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Gastrointestinal biofilms in health and disease

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Abstract | Microorganisms colonize various ecological niches in the human habitat, as they do in nature. Predominant forms of multicellular communities called biofilms colonize human tissue surfaces. The gastrointestinal tract is home to a profusion of microorganisms with intertwined, but not identical, lifestyles: as isolated planktonic cells, as biofilms and in biofilm-dispersed form. It is therefore of major importance in understanding homeostatic and altered host–microorganism interactions to consider not only the planktonic lifestyle, but also biofilms and biofilm-dispersed forms. In this Review, we discuss the natural organization of microorganisms at gastrointestinal surfaces, stratification of microbiota taxonomy, biogeographical localization and trans-kingdom interactions occurring within the biofilm habitat. We also discuss existing models used to study biofilms. We assess the contribution of the host–mucosa biofilm relationship to gut homeostasis and to diseases. In addition, we describe how host factors can shape the organization, structure and composition of mucosal biofilms, and how biofilms themselves are implicated in a variety of homeostatic and pathological processes in the gut. Future studies characterizing biofilm nature, physical properties, composition and intrinsic communication could shed new light on gut physiology and lead to potential novel therapeutic options for gastrointestinal diseases.

There is evidence that bacteria have been able to form sessile communities from the beginning of life on Earth. Still, it is less than 50 years since Costerton et al. coined the term 'biofilm' as a simple expression to describe the immense variety of microbial aggregates on surfaces[1]. Environmental microbiologists have since reported the presence of biofilms in almost all natural and industrial ecosystems[2,3]. The dominance of biofilms over the free-living (planktonic) mode of life on Earth was quantitatively assessed in 2019 (REF.[3]). Even in liquid natural environments, microbial biomass is almost exclusively found under a biofilm phenotype rather than freely swimming or floating[3].

Biofilms are extremely complex microbial ecosystems that form a 'biological film' on surfaces. Members of this community are characterized by distinct gene expression profiles, growth rate, interacting behaviour and/or structural appearance compared with single isolated cells (that is, planktonic)[2–5]. Natural biofilms vary greatly in structure and composition from one environmental niche to another. The biofilm matrix might be of microbial origin or contain non-cellular materials such as mineral or organic particles[2,3] as well as host components for biofilms interacting with live surfaces[6]. The definitions of biofilms in the literature vary, ranging from the structural (such as 'surface-attached matrix-embedded community')[2,5] to the ecological (such as 'complex differentiated communities'[4], in which communicative networks lead to a higher level of organization than isolated cells[3]). In agreement with a number of reports[3,6–11], the definition of gut biofilms used in this Review is kept general: aggregates of microorganisms embedded in a biopolymer matrix composed of host and microbial compounds, and adherent to food particles, mucus or epithelia.

Abnormal and deleterious biofilms in contact with mucosal tissues have long been associated with human diseases, including surgical implant infections, gum diseases, catheter-induced urinary tract and lung infections[11] and some intestinal diseases. Nevertheless, the biofilm phenotype also contributes to homeostasis in the gut, organizing colonization resistance, community stability and resilience, host defence maturation, food digestion and chemical drug modifications. These beneficial roles of biofilms have yet to be fully incorporated into our current perception of the gut microbiota. Several studies have now clearly distinguished between faecal and mucosa-attached microbial communities in terms of composition, genetics and behaviour[12–15]. Thus, the mucosal polymicrobial community is particularly

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Key points

- Bacteria adopt different lifestyles in their natural habitats, from single planktonic cells to biofilm communities.
- Polymicrobial biofilms naturally grow throughout the gastrointestinal tract, both at the epithelial surface and in the lumen as mucin-attached and food particle-attached colonies.
- The biofilm lifestyle influences metabolic behaviour of the microbiota but more research is needed to characterize gut biofilm-specific metabolites and their effects on the host response in health and disease.
- Polymicrobial and trans-kingdom interactions occur in gut biofilms; deciphering the nature of such interactions might improve our current understanding of the homeostatic relationship between the host and its gut microbiota.
- Abnormal biofilm features are associated with gastrointestinal diseases; characterization of biofilm alterations and cause-to-effect studies are warranted to elucidate their role in pathophysiology.
- Investigating biogeographical redistribution of biofilms at mucosal surfaces might provide new tools to characterize microbial alterations associated with gastrointestinal diseases and options for therapeutic intervention.

Biofilm
A microbial lifestyle in which microorganisms are embedded in a biopolymer matrix, attached to surfaces, engaged in collective behaviour (for example, communication, cooperation, competition and differentiation) and able to persist even in hostile environments.

Planktonic
Free-swimming, free-floating, single-cell mode of life of microorganisms.

Biofilm matrix
The biopolymer substance containing communities of microorganisms assembled as a biofilm. The chemical complexity of the matrix is not fully appreciated for in vivo biofilms.

Polymicrobial
A microbial community that harbours diversity in terms of species and/or strain content.

Inflammatory bowel disease
(IBD). Chronic inflammatory disorder of the gastrointestinal tract, with multifactorial aetiology that includes genetic susceptibility and environmental factors. Associated with a displacement of gut ecology and an uncontrolled activation of the immune system.

Oxygen tension
Variable oxygenation profile over the intestinal landscape, affecting host immunity and gut microbiota.

important for the search for novel biomarker signatures of diseases (for example, colorectal cancer (CRC) and inflammatory bowel disease (IBD)). Moving from correlations to a potential causal aetiology in these diseases will require further studies of mucosal communities, focusing on the interactions between the host and the microbiota under its natural biofilm phenotype. Hence, studying biofilms in the context of a healthy and diseased gut could initiate a paradigm shift in the field of gastroenterology. In this Review, tools and methods to study biofilms are assessed, together with the biological and metabolic characteristics of gut mucosal biofilms. Host regulation by biofilms and biofilm regulation by the host are discussed in the context of gut health and disease, highlighting the importance of further studies on biofilm communities in the development of new therapeutic strategies.

Tools and methods to study gut biofilms

Methods and technologies to study biofilms (TABLE 1) have evolved considerably as a consequence of the development of technology, including new imaging techniques and engineered microfluidic ecosystem tools[16]. It is now possible to obtain the 3D structure of biofilms, as well as detailed knowledge on the interaction of molecules in situ, down to the micrometre and atomic levels. With the help of both traditional and newer models, we can achieve a deeper understanding of the overall genotypic and phenotypic characteristics of microorganisms within a biofilm community, such as the biofilm metabolome[17,18], proteome[19–22] and transcriptome[23–25]. Owing to the acquisition of such complex and numerous data, extensive use of bioinformatics tools will have to be promoted. In addition, models are now evolving towards devices that better mimic in vivo gut conditions (for example, shear stress, oxygen tension and host cells) to help understand the interplay of mucosal tissue with its surrounding biofilm. These models are expected to provide comprehensive knowledge and new possibilities to improve our current understanding of the homeostatic relationship between a host and its microbial biofilms.

In vitro models. A large number of studies have used microtitre plates since the ability of various microorganisms to form biofilms on their surface was described in 1998 by O'Toole and Kolter[26]. The microtitre-derived model, such as the Calgary biofilm device, was then designed to facilitate active biofilm formation at the air–liquid interface[27]. This device has been used to grow human colon-associated microbiota ex vivo in its polymicrobial biofilm phenotype[18,28–31]. Drip-flow[32] and rotating reactors[33] reduce the risks of nutrient exhaustion and introduce dynamic forces into biofilm models. Fermenter models were originally developed as a large-scale platform to continuously grow intestinal microbiota under a biofilm phenotype[34]. Newer fermenter-based platforms enable multi-compartment systems and tunable conditions of anaerobiosis, shear forces, temperature and pH[35–37]. Microfluidic devices[38,39] and chip-based chemostat models[40–42] are miniaturized forms of previously described flow systems (TABLE 1).

In vivo models. The evolutionarily conserved mucosal defence towards gut biofilm factors is studied using non-mammalian models with a reduced host defence complexity: *Caenorhabditis elegans*[18,43,44], *Drosophila melanogaster*[45], honeybee[46] and *Danio rerio* (zebrafish)[47,48]. However, these models cannot be used for gut bacterial species whose optimal growth conditions (such as temperature, oxygen and pH) differ in these organisms and in the mammalian intestine. Although not directly related to the gastrointestinal tract, various models of device-related biofilms have been developed in rodents[49], larger mammals (such as sheep)[50], and non-human primates[51]. All of these approaches reveal that in vivo biofilms are probably composed not only of microbial elements but also of host molecules such as phagocytes, nucleic acid elements, fibrin meshes and host immunoglobulins[29,52–55]. Imaging studies using taxon-specific 16S ribosomal DNA (rDNA) fluorescence in situ hybridization (FISH), mucus staining (such as immunostaining of mucins, non-specific glycoprotein staining or periodic acid–Schiff–Alcian blue staining) and electron microscopy have provided insights into the spatial organization of in vivo biofilms in the gastrointestinal tract of animals and humans[18,29,31,53,56–66].

Ex vivo models. In vitro and in vivo models have limitations, which motivated the development of more sophisticated ex vivo models using human cells, tissues or organs extracted from an organism and placed in an artificial environment[67,68]. Such ex vivo models have enabled, for instance, a better understanding of the contribution of mucins to microbial biogeography using human colonic explants[69,70]. In contrast to in vivo models, the environmental conditions of these ex vivo models are controllable, providing a good alternative to the use of living organisms[71–73]. These tissue-based models are currently evolving towards engineered chip-based models, incorporating human tissue and cells together with their mucosal microbiota grown under its sessile biofilm phenotype[74,75]. These models will surely represent the next generation of platforms for biologically relevant studies on gut biofilms and on the gut microbiota more generally.

Table 1 | **Characteristics and critical description of models for gut biofilm studies**

Model	Features or species	Possible biofilm measures			Limitations	Possible improvements
		Imaging	Total biomass	Biofilm-dispersed recovery		
Microtitre plates	Biofilm growth in the bottom of the plate Media volume low Static model	Yes[a]	Low	Yes	Not dynamic Nutrient starvation Sedimentation of bacteria Not suitable for long-term cultures	Possible coating of surfaces with abiotic or biotic elements
	Air–liquid interface biofilm (Calgary biofilm device) Media volume low	Yes	Low	Yes	Not dynamic Nutrient starvation Not suitable for long-term cultures of biofilm	
Microscopy slides	Air–liquid interface biofilm Media volume medium	Yes	Medium	Yes	Not dynamic Nutrient starvation Limited sample replicates	Possible coating of surfaces with abiotic or biotic elements
Drip-flow and rotating reactors	Biofilm growth on coupons Media volume high	Yes	High	No	Only one surface per experiment Extensive equipment Few commercial supplies	Anaerobic cultures Culture of polymicrobial communities
Chemostat-based models	Biofilm growth on recipient surfaces Media volume high to very high	Yes	Very high	No	Limited access to biofilms Extensive equipment Few commercial supplies	Size reduction Possible coating with cells
Microfluidic devices	Biofilm growth on various surface materials Media volume low to high	Yes	Very low	No	High technical skills required Few commercial supplies Mostly 2D biofilms	Possible coating with cells Possibilities for probing pH, O_2 Cost reduction
Chip-based models	Biofilm growth on various surface materials Media volume low to high	No	Very low	No	High technical skills required Limited access to biofilms Expensive No commercial supplies	3D printing of organ architecture Standardization of protocols Use of primary cells and/or organoids for the host compartment
Non-mammalian	*Drosophila melanogaster* Zebrafish (*Danio rerio*) *Caenorhabditis elegans*	Yes (in situ)	NA	NA	Non-human Simplified host immune defence	Host genetic manipulation
Mammalian	Rodents	Yes (in situ)	NA	NA	Non-human	
Intestinal loop	Rodents	Yes (in situ)	NA	NA	Non-human	Regulation and probing of tissue environment
Tissue explants	Humans, rodents	Yes (in situ)	NA	NA	Ethics and safety concerns for human collection Short-term experiments	Developing conditions for longer term cultures

NA, not applicable. [a]Need for microscopy-compatible material for imaging.

Mucins
A class of epithelial gel-forming and non-gel-forming proteins that confer to mucus its viscous hydrophobic property, making it a physical barrier to microorganisms.

Gut: a natural support for biofilms

As they do in nature, intestinal microorganisms can use all of their possible lifestyles in the gut (FIGS 1,2). They can be fully embedded in biofilms, they can be fully plank-tonic, or they can have been recently dispersed from bio-films. This latter status can be considered as a distinct phenotype that naturally occurs between biofilms and the planktonic lifestyle[23,24]. Several factors (for exam-ple, fatty acid signalling, oxygen, nutrient availability, nitric oxide, iron and proteases) are known to induce biofilm bacteria dispersion[76]. However, the precise mechanisms and inducers leading to dispersion of bio-films in the gastrointestinal environment have to be fully investigated. Important questions to ask when consider-ing gut microorganisms living on intestinal surfaces are whether there is a specific host response to biofilms and whether biofilm-dispersed bacteria have a different phenotype to biofilms or purely planktonic bacteria.

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Microbial planktonic cells in suspension can be viewed as a temporary state of a population actively searching for a new habitat for biofilm formation[4] or even as an artificial phenotype generated by extremely favourable laboratory in vitro culture conditions[77]. Although not yet investigated for gut microbiota, examples of how the host might respond differently to biofilms versus planktonic cells include in vitro studies in which immune cells demonstrated reduced oxidative burst or neutrophil extracellular trap responses to biofilms[78–80].

The biofilm-dispersed phenotype has strikingly different characteristics from its biofilm and planktonic counterparts, including increased antimicrobial resistance, iron intake capacity and overall virulence[18,23,24,81,82]. This observation is likely to be of large importance for human gut diseases that are associated with an alteration of commensal biofilms. Thus, it is crucial to identify the factors that can induce bacterial dispersion from biofilms, whether these factors originate from the host (for example, from immune cells, neuronal cells, fibroblasts or enterocytes) or from the environment (for example, from the diet, pollutants or invading pathogens). Such factors can be associated with pathologies, but they can also be released in health.

Biofilms interacting with gastrointestinal surfaces can be composed of hundreds to thousands of cells encased in a mucin-rich matrix, but they can also contain fewer cells arranged as small clusters and aggregates around mucin aggregates in the lumen or attached to food particles[10,53,55,63,83–85] (FIG. 2). Even in regions of the gastrointestinal tract in which the overall taxonomic diversity is poor (that is, the stomach and upper gastrointestinal tract), biofilms can still be heterogeneous and be composed of cells with different phenotypes, several genotypic variants of a strain and/or different strains of the same species[3–5]. This heterogeneity, along with the complex spatial structure of biofilms, leads to cell–cell interactions and the emergence of social behaviours, such as cooperation, competition and cheating[86], all of which are important to our understanding of microbiota-associated health and disease.

Specific taxonomy at the mucosal surface. Today, sequencing gut microbiota composition in faeces is routine[87], and it is well established that mucosal and faecal microbiota are different in terms of composition and repertoire of microbial genes[12,13,15,88,89]. The taxonomy of mucosal biofilms has to be inferred from mucosal sampling, as opposed to faecal sequencing. The oropharynx tissue of a healthy human adult is usually colonized with anaerobic commensal genera such as *Veillonella*, *Prevotella*, *Leptotrichia* and *Fusobacterium* as well as with potential pathobionts such as *Streptococcus*, *Haemophilus* and *Neisseria*[90,91]. In the mouse small intestine, metabolism of polysaccharides and amino acids is favoured by facultative anaerobes such as proteobacteria and Lactobacillales[92]. Laser capture microdissection in the mouse colon identified mucosal communities enriched in members of *Clostridium* cluster XIVa (such as species of the Lachnospiraceae and Ruminococcaceae families) and to a lesser extent in Bacteroidaceae, Enterococcaceae and Lactobacillaceae[15]. Similar approaches in the human colon revealed that healthy mucosal biopsy samples were enriched in proteobacteria (in the ascending colon), proteobacteria and actinobacteria (in the left descending colon) and Firmicutes (albeit less abundant than in the faecal samples)[14]. These findings were confirmed by microscopy, as colon biopsy samples from healthy humans were found to be covered by thin biofilms consisting mostly of Bacteroidetes, Lachnospiraceae and Enterobacteriaceae in the right ascending colon, and Bacteroidetes and Lachnospiraceae in the left descending colon[60].

Stability and resilience. In addition to taxonomic considerations, microbiota stability and microbiota resilience is of crucial interest[93]. From an ecological perspective, this perpetual competition within a biofilm leads to stability of the overall community, in part due to partitioning of the available ecological niches[94,95]. In multispecies biofilm settings, interactions between bacteria have a key role in the successful outcome of the community as some organisms depend on the metabolic activity of
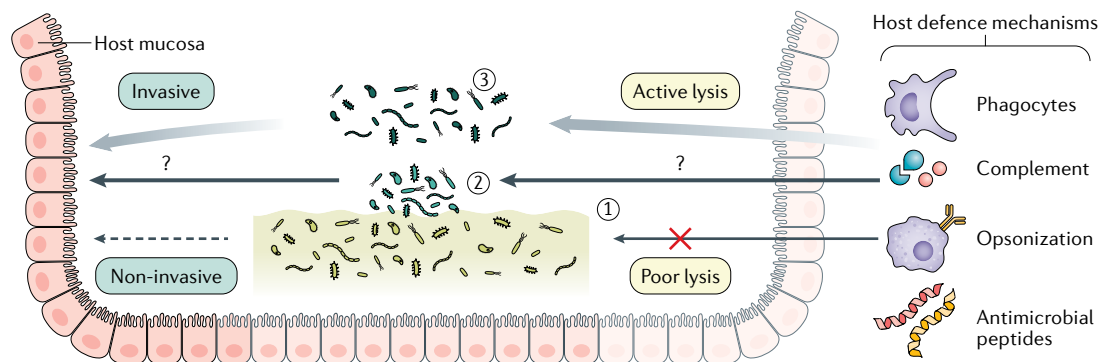

Fig. 1 | **Schematic representation of the three possible bacterial lifestyles at the gut mucosal surface under healthy conditions and their relationship with the host.** Microbiota living at the gastrointestinal surface might adopt one of three different lifestyles. They can be organized as a network of communicating cells leading to a community behaviour, known as the biofilm phenotype (lifestyle 1). They can be recently dispersed from a biofilm and migrate towards the lumen or the host, known as biofilm-dispersed (lifestyle 2). Or they can engage in a free-living, free-floating planktonic phenotype (lifestyle 3). These microbiota lifestyles are associated with inherent properties (motility, adherence and metabolism) that can influence a different host response towards them.
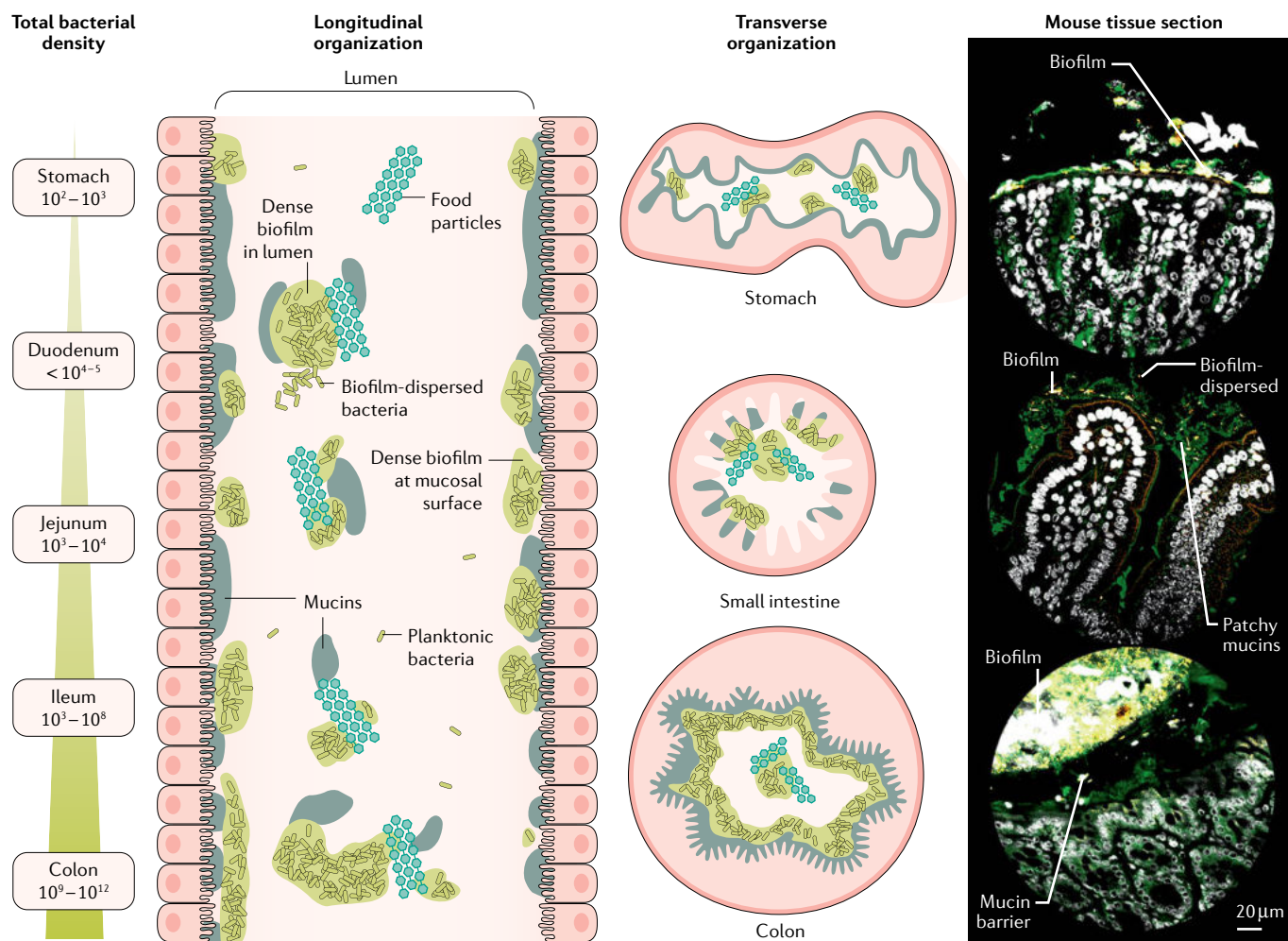
Total bacterial density

- Stomach $10^2 – 10^3$
- Duodenum $< 10^{4–5}$
- Jejunum $10^3 – 10^4$
- Ileum $10^3 – 10^8$
- Colon $10^9 – 10^{12}$

Longitudinal organization

Lumen

Dense biofilm in lumen — Food particles

Biofilm-dispersed bacteria

Dense biofilm at mucosal surface

Mucins — Planktonic bacteria

Transverse organization

Stomach

Small intestine

Colon

Mouse tissue section

Biofilm

Biofilm — Biofilm-dispersed

Biofilm — Patchy mucins

Mucin barrier — 20 µm

Fig. 2 | **Homeostatic gut microbiota biofilms throughout the gastrointestinal tract.** Over the landscape of the entire digestive tract, the commensal microbiota living at the mucosal surface consists of communities that are biogeographically stratified along the longitudinal (centre-left panel) and transverse (centre-right) axes. The microbial density and diversity increase from the stomach to the colon (left panel). Mucosal microbiota form scattered biofilm aggregates of various sizes in the stomach and small intestine and a rather dense and uniform biofilm community at the beginning of the large intestine. The mucus layer at the mucosal surface also varies across the digestive tract as it forms a continuous gel-forming layer in the stomach and colon, with more loose adherent aggregates in the small intestine (right inserts, from top to bottom: stomach, duodenal and colon sections). In the lumen, and throughout the entire digestive tract, microbial biofilms are intimately linked with mucin aggregates and food particles. Microscopy inserts are representative samples from the mouse digestive tract. In grey are host nuclei, in green are glycoproteins, and in yellow are bacteria stained by 16S ribosomal DNA fluorescence in situ hybridization.

Probiotic
Microorganisms providing health benefits through direct or indirect effects on intestinal pathobionts, pathogens or host cells.

other organisms to grow[94,95]. Indeed, anaerobe isolates of *Bacteroides*, *Clostridium*, *Fusobacterium*, *Finegoldia*, *Prevotella*, and *Veillonella* recovered from human faecal samples differentially adhere and form mono-species biofilms in vitro. Interestingly, this work using gut-associated taxa suggests that bacterial species that would not form a biofilm by themselves could benefit from living in a mixed biofilm community along with other strong biofilm-forming species[96]. Mucosa-associated communities are likely to have a key role in promoting microbiome resilience after antibiotic treatment, faecal microbiota transplantation, and probiotic colonization[64,97,98]. In addition to theories about the role of biofilms in the human appendix[62,99] (BOX 1), we can expect important discoveries regarding the significance of biofilm lifestyle in stability and resilience properties of the microbiota associated with gut homeostasis to follow.

Colonization resistance. A biofilm is a physical structure with a chemical composition and specific metabolism that functions as a protective barrier against environmental molecules, immune cells, predatory protists and bacteria[2,77] (FIG. 1). Commensals are indeed armed with many competitive strategies, such as rapid growth to gain access to nutrients and direct aggression to exclude other competitive species from their environment, to benefit from or to exploit other competitors[94]. This biofilm-induced protection against invaders is exemplified by the colonization resistance of intestinal microbiota against enteropathogens[100]. This resistance is mediated in vivo in mice by both direct mechanisms (such as production of bacteriocins, production of short-chain fatty acids (SCFAs), conversion of bile acids, and expression of type VI secretion system) and indirect mechanisms (modulation of host physiology and immunity)[101]. Commensals also compete in vivo

Box 1 | **The special case of the human appendix**

The human appendix harbours a very dense biofilm compared with other areas of the human colon, which has led to speculation with regard to its function. The appendix biofilms are at a protected location, in low contact with food particles in faeces and spared from diarrhoeal clearance. Indeed, some researchers have proposed that biofilms in the appendix might act as a 'safe house' for commensal bacteria, playing a key part in reconstitution of a normal microbiota, for instance after a gastrointestinal infection[62,99]. As the human appendix is frequently removed, this proposed evolutionary function might not be vitally important[99]. However, the human appendix cannot be considered a vestigial organ. The high density of appendix mucins and secreted immunoglobulin A assists biofilm formation by increasing adhesive growth of agglutinated microorganisms, and could therefore aid the development of the immune system in the early days of life. Indeed, one hypothesis is that commensal biofilms in the human appendix stimulate B cells in germinal centres to produce antibodies, ensuring the normal development of the immune system postnatally[99].

with pathogens for nutrients and access to metals and for sequestration of residual oxygen[100–102]. Finally, in the mouse colon, the biofilm phenotype of commensal strains (specifically, *Escherichia coli*) drives colonization resistance against related taxa (in this case, enteroaggregative *E. coli* and *Klebsiella pneumoniae*) occupying a similar ecological niche[103].

Polymicrobial and trans-kingdom interactions. The microbiota associated with gastrointestinal surfaces contains all three domains of life (Archaea, Prokarya and Eukarya), and viruses[87]. Current knowledge is almost exclusively focused on Prokarya, although scientific interest in the role of other kingdoms in homeostasis and disease is emerging[104–106]. Co-evolution has generated powerful mechanisms, with many species existing only in association with human hosts. Bacteria within intestinal biofilms interact with each other and with human cells. These trans-kingdom interactions probably have important roles in maintaining digestive health, as well as in disease when these homeostatic interactions are disrupted (known as dysbiosis). The mechanisms by which this occurs remain largely obscure, although it is becoming clear that these microbiota–host interactions are highly dependent on the nature and spatial organization of bacterial communities as biofilm structures[6,8,9,61,107].

Biogeography. Variability in microbial composition is observed between each individual, but also across the landscape of a single human organ such as that in the digestive tract[87]. Studies in mice and non-human primates using microscopy and sequencing approaches have demonstrated that the commensal microbial communities are indeed geographically stratified throughout the gastrointestinal tract, on different spatial scales and axes[12,85,107] (FIGS 1,2). The highest microbial density is generally found in mucus-rich regions, near the gastrointestinal epithelium and around food particles, forming patchy aggregates in the lumen (FIG. 2). Hence, gut biofilms utilize the gel-forming mucus layers as a matrix and a substratum for their attachment in vivo[10,29,56,65]. At micrometre scale, each microhabitat is occupied by communities of mixed taxa, which physically agglutinate to each other. This organization is unlikely to be random, although its biological importance is unclear[61,107]. Overall, the biogeography in mice is believed to be

Dysbiosis
A term used to describe taxonomic, metabolic or structural imbalances that characterize the microbiota associated with a disease condition.

Substratum
The biotic or abiotic surface on which a biofilm can form.

under the control of dynamic factors including motility, flux of mucus, gastrointestinal epithelial cell secretions, and affinities for host and food particles, as well as ecological interactions between the microorganisms[61]. Mucosa-associated microbiota biogeography, composition and metabolic activities are also subject to daily oscillations that help synchronize intestinal physiology around the circadian clock[108]. New methods for imaging thicker tissue sections, enabling full visualization of crypts and biopsy samples, have helped obtain 3D images of mucosa-associated biofilms[109]. Now, important work remains to precisely describe microbial microhabitats throughout the gastrointestinal tract, to define the exact localization of each taxa within mucosal biofilms, and to better understand the physiological contribution of microbial biogeography to intestinal health (FIG. 3).

Biofilm components inducing host response

Intestinal biofilms stimulate a unique mucosal response that we are only just beginning to understand. Host mucosal defences recognize and respond differently to each component of gut biofilms, even if these molecules can be hidden within a biofilm matrix. In the context of homeostasis, these interactions probably have a substantial role in educating host defence and in shaping gut physiology in general. Current knowledge was made possible by studies using various models of in vitro biofilms, gut-relevant bacterial species, host cells, tissues and animals. Whether in vivo biofilm components induce host responses equivalent to those observed in in vitro models is a complicated but important question that still needs to be explored.

Polysaccharides. A biofilm matrix can be composed of various families of polysaccharides that are involved in numerous structural and metabolic functions[110]. Cellulose could serve as a public good for *Salmonella enterica* subsp. *enterica* ser. Typhimurium biofilms and act as a barrier to keep away non-producers of matrix components from established biofilms, as has been demonstrated in vitro[111]. Glycosaminoglycans constitute a molecular camouflage for pathogens (for example, *E. coli*, *Pasteurella multocida* and *Streptococcus* spp.) during infection in mice[112]. Secreted polysaccharides (also known as exopolysaccharides) from *Lactobacillus plantarum*, *Burkholderia cepacia* and *Salmonella enterica* subsp. *enterica* ser. Typhi decreased the production of cytokines and inhibited chemotaxis and oxygen burst response of human and porcine immune cells in vitro[113,114] and in human colonic tissue explants[115]. Identifying polysaccharides that are naturally produced by gut biofilms in vivo, and understanding the host response towards such components, could be useful for the development of new therapies to prevent pathogenic biofilm growth at mucosal surfaces.

Proteins. Matrix-associated proteins are important components of biofilm matrix in vitro, with a total biomass largely equivalent to that of polysaccharides[110]. They have a structural function, promote bacterial dispersion, protect against host mediators and participate in cell–cell communications[116,117]. The matrix-associated proteome

of a given bacteria growing as a biofilm is different from the secretome of equivalent planktonic cultures[118]. Expression of virulent proteins, for instance, is upregulated in biofilms, with an expression profile that differs between biofilms grown as monospecies or polymicrobial with *Candida albicans*[118,119]. The host response to protein components of the in vivo biofilm can be divided into innate and adaptive mechanisms. The innate response

and antimicrobial defence proteins can be mediated by activation of the formyl peptide receptors, which can recognize biofilm-associated oligopeptides containing *N*-formylmethionine derivatives in vitro[120]. Both innate and adaptive immune responses against *Staphylococcus aureus* were proven to be different upon stimulation by biofilms compared with planktonic cultures of the same pathogen, as demonstrated in mouse leukocytes
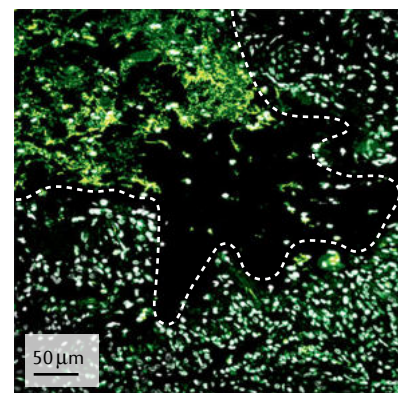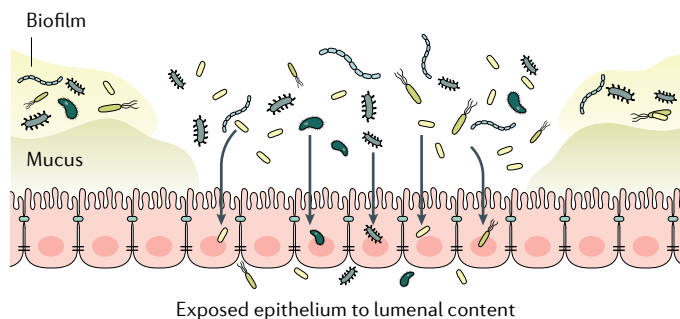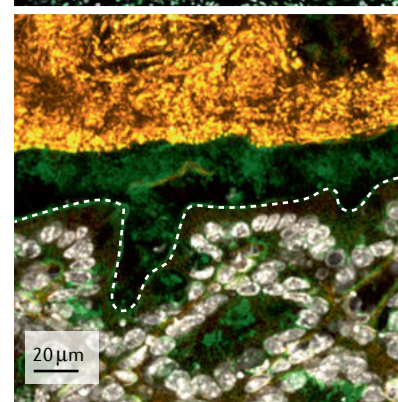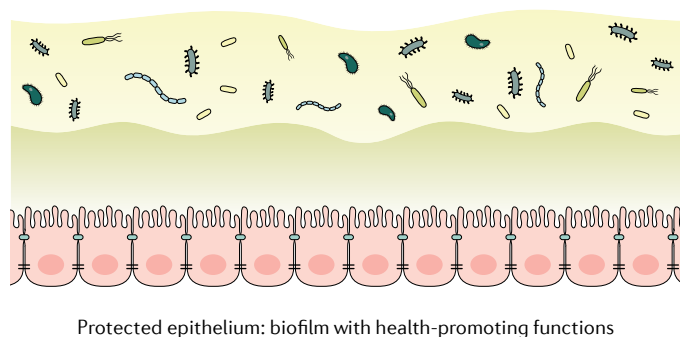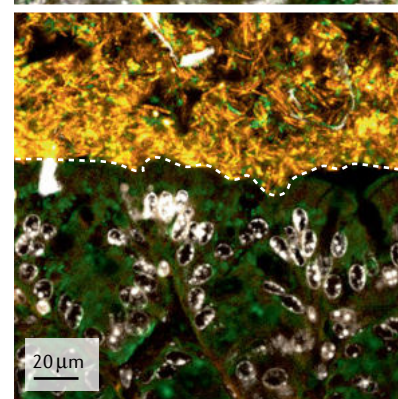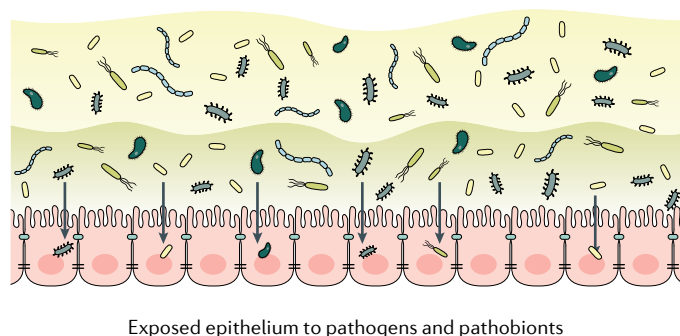

Fig. 3 | **Schematic of biofilm biogeography: a marker of mucosal health in the distal colon.** In healthy circumstances, an ecologically stable microbial biofilm interacts with a sterile epithelium and mucus layer (section of a healthy mouse distal colon). This symbiotic relationship and organization are central to numerous health-promoting functions (middle panel). Two possible scenarios of an altered biofilm organization can occur and be associated with disease. On the one hand, the mucosal biofilm can be completely altered and form aggregates of various sizes, some of which might abnormally make contact with the host (section of a dinitrobenzene sulfonic acid-exposed rat colon) (top panel). On the other hand, a dense biofilm might be visible, but bacteria can colonize the inner sterile mucus layer and can potentially come into contact with tissues (section of a thrombin inhibitor-exposed mouse colon) (bottom panel). Both disease-associated biogeographic changes of the microbiota might predispose the host epithelium to luminal contents, enteropathogens and pathobionts, all of which can play a triggering or contributing role in diseases. Microscopy inserts are representative samples from rat and mouse digestive tract. In grey are host nuclei, in green are glycoproteins, and in yellow are bacteria stained by 16S ribosomal DNA fluorescence in situ hybridization. Dashed white lines delimit the tissue surface.

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and in rabbits. These studies demonstrated that twenty *S. aureus* biofilm-specific proteins are immunogenic and elicit a specific host response[121,122]. Interestingly, a set of microbial intracellular proteins (specifically, ribosomal proteins, RNA polymerase and arginine deiminase) encased within biofilm matrix of human gut polymicrobial biofilms grown in vitro can also be cleaved by the intestinal epithelial protease thrombin[31]. This finding could suggest that biofilm-associated bacteria use host proteases to cleave microbial proteins encased within biofilm matrices. Whether the purpose of this cleavage is the activation or the degradation of microbial proteins still has to be investigated. However, this observation provides a potential additional example of protein-based biofilm–host signalling.

Membrane vesicles and hydrophobic compounds. Membrane vesicles (also known as outer membrane vesicles) are released by bacteria and archaea and are important components of their biofilm matrices[123]. Matrix-associated proteins largely derive from membrane vesicles, as has been illustrated for *Pseudomonas aeruginosa* biofilms in vitro[19]. Membrane vesicle production is dependent on the bacterial stress response. The putative biological role of membrane vesicles in vivo could be to protect secreted bacterial molecules from degradation or to act as a decoy for antimicrobials[124]. Activation of human macrophages is enhanced in vitro by *P. aeruginosa* membrane vesicles compared with their activation with soluble molecules[125]. Although poorly illustrated in the context of intestinal physiology, it is expected that studies of biofilm membrane vesicles will enhance our understanding of the contribution of intestinal biofilm components to gut homeostasis. Other hydrophobic compounds present in biofilm matrices are glycolipids and peptidolipids. Rhamnolipids, a class of glycolipid surfactants (such as viscosin and surfactin), are present in proteobacteria biofilms. They mediate swarming (a collective motility behaviour) and increase biofilm dispersion in vitro[126,127]. Peptidolipids produced in vitro by *Staphylococcus* spp. participate in the hydrophobicity of their biofilm surface[128]. This process has a role in establishing the impenetrability of biofilms to environmental molecules[5,110].

Nucleic acids. Extracellular nucleic acids function as structural scaffolds for biofilm matrix in several gut-relevant genera such as *Escherichia*, *Citrobacter*, *Listeria*, *Enterococcus*, *Streptococcus*, *Pseudomonas* and *Neisseria*[129]. They facilitate horizontal gene transfer and contribute to bacterial adhesion and aggregation to surfaces[110,129]. Unmethylated cytosine–phosphate guanine (CpG) motifs in extracellular DNA from *P. aeruginosa* biofilm matrix can trigger activation of TLR9 in human neutrophils in vitro[130]. As demonstrated in vitro for *P. aeruginosa*, extracellular DNA bound to polysaccharides creates a cation-limited environment to protect biofilms from lysis by antimicrobial peptides (AMPs)[131]. Extracellular DNA (of unclear microbial or host origin) surrounding bacteria is found in damaged gut biofilm during colitis in mice and rats[29] and in medical device-associated biofilms upon implantation into the

rabbit peritoneal cavity[52]. Secreted RNAs from pathogenic proteobacteria (*P. aeruginosa* and *E. coli*) can reach the host mucosa via membrane vesicles and dampen immune responses in mouse bladder (specifically, uropathogenic *E. coli*), in human airway epithelial cell lines and in mouse lungs (specifically, *P. aeruginosa*)[132,133].

Quorum sensing. When a community of bacteria reaches a threshold number, the bacteria can synchronize their metabolism and engage in a community-like behaviour to form a biofilm. This mechanism is known as quorum sensing[134]. Quorum sensing is mediated by small amphiphilic molecules in Gram-negative bacteria and small peptides in Gram-positive bacteria[134]. *Pseudomonas aeruginosa*-derived quorum-sensing molecules (for example, 3-oxo-C12-HSL) can modify in vitro cytokine production and chemotaxis in immune cells and lung epithelial and endothelial cell apoptosis[135–137]. A similar effect of *P. aeruginosa* quorum-sensing molecules in the intestine remains to be demonstrated. Conversely, gut hormones such as adrenaline and noradrenaline are recognized by *E. coli* through quorum-sensing pathways in vitro[138–140]. Opioids (for example, endorphins and dynorphins) can also be recognized by *P. aeruginosa* in mice and act as quorum-sensing molecules[141]. Interestingly, noradrenaline enhances the capacity of *Brachyspira pilosicoli* to adhere to and attach to human intestinal epithelial cell lines[142], a property that could explain biofilm formation on the colonic epithelium during intestinal spirochaetosis in vivo in humans[143]. Several other gut-relevant taxa such as *Salmonella typhi*, *Listeria monocytogenes*, *Citrobacter freundii*, *Cronobacter sakazakii* (previously known as *Enterobacter sakazakii*), *Enterococcus faecalis*, *Helicobacter pylori*, *Campylobacter jejuni*, *Fusobacterium* spp. and *Prevotella* spp. are responsive to human gut hormones (such as noradrenaline, dopamine and adrenaline), which directly influence the outcome of infection in animal models[144]. Altogether, a trans-kingdom dialogue clearly exists between the host and the gut microbial biofilms through pathways linked to quorum sensing. Future investigations of polymicrobial biofilms in vivo will bring important new knowledge into this still very young field of 'microbial endocrinology'.

Host factors controlling biofilms
The host mucosa is equipped with a great arsenal of defence mechanisms that could shield deleterious interactions and contacts between epithelia and gut biofilms. The diversity of biofilm organization along the gastrointestinal tract under physiological conditions (FIG. 2) might also dictate the regional expression and function of host factors dedicated to biofilm control. Overall, host factors controlling biofilms depend on the host genetic and immune status, but also on the taxonomic composition of biofilms and their metagenomes. Alterations of this delicate equilibrium, on the host and/or microbial side, could be a substantial driver of intestinal diseases and could explain the dysbiosis that is associated with a number of intestinal pathologies (TABLE 2). To develop improved therapeutics aimed at restoring intestinal homeostasis, it is important to identify the actors and

Table 2 | **Factors involved in host–biofilm interactions: contribution and significance in gut health and disease**

Factors	Role in homeostasis	Role in disease
Biofilm factors		
Polysaccharides	Key role in biofilm matrix scaffold and physicochemical properties[110]	Molecular camouflage for pathogens (e.g. *Escherichia coli*, *Pasteurella multocida*, *Streptococcus*)[112]
	Influence spatial organization of taxa within polymicrobial communities[111]	Modify the production of inflammatory cytokines and oxygen burst responses[113–115]
	Train the immune system (e.g. via TLRs)[113–115]	
Proteins	Key role in biofilm matrix scaffold[21,110]	Virulence factors[118,119]
	Improve nutrient access (for matrix-associated enzymatic proteins)[116,117]	Effect on drug efficacy and bioavailability[280,282–285]
	Participate in biofilm matrix restructuring (for degradative enzymes)[116,117]	Stimulate innate and adaptive defence mechanisms (e.g. via TLRs, FPRs)[120–122]
	Degrade and eliminate xenobiotics[110] (not specifically demonstrated for gut bacteria)	
	Train the immune system[120–122]	
Membrane vesicles	Bacteria-to-bacteria signalling within gut biofilms[123]	Disseminate virulence factors at distant sites[123]
	Bacteria-to-host cell signalling[123]	Mask virulence factors[124]
	Cargo for protein (intracellular and membrane-bound) release[19]	Trigger higher immunogenic properties compared with soluble antigenic molecules[125]
Nucleic acids	Bacteria-to-bacteria signalling and genetic transfer within gut biofilms[110,132,133]	Facilitate acquisition of antibiotic resistance genes (i.e. resistome)[244]
	Key role in biofilm matrix scaffold[129]	Stimulate innate and adaptive immune response (e.g. via TLR9)[129,132,133]
	Train the immune system[129,132,133]	
Quorum sensing molecules	Key signal to trigger community assembly[134]	Induce apoptosis in various host cells, including epithelia[135–137]
	Recognize host factors as quorum-sensing signals (e.g. opioids, adrenaline and noradrenaline)[141]	Modify innate defence mechanisms[135]
Host factors		
Mucins	Safe house and pantry for gut microorganisms[56,145]	Targets for pathobiont to gain advantageous access to epithelia[145,231,232]
	Key factor in microbiota biogeography[149,151]	Used by pathogens to turn on virulence factor expression[146–148]
	Stimulate biofilm formation and/or dispersal[152]	
Antimicrobial peptides	Contribute to microbial species selection and microbiota biogeography[156,157]	Host defence factors against pathogenic biofilms[154,155]
Gaseous mediator H_2S	Promote mucus layer secretion and gut biofilm formation[29]	Promote epithelial healing and has anti-inflammatory activities[160]
	Role in microbiota biogeography[29]	High production is found in diseases associated with abnormally epithelium-adherent gut biofilms (i.e. colorectal cancer)[163,266,267]
		Has antimicrobial activity against planktonic bacteria, viruses and parasitic eukarya[29,165]
		Prevent in vivo biofilm formation on implanted devices[169]
Immunoglobulins	Promote biofilm formation and microbial colonization in the gut (IgA-dependent)[54]	Immune-exclusion of gut microbiota, due to IgA coating and clearance of planktonic bacteria[166,167]
	Promote commensal adhesion through N-linked and O-linked oligosaccharide chains of secretory IgA[171,172]	
Proteases	Promote physical exclusion of gut biofilms from host tissues[31,175]	Prevent biofilm adherence to surfaces (e.g. surgical implants, chronic wound infections)[174,176–178]
		Are targets of biofilm matrix-associated inhibitors (e.g. ecotin)[179,180]

FPR, formyl peptide receptor; TLR, Toll-like receptor.

pathways through which host tissues control the growth of gut biofilms at mucosal surfaces (FIG. 4).

Mucins. Mucins are a family of highly glycosylated proteins secreted by epithelial goblet cells. They constitute the major proteinaceous component of the mucus barrier overlying the intestinal epithelium. The composition and barrier properties of this mucus layer vary in different portions of the gastrointestinal tract (FIG. 2). Commensal as well as pathogenic bacteria have evolved several mechanisms that enable them to adhere to mucus and to compete with one another to exploit it as a beneficial habitat[145]. Pathogens such as *C. jejuni* and *Vibrio cholerae* utilize mucin proteins as a signal to
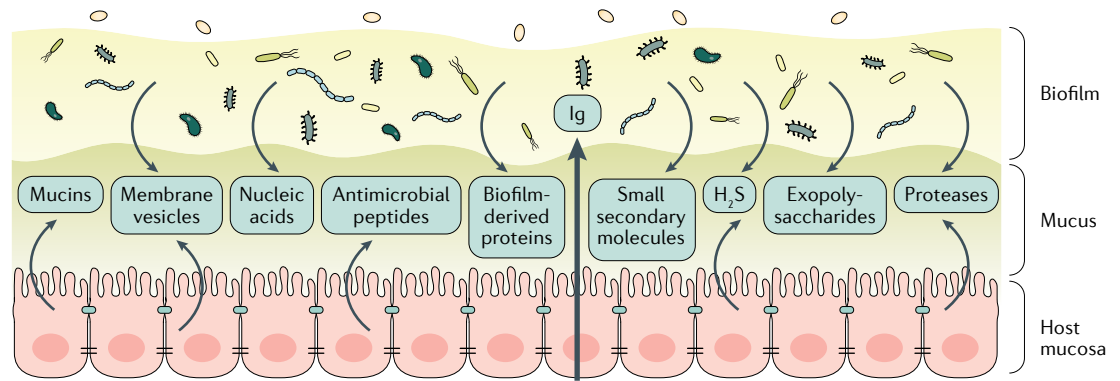
Fig. 4 | **Endogenous actors in biofilm–host interactions.** Numerous factors and pathways are involved in the symbiotic relationship between the host tissues and their mucosal biofilms in vivo. On the one hand, the host can exert a direct and persistent influence on its mucosal biofilm via mucin secretion, membrane vesicles, antimicrobial peptides, immunoglobulins, hydrogen sulfide and proteases. On the other hand, secreted proteins, polysaccharides, proteases, hydrogen sulfide, small secondary metabolites, membrane vesicles and nucleic acids are all components of the mucosal biofilm, which might in turn trigger host defence mechanisms.

promote expression of virulence factors and induce biofilm formation in vitro[146,147] and in mice[148]. In the healthy distal colon, the mucus layer is composed of an inner layer that is dense, free of bacteria and firmly attached to the epithelium. The loosely adherent outer layer houses most of the bacteria communities[56]. The mucus layer might be more intimately attached to biofilm communities rather than to the intestinal epithelium itself[149]. As a result of this feature, in vivo gut biofilms could be defined as mucus-embedded, mucus-adherent microbial aggregates[10,63,65,83–85]. The gastrointestinal tract naturally harbours taxa of non-mucolytic bacteria. As mucin consumption requires a large combination of enzymatic activities[150], non-mucolytic gut bacteria would collectively benefit from the metabolic properties of the polymicrobial biofilm lifestyle. Finally, it is widely assumed that the mucin layer in the gastrointestinal tract prevents pathogens as well as commensals from reaching and contacting the intestinal epithelium[56,69,151]. However, mucus layer composition, thickness and viscoelasticity vary considerably along the gastrointestinal tract[145]. Contacts between microbial aggregates and the epithelium are frequent in the upper intestinal tract and proximal colon (FIG. 2). An in vitro study suggested that stomach-derived mucins cause dispersal of *P. aeruginosa* biofilms via the induction of flagellar motility[152]. The precise role of lower gastrointestinal tract mucins (such as MUC2) on gut biofilms remains to be clarified. Although a role for mucins in preventing contacts between biofilms and the mucosal surface is clearly established in the colon, such mucins might also have specific (but yet undiscovered) effects on biofilm-embedded bacterial species.

Antimicrobial peptides. AMPs are a class of host defence peptides that are widely distributed in nature. They are produced by fungi, insects, amphibia, mammals and Prokarya (known as bacteriocins). Most AMPs are cationic, a property that facilitates the killing of planktonic bacteria through membrane disruption, pore formation, penetration and inhibition of bacterial intracellular molecules and enzymes, and inhibition of cell wall

synthesis[153]. AMPs exhibit strong anti-biofilm activities in vitro against multidrug resistant as well as clinically isolated bacterial taxa[154]. They disperse biofilms in vitro by reducing their adhesive forces to surfaces, killing embedded bacteria or directly interfering with metabolic pathways involved in biofilm formation[154,155]. Human cathelicidin peptides can impede the twitching motility of *P. aeruginosa* biofilms in vitro through interfering with quorum-sensing pathways[156,157]. Studies involving in vivo experiments have revealed that defensins (for example, human α-defensin 6) can form self-polymerized structures called 'nanonets', which can then trap bacteria and prevent their physical contact with the intestinal epithelium[158]. Interestingly, this polymerization can be finely tuned by redox conditions that differ between the intestinal crypts and the top of the villi[159]. Overall, AMPs can be considered as good alternatives to conventional antibiotics to fight deleterious biofilms. But, maybe more importantly from a gut perspective, they could have a key role in homeostasis by selecting a particular taxonomy (due to their killing defence mechanisms) and by physically preventing biofilms invading gut tissues.

Hydrogen sulfide. Hydrogen sulfide (H_2S) is a mediator of inflammation, homeostasis and repair in the gastrointestinal tract[160]. The commensal gut microbiota is a substantial source of H_2S, some of which acts as an energy source, as confirmed in human intestinal epithelial cell cultures[161]. Evidence implicates microbiota-derived H_2S in inflammatory flares in patients with colitis[162], and it might also be implicated in the development of CRC according to an in vitro study[163]. The colonic epithelium itself also produces H_2S by means of cystathionine-β-synthase, cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfotransferase[160]. Using mice deficient in the CSE enzyme, colonic endogenous H_2S production has been shown to contribute to the promotion of healthy colonic microbiota biofilm formation and to mucus barrier function[29]. Administration of H_2S-releasing compounds directly into the mouse

colon during colitis has been shown to promote normal production of mucus and to restore healthy microbiota biofilm biogeography[29]. Interestingly, H₂S-rich dietary compounds from garlic have demonstrated antibacterial, antifungal and antiparasitic properties in vitro against various pathogens (such as *S.* Typhi, *S. aureus*, *Plasmodium falciparum*, *Trypanosoma brucei* and *C. albicans*)[164,165]. Moreover, an H₂S-releasing anti-inflammatory drug (ATB-429; Antibe Therapeutics) reduces the virulence of biofilms from patients with IBD in vitro[18]. Nevertheless, the precise role for H₂S on the physiological gut biofilm needs further investigation if translational applications for H₂S-based therapeutics are to be realized in humans[160].

Immunoglobulins. Secretion of immunoglobulins (IgA, specifically) in the intestinal lumen neutralizes microbial toxins and coats bacteria to prevent them adhering to epithelial cells[166]. High-affinity IgA coating indicates a subset of inflammatory bacteria with increased abilities to invade the mucus layer, activating inflammasome pathways and thereby driving intestinal diseases such as IBD[167]. Human monoclonal IgG antibodies that bind to amyloid-β protein oligomers and fibrils[168] destroy biofilms of *S.* Typhimurium[169]. This property is due to a direct inhibition of the fibrilization of microbial curli proteins (an extracellular amyloid fibre produced by enterobacteria), hence altering the stability of biofilm matrices in vitro[170]. This mechanism was confirmed in mice infected with catheter-associated *Salmonella* biofilms, in which human monoclonal IgG antibodies that bind to amyloid fibrils not only led to biofilm dissociation but also to improved biofilm eradication by antibiotics[169]. Because many other gut-relevant bacteria produce curli or curli-like amyloids in their biofilm matrices (for example, *E. coli* and *P. aeruginosa*)[170], this novel biofilm-specific immunotherapy has the potential to be applied to a wide variety of pathogenic biofilms.

Although bacterial growth is typically impaired by IgA coating, commensal microorganisms can be coated with IgA in vitro without substantial alterations to their growth[166]. Indeed, IgA can favour microbial colonization in the gut, as intestinal bacteria (*E. coli*[171] and *H. pylori*[172]) express receptors that recognize IgA glycoprotein motifs. The binding of IgA to these surface receptors can indeed facilitate initial bacterial adhesion to the host surface, such as in the dental plaque biofilm[173]. Enteric biofilm formation at the intestinal epithelial cell surface (as demonstrated in human cell line monolayers) is also helped by the addition of secretory IgA to the milieu (but not by IgG addition)[54]. Conversely, biofilm formation in vitro is prevented by an IgA-specific protease[54].

Thus, immunoglobulins can exert both immune-exclusive and immune-inclusive functions against gut biofilms. They can promote a biofilm phenotype in the gut lumen, while at the same time preventing planktonic elements, or eventually biofilm-dispersed elements, from invading the mucus layer and contacting and crossing the epithelial barrier.

Proteases. Eukaryotic proteases, such as the chymotrypsin derived from *Lucilia sericata* maggots, present in the gut lumen have a negative effect against bacterial biofilm formation in vitro[174]. Interestingly, a host trypsin-like protease disseminates *E. faecalis* biofilm formation in the urinary tract of mice[175]. Another study added epithelia-derived thrombin to the list of matrix-degrading antibiofilm agents[31]. In that study, epithelial thrombin was identified as a pivotal actor in homeostatic biofilm containment at the colonic mucosal surface, using both an in vivo approach (a mouse model) and an in vitro human intestinal microbiota biofilm culture. Targeting the matrix-associated protein backbone of biofilms, for example via enzymatic lysis caused by proteases, seems to be a promising approach for biofilm control or eradication on medical and host surfaces[176–178]. Interestingly, Enterobacteriaceae such as *P. aeruginosa* and *E. coli* can release the protease inhibitor ecotin in their biofilm matrix to protect it from neutrophil elastase lysis[179,180]. The gastrointestinal tract hosts a wide variety of proteases from multiple sources, and for many of them their production and activity is altered in disease conditions[181]. Whether a protease-based approach can be used to eliminate deleterious epithelia-adherent colonies and restore proper host–biofilm homeostasis is therefore an exciting road to explore.

Clinical importance of gut biofilms

The clinical relevance of biofilm-associated infections is important, as the vast majority of persistent infections in the human body derive from biofilms[11,182]. Alterations of biofilm features are associated with IBD, cancer and infectious diseases, and are related to taxonomic composition changes, biogeography redistribution, antibiotic tolerance or resistance, and biofilm-dispersed pathobiont emergence. The field of antibiofilm research is prolific, and future strategies are expected to produce important tools for the control of deleterious biofilms in vivo[183]. However, because biofilm is a natural lifestyle of microorganisms in the gut habitat and is not necessarily a marker of disease (BOX 2), future therapeutic directions could focus on restoring host–biofilm

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Box 2 | Gut biofilms are not necessarily disease markers

Imaging studies investigating the spatial organization of resident gut mucosal microbiota have failed to show, or have underestimated, the number of matrix-embedded biofilms because common washing and fixing methods can easily remove these structures. This observation led to the belief that the mucosal surface of the healthy colon is devoid of microbial biofilms[60] and that the presence of biofilms at the mucosal surface of the intestine might be associated with gut disease (inflammatory diseases such as inflammatory bowel disease (IBD) in particular)[9]. However, bacterial biofilms have been visualized at various healthy gastrointestinal surfaces embedded within a mucin-rich matrix, such as in honeybees[46], fish[48], amphibians[66], rats[53,55], mice[29,57,63,151], primates[53], and finally in the human appendix and colon[53,58,62,99]. After various external challenges, species with almost total depletion of faecal communities can later be recovered, suggesting that some bacterial reservoirs intimately linked with tissues can help with stability and resilience of the human gut microbiota[93]. Hence, rather than simply the presence or absence of biofilms, the presence of abnormal biofilm characteristics during disease might reflect an altered microbial phenotype and a disease state. For instance, an increased epithelial adherence of biofilms in the distal colon is rarely encountered in healthy states and could be considered a marker of disease, but the presence of a biofilm itself certainly could not. However, the mechanisms and aetiological causes involved in biofilm adherence to the epithelium during disease have yet to be fully understood.

Research on the microbiota has extensively characterized the taxonomy of faecal communities and their relative abundance in health and disease. Unfortunately, this research has not yet translated to the clinic. Much less attention has been given to the phenotype of these consortia under a biofilm organization. Preclinical studies in humans would benefit from the use of novel gut biofilm biomarkers that could help improve stratification of patients and to assess drug responses of a patient's microbiota under its natural biofilm phenotype. A better understanding of the mechanisms by which bacteria interact with each other in a polymicrobial context could help improve the efficacy of existing approaches such as faecal microbiota transplantation. Because the spatial structure of the gut biofilm is important for gut homeostasis, drugs that disrupt or restore its biogeography could prove to be useful. Host or microbial serine proteases are tools that could digest structural components of biofilms[31,174,176–178]. Understanding and modulating their actions could improve human drug efficacy. Similarly, a better understanding of the effects of antimicrobial peptides and antibiotics on gut biofilms could help in the design of more effective drugs. Finally, drug development could benefit from information on the effects of specific microbiota biofilm compositions in a diseased or healthy individual and from information on the effect of microbiota biofilm composition on the active component of a drug. In that context, it would be important to address these questions with the microbiota considered as biofilms and not only as an in vitro planktonic culture. One can envision future tests for drug metabolism by advantageously using specific gut biofilms. This application could even lead to personalized medicine, in which an individual's microbiota composition at a given time of treatment is considered.

homeostasis and not necessarily eliminating biofilms at mucosal surfaces. Because the organization of biofilms throughout the gastrointestinal tract is likely to vary, these approaches would also need to be region-specific. Studying biofilm behaviour in diseases, and reconsidering the dogmatic view of biofilms being harmful (BOX 3), are therefore likely to bring a set of discoveries of substantial clinical importance in gastroenterology. However, one major question remains. Despite all the evidence that is summarized in this section suggesting a role for biofilms in intestinal diseases, we do not have clear answers as to whether altered biofilms are a cause or consequence of the disease. Should we consider biofilm alterations as markers of disease? Or as potential targets for therapeutic intervention? Or both? These are questions to be answered in the coming years.

Gastrointestinal infections. The human gastrointestinal tract can be colonized by various enteropathogens that can be responsible for various conditions ranging from asymptomatic colonization (for example, *H. pylori*[184] and spirochaetes[143,185]) to mildly symptomatic and self-limiting (for example, *Campylobacter* spp.[186], spirochaetes[187] and *H. pylori*[184]) and to potentially life-threatening chronic infections (for example, healthcare-associated infections by drug-resistant *Enterococcus* spp.[188] and *Clostridioides difficile*[189]). Several studies have clearly demonstrated that these enteropathogens are well-equipped to form biofilms in vitro (*H. pylori*[190], *C. jejuni*[191,192], *Enterococcus* spp.[193], *C. difficile*[194] and *Streptococcus gallolyticus*[195]). Animal models of gastrointestinal infection present histological characteristics of deleterious biofilm colonies that are densely packed and adherent to the epithelial surface (for example, *H. pylori* biofilm in gastric pits[196,197], *C. jejuni*[198–200] and *C. difficile* biofilms in the colon[201,202]). In human intestinal biopsy samples, histological and

microscopy staining of the microbiota reveals the presence of dense mucosa-associated biofilms covering tissues (for example, *H. pylori* biofilms in stomach ulcers[203] and spirochaete biofilms in the rectum[143,187]). Although studies have established a link between biofilm-forming enteropathogens and chronic infectious diseases, the contribution of the biofilm lifestyle *per se* to the chronicity and persistence of infections has yet to be fully understood, and biofilm-specific strategies to combat such conditions remain to be fully evaluated. Moreover, several of these enteropathogens are incidentally associated with other conditions such as sepsis (for example, *H. pylori*[184] and spirochaetes[204,205]), IBD (for example, *C. jejuni*)[186,206–208], colonic eosinophilia and irritable bowel syndrome (IBS) (for example, spirochaetes)[204,205] or cancer (for example, *H. pylori*[184], *S. gallolyticus*[209], genotoxin-expressing *C. jejuni*[198,199] and *E. coli*[67,210–212]).

Cancer. Biofilms have been linked to cancer initiation and development in the stomach, small intestine and colon (reviewed previously[7,9]). Biofilms that are adherent to the intestinal epithelium can be visualized in healthy human colon tissues by microscopy approaches[60]. Still, thick polymicrobial biofilms are more prevalent in patients with CRC than in healthy individuals as controls, especially in the ascending right colon[17,60,210,212]. Strains frequently recovered in mucosa-associated microbiota from patients with CRC are *Fusobacterium nucleatum*[213,214], enterotoxigenic *Bacteroides fragilis* and genotoxin-producing *E. coli*[67,210–212] and *S. gallolyticus*[209]. Patients with familial adenomatous polyposis also harbour abnormally adherent bacterial biofilms in areas close to polyps, which are predominantly composed of *E. coli* strains coding for the genotoxin colibactin and enterotoxigenic *B. fragilis*[210]. Interestingly, in that study, the taxonomic composition of these inherited biofilms seemed to be different from those detected on sporadic colorectal tumours or healthy hosts (notably enriched in mucus-invasive proteobacteria and *Bacteroides* compared with sporadic CRC). Regarding the clinical contribution of biofilms to CRC, studies using mouse models and human colonic tissues suggest that biofilms can be directly carcinogenic but also that they can participate in tissue transformation in the context of an inflammatory milieu and a genetically predisposed host[7,67,213–216]. Studies using *Apc*[Min] germ-free mice suggested that invasive biofilms are tumorigenic through alteration of host mRNA or microRNA, and that the contribution of specific taxa is essential for the replication of tumorigenesis[67,216]. *Fusobacterium nucleatum* can directly contribute to colorectal carcinogenesis via the recruitment of tumour-infiltrating immune cells in a genetically predisposed individual, as evidenced by studies using the *Apc*[Min] mouse model and human cell lines[213,214]. Finally, enterotoxigenic *B. fragilis* could favour CRC tumour initiation, in part through secretion of a metalloproteinase toxin leading to overactivation of a T helper 17 (Th17) cell-dependent response and production of genotoxic oxygen radicals[217]. Other potential mechanisms for biofilm-induced tumorigenicity include metabolomic changes in polyamine (spermine and spermidine) host pathways[17] as well as

Gut biofilm biomarkers
A molecule, gene or other biological characteristic of gut biofilms that might be used to predict a specific intestinal disease status.

in silico prediction of functional changes in the mucosa-associated microbiome[212]. Beneficial commensal microorganisms are out-competed by opportunistic bacteria better adapted to the tumour microenvironment[211,218,219]. Finally, it is also becoming clear that microbiota-induced cancer might not be attributable to a single microorganism, but instead requires a complex bacterial community assembled in a biofilm setting, in which beneficial commensals are supplanted by pathobionts (such as those previously discussed) that are better adapted to the tumour microenvironment and that can play the part of a keystone pathogen[67,210–212,215].

Inflammatory bowel disease. Accumulating evidence now supports the idea that IBD-specific mucosal biofilms elicit pro-inflammatory responses in host tissues through multiple pathways[18,58,60,67,220]. Differences in taxonomy and a decrease in the overall diversity of the mucosa-associated microbiota are associated with IBD (specifically, decreased abundance of *Faecalibacterium prausnitzii* in Crohn's disease[221,222], increased abundance of virulent *E. coli* in Crohn's disease, and enterotoxigenic *B. fragilis* and *P. aeruginosa* in ulcerative colitis and Crohn's disease[57,223–225]). Several studies now point to the fact that microbial communities living in close contact with the mucosa have a different behaviour. Similar to what is observed in cancer, the prevalence of epithelia-adherent biofilms is elevated in human biopsy samples from patients with IBD compared with those from healthy individuals as controls[58,222,226]. Mucosal microbiota from patients with IBD generates larger biofilms ex vivo compared with microbiota from healthy tissues[18]. *Enterococcus* spp. as well as adherent-invasive *E. coli* isolates from patients with Crohn's disease have an increased ability to form a biofilm on plastic surfaces and on intestinal epithelial cell line cultures[223,227,228]. Moreover, biofilm-dispersed bacteria from IBD-associated biofilms can become invasive pathobionts in vitro and in vivo, a phenomenon that could have a causative role in the pathophysiology of IBD[18,29,30,44,82]. Altered abundances of mucolytic commensal microorganisms (for example, *Akkermansia muciniphila* and Ruminococcaceae)[229] and biogeographic repositioning of bacteria that release proteases and glycosidases (for example, *Porphyromonas* spp., adherent-invasive *E. coli*, and *Bacteroides thetaiotaomicron*) might also facilitate access to the intestinal epithelial surface for neighbouring commensal microorganisms[17,230–233]. Altogether, the mechanisms by which spatial redistribution of microorganisms, and dispersal of specific pathobionts, occurs in IBD are largely unknown. Thus, future studies would need to identify whether these changes are due to the impairment of host factors (for example, mucus barrier defects or depletion[69,151,229], immune system activation, and impaired protease–antiprotease balance) or due to the bacteria within biofilms being more invasive.

Other clinical conditions. In situ microscopy analysis of colonic samples from patients with IBS has revealed similar features of microbiota biofilm disorganization to those observed in samples from patients with IBD, in particular abnormal growth at the site of epithelial contact[58]. The relative abundance of a well-known biofilm-forming species, *P. aeruginosa*, is increased in mucosal samples from the duodenum of patients with IBS[234] and coeliac disease[235]. In post-infectious IBS, it might not be the pathogen itself that precipitates the disease but rather its detrimental effect on commensal biofilm integrity, and on a change of the commensal biofilm behaviour[30,206,207]. Moreover, exposure to an enteropathogen might also increase pathobiont properties of otherwise non-invasive commensals (for example, the effects of *Giardia duodenalis* and *C. jejuni* on *E. coli*)[44,236]. It is therefore expected that other colonic disorders that are not necessarily associated with severe tissue damage (for example, IBS, mild forms of coeliac disease, enteric neuropathies and self-limiting colitis) could also be linked with abnormal characteristics of the gut biofilm.

Use of biofilm control for therapy. As discussed above, microbial biofilm communities help maintain various aspects of homeostasis throughout the gastrointestinal tract. However, in some clinical contexts these biofilms need to be preserved or repaired when disrupted, and in others epithelium-adherent biofilms might need to be eradicated. Hence, enormous research effort has been expended in the attempt to develop therapeutic biofilm-specific control strategies[183].

One approach is the prevention of initial biofilm formation, for example by developing drugs that impede surface attachment. For instance, human monoclonal antibodies that neutralize curli-like proteins can prevent the formation of biofilms on implanted devices in mice[168,169]. Another example is that probiotic strains can form a safe (as in, non-pathogenic) biofilm on the gut mucosa, thereby blocking the adhesion of pathogens[10]. Biofilms enriched in *Lactobacillus* genera were indeed visualized in the stomach of a healthy horse[237] and in the forestomach of mice[63,238]. The capacity to form a biofilm at the gut mucosal surface led to the development of nanomaterials coated with a *Lactobacillus reuteri* biofilm (*L. reuteri* 'bioparticles') to deliver local oral drugs in a mouse model of CRC[239]. Despite promising studies in animal models, polymicrobial biofilms (such as in the colon) are usually impermeable to newcomer microorganisms[100]; thus, the therapeutic effectiveness of probiotics is likely to depend on a precise population composition and on the nature of the probiotic, as well as other environmental factors including diet[240]. Most clinical studies suggest that shedding of probiotic bacterial strains in stool samples diminishes drastically following cessation of probiotic intake[240]. Moreover, oral intake of probiotics in humans after antibiotic perturbations caused a detrimental delay in the return of resilient mucosa-associated microbiota during faecal microbiota transplantation[98]. Overall, these studies raise important questions about the beneficial or sometimes detrimental effects of probiotics under certain circumstances. One might also question whether the therapeutic effects of ingested probiotics in the clinic require a biofilm lifestyle. Although conceptually easy to apply on implanted materials, prophylactic approaches against deleterious epithelium-adherent biofilms in the gut, with drugs,

immunoglobulins or probiotics, clearly need further research before being applicable in the clinic.

The second strategy aims to kill bacteria. This strategy can be achieved with antibiotics, although they will not completely eradicate in vivo biofilms[11]. Also, this strategy can lead to persistence of biofilms, a condition in which bacteria survive but without necessarily growing. The mechanisms underlying this phenomenon are multifactorial and are connected directly to the biofilm lifestyle and responsible for so-called antibiotic tolerance (for example, restricted penetration of molecules in the biofilm matrix[110], reduced metabolism or metabolically distinct subpopulations[5], and emergence of persistent cells[49]). These tolerance mechanisms can then provide conditions for the emergence of antibiotic resistance due to increased mutagenesis and natural selection (for example, efflux pumps that extrude antimicrobials)[241]. The gut is a complex polymicrobial environment that might be the perfect habitat for genetic transfer and selection of hypermutable subpopulations[242–244]. In conditions associated with polymicrobial biofilm alterations (such as CRC and IBD), the link between biofilm persistence and the outcome of disease have yet to be fully appreciated. But the persistence of biofilms in response to antibiotic treatment might still be relevant to chronic gastrointestinal infections.

The last approach is aimed at weakening the biofilm. Bacteriophages (prophages or phages) are viruses that infect bacteria, and eventually kill them by lysis. They can eradicate biofilms on medical devices, and have been investigated in various models of infection in vivo (for example, skin, lung and bone infections[245], and sepsis[246,247]). T7 phages genetically engineered to promote expression of the glycosidase dispersin B eradicate *E. coli* biofilms more efficiently than non-enzymatic phages alone[248]. Bacteriophages seem to be promising tools against biofilm infections, but a number of issues still need to be solved before applying such tools against

deleterious biofilms in the gut. Such issues include narrowing the host range of bacteriophages (such as identifying which bacteriophage will work better on a specific strain of bacteria), understanding the risks of phage resistance, determining whether host factors inactivate bacteriophage properties, and determining the long-term safety of phage preparations in humans[245]. The use of enzymes, such as proteases, that degrade the proteinaceous matrix backbone of biofilms is another potential strategy to weaken biofilms[31,176–178]. As previously discussed, epithelial AMPs and host proteases produced under healthy conditions are able to naturally disperse bacteria from biofilms[31,154,155]. However, a cautionary point has to be raised concerning biofilm reduction at gastrointestinal mucosal surfaces. Such targeting of biofilms might cause the release of biofilm-dispersed elements, which could pose a health risk, particularly in the gut where pathobionts might be present. Studies investigating the contribution of epithelium-derived factors to abnormal dispersion of bacteria in disease conditions should produce important findings.

Biofilm as a metabolic factory

The interactions between gastrointestinal luminal compounds, biofilms and the host are complex and need to be considered in both health and disease.

The biofilm can manage gastrointestinal luminal compounds in a number of different ways (Supplementary Fig. 1). Luminal compounds, whether they are microorganisms, dietary molecules, contaminants or xenobiotics, can either diffuse freely across the biofilm and reach the host epithelium or be blocked by the biofilm, which acts as a tight barrier[249]. These luminal compounds can also be filtered by the biofilm, which can permit the passage of only certain molecules, thereby acting as a selective filter. For some luminal molecules, as the biofilm metabolizes some of them (such as carbohydrates)[34,84,250], it is expected that the gut biofilm allows the passage of a diluted form of the luminal compounds. Interestingly, in that specific case, one can hypothesize that the biofilm could serve as a detoxifier, reducing the penetration of potentially harmful concentrations of luminal molecules. Some dietary molecules issuing from digestion processes, xenobiotics or contaminants can also be chemically transformed by the biofilm factory (FIG. 5), which then produces new compounds that are released close to host cells. Whether these biofilm-transformed compounds might be useful or harmful to the host depends, of course, on the biofilm composition and its metabolism. Finally, the host and the biofilm might compete with one another for luminal compounds. Because the biofilm is in closer contact with the contents of the lumen, it might have an advantage over the host. However, in the upper gastrointestinal tract, some regions of the epithelium seem to be in close, if not direct, contact with luminal compounds[57,61,85,185] (FIG. 2).

Taken together, it is logical to think that depending upon the composition of the biofilm, and more importantly on its metabolism, the luminal compounds that are recognized by the host might be of different natures and different concentrations. These molecules could be beneficial when they originate from a healthy
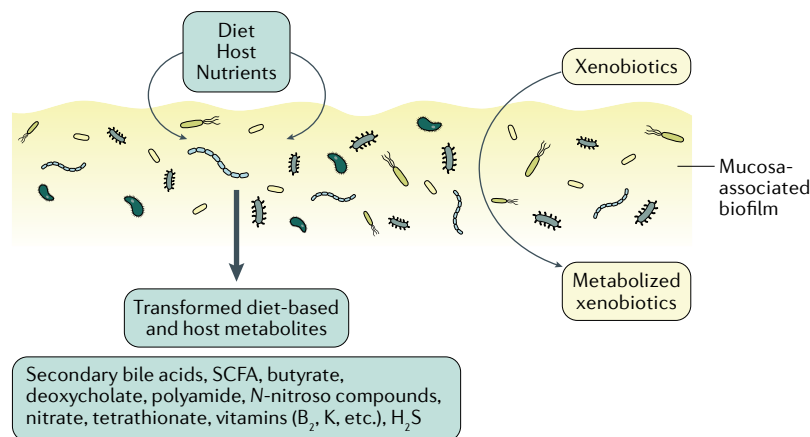

Fig. 5 | **Mucosa-associated biofilms: a biological factory.** Mucosal biofilm can transform digestive leftovers, host metabolites and xenobiotic chemicals from virtually any class of dietary compound, including complex polysaccharides, lipids, proteins and phytochemicals. This metabolic function leads to the productions of novel microbiota-derived metabolites that could exert health-promoting effects (for example, vitamins and short-chain fatty acids (SCFA)), could help the clearance of these compounds from the body (conjugation of chemicals) and alternatively could lead to compounds with novel biological functions (metabolism of drugs).

Labels in figure: Diet Host Nutrients; Xenobiotics; Mucosa-associated biofilm; Metabolized xenobiotics; Transformed diet-based and host metabolites; Secondary bile acids, SCFA, butyrate, deoxycholate, polyamide, N-nitroso compounds, nitrate, tetrathionate, vitamins (B_2, K, etc.), H_2S

ecologically stable biofilm, or detrimental when they originate from an unhealthy unstable biofilm. It is therefore of major importance to understand, in combination, the composition of biofilms, their metabolic activity, and their spatial distribution.

Biofilm versus planktonic metabolism. Studies in human have demonstrated that mucosa-associated and food particle-associated microbial aggregates are unique, not only from a taxonomic point of view but also from a metabolic perspective[34,84,88,97]. Microenvironments within the biofilm itself create a considerable spectrum of gene expression profiles and microbial behaviour[25,251–253]. Diverse quantitative and imaging technologies have been applied to biofilm metabolomic research[16–18,22,254]. Because different bacteria might have different metabolic capabilities when cells switch from planktonic to biofilm growth, metabolomic studies have to also consider the microbial lifestyle for gut-relevant taxa and for in vivo biofilms. Notably, the strict anaerobe *F. prausnitzii*, which is an important member of the mucosal microbiota, engages metabolic functions (such as extracellular flavin–thiol electron transfer pathways) that are not expressed in standard in vitro and anaerobic cultures to survive in the oxygenated gut environment[255]. Thus, it is of major importance, now that microbial taxonomy can be addressed relatively easily, to understand the identity and conditions for production of key metabolites that are produced by gut biofilms and that could be involved in digestive health and disease.

Diet metabolism. The colon could be viewed as a central fermenting organ involved in the genesis and processing of digestive leftovers[34,84]. The gut microbiota can modify virtually all classes of dietary compounds, including complex polysaccharides, lipids, proteins, and phytochemicals (FIG. 5). Early observations demonstrated that there are differences in bacterial growth between the centre of the faeces, on the mucus–faeces interface and on food particle-attached microbial communities[84,256]. These studies provided useful information on the contribution of the biofilm phenotype to microbial colonic fermentation.

Metabolism of undigested carbohydrate complexes (such as in the diet, as well as those linked with mucin) by intestinal biofilms can lead to the production of SCFAs, the more prevalent being acetate, propionate and butyrate[34,84,256–258]. Other sources for SCFAs are amino acids, such as valine, leucine and isoleucine, resulting from the breakdown of proteins. The biological effects of SCFAs in the gut are numerous: they can serve as an energy source for the intestinal epithelium, help to reduce luminal pH, directly inhibit growth of pathogenic bacteria, promote differentiation of T regulatory cells, and improve epithelial tight junction integrity[83,84,256–258]. Interestingly, the concentrations of SCFAs in the gut lumen in a model pig fed with various sources of fibre decreased from the proximal to the distal section of the colon[259]. This observation is somewhat surprising, because bacterial density is higher in the distal colon than in the proximal colon (FIG. 2). As illustrated in an in vitro model of human oral biofilms, local production

of SCFAs by *Porphyromonas gingivalis* and *F. nucleatum* promotes biofilm formation of other commensal partners, *Actinomyces oris* and *A. naeslundii*[260,261]. Alternatively, a structurally SCFA-related molecule produced by *P. aeruginosa* has been demonstrated to cause dispersal of in vitro biofilms formed by a range of proteobacteria[262]. This finding suggests that SCFAs might have direct effects on gut biofilms through mechanisms that remain to be discovered.

The biofilm phenotype provides cells with favourable conditions to metabolize amino acids in their environment, a specific property that has been exploited in industrial biotechnological processes[263]. Microbial metabolism of proteins can lead to the formation of end products such as polyamines (from arginine, lysine, tyrosine or histidine), H_2S (from methionine and cysteine), phenolic and indolic compounds (from tryptophan)[264], as well as the production of N-nitroso compounds (nitrosamines and nitrosamides), which are potent inducers of intestinal tumours in animal models[265]. In addition, several reports suggest that microorganism-derived generation of H_2S (by sulfate-reducing bacteria as well as other intestinal strains such as *E. coli*, and *Clostridium* and *Enterobacter* species) is genotoxic and might have a role in the pathophysiology of CRC and ulcerative colitis[162,163,266,267]. Alternatively, local actions of H_2S can also exert some beneficial effects on gut tissues. For instance, H_2S inhibits the activation of NF-κB, has antioxidant activity and inhibits caspase-3 cleavage, thereby limiting apoptosis[160]. Studies have also demonstrated that H_2S preserves healthy distal colon biofilm organization[29,268].

Vitamin B_{12} (a key vitamin for DNA synthesis, fatty acid and amino acid metabolism) and vitamin K (a key vitamin for the synthesis of coagulation cascade proteases) are synthesized by a small percentage of taxa within the gut microbiota community[269]. *Pseudomonas* spp. synthesize all vitamers of vitamin B_{12} (also known by the generic term cobalamin), including cyanocobalamin, hydroxycobalamin, adenosylcobalamin and methylcobalamin[270,271]. *Pseudomonas* spp. also utilize vitamin B_{12} for methionine and ribonucleotide biosynthesis during biofilm formation through oxygen-dependent pathways[270,271]. Therefore, the reduced availability of vitamin B_{12} that is observed in human IBD[272] could be explained in part by the increased mucosal abundance of proteobacteria combined with an altered oxygen tension during inflammation. Vitamin K deficiency occurs rapidly in humans treated with high doses of antibiotics, and this deficiency is associated with severe gastrointestinal damage (bleeding and ulcers)[273]. The biofilm phenotype of *Bacillus subtilis* favours a specific fermentation pathway in vitro that substantially improves the production of vitamin K[274,275]. Despite its critical importance for haemostasis and for the physiology of the mucosal tissue itself[31], the contribution of the biofilm phenotype to the production of vitamin K remains to be elucidated.

Finally, we currently lack a thorough understanding of the extent to which biofilm-associated metabolism varies in health and disease. This knowledge will pave the way for more efficient interventions on the

Box 4 | Outstanding questions and future directions

- Are disease-associated biofilm perturbations (biogeography, stability, resilience and taxonomy) a cause or a consequence of the disease?
- What factors (host or environmental) induce the detachment of bacteria from gut mucosa-associated biofilms?
- Do probiotics need to be part of the mucosa-associated biofilms to exert efficient beneficial effects?
- Understand the role of biofilm lifestyle in the resilience and stability of gut mucosa-associated microbiota.
- Define biofilm metabolism in health and disease.
- Develop assays to assess the effects of human gastrointestinal biofilms on drug metabolism.
- Develop new therapeutic tools to specifically restore host–biofilm homeostasis in gastrointestinal diseases.

gut microbiota for therapeutic purposes. A thorough understanding of how gut biofilms process dietary components will be essential for a rational use of functional foods, prebiotics and probiotics to treat conditions such as metabolic disease and malnutrition, as well as functional, infectious or inflammatory gut diseases[276].

Drug metabolism. Therapeutic drugs have been reported to alter the composition of the gut microbiota in animal models and in humans, but the gut microbiota itself is also involved in drug processing[277,278]. Although host metabolism generally eliminates xenobiotics from the body, intestinal microorganisms could use these compounds as nutrients and energy sources[279]. The gut microbiota can indeed directly metabolize xenobiotics (for example, amiodarone[280], tacrolimus[281], digoxin[282] and paracetamol[283]), thereby modifying their chemical properties, stability, bioavailability and potential biological effects. Consequently, microbiota-driven processing of xenobiotics can lead to unwanted adverse effects, to loss of efficacy of molecules, or inversely to more active and efficient molecules[284,285]. This observation raises serious questions about the recommendations and dosage of drugs for human use, especially considering the fact that some of them must be carefully monitored because of their toxicity. It is increasingly clear that the metabolic repertoire of the gut microbiota is larger than in human cells. Unfortunately, in the vast majority of cases, the specific microorganism or community of microorganisms, and the enzymes that mediate these reactions, are unknown. Moreover, the different habitats within the gastrointestinal tract can lead to various taxonomic compositions as well as various metabolic processes[85,107]. This observation adds to the complexity of trying to address the effects of the microbiota on xenobiotic or drug processing. Knowledge of microbiota-associated metabolic function of xenobiotics is in its infancy, and is clearly an area of great interest for the future.

Conclusions

The biofilm lifestyle is predominant in every natural habitat on Earth, including gastrointestinal surfaces[3,8,9,83]. Nevertheless, the concept of biofilms in medicine is only 50 years old and should be viewed not only as a pathological chronic infection but also as an ordinary lifestyle of microorganisms living on mucosal surfaces. Microbial biofilms are central to the pathophysiology of many intestinal disorders, but they are also key contributors to the homeostatic development of the gut. The modulation of biofilms in the gut could hold the key to new therapies. Despite having important translational significance, strategies focusing on metagenomic faecal communities have usually failed to consider the phenotype of the microbiota that is interacting with gut mucosal tissues. Thus, we need to reconsider classic views of gut microorganisms as isolated actors in host–microorganism interactions in the gut and consider the microbiota as a biofilm community of microbial aggregates constantly interacting with each other as much as with host cells. The evidence presented in this Review on the contribution and importance of biofilms to intestinal homeostasis and disease warrants further investigations of the gut biofilm and of the means of controlling biofilms (BOX 4). First, the gut biofilm composition and nature in vivo need to be fully characterized to have a better view of the different structural and functional characteristics of disease-associated biofilms compared with their healthy counterparts. This knowledge will help identify whether and how the host engages specific metabolic programmes in response to each of these biofilms. We also need to translate important concepts of microbial ecology into our current perception of gut physiology and of host–microbiota interactions. These concepts include microbiota stability, resilience and microbial biogeography and require us to determine how they can influence gastrointestinal health. Opportunities exist to use biofilms with metabolic capabilities beyond those described in planktonic cultures to help metabolize xenobiotics to our advantage. Future research will have to better understand the polymicrobial diversity and complexity in gut mucosal habitats. This understanding constitutes the essential step in developing better biomarkers and therapeutics for intestinal diseases (BOX 3). To embrace this challenge, we would benefit from transdisciplinary collaborations, not only among microbiologists, physiologists and clinicians, but also with biophysicists for the development of clinically relevant biofilm models, bioinformaticians for analysing large datasets, and microbial ecologists for their theoretical frameworks to understand such an extraordinarily complex habitat.

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Author contributions
J.-P.M. and N.V. researched data for the article, made substantial contributions to discussion of content, wrote the article, and reviewed/edited the manuscript before submission. C.D. made a substantial contribution to discussion of content, and reviewed/edited the manuscript before submission. J.L.W. and A.G.B. reviewed/edited the manuscript before submission.