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Modeling the Complex Human Intestinal Mucus-Microbiome Nexus In Vitro

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Mucus plays a pivotal role in the gastrointestinal (GI) tract, serving as an integral component of the host's defense system and contributing to the orchestration of a symbiotic relationship with the resident microbiome. Mucus is a gel-like substance that forms a protective barrier along the epithelial lining of the gut, acting as a first line of defense against pathogens and environmental insults (Figure 1).[1,2] Intestinal mucus represents a complex biological milieu composed of goblet cell-secreted mucins mixed with antimicrobial peptides/proteins secreted from enterocytes and Paneth cells localized to the bases of intestinal crypts.[3,4] Mucins are large glycoproteins that form a polymeric mesh within the mucus, which provides viscoelasticity and structure to this protective layer.[5] Beyond their physical barrier function, mucin glycans also serve as a nutrient source for microbes, fostering the growth of commensal bacteria that contribute to gut homeostasis.[6] Additionally, mucins are immune modulators influencing a host's response to microbial colonization and contribute to the maintenance of a balanced and tolerant immune environment.[3] The intricate interplay between mucus, mucins, and the gut microbiome highlights their collective significance in preserving intestinal health and underscores the importance of understanding these dynamic interactions for therapeutic interventions in conditions associated with dysbiosis and gastrointestinal diseases.

Colonic mucus, dominated by the mucin glycoprotein MUC2, is organized as a functional bi-layer comprised of a dense inner and loose outer layer. The inner mucus layer, adjacent to the epithelial cells, serves as a physical barrier that prevents direct contact between microbes and the host epithelia. Composed of tightly packed, highly glycosylated mucin proteins, this layer acts as a physical mesh that prevents the diffusion of pathogens yet allows nutrients to permeate to the epithelial cells. The less dense and more penetrable outer mucus layer creates a habitat rich in nutrients, promoting the colonization and growth of beneficial microbes. Together, these mucus layers orchestrate a finely tuned spatial arrangement that not only protects the host from harmful pathogens but also nurtures a diverse and stable microbial community. Disruptions in the integrity or composition of these layers

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can have profound implications for host-microbiome interactions, contributing to inflammatory conditions and dysbiosis in the gut. Understanding the nuanced roles of the mucus layer is essential for understanding the complexities of the intestinal ecosystem and devising strategies to therapeutically restore the relationship between the host and its microbiome. This review focuses on the complexity of host-microbiome interactions and recent advances in the field to model these interactions in vitro using human-based model systems.

The Microbiome: A Dynamic Community in the GI Tract. The gastrointestinal microbiome, comprised of bacteria, archaea, fungi, and viruses, is a remarkably complex and rich ecosystem inhabited by an estimated 10^{13} bacterial cells made up of more than 1000 identified species.[7,8]

The symbiotic relationship between the commensal flora and host is driven by nutrients and substrates from the host, provided by both diet and the carbohydrate-rich outer mucus layer, which provide rich carbon and nitrogen sources to fuel bacterial growth and metabolism. The bacterial composition in the gut is directly influenced by the mucus layer composition and, more specifically, the O- glycosylation profile of mucins, which varies among species and hosts.[10] As an example, mucins regionally display the ABH antigens, which serve as blood-type specific gut selectivity filters to direct engraftment and growth of resident flora in the GI lumen. As such, specific microbial species possess enzymes enabling them to replicate in a host-specific manner, suggesting a unique gut microbiota for every individual. Mouse studies have revealed that glycan-modifying enzymes, including glycosyltransferases and fructosyltransferases, are essential for establishing a healthy microbiome.[10] Conversely, the gut microbiota contributes to the breakdown of complex carbohydrates and fibers that human enzymes alone cannot digest. Bacterial-derived metabolites, such as bile acids and short-chain fatty acids (SCFAs), contribute to host nutrient absorption, cellular differentiation, immune regulation and provide an energy source for the GI epithelial cells.[11] The gut microbiota also confers colonization resistance against pathogenic microorganisms by competing for resources, producing antimicrobial substances, and enhancing the mucosal barrier.[9] Furthermore, the microbiome reciprocally regulates mucin expression and mucus secretion/composition from secretory intestinal cells. For example, beneficial bacteria stimulate mucus secretion, and occupy mucus binding sites (mucin glycans), ultimately protecting the epithelium from pathogen adhesion and infection.[5] Collectively, these findings suggest that commensal bacteria play a role in shaping the mucin glycosylation profile, which in turn influences bacterial composition.

Disruptions in the Microbiome-Mucus Axis: Implications for Intestinal Diseases. Microbiota dysbiosis, characterized by an imbalance in the composition and function of the gut microbial community, is increasingly recognized as a contributing factor to the pathogenesis of IBD and many other chronic inflammation-related disorders[12]. Growing evidence suggests a role for mucous disruptions in these disorders. Patients with ulcerative colitis (UC), an IBD, have an increased bacterial load, specifically mucolytic bacteria with potent mucin-degrading activity, such as

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Ruminococcus gnavus, Ruminococcus torques and Prevotella spp.[13] Perturbation of the microbiota can lead to several detrimental outcomes in IBD patients: (1) adherence and invasion of bacteria into the mucosal layer of the intestine leading to PAMP recognition; (2) changes to microbial metabolism, affecting the production of SCFAs and other metabolites that are important for gut health; and (3) disrupt the development and maintenance of immune tolerance leading to an inappropriate immune response, chronic inflammation, and tissue damage, leading to GI epithelial remodeling and fibrosis.[12]

The interplay between the protective mucus layer and the intricate balance of gut microbiota influences integrity of the gut mucosal surface and immune responses within the GI tract.[14] Biopsies from patients with UC revealed contact between luminal bacteria and the epithelium, indicating the structural integrity of the mucus layer had been compromised.[15] Several underlying mechanisms have been identified that account for increased mucus penetrability in UC including inflammation, alteration of the microbiota, defects in glycosylation, and impaired MUC2 biosynthesis.[14,16] Inflammation can directly alter goblet cell function, disrupting mucin secretion as well as glycosylation patterns. In the non-inflamed intestine, the MUC2 protein structure is stable and resistant to bacteria proteases. In GI disease states, bacterial mucin degradation provides a rich source of nutrients for pathogens, as well as results in the impairment/loss of the physical mucus barrier, leaving the intestinal epithelium vulnerable to direct interactions with bacteria/bacterial products that drive pro-inflammatory TLR signaling pathways[14]. Chronic GI inflammation, in turn, can lead to detrimental goblet cell compensatory responses, including hyper-stimulated MUC2 biosynthesis resulting in mucin mis-folding and endoplasmic reticulum stress, further propagating and sustaining injury to the intestinal epithelium[16]. The disruption of the mucin layer in IBD is a complex and multifaceted process that involves interactions between the immune system, gut microbiota, and the intestinal epithelium. Understanding these mechanisms is essential for developing targeted therapeutic approaches to restore mucosal integrity in individuals with IBD.

Modeling Host-Microbiome Interactions In Vitro: Cell-based models are crucial for addressing the challenges associated with studying host-microbiome interactions in the intricate intestinal environment. The complexity of the intestinal ecosystem poses significant hurdles for in vivo animal studies, making cell-based models essential for providing a controlled and accessible platform. These models, derived from human intestinal cells, help researchers overcome challenges related to isolating specific variables and deciphering the complex molecular mechanisms governing host-microbiome relationships. Despite their utility, challenges persist, such as accurately replicating the intestinal mucus layer, the three-dimensional tissue structure, and the diverse microbial communities that inhabit the GI lumen. Addressing these challenges is vital for enhancing the relevance and reliability of cell-based models in studying the dynamic interplay between the host and its resident microbiota. Overcoming these obstacles will contribute to a more comprehensive understanding of

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intestinal health and pave the way for improved therapeutic interventions targeting the gut microbiome.

As mucus serves as the primary interface between the intestinal epithelium and gut microbiome, the production of purified GI mucus, free from bacteria, contaminants, and antibiotics, represents an important biomaterial and substrate for microbiome research, providing the physiologic source of nutrients for specific microbes. Historically, the most used and available sources of purified mucins are obtained via industrial purification from whole pig stomach, utilizing pepsin digestion followed by ethanol precipitation. While pig gastric mucins (PGM) represent commercially abundant sources of glycoprotein, these crude commercial preparations fail to form viscoelastic hydrogels, are largely composed of non-mucin proteins, lose their anti-pathogen activities, and are not of human origin, which are all essential factors for studying the human microbiome[17]. As an alternative, it was more recently shown that primary cultures of human colon, grown at an air-liquid interface to promote goblet/mucus cell differentiation, could serve as a source to produce and harvest "native" human GI mucus[18]. The secretions derived and harvested from stem-cell based human colonic cultures were shown to reprise an intact and complex human GI mucus, retaining biophysical properties and composition comparable to ex vivo mucus collected from human donors during colonoscopies[18]. As these mucus preparations are 1) human-derived; 2) sterile; and 3) produced in the absence of antibiotics, human culture produced mucus offers a potentially pristine mucus sample ideal for use as a growth substrate for the study of the human microbiota, GI pathogens, and therapeutic probiotic strains. Furthermore, mucus derived from intestinal cultures of diverse tissue donors enables studies to explore the impact of demographic and genetic diversity on mucus composition and, ultimately, microbial diversity[1].

In addition, cell-based model systems, capable of reprising the axis between the luminal/mucus environment along with intestinal epithelial and immune cells represent critical research tools for understanding the intricate dynamics between the host and its resident microbial communities. Innovative approaches, including microphysiological systems (MPS), offer in vitro platforms that seek to mimic the physiological conditions of the gastrointestinal microenvironment[19]. MPS, or "organ-on-a-chip" platforms, derived from human stem cells, provide a dynamic environment which includes a physiologic mucus layer, that can integrate the intestinal epithelium and microbiota in a single model. These MPS models are established by reprising key physiologic gradients of growth factors, nutrients, and oxygen, often through matrix bioengineering and/or microfluidics. For example, systems developed by Wang et al.[20], Shin et al.[21], and Zhang et al.[22] represent novel MPS capable of establishing anaerobic oxygen gradients that enable the maintenance of simple and complex bacterial growth on human intestinal cultures, which are maintained under physiologic/normoxic conditions. These systems enable the real-time observation of host-microbe interactions, providing important tools to unravel the complexities of microbial colonization and the impact of environmental factors on the gut ecosystem. Despite challenges in fully recapitulating the

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in vivo complexity, in vitro models are increasingly contributing valuable insights into the crosstalk between the intestinal epithelium and the microbiome, offering a controlled and reproducible platform for studying the factors influencing gut health and disease.

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