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Organoids and derived models to study the microenvironments of bacterial infections

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The microenvironment of an infection is the biological space surrounding the interaction between the pathogen and the host. Focusing on epithelial barriers, the apical microenvironment corresponds to the lumen of the organ, where the pathogen must survive amidst body fluids, microbiota, and cellular secretions. On the opposite side, the basal microenvironment includes stromal cells, endothelial cells of blood vessels, and immune cells recruited to combat infection. The first distinguishing element between the apical and basal domains is the epithelium itself, which consists of polarized cells that secrete different molecules to their apical and basal domains. Organoids and other stem cell-derived culture systems have emerged as valuable models for studying epithelial barriers and their capacities for pathogen recognition, inflammatory signalling, and differentiation. By mimicking multiple aspects of epithelial biology *in vitro*, organoids provide an opportunity to investigate infections from the initial attack to the subsequent defences. This review explores how organoids, stem cell-derived planar cultures, and micro-physiological systems are transforming our understanding of infection microenvironments.

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Introduction

The epithelium is the outermost part of a mucosa that covers every organ in the human body. Its composition, structure, and activities vary by function and anatomical location. For example, epithelial barriers are involved in digestion in the gastrointestinal tract as well as in the transport of oxygen in the lung. Importantly, epithelial barriers also protect the host from invasion by pathogens, which can exploit host adhesion molecules to attach/enter targeted cells. Detection of pathogen-associated antigen triggers an inflammatory response which modifies cellular secretions and, subsequently, the microenvironments on both sides of the epithelium. By modifying cellular secretion, inflammation substantially impacts the microenvironment at both the apical and basal sides of the epithelium. Organoids and other stem cell-driven models have expanded opportunities to model epithelial barrier functions with higher complexity compared to cell lines. Organoids can be generated using adult stem cells (ASCs), embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs). ASCs are generally preferred for gastrointestinal research due to their relative ease and ability to retain tissue-specific traits, whereas iPSCs offer flexibility for studying tissues (such as the heart and the brain) that are more invasive to sample. Since the invention of organoids [1], other stem cell-driven cultures have emerged, including planar cultures and micro-physiological systems (which can be populated with primary or organoid derived cells). Each of these epithelial models has technological advantages resulting from the balance between throughput and culture complexity.

Methodologies to study the infection microenvironment

Traditionally, organoid models feature an outward-facing basolateral domain and an inward-facing apical domain. To mimic the route of infection, a pathogen can be microinjected into the lumen—a process that can be streamlined with robotic systems [2] or combined with disaggregation protocols for luminal pathogen capture [3]. Notably, organoid injection is technically challenging and can induce local inflammatory processes. It is also possible to invert organoid cell polarity to initiate apical-out culture [4]. These methodologies are limited, however, in their capacity to investigate both apical and basal microenvironments due to small scale and closed topology, which limits analysis of luminal contents. Organoid-derived planar cultures overcome this limitation and can be generated by disaggregating organoids into single cells or fragments and culturing them on Transwell inserts featuring porous membranes separating apical and basolateral chambers [5]. To preserve

the apical secretion, apical media can be removed ("airlifting") to initiate culture at the air-liquid interface (ALI). Originally a technique for mimicking physiological airway conditions [6,7], ALI has continued to be successfully applied to different tissues [8,9]. Due to their open configuration, planar cultures present a major advantage for accessing the apical microenvironment of an infection. Secreted mucus—an essential component of the apical microenvironment—creates a sticky shield over cells to trap potential invaders, and such cultures are often referred to as mucosoids [10–12]. Planar culture offers the additional advantage of studying basal cytokine secretion and communication with stromal or immune cells [13]. Finally, there are micro-physiological systems, in which cells are cultured on a chip that includes multiple physiological features such as the presence of stromal or endothelial cell types [14], tissue architecture [15], nutrient flow [16], signalling molecules [17], and gases [18]. Sometimes referred to as organs-on-a-chip, these were originally populated with commercial cell lines but have recently been developed with organoid-derived cells [19]. In each paragraph of this review, we explore organoids, planar cultures and micro-physiological systems in modelling infection of apical and basal microenvironments.

Modelling infection: epithelial barrier disruption

As organoid cells often originate from adult tissues, they maintain the ability to display molecules that are exploited by pathogens for adhesion and internalisation, as well as capacity to recognize pathogens and activate barrier response to infection [20]. Murine endometrial organoids have been microinjected to model infection with *Chlamydia trachomatis*, a gram-negative bacterium linked to endometriosis and infertility [21]. This infection impairs polarity and barrier function, specifically by recruiting ZO-1 tight junction proteins and β-catenin to the invasion site [21]. Murine organoids have also been used to study translocation of injected *Listeria monocytogenes* - gram-positive bacilli that cause foodborne illness, across the intestinal epithelium via interaction of bacterial internalins with host E-cadherin. *L. monocytogenes* exploits E-cadherin recycling pathways, involving dynamin-mediated endocytosis and microtubules. Inhibitors of the latter pathways (such as dynasore and colchicine) reduced bacterial translocation, while key intracellular trafficking protein Rab11 was essential for basolateral bacterial release [22].

Human organoid-derived planar cultures have been used to study Enteroaggregative *Escherichia coli* (EAEC) infection of the colon, which causes diarrhoea and foodborne outbreaks. Here, stem cell maintenance factors WNT3A and RSPO1 were eventually removed from the culture media to induce differentiation of mucus-secreting goblet cells. EAEC employed pilin subunit AAF/II, flagella, dispersin, and the common pilus to achieve host cell adhesion [23]. Such heparin sulphate receptor-mediated adhesion is also dependent upon the mucus layer [24]. Similar human planar cultures were used to model *Shigella flexneri* infection, another foodborne disease. Though it can access the apical domain by disrupting tight junctions, *S. flexneri* preferentially invades basolaterally and replicates in the host cytosol, triggering a strong inflammatory response via detection of IL-8 and other proinflammatory cytokines by neighbouring cells [25].

Finally, human bronchial planar cultures were used to study *Pseudomonas aeruginosa* infection, a leading cause of hospital-acquired pneumonia. *P. aeruginosa* uses Type VI secretion systems to extrude goblet cells and access the basal domain (Figure 1) [26]. Transposon insertion sequencing further revealed that while *P. aeruginosa* biofilm survival relies on sugars and lactate, amino acids are sourced directly from the mucin-rich host microenvironment [27]. Another study used RNA-sequencing of a planar human airway model to highlight the role of quorum sensing molecules as virulence factors in biofilm formation on pseudostratified primary epithelium. Notably, *in vitro* data was compared with data from clinical samples of *P. aeruginosa* infected airways, confirming the reliability of the model [28]. By maintaining key physiological features, such as polarization and pathogen recognition, these studies showcase the power of organoid-derived models in reproducing the pathogen-driven disruption of the epithelial barrier *in vitro*.

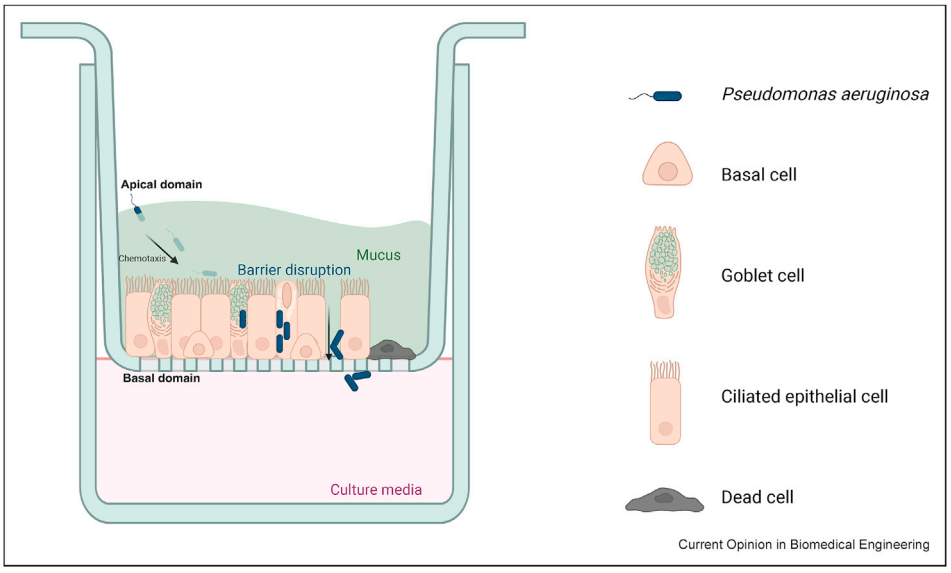
Modelling the apical microenvironment

The microenvironment of a pathogen approaching the epithelial barrier consists of mucosa-associated microbiota and body fluids (e.g., mucus, bile, saliva, urine, tears). To establish infection, pathogens must adapt to this environment which is influenced by cellular secretions during infection. The polarity and structure of organoid-derived models provide new opportunities to study the apical microenvironment of infections.

Helicobacter pylori—a stomach pathogen associated with gastritis, ulcers and cancer—was among the first infections modelled in organoids [29]. Hydrochloric acid secretion, a major gastric defence mechanism to kill bacteria, has been assessed in the human gastric organoid lumen using pH-sensitive dyes [30,31] and real-time pH microsensing [32]. Similarly, analyses of the human intestinal organoid lumen have demonstrated that the *E. coli* RspoS regulatory system, essential for adaptation to environmental stress, mediates bacteria colonization [33].

Additionally, the protective role of the microbiome has been modelled in murine intestinal organoids infected

Figure 1



Legend: Bronchial epithelial progenitor cells were cultured at the air-liquid interface. The model demonstrates differentiation into a pseudostratified mucociliary epithelium resembling human lung tissue, including ciliated, goblet, and basal cells. Planar culture enables dynamic multiphase analysis of infection with *P. aeruginosa,* elucidating mechanisms behind its capacity for chemotaxis, epithelial barrier invasion, and intracellular behaviour. Such a model enables the modelling of pathological features such as goblet cell hyperplasia, making it a versatile model for disease outcomes. Adapted from Swart, A.L. et al., 2024 [26].

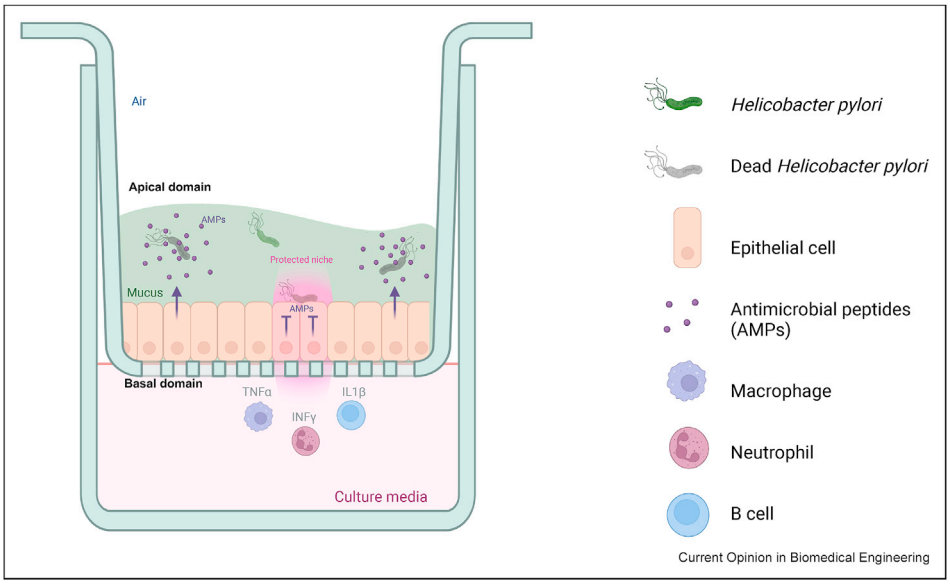
with *Salmonella typhimurium,* a foodborne pathogen. While this infection disrupts organoids, the presence of *Lactobacillus acidophilus* was found to reduce this disruption, suggesting a protective role of this commensal [34]. The same protective role for commensal *Cutibacterium acnes* was shown in an iPSC-derived ALI culture to study *Staphylococcus aureus* infection. This model includes dermal, hypodermal, and stratified squamous epidermal layers representing human skin. *S. aureus* can penetrate the dermis and epidermis, but the presence of *C. acnes* plays an important role in regulating homeostasis and preventing skin damage [35].

Human primary bronchial epithelial cells cultured at the ALI have secreted mucus with protective mechanical properties such as viscoelastic behaviour and shear-thinning, critical for preventing bacterial penetration and responding to peristalsis [6]. Rheological analyses, including macro- and microrheology, provide insight into mucus layer mechanics, including microscale heterogeneities relevant to human–pathogen interactions [36]. The impact of apical mucus has also been shown in human gastric mucosoid cultures [10]. While this mucus is mainly composed of the mucin proteins MUC5AC and MUC6 [10,37], this is altered in inflammatory states. When mucosoids are treated with pro-inflammatory cytokines, epithelial cells secrete antimicrobial peptides into the mucus layer. While antimicrobials provide partial protection, *H. pylori* can evade this defence after colonization [12](Figure 2). These immunological activities of epithelial cells underscore the important role of the apical microenvironment in host–pathogen interactions.

Micro-physiological systems have advanced apical microenvironment modelling by introducing physiological features such as nutrient flow and waste removal, as demonstrated by the gastric organoid flow chip (GOFlowChip) [16]. This device improves human organoid longevity while also enabling infection studies. A further advancement in modelling of the gut lumen was achieved by combining flow with biomimetic architectural patterns. This was achieved via engineered growth scaffolds and techniques such as laser ablation of mixed extracellular matrices or moulding using polydimethylsiloxane stamps [38] to replicate glands, villi, and crypts in the gut [38–40]. These models, capable of maintaining apical-basal compartmentalization, have also proven invaluable for studying parasitic infections [38,39] and the complex interplay between infections and microbiome [40].

As gut microbiota are facultative or obligate anaerobes, another important aspect of the luminal microenvironment is oxygen compartmentalization. This was achieved in the GuMI physiome platform, which supports six simultaneous cultures with separately controlled apical and basolateral microenvironments [41]. By depriving the apical chambers of oxygen, it was possible to obtain oxygen-sensitive anaerobe colonisation by *Faecalibacterium prausnitzii* [41]. Similarly, a

Figure 2



Legend: Gastric epithelial planar cultures at the air-liquid interface secrete antimicrobial peptides (AMPs) as a defence against *Helicobacter pylori* invasion. The accessible apical domain enables the addition of *H. pylori* and apical washes for quantification of AMPs. Access to the basal domain enables the quantification of pro-inflammatory cytokines. Adapted from Vllahu et al., 2024 [12].

system incorporating oxygen gradients within a 3D scaffold facilitated epithelia-microbiota co-culture while preserving luminal curvature [15].

In lung-on-chip models, ALI culture has been used to study *Mycobacterium tuberculosis* infection, where apically-secreted pulmonary surfactant provided significant protection against bacterial invasion [42].

These systems provide unparalleled opportunities to investigate the apical microenvironment of an infection. The continued incorporation of key apical environmental factors such as mucus, oxygen, pH, and tissue architecture will advance the study of diverse pathogens and commensals under conditions closely resembling those *in vivo*.

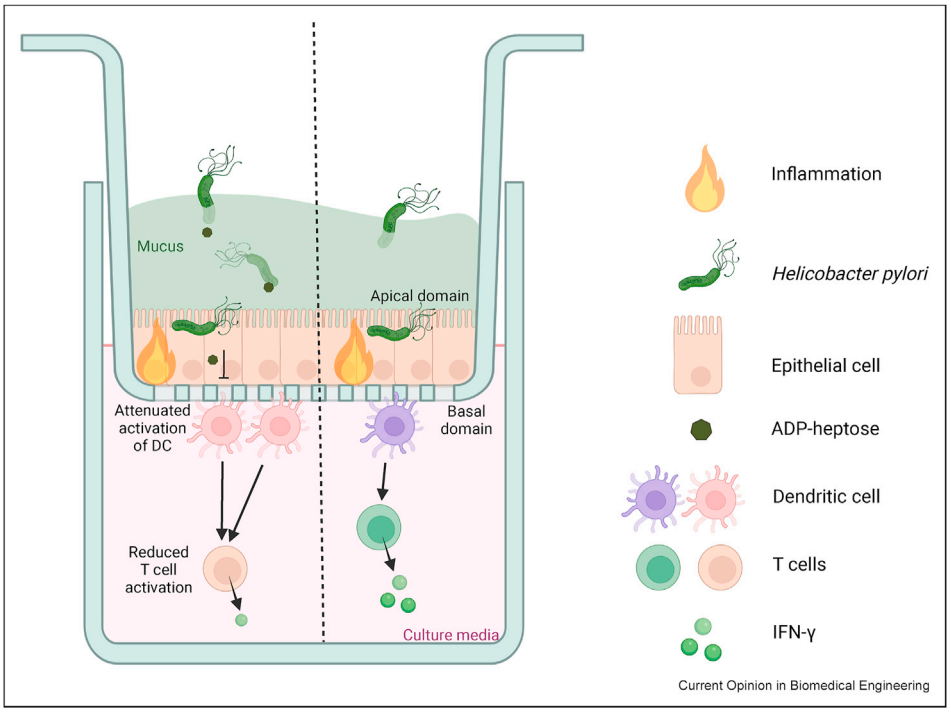
Modelling the basal microenvironment

Modelling the basal microenvironment during infection is crucial to understanding how epithelial cells coordinate the recruitment of underlying immune cells through the use of chemokines and activate inflammation in other cell types, such as endothelial and mesenchymal cells (i.e., fibroblasts). Rich in extracellular matrix and connected to the bloodstream, this environment hosts coordinated responses to combat infection. Co-culturing organoid-derived systems with other cell types enables interrogation of epithelial interactions with other basal mucosal cells. Human gastric organoid co-cultures with immune cells and *H. pylori* have elucidated complex immune interactions in the basal microenvironment, where monocyte-derived dendritic cells (MoDCs) demonstrate their critical role in immunosurveillance. MoDCs exhibit phagocytic activity depending on infection severity, engulfing *H. pylori*-infected cells which secrete CXCL1, CXCL16, CXCL17, and CCL20 in response [43]. Another study has used human stomach mucosoid co-culture with DCs to uncover a sophisticated immune evasion mechanism employed by *H. pylori* (Figure 3) [13]. ADP-heptose—a bacterial metabolite and intermediate in lipopolysaccharide biosynthesis—activates inflammation through binding to the ALPK1 receptor while paradoxically suppressing DC activation, resulting in an impaired Th1 response [44]. Sequential co-culture systems involving epithelia, DCs, and T-cells demonstrated that while *H. pylori* triggers immune recognition and inflammation [45], the host immune response remains insufficient, enabling *H. pylori* to establish lifelong persistence in the stomach unless eradicated with antibiotics.

Intestinal co-cultures with neutrophils have elucidated essential immune responses to *S. flexneri* infection, including basal cytokine secretion and neutrophil transmigration into the lumen—reaction cascades that cannot be observed without apical-basal compartmentalisation [46]. Another neutrophil co-culture with murine endometrial organoids revealed *C. trachomatis* immune evasion through chlamydial proteasome-like activity factor, limiting neutrophil activation and cell death [21].

A model combining human ileal organoids infected with *Salmonella enterica* serovar Typhi, fibroblasts, and

Figure 3



Legend: Gastric epithelial planar cultures at the air-liquid interface were infected by *Helicobacter pylori*, while dendritic cells were cultured in Matrigel at the basal domain prior to the infection. Through co-culturing with DCs, this study revealed that the DCs are actively recruited to the infection site, and there are increased expressions of CD80, CD80 and PD-L1 from the epithelial. Further exploration pinpointed ADP-heptose, a metabolite from *H. pylori* had impacted the DCs activity, by impairing the following activation of Th1 T helper cells. Adapted from Neuper et al., 2024 [13].

peripheral blood mononuclear cells (PBMCs) revealed epithelial-immune cell crosstalk—specifically, increased expression of TNF-α, IL-18, IL-6, and IL-23. This study also suggests the potential role of epigenetic factors in shaping host immune responses [47].

Co-cultures have also shed light on the benefits of commensal bacteria in maintaining intestinal homeostasis. *Lactobacillus reuteri* promotes DC production of IL-10—a key anti-inflammatory cytokine—but interestingly decreases this production when enteroids are microinjected with the bacterium, suggesting that an intact epithelial barrier hinders immune cell stimulation [48]. Furthermore, *L. murinus* stimulates IL-10 release from macrophages, protecting the intestinal epithelium from hypoxia-reoxygenation injury via TLR-2 [49].

Finally, a more complex human organoid blood–brain barrier (BBB) model has been developed in which brain endothelial cells, pericytes, and astrocytes self-organise within an extracellular matrix. This model has properties typical of the BBB, such as tight junction formation, polarized endothelial layers, and selective permeability, providing a physiologically relevant platform to study Lyme disease caused by *Borrelia burgdorferi*. This system demonstrates both infectious activity and barrier integrity loss, offering a platform for studying host–pathogen interactions and testing therapeutics [50].

Newer advancements will allow exploration into the role of tissue-resident immune cells, which exhibit critical phenotypic and functional differences compared to PBMCs. A recent autologous study combining intestinal organoids and resident memory T cells reported differences in resident and blood-derived lymphocyte migration and interaction with organoids [51].

With the advancements of co-culture and studies on cell signalling, organoid-derived models have significantly advanced our knowledge in bacteriology and physiology. These models have provided a unique opportunity to study host–pathogen interactions while maintaining specific architectures of the cells.

Conclusions

The advent of multi-omics integration will also open new avenues for more comprehensive studies of the interactions between the epithelium and luminal microbiota. Newly emerged murine assembloid systems—yet to be optimized for human cells—enable modelling of the interface between the epithelial and stromal tissue compartments [52].More research groups

are beginning to analyse such advanced models with single-cell sequencing, spatial transcriptomics, and proteomics to provide deeper insights into cell-specific responses and interactions during infections.

Considering the wide range of currently available culture technologies, planar cultures remain essential for the study of epithelial infection due to their accessibility, scalability, and suitability for high-throughput drug screening. Despite microphysiological systems gaining attention for their incorporation of *in vivo*-like conditions such as oxygenation and nutrient flow, microphysiological systems still lack widespread use of organoid-derived cells. Recent advancements in micropatterning and bioprinting of planar cultures [53] have demonstrated the ability to recreate tissue architectures and longevity of infection *in vitro*. In these settings, the addition of other cell types (immune, stromal, or endothelial) may represent a promising direction for better mimicry of microscale tissue organization infection modelling.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Katrina Lyon: Writing, visualization, review & editing. **Kai Yee Eng**: Writing, editing. **Francesco Boccellato**: Conceptualization, review & editing, supervision. **Antonella D'Amore**: Writing, review & editing, visualization, supervision.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to check the grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Data availability

No data was used for the research described in the article.

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