BMB Reports - Manuscript Submission

Manuscript Draft

DOI: 10.5483/BMBRep.2022-0182

Manuscript Number: BMB-22-182

Title: Intestinal organoids as advanced modeling platforms to study the role of host-microbiome interaction in homeostasis and disease

Article Type: Mini Review

Keywords: : intestinal organoid; microbiome; symbiont; pathobiont; probiotics

Corresponding Author: Hyung-Sik Kim

Authors: Hyung-Sik Kim[1,2,3,*], Ji-Su Ahn[1,2,3], Min-Jung Kang[1], Yoojin Seo[1]

Institution: [1]Department of Oral Biochemistry, Dental and Life Science Institute, School of Dentistry, Pusan National University, Yangsan 50612, Republic of Korea,
[2]Department of Life Science in Dentistry, School of Dentistry, Pusan National University, Yangsan 50612, Republic of Korea,
[3]Education and Research Team for Life Science on Dentistry, Pusan National University, Yangsan 50612, Republic of Korea,

Manuscript Type: Mini Review

Title: Intestinal organoids as advanced modeling platforms to study the role of host-microbiome interaction in homeostasis and disease

Author's name: Ji-Su Ahn[1, 2, 3, #], Min-Jung Kang[1, #], Yoojin Seo[1, *], Hyung-Sik Kim[1, 2, 3, *]

Affiliation:

[1] Department of Oral Biochemistry; Dental and Life Science Institute, School of Dentistry, Pusan National University, Yangsan 50612, Republic of Korea

[2] Department of Life Science in Dentistry, School of Dentistry, Pusan National University, Yangsan 50612, Republic of Korea

[3] Education and Research Team for Life Science on Dentistry, Pusan National University, Yangsan 50612, Republic of Korea

Running Title: in vitro modeling of gut-microbiome interaction with intestinal organoids

Keywords: intestinal organoid, microbiome, symbiont, pathobiont, probiotics

[#]These authors equally contributed to this work

[*]Authors share co-corresponding authorship

Corresponding Author's Information:

Yoojin Seo, D.V.M, Ph.D.

E-mail: amaicat24@pusan.ac.kr

Department of Oral Biochemistry, Dental and Life Science Institute, School of Dentistry, Pusan

National University, Yangsan, 50612, Republic of Korea

Tel: +82-51-510-8235

Hyung-Sik Kim, D.V.M., Ph.D.

E-mail: hskimcell@pusan.ac.kr

Department of Oral Biochemistry, Dental and Life Science Institute, School of Dentistry, Pusan National University, Yangsan, 50612, Republic of Korea

Tel: +82-51-510-8231

ABSTRACT

After birth, animals are colonized by a diverse community of microorganisms. The digestive tract is known to contain the largest number of microbiome in the body. With emergence of the gut-brain axis, the importance of gut microbiome and its metabolites in host health has been extensively studied in recent years. The establishment of organoid culture systems has contributed to studying intestinal pathophysiology by replacing current limited models. Owing to their architectural and functional complexity similar to a real organ, co-culture of intestinal organoids with gut microbiome can provide mechanistic insights into the detrimental role of pathobiont and the homeostatic function of commensal symbiont. Here organoid-based bacterial co-culture techniques for modeling host-microbe interactions are reviewed. This review also summarizes representative studies that explore impact of enteric microorganisms on intestinal organoids to provide a better understanding of host-microbe interaction in the context of homeostasis and disease.

INTRODUCTION

Growing evidence supports that microorganisms and their byproducts can affect an individual's phenotype and vice versa (1). With advances in high-throughput sequencing technology in the last decade, great efforts have been devoted to understanding host-microbiome interactions. Numerous works have demonstrated that the microbiome not only shapes the host immune system, but also correlates with tissue homeostasis and pathophysiology of diseases (2). Of note, the gut is the most heavily colonized organ. It contains over 70% of total symbionts. Significant dysbiosis has been found in gut luminal and fecal microbiota according to disease cohort studies, suggesting a causal relationship between the gut and its microbiota in host health (3-5). For a mechanistic study, a germ-free mouse model has been widely used to assess impact

of the microbiome on disease progression. However, significant differences in microbial tropism, cellular composition, and microenvironment cues such as metabolic pathways between human and mouse often hinder interpretation of results (6). In this aspect, organoid technology has brought great advances in modeling of host-microbiome interaction *in vitro*. Organoids are self-organizing 3D structures with multiple differentiated cells derived from tissue-specific stem cells (7). With support of niche factors and extracellular matrix (ECM), LGR5-expressing crypt columnar cells can generate intestinal organoids (IOs), the first established epithelial organoids that could recapitulate the crypt-villus axis and lumen structures (8). Besides structural similarity, they can mimic several physiological properties of the gut such as selective absorption, barrier function, and mucus production. In addition, optimized culture conditions ensure robust generation and establishment of personalized- or genetically manipulated IOs (9, 10), overcoming limitations of conventional *in vitro* models. In this mini-review, we will briefly introduce current methodology for microbe-IO co-culture. We then summarize representative findings describing the impact of symbiont and pathobiont as well as probiotic candidates in host health using co-culture systems to provide insights into the importance of cross-talk between host and microorganisms.

1. Organoid-microbial co-culture technology

To investigate interactions between IOs and microbes, it is essential to mimic the naïve gut environment harboring microbes. In the gastrointestinal tract, microbes exist within the lumen and directly interact with the intestinal epithelium through the apical side. In contrast, typical IOs have basal-out structures. Therefore, co-culture methods that allow physiologically relevant interaction are required for modeling microbial infections.

1-1. Microinjection

Microinjection of bacteria directly into the lumen of IOs can facilitate bacterial contact with the apical side of the epithelium (11, 12). Since the closed lumen has low oxygen tension, microinjection can improve the infection efficiency of anaerobic bacteria (13). Given that manual injection is a highly labor-intensive, time-consuming procedure (14, 15), a high-throughput organoid microinjection platform has been developed (16). However, since it is not a perfect anaerobic co-culture system, maintenance of a long-term culture of IOs with anaerobic bacteria is limited.

1-2. Suspension culture with IOs

Microbes can be simply treated to organoid growth media or embedded with ECM during IO culture. It is the most common method to study host-microbe interaction so far. Organoids can be cultured with live- and heat-killed (HK) bacteria or with conditioned media containing their byproducts, including bacterial toxins and metabolites (17-19). However, this method restricts the access of bacteria to the apical side of IOs. To overcome this limitation, IOs can be mechanically shredded to expose the luminal side and then re-seeded into ECM following co-culture with live bacteria (20, 21).

1-3. Organoid-derived monolayers (ODMs)

ODMs are established by seeding dissociated 3D-grown IOs on a Transwell plate to expose the apical surface upward with media (22, 23). ODMs can recapitulate cell compositions of gastrointestinal epithelium such as enterocytes, goblet cells, Paneth cells, and other cell populations (24). This monolayer culture provides practical advantages of easy microbial access to the luminal side and convenient sample collection compared to ECM-embedded

classical IOs. However, 2D-grown epithelial stem cells usually undergo differentiation. They cannot be sub-cultured or maintained for a long time. Thus, many cells are needed each time. In addition, it is hard to obtain morphological information with ODM method (25). ODM culture technique can be further modified by exposing the upper side of the layer to air to generate an oxygen gradient. With the air-liquid interface (ALI) culture method, in which the basolateral side and the apical side contact with media and surrounding air, respectively, ODMs can differentiate into more mature epithelial cells such as mucus-secreting goblet cells than 3D organoids (26, 27). In addition, IHACS (intestinal hemi-anaerobic co-culture system), composed of a hypoxic apical chamber sealed with a rubber plug and a basal chamber in normal oxygen concentration can facilitate the survival of both epithelial cells and microbes (28, 29).

1-4. Organoids with reversed polarity

The apical-out IO model is an alternative to microinjection which has limitations of laborious processes and requirement of special equipment. Reversion of epithelial polarity is performed by removing ECM and maintaining suspended IOs in the low-attachment plate, where spontaneous polarity changes from basal-out to apical-out occur (30). Apical-out IOs allow direct interactions between the epithelium and microbes. Functional assays for nutrient uptake and epithelial barrier integrity can also be performed since epithelial cells within apical-out IOs can differentiate into a more mature state than conventional IOs (30).

2. Culturing IOs with microorganisms to study host-microbiome interactions

2.1. Co-culture with pathobionts

Pathobiont has a significant impact on host health. Enteric infections by bacterial pathogens

are responsible for various diarrheal diseases, particularly in developing countries. Dysbiosis in the gut microbiome can lead to several enteric and systemic disorders (31). Moreover, the rapid increase of antibiotic-resistant pathogens has become a critical threat in recent years (32). Thus, numerous efforts have been made to evaluate and understand the detrimental impact of pathobionts on the gut using IOs (Table 1).

a. *Shigella flexceri*

Shigella flexneri infection is a leading cause of acute diarrhea, fever, and stomach pain in humans, particularly young children (33). ODM culture is the most frequently used *in vitro* model to study the role of *S. flexneri* in intestinal epithelial injury. After administration, *S. flexneri* exhibits high bacterial adherence rates in ODMs and induces maturation of M cells with upregulation of pro-inflammatory signals (24, 34, 35). Infection by *S. flexneri* can also trigger IL-8 secretion and mucin glycoprotein MUC2 expression (24, 35). *S. flexneri* can invade ODMs via the basolateral side, while disruption of the tight junctions in the epithelial barrier allows *S. flexneri* to invade ODMs via the apical surface (36). In addition, the killing potency of bacteriophages targeting *S. flexneri* has been tested in an ODM-based co-culture model to find alternatives to current antibiotics (34). Interestingly, bacterial adherence and invasion capacity in ODMs are significantly inhibited by the presence of bacteriophage.

b. *Salmonella enterica*

Salmonella enterica is a major pathogen for food-borne diarrheal diseases such as typhoid fever known to be caused by *S. enterica* serovar Typhimurium (37). Zhang *et al*. have investigated *S*. Typhimurium pathogenesis in the intestine using a microinjection method (38). In their study, disruption of epithelial tight junction and increment of inflammatory cytokine by NF-κB

activation were observed in IOs upon administration of *S.* Typhimurium. Moreover, infection by *Salmonella* led to reduced Lgr5 expression, suggesting suppression of stem cell population. In an apical-out organoid model, *S.* Typhimurium invaded the apical epithelial surface more efficiently than invading the basolateral surface, which induced actin ruffles (30). Of note, key components for bacterial invasion have been revealed with a co-culture system. For instance, colonization of *S.* Typhimurium after microinjection into the lumen of IOs is dependent on an important virulence factor, type III secretion system 1 (TTSS-1), and its flagellar motility (39). The invasion capacity of *S.* Typhimurium is also mediated by invA (40) and Pathogenicity Island (SPI)-derived Type 3 secretion systems (T3SS) (41). Interestingly, a deficiency of phospholipid transporter YrbE in *S. enterica* serovar Typhi can lead to upregulation of flagellin, which enhances pro-inflammatory IL-8 expression in ODM (42).

c. *Escherichia coli*

Most *Escherichia coli* strains are commensal bacteria in the large intestine. However, several pathogenic strains are important causes of diarrheal illness and food poisoning (43). For example, enterohemorrhagic *E. coli* (EHEC) serotype O157:H7 is responsible for fatal foodborne diarrheal diseases (44, 45). After co-culturing with human ODMs, EHEC can readily colonize differentiated human ODMs. It especially targets MUC2 and microvillar resident protein PCDH24 at the early stage of infection (46). EHEC can also secrete extracellular serine protease EspP that exhibits enterotoxin activity by stimulating an electrogenic ion transporter, leading to reduced PCDH24 and brush border damage, while the enterotoxin-producing activity of EHEC in human IOs is independent of EspP activity (47).

Meanwhile, enterotoxigenic *E. coli* (ETEC) infection is associated with high mortalities in developing countries (48). ETEC can secrete heat-stable enterotoxin (ST) and heat-labile

enterotoxin (LT) into the intestinal epithelium, which can induce cGMP and cAMP stimulation (49). Indeed, ST-induced cGMP synthesis followed by apical efflux of cGMP into the basolateral space has been observed in the human ODM model. However, disruption of phosphodiesterase PDE5 can reverse this phenomenon, revealing that cyclic nucleotide export and degradation can be initiated by enterotoxins (50). Given their importance in domestic animal farms, IOs have been established from porcine small intestine to generate ODM to investigate ETEC pathogenesis in pig gut (51). ETEC exhibits F4 fimbriae-mediated adhesion to porcine ODM as observed *in vivo*, indicating the utility of porcine IOs and co-culture systems to study enteric pathobionts in industrial animals.

Recent studies have shown that genotoxic colibactin-secreting *E. coli* strains are more abundantly detected in colorectal cancer (CRC) tissues than in healthy ones (52) and that *pks* genome is responsible for colibactin production (53). Indeed, long-term exposure (over five months) of human IOs to *pks*+ *E. coli* performed by luminal microinjection can lead to accumulation of genetic mutations in epithelial cells (54). Interestingly, organoids upon short-term exposure to *pks*+ *E. coli* also exhibit DNA damage, enhanced proliferation, and Wnt-independent abnormal growth (21). These studies demonstrate a hypothesis regarding the *pks*+ *E. coli* can mediate the tumorigenic process in CRC development.

d. *Clostridium difficile*

Clostridium difficile accounts for a significant proportion of antibiotic-induced diarrhea and colitis (55). Microinjection of *C. difficile* into IOs can lead to a reduction of Na(+)/H(+) exchanger 3 (NHE3) with organoid swelling, which can recapitulate the *in vivo* situation of *C. difficile*-induced chronic diarrhea (56). Main virulence factors of *C. difficile* including *C. difficile* toxin A (TcdA), *C. difficile* toxin B (TcdB), and *C. difficile* transferase (CDT) can

collapse the adherens junction through disruption E-Cadherin and actin-cytoskeleton (18, 57). Similarly, human ODMs express a high level of TcdA receptor (58). TcdA can disrupt the barrier function of human IOs upon *C. difficile* microinjection into the lumen for up to 12 h (11). TcdB can further inhibit epithelial regeneration by impairing stem cell functions in IOs established from *C. difficile*-infected mice (57). In this context, several studies have targeted *C. difficile* toxins to neutralize the harmful impact of *C. difficile* and found that administration of human serum albumin (HSA) and antibiotic bacitracin could prevent the toxic effect of TcdA and TcdB in IOs (59, 60). Co-culture of *C. difficile* and IOs has also provided insights into the physiological response of epithelial cells to resist microbial infection as reported by Liu *et al.* (19). In their work, the protective role of Paneth cells during *C. difficile* infection was investigated in murine and human IOs. It was shown that constitutive activation of signal transducer and activator of transcription 5 (STAT5) signaling could potentiate anti-bacterial and niche-supporting functions of Paneth cells in response to inflammatory cytokines and bacterial toxin, thus reducing *C. difficile* cytotoxicity.

e. *Vibrio cholerae*

Vibrio cholerae is an important cause of epidemic diarrhea, which is mediated by cholera toxin (CT) (61). Several studies have reported that treatment of IOs with CT can activate cAMP pathway, which induces acute swelling of IOs due to fluid accumulation in the lumen (62, 63). In this aspect, organoid swelling assay has been used for testing CT inhibitors (63). Interestingly, IOs derived from O-blood group show more elevated cAMP response upon CT administration than IOs derived from A-blood groups (64).

f. *Listeria monocytogenes*

Listeria monocytogenes is an opportunistic food-borne pathogen that causes listeriosis in immunocompromised individuals (65). *In vitro* infection of *L. monocytogenes* in IOs can stimulate organoid growth and induce differentiation into Paneth cells by regulating the expression of transcriptional factors *Math1* and *Sox9* (20). Another study has shown that *L. monocytogenes* can lead to Paneth cell induction in IOs by inhibiting Notch signaling and activating the toll-like receptor (TLR) 2/4 pathway with upregulation of opsonin protein CCN1 (66, 67). When the infection efficiency of *L. monocytogenes* was assessed in IOs exhibiting different polarity, basal-out IOs were more susceptible to *L. monocytogenes* invasion than apical-out ones because *L. monocytogenes* could bind to basolateral receptors after targeting cell extrusion regions (30). Interestingly, luminal microinjection of *L. monocytogenes* into IOs also demonstrated that crosslinking between bacterial internalin A (InlA) and E-cadherin on goblet cells could mediate the invasive process of *Listeria*, allowing its entry from lumen to the basal side despite the presence of epithelial barriers (68). Meanwhile, quantitative proteomic analysis has revealed that *L. monocytogenes* could differentially regulate the transcriptional activity and metabolism of IOs depending on their strains and serotypes (69).

g. Other pathogenic bacteria

A positive correlation between the abundance of *Campylobacter* species in CRC tissues and CRC development has been confirmed (70). Specifically, *Campylobacter jejuni* can induce DNA damage and intestinal inflammation by producing genotoxin (71). In line with previous studies, treatment with bacterial lysate from *C. jejun*i can lead to accumulation of DNA damage with increased rH2AX induction in human IOs, while ablation of cytolethal distending toxin (CDT) can abrogate pro-inflammatory- and genotoxic impact of *C. jejuni* (72).

Since the gut is part of the digestive tract, oral pathogenic bacteria can be detrimental to the

intestine. For instance, *Fusobacterium nucleatum* in the gastrointestinal tract is positively associated with the development of gut inflammation and CRC (73). Interestingly, treatment with outer membrane vesicles (OMV) produced by *F. nucleatum* subsp. *polymorphum* can promote proinflammatory responses by stimulating tumor necrosis factor (TNF) secretion and NF-κB activation in human ODMs (74). Lipopolysaccharides (LPS) derived from *Porphyromonas gingivalis*, another major oral bacterium responsible for periodontitis, can regulate differentiated epithelial cell marker expression in murine IOs (75).

Table 1. Studies reporting host-pathogenic bacteria interactions using organoids

Bacteria	Source of organoid	Culture system	Key findings	References
Shigella flexneri	Human small intestine, colon	ODM	↑ IL-8 secretion	(24)
	Human (unspecified)	ODM	↑ IL-8 secretion ↑ Muc2 expression	(34)
	Human small intestine	ODM	Basolateral infection, ↑ Pro-inflammatory signals	(35)
	Human small intestine, colon	ODM	Testing the therapeutic effect of bacteriophage	(36)
Salmonella	Human small	3D-	↓ Organoid growth	(38)

enterica	intestine	microinjection	↑ NF-kB signaling ↑ Pro-inflammatory cytokine, ↓ LGR5 expression	
	Human/mouse small intestine	3D-microinjection	Recapitulation of early infection cycle, TTSS-1 is required for colonization	(39)
	Human iPSC	3D-microinjection	↑ Proinflammatory cytokines, InvA-dependent invasion	(40)
	Human ESC	3D-microinjection	T3SS-1-dependent invasion, ↑ Inflammatory chemokine	(41)
	Human small intestine, colon	Apical-out	Cytoskeletal rearrangement	(30)
	Human small intestine	ODM	YrbE-dependent inflammatory response	(42)

EHEC	Human colon	ODM	↓ Colonic mucus Brush border damage	(45)
	Human colon	ODM	Change in active ion transport	(47)
ETEC	Human small intestine	ODM	PDE5-mediated restriction of intracellular cGMP accumulation	(50)
	Pig small intestine	ODM	F4-mediated adhesion	(51)
pks+ E. coli	Human colon	ODM	Long-term exposure caused mutational signature	(54)
	Human/mouse colon	ODM/ Shredded 3D	↑ Proliferation Wnt-independent growth	(21)
Clostridium difficile	Human iPSC, ESC	3D-microinjection	↓ Epithelial barrier function	(11)
	Human iPSC	3D-microinjection	↓ NHE3 expression	(56)
	Human/mouse colon	3D derived from infected	↓ Adherens junction,	(57)

		mice/ 3D-toxin treatment	↓ Epithelial regeneration	
	Human small intestine	ODM	Adherence mechanism in human ODM model	(58)
	Human iPSC	3D-toxin treatment	↓ Transmembrane adhesion protein	(18)
	Human iPSC	3D-toxin treatment	Protective effect of HSA	(59)
	Human (unspecified)	3D-toxin treatment	Protective effect of antibiotic Bacitracin	(60)
	Human/mouse iPSC	3D-microinjection	Protective effect of Paneth cells on *C. difficile*-infected IO	(19)
Vibrio cholerae	Mouse small intestine	3D-toxin treatment	↑ cAMP pathway	(62)
	Human small intestine	3D-toxin treatment	Testing CT inhibitor with swelling assay	(63)
	Human small intestine	3D-toxin treatment	O-blood group exhibited different responses to CT	(64)
Listeria	Mouse small	Shredded 3D	↑ Organoid growth	(20)

monocytogenes	intestine		↓ Lgr5+ ISCs ↑ Paneth cells	
	Mouse small intestine	Shredded 3D	↑ TNFa ↑ Paneth cell, goblet cell ↓ Notch signaling	(66)
	Mouse small intestine	Shredded 3D	↑ TLR 2/4 signaling	(67)
	Human small intestine, colon	Apical-out	Binding with basolateral receptor	(30)
	Mouse small intestine	3D-microinjection	InlA-Ecad-dependent translocation through goblet cells	(68)
	Mouse (unspecified)	Shredded 3D	TMT-based quantitative proteomic analysis in different strains	(69)
Campylobacter jejuni	Mouse small intestine	3D-bacterial lysate	DNA damage	(72)
Fusobacterium nucleatum	Human (unspecified)	ODM-OMV treatment	↑ TNF, NF-κB, MAPK signaling	(74)

Porphyromonas gingivalis	Mouse small intestine	3D	Regulation of cell composition	(75)

2.2. Co-culture with commensal bacteria and probiotics

Recent evidence has shown that probiotics can provide beneficial effects on the host by improving the balance of gut microbiota composition and promoting intestinal mucosal barrier function, indicating their therapeutic potential to treat a variety of intestinal disorders as shown below (76, 77) (Table 2).

a. *Lactobacillus*

Most *Lactobacillus* species including *L. reuteri*, *L. rhamnosus*, and *L. acidophilus* are regarded as important probiotics in the intestine. They have been reported to be able to improve proliferation, regeneration, and maturation of IOs (78).

L. reuteri can enhance the recovery of Lgr5+ cells and epithelial barrier after TNF-induced intestinal damage by activating the Wnt/β-catenin pathway in IOs (79). When *L. reuteri D8* was co-cultured with murine IOs in the presence of lamina propria lymphocytes (LPLs), *L. reuteri D8* could stimulate the proliferation of stem cells and Paneth cell induction by up-regulating the secretion of IL-22 mediated by STAT3 signaling activation (80). The role of *L. reuteri* in modulating host immunomodulation has also been investigated in an IOs-dendritic cell (DC) co-culture system (81). It was verified that both *L. reuteri* and its bacterial surface components could promote IL-10 production and DC maturation (81).

Administration of *L. rhamnosus* GG (LGG) to an IO culture system can provide some protection against rotavirus infection by up-regulating antiviral secretory factors such as interferon-α (IFN-α) and CXC motif chemokine ligand 1 (CXCL1) via activation of TLR3

pathway in epithelial cells (82). Han *et al*. have also investigated the therapeutic potential of LGG in irritable bowel syndrome (IBS) using IO-based co-culture systems with a focus on barrier function (83). Interestingly, they found that LGG significantly upregulated junctional marker expression and prevented the increase in organoid permeability in response to treatment with IFN-γ or fecal supernatants obtained from IBS patients, indicating a beneficial role of LGG in the maintenance of gut barrier integrity.

The protective effect of *Lactobacillus* species on intestinal epithelial damage has been also exhibited by *L. acidophilus*. To evaluate whether *L. acidophilus* could suppress the detrimental impact of pathobionts on IOs, *L. acidophilus* and *S. typhimurium* were treated simultaneously to dissociated IOs. Cellular properties of IOs were then assessed (84). In that work, *S.* Typhimurium significantly reduced the organoid-forming efficiency, which could be reversed by co-treatment with *L. acidophilus*. *L. acidophilus* could also enhance the differentiation into secretory lineage cells and, which in turn increased the production of mucus and antibacterial peptides to strengthen the mucosal barrier by regulating *S. typhimurium*-mediated hyperactivation of TLR2- and Wnt/β-catenin signaling pathways. Of note, Sittipo *et al*. have revealed dynamic changes in the prevalence of *Lactobacillu*s species in murine stool samples after irradiation exposure using 16S rRNA-based oligotyping analysis (85) and reported the contribution of *L. acidophilus* to functional recovery of radiation-induced epithelial injury both *in vitro* and *in vivo*. Treatment of irradiated IOs with HK *L. acidophilus* can significantly enhance the organoid formation capacity with goblet cell enrichment, suggesting that administration of *L. acidophilus* and its derivatives might be beneficial to restoring intestinal homeostasis and barrier function impeded by pathogenic bacterial infection or irradiation.

b. *Bifidobacterium*

Bifidobacterium is another commensal probiotic bacterium that participates in the homeostasis of the gut microorganism community (86). *Bifidobacterium*, the dominant species in the large intestine, requires an anaerobic environment (78). In this context, IHACS, which mimics physiological gut anaerobic conditions *in vitro*, is useful for culturing IOs with *Bifidobacterium* (29). With this oxygen-controlled co-culture system, *B. adolescentis* could be successfully propagated with ODM. Importantly, only live *B. adolescentis,* not heat-killed bacteria or bacterial culture supernatant, could increase the expression of stem cell and goblet cell markers of ODM upon co-culture, demonstrating the importance of viability of bacteria in studying host-microbe interactions.

c. *Escherichia coli*

Benefits of commensal bacteria *E. coli* Nissle against dysbiosis have been confirmed in IOs (87). *E. coli* Nissle can protect human IOs from pathogenic *E. coli*-mediated disruption of the epithelial barrier, increased oxidative stress, and apoptosis. In addition, microinjection of a non-pathogenic *E. coli* strain ECOR2 into human IOs can lead to transient changes in the oxygen concentration without causing any harmful impact on epithelial cells (88). After colonization, *E. coli* strain ECOR2 can increase the production of antimicrobial peptides and improve tissue maturation of IOs.

Table 2. Studies reporting host-probiotic bacteria interactions using organoids

Bacteria	Source of organoid	Culture system	Key findings	References
Lactobacillus reuteri D8	Mouse small intestine	3D	↑ Intestinal epithelial regeneration	(79)

	Mouse small intestine	3D	↑ Proliferation of intestinal epithelial stem cells	(80)
Lactobacillus reuteri	Mouse small intestine	3D	↑ Dendritic cell maturation and IL-10 production	(81)
Lactobacillus rhamnosus GG	Mouse small intestine	3D	↑ Expression of TLR3	(82)
	Mouse small intestine, colon	3D-microinjection	↑ Epithelial barrier function	(83)
Lactobacillus acidophilus	Mouse small intestine	3D	↑ Protects the intestinal mucosa against pathogen	(84)
	Mouse small intestine	3D -HK bacteria	↑ Intestinal epithelial function and differentiation	(85)
Bifidobacterium adolescentis	Human colon	ODM	↑ Differentiation of goblet cell and stem cell	(29)
Escherichia coli Nissle	Human small intestine	3D-microinjection	↑ Epithelial barrier function	(87)

Nonpathogenic *E. coli*	Human small intestine	3D-microinjection	↑ Epithelial proliferation & secretion of anti-microbial peptide	(88)

FUTURE PERSPECTIVES

Organoid technology holds great potential to overcome limitations of conventional models such as 2D cell lines and experimental animals for modeling human anatomy and physiology. However, several challenging issues have to be solved to achieve advanced modeling of host-microorganism interactions with IOs. First, the absence of other cellular components except for epithelial cells is the main limitation of present IOs. Compared to pluripotent stem cell-derived organoids, which exhibit diverse cellular complexity, most adult stem cell-derived organoids like IOs consist of restricted lineage-derived cells that impede modeling of naïve microenvironment (89). Considering that microorganisms usually elicit an immune response and a repair process, which are predominantly mediated by regional immune cells and stromal cells respectively, the addition of these cells to an IO-microbe co-culture system would be necessary to recapitulate *in vivo* circumstances. Optimization of culture conditions is another important challenging issue. Although IOs are generally maintained under neutral, normoxic conditions, a dynamic range of oxygen concentration and pH found in the gut can significantly influence bacterial colonization patterns (90). Since oxygen availability strictly regulates bacterial behavior including growth, metabolism, and stress resistance, providing adequate oxygen gradient to both mammalian cells and microorganisms using advanced techniques such as microfluidic systems would be required to recapitulate *in vivo* situations. These improvements can also help us evaluate IO responses to a polymicrobial infection, which will provide clues about the role of dysbiosis in enteric disorder progression and greatly contribute

to the understanding of disease mechanisms and establishing an effective therapeutic strategy.

ACKNOWLEDGMENTS

This study was supported by the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (2018-R1A5A2023879, 2019-R1A2C2085876) and the Ministry of Education (2021-R1I1A1A01055654). Korean Fund for Regenerative Medicine (KFRM) grant funded by the Ministry of Science and ICT and the Ministry of Health & Welfare) (RS-2022-00070468) also supported this project.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Ley RE, Peterson DA and Gordon JI (2006) Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124, 837-848
2. Lynch JB and Hsiao EY (2019) Microbiomes as sources of emergent host phenotypes. Science 365, 1405-1408
3. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y and Andoh A (2018) Gut microbiota in the pathogenesis of inflammatory bowel disease. Clinical Journal of Gastroenterology 11, 1-10
4. Baumler AJ and Sperandio V (2016) Interactions between the microbiota and pathogenic bacteria in the gut. Nature 535, 85-93
5. Park EM, Chelvanambi M, Bhutiani N, Kroemer G, Zitvogel L and Wargo JA (2022) Targeting the gut and tumor microbiota in cancer. Nature Medicine 28, 690-703
6. Nguyen TLA, Vieira-Silva S, Liston A and Raes J (2015) How informative is the mouse for human gut microbiota research? Disease Models & Mechanisms 8, 1-16
7. Kim J, Koo BK and Knoblich JA (2020) Human organoids: model systems for human biology and medicine. Nature Reviews Molecular Cell Biology 21, 571-584
8. Sato T, Vries RG, Snippert HJ et al (2009) Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 459, 262-U147
9. Menche C and Farin HF (2021) Strategies for genetic manipulation of adult stem cell-derived organoids. Experimental and Molecular Medicine 53, 1483-1494
10. Min S, Kim S and Cho SW (2020) Gastrointestinal tract modeling using organoids engineered with cellular and microbiota niches. Experimental and Molecular Medicine 52, 227-237
11. Leslie JL, Huang S, Opp JS et al (2015) Persistence and toxin production by Clostridium difficile within human intestinal organoids result in disruption of epithelial paracellular barrier function. Infect Immun 83, 138-145
12. Zhang Y and Yu LC (2008) Microinjection as a tool of mechanical delivery. Curr Opin Biotechnol 19, 506-510
13. Poletti M, Arnauts K, Ferrante M and Korcsmaros T (2021) Organoid-based Models to Study the Role of Host-microbiota Interactions in IBD. J Crohns Colitis 15, 1222-1235
14. Ginga NJ, Slyman R, Kim GA et al (2022) Perfusion System for Modification of Luminal Contents of Human Intestinal Organoids and Realtime Imaging Analysis of Microbial

Populations. Micromachines (Basel) 13

15. Puschhof J, Pleguezuelos-Manzano C, Martinez-Silgado A et al (2021) Intestinal organoid cocultures with microbes. Nat Protoc 16, 4633-4649
16. Williamson IA, Arnold JW, Samsa LA et al (2018) A High-Throughput Organoid Microinjection Platform to Study Gastrointestinal Microbiota and Luminal Physiology. Cell Mol Gastroenterol Hepatol 6, 301-319
17. Chen Y, Cao K, Liu H et al (2021) Heat Killed Salmonella typhimurium Protects Intestine Against Radiation Injury Through Wnt Signaling Pathway. J Oncol 2021, 5550956
18. Fischer S, Uckert AK, Landenberger M et al (2020) Human peptide alpha-defensin-1 interferes with Clostridioides difficile toxins TcdA, TcdB, and CDT. FASEB J 34, 6244-6261
19. Liu R, Moriggl R, Zhang D et al (2019) Constitutive STAT5 activation regulates Paneth and Paneth-like cells to control Clostridium difficile colitis. Life Sci Alliance 2
20. Huang J, Zhou C, Zhou G, Li H and Ye K (2021) Effect of Listeria monocytogenes on intestinal stem cells in the co-culture model of small intestinal organoids. Microb Pathog 153, 104776
21. Iftekhar A, Berger H, Bouznad N et al (2021) Genomic aberrations after short-term exposure to colibactin-producing E. coli transform primary colon epithelial cells. Nat Commun 12, 1003
22. Sato T, Stange DE, Ferrante M et al (2011) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology 141, 1762-1772
23. Thorne CA, Chen IW, Sanman LE, Cobb MH, Wu LF and Altschuler SJ (2018) Enteroid Monolayers Reveal an Autonomous WNT and BMP Circuit Controlling Intestinal Epithelial Growth and Organization. Dev Cell 44, 624-633 e624
24. Nickerson KP, Llanos-Chea A, Ingano L et al (2021) A Versatile Human Intestinal Organoid-Derived Epithelial Monolayer Model for the Study of Enteric Pathogens. Microbiol Spectr 9, e0000321
25. Zhang J, Hernandez-Gordillo V, Trapecar M et al (2021) Coculture of primary human colon monolayer with human gut bacteria. Nat Protoc 16, 3874-3900
26. Nossol C, Diesing AK, Walk N et al (2011) Air-liquid interface cultures enhance the oxygen supply and trigger the structural and functional differentiation of intestinal porcine epithelial cells (IPEC). Histochem Cell Biol 136, 103-115
27. Sachs N, Papaspyropoulos A, Zomer-van Ommen DD et al (2019) Long-term expanding human airway organoids for disease modeling. EMBO J 38
28. Kim R, Attayek PJ, Wang Y et al (2019) An in vitro intestinal platform with a self-sustaining oxygen gradient to study the human gut/microbiome interface. Biofabrication 12, 015006
29. Sasaki N, Miyamoto K, Maslowski KM, Ohno H, Kanai T and Sato T (2020) Development of a Scalable Coculture System for Gut Anaerobes and Human Colon Epithelium. Gastroenterology 159, 388-390 e385

30. Co JY, Margalef-Catala M, Li X et al (2019) Controlling Epithelial Polarity: A Human Enteroid Model for Host-Pathogen Interactions. Cell Rep 26, 2509-2520 e2504
31. Collaborators GBDDD (2018) Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis 18, 1211-1228
32. Hazen TH, Michalski J, Nagaraj S, Okeke IN and Rasko DA (2017) Characterization of a Large Antibiotic Resistance Plasmid Found in Enteropathogenic Escherichia coli Strain B171 and Its Relatedness to Plasmids of Diverse E. coli and Shigella Strains. Antimicrob Agents Chemother 61
33. Livio S, Strockbine NA, Panchalingam S et al (2014) Shigella isolates from the global enteric multicenter study inform vaccine development. Clin Infect Dis 59, 933-941
34. Llanos-Chea A, Citorik RJ, Nickerson KP et al (2019) Bacteriophage Therapy Testing Against Shigella flexneri in a Novel Human Intestinal Organoid-Derived Infection Model. J Pediatr Gastroenterol Nutr 68, 509-516
35. Ranganathan S, Doucet M, Grassel CL, Delaine-Elias B, Zachos NC and Barry EM (2019) Evaluating Shigella flexneri Pathogenesis in the Human Enteroid Model. Infect Immun 87
36. Koestler BJ, Ward CM, Fisher CR, Rajan A, Maresso AW and Payne SM (2019) Human Intestinal Enteroids as a Model System of Shigella Pathogenesis. Infect Immun 87
37. Jajere SM (2019) A review of Salmonella enterica with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. Vet World 12, 504-521
38. Zhang YG, Wu S, Xia Y and Sun J (2014) Salmonella-infected crypt-derived intestinal organoid culture system for host-bacterial interactions. Physiol Rep 2
39. Geiser P, Di Martino ML, Samperio Ventayol P et al (2021) Salmonella enterica Serovar Typhimurium Exploits Cycling through Epithelial Cells To Colonize Human and Murine Enteroids. mBio 12
40. Forbester JL, Goulding D, Vallier L et al (2015) Interaction of Salmonella enterica Serovar Typhimurium with Intestinal Organoids Derived from Human Induced Pluripotent Stem Cells. Infect Immun 83, 2926-2934
41. Lawrence AE, Abuaita BH, Berger RP et al (2021) Salmonella enterica Serovar Typhimurium SPI-1 and SPI-2 Shape the Global Transcriptional Landscape in a Human Intestinal Organoid Model System. mBio 12
42. Verma S, Prescott RA, Ingano L et al (2020) The YrbE phospholipid transporter of Salmonella enterica serovar Typhi regulates the expression of flagellin and influences motility, adhesion and induction of epithelial inflammatory responses. Gut Microbes 11, 526-538
43. Blount ZD (2015) The unexhausted potential of E. coli. Elife 4
44. Kaper JB, Nataro JP and Mobley HL (2004) Pathogenic Escherichia coli. Nat Rev Microbiol 2, 123-140

45. Takeda Y (1997) Enterohaemorrhagic Escherichia coli. World Health Stat Q 50, 74-80
46. In J, Foulke-Abel J, Zachos NC et al (2016) Enterohemorrhagic Escherichia coli reduce mucus and intermicrovillar bridges in human stem cell-derived colonoids. Cell Mol Gastroenterol Hepatol 2, 48-62 e43
47. Tse CM, In JG, Yin J et al (2018) Enterohemorrhagic E. coli (EHEC)-Secreted Serine Protease EspP Stimulates Electrogenic Ion Transport in Human Colonoid Monolayers. Toxins (Basel) 10
48. Kotloff KL, Nataro JP, Blackwelder WC et al (2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 382, 209-222
49. Evans DJ, Jr. and Evans DG (1973) Three characteristics associated with enterotoxigenic Escherichia coli isolated from man. Infect Immun 8, 322-328
50. Foulke-Abel J, Yu H, Sunuwar L et al (2020) Phosphodiesterase 5 (PDE5) restricts intracellular cGMP accumulation during enterotoxigenic Escherichia coli infection. Gut Microbes 12, 1752125
51. Vermeire B, Gonzalez LM, Jansens RJJ, Cox E and Devriendt B (2021) Porcine small intestinal organoids as a model to explore ETEC-host interactions in the gut. Vet Res 52, 94
52. Dejea CM, Fathi P, Craig JM et al (2018) Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. Science 359, 592-597
53. Escobar-Paramo P, Le Menac'h A, Le Gall T et al (2006) Identification of forces shaping the commensal Escherichia coli genetic structure by comparing animal and human isolates. Environ Microbiol 8, 1975-1984
54. Pleguezuelos-Manzano C, Puschhof J, Rosendahl Huber A et al (2020) Mutational signature in colorectal cancer caused by genotoxic pks(+) E. coli. Nature 580, 269-273
55. Chandrasekaran R and Lacy DB (2017) The role of toxins in Clostridium difficile infection. FEMS Microbiol Rev 41, 723-750
56. Engevik MA, Engevik KA, Yacyshyn MB et al (2015) Human Clostridium difficile infection: inhibition of NHE3 and microbiota profile. Am J Physiol Gastrointest Liver Physiol 308, G497-509
57. Mileto SJ, Jarde T, Childress KO et al (2020) Clostridioides difficile infection damages colonic stem cells via TcdB, impairing epithelial repair and recovery from disease. Proc Natl Acad Sci U S A 117, 8064-8073
58. Engevik MA, Danhof HA, Chang-Graham AL et al (2020) Human intestinal enteroids as a model of Clostridioides difficile-induced enteritis. Am J Physiol Gastrointest Liver Physiol 318, G870-G888
59. di Masi A, Leboffe L, Polticelli F et al (2018) Human Serum Albumin Is an Essential Component of the Host Defense Mechanism Against Clostridium difficile Intoxication. J Infect Dis 218, 1424-1435

60. Zhu Z, Schnell L, Muller B, Muller M, Papatheodorou P and Barth H (2019) The Antibiotic Bacitracin Protects Human Intestinal Epithelial Cells and Stem Cell-Derived Intestinal Organoids from Clostridium difficile Toxin TcdB. Stem Cells Int 2019, 4149762
61. Sigman M and Luchette FA (2012) Cholera: something old, something new. Surg Infect (Larchmt) 13, 216-222
62. Foulke-Abel J, In J, Yin J et al (2016) Human Enteroids as a Model of Upper Small Intestinal Ion Transport Physiology and Pathophysiology. Gastroenterology 150, 638-649 e638
63. Zomer-van Ommen DD, Pukin AV, Fu O et al (2016) Functional Characterization of Cholera Toxin Inhibitors Using Human Intestinal Organoids. J Med Chem 59, 6968-6972
64. Kuhlmann FM, Santhanam S, Kumar P, Luo Q, Ciorba MA and Fleckenstein JM (2016) Blood Group O-Dependent Cellular Responses to Cholera Toxin: Parallel Clinical and Epidemiological Links to Severe Cholera. Am J Trop Med Hyg 95, 440-443
65. Radoshevich L and Cossart P (2018) Listeria monocytogenes: towards a complete picture of its physiology and pathogenesis. Nat Rev Microbiol 16, 32-46
66. Zhou C, Zhang Y, Bassey A, Huang J, Zou Y and Ye K (2022) Expansion of Intestinal Secretory Cell Population Induced by Listeria monocytogenes Infection: Accompanied With the Inhibition of NOTCH Pathway. Front Cell Infect Microbiol 12, 793335
67. Zhou C, Zou Y, Zhang Y, Teng S and Ye K (2022) Involvement of CCN1 Protein and TLR2/4 Signaling Pathways in Intestinal Epithelial Cells Response to Listeria monocytogenes. Int J Mol Sci 23
68. Kim M, Fevre C, Lavina M, Disson O and Lecuit M (2021) Live Imaging Reveals Listeria Hijacking of E-Cadherin Recycling as It Crosses the Intestinal Barrier. Curr Biol 31, 1037-1047 e1034
69. Zhou C, Zou Y, Huang J et al (2022) TMT-Based Quantitative Proteomic Analysis of Intestinal Organoids Infected by Listeria monocytogenes Strains with Different Virulence. Int J Mol Sci 23
70. Wu N, Yang X, Zhang R et al (2013) Dysbiosis signature of fecal microbiota in colorectal cancer patients. Microb Ecol 66, 462-470
71. Phongsisay V (2016) The immunobiology of Campylobacter jejuni: Innate immunity and autoimmune diseases. Immunobiology 221, 535-543
72. He Z, Gharaibeh RZ, Newsome RC et al (2019) Campylobacter jejuni promotes colorectal tumorigenesis through the action of cytolethal distending toxin. Gut 68, 289-300
73. Shang FM and Liu HL (2018) Fusobacterium nucleatum and colorectal cancer: A review. World J Gastrointest Oncol 10, 71-81
74. Engevik MA, Danhof HA, Ruan W et al (2021) Fusobacterium nucleatum Secretes Outer Membrane Vesicles and Promotes Intestinal Inflammation. mBio 12
75. Seo Y, Oh SJ, Ahn JS, Shin YY, Yang JW and Kim HS (2019) Implication of Porphyromonas gingivalis in colitis and homeostasis of intestinal epithelium. Lab Anim Res 35, 26

76. Khan R, Petersen FC and Shekhar S (2019) Commensal Bacteria: An Emerging Player in Defense Against Respiratory Pathogens. Frontiers in Immunology 10
77. Lordan C, Thapa D, Ross RP and Cotter PD (2020) Potential for enriching next-generation health-promoting gut bacteria through prebiotics and other dietary components. Gut Microbes 11, 1-20
78. Park NY and Koh A (2022) From the Dish to the Real World: Modeling Interactions between the Gut and Microorganisms in Gut Organoids by Tailoring the Gut Milieu. Int J Stem Cells 15, 70-84
79. Wu H, Xie S, Miao J et al (2020) Lactobacillus reuteri maintains intestinal epithelial regeneration and repairs damaged intestinal mucosa. Gut Microbes 11, 997-1014
80. Hou QH, Ye LL, Liu HF et al (2018) Lactobacillus accelerates ISCs regeneration to protect the integrity of intestinal mucosa through activation of STAT3 signaling pathway induced by LPLs secretion of IL-22. Cell Death and Differentiation 25, 1657-1670
81. Engevik MA, Ruan W, Esparza M et al (2021) Immunomodulation of dendritic cells by Lactobacillus reuteri surface components and metabolites. Physiol Rep 9, e14719
82. Aoki-Yoshida A, Saito S, Fukiya S et al (2016) Lactobacillus rhamnosus GG increases Toll-like receptor 3 gene expression in murine small intestine ex vivo and in vivo. Benef Microbes 7, 421-429
83. Han X, Lee A, Huang S, Gao J, Spence JR and Owyang C (2019) Lactobacillus rhamnosus GG prevents epithelial barrier dysfunction induced by interferon-gamma and fecal supernatants from irritable bowel syndrome patients in human intestinal enteroids and colonoids. Gut Microbes 10, 59-76
84. Lu X, Xie S, Ye L, Zhu L and Yu Q (2020) Lactobacillus Protects Against S. Typhimurium-Induced Intestinal Inflammation by Determining the Fate of Epithelial Proliferation and Differentiation. Mol Nutr Food Res 64, e1900655
85. Sittipo P, Pham HQ, Park CE et al (2020) Irradiation-Induced Intestinal Damage Is Recovered by the Indigenous Gut Bacteria Lactobacillus acidophilus. Front Cell Infect Microbiol 10, 415
86. Pino A, Benkaddour B, Inturri R et al (2022) Characterization of Bifidobacterium asteroides Isolates. Microorganisms 10
87. Pradhan S and Weiss AA (2020) Probiotic Properties of Escherichia coli Nissle in Human Intestinal Organoids. mBio 11
88. Hill DR, Huang S, Nagy MS et al (2017) Bacterial colonization stimulates a complex physiological response in the immature human intestinal epithelium. Elife 6
89. Kim J, Koo BK and Knoblich JA (2020) Human organoids: model systems for human biology and medicine. Nat Rev Mol Cell Biol 21, 571-584
90. Lee JY, Tsolis RM and Baumler AJ (2022) The microbiome and gut homeostasis. Science 377, eabp9960