elements to immunity that are necessary to model: primary immune tissues and peripheral tissues with immune components. Here, we systematically review progress made along three strategies to modeling immunity: ex vivo cultures, which preserve native tissue structure; microfluidic devices, which constitute a versatile approach to providing physiologically relevant fluid flow and environmental control; and engineered tissues, which provide precise control of the 3D microenvironment and biophysical cues. While many models focus on disease modeling, more primary immune tissue models are necessary to advance the field. Moving forward, we anticipate that the expansion of patient-specific models may inform why immunity varies from patient to patient and allow for the rapid comprehension and treatment of emerging diseases, such as coronavirus disease 2019.

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1. INTRODUCTION

Interest in the immune system has grown exponentially in recent years, spreading from traditional immunologists to the realm of engineers and physical scientists. The success of cancer immunotherapy, named the *Science* Breakthrough of the Year in 2013 (1), heralded a new era of heightened attention to immunity throughout the scientific community. While much work has focused on immunoengineering in vivo responses, for example, via engineered cells, vaccines, and immunotherapies (2), efforts to generate faithful and robust models of immunity in vitro and ex vivo are less developed.

With human data mostly limited to the cells and molecules present in the bloodstream, experimental insights into tissue-level immunity largely come from in vivo, ex vivo, and in vitro models of immunity. At varying levels of reductionism, ex vivo and in vitro models offer the unique opportunity to dissect individual cell functions, cell–cell interactions, and tissue-level mechanisms of immunity. Such models range from top-down, ex vivo cultures of intact human and animal tissues to bottom-up, engineered cocultures in traditional well plates or advanced microfluidic systems. The models can synergize with a growing suite of computational models of immunity (3, 4) to both inform the models and test their hypotheses in ways not possible in vivo. Applications of in vitro and ex vivo models of immunity range from drug screening and toxicity studies to mechanistic models of development and disease.

Despite a surge of recent progress, many aspects of the immune system remain unexplored and rarely or inadequately modeled in vitro. Below, we briefly outline the fundamentals that should be considered when creating a model of a particular facet of the immune system. Then, we present a summary of existing technologies and recent progress, while pointing out the many gaps that still remain.

1.1. Elements of Immunity

The immune system in the body is incredibly complex, with multiple cell types at varying states of differentiation occupying multiple scales in length and time. Here we provide a brief overview of

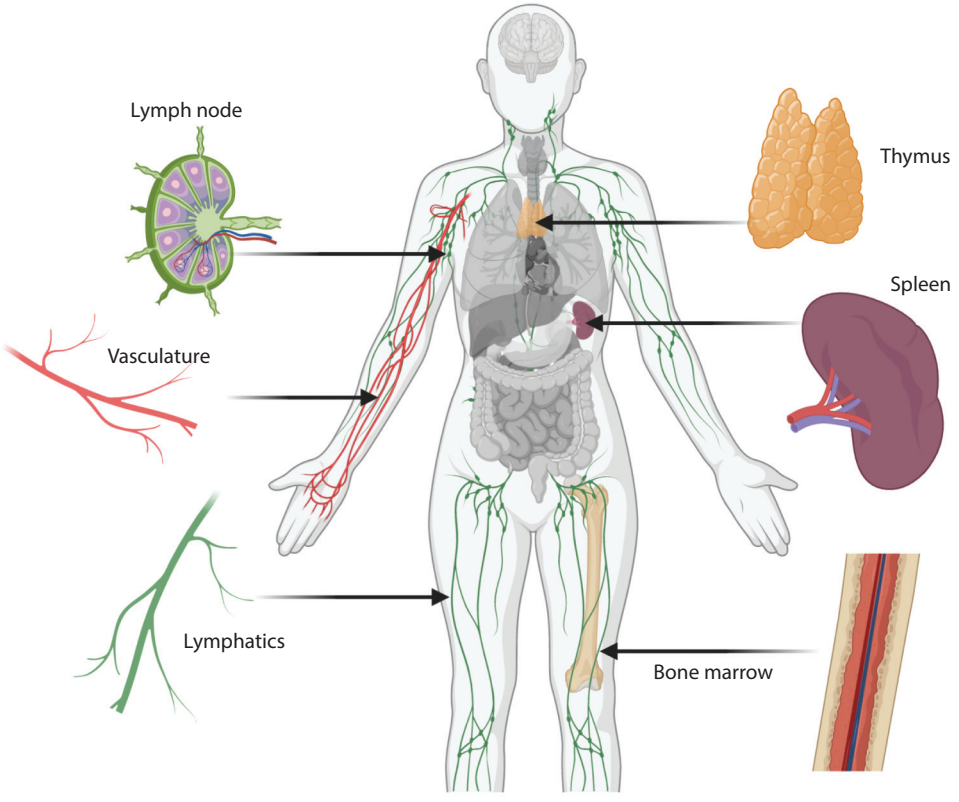

Figure 1

Overview of organs and vasculature central to immunity. Immune compartments are found throughout the body and are connected to each other and peripheral tissues through lymphatic and blood vasculature, forming a sophisticated and effective immune system. The primary and secondary immune organs discussed in this review are shown here, including the lymph node, thymus, spleen, and bone marrow. Figure adapted from images created with BioRender.com.

three aspects that need to be considered when generating an in vitro or ex vivo model of immunity: immune compartments, types and timescales of immunity, and biophysical and biochemical inputs.

1.1.1. Compartments. Development of immune cells begins in the bone marrow, where progenitors of T cells, B cells, natural killer (NK) cells, and myeloid cells such macrophages and dendritic cells (DCs) are formed (**Figure 1**). Thus, the bone marrow is considered a primary immune tissue. From there, T cell progenitors migrate to the thymus, another primary immune tissue, where those of suitable antigen specificity and affinity are selected for survival. Naive T cells and naive B cells enter circulation in the bloodstream, periodically exiting to pass through the lymph nodes and spleen (5, 6). While there, lymphocytes sample cells called antigen-presenting cells (APCs; e.g., DCs and macrophages) to determine which, if any, APCs are presenting their cognate (matching) antigen. Those antigens would have arrived in the lymph node from peripheral tissues via the lymphatic system, while the spleen collects only blood-borne antigens. These organs where the immune cells mature and perform their expected functions are referred to as secondary immune tissues.

If a naive T cell finds its antigen on an activated APC, it becomes activated and begins a program of differentiation and/or proliferation that ultimately leads to an effector immune response. For antibody-mediated immune responses (humoral immunity), specialized antigen-specific T cells called follicular helper T cells interact with B cells (7, 8), which eventually mature and expand into memory B cells or antibody-producing plasma cells. Once activated, T cells and B cells travel via the bloodstream to other peripheral organs (e.g., skin, lungs, brain, gut) to respond to pathological conditions. There, they interact with tissue-resident immune cells such as macrophages as well as other white blood cells recruited during the initial innate immune response and inflammation (e.g., eosinophils, monocytes, NK cells, neutrophils). After the initial threat is cleared, memory cells reside in the infected tissue, the lymph node, or the bone marrow, where they serve as a pool for rapid reactivation upon future infection or antigen exposure (9–12).

1.1.2. Timescales: adaptive versus innate immunity. The immune response can be broken down into two phases, innate and adaptive (13). The innate response initiates rapidly, within the first hours of exposure to an immunogenic stimulant. This response is not pathogen-specific and relies on the recognition of conserved motifs to initiate activation (14, 15). Motifs include pathogen-associated molecular patterns, such as bacterial peptidoglycans and lipopolysaccharide (LPS), as well as damage-associated molecular patterns. Adaptive immunity, on the other hand, is highly specific to the antigens that initiated it. Antigen transport to the lymph node requires hours or days, and it may be weeks before T cells and B cells are sufficiently activated, differentiated, and proliferated to counter the invading threat.

1.2. Multifaceted Inputs

From a physical science and engineering perspective, the immune response is a fascinating integration of both biochemical and biophysical inputs (**Figure 2**). Cells of the immune system communicate largely via receptor-ligand binding, either via physical cell–cell contact or by exchange of secreted proteins, vesicles, and small molecules. Each type of immune cell secretes a dynamic combination of cytokines and chemokines, small proteins (most less than 20 kDa) that act in both autocrine and paracrine fashions to control cellular chemotaxis, differentiation, proliferation, and apoptosis. After secretion, cytokines and chemokines undergo diffusion, convection, active transport, and binding to the extracellular matrix (ECM) (16, 17); their local concentrations heavily

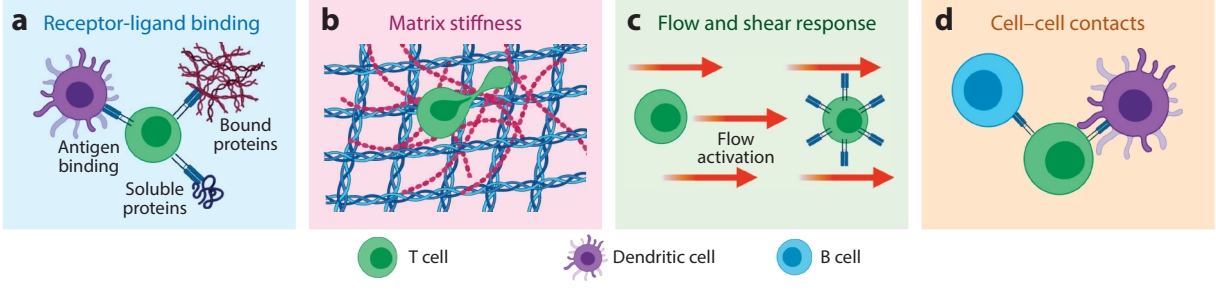

Figure 2

Cells of the immune system integrate biochemical and biophysical cues. (*a*) For example, T cells and other cells experience receptor-ligand binding to antigens, bound proteins, and soluble proteins. Immune cells are also responsive to biophysical cues, including (*b*) local tissue stiffness, (*c*) the presence or absence of interstitial fluid flow, and (*d*) physical contact with neighboring cells. For example, T cells physically engage with antigen-presenting dendritic cells to initiate an adaptive immune response or engage with B cells to initiate the cascade that leads to antibody production. Figure adapted from images created with BioRender.com.

influence tissue-level immune function (18). Thus, composition of the ECM and the distribution or transport of secreted factors are critical elements of any in vitro model of immunity.

The role of the microenvironment does not stop at biochemical composition but also includes biophysical aspects such as stiffness and fluid flow. T cells and other immune cells are responsive to the stiffness of their local microenvironment, a finding that is still underexplored in engineered models (19). Immune cells are also responsive to flow-induced shear stress. T cells can respond to flow by proliferation (20) or by migration against the direction of flow (21). Macrophages similarly migrate against the direction of flow and are polarized toward an M2 anti-inflammatory phenotype (22). In contrast, neutrophils are shown to migrate in the direction of flow via the αvβ3 integrin (23), demonstrating the varying responses of immune cells to flow.

1.3. Scope of Review: Three Approaches Times Two Contexts

In this review, we take a critical look at the state of ex vivo models of the immune system. We have identified two major contexts for which models are needed: models of primary immune tissues and models of peripheral tissues (healthy or pathological) with an immune component. Within each context, we systematically review the development of three types of approaches: ex vivo cultures, microfluidic devices, and engineered models of tissue (**Figure 3**). The use of ex vivo cultures, including explants and slices, provides an important bridge between in vivo animal models and in vitro cell cultures. These tissues maintain the original structure and organization of the organ while providing increased experimental accessibility. Removal of the organs from the body, however, also removes any native fluid flow. Microfluidic chips offer the possibility to add fluid flow and a controlled microenvironment to ex vivo and in vitro cultures and provide a versatile experimental platform to study immune function (24, 25). However, many chips are often designed for one specific function, meaning that each biological question requires a specific chip design. Tissue engineering offers the most flexible experimental platforms to study a variety of immune cell functions (24, 26). By creating a model tissue from scratch, researchers may investigate specific cell–cell and cell–matrix interactions in a strictly controlled environment. These models offer the

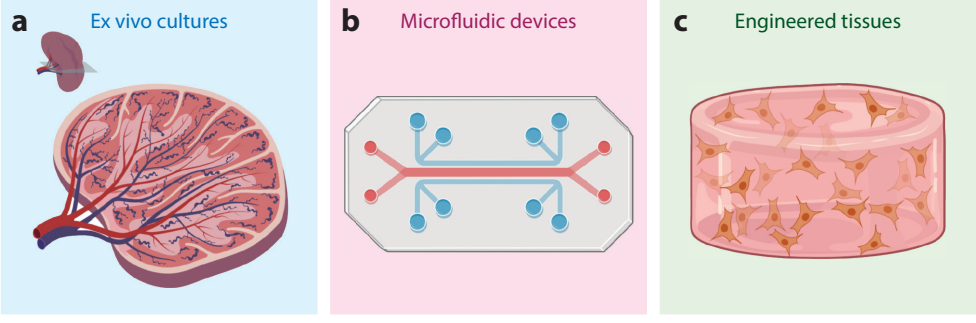

Figure 3

Overview of the three approaches to modeling immunity in vitro: (*a*) ex vivo cultures, (*b*) microfluidic devices, and (*c*) engineered tissues. Ex vivo cultures, including slices and explants, preserve the tissue structure by removing live tissues from in vivo animals and culturing them. Microfluidic devices are versatile, miniaturized engineered platforms that incorporate fluid flow and provide dynamic control over the microenvironment. Engineered models can range from bioreactors to hydrogels but typically incorporate a 3D extracellular matrix component and can include multiple cell types. Microfluidic devices can bridge the gap by incorporating slices or engineered tissues. Figure adapted from images created with BioRender.com.

chance to manipulate many of the fundamental components that are inherent in ex vivo or microfluidic platforms, including the ECM and fluid dynamics. The introduction of novel techniques to model immunity offers the potential for new insights into its dynamic complexity and to inform new immunotherapeutic strategies.

2. CONTEXT I: MODELS OF PRIMARY IMMUNE TISSUES

Modeling primary and secondary immune tissues gives insight into the development, maturation, and activation of immune cells and adaptive immune responses. Here we discuss models of immune tissues, including the bone marrow, thymus, lymph node, and spleen. We also include models of lymphatic vessels here.

2.1. Bone Marrow

The bone marrow stem cell niche is vital for maintaining multipotent hematopoietic stem and progenitor cells (HSPCs), which are the source for all blood cell types, including lymphocytes, DCs and macrophages, NK cells, and granulocytes (27). It is also the residence of long-lived plasma cells, which may live for decades in the bone marrow niche to preserve humoral memory.

2.1.1. Ex vivo cultures. Most experimental work with bone marrow begins with cell suspensions collected from bone marrow aspirates or biopsies; intact bone marrow samples are usually fixed for immunohistochemistry (28). Thus, live slices and explants have not yet been used to study immunological functions of the bone marrow, although ex vivo cultures of bone, developed to study osteogenesis (29), may potentially prove useful to study hematopoiesis as well.

2.1.2. Microfluidic chips. While there are many bone marrow-on-a-chip models, most are focused on developing a biomimetic scaffolding and culturing HSPCs rather than the differentiation into immune cells, one of the main functions of bone marrow. In a study by Sieber et al. (30), a multiorgan chip (MOC) developed previously (31) was used to culture human HSPCs in a ceramic scaffold. The MOC consists of a single looped channel with recirculating flow and a compartment to culture the bone marrow niche model. To confirm proper HSPC function within the device for long-term culture (up to 28 days), bone marrow niche–related genes were expressed and compared with off-chip cells without the ceramic scaffold (30). After a period of 4 weeks on the device, the cells were removed and tested and were seen to maintain their ability to differentiate into different cell types (erythrocytes, granulocytes, and macrophages) in a manner comparable with that of freshly isolated HSPCs (30). This bone marrow niche model could be cultured with additional organ models to generate a functioning MOC.

Torisawa et al. (32) tested responses to radiation countermeasure drugs in a microfluidic device. By integrating engineered bone marrow into a polydimethylsiloxane (PDMS) device, HSPCs could be cultured for at least 2 weeks, and normal bone marrow function was maintained with an increase in leukocytes and red blood cells (RBCs) over time (32). In addition to modeling healthy bone marrow function, the bone marrow-on-a-chip was used as a model to test novel drugs through exposure to gamma radiation. Initially, the gamma radiation significantly decreased the production of leukocytes, but the introduction of two potential therapeutics induced significant increases (32). Along with generating a bone marrow model that integrated engineered tissue, Torisawa et al. (33) successfully tested the effect of novel drugs on cells exposed to radiation.

Chou et al. (34) expanded the bone marrow-on-a-chip model to include a vascularized channel beneath the bone marrow niche 3D culture. Bone marrow–derived stromal cells and $CD34^+$ stem

cells were cocultured in a hydrogel channel, separated from a vascularized channel by a porous membrane. Similar to the model used by Torisawa et al. (32), this bone marrow model was cultured long-term and exposed to radiation and chemotherapeutic drugs. The authors integrated cells from patients with Shwachman-Diamond syndrome, a genetic disorder that results in bone marrow failure, which resulted in neutrophil-maturation abnormalities (34). In a 2019 study by Aleman et al. (35), a bone marrow niche-on-a-chip integrated four major niche constructs (periarterial, perisinusoidal, mesenchymal, and osteoblastic) within a single bone marrow microenvironment. Homing and retention of both healthy HSPCs and malignant lymphoma and leukemic cells were monitored in real time on the niche-on-a-chip platform (35). These approaches may provide a platform to test novel therapeutics on patient-specific cells and eventually reduce, though not eliminate, the need for animal testing.

2.1.3. Engineered tissues. Modeling bone marrow through engineered tissues is primarily achieved via static 3D culture of hematopoietic or mesenchymal stem cells followed by proof of immune cell production. This strategy encapsulates the spongier aspect of bone marrow. Mortera-Blanco et al. (36) created a bone marrow model by expanding cord blood mononuclear cells in type I collagen–coated polyurethane scaffolds, creating a dynamic culture that contained erythroid precursors, HSPCs, maturing myeloid cells, T lymphocytes, and megakaryocytes. More recently, perfusion has been introduced via bioreactor, enabling the study of bone marrow models in dynamic conditions that recapitulate in vivo physiology. Nichols et al. (37) cultured hematopoietic stem cells in a 3D polyacrylamide scaffold with inverted colloidal crystal geometry in a rotating wall vessel and showed the production of B lymphocytes. HSPCs have also been cultured in a hydroxyapatite bone-like scaffold under perfusion via bioreactor and shown to produce NK cells (38).

Ultimately, bone marrow models alone do not provide a complete picture of immunity and have thus far been mostly limited to modeling immune cell production. However, they provide a useful system for the study of diseases such as leukemia and multiple myeloma. In one such study examining multiple myeloma, Braham et al. (39) developed a bone marrow model consisting of mesenchymal stem cells (MSCs), osteogenic MSCs, and endothelial progenitor cells within Matrigel®. Myeloma cells were added to the culture and engineered T cells were tested as a treatment to observe the killing response and how they migrated through the model (39). Therefore, in vitro bone marrow models could provide a disease modeling platform for testing therapeutics.

2.2. Thymus

The thymus is a primary immune organ where naive T cells are screened for antigen recognition. Removal of the thymus during development is lethal after 2–4 months (40), and even in adulthood the removal of the thymus has been shown to correlate with a drop in circulating immune cells (41), thus indicating the crucial role of the thymus, not only in immune development but also throughout the lifetime of the organism. Interestingly, we were unable to identify any microfluidic models of the thymus, so the sections below focus on ex vivo cultures and engineered tissue models.

2.2.1. Ex vivo cultures. Ex vivo thymus culture has provided invaluable insight into important characteristics of T cell development and tolerance. Mouse fetal thymus organ culture in particular set the stage for many early studies of the requirements for healthy T cell development (42–46). Slices of mouse thymus tissue have allowed for the dynamic nature of T cell tolerance to be better understood (47–52). By carefully tracking CD8 single positive thymocytes through the process of positive selection, Ross et al. (47) identified distinct phases of selection using thymic slices.

Phases were distinguished by changes in thymocyte motility, surface marker and T cell receptor expression, and Ca^{2+} signaling, as well as the location of the thymocytes within specific regions of the tissue (47). This information has led to a comprehensive toolbox to study T cell development in mouse models; recently, slices of human thymus tissue have also been introduced to begin studying human T cells (53). As new questions about T cell development come to light, ex vivo slices and culture will continue to provide a platform to help answer them.

2.2.2. Engineered tissues. Attempts to engineer the thymus have been largely limited to organoids with a focus on T cell generation. Poznansky et al. (54) seeded thymus fragments from mice onto cell foam until stromal cells had reached 80% confluency. Then, human bone marrow–derived progenitor cells were added and shown to efficiently differentiate into T cells within 14 days. The authors suggest a potential use of their system for ex vivo generation of T cells for the treatment of immunodeficiency. Seventeen years later, Seet et al. (55) aggregated stromal cells and HSPCs into organoids that were also capable of producing T cells, in this case, antigen-specific T cells. These authors suggested their platform could be used for testing engineered T cells, demonstrating how the goals of modeling immunity have changed over time.

Instead of using organoid technology, Bredenkamp et al. (56) approached treating immunodeficiency via cell transfection and implantation for the in vivo generation of T cells. Fibroblasts were transfected with the transcription factor forkhead box N1 (FOXN1) and reprogrammed into FOXN1-induced thymic epithelial cells (iTECs). The iTECs were able to produce $CD4^+$ and $CD8^+$ T cells from early T lineage progenitors in vitro. When iTECs were combined with thymocytes and transplanted into mice, an organized and functional thymus was formed. The authors note that this iTEC system has the potential to serve as the basis for thymus transplantation therapies to treat immunocompromised patients.

2.3. Lymph Node

In addition to the spleen, the lymph node is the primary location where adaptive immune responses initiate and mature. This organ is highly organized in distinct regions and changes drastically during the course of an immune response (57). Interactions within the lymph node help direct the strength of the immune response generated, including T cell polarization (58, 59), antibody specificity, and adequate memory formation. Models of the lymph node may further our understanding of how immune responses are generated.

2.3.1. Ex vivo cultures. The lymph node is a complex organ, and appreciation of its dynamic cell–cell interactions, lymphocyte motility, and sophisticated mechanisms of antigen recognition first arose from live fluorescence imaging of explanted whole lymph nodes from mice and humans (**Figure 4*a***) (60–62). Culture of these organs is facilitated by their small size (1–2 mm in mouse; 1–2 cm in human), particularly in mice, whose lymph nodes can be kept viable for several hours ex vivo by perfusing with oxygenated solution. When deeper portions of the organ must be visualized or manipulated, live slices 250–400 μm thick may be produced using a vibratome and similarly monitored by live imaging, though this technique is less common for lymph nodes than it is for thymus (63, 64). One of the greatest strengths of tissue slice platforms is the ability to visualize individual cells over time (**Figure 4*b***), while also allowing for monitoring of gross changes in surface marker expression and measuring bulk cytokine secretion from the intact slice or explant to evaluate immune responses (65–70). While ex vivo culture retains the spatial organization of the in vivo system, removing the tissue from the body does cut off blood and lymphatic flow and prevents additional cells from homing to the tissue. Most ex vivo lymph node

cultures are used for short-term studies of a few hours or overnight, in part because motile lymphocytes largely exit from open-face slices after 24–48 h. However, human tonsil slices have been maintained for 4–7 days for informative models of viral infections, including human immunodeficiency virus, measles, and West Nile virus, despite cell egress (67). Recently, human tonsil slices were cleverly used to demonstrate that the lymph node stromal microenvironment inhibited T cell proliferation in response to stimulation, confirming the predictions of in vitro cell cultures (71). Using a multicompartment microfluidic device, Shim et al. (72) modeled immunosuppression by cocultured lymph node slices with tumor slices in recirculating fluid flow that allowed

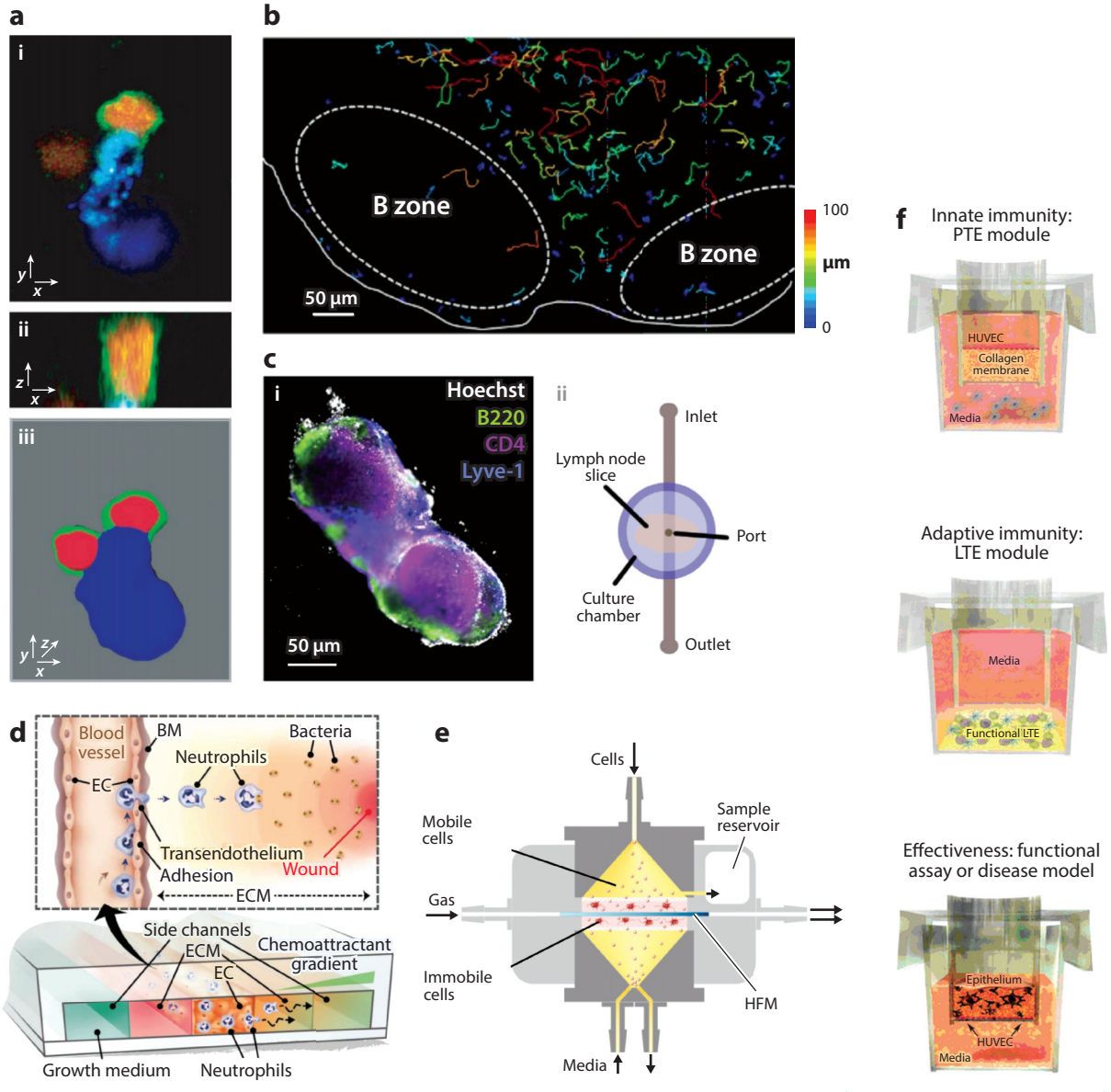

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Figure 4 (*Figure appears on preceding page*)

Experimental models of the lymph node. (*a*) Intact explanted lymph nodes provide a view of the molecular organization of the immune synapse between a dendritic cell (*blue*) and a T cell (*red* cytoplasmic label; *green* on membrane-bound CD43), shown (*i*,*ii*) in confocal cross sections and (*iii*) in 3D view. Panel adapted with permission from Reference 61. (*b*) Ex vivo lymph node slices have been used to track T cell motility outside of the B cell follicles (B zone). Colored lines indicate cell tracks during time-lapse microscopy. Panel adapted with permission from Reference 63. (*c*) Live lymph node slices were combined with microfluidics to deliver local stimulation to the tissue: (*i*) tissue immunostained for B220 (B cells, *green*), CD4 (T cells, *purple*), and Lyve-1 (lymphatics, *blue*), with Hoechst nuclear counterstain (*white*); (*ii*) top-view schematic of the microfluidic device used for local fluid delivery and analysis of diffusion. Panel adapted with permission from Reference 73. (*d*) A schematic of a microfluidic device modeling neutrophil migration in the presence of a chemoattractant gradient. Panel adapted with permission from Reference 78. (*e*) The human artificial lymph node bioreactor. Gas perfuses the system horizontally in an HFM surrounded by a hydrogel matrix that contains antigen-presenting cells, forming the central culture space (*pink*). The peripheral culture space (*yellow*) contains media flowing vertically and a cycling B cell suspension. Panel adapted with permission from Reference 82. (*f*) The MIMIC® system to model immunity in vitro, with three of four main components shown. The innate immunity module, or PTE module, consists of a HUVEC-coated hydrogel and peripheral blood mononuclear cells. The adaptive immunity module, or the LTE module, consists of a sequential application of dendritic cells, follicular dendritic cells, T cells, and B cells. The functional assay modules are customized for each experimental design to assess the responses of the PTE and LTE against a stimulus. Panel adapted with permission from Reference 89. Abbreviations: BM, basement membrane; EC, endothelial cell; ECM, extracellular matrix; HFM, hollow fiber membrane; HUVEC, human umbilical vein endothelial cell; LTE, lymphoid tissue equivalent; MIMIC®, modular immune in vitro construct system; PTE, peripheral tissue equivalent.

interorgan communication via secreted factors. Lymph node slices cultured with the breast cancer tissue secreted more immunosuppressive cytokines than those cultured with healthy tissue, showing the initial effects of cancer on the peripheral immune organs and subsequently the immune system (72). Thus, studies in ex vivo lymph node tissue provide visualization of immune system dynamics and a deeper understanding of early events in the immune response.

2.3.2. Microfluidic chips. Microfluidic chips have been used to model the lymph node both in combination with slice cultures and by supporting cellular-scale studies. Combining lymph node slices with chips provides a way to access the full range of cell types and tissue organization, along with fluid flow control. So far, work has focused on local delivery and diffusion within the tissue slice, not whole organ immune function. In a study from 2017, Ross & Pompano (73) developed a user-friendly microfluidic platform, dubbed microfluidic integrated optical imaging, that was used to study diffusion of cytokines through the lymph node slice to gain a better understanding of cell–cell communication and drug efficacy (**Figure 4*c***). The simple PDMS-based device was used to deliver picograms of fluorescently labeled cytokines [tumor necrosis factor-α (TNF-α), interferon-γ, and interleukin-2 (IL-2)] to specific regions of the tissue slice through a small port beneath the slice. The diffusion of each individual cytokine was quantified via wide-field fluorescence microscopy and was the first method to directly measure cytokine transport in live tissue slices (73). A second generation of the device provided user-defined locations for the delivery and stimulation by incorporating movable elements (74).

Other microfluidic models have focused on the level of lymphocyte function rather than whole organ function. In a study by Moura Rosa et al. (75), a microfluidic device was used to examine perfusing T cells as they interacted with adherent DCs as well as the effects of varying shear stress within the device. This device aimed to model the T cell region within the lymph node, where DCs prime and activate T cells during an immune response. Another main function of T cells, and other lymphocytes such as neutrophils, is migration in the presence of a chemoattractant gradient. The immune synapse was further studied by Faley et al. (76), where T cell–APC interactions were observed in a microfluidic cell trap device. Various bucket-like structures within the device passively trapped perfusing cells, where cell–cell interactions can be observed for up to 24 h. To mimic the immune synapse, primary T cells and LPS-matured DCs were introduced into the device and shown to increase calcium signaling in T cells postinteraction (76). A 2006 study by

Lin & Butcher (77) used a simple Y channel in a microfluidic device to study T cell chemotaxis and migration in the presence of single and competing gradients of two cytokines, CCL19 and CXCL12.

While chemotaxis is a main function of the adaptive immune response, transendothelial migration (TEM) of leukocytes is crucial for both innate and adaptive immunity. As with the Lin and Butcher device, TEM of neutrophils has been modeled on a microfluidic device via culture of the leukocytes in the presence of a chemoattractant gradient (78, 79). In a study by Han et al. (78), neutrophils were housed in a central channel surrounded by hydrogel ECM, with perfusing media and a chemoattractant gradient running parallel (**Figure 4*d***). As neutrophils responded to the gradient and migrated toward the regions with higher concentrations, they could be tracked within the device. In a 2015 study, Wu et al. (79) further integrated endothelial cells into the hydrogel as well as competing chemoattractant gradients to develop a more biomimetic model of neutrophil TEM. In a 2013 study, Mitra et al. (80) integrated DC chemotaxis with T cell activation by coculturing these cell types in adjacent compartments on a PDMS-based microfluidic device. The DC migration was observed in the presence of a chemokine gradient, and T cell activation was quantified by measuring cellular calcium levels. This device was used to study various stages of the immune response, from DC maturation and migration to T cell activation (80). While these devices modeled key components of the lymph node immune response, there is still no integrated microphysiological (organ-on-chip) model of a lymph node that replicates immune function of the organ, though several groups are working toward that goal.

2.3.3. Engineered tissues. Interestingly, engineered tissues for the study of immunity have largely focused on replicating specific functions, as compared with other engineered systems that focus on tissue structure (26). As such, lymph node organoids are designed after organic development from precursor cell types and aim to mimic specific functions, such as antibody production. These organoids provide a platform to control the cell and matrix elements of the microenvironment around cultured immune cells and to better replicate in vivo conditions, although they usually do not incorporate fluid flow. A 2017 study by Purwada & Singh (81) produced ex vivo immune organoids, consisting of B cells from mouse spleen, that replicated the germinal center reaction of secondary lymphoid organs. In vivo, B cells are activated through a variety of signals, including the CD40L ligand presented by follicular T helper cells, the B cell activating factor presented by follicular DCs, and the cytokine IL-4. To replicate these signals without incorporating T cells or true follicular DCs, fibroblasts were transduced with CD40L and B cell activating factor, and the culture was supplemented with IL-4. The ECM was designed to mimic in vivo conditions by using cross-linked, RGD-presenting gelatin. In this immune organoid platform, the naive B cells were successfully differentiated into the germinal center phenotype and were capable of antibody class switching. Further, they were more proliferative and viable compared with 2D systems, providing a successful platform to study B cell development in secondary lymphoid organs (81). This study demonstrates the advantages of organoid technology: allowing precise control of ECM and cell–cell contacts to replicate in vivo immune organs.

Adding another layer of complexity to immune organoids, bioreactors introduce the element of flow. The HuALN (human artificial lymph node) bioreactor, developed by Giese et al. (82), is used extensively as an in vitro model of the lymph node. The HuALN incorporates several cell types (T cells, B cells, DCs), a hydrogel matrix, and micro-organoid structures. It has enabled reproduction of functions such as antigen-specific antibody production and proinflammatory and anti-inflammatory cytokine secretion (**Figure 4*e***) (82). The HuALN bioreactor has also been applied to the study of immunogenicity of protein aggregates. Patients can produce antidrug antibodies when a drug has high immunogenicity. Such production can be impacted by

patient-specific factors, such as allergies and the route of administration, but also by drug formulation and protein aggregates within the drug, as found in a study by Kraus et al. (83). Two monoclonal antibodies—adalimumab (also known as Humira®, an immunosuppressive drug used to treat arthritis, psoriasis, and Crohn's disease) and bevacizumab (also known as Avastin®, a drug used to treat colorectal, lung, glioblastoma, kidney, cervical, and ovarian cancer)—were turned into protein aggregates by exposure to light, mechanical stress, and heat stress. A proinflammatory immune response was triggered by bevacizumab after heat exposure. Though this result has not been proven to have clinical relevance yet, the authors point out that with further optimization, the HuALN can be a useful predictive tool for evaluating immunogenicity in humans (83). Another bioreactor system—designed for bone marrow cultures—was adapted to culture immune cells isolated from tonsils. Immune cells including monocytes, DCs, B cells, and T cells were viable over several weeks without the addition of specific growth factors, produced antigen-specific antibodies, and self-organized into cell-specific aggregates (84). These results demonstrate the importance of fluid flow to immune function.

Despite being a quintessential tissue engineering approach, cells in hydrogels have been limited in their use as models of the lymph node. Interestingly, 3D hydrogels have been mainly applied to modeling fibroblastic reticular cells in the T zone of the lymph node. In vivo, T zone fibroblastic reticular cells (FRCs) deposit and remodel ECM to guide lymphocyte migration and secrete CCL19 and CCL21 to direct immune cell interaction. Tomei et al. (85) encapsulated FRCs in type I collagen and Matrigel on macroporous polyurethane scaffolds and applied interstitial flow (1–23 μL/min). This 3D in vitro system recapitulated the morphology of FRCs in vivo and confirmed the importance of flow for lymph node function, showing that flow enhanced FRC organization, and in its absence, the FRCs did not produce CCL21 (85). Since that study, more work using 3D hydrogels to model the T zone and stromal cells of the lymph node has been published (86, 87), validating the benefits of a 3D matrix format that is less dense than organoid cultures. In a patient-specific model developed by Votanopoulos et al. (88), matched lymph node and melanoma cells were aggregated together into immune-enhanced patient tumor organoids (iPTOs) for testing cancer drug efficacy. The iPTOs were able to replicate 85% (six out of seven) of patient immune responses. The iPTOs were then used to activate naive T cells before transfer to melanoma-only tumor organoids, where they were able to promote tumor cell death.

Apart from bioreactors and 3D hydrogels, the modular immune in vitro construct (MIMIC®) system provides a sophisticated way to recapitulate patient-specific immune response (89). The MIMIC system has four components: a leukocyte module, a peripheral tissue equivalent (PTE) module to simulate innate immune responses, a lymphoid tissue equivalent (LTE) module to simulate adaptive immune responses, and a functional assay module (**Figure 4*f***). In the first module, leukocytes are collected from donors and cryopreserved. In the PTE module, human umbilical vein endothelial cells are cultured to confluence above a layer of collagen, after which donor peripheral blood mononuclear cells are applied and differentiated into DCs, APCs, and macrophage-like cells. In the LTE module, DCs, follicular DCs, T cells, and B cells are applied sequentially. Finally, the functional assay module is customized for each study to confirm how effective the immune responses from the PTE and LTE modules are against the antigen (89). MIMIC incorporates multiple elements of immunity, is highly patient-specific, and serves as a useful in vitro clinical trial system. So far, engineered lymph node models have focused on recapitulating immune function and have proven successful. However, future models with a focus on structure would enable the investigation of lingering biological questions about lymph node structure and function.

2.4. Spleen

The spleen is the single largest secondary immune organ in the body and is often the preferred source of primary immune cells from animal models for in vitro culture. The organ itself acts as a filter for blood pathogens and maintains roles in hematopoiesis and RBC clearance (6). With a unique cellular organization that is somewhat distinct from that of the lymph node, the spleen also functions as the primary site for immune activation against blood-borne pathogens. Therefore, models of the spleen and its immune functions may inform novel therapies for blood-borne infections.

2.4.1. Ex vivo cultures. Protocols to slice mouse spleen were described during the 1970s (90), and recently renewed interest in this organ has resulted in updated technologies to study spleen biology (91). Human spleen sections have also been described, and interestingly, significant differences in cytokine secretion were observed between bulk splenocyte culture and slices of tissue, similar to those described for the lymph node above (65, 66). The difference between slices and suspension cultures may be attributable to the preservation of the spatial organization of the tissue or to preservation of rare or matrix-adhesive cell types. Organotypic cultures of spleen sections supplemented with different amino acids have been shown to have distinct proliferation and differentiation profiles (92), highlighting the need for further studies of the spleen, especially within intact tissue samples, to fully place the organ in the appropriate immune context.

2.4.2. Microfluidic chips. Efforts to model the spleen on a microfluidic device have focused primarily on the main function of the spleen, filtering RBCs, rather than on the immune functions of the splenocytes. To model spleen function, microconstraints are integrated into microfluidic devices in the form of small posts that healthy RBCs can fit through by deforming, while stiff, unhealthy RBCs are trapped (93, 94). In a 2014 study by Rigat-Brugarolas et al. (94), the spleen-on-a-chip model was used to filter out malaria-infected RBCs from healthy RBCs; future integration of immune functions into such a model may enhance the ability to study the outcomes and mechanisms of blood-borne infections. Consistent with the lack of microfluidic models of immune function in the spleen, engineered tissue models also remain unexplored in this area.

2.5. Lymphatic Vessels

An emphasis on lymphatic-immune interactions is an essential step toward recapitulating immunity in vitro. Lymphatic vessels run through tissue alongside blood vessels and act as paths for lymphatic fluid and immune cells to travel between organs. These vessels were traditionally viewed as maintaining a passive role in the immune response or were even excluded as a formal part of the immune system. Therefore, models of the lymphatics so far have focused primarily on fluid flow and biomechanics, as reviewed previously by Nipper & Dixon (95). However, new studies are highlighting the important roles that lymphatic endothelial cells play in presenting antigen to the immune system and regulating cell entry to the lymphatic network (96, 97). Due to the lack of basement membrane, initial lymphatic vessels are highly responsive to interstitial fluid flow—a benefit that allows for easy immune cell trafficking (98). Lymphatics have now been implicated in antitumor immune responses, autoimmune diseases, and the regulation of inflammation (98). Indeed, lymphatics have been added to cocultures of breast cancer cells and mammary fibroblasts to recreate initial lymphatic–tumor cell interactions during metastatic spread, showing that lymphatic endothelial cells (LECs) reduce therapeutic efficacy (99). No

ex vivo model of the full immune system would be complete without considering the role of lymphatic vessels, but so far this aspect of immune modeling is highly incomplete.

2.5.1. Ex vivo cultures. Experimental models to investigate lymphatic vessels ex vivo were recently reviewed (100). While many excellent studies have focused on the physical functionality (e.g., contractility, fluid flow) of the vessels under different pressurized conditions (101), lymphatic vessels have not been examined in the context of the whole tissue ex vivo, with most studies focusing on single explanted lymphatic vessels. The lymphatic system is intimately involved in immune responses, and LECs, the fundamental unit of vessels, are immunologically active cells (96). Thus, further study into how immune responses interact with, and depend upon, the lymphatic system is required to help provide a full picture of the systemic nature of immunity.

2.5.2. Microfluidic chips. With its control over fluid flow and cellular-scale microenvironments, microfluidic technology is naturally suited to model vascular networks. Models of the lymphatic system often use hydrogels as scaffolding and can be used to study lymphatic drainage, lymphangiogenesis (LA), and barrier function. Primarily, due to these approaches, initial lymphatics have been modeled, with little technology existing to model the collecting lymphatics, which feature pumping mediated by smooth muscle cells and an intricate valve system. One established and robust strategy to micropattern lymphatic vessels is to insert a rod or a wire into liquid hydrogel and remove it after the hydrogel has solidified, leaving behind a physiologically relevant circular hydrogel channel that is ideal for endothelial cell culture. Thompson et al. (102) formed lymphatic vessels in this manner in collagen scaffolds with and without lymphatic endothelial cells, leaving one end of the channel closed to model drainage under lymphatic-like pressure. A 2019 study by Gong et al. (103) further studied lymphatic drainage and barrier function in a microfluidic device fabricated in a similar style, called μLYMPH. A lumen rod was used to intersect the hydrogel region, and once the tubular channel had been formed, the LECs were seeded, forming a lymphatic vessel model that mimicked physiological structure and function. This study began to address the immunological function of the lymphatics by quantifying the secretion of various growth factors and inflammatory mediators (e.g., follistatin, granulocyte colony-stimulating factor, hepatocyte growth factor) as well as the vessel response in the presence of exogenous stimuli [vascular endothelial growth factor (VEGF)-C, VEGF-D, and IL-6] (103).

Kim et al. (104) cultured LECs in two fluidic chambers that contained perfusing media, and the chambers were separated by a central fibrin gel matrix. On either side of the LEC fluidic chambers, stromal fibroblasts were cultured within a separate fibrin gel matrix. This microfluidic device closely mimicked the interstitial flow, biochemical cues, and cell–cell interactions found in the lymphatic vessel microenvironment in vivo and was used to model sprouting LA. In the presence of pro- and antilymphangiogenic stimuli, increased and decreased LEC sprouting, respectively, were observed in the fibroblast gel channels (104). LEC-lined channels or monolayers also can serve as interfaces in which to examine the role of the lymphatic system in more complex tissues and microenvironments, including its interaction with blood vessels (105) and the tumor microenvironment (106, 107).

2.5.3. Engineered tissues. Engineering of lymphatics in vitro is structure focused and has been performed in both decellularized scaffolds and hydrogels. While fluid flow in the lymphatic system is highly complex and governed by biological and mechanical phenomena (95), engineered lymphatic systems are more often simple models that focus on whether cells or vessels assemble into the correct morphology. For example, LECs have been cultured in polyglycolic acid scaffolds (108), in VEGF-fibrin-collagen matrices (109), and in collagen alone (110) to quantify the

extent of networking and morphology of LECs. These systems did not form lymphatic vessels with open lumens or luminal flow, limiting their physiological relevance. However, when exposed to interstitial flow through the culture matrix, the LECs did show drastic morphological differences compared with blood endothelial cells (BECs), forming large vacuoles and long extensions, thus providing insight into the fundamental behavior of LECs (110). In a more macroscopic study, human adipose-derived stem cells were differentiated into LECs and cultured on decellularized arterial scaffolds and maintained vessel-like morphology for 10 days (111). The lymphatic models discussed above demonstrate the feasibility of culturing LECs into lymphatic vessels in vitro. Through the addition of fluid flow and immune cells, such as T or B cells, these models may be used to address and understand lymphatic-immune interactions.

3. CONTEXT II: MODELS OF PERIPHERAL TISSUES WITH IMMUNE ELEMENTS

All tissues interact with immune cells, not just the primary and secondary organs of the immune system discussed above. Each tissue contains populations of tissue-resident APCs for immunosurveillance, for example, the so-called conventional DCs and macrophages in peripheral organs and the microglia in the brain (112). In addition, each organ experiences a constant flux of circulating naive T and B cells that patrol tissues in search of their cognate antigen. Effector T and B cells are recruited to the sites of inflammation or infection, along with innate cells such as NK cells, neutrophils, and macrophages via the bloodstream.

So far, efforts to incorporate components of the immune system into models of tissues have focused most frequently on the incorporation of macrophages or cytokines into preexisting tissue models, of both normal and pathological conditions. More recently, efforts have been made to include elements of adaptive immunity as well, but this area is in its infancy. The addition of an immune component to models of healthy tissue has the potential to reveal not only the importance of the immune system in maintaining the tissue of interest but also the dynamic cross talk between immunity and other body systems. Here we discuss in more detail models of the gut, brain, lungs, and vasculature with an integrated immune element. The role of macrophages has also been examined in models of osteogenesis and muscle repair (113–117) but is outside the scope of this review.

3.1. Gut

The gut not only digests the food that you eat but also plays an important role in maintaining immunity. The large mucosal surface area, passage of foodborne antigens, and the presence of commensal bacteria populations mean the intestinal immune system interacts with more potential antigens than any other part of the body. The immune system of the gut is large enough to merit its own name, the gut-associated lymphoid tissue, and includes specialized lymph-node-like structures known as Peyer's patches (118). The gut-associated lymphoid tissue provides balance and triggers protective immunity in the presence of commensal and pathogenic microbiota and is a critical component to modeling immune homeostasis in the gut.

3.1.1. Ex vivo cultures. There is a close relationship between the gut and the immune system; however, the use of explanted gut tissue to investigate this relationship is rare, despite its previous popularity as a platform to study toxicology and drug development (119, 120). In one recent study, mouse gut explants were used to examine Peyer's patches and the gut-brain-immune axis (121). The organotypic tissue cultures maintained the structural integrity of the intestine, Peyer's

patches, and neurons ex vivo, potentially allowing for future studies into complex gastrointestinal function. Samples of human gut tissue can be collected during colonoscopies and can thus provide a valuable platform for translational study. For example, Pender et al. and Schwerdtfeger et al. (122, 123) used human gut cultures to investigate the effects of antibiotics and immunoprotection on exposure to *Salmonella enterica* bacteria. Thus, ex vivo gut culture has the potential to facilitate deep investigations of the complex interactions between the gut and the immune system.

3.1.2. Microfluidic chips. While many gut-on-a-chip microfluidic models have been developed, they originally focused on replicating the intricate structural and barrier functions of the gut. Researchers have built on that foundation in a more recent push to include immunity in these models (124). Maurer et al. (125) developed a microphysiological model of the intestine that integrated resident innate immune cells, including mucosal macrophages and DCs. Endothelial and epithelial cell layers were separated by a porous membrane within the device and formed villus- and crypt-like structures normally found within the human intestine (125). Mucosal macrophages and DCs were integrated into the endothelial and epithelial cell layers, respectively. This model was used to quantify changes in barrier functionality and immunotolerance in response to LPS, for example, to model endotoxemia (125), thus opening the door to future studies with more complex immune-related challenges. A 2013 study by Ramadan et al. (126) integrated immune cells into a miniaturized artificial gastrointestinal tract, titled NutriChip, to study the immunomodulatory function of dairy in the diet. The NutriChip device cocultured an epithelial monolayer with macrophages, which were separated by a permeable membrane, with an on-chip sandwich immunoassay located downstream to detect proinflammatory cytokine secretion. In the future, the authors plan to use the device to test the inflammatory response in the presence of dairy to evaluate the influence of food on human health (126).

While there are many gut-on-a-chip models, so far there are only a few that integrate immunity with a specifically disease-focused gut model, for example, to study the inflammatory events in inflammatory bowel disease (IBD). Kim et al. (127) studied the different contributing factors of inflammation and villus damage using a gut-on-a-chip model that incorporated elements from the gut microbiome, immune cells, and epithelial deformation to model a diseased state. Human epithelial cells were cultured in a microfluidic device with luminal-like fluid flow that encourages villi formation. When immune cells and LPS endotoxin were introduced into the system, the epithelial barrier produced proinflammatory cytokines that induced villus damage and compromised the barrier integrity; this result is similar to those produced by in vivo studies (127).

3.1.3. Engineered tissues. Similar to chip-based models, many tissue-engineered bowel models focus on recapitulating the inflammation associated with IBD. Roh et al. (128) fabricated a 3D model of the large intestine using spongy silk materials. The inner layer of the scaffold was seeded with nontransformed human colon organoid cells, and the outer layer of the scaffold was seeded with monocyte-derived macrophages. The engineered bowel formed microvilli and mucus layers. The presence of macrophages enhanced the engineered bowel's response to inflammatory stimuli, including *Escherichia coli*, LPS, and interferon-γ, specifically upregulating the secretion of inflammatory cytokines associated with IBD: CXCL10, IL-1β, IL-6, monocyte chemoattractant protein-2, and macrophage inflammatory protein-1β (128). Another area of interest in bowel tissue engineering is modeling the inflamed intestinal mucosa for drug screening and safety testing. The Lehr group (129–131) utilized a 3D coculture system of macrophages, DCs, and Caco-2 epithelial cells to test inflammatory responses to bacteria, drugs, and nanomaterials. In vitro 3D models of the inflamed bowel have been shown to be an effective and easily implemented tool for the study of inflammation and drug screening in the bowel.

BLOOD-BRAIN BARRIER

Normal brain function is maintained by preserving the ionic and cellular composition of the neural environment and by a unique anatomical and physical barrier called the blood-brain barrier (BBB) (132–134). This barrier separates the central nervous system from peripheral blood circulation by tightly regulating the passive and active transport of cells, molecules, and ions (133, 134). Along with the physical barrier, there is an immunological barrier that regulates the recruitment and transport of leukocytes and other innate immune elements (134). The BBB is composed of a highly specialized monolayer of endothelial cells (ECs) and is a part of a larger structure called the neurovascular unit (134). The ECs are linked by tight junction proteins and form a capillary surrounded by the basal lamina, a collagen-based basement membrane (132–134). Surrounding the basement membrane are pericytes, astrocytes, microglia, and neurons, all forming the highly regulated barrier that is essential for maintaining cerebrospinal fluid homeostasis (132, 134). In the presence of locally secreted inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α, the tight junctions between the ECs are disrupted, leading to increased leukocyte adhesion and migration (135). There is increasing evidence that inflammation in the brain is involved in the pathogenesis of neurological diseases such as Alzheimer's disease and multiple sclerosis (135).

3.2. Central Nervous System

The brain is an immunoprivileged organ, with naive peripheral immune cells being largely excluded from the brain tissue. Adaptive immune cells found within the brain are associated with disease. The brain parenchyma contains a tissue-resident macrophage counterpart, microglia, which populate the brain tissue during development and provide immune function as innate immune cells for immune protection throughout the life of the organism. Immune-brain interactions are increasingly examined experimentally, due to recent findings of more complex neuroimmune connections in vivo. Immune cells moving into the brain must cross through one of the barriers, be it through the blood vasculature and the blood-brain barrier (BBB), the choroid plexus, or the newly discovered meningeal and subdural lymphatics (see the sidebar titled Blood-Brain Barrier).

3.2.1. Ex vivo cultures. Brain slices are used extensively in the neuroscience field to study brain function and biology. With the increased interest in neuroimmunity, microglia in particular are a recent intensive focus of investigation in both rat and mouse brain slices (136–141). Neural inflammation is a concern for many brain diseases, and the immune response to inflammatory stimuli has been investigated in brain slices (142, 143). T cell interactions with neurons are also a topic of interest in brain slice studies (144, 145). For example, Nitsch et al. (144) monitored interactions between activated proteolipid protein-specific T cells and neurons in a model of multiple sclerosis and reported that these interactions resulted in neuron death. The ability to clear viruses from the brain has also been described for both cytomegalovirus (146) and the Zika virus (147). Lyme disease clearance has also been studied in brain slices (148). Through the study of how tissues both clear pathogens and respond to self-antigens, more targeted therapies can be designed, off-target damage can be limited, and patient-specific effects may be determined. These studies highlight the importance of investigating all components of the immune system when preparing models of brain immunity to provide insight into normal homeostatic and disease-specific states.

3.2.2. Microfluidic chips. Immune cell trafficking into the central nervous system via the BBB has been a primary focus for microfluidic models of immunity in the brain (135, 149). In a 2011 study, Cucullo et al. (135) altered their existing dynamic in vitro BBB model to introduce immune cell trafficking. The device utilized artificial capillaries to culture a layer of endothelial cells with

abluminal astrocytes to form a biomimetic BBB model. Microholes were formed in the artificial capillary, allowing monocyte extravasation across the vascular endothelial layer, shown by a significant increase of cytokine levels as well as a loss of barrier integrity (135). Another BBB model from Mossu et al. (149) cultured brain-like endothelial cells on a nanoporous silicon nitride membrane to form an in vitro cerebrovascular barrier (CVB) model, termed μSiM-CVB. While the device incorporated only brain-like endothelial cells and not additional cell types found in the neurovascular unit, pericyte-conditioned media were used to make a more physiologically relevant model. When cocultured with T cells, μSiM-CVB was able to capture the multistep migration process using live imaging (149).

3.2.3. Engineered tissues. While the microfluidic models focused on barrier function at the BBB, engineered tissue models using hydrogels for 3D culture are well suited to model the brain parenchyma. Modeling immunity in tissue-engineered brain models has focused largely on microglial function and inflammatory response. Many studies have observed microglia in 3D hydrogels and their response to inflammation induced by LPS (150–153). These studies confirmed that microglial function in 3D more closely resembles in vivo responses compared with 2D cultures. Though these models included only one cell type, they provide a simple platform to study microglial activation in response to inflammatory stimuli and validate the necessity of 3D cultures for in vitro studies of brain immunity. A more multicellular model, presented by Abreu et al. (154), utilized induced pluripotent stem cell–derived micro brain spheres (μBS). Neural progenitor cells are aggregated into spheroids with and without immortalized microglia and tested for inflammatory response and cell viability. The μBS were shown to contain astrocyte, oligodendrocyte, and neuronal populations. The μBS with and without microglia were treated with LPS for 24 h (154) so that inflammatory response could be studied. The inflammatory response to LPS differed greatly on the basis of which cell types were included in the model. In the absence of microglia, the μBS had a very minimal response. Microglia-only spheroids upregulated CCL2 and TNF-α at 12 h. In combination microglia-μBS cultures, IL-1β, IL-6, and IL-10 were upregulated at 12 h and downregulated at 24 h, and the microglia-only response was not observed (154). The authors point to cross talk between microglia and the other cell populations as the cause for these altered responses. The LPS treatment also caused an increase in dividing microglia and a decrease in viability in both microglia and the μBS cells (154). A recent study performed a triculture of neurons, microglia, and astrocytes in a 3D microfluidic platform (155). This model successfully recapitulated important aspects of Alzheimer's disease, including beta-amyloid aggregation, phosphorylated tau accumulation, and neuroinflammatory activity, and can be used in the future to study the pathogenesis of Alzheimer's disease and to develop therapeutics. Bioprinted glioblastoma models that incorporated microglia or macrophages were shown to recapitulate patient survival profiles, including tumor invasion and drug resistance (156). When compared with 2D monolayer cultures, glioblastoma cells in bioprinted glioblastoma models with macrophages were more resistant to drugs (157). More complex tissue-engineered models of the brain incorporating microglia allow more physiologically relevant inflammatory responses while enabling the examination of cell–cell cross talk to answer more specific mechanistic questions.

3.3. Lungs

Similar to the gut, the lungs are a mucosal layer that is exposed to foreign particles on a daily basis. Many airborne pathogens enter the body through the lungs, including viruses and solid

particulates. These organs pose a particular challenge to regulating immunity, as collateral damage to the surrounding tissue as a result of an excessive immune response can severely affect their critical role as a gas exchange surface. Immunity within the lung is a closely regulated balance between the innate and adaptive responses, and understanding this balance in healthy tissue is important for designing effective vaccines against airborne pathogens (158).

3.3.1. Ex vivo cultures. Lung immunity is an area of intense study; as a result, precision-cut lung slices have been well characterized for immune-related functions (159–161). In one application, lymphatic vessels were visualized within healthy tissue to help further our understanding of immune cell trafficking. Kretschmer et al. (160) investigated the role of CD90/Thy-1 as a marker for both healthy and inflamed lymphatic vessels in lung slices (**Figure 5*a***). By using this marker, the researchers were able to highlight a possible route for immune cells to leave the lung, by tracking T cell infiltration in response to dust mite antigen (160). Human lung sections have even been characterized to act as a screening platform for vaccines and pharmaceuticals (162, 163). Additional studies have been completed in the context of infection, as ex vivo tissue culture is often the only means to study the human immune response in a fully intact tissue setting. This is particularly timely with the current investigation of immune responses related to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, which causes coronavirus disease 2019 (COVID-19). Respiratory infections have been extensively modeled in lung slices, including responses to the flu (164) and to flu vaccines (165, 166).

3.3.2. Microfluidic chips. Immunity is crucial for maintaining health and proper function within the lungs, as this tissue is exposed to foreign particles daily and immune cells are naturally found throughout this tissue. While there are many microfluidic devices that model healthy and diseased lung tissue (167, 168), they don't include an immune element, which would be crucial for making a lung model that accurately mimics in vivo function.

3.3.3. Engineered tissues. Engineered models of the lung aim to recapitulate the respiratory tract to study the effects of particles, such as potentially toxic nanoparticles released by industrial processes (169, 170) or gold nanoparticles that have potential for drug delivery (171). Harrington et al. (172) developed an immunocompetent model of the respiratory tract that incorporated epithelial cells, fibroblasts, and DCs onto electrospun polyethylene terephthalate scaffolds. Stimulation with LPS and papain, an allergen that disrupts tight junctions in the respiratory tract, caused DC migration and mimicked the in vivo response (172). More recently, lung organoids have been applied to the global COVID-19 pandemic caused by the novel SARS-CoV-2 virus (173, 174). These models provide useful tools to investigate drug delivery and the effects of allergens or harmful particles in the lung.

3.4. Vasculature

The body is connected by an intricate vascular system composed of blood vessels of varying sizes, from the aorta down to the capillaries. The immune system and vasculature interface in many contexts. During an immune response, immune cells travel to an inflamed site through the vasculature. Many in vitro models focus on elucidating and recapitulating the migration, adhesion, and extravasation of immune cells in blood vessels. Immune cells have also been implicated in regulating angiogenesis, the formation of new blood vessels (175).

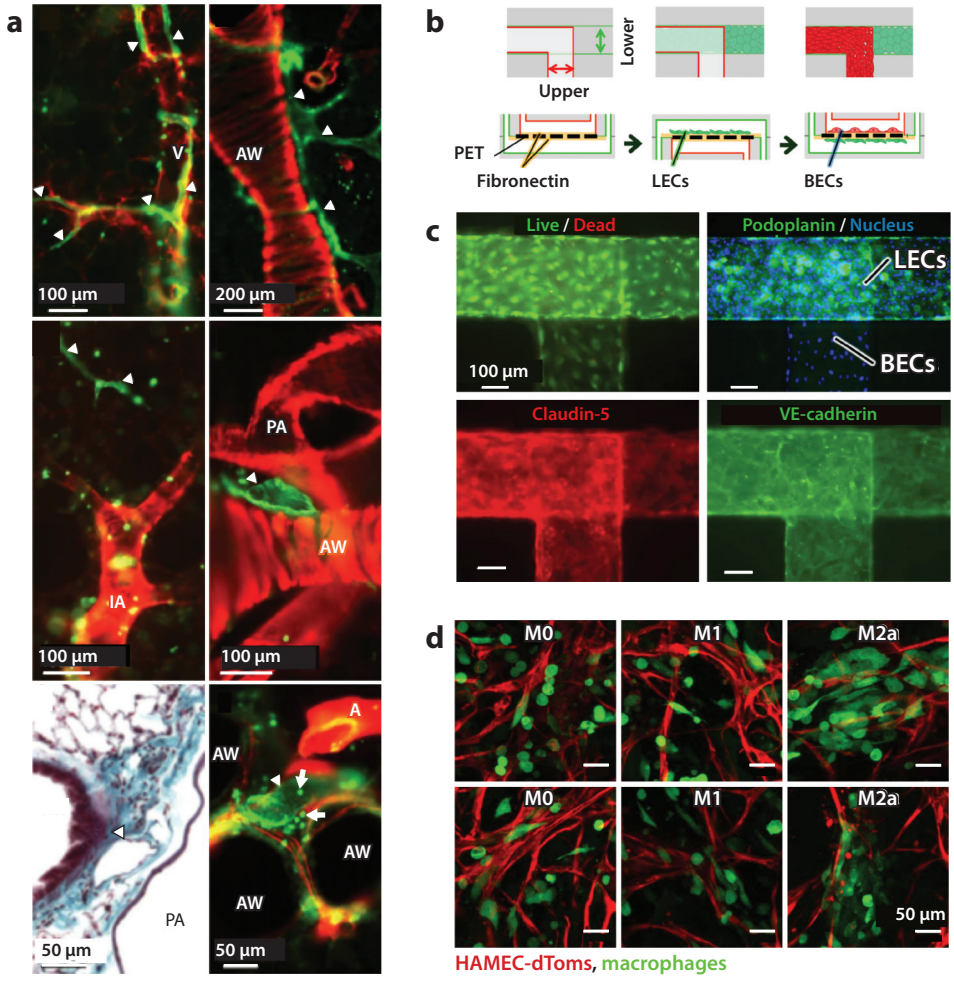

Figure 5

Experimental models of immune-related vasculature. (*a*) Visualization of lymphatic vessels in slices of lung tissue. Double labeling of precision-cut lung slices with anti-CD90/Thy-1 (*green*) and anti-α-smooth muscle actin (*red*). Lymphatic vessels (*white arrowheads*) were found near veins (V), muscular airways (AW), arteries (A), and pulmonary arteries (PA) and were not associated with intra-acinar arteries (IA). The bottom left panel was paraffin-embedded and stained with Masson-Goldner stain. Panel adapted with permission from Reference 160. (*b*) A microcirculation-on-a-chip model was developed with lymphatic endothelial cells (LECs) in the lower channel and blood endothelial cells (BECs) in the upper channel, separated by a polyethylene terephthalate (PET) membrane. The membrane was initially coated with fibronectin before coculturing the LECs and BECs. Panel adapted with permission from Reference 176. (*c*) Visualization of LECs and BECs within the microfluidic device. (*Upper left*) High cell viability was shown using calcein acetoxymethyl (*green*) and ethidium homodimer (*red*). (*Upper right*) Both LEC and BEC nuclei were labeled with Hoescht 33342 (*blue*), and LECs were immunostained for podoplanin (*green*). (*Lower left*) BECs were labeled with claudin-5 (*red*). (*Lower right*) Both channels were stained for an endothelial-specific adhesion molecule, vascular endothelial (VE)-cadherin (*green*), to check for barrier stability. Panel adapted with permission from Reference 176. (*d*) The effects of macrophages of different polarizations on angiogenesis in a 3D coculture model. Engineered blood vessels (*red*) were formed on Gelfoam® by culturing human microvascular endothelial cells expressing tdTomato (HAMEC-dToms) and mesenchymal stem cells. After 3 days, suspensions of M0, M1, or M2a macrophages (*green*) were added to the vessels, and the vessels were imaged 1 day later. Panel adapted with permission from Reference 182.

3.4.1. Ex vivo cultures. Regarding ex vivo systems, studies of immune-related vascular angiogenesis (VA) and LA have been confined to immunostaining slices of fixed tissue.

3.4.2. Microfluidic chips. Many vascular microfluidic models integrate immunity by studying blood vessel function and interaction alongside lymphatic vessels. The interactions of both blood and lymphatic vessels may be keys to gaining a further understanding of inflammation and cancer metastases (105). To study the cooperative effects of VA and LA, Osaki et al. (105) modeled blood and lymphatic vessels side by side on a microfluidic device. The in vitro vessels were fabricated using sacrificial molding to form two circular channels in PDMS. To induce VA and LA in the microfluidic device, phorbol 12-myristate 13-acetate and VEGF-C or VEGF-A were introduced. Angiogenic sprouting for both the blood and lymphatic vessels was observed. In addition, antiangiogenic factors such as VEGF-R3, a VEGF antagonist, were introduced, resulting in reduced angiogenic sprouting (105). A 2015 study by Sato et al. (176) developed a microcirculation-on-a-chip that cocultured BECs and LECs in upper and lower channels, separated by a porous membrane (**Figure 5*b***). Both claudin-5 and vascular endothelial–cadherin were used to detect cell–cell junctions of both the lymphatic and blood vessel channels, confirming barrier integrity (**Figure 5*c***). Histamine is a compound generally released by cells as a part of the inflammatory response and was introduced to the microcirculation model. This reduced tightness of the cell–cell junctions of both the BECs and the LECs, leading to an increase in vascular permeability (176).

Vasculature-on-a-chip has also been used to study migration and adhesion of lymphocytes and other immune cells to endothelial cells. In a 2011 study, Srigunapalan et al. (177) developed a microfluidic device that modeled the vascular microenvironment, incorporating hemodynamic shear stress via perfusion, circulating cytokines, extracellular matrix proteins, and monocyte interactions. A monolayer of aortic endothelial cells was cultured across a membrane within the device, and monocytes were introduced into perfusing media to quantify cell adhesion and transmigration (177). Monocyte adhesion and transmigration can be increased with the introduction of specific inflammatory cytokines (TNF-α) and chemoattractant proteins (monocyte chemoattractant protein-1). To gain a better understanding of leukocyte adhesion to endothelial cells, Kim et al. (178) cultured an endothelial monolayer in a microfluidic channel to model in vitro vasculature to study cell–cell binding with activated and inactivated T cells. Immunosuppressive drugs were also perfused through the device, decreasing the frequency of T cell–endothelial cell interactions (178).

Utilizing a similar device design, Zhang et al. (179) cultured endothelial monolayers within a multichannel microfluidic device. By coupling this device with a multiplexed perfusion platform, they could run up to four devices at once. The branched channels within each device better mimicked the geometric configuration of natural blood vessels. Immune cell adhesion was tested with this platform to validate proper function by introducing monocytes with and without TNF-α (179). Wu et al. (180) coupled another function of VA, endothelial migration, with neutrophil migration in a hydrogel scaffold on a microfluidic chip. A controllable chemical gradient that was run in parallel to the cultured cells induced cell migration through the gel support, showing that this model can be used to gain a better understanding of dynamic cellular interactions (180).

3.4.3. Engineered tissues. It is well established that macrophages are beneficial to angiogenesis. A 2019 review by Moore & West (181) summarized how macrophages have been utilized in tissue engineering of vasculature. Graney et al. (182) recently used an engineered vascular system to test the importance of the macrophage phenotype (see the sidebar titled Macrophage Polarization) in regard to endothelial cell behavior. When added to 3D tissue-engineered blood

MACROPHAGE POLARIZATION

Macrophage subtypes offer differential effects in tissues, and though recent evidence presents these phenotypes as existing on a spectrum, the defined polarizations are useful for categorizing behaviors. The classically activated M1 phenotype is considered proinflammatory, while the alternatively activated M2 phenotype is considered anti-inflammatory (182). However, M2 macrophages themselves have recently been identified to have varying subtypes. M2a macrophages are implicated in stabilizing blood vessels (183), M2c macrophages secrete matrix metalloproteinases (184), and M2f macrophages secrete anti-inflammatory signals in response to phagocytosis of apoptotic cells (185).

vessels, M1 macrophages were elongated and found in proximity with the leading front of sprouts (**Figure 5*d***). Further, M1 macrophages supported vascularization at day 1 but caused vessel regression by day 3. M2 macrophages had a paracrine effect of upregulated pericyte differentiation genes and were found wrapped around blood vessels in the 3D tissue-engineered model (**Figure 5*d***). Overall, this study showed that macrophage impact on endothelial cell behavior relies greatly on phenotype and raises questions of temporal regulation of angiogenesis via macrophage polarization. Angiogenesis can be triggered by hypoxia, and hypoxic conditions lead to inflammation and immune cell invasion. Therefore, the immune system plays an important role in blood vessel formation. Modeling the interaction between blood vessels and immune cells in vitro provides a platform that could be used to study hypoxic tissues, tissue expansion, or tissue regeneration.

4. CONCLUSIONS AND FUTURE DIRECTIONS

Overall, the development of in vitro and ex vivo models of immunity is expanding, especially in recent years. We have described here several types of systems in which to study and model immune interactions, including ex vivo cultures, chips, and engineered tissues. Many of these systems overlap, with the inclusion of engineered tissues or slices with microfluidics, resulting in multimodal systems that recapitulate more complex immune interactions. Interestingly, we found that the majority of systems that incorporate immunity are focused on a narrow set of disease states (e.g., tumors and infection) or incorporation of only a single aspect of immunity (e.g., macrophages or T cells). While such models are important, the in vitro systems are difficult to validate without a definitive knowledge of their fundamental immune-tissue or immune-organ interactions and functions. In addition, the use of a small number of macrophage cell lines to replicate the vast diversity of APCs is a practical simplification that is useful in the short term but will hinder the biological relevance of the models for more complex scenarios. Thus, we propose that development of primary immune organs and healthy tissues that adequately recapitulate native immune functions, including use of primary or stem cell–derived cell types, is vital to the future creation and use of immune-disease systems. Currently, pluripotent stem cell protocols are insufficient to derive functional immune cells such as lymphocytes, APCs, and stromal cells but after optimization could prove crucial to building patient-specific models (186). In addition, most models so far focus on immunity within a single organ; additional models that replicate the systemic and multiorgan nature of immune responses will be valuable advances.

In vitro and ex vivo models of immunity may be best grounded in the use of patient-specific tissues, both for comparison to validate the model's functions and as a source of a diversity of cells. In patients, we know there are myriad baseline immune states, immune responses, and immune profiles that we are only just beginning to understand. The use of in vitro systems to model

patient-specific organs and tissues will expand our knowledge of how and why immunity varies from patient to patient. With the recent pandemic crisis, where we see that patient-to-patient responses are widely varied with resultant tragic health consequences (187), the development of these types of high-throughput, immune-incorporating, rapidly usable in vitro systems is now more important than ever.

DISCLOSURE STATEMENT

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Errata

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