Title: Vasculature-on-a-chip Technologies as Platforms for Advanced Studies of Bacterial Infections

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Abstract

Bacterial infections frequently occur within or near the vascular network as the vascular network connects organ systems and is essential in delivering and removing blood, essential nutrients, and waste products to and from organs. In turn, the vasculature plays a key role in the host immune response to bacterial infections. Technological advancements in microfluidic device design and development have yielded increasingly sophisticated and physiologically relevant models of the vasculature including vasculature-on-a-chip and organ-on-a-chip models. This review aims to highlight advancements in microfluidic device development that have enabled studies of the vascular response to bacteria and bacterial-derived molecules at or near the vascular interface. In the first section of this review, we discuss the use of parallel plate flow chambers and flow cells in studies of bacterial adhesion to the vasculature. We then highlight microfluidic models of the vasculature that have been utilized to study bacteria and bacterial-derived molecules at or near the vascular interface. Next, we review organ-on-a-chip models inclusive of the vasculature and pathogenic bacteria or bacterial-derived molecules that stimulate an inflammatory response within the model system. Finally, we provide recommendations for future research in advancing the understanding of host-bacteria interactions and responses during infections as well as in developing innovative antimicrobials for preventing and treating bacterial infections that capitalize on technological advancements in microfluidic device design and development.

1. Introduction

The vascular network is essential for the transportation of blood and delivery of oxygen and nutrients to tissues as well as in the removal of cellular and metabolic waste products throughout the body [1]. Additionally, the vascular network serves as a connecting network between organs. The vasculature and the blood cells contained within the vascular network are essential components of the host immune response to bacterial infections.

During infections, pathogenic bacteria interface with the vasculature in direct and indirect ways. Bacteria come into direct contact with the vasculature during catheter-associated infections, bloodstream infections, secondary infections, infections of deep wounds and surgical incisions, infective endocarditis, and bacterial meningitis (Fig. 1). The adhesion of bacteria to the vasculature is mediated by surface proteins of both bacteria and the vascular endothelium [2] as well as fluid flow within the vascular network [3]. Bacteria located within tissues can also cue an inflammatory response of the vascular endothelium that signals the recruitment of immune cells to an infection site [4]. Additionally, bacterial-derived molecules within the bloodstream or within host tissues can activate an inflammatory response from the vascular endothelium [2]. Bacterial-derived molecules or components with structural motifs recognized by host cell receptors are known as pathogen-associated molecule patterns (PAMPs) [5].

The first studies of bacterial-vascular interactions used parallel plate flow chambers or microfluidic flow cells inclusive of vascular endothelial cell monolayers to reveal mechanisms of bacterial adhesion to the vasculature. Technological advancement in microfluidic device design and fabrication has enabled the development of more sophisticated *in vitro* models of the vasculature and in turn advanced studies of bacterial infections at or near the vascular interface. Recent developments in vascularized bacterial infection models include three-dimensional

vasculature-on-a-chip microphysiological systems and organ-on-a-chip models with vascular monolayers interfacing with relevant host tissues that incorporate bacteria or bacterial-derived molecules. The incorporation of bacteria or bacterial-derived molecules into technologically advanced vascularized microfluidic models creates opportunities for gaining new mechanistic insight into how bacteria interact with the vasculature during infection and in developing novel antimicrobial therapeutics to prevent and treat bacterial infections.

The overall goal of this review article is to provide perspective on how technological advancements in microfluidics have enabled new insights into the role of the vasculature in the host response to bacterial infections and highlight how continued innovation in microfluidic technologies will pave the way for new directions of research in the pathogenesis of bacterial infections and the development of bacterial infection prevention and control strategies. This review starts by introducing research insights gained by studying bacterial adhesion to the vascular endothelium in parallel plate flow chambers and microfluidic flow cells (Section 2). Next, we review microfluidic models of the vasculature utilized to study the response of the vascular endothelium and immune cells to bacterial infections at or near the vascular interface (Section 3). We then highlight organ-on-a-chip technologies inclusive of the vasculature that have modeled bacterial infections (Section 4). Finally, we discuss future opportunities for utilizing vascularized microfluidic models to advance the scientific understanding of the pathogenesis of bacterial infections and inform the development of innovative infection prevention and control technologies (Section 5).

2. Parallel plate flow chambers and flow cells for studying bacteria-vascular interactions

Parallel plate flow chambers for studying the vascular response to fluid flow were first developed in the 1970s [6]. Parallel plate flow chambers generally consist of a lower plate containing a glass slide for cell culture and an upper polycarbonate plate with inlet and outlet ports for media flow. The two plates are separated by a silicon gasket of ~200-250 μm. Parallel plate flow chambers and commercial flow cells with similar geometries have been the primary models for advancing the understanding of bacterial adhesion to the vasculature under flow as these models enable study of bacterial adhesion to vascular monolayers at physiological shear stress. As vascular cells are sensitive to shear stress their properties are maintained when grown at physiological shear stress, including cellular morphology, alignment, barrier properties, and signaling pathways[7,8,9]. The inclusion of a vascular endothelium with physiologically relevant properties is important in studies of bacterial-vascular interactions.

Bacterial adhesion to the vasculature while undergoing shear stress has been studied using both parallel plate flow chambers and flow cells. Parallel plate flow chambers have revealed that *Staphylococcus aureus* adhesion to the vascular endothelium significantly decreases at physiological shear stress on a non-activated vascular endothelial monolayer [10, 11]. However, when the endothelial monolayer is activated, von Willebrand factor expression on the endothelial surface mediates the binding of *S. aureus* [12, 13, 14] and *Streptococcus pneumoniae* [15] to the vascular endothelium at physiological shear stress. Parallel plate flow chambers have also been used to demonstrate that adhesion of *Neisseria meningitidis* to the vascular endothelium occurs at the low shear stresses found in capillaries but not in the higher shear stresses typical of the bloodstream, which aligns with clinical observations of the localization of meningococcus attachment to capillaries during meningitis [16]. Collectively, these studies demonstrate how parallel plate flow chambers and flow cells have been used to show how shear stress, vascular

response, and bacterial proteins contribute to preventing or promoting bacterial adhesion to the vasculature.

In addition to direct interactions with bacteria, the vascular endothelium is responsive to bacterial-derived molecules, such as the PAMP lipopolysaccharide (LPS). The response of the vascular endothelium to LPS and shear stress has been studied using parallel plate flow chambers. Zeng et al. found that at high physiological shear stress the vascular endothelium can resist LPS-induced apoptosis as compared to static cultures using the same concentration of LPS to stimulate the vascular endothelium [17]. Additionally, they found that shear stress reduced the expression of the pro-inflammatory cytokine interleukin 6 (IL-6) by the vascular endothelium during LPS stimulation [17]. In another study, Ploppa et al. revealed that stimulation of both the vascular endothelium and neutrophils by LPS resulted in reduced neutrophil adhesion to the vascular endothelium at high shear stress [18]. These studies show that parallel plate flow chambers are useful for identifying how shear stress modulates the response of the vascular endothelium to LPS stimulation.

Parallel plate flow chambers are a beneficial tool for studying bacterial-vasculature interactions as they enable the development of well-defined uniform shear stresses, direct visualization of bacteria and the vasculature, and straightforward sampling of secreted factors [19]. One limitation of parallel plate flow chambers is the two-dimensional geometry of the vascular endothelial monolayer. Another limitation specific to using parallel plate flow chambers for studying bacteria is that they require large volumes of media to maintain high shear stress during experiments, as media cannot be recirculated in parallel plate flow chambers containing bacteria. In experiments with only vascular cells, media can be recirculated within the flow chambers to mitigate the use of high media volumes while maintaining high shear stress. Recirculation of

media in a system containing bacteria for studies longer than the doubling time of the bacteria is problematic as bacteria will be continually introduced to the system from the media reservoir. Thus, parallel plate flow chambers are valuable for studies of bacterial adhesion but not suitable for studies of the progression of bacterial infections.

3. Vasculature-on-a-chip devices for studying Bacterial-Vascular Interactions

This section highlights microfluidic models that incorporate either intact bacteria or bacterial-derived molecules at or near the vascular interface for investigating how bacterial interactions with the vascular endothelium prompt inflammation and immune cell recruitment.

3.1 Microfluidic models containing bacteria and the vasculature

There are a limited number of microfluidic models that have been developed to study the interactions between intact bacteria and the vasculature. During an infection, bacterial cells can be found within bloodstream, at the vascular interface or at an infection site, such as a specific tissue or medical device. Microfluidic models including intact bacteria have recapitulated bacterial transmigration from the bloodstream across the vasculature as well as immune cell recruitment from the bloodstream to an infection site (Fig. 2A-B, Table 1).

Utilizing transwell dishes as design inspiration, Bergevin et al. developed a transmembrane microfluidic device that enabled the direct visualization of bacterial extravasation [20]. The device was designed such that endothelial cells could be grown at physiological shear stresses on the bottom surface of a polyethylene terephthalate (PET) membrane that was located between a lower channel and an upper observation chamber [20]. Bacteria were added to the device via the lower channel containing endothelial cells and direct

visualization of bacteria transmigration across the endothelial monolayer was observed [20]. This study enabled advances in understanding bacterial transendothelial migration out of the bloodstream and into the surrounding tissue [20].

Intact bacteria have also served as a cue for stimulating the migration of neutrophils across the endothelial lumen. Hind et al. developed a model of neutrophil migration across a three-dimensional, endothelial cell lined lumen [21]. The vessel was embedded within an extracellular matrix (ECM) and neutrophils migrated toward an intact *Pseudomonas aeruginosa* bacterial source located exterior to the extracellular matrix [21]. This model revealed that neutrophils can migrate towards bacteria for up to 24 hours and that endothelial cell secretion of IL-6, a pro-inflammatory cytokine, was important for mediating neutrophil migration as blocking IL-6 decreased neutrophil migration [21]. The endothelial lumen was required to see neutrophil response, as there was negligible migration when the vessel was not vascularized [21]. Additionally, using intact bacteria in the model increased the overall number of migrating neutrophils and the distance of neutrophil migration compared to the use of the chemoattractants N-Formyl-methionyl-leucyl-phenylalanine (fMLP) and interleukin 8 (IL-8) [21]. This model enabled scientific advancement of the understanding of how the vascular endothelium contributes to neutrophil migration and survival during bacterial infections [21].

3.2 Microfluidic models containing bacterial-derived molecules and the vasculature

Bacterial-derived molecules have been incorporated into microfluidic devices containing vascular monolayers to stimulate the inflammatory response of the endothelium or as a chemoattractant for recruiting immune cells across the vascular endothelium (Fig. 2C-G, Table 2). The first scenario represents the release of bacterial products into the bloodstream, whereas

the second scenario represents the recruitment of immune cells to an infection site. LPS, an inflammatory PAMP derived from the outer membrane of Gram-negative bacteria [22], and fMLP, a chemoattractant for neutrophils derived from Gram-negative and Gram-positive bacteria [23], are the primary molecules used within these two types of infection models.

Lipopolysaccharide (LPS) within microfluidic models of the vasculature

LPS has been used to stimulate the inflammatory response of endothelial cells within microfluidic models of the vasculature. These models have provided new insights into immune cell recruitment to the vascular endothelium during inflammation and factors that protect the barrier integrity of the vascular endothelium during activation by LPS.

Immune cell recruitment due to LPS gradients outside of the vasculature has been modeled experimentally in a microfluidic device. Nam et al. developed a three-dimensional microfluidic model with three channels, where channel one contained an endothelial cell monolayer, channel two contained a collagen hydrogel, and channel three was a media reservoir with LPS [24]. In this study, monocytes were introduced within the endothelial cell lumen and migrated across the endothelial cell monolayer through the collagen toward the LPS [24]. Monocyte migration distance through the ECM increased in the presence of LPS [24]. Additionally, barrier function of the vascular endothelium was evaluated as a marker of inflammatory response, where barrier integrity was disrupted when LPS was present as indicated by downregulation of vascular endothelial cadherin (VE-cadherin), a cell-cell adhesion molecule between endothelial cells [24]. Intercellular adhesion molecule 1 (ICAM-1), an adhesion molecule necessary for immune cell adhesion to the vasculature prior to transmigration through the vasculature to an infection site, was also upregulated when LPS was present within the system [24].

This study recapitulated the inflammatory response of blood vessels and monocyte migration across blood vessels due to diffusion of LPS through the extracellular matrix on the exterior of a blood vessel [24].

Microfluidic models have also been utilized to evaluate endogenous mechanisms and pharmaceutical treatments for preserving endothelial cell barrier integrity in response to inflammation by LPS. The glycocalyx is the outermost surface layer of the endothelium [25]. Glycocalyx shedding contributes to the breakdown of the integrity of the endothelium barrier and can occur during bloodstream infections. Kiyan et al. established a microfluidic model for evaluating the protective nature of heparanase-2, an endogenous inhibitor of an enzyme that targets heparan sulfate, in preserving the integrity of the endothelial glycocalyx during activation of endothelial cells by LPS [26]. In a simple flow cell model inclusive of an endothelial monolayer on a coverglass, heparinase-2 was shown to help maintain the endothelial cell barrier, as evidenced by preservation of VE-cadherin cell-cell contacts when heparinase-2 is over-expressed by endothelial cells, reduction of pro-inflammatory cytokine expression by endothelial cells and reduction of toll-like receptor 4 (TLR-4) activation by LPS [26]. This study revealed a protective role of an endogenous enzyme, heparinase-2, in microvascular inflammation. In another flow cell model, Liao et al. demonstrated that the medication dexmedetomidine (DEX) was able to ameliorate the degradation of the glycocalyx and inhibit the loss of VE-cadherin expression typically observed during LPS treatment [27]. This study revealed that DEX can serve as a protective agent for the glycocalyx and endothelium during LPS exposure [27].

N-Formyl-methionyl-leucyl-phenylalanine (fMLP) within microfluidic models of the vasculature

fMLP has been used as a neutrophil chemoattractant within microfluidic models to study neutrophil chemotaxis in the presence of endothelial cells and transendothelial migration of neutrophils through an endothelial cell monolayer. These models shed new light on neutrophil-endothelial interactions during neutrophil recruitment to an infection site.

In the simplest microfluidic models of neutrophil chemotaxis, a chemoattractant gradient is created in a solution adjacent to an endothelial monolayer. In one such model, Kim et al. used serpentine channels to create a fMLP concentration gradient within media flowing across an observation channel containing endothelial cells and revealed that neutrophil chemotaxis occurs towards the fMLP with and without a competing chemoattractant gradient [28]. In another microfluidic model, Soroush et al. utilized digitized microvascular networks to create channel geometries based on the microvasculature within mouse cremaster muscles [29]. The networked channels were then divided into three parallel compartments with the first compartment containing vascular cells, the second compartment consisting of a porous barrier, and the third compartment acting as a mock "tissue compartment" filled with media containing a chemoattractant [29]. This microfluidic model was used to demonstrate that protein kinase C ∂ plays a key role in regulating neutrophil migration by decreasing the adhesion and migration of neutrophils across endothelial cells in response to fMLP [29].

Three-dimensional models of neutrophil chemotaxis generally include a vascular monolayer adjacent to an extracellular matrix containing a concentration gradient of chemoattractant. The specific geometry of the devices and the orientation of the chemoattractant gradients vary by model. In one three-dimensional model, Han et al. found that a vascular endothelial monolayer was required for significant neutrophil migration into a collagen hydrogel indicating that endothelial-neutrophil interactions are critical for neutrophil migration to an

infection site [30]. In a different three-dimensional model of neutrophil chemotaxis, Wu et al. learned that neutrophil migration across endothelial cells into a collagen hydrogel is dependent on the spatial gradient and concentration of the chemoattractant within the extracellular matrix material, and demonstrated synergistic effects between different chemoattractants [31]. In another three-dimensional model inclusive of microchannels with bifurcations and curvatures, Menon et al. found that chemoattractant gradients across a collagen hydrogel significantly increased neutrophil migration [32]. Neutrophils consistently are recruited across the endothelial-extracellular matrix interface when a chemoattractant gradient is present in the extracellular matrix.

Three-dimensional models of neutrophil chemotaxis toward fMLP have also been employed to reveal how primary vascular cells and extracellular matrix influence neutrophil migration through the vascular endothelium. Ingram et al. utilized a microfluidic model containing a three-dimensional endothelial cell lumen embedded within a collagen hydrogel with fMLP added to a channel on the exterior of the hydrogel as a chemoattractant to show neutrophil extravasation and migration responses [33]. A unique feature of this model was the use of induced pluripotent stem cells (iPSC)-derived cells as a more physiologically relevant source of endothelial cells [33]. Additionally, differences between purified neutrophil migration and neutrophil migration from whole blood were assessed in this model, where purified neutrophil migration distances were reduced compared to the migration distances of neutrophils from whole blood when fMLP was used as the model chemoattractant [33]. In another study, Riddle et al. used a three-dimensional model with an endothelial vessel in contact with extracellular matrix to investigate differences in neutrophil transmigration toward fMLP through endothelial cells surrounded by either collagen or geltrex matrices [34]. This study determined that fMLP was

255 required for neutrophil transmigration into collagen, whereas transmigration occurred without
256 fMLP in the geltrex matrix indicating that extracellular components play a key role in
257 influencing cellular behaviors [34].
258
259 **4. Organ-on-a-chip infection models inclusive of vasculature**
260 Organ-on-a-chip models have emerged as a novel method for recapitulating organ
261 behaviors *in vitro* and are beginning to be used to study host-pathogen interactions during the
262 progression of infectious disease. The initial use of organ-on-a-chip models to study interactions
263 between pathogenic bacteria and the gut, lung, bladder, and placenta has been reviewed
264 elsewhere [35, 36, 37, 38]. As the vascular system interfaces with organs throughout the body, many
265 organ-on-a-chip models include an interface within the microfluidic device between the specific
266 organ tissue being modeled and the vascular endothelium. This section of our review focuses on
267 providing an overview of organ-on-a-chip models inclusive of vasculature that study the
268 responses of organ systems to intact bacteria or bacterial-derived molecules. Organ-on-a-chip
269 models inclusive of the vasculature and pathogenic bacteria or bacterial-derived molecules have
270 been developed for the lung, gut, brain, lymphatic system, liver, bladder, and placenta (Fig. 3).
271 Scientific findings utilizing these microfluidic models range from the effects of bacterial
272 infections or bacterial-induced inflammation on organ tissues to specific biological mechanisms
273 tied to cellular responses to infection.
274
275 **4.1 Organ-on-a-chip infection models inclusive of vasculature and bacteria**
276 Organ-on-a-chip models inclusive of the vasculature have been used to investigate the
277 effect of intact bacteria on immune cell recruitment and cellular responses during both

bloodstream and organ specific infections (Fig. 4, Table 3). These studies frequently include reports on the effects of bacteria on the barrier function of the vascular endothelium and cellular expression of inflammatory markers. This section highlights studies of neutrophil transmigration and macrophage responses to intact bacteria using various organ-on-a-chip models. Additionally, this section reviews an organ-on-a-chip model with added functionality for monitoring cellular response to bacterial infection and a disease-on-a-chip model for examining interactions between bacteria and diseased organs.

Neutrophil transmigration from the vascular endothelium to an infection site has been studied using organ-on-a-chip models of the lung, lymphatic system, and bladder. Huh et al. developed a lung-on-a-chip representative of the alveolar-capillary interface with alveolar epithelial cells and vascular endothelial cells grown on opposite sides of a thin, porous membrane and studied transmigration of neutrophils from the endothelial microchannel into the alveolar channel during an *Escherichia coli* infection of the alveolar channel [39]. After five hours of infection, the vascular endothelium was activated as evidenced by the transmigration of neutrophils across the capillary-alveolar interface [39]. After transmigration, the neutrophils migrated toward the *E. coli* and engulfed the bacteria until most bacteria were cleared from the channel, recapitulating an integrated cellular immune response to lung infections [39]. In another study of neutrophil extravasation during bacterial infection using an organ-on-a-chip model, McMinn et al. created a microfluidic model of the lymphatic system that included parallel lumens of lymphatic endothelial cells and vascular endothelial cells embedded in a hydrogel, where *Pseudomonas aeruginosa* was added to the lymphatic endothelial lumen and neutrophils were added to the vascular endothelial lumen [40]. In this lymphatic system model, neutrophil extravasation occurred across the vascular endothelium and neutrophils subsequently migrated

toward *P. aeruginosa* [40]. There were variations in the occurrence of transendothelial migration based on the cells present in the model, indicating that modulation of cellular secretion factors transpires due to crosstalk between the lymphatic and vascular endothelium [40]. Sharma et al. studied the dynamics of neutrophil recruitment to the site of infection from the bloodstream using a bladder-on-a-chip model representative of a urinary tract infection[41]. The model was comprised of the epithelial-vascular interface where bladder epithelial cells and vascular endothelial cells were cultured on opposite sides of a porous membrane with *E. coli* added to the channel with bladder cells and neutrophils added to the channel with endothelial cells [41]. The neutrophils in the bladder-on-a-chip model migrated across the endothelium to the bacteria source and subsequently formed neutrophil extracellular traps [41]. There was also an increase in ICAM-1 expression by the endothelial cells and an increase in the release of inflammatory cytokines from the bladder and endothelial cells in response to the bacteria in the bladder-on-a-chip model [41].

Evaluation of macrophage response and recruitment to bacterial infections has been performed using organ-on-a-chip models of the liver, lung, and placenta. Siwczak et al. developed a liver-on-a-chip with endothelial cells and hepatocytes grown on opposite sides of a porous membrane and macrophage at the interface between the endothelial cell layer and the parenchymal cell layer formed by the hepatocytes [42]. *Staphylococcus aureus* was introduced to the vascular channel on the liver-on-a-chip to represent a bloodstream infection [42]. *S. aureus* cells were sequestered to the macrophage and cleared from circulation within the model [42]. As a result, the macrophage response protected the endothelial cells and hepatocytes from becoming infected with bacteria [42]. M2 polarization of macrophage, prior to infection, resulted in the highest bacterial cell counts within macrophage with a preference for small colony variants of *S.*

aureus [42]. This study revealed a specific role of M2 macrophage as a niche for facilitating *S. aureus* persistence during bacterial infection of the liver [42]. Deinhardt-Emmer et al. created an alveolus-on-a-chip model inclusive of macrophage at the alveolar-endothelial interface to study co-infections of *S. aureus* and influenza virus [43]. Infection of the alveolar channel induces an inflammatory response of the endothelium in both bacterial infections and bacterial and influenza co-infections [43]. Additionally, bacteria are found to translocate from the alveolar channel to the endothelial channel during co-infections [43]. In another organ-on-a-chip infection model containing macrophage, Zhu et al. established a placenta-on-a-chip to study the response of the placenta to an *E. coli* infection originating on the maternal side of the placenta [44]. The placenta-on-a-chip consisted of trophoblasts and vascular endothelial cells grown on opposite sides of a thin, porous membrane to represent the maternal and fetal side of the placental barrier, respectively, with *E. coli* added to the trophoblast channel [44]. The addition of *E. coli* to the model resulted in significant cell death of both the trophoblasts and endothelial cells [44]. This also corresponded with an increase in inflammatory cytokine expression and barrier disruption of the fetal endothelial cells when the trophoblasts were present in the model, indicating cell-to-cell communication between the maternal and fetal cells [44]. In addition, macrophage added to the maternal channel after *E. coli* infection migrated and attached to the trophoblast surface, which increased inflammatory cytokine expression in response to infection [44].

Cellular responses can be monitored in real-time on microfluidic devices by integrating biosensors into the chip. One example of an infection model with an integrated biosensor is a neurovascular-unit-on-a-chip, where a microelectrode array (MEA) was integrated into the chip design to enable monitoring of the neuronal response to a bacterial infection representative of bacterial meningitis [45]. The neurovascular-unit-on-a-chip consisted of two chambers separated

by a porous membrane with vascular endothelial cells cultured directly on the porous membrane in the upper chamber and cortical neurons cultured on the MEA situated at the lower wall of the lower chamber [45]. After *E. coli* was added to the vascular chamber, the barrier permeability of the endothelial layer was reduced within four hours, while the neuronal electrical activity decreased 20 hours after infection [45]. The duration of time for the decrease in neuronal electrical activity was five times longer when the endothelial vasculature was included with the cortical neurons in the model indicating a protective role of the vasculature during bacterial meningitis [45].

Bacterial infections of healthy and diseased lungs were recapitulated using lung-on-a-chip models. Plebani et al. developed a model of cystic fibrosis (CF) by modifying a lung-on-a-chip model to include cystic fibrosis bronchial epithelial cells [46]. Bronchial epithelial cells and vascular endothelial cells were grown in two channels on opposite sides of a porous membrane [46]. *P. aeruginosa* was added to the channel with bronchial epithelial cells and polymorphonuclear leukocytes (PMNs) were added to the vascular endothelial channel [46]. In the CF model, there were a higher number of bacteria embedded in the mucus layer of the epithelium and a higher number of PNMs adhered to the endothelium than in the healthy model; however, there was an increase in inflammatory cytokine expression by vascular endothelial cells on both microfluidic chips [46].

4.2 Organ-on-a-chip infection models inclusive of vasculature and bacterial-derived molecules

Inflammation and inflammatory responses of organ tissues and vasculature due to bacterial-derived molecules have been studied using organ-on-a-chip models (Fig. 5, Table 4). Bacterial-derived molecules, such as LPS, can be added to the vascular channel or to the organ

tissue channel of a device to mimic bacterial-derived inflammatory cues within the bloodstream or to represent an infection within an organ, respectively. Organ-on-a-chip models inclusive of bacterial-derived molecules have been used to investigate immune cell recruitment and response during infection, mechanistic insights into the inflammatory response of the vascular endothelium, pulmonary thrombosis, and differences between the responses of healthy and diseased organ tissue to bacterial-derived inflammatory cues.

Neutrophil recruitment due to stimulation with LPS in a liver-on-a-chip shows evidence of crosstalk between liver cells during neutrophil recruitment. Du et al. developed a liver-on-a-chip model consisting of two channels separated by a porous membrane with liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs) co-cultured on the membrane in the upper channel, hepatic stellate cells cultured on opposite side of the membrane in the lower channel, and hepatocytes cultured on the far wall of the lower channel. [47]. LPS and neutrophils were both added to the endothelial cell channel [47]. LPS stimulation of the endothelium in the liver-on-a-chip increased neutrophil adhesion to the endothelial surface by 63% and increased neutrophil aggregate size compared to neutrophil adhesion to a monoculture of LSECs stimulated by LPS [47]. This indicates that cross talk between liver cells increases neutrophil recruitment to the vascular endothelium [47].

Monocyte response to infection has also been investigated in organ-on-a-chip models. Kim et al. studied interactions between LPS stimulation and peripheral blood mononuclear cells (PBMCs)—a combination of monocytes, macrophages, lymphocytes, Natural Killer cells, and dendritic cells [48]—in a gut-on-a-chip model [49]. The gut-on-a-chip model included intestinal epithelial cells and vascular endothelial cells on opposite sides of a flexible, porous membrane [49]. LPS was added to intestinal epithelial chamber and PBMCs were added to the endothelial cell

chamber [49]. Inclusion of LPS and PBMCs in the model was necessary for activation of endothelial cells and PBMC adhesion to the endothelium, demonstrating cellular cross talk is important for the initiation of an immune response in the gut-on-a-chip model [49]. In a study of specific roles of monocytes in sepsis-related liver dysfunction, Gröger et al. stimulated a liver-on-a-chip containing monocytes with LPS and found that monocytes attenuate inflammation-related cell responses in the liver model [50]. The liver-on-a-chip model included two channels separated by a porous membrane [50]. The upper channel contained vascular endothelial cells co-cultured with macrophage on a suspended porous membrane and the lower channel contained a hepatic cell layer comprised of hepatocytes and hepatic stellate cells cultured on the bottom channel wall with a media-filled space between the porous membrane and the hepatic cells in the lower channel [50]. After LPS addition to the liver-on-a-chip, monocytes are recruited to the endothelium and transmigrate to the hepatic compartment, the endothelium barrier is disrupted, and the endothelium increases inflammatory cytokine expression [50]. The presence of the endothelial cells and macrophage in the liver-on-a-chip decreases the susceptibility of hepatocytes to LPS stimulation, indicating that cellular cross talk between macrophage, the vasculature, and hepatic cells contributes to the inflammatory response to LPS in the liver [50].

In a more mechanistic study, a blood-brain-barrier-on-a-chip was utilized to demonstrate that LPS stimulation of the vascular channel adjacent to human astrocytes reduced the barrier integrity of the vasculature [51]. Additionally, this study revealed that LPS stimulation of vasculature increased expression of miR-146a, a microRNA expressed in the central nervous system associated with an inflammatory response [51].

Organ-on-a-chips can be used to evaluate the response of both diseased and healthy tissues to inflammation due to bacterial-derived molecules. A lung-on-a-chip model of

pulmonary thrombosis incorporated whole blood into the model to study the development of thrombosis during LPS stimulation [52]. The lung-on-a-chip included two microchannels separated by a thin membrane with primary human lung alveolar epithelial cells grown on the thin membrane in the upper channel and vascular endothelial cells on all four channel walls to create a three-dimensional microvessel in the lower channel [52]. After LPS addition to the channel with alveolar epithelial cells, the permeability of the tissue-tissue interface increased, platelet binding to the endothelium increased, and inflammatory cytokine expression increased; however, this did not occur when LPS was added to the endothelial cell channel, indicating that tissue-tissue interactions between the lung alveolar epithelium and the vascular endothelium contribute to the induction of pulmonary thrombosis by LPS [52]. The response of healthy and diseased lungs to a bacterial-derived molecule can be elucidated using lung-on-a-chip models. In one such example, Benam et al. developed a chronic obstructive pulmonary disease (COPD) model, where COPD-patient derived primary human airway epithelial cells and human lung microvascular endothelial cells were cultured on opposite sides of a porous membrane to create a tissue-tissue interface within a microfluidic device [53]. Comparisons between COPD and healthy lung-on-a-chip models revealed that LPS exacerbated cells on the COPD chip as indicated by the upregulation of inflammatory cytokines on the COPD chip [53].

5. Future Directions and Opportunities

The measurable advancements in microfluidic models of bacterial infections at the vascular interface open the door to future research directions in infection pathogenesis, device development, and antimicrobial discovery. Variations in the biological elements included within

the devices, such as the bacterial strains, bacterial-derived molecules, or mammalian cell sources, integration of technological advancements realized through the development of other microphysiological systems, and application of the model to develop and evaluate novel antimicrobial solutions are all future opportunities for utilizing microfluidic models of bacterial infections at the vascular interface.

Bacteria included in existing microfluidic models of bacterial infection at the vascular interface are primarily common laboratory strains of prevalent pathogenic bacteria. Bacterial species and strains vary significantly in their genotypes and phenotypes. For a more generalized understanding of the vascular response to infection, models should incorporate additional bacterial strains, including clinical isolates of bacteria. Current models have also been limited to the study of the planktonic phenotype of bacteria. Bacterial infections are not always caused by planktonic bacteria. The bacterial biofilm phenotype is a significant clinical problem as biofilms are recalcitrant—resistant or tolerant—to antimicrobial treatment [54]. Bacterial biofilms—structured communities of cells encapsulated in self-produced matrix materials, including polysaccharides, proteins, and DNA [55]—are estimated to be involved in 65-80% of infections annually [56]. Biofilm formation can occur at the vascular interface during catheter-associated infections, bloodstream infections, infective endocarditis, deep wound or surgical site infections, and bacterial meningitis. There are not currently microfluidic models of biofilm infections at or near the vascular interface. The inclusion of biofilms within microfluidic models requires microfluidic model design that enables the co-culture of biofilms and mammalian cells on relevant time scales. For biofilm development, bacteria will need to be included within the microfluidic system for time periods of at least 24 hours and biofilm growth will need to be optimized in environments that maintain the physiologically relevant properties of mammalian

cells within the model (e.g. mammalian culture media, physiological shear stress). Establishing microfluidic models of biofilm development at the vascular interface will enable scientific questions to be addressed, such as how the biofilm microenvironment and nutrient transport is modulated by the vascular endothelium or how the vascular endothelium influences biofilm recalcitrance to antimicrobial treatments. In addition to developing models of single species biofilm communities in microphysiological systems, multispecies biofilms are also a prevalent source of infection that exhibit behaviors that could be further understood utilizing microfluidic models. Additionally, free-floating bacterial aggregates are emerging as an infection phenotype [57, 58] that could be studied in these microfluidic systems.

Bacterial-derived molecules, such as LPS or fMLP, have been integrated into microfluidic models to stimulate the inflammatory response of the vascular endothelium or the recruitment of immune cells to an infection site. These are only two of many bacterial-derived cues that are known to stimulate host responses. There are a variety of PAMPs produced by both Gram-negative and Gram-positive pathogens that can trigger an inflammatory response from the vascular endothelium [59]. Integrating multiple PAMPs or bacteria and specific PAMPs into microfluidic models in a controlled manner will enable mechanistic studies of the specific roles of PAMPs and bacteria in the pathogenesis of bacterial infections.

In addition to consideration of bacteria and bacterial-derived molecules within infection models, the selection of the biological elements representative of the host is important to the design of microfluidic models of bacterial infection. The use of cells with patient specificity within microfluidic models of organ systems is a key consideration in device design [60]. Primary cells or patient-derived stem cells could be used within microfluidic models to increase the patient specificity of the microfluidic models for the endothelial cells, immune cells, or modeled

organ tissue cells found within bacteria infection models. Additionally, immune cell response in current models has focused on the initial recruitment of neutrophils or macrophage to an infection site. However, the host immune response is not isolated to individual immune cells. Vasculature-on-a-chip and organ-on-a-chip models provide a unique platform for isolating the individual and cooperative responses of different immune cells to determine the role of cell-to-cell communication between the vascular endothelium and bacteria in the host immune response to infection. Host-derived cues of infection are also important to consider within models of bacterial infection. When host cells are damaged or dying due to bacterial infections, they release cues known as damage-associated molecular patterns (DAMPs) that signal the inflammatory and innate immune response to infections [61]. Microfluidic bacterial infection models that incorporate the vasculature are a promising platform for evaluating how isolated DAMPs as well as the interactions between PAMPs and DAMPs influence the host response to bacterial infections.

Recent advancements in vasculature-on-a-chip and vessel-on-a-chip device development have recapitulated a variety of geometries and biological features that can be employed for studying bacterial infections at the vascular interface. Device geometry has moved from two-dimensional to rectangular to semi-circular to circular endothelial monolayers [62]. Biological layers across the vessel wall including endothelial cells, the extracellular matrix, fibroblasts, and smooth muscle cells have been incorporated into microfluidic models of the vasculature to better represent physiological environments [62]. Stretchable and deformable vessels have been engineered to enable the recapitulation of pulsatile blood flow in vessel-on-a-chip models [62]. Additionally, microfluidic models of reversed flow due to bifurcations, sharp curves, or local expansion of vessel geometry have been developed [62]. There are also vascular specific disease or

injury states that may contribute to the recalcitrance of bacterial infections that have been modeled using microfluidic devices, including vessel injury, endothelial dysfunction, thrombosis, and vascular inflammation [62, 63]. In addition to modifying device geometries and biological features, bacterial infections in specific vascular microenvironments can be recapitulated in future infection models, such as central venous catheter infections, intravenous catheter infections, or infective endocarditis. Endothelial cell type and shear stress are two critical components of the microenvironment that vary throughout the vasculature and are important design considerations for developing site-specific infection models. Vessels in microfluidic devices have been formed with aortic [64], microvascular [65], vein [66], and lymphatic [67] endothelial cells depending on the type of vessel being modeled. Shear stress within the vascular system varies from 0.1 – 9.5 Pa, with lowest shear stresses in large capillary and large venous vessels and highest shear stresses in small capillary and small arterial vessels [68, 69, 70]. Shear stress used within a microfluidic model can be modulated according the location, size, and health of the vasculature being modeled [70]. Existing vessel-on-a-chip models could be adapted to represent specific infection sites by varying endothelial cell selection and shear stress within the model. For example, a central venous catheter infection could be developed by including venous endothelial cells and utilizing a shear stress of 0.35 Pa, which is the shear stress of a mid-sized vein [71], while an infective endocarditis model could be developed using valvular endothelial cells and a shear stress of 2 Pa, which is the average shear stress at the aortic valve [72]. As discussed here, there are many opportunities for existing microphysiological models of the healthy and diseased vasculature to be adapted for studying bacterial infections at the vascular interface.

As was highlighted above in studies of bacterial infections in disease-on-a-chip models of CF and COPD [46, 53], there are research opportunities available for studying how bacterial

infections progress in diseased or injured organs as diseased and injured tissue typically respond differently to treatment than healthy tissue. Disease specific organ-on-a-chip models are rapidly being developed across organ systems [73]. Microfluidic wound models inclusive of the vasculature have been developed for studying wound healing and inflammation of injured tissue [74, 75, 76]. However, microfluidic models of wound infections inclusive of the vasculature are yet to be utilized for studying bacterial infections. This is an important direction of research as injured tissue, such as the tissue in chronic wounds and burns, has an increased susceptibility to bacterial infection [77, 78]. Future research opportunities in infectious disease research utilizing wound-on-a-chip models include evaluation of host-pathogen interactions, the influence of bacteria on wound closure and healing, and the progression of bacterial infections in deep wounds, chronic wounds, and surgical incision sites. As more disease and injury specific-organ models emerge, bacterial infections within these models can be investigated and a mechanistic understanding of why bacterial infections are more difficult to treat for patients with specific diseases and injuries can be revealed.

In addition to the utilization of organ-on-a-chip models for studying bacterial infections, there are parallel research efforts in utilizing organ-on-a-chip models for studying viral infections, as reviewed elsewhere [35, 36, 79, 80, 81]. Microfluidic viral infection models inclusive of the vasculature have been developed using lung-on-a-chip models [82, 83], a skin-on-a-chip model [84], and a liver-on-a-chip model [85]; these microphysiological systems have been used to study viral pathogenesis, host immune response, and antiviral efficacy. The approaches and methods used to study viral infections in organ-on-a-chip models can be adapted for informing the development of bacterial infection models and vice versa, especially when infection models have only been developed using one class of infectious agent. For example, Nawroth et al. developed

553 an airway lung chip inclusive of vascular endothelial cells and neutrophils to probe mechanisms
554 leading to human rhinovirus (HRV)-induced asthma exacerbation [83]. Bacteria, including
555 *Streptococcus pneumoniae, Hemophilus influenza,* and *Moraxella catarrhalis*, are also known to
556 exacerbate asthma [86]. Therefore, Nawroth et al.'s airway lung chip modeling HRV-induced
557 asthma exacerbation [83] could be adapted for the study of bacterial-induced asthma exacerbation.
558 Similarly, Sun et al.'s vascularized skin-on-a-chip model of a herpes simplex virus (HSV)
559 infection that replicated host inflammatory and immune responses to HSV ulcerations[84] could be
560 adapted for studying bacterial infections of skin lesions, such as ulcers or wounds. Thus,
561 microfluidic models of viral infections can be utilized to inspire the future development of
562 bacterial infection models.

563 Multi-organ-on-a-chip and body-on-a-chip microphysiological systems are being
564 developed to enable systemic studies of interactions and crosstalk between organs using
565 physiologically relevant *in vitro* models [60, 87, 88]. In one such multi-organ chip model inclusive of
566 vasculature, heart, liver, bone, and skin tissues are connected by vasculature with recirculating
567 flow [89] . This multi-organ-on-a-chip model showed improved predictive values of cardiotoxicity
568 biomarkers relative to isolated tissues. There is immense potential for exploiting body-on-a-chip
569 models to investigate the systemic response of the host to bacterial infections during specific
570 disease states, such as sepsis, bloodstream infections, or the spread of secondary infections as
571 well as in evaluating the efficacy of innovative antimicrobial treatments.

572 The rise of antimicrobial resistance in bacterial strains demands new antimicrobials and
573 approaches for treating bacterial infections. Predictions estimate that by 2050 up to 10 million
574 deaths may be attributed to antimicrobial resistance annually [90]. Vasculature-on-a-chip and
575 organ-on-a-chip models inclusive of vasculature can be applied in the development of innovative

antimicrobial solutions for treating bacterial infections. The efficacy of antimicrobial agents and biomaterials can be tested using microphysiological models [60]. This is exemplified by the previously highlighted urinary tract infection model, which observed intracellular bacterial communities contribute to the dynamic persistence of infection during antibiotic treatment [41]. Additionally, emerging therapeutic targets for preserving or restoring vascular function after degradation due to bacterial sepsis, such as therapeutic interventions to restore the endothelial glycocalyx [91], can be evaluated in these model systems. The devices can also be modified to include patient-specific bacteria or patient-derived cells for personalized evaluation of antimicrobial solutions in microfluidic systems [73]. Finally, scale-up of the microfluidic models will be required to enable high-throughput approaches for pre-clinical predications of antimicrobial effectiveness, which would reduce cost, amount of time, and number of animal models used in antimicrobial development.

6. Conclusions

This review considers microfluidic technologies for studying bacterial infections at or near the vascular interface. Current microfluidic models of bacteria and bacterial-derived molecules at or near the vascular interface have enabled studies of the inflammatory response of the vasculature as well as the initial host immune response to bacterial infections. Existing models contain a limited number of pathogenic bacteria, bacterial-derived molecules, and host cells. Extending current models to include different biological elements will provide new insights into how bacterial infections progress at and near the vasculature. The latest technological developments in vasculature-on-a-chip, organ-on-a-chip, and body-on-a-chip models provide numerous opportunities for advancing the understanding of bacterial interactions

with the vasculature during infections. In addition, studying bacterial infections using disease-on-a-chip models will enable newfound understanding of why bacterial infections are more prevalent in certain disease states. Current models can also be modified for the development and testing of novel antimicrobial treatments for bacterial infections. Furthermore, the use of patient-derived bacteria, vascular cells, or immune cells within microfluidic models could enable the development of personalized antimicrobial treatments. Overall, technological advancements in the microfabrication of microfluidic systems inclusive of the vasculature have led to measurable advances in the understanding of how bacteria and bacterial-derived molecules inform the host response to infection. Emerging microfluidic technologies offer additional promise for transforming the scientific understanding of bacterial pathogenesis and supporting the development of innovative antimicrobial solutions in the future.

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Author Contributions Statement

Lily Isabelle Gaudreau: Conceptualization (supporting); Visualization (lead); Writing- original draft (equal); Writing- review & editing (equal). **Elizabeth J. Stewart:** Conceptualization (lead); Visualization (supporting); Writing- original draft (equal); Writing- review & editing (equal); Project Administration (lead); Supervision (lead); Funding Acquisition (lead).

Conflict of Interest Statement

622 The authors declare no competing financial interests.

623

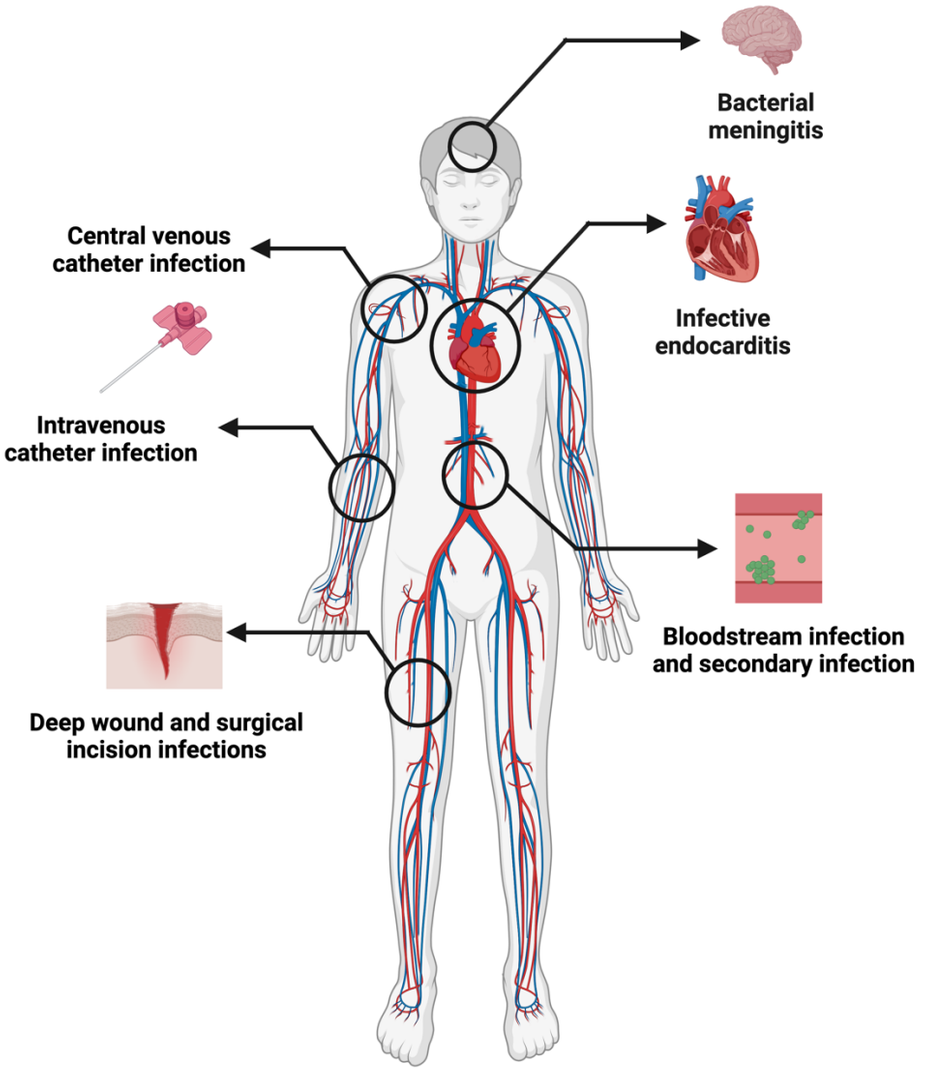
624 **Data Availability Statement**

625 Data sharing is not applicable to this article as no new data were created or analyzed in this

626 study.

627

628 **Figures**



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630 **Figure 1. Bacterial infections that occur at the vascular interface throughout the body.**

631 Schematic indicating the various locations and types of infections where bacteria directly interact

632 with the vasculature.

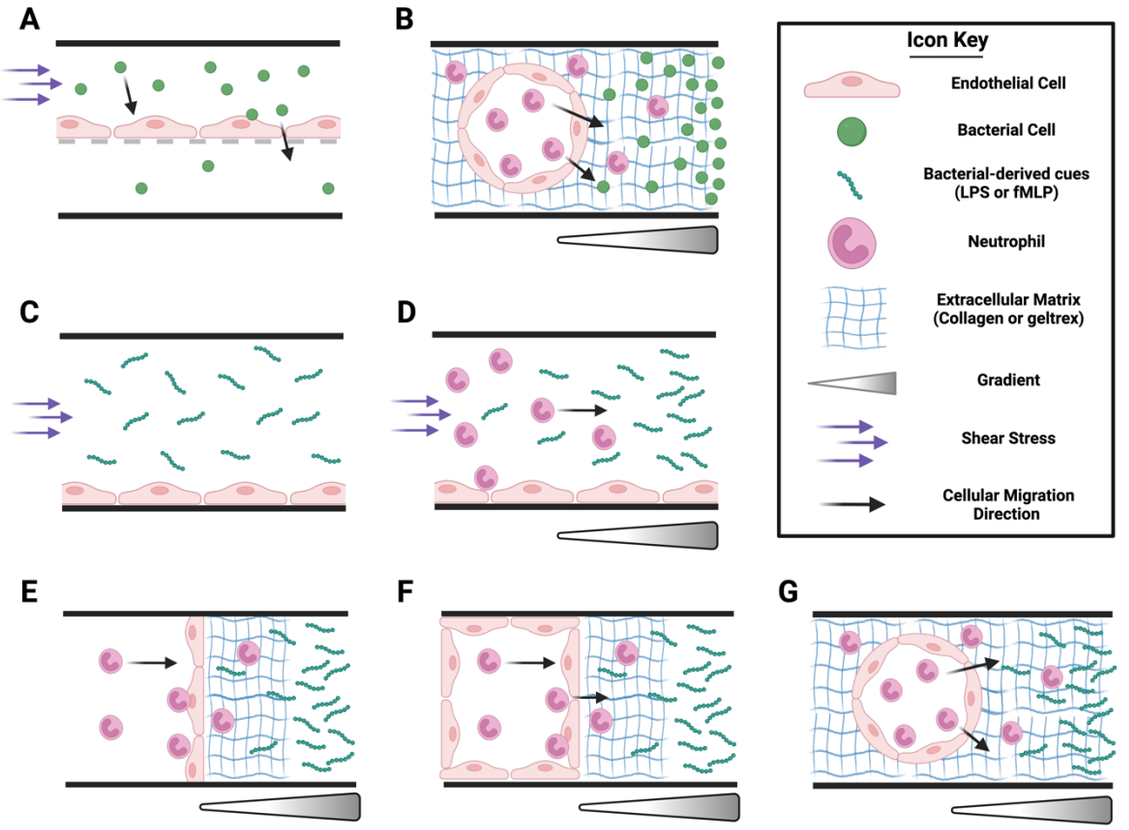

Figure 2. Vasculature-on-a-chip models inclusive of bacteria and bacterial-derived molecules. Vasculature-on-a-chip devices that include intact bacteria have modeled (A) bacterial extravasation from the blood stream via transendothelial migration across a 2D endothelial monolayer and (B) neutrophil transendothelial migration from a 3D cylindrical vessel through ECM toward intact bacteria. In addition, microfluidic models of the vasculature have modeled endothelial response to bacterial-derived cues in the bloodstream (C) without and (D) with neutrophils. Microfluidic systems have also modeled neutrophil transendothelial migration toward bacterial-derived cues located in the host tissue by including the ECM in the model with endothelial geometries varying in complexity from (E) a 2D monolayer to (F) a 3D rectangular vessel to (G) a 3D cylindrical vessel. Shear stress in models depicted in 2B, 2E, 2F, and 2G is perpendicular to the plane of view.

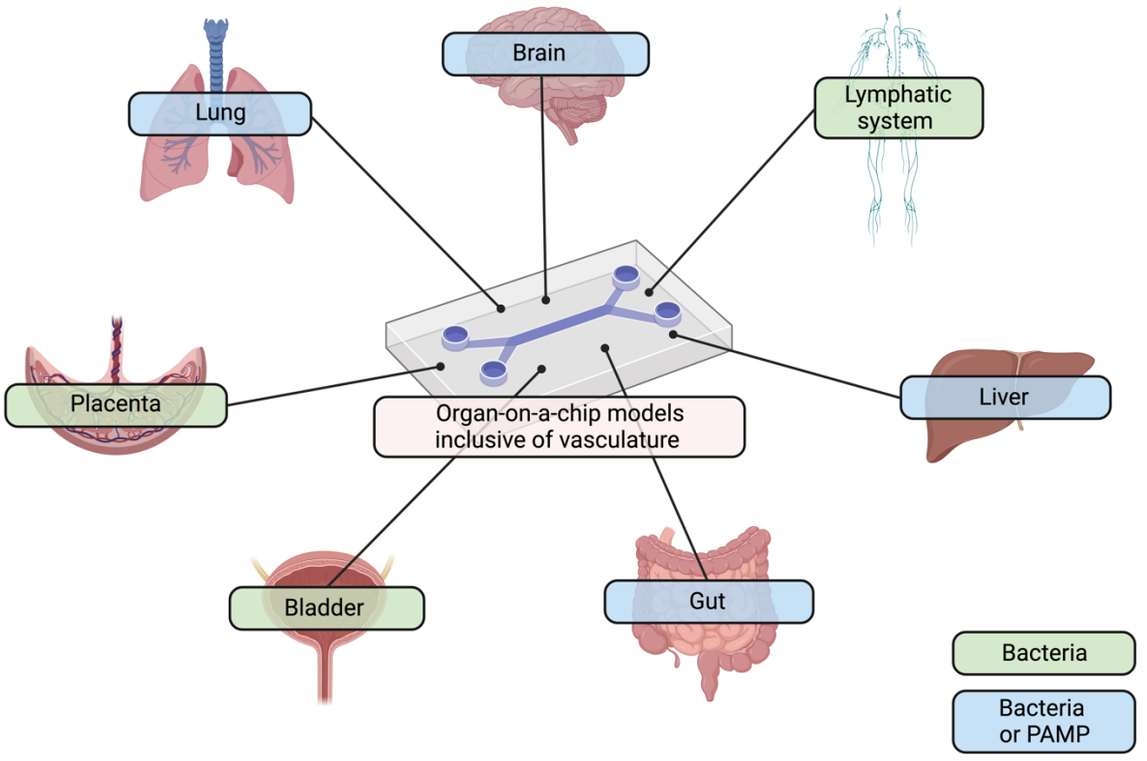

Figure 3. Organs represented in organ-on-a-chip models inclusive of vasculature and bacterial infections. Bacterial infections in the lung, brain, lymphatic system, liver, gut, bladder, and placenta have been modeled in organ-on-a-chip devices inclusive of the vasculature. Organ labels shaded in green have investigated intact bacteria in the models. Organ labels shaded in blue have included intact bacteria or a PAMP in the model.

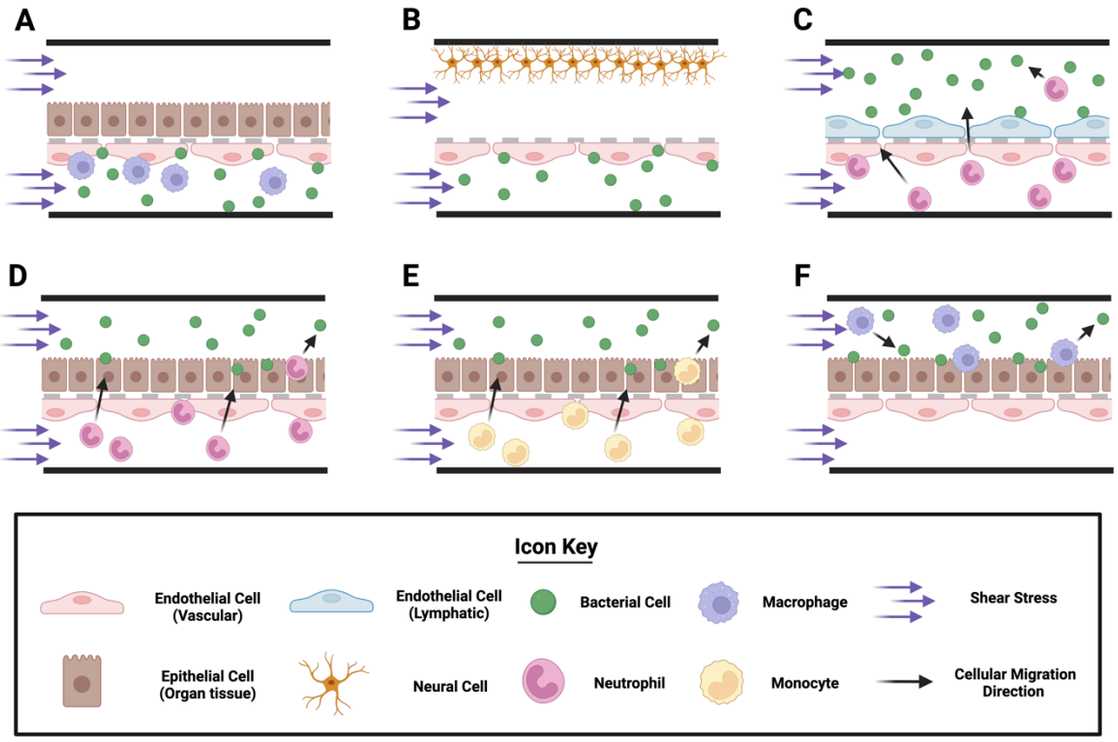

Figure 4. Organ-on-a-chip models inclusive of vasculature and bacteria representing either bloodstream or organ infections. Organ-on-a-chip systems that included intact bacteria in the bloodstream have modeled: (A) macrophage response to bacteria in a blood vessel adjacent to the liver and (B) bacterial infections at the blood-brain interface. Organ-on-a-chip systems that included intact bacteria within organ tissues have modeled: (C) neutrophil transendothelial migration from the bloodstream to a lymphatic vessel containing intact bacteria, (D) neutrophil transendothelial migration from the bloodstream towards a lung or bladder infection, (E) monocyte transendothelial migration from the bloodstream to a lung infection, and (F) macrophage response to bacterial infections in the lung or placenta.

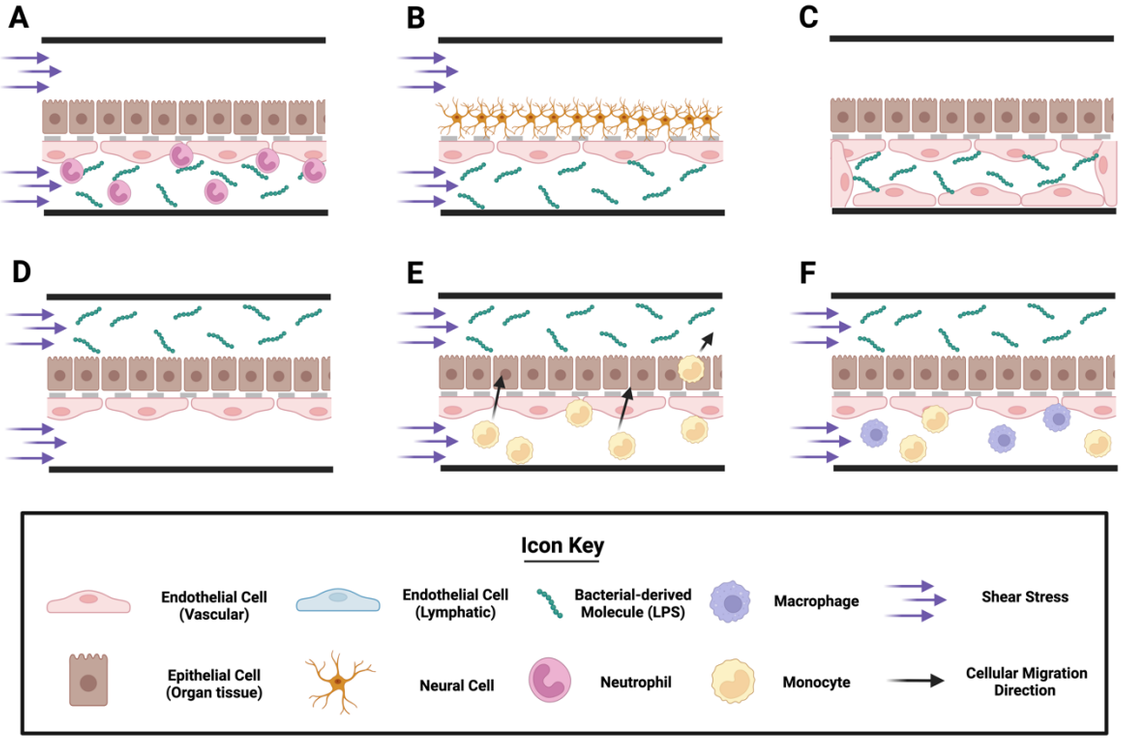

Figure 5. Organ-on-a-chip models inclusive of vasculature and bacterial-derived molecules. Organ-on-a-chip systems that include the bacterial-derived molecule lipopolysaccharide (LPS) in the bloodstream have modeled: (A) neutrophils and LPS in a blood vessel adjacent to the liver, (B) LPS in the bloodstream at the blood-brain barrier, and (C) LPS in a 3D blood vessel adjacent to the lung. Organ-on-a-chip systems that included LPS within the host tissue have modeled: (D) LPS in the lung, (E) monocyte transendothelial migration from the bloodstream to LPS in the liver, and (F) the response of monocytes and macrophage in the bloodstream to LPS in the gut. Shear stress in the model depicted in 2C is perpendicular to the plane of view.

Tables

Table 1. Vasculature-on-a-chip models used to study the vascular response to bacterial infection.

Pathogen	Biological Process	Device Design	Key Scientific Findings	Ref.
Borrelia burgdorferi	Bacterial extravasation out of the bloodstream	***Device Design:*** Two channels separated by a PET membrane with 3 µm pores in a microfluidic device that mimicked a transwell dish ***Endothelium Geometry:*** 2D ***ECM:*** No	Bacterial transendothelial migration while undergoing shear stress is initially delayed compared to static conditions, but after ~1 hour bacterial migration rates become similar under shear and static conditions.	[20]
Pseudomonas aeruginosa	Neutrophil migration from the blood vessel toward bacteria	***Device Design:*** Large PDMS chamber filled with a collagen hydrogel containing a suspended cylindrical channel lined with endothelial cells ***Endothelium Geometry:*** 3D, cylindrical vessel ***ECM:*** Yes, collagen	An endothelial monolayer is needed for persistent neutrophil migration out of the vessel into the ECM toward intact bacteria. Neutrophil migration time toward infection was much longer than expected (up to 24 hours).	[21]

Abbreviations: Polyethylene terephthalate (PET); Polydimethylsiloxane (PDMS); Extracellular matrix (ECM)

673 **Table 2.** Vasculature-on-a-chip microfluidic models used to study the vascular response to

674 bacterial-derived molecules.

Bacterial-Derived Molecule	Biological Process	Device Design	Key Scientific Findings	Ref.
LPS	Vascular inflammation and monocyte transendothelial migration	***Device Design:*** PDMS chip with three channels for endothelial cells, a hydrogel, and media with LPS to create a concentration gradient across the hydrogel. ***Endothelium Geometry:*** 3D rectangular vessel ***ECM:*** Yes, collagen I	- ICAM-1 expression increased 136% and 289% and VE-cadherin expression decreased 43% and 37% after 4 and 8 hours of LPS treatment, respectively. - The number of adhered and transmigrated monocytes increased 57% and the distance migrated into the ECM increased by 95% with LPS treatment.	[24]
LPS	Endothelial glycocalyx degradation	***Device Design:*** PDMS flow cell. ***Endothelium Geometry:*** 2D ***ECM:*** No	- LPS treatment activates endogenous enzyme, HSPE1, which causes glycocalyx shedding and signals the production of the inflammatory cytokine IL-6 - Overexpression of endogenous inhibitor HPSE2 downregulated inflammatory pathways within endothelial cells and protected the vascular endothelium from endothelial glycocalyx shedding due to LPS.	[26]
LPS	Endothelial glycocalyx degradation	***Device Design:*** PDMS chip with single rectangular channel or branched channel network. ***Endothelium Geometry:*** 2D ***ECM:*** No	- Glycocalyx and VE-cadherin expression decreased by 18% and 22%, respectively, due to LPS treatment. - DEX inhibited glycocalyx degradation and VE-cadherin expression induced by LPS.	[27]
fMLP	Neutrophil migration	***Device Design:*** PDMS chip with serpentine channels for generating a chemoattractant gradient that interfaces with a large rectangular observation channel. ***Endothelium Geometry:*** 2D ***ECM:*** No	- Neutrophils preferentially migrate toward fMLP with and without a competing chemoattract gradient, showing prioritization of chemotaxis toward fMLP.	[28]
fMLP	Neutrophil adhesion and transendothelial migration	***Device Design:*** PDMS chip with a physiological microvascular channel network and channels with three parallel compartments. ***Endothelium Geometry:*** 3D, semi-circular vessels ***ECM:*** No	- Neutrophil migration across an activated endothelium toward fMLP was inhibited 85-92% after treatment with protein kinase C δ (PKCδ). - The number of neutrophils adhered to an activated endothelium decreased by 46% after treatment with PKCδ.	[29]
fMLP	Neutrophil transendothelial migration	***Device Design:*** PDMS chip with five rectangular channels that enable measurements of chemoattractant driven neutrophil transendothelial migration and ECM migration ***Endothelium Geometry:*** 3D, rectangular vessel ***ECM:*** Yes, collagen	- An endothelial monolayer was required for neutrophil transendothelial migration toward fMLP into a collagen ECM as there was negligible neutrophil migration into the ECM without an endothelial monolayer. - Lower collagen ECM stiffness increased the speed and distance of neutrophil migration under optimal fMLP concentrations.	[30]
fMLP	Neutrophil transendothelial migration	***Device Design:*** Upper chamber with endothelial cells and lower ECM chamber with varied curvatures and bifurcations. ***Endothelium Geometry:*** 3D, rectangular vessel ***ECM:*** Yes, collagen	- Neutrophil transendothelial migration within a hydrogel was ~600% greater with fMLP present.	[32]
fMLP	Neutrophil transendothelial migration	***Device Design:*** PDMS chip with two side channels, one bottom channel, and a central gel chamber where cells are cultured against a hydrogel.	- High concentrations of chemoattractants induce steep chemoattractant gradients that result in significant neutrophil transendothelial migration. - Multiple chemoattractants show synergistic effects in neutrophil transendothelial migration.	[31]

		Endothelium Geometry: 2D, vertical plane ***ECM:*** Yes, collagen I	- Neutrophils do not migrate into the collagen hydrogel without endothelial cells within the device.	
fMLP	Neutrophil transendothelial migration	***Device Design:*** PDMS chamber with a cylindrical channel lined with endothelium derived from iPSCs suspended in a collagen hydrogel. ***Endothelium Geometry:*** 3D, cylindrical vessel ***ECM:*** Yes, collagen	- Neutrophil transendothelial migration toward fMLP was stronger from whole blood than from purified neutrophils.	[33]
fMLP	Endothelial inflammation and neutrophil transendothelial migration	***Device Design:*** Three adjacent channels separated by Phaseguides to enable endothelial vessels to grow in direct contact with ECM. ***Endothelium Geometry:*** 3D, rectangular vessel ***ECM:*** Yes, collagen I and geltrex	- Neutrophils migrated through geltrex without fMLP but were unable to migrate into collagen I ECM without the presence of endothelial cells and fMLP stimulation. - Neutrophils readily migrated across a geltrex ECM toward fMLP, but 58% fewer neutrophils migrated into a collagen I ECM toward fMLP with some neutrophils stalling within the ECM.	[34]

Abbreviations: Polydimethylsiloxane (PDMS); Extracellular matrix (ECM); Lipopolysaccharides (LPS); N-formyl-methionyl-leucyl-phenylalanine (fMLP); Endo-β-D-glucuronidase heparanase-1 (HPSE1); HPSE1 inhibitor heparanase-2 (HPSE2); Dexmedetomidine (DEX); Induced pluripotent stem cells (iPSCs); Intercellular adhesion molecule-1 (ICAM-1); Vascular endothelial cadherin (VE-cadherin)

679 **Table 3.** Organ-on-a-chip microfluidic models used to study vascular and host tissue responses

680 to bacterial infections.

Model and Cell Types	Device Design	Pathogen & Infection Site	Immune Cell (Y/N; type; location)	Vascular and Immune Responses to Infection	Ref.
Model: Lung ***Mammalian Cells:*** - Human alveolar epithelial cells - Human pulmonary microvascular endothelial cells	- PDMS chip with two channels separated by a flexible membrane with 10 μm pores - Vacuum channels utilized to recapitulate deformation during respiration	***Bacteria:*** *Escherichia coli* ***Infection Site:*** Host tissue (epithelial channel)	Yes - Neutrophils; Bloodstream (endothelial channel)	- Endothelium captures circulating neutrophils indicative of an increase in endothelial ICAM-1 expression during infection. - Neutrophils transmigrate from the endothelial channel into alveolar chamber with directed movement toward bacteria. Neutrophils then clear bacteria via engulfment.	[39]
Model: Lymphatic System ***Mammalian Cells:*** - Lymphatic endothelial cells (HLECs) - Human Umbilical Vein Endothelial Cells (HUVECs)	- Large PDMS chamber filled with collagen or fibronectin hydrogel in which a cylindrical channel is cast and lined with endothelial cells (LumeNEXT device)	***Bacteria:*** *Pseudomonas aeruginosa* ***Infection Site:*** Lymphatic System (Lymphatic endothelial channel)	Yes - Neutrophils; Bloodstream (vascular endothelial channel)	- Bacterial infection resulted in increased endothelial expression of the proinflammatory cytokines IL-1α and IL-6 as well as the anti-inflammatory cytokine IL-10. - Lymphatic endothelial (LECs) vessels increase neutrophil migration to bacteria by 157%, but presence of both HUVEC and LECs vessels results in only a 27% increase, indicating cross talk between the vessels.	[40]
Model: Bladder ***Mammalian Cells:*** - Bladder epithelial cells - Bladder microvascular endothelial cells	- PDMS chip with two rectangular channels separated by a stretchable porous membrane - Negative pressure used to induce strain on the membrane, simulating bladder filling and voiding	***Bacteria*:** Uropathogenic *Escherichia coli* (UPEC) ***Infection Site:*** Host tissue (epithelial channel)	Yes - Neutrophils; Bloodstream (endothelial channel)	- Increased expression of ICAM-1 on endothelial cells enabled for robust neutrophil attachment to endothelial layer and mediated diapedesis of neutrophils during bacterial infection. - UPEC infection model creates a strong proinflammatory cytokine gradient of IL-1α, IL-1β, IL-6, and IL-8 to drive neutrophil recruitment.	[41]
Model: Liver ***Mammalian Cells:*** - Liver epithelial cells - Human Umbilical Vein Endothelial Cells (HUVECs)	-PDMS chip with two rectangular microfluidic channels separated by a thin membrane with 8 μm pores	***Bacteria:*** Methicillin resistant *Staphylococcus aureus* ***Infection Site:*** Bloodstream (endothelial channel)	Yes - Macrophages; Bloodstream (endothelial channel)	- Macrophages increase the concentrations of IL-1β, IL-18, IL-6, and IL-10 proinflammatory cytokines within liver-on-a-chip model.	[42]
Model: Lung ***Mammalian Cells:*** - Lung epithelial cells (NCI-H441) - Human Umbilical Vein Endothelial Cells (HUVECs)	-PDMS chip with two channels separated by a membrane with 8 μm pores	***Bacteria:*** *Staphylococcus aureus* (with influenza virus) ***Infection Site:*** Host tissue (epithelial channel)	Yes – Macrophages; Host tissue (epithelial channel)	- Coinfection of bacteria and a virus led to a significant reduction of VE-cadherin expression and a disrupted endothelial barrier that enabled bacteria to translocate from the lung epithelial channel to the endothelial channel.	[43]
Model: Placenta ***Mammalian Cells:*** -Trophoblasts (placental epithelial cells) - Human Umbilical Vein Endothelial Cells (HUVECs)	-PDMS chip with two rectangular channels separated by a semipermeable membrane with 0.4 μm pores	***Bacteria:*** *Escherichia coli* ***Infection Site:*** Host tissue (epithelial channel)	Yes – Macrophages; Host tissue (epithelial chamber)	- The vascular endothelium has a loss of VE-cadherin, barrier integrity, and some cell death via apoptosis during a bacterial infection. - Bacteria also increased the production of pro-inflammatory cytokines IL-1α, IL-1β, IL-6, IL-8 in the placenta-on-a-chip model.	[44]

Model: Brain ***Mammalian Cells:*** - Neuronal hippocampal cells - Human Umbilical Vein Endothelial Cells (HUVECs)	- Transwell platform with a 3D printed insert chip - 3D printed insert chip is made of a clear resin with a porous membrane as the base	***Bacteria:*** *Escherichia coli* ***Infection Site:*** Bloodstream (endothelial channel)	No	- Bacterial infection resulted in decreased VE-cadherin expression and increased permeability of the endothelium as well as increased expression of the proinflammatory cytokine IL-6 expression and von Willebrand Factor by the endothelial cells.	[45]
Model: Lung ***Mammalian Cells:*** - Cystic Fibrosis bronchial epithelial cells - Pulmonary microvascular endothelial cells	-PDMS chip with two parallel, linear channels separated by a membrane with 7 µm pores	***Bacteria:*** *Pseudomonas aeruginosa* ***Infection Site:*** Host tissue (epithelial channel)	Yes - Polymorphonuclear leukocytes (PMNs); Bloodstream (endothelial channel)	- Bacterial infection in a CF model showed increases in PMN adhesion to the endothelium, but no increase in the transmigration of PMNs. - Bacterial infection increased endothelial expression of the proinflammatory cytokine IL-6.	[46]

Abbreviations: Polydimethylsiloxane (PDMS); Intercellular adhesion molecule-1 (ICAM-1); Vascular endothelial cadherin (VE-cadherin)

Table 4. Organ-on-a-chip microfluidic models used to study the vascular and host responses to bacterial-derived molecules.

Model and Cell Types	Device Design	PAMP & Infection Site	Immune Cell (Y/N; type; location)	Vascular and Immune Responses to Infection	Ref.
Model: Liver ***Mammalian Cells:*** -Murine hepatic epithelial cells - Liver sinusoidal endothelial cells - Kupffer cells	-PDMS chip with two rectangular channels separated by a membrane with 0.4 µm pores	***PAMP*:** LPS ***Infection Site:*** Bloodstream (endothelial channel)	Yes - Neutrophils; Bloodstream (endothelial channel)	- LPS treatment resulted in increased neutrophil adherence to the endothelium and increased neutrophil aggregate size.	[47]
Model: Gut ***Mammalian Cells:*** - Intestinal epithelial cells - Lymphatic microvascular endothelial cells	- PDMS chip with two rectangular channels separated by a thin membrane with 10 µm pores - Pneumatics used to apply cyclic strain to cell culture chamber	***PAMP:*** LPS ***Infection Site:*** Host tissue (epithelial channel)	Yes - Monocytes and Macrophages; Bloodstream (endothelial channel)	- Treatment with LPS, monocytes, and macrophage resulted in increased ICAM-1 expression on endothelium surface.	[49]
Model: Liver ***Mammalian Cells:*** - Hepatocytes, liver epithelial cells - Human Umbilical Vein Endothelial Cells (HUVECs)	- MOTiF biochip made from polystyrol with two chambers separated by a membrane with 8 µm pores	***PAMP:*** LPS ***Infection Site:*** Host tissue (epithelial chamber)	Yes - Monocytes; Bloodstream (endothelial chamber)	- LPS treatment resulted in monocyte recruitment to the endothelium and transendothelial migration of monocytes to the hepatic compartment. - LPS treatment reduces endothelial barrier integrity as evidenced by loss of VE-cadherin and ZO-1.	[50]
Model: Brain ***Mammalian Cells:*** - Human astrocytes - Human Umbilical Vein Endothelial Cells (HUVECs)	-PDMS chip with two channels separated by a polycarbonate membrane with 0.4 µm pores	***PAMP:*** LPS ***Infection Site:*** Bloodstream (endothelial channel)	No	- LPS treatment reduced barrier integrity of vasculature as shown by the discontinuous distribution of tight junction protein, ZO-1, throughout the endothelium after 12 hours of LPS treatment and reduced expression of ZO-1 over time.	[51]
Model: Lung ***Mammalian Cells:*** - Alveolar epithelial cells - Human Umbilical Vein Endothelial Cells (HUVECs)	-PDMS chip with two parallel rectangular microchannels separated by a thin membrane	***PAMP*:** LPS ***Infection Site:*** Host tissue or Bloodstream (epithelial or endothelial channel)	No	- LPS treatment of alveolar epithelial channel resulted in ~5.5x increase in tissue-tissue permeability, but not when LPS was added to the endothelial channel. - LPS treatment increased endothelial expression of ICAM-1 and proinflammatory cytokine expression of IL-6, IL-8, and MCP1.	[52]
Model: Lung ***Mammalian Cells:*** -Human alveolar epithelial cells - Primary human lung microvascular endothelial cells	-PDMS with two rectangular channels separated by a membrane with 0.4 µm pores	***PAMP***: LPS ***Infection Site:*** Host tissue (epithelial channel)	No	- LPS treatment increased endothelial expression of proinflammatory cytokine IL-8 and macrophage stimulating factor (M-CSF) in COPD model as compared to a healthy lung model.	[53]

Abbreviations: Polydimethylsiloxane (PDMS); Pathogen-associated Molecular Pattern (PAMP); Lipopolysaccharides (LPS); Intercellular adhesion molecule-1 (ICAM-1); Vascular endothelial cadherin (VE-cadherin); Zonula occludens tight junction protein-1 (ZO-1); Chronic obstructive pulmonary disease (COPD)

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