Comparative Analysis of Intestinal Tract Models

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Abstract

The human gut is a complex ecosystem occupied by a diverse microbial community. Modulation of this microbiota impacts health and disease. The definitive way to investigate the impact of dietary intervention on the gut microbiota is a human trial. However, human trials are expensive and can be difficult to control; thus, initial screening is desirable. Utilization of a range of in vitro and in vivo models means that useful information can be gathered prior to the necessity for human intervention. This review discusses the benefits and limitations of these approaches.

1. INTRODUCTION

The human gut is an extremely complex and dynamic environment and has numerous important roles in human physiology, including digestion of nutrients, protection against invading pathogens (a process known as homeostasis), and immune enhancement. These roles are, in part, performed by the microbiome, which has coevolved with humans in a symbiotic relationship (Kamada et al. 2013). Indeed, the human gut hosts approximately 10^{13} to 10^{14} microorganisms, whose collective genome contains roughly 100 times more genes than that of the human host (Gill et al. 2006). The contribution of the microbiome to overall health of the host has been well-documented, with dysbiosis being linked to numerous disease states including obesity, diabetes, inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS) (see review by Gerritsen et al. 2011). Thus, the concept of modulating the composition of the microbiome, particularly that of the gut, is an important avenue of research and has led to the use of key approaches in maintaining gut health, for example, through probiotics and prebiotics (see review by Quigley 2010). Due to the inherent limitations involved in sampling the human gastrointestinal tract, ethical restrictions implicated in human trials, as well as variable compliance, a substantial degree of effort has been put into the development of in vitro, ex vivo, in silico, and animal systems that model the human gastrointestinal tract. These models vary in complexity and applicability of data to in vivo situations. Although none of these models can be used as a complete replacement of human trials, they do provide powerful tools for proof of concept studies, before in vivo validation (Petrof et al. 2013). This review provides an overview of different gastrointestinal models that are currently available to study the human gut microbiome.

1.1. Complexity of the Gut Microbiota

To effectively model the human gastrointestinal tract, one must appreciate the dynamic physiology present along the gut, which governs the composition of microbes therein. The entire gastrointestinal tract is populated by microorganisms; however, numbers and species composition varies greatly according to anatomical region. The stomach is not heavily colonized due to its low pH, and typically harbors up to 10^3 CFU g^{-1} consisting mainly of lactobacilli, streptococci, and yeasts (Bernhardt et al. 1995, Holzapfel et al. 1998). The duodenum also has low microbial populations due to its rapid transit time and the secretion of bile salts and pancreatic enzymes, which create a hostile environment. However, there is a progressive increase in both numbers and species along the jejunum and ileum, from approximately 10^4 to 10^{6-7} at the ileocaecal region (Salminen et al. 1998), with the appearance of gram-negative facultative organisms and obligate anaerobes. Beyond the ileocaecal valve, in the colon, bacterial numbers reach approximately 10^{11} g^{-1} of contents, with strict anaerobes predominating in the colon (Holzapfel et al. 1998).

1.2. Difficulties in Sampling the Human Gastrointestinal Tract

Several methods are available to detect changes in the growth and metabolism of human gut microbiota in response to, e.g., disease states, dietary interventions, and drug treatment. The approaches range from simple culture-based approaches to high-throughput molecular-based methods. However, all such methods are limited by the inaccessibility and ethical issues surrounding sampling the gastrointestinal tract in human subjects. At the extremities of the digestive system, sampling is relatively straightforward, with chew/spit methods employed to simulate ingestion and mechanical/enzymatic processing of food as it enters the mouth, as well as postdigestion sampling of stools to provide endpoint data. However, sampling of the main internal digestive

organs requires far more invasive approaches. Sampling of the stomach and duodenum requires nasogastric/duodenal aspirations, which are limited as only liquid meals can be collected in this way. Patients with ileostomy bags provide an accessible means of sampling the ileum, allowing collection of digest prior to large bowel fermentation. However, the ileostomy gut is unlikely to be fully representative of a "normal" gut, but it currently remains a useful option for sampling. Colonoscopy offers a means of providing biopsies and lumen samples from the ileum through to the sigmoid colon in the large intestine. Thus, due to inherent restrictions in human volunteer trials, in vitro, ex vivo, in silico, and animal gut model systems provide an alternative approach for studying the gut.

2. IN VITRO MODELS

Because of the above-mentioned difficulties in sampling different regions of the gut, human in vivo studies predominantly rely on endpoint data alone, usually derived from fecal samples. This means that dynamic monitoring of the gut microbiota along the gastrointestinal tract is not always possible, thus making it difficult to determine where a particular intervention exerts its effects. Furthermore, the presence of host-derived metabolites means that determination of microbial biochemical reactions is limited.

In vitro gut model systems provide a quick, easy, and cost-effective means of studying the gut microbiome, in one or more gut compartments, or along the entire gastrointestinal tract (Venema et al. 2012). All in vitro gut models have their limitations, mainly related to their reduced physiological relevance. Such systems do not always provide accurate models of what occurs in vivo, as they lack an epithelial mucosa, host immunological interactions, and neuroendocrine system functionality (Boureau et al. 2000). They do, however, enable monitoring of changes in microbiota, in terms of numbers and metabolism, attributable to the substrate, inhibitor, or disease state that is to be assessed. Such models range from pure cultures and simple single vessel batch cultures, to more complex single- or multistage pH-regulated continuous cultures. **Table 1** provides a summary of in vitro gut model system designs.

2.1. Batch Fermentation Models

Batch cultures constitute the simplest forms of in vitro models used to study the human gut microbiota. These are usually composed of a single bioreactor vessel with basal media, supplemented with the test substrate/inhibitor, incubated under constant physiological temperature (37°C) and anoxic atmosphere (usually N_2 flushed), and typically run for short periods of up to 72 h (usually 24–48 h) (Rumney & Rowland 1992, Wang & Gibson 1993, Barry et al. 1995, Oufir et al. 2000). The short period of incubation is due to rapid progression to the stationary phase as a result of nutrient depletion and accumulation of inhibitory bacterial metabolites (Macfarlane et al. 1992). For this reason, the distal colon is usually modeled using this system (controlled at pH 6.8). The efficacy of probiotics (Likotrafiti et al. 2014), dietary prebiotics such as fructans, and other complex carbohydrates has been studied in such batch fermentation models (Pompei et al. 2008, Lesmes et al. 2008, Gietl et al. 2012). Additionally, a range of food ingredients have been studied as substrates to investigate bacterial growth, metabolism [e.g., short-chain fatty acid (SCFA) production], and gas profiles in batch fermentations with human fecal microbiota (Macfarlane & Macfarlane 2007, Beards et al. 2010, Gumienna et al. 2011). Because of their simplicity, batch cultures are usually employed before conducting more lengthy multivessel continuous fermentation experiments.

Table 1 Comparison of parameters employed in various in vitro gut model systems

In vitro model	Target organ	Vessel volumes	pH	Running time	Peristaltic pumping	Absorption	Mucus	Cell lines	Reference
Batch fermenter	Any region of GI tract (usually distal colon)	Varies	Varies (usually 6.8)	≤48 h	No	No	No	No	Wang & Gibson 1993
Three-stage continuous	V1 = proximal, V2 = traverse, V3 = distal colon	V1 = 80, V2 = 100, V3 = 120 ml	V1 = 5.5, V2 = 6.2, V3 = 6.8	16 days to steady state (8 turnovers)	No	No	No	No	Gibson et al. 1988
Enteromix	Colon	6–15 ml	5.5–7.0	2 days	No	No	No	No	Mäkivuokko et al. 2006
PolyFermS	Proximal colon	300 ml	5.5	6 days	Yes	No	No	No	Berner et al. 2013
SHIME	Stomach to colon	300–1,600 ml	2.0–7.0	Typically 30 days per cycle	No	Yes	No	No	Molly et al. 1993
M-SHIME	Stomach to proximal colon	300–1,600 ml	2.0–7.0	Typically 30 days per cycle	No	Yes	Yes	No	Van den Abbeele et al. 2012
TIM-1	Stomach to ileum	200 ml	1.8–6.5	∼1 day	Yes	Yes	No	No	Minekus et al. 1995
TIM-2	Proximal colon	200 ml	5.8	∼3 days	Yes	Yes	No	No	Minekus et al. 1999
HMI	Colon		5.6–5.9	48 h	Yes	Yes	Yes	Yes	Mazorati et al. 2014
Gut-on-a-chip	Colon	700 μl	Not controlled	96 h	Yes	Yes	Yes (gel layer)	Yes	Kim et al. 2012

Abbreviations: GI, gastrointestinal; HMI, host-microbiota interaction; M, mucus; PolyFermS, Polyfermentor Intestinal Model; SHIME, simulator of the human intestinal microbial ecosystem; TIM, TNO intestinal model.

2.2. Continuous Culture Fermentation Models

Gut models, which incorporate continuous fermentation systems, provide an environment that more closely resembles physicochemical conditions found within the gastrointestinal tract, such as a constant influx of nutrients and efflux of waste products over a defined retention time. Moreover, pH, temperature and atmospheric control are required. Such models are based on Wolin and colleagues' (Rufener et al. 1963, Slyter et al. 1964) original in vitro semicontinuous cultures, developed to investigate rumen microbial communities, as well as Pirt's fed batch cultures (1974). Miller & Wolin (1981) adapted the design further for in vitro maintenance of the microbial communities present within the human large intestine. Several groups have been involved in the development of artificial colonic and entire digestive systems that simulate the gut on both structural and functional levels.

2.2.1. Three-stage colonic model system. Several gut model systems base their design on Gibson et al.'s (1988) three-stage colonic model system. The in vivo relevance of this model has been validated against intestinal contents from sudden death victims. Both bacterial and chemical SCFA profiles from the in vitro model and human intestinal samples were comparable (Macfarlane et al. 1998; see also **Figure 1*a***). Therefore, although this system does not incorporate host factors,

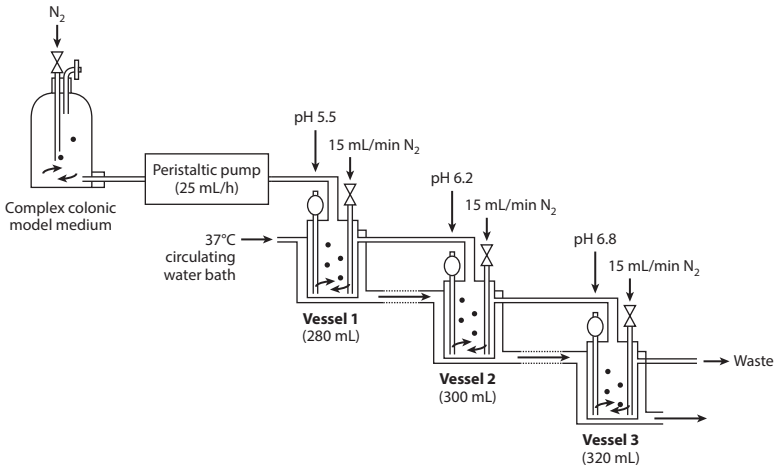
such as intestinal secretions, immunology, or absorption, it does offer an inexpensive and reliable tool for modeling the microbial ecology and activity of the colon.

Gibson et al.'s (1988) system models the different environments encountered along the human colon. The proximal (ascending) colon is typically an acidic and nutrient-rich environment, resulting from bacterial fermentation processes. It becomes progressively more alkaline and nutrient-depleted toward the transverse (middle) and reaches near pH-neutral in the distal (descending) colon. The system uses three fermentation vessels connected sequentially to mimic these distinct regions of the colon (**Figure 1*a***). A typical experimental setup using the model involves inoculating each vessel with human fecal slurry, containing the entire colonic bacterial diversity. Each vessel is then incubated as an overnight batch culture to increase the initial microbial biomass. Following this, fresh growth medium is pumped into vessel 1 (V1) via a peristaltic pump, at a rate corresponding to the desired retention time, often 48 h. This represents a typical transit time through the human colon and overcomes washout of key bacterial species, ensuring a stable microbial community over time (Macfarlane et al. 1998). Overflow from V1 is then transferred to V2 and from V2 to V3 via gravitational flow. Overflow from V3 is collected as waste. The system is run until the microbial communities reach steady state (this typically takes 8 turnovers or 16 days). Steady state is confirmed after obtaining consistent SCFA measurements on three consecutive days (Macfarlane et al. 1998). Thereafter, a specific test substrate/probiotic may be tested until a second steady state is reached (again based on stable SCFA measurements), which is then compared to the original steady state. This may be followed by a washout period to determine how long changes induced by the intervention can be sustained (Macfarlane et al. 1998).

The overall length of any continuous gut model experiment is usually governed by composition of the initial fecal inoculum. Experiments employing liquid fecal inocula, usually diluted to 20% (w/v) before inoculation into reaction vessels, are usually time-limited due to washout of less competitive planktonic and sessile fecal bacteria during the stabilization period (Payne et al. 2012). Furthermore, fecal bacterial density is usually lower than colonic contents by a factor of 10 (Cinquin et al. 2006). To perform more lengthy experiments, fecal samples may be immobilized within beads composed of a porous polysaccharide matrix (Cinquin et al. 2004). These beads are then introduced into V1 of the three-stage continuous fermentation system, and bacteria dislodged from the beads (as a result of microbial growth) are passed sequentially from V1 to V3, ensuring a continuous, high-density supply of bacteria.

2.2.2. Other colonic simulators. The EnteroMix® model is a modified, semicontinuous culture colon simulator that has four parallel units, each comprising four glass vessels to mimic the ascending- (V1), transverse- (V2), descending- (V3), and sigmoid-colon/rectum (V4) areas (Mäkivuokko et al. 2005). The pH levels are controlled at 5.0, 6.0, 6.5, and 7.0, and the initial working volume is 3, 5, 7, and 9 ml, respectively. Three hours after inoculation of the fecal sample, 3 ml of fresh medium with (three test channels) or without (one control channel) the test substance is pumped to the first vessel. The contents of V1 are allowed to ferment for 3 h before being transferred to V2 with simultaneous transfer of 3 ml fresh medium into V1. The same process is repeated for V3 and V4, with system setup completed at 15 h postinoculation. The fermentation lasts for 48 h, after which samples are collected from each vessel and the simulation is terminated. The model was designed specifically to study the effects of carbohydrate fermentation by the colonic microbiota (Mäkivuokko et al. 2006, Mäkeläinen et al. 2007, 2009). An advantage of the EnteroMix® model is the ability to run four parallel experiments using the same fecal sample as the inoculum. However, the small working volumes and semicontinuous nature of the system mean that only short-term experiments can be performed, thus reducing in vivo relevance. Despite this, the model has good correlations with in vivo studies whereby a bifidogenic effect was

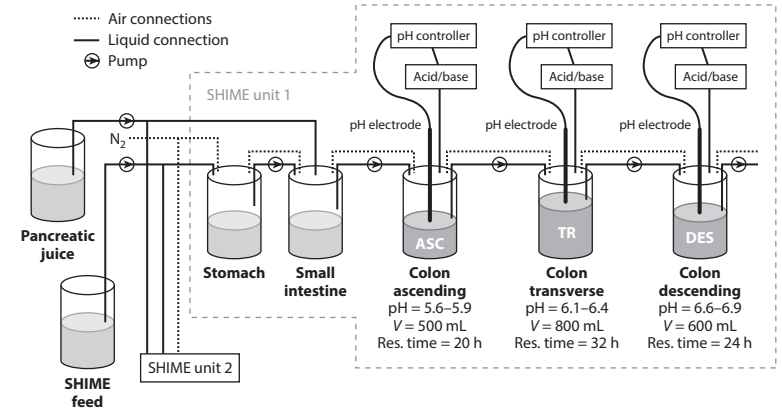
a



Three-stage continuous colonic model system (Macfarlane et al. 1998)

- Colonic contents of four sudden death victims compared in the in vitro against a fecal sample from a healthy donor
- Microbiological (culture-dependent) and chemical (SCFA) analyses
- Bacterial counts in the colon were similar to those obtained in the in vitro model
- Good correlation in distribution of SCFAs in vivo and in vitro (maximal in proximal colon)

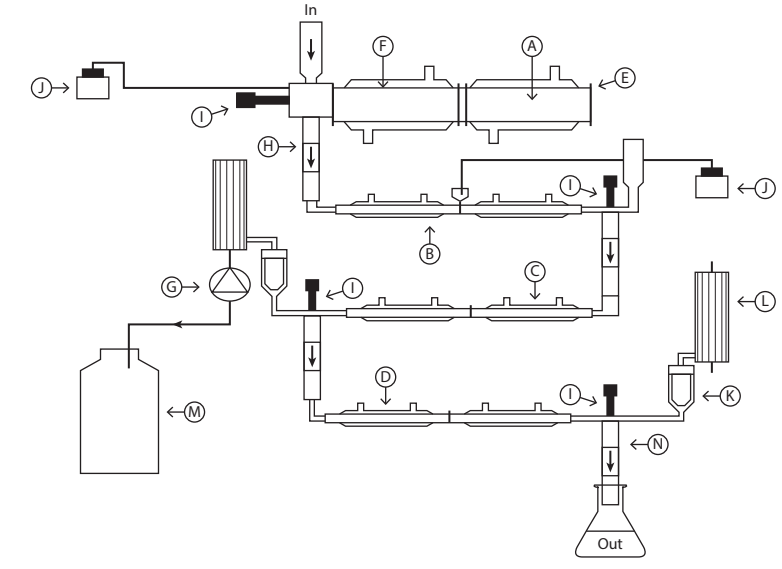
b



SHIME model (Molly et al. 1993)

- In vitro data compared to published in vivo data (Cummings & Macfarlane 1991)
- Microbiological (culture-dependent), chemical (SCFA, NH_4), and gas-phase analyses
- 1-log higher cell counts in vitro
- No significant differences in molar ratios of SCFAs (medium #4 only), but significantly less butyric acid produced in vitro
- NH_4 levels correlated well with in vivo data
- No H_2 found in the descending colon reactor, contradicting in vivo data
- Overall fair correlation between in vivo and in vitro data

c



TIM-1 (Marteau et al. 1997)

- Survival of probiotic strains (*B. bifidum*, *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus*) was compared to an in vivo study by Marteau et al. 1997
- Microbiological (culture-dependent analysis)
- Survival of *B. bifidum* and *L. acidophilus* in the TIM-1 system did not differ significantly from data derived from humans under similar conditions
- Good correlation between in vitro and in vivo data

observed following a synbiotic treatment consisting of a combination of *Lactobacillus acidophilus* NCFM (National Collection of Food Microorganisms) and lactitol (Björklund et al. 2012).

The Polyfermentor Intestinal Model (PolyFermS) system also circumvents problems of reproducibility and biological replication associated with gut modeling, allowing the study of parallel effects of different treatments on the same complex colonic microbiota (Berner et al. 2013). This proximal colon model consists of an initial inoculum reactor containing an immobilized fecal inoculum. The contents of this vessel are then transferred by continuous flow into second-stage vessels including a control reactor and up to three test reactors, all of which are maintained at pH 5.5. High intramodel metabolic and phylogenetic stability has been observed between all parallel reactors in the system, sharing similarities to the temporal stability reported for human intestinal microbiota of healthy individuals. The model therefore provides a robust system for comparing the effects of different treatments on the microbiome of the proximal colon against that of the control reactor in parallel.

2.3. Artificial Digestive Systems

As an extension of the previously described colonic model system design, a new generation of gut model systems has been developed to mimic physicochemical conditions found along the entire gastrointestinal tract. Such systems allow modeling of the dynamic environment of the gut providing an influx of gastric juice, pancreatic juice, bile salts, peristaltic motility, absorption capacities, high shear forces, and host-microbiota interactions.

2.3.1. Simulator of the human intestinal microbial ecosystem. SHIME, originally developed and validated (see **Figure 1*b***) by Molly et al. (1993), is a dynamic model of the human gut. It is comprised of five reactors respectively simulating the stomach; small intestine; and ascending, transverse, and descending colon. The first two reactors mimic the enzymatic and physicochemical environment by controlling pH, residence time, and dosing with culture medium, including enzymes and bile salts (Molly et al. 1993). These two reactors have a fill-and-draw system with a dialysis filter used to simulate the absorptive processes occurring in the stomach and the small intestine (Vermeiren et al. 2011). The last three-stage reactors, which simulate the large intestine, are continuously stirred vessels inoculated with fresh fecal samples corresponding to the in vivo situation in terms of metabolic activity and community composition, based on the aforementioned Gibson et al. (1988) model. The typical stabilization period before treatment/washout regimes can begin is around two weeks.

Applications of the SHIME model include determination of bioavailability of various toxic compounds such as PAHs, arsenic or isoxanthohumol, in the study of microbial metabolism in the gastrointestinal tract (Van de Wiele et al. 2003, Laird et al. 2007, Possemiers et al. 2006). The SHIME model has also been used to study the effects of various probiotics and prebiotics on the gut microbial ecosystem (Kontula et al. 1998; Alander et al. 1999a; Van de Wiele et al. 2004,

Figure 1

Schematic representation and parameters used in the validation of in vitro gut model systems. (*a*) Three-stage continuous colonic model system (Sannasiddappa et al. 2011), (*b*) SHIME model (Van den Abbeele et al. 2010; panel *b* reproduced with permission from American Society for Microbiology), and (*c*) TIM-1 model (Reis et al. 2008). Abbreviations: ASC, ascending; DES, descending; V, vessel; Res., residence; A, gastric compartment; B, duodenal compartment; C, jejunal compartment; D, ileal compartment; E, glass jacket; F, flexible wall; G, rotary pump; H, pyloric valve; I, pH electrodes; J, secretion pump; K, prefilter; L, hollow fiber membrane; M, filtrate from jejunum; N, ileal delivery valve; SHIME, simulator of the human intestinal microbial ecosystem; SCFA, short-chain fatty acid; TIM, TNO intestinal model; TR, transverse. Figure reprinted with permission.

2007). An extension of the SHIME model, called the TwinSHIME® system, has been developed, whereby two SHIME systems are run in parallel, allowing comparisons between different treatments (Grootaert et al. 2009). Other extensions include the mucus (M)-SHIME system, whereby mucin-coated microspheres are included in the model to allow adherence of bacteria, thus more closely resembling the human colon and the host-microbiota interaction (HMI) module, incorporating epithelial cell lines (see Section 2.4).

2.3.2. TNO intestinal model. Minekus et al.'s (1995, 1999) TNO intestinal model (TIM) is comprised of two complementary components, TIM-1 and TIM-2. The TIM-1 system contains eight glass modules mimicking the stomach, duodenum, jejunum, and ileum, which encompass peristaltic movements, pH control of each compartment, enzyme secretion and activity, concentrations of bile salts, physiological gut transit times, and absorption capacities (Minekus et al. 1995). The TIM-2 system consists of four glass modules in a loop mimicking the proximal colon with peristaltic mixing, water, and metabolite absorption using a hollow fiber membrane (Minekus et al. 1999). TIM differs from other models in two main aspects: (*a*) Fluid transportation from vessel to vessel happens via peristaltic valve pumps, and (*b*) there is a constant absorption, although passive, of water and fermentation products through dialysis membranes. TIM-1 has two integrated 5-kDa dialysis membranes, next to jejuna and ileal modules, and TIM-2 has one hollow-fiber membrane that has a molecular mass cut-off of 50 kDa (Minekus et al. 1995, 1999).

The TIM-1 stomach/small intestine model can be validated against a relevant human study (see **Figure 1*c***); as such, numerous studies have employed this model to study the gut ecosystem. For example, the absorptive capacity of the system has been used to predict the in vivo performance of pharmaceutical drugs and drug products. Naylor et al. (2006) showed that use of TIM-1 together with the GastroPLUS™ software, which simulates the absorption and pharmacokinetics of drugs, provides an excellent tool for drug formulation selection. In this study, the system provided estimates of drug in vivo dissolution, which when compared with the conventional USP II standard profile, had higher accuracy against measured pharmacokinetics. TIM-1 has also been used to study the survival of probiotics through the upper gastrointestinal tract (Marteau et al. 1997, Maathuis et al. 2010) and their subsequent effects on the human colonic microbiome in TIM-2 (Hatanaka et al. 2012).

Van den Abbeele et al. (2013) conducted a recent study into the reproducibility between different artificial gut model systems. Here, fermentation patterns of the candidate prebiotics arabinoxylan and inulin were compared in the TIM-2 colon model and the SHIME system. Although these in vitro models differ in their designs, both systems showed that inulin and arabinoxylan stimulated the production of butyrate and had a bifidogenic effect. These results also compared favorably with an in vivo animal trial of inulin and arabinoxylan in humanized rats (Van den Abbeele et al. 2011).

2.4. Mucosal Models

None of the above-mentioned models takes into account the effects of the host epithelium in structuring the microbial community (Marzorati et al. 2010). Host responses can be simulated in vitro through mucus- or mucosal-associated models. The simplest of such studies incorporate immobilized mucus, e.g., mucin beads, into existing gut model systems as a substrate for bacterial adhesion. Incorporating in vitro maintained enterocyte-like cells introduces an extra level of complexity, permitting the study of host-microbiota cross-talk. One of the most extensively used in vitro cell lines is the Caco-2 colonic adenocarcinoma cell line. Caco-2 cells grown as a monolayer spontaneously differentiate to resemble small intestine epithelial cells, exhibiting microvilli (including tight

junctions), and express small intestinal hydrolase activity (Pinto et al. 1983). Other studies utilize ex vivo methods that involve maintaining viability of live tissue, e.g., human intestinal biopsy samples or animal organs, in vitro over extended time periods, thus providing an in vivo–like environment.

2.4.1. Immobilized mucus models. Ouwehand et al. (1999) studied the influence of normal fecal microbiota on the adhesion of a probiotic to the mucosa using an immobilized mucus in vitro model. They also studied the adhering potency of a candidate probiotic in a new model with colonic tissue (Ouwehand et al. 2002). Probert & Gibson (2004) developed a fermentation model of the proximal colon that incorporated mucin beads to mimic the mucus gel layer microhabitat with a dialysis membrane. Macfarlane et al. (2005) used sterile porcine mucin gels in small glass tubes to determine how intestinal bacteria colonize and degrade mucus in a two-stage continuous culture system. These tubes can be placed in a fermenter simulating a specific area of the gastrointestinal tract and removed over a period of 48 h for further analyses of the biofilm. Van den Abbeele et al. (2009) studied the adhesion of the mucin colonization of bacteria from the SHIME model. Bahrami et al. (2011) studied adherence and cytokine induction in Caco-2 cells by bacterial populations from a three-stage continuous fermentation model. Furthermore, the M-SHIME model (Van den Abbeele et al. 2012) has been validated by comparison against in vivo adhesion of *L. rhamnosus* GG in a human study by Alander et al. (1999b). In both the in vitro and in vivo experiments, *L. rhamnosus* GG remained associated with the mucin microspheres in the M-SHIME model and the mucosa in the in vivo experiment for 3 days and 1 week, respectively, after administration of the probiotic. However, further work is required to determine how other bacterial groups colonize this in vitro model (Van den Abbeele et al. 2012).

None of the aforementioned mucus-based models provide a means of studying the mechanisms of bacterial adhesion in response to host signals and the subsequent reciprocal cross-talk (Marzorati et al. 2010). The HMI model and gut-on-a-chip models provide a means of bridging this gap: simulation of a higher degree of gastrointestinal complexity, thus closer mimicking of the gut physiology.

2.4.2. Host-microbiota interaction model. The HMI model consists of two compartments separated by a functional double layer composed of an upper luminal region coated with a mucus layer and a lower semipermeable membrane (Marzorati et al. 2014). Epithelial cells and/or other cell types are grown in the basal compartment of the module with the semipermeable membrane allowing secretion of metabolites from the artificial mucus layer. Incorporation of this mucus layer reduces cytotoxicity resulting from direct interaction between a mixed bacterial community and the epithelial monolayer, allowing extension of experiments from a few hours to 48 h. Furthermore, use of two separate compartments allows the establishment of different oxygen pressures on both sides of the membrane to establish the optimal conditions for aerobic epithelial cells in the basal compartment and the anaerobic microorganisms in the luminal compartment. These combined features provide a tool for investigating the role of microbial metabolism on the biotransformation of active compounds and studies related to new drug development (Vermeiren et al. 2011). The model can be combined with the SHIME system, thus introducing an additional host element by allowing analysis of mixed bacterial communities (Marzorati et al. 2010). Marzorati et al. (2014) validated the HMI module against previously published data, as follows: (*a*) Adhesion parameters of *L. rhamnosus* GG to the mucus layer of the HMI module were consistent with previously acquired data from the M-SHIME model. (*b*) Caco-2 cells in the HMI module were able to withstand up to 48 h exposure to gut microbiota, which corresponds to the average in vivo exposure time of colonocytes. (*c*) An inverse relationship between metabolite size and permeation, with coefficients being within the same range as previous experiments with Caco-2 cells, perfused animals and

ex vivo human colon tissues. (*d*) Finally, an in vivo–like oxygen gradient across the epithelium, providing microaerobic conditions on the luminal side, allows colonization of distinct bacterial communities, including *Faecalibacterium prausnitzii* and bifidobacteria, previously shown to be associated with the mucus layer.

2.4.3. Gut-on-a-chip. The gut-on-a-chip model system offers a microdevice platform for studying host-microbe interactions. The model consists of two microfluidic channels, which are separated by a porous (10 μm) membrane coated with extracellular matrix, upon which Caco-2 cells are grown as a monolayer (Kim et al. 2012). Continuous peristaltic pumping of culture medium (flow rate = 30 μl/h) over this cell line monolayer accelerated epithelial cell differentiation, increased intestinal barrier function, and induced development of 3D villi-like structures. This was the first demonstration of spontaneous differentiation of Caco-2 cells into intestinal villi in vitro, a phenomenon that was directly proportional to the flow rate of culture medium over the monolayer. It was later shown that the cells that line these villi are linked by tight junctions, display brush border morphology, and are covered with mucus (Kim & Ingber 2013). In addition, the cells that line the crypts between villi consist of four types of differentiated epithelial cells (i.e., absorptive, mucus-secretory, enteroendocrine, and Paneth cells), which occupy similar positions expected in the human small intestine. Parallel experiments using conventional static transwell inserts failed to replicate these conditions. Furthermore, coculture of Caco-2 cells with *L. rhamnosus* GG in this system enhanced epithelial integrity and provided microenvironment cues that promoted normal epithelial functions, such as secretion of mucin, which further promotes in vivo–like interactions between the epithelium and gut microbiota. Again, such dynamic interactions are not observed in static cell line–based systems, where bacterial overgrowth causes disruption of epithelial integrity, meaning that only short-term experiments are possible. Experimental durations of more than one week are possible in the gut-on-a-chip system. The microplatform nature of this gut model also provides a more user-friendly system to facilitate the study of host-microbe interactions and intestinal physiology as well as drug screening and development.

3. EX VIVO SYSTEMS

Despite significant advances in artificial in vitro intestinal cell line models, in vivo experiments are still required due to their dynamic physiological, biochemical, and immunological complexity. Such experiments have been conducted in anaesthetized mice using two-photon microscopy to penetrate the gut wall and examine inside the intestine using fluorescence (Klinger et al. 2012). However, in addition to having different physiology from humans, blood flow and breathing motions of the animal are often problematic, causing image artifacts; furthermore, anaesthetization of the animal is known to perturb certain physiological processes (see review by Tremoleda et al. 2012 and Weigert et al. 2013). Such approaches are expensive and require specialized setups; alternatively, ex vivo systems have been used. These models involve maintaining the viability of human or animal biopsy samples in vitro by mounting small organ sections (~2–3 mm) on agarose plugs saturated with buffered culture medium and gassed with 95% O_2–5% CO_2 on a rocking platform (Hicks et al. 1996). Under these conditions, organ specimens can maintain viability for up to 48 h and have been used to study celiac disease, pathogen challenge tests (e.g., *Campylobacter jejuni* infections), and effects of probiotics (Grant et al. 2006). Tubular inserts have been incorporated onto the apical surface of the organ using surgical glue, which ensures that intestinal bacteria do not bypass the epithelium and penetrate the basolateral side (Tsilingiri et al. 2012). Further advances on this design include development of an organ perfusion chamber, for example, the parabiotic chamber developed by Gabridge (1974). Their design has since been

modified by Wieser et al. (2011) for the study of hollow organs, e.g., the stomach, small intestine, and colon. This modified water and gas-tight chamber consists of apical and basal compartments that are sealed and separated by the organ itself. Both compartments have inlets and outlets, which allow flow of oxygen-saturated medium on the basal side and flow of luminal medium on the apical side. An additional inlet on the apical side allows introduction of a pathogen, substrate, or inhibitor of interest into the organ for analysis. Both compartments have separate outlets, from which the medium flow-through can be sampled for enumeration of unattached organisms or cytokine measurements. A window, positioned on both the apical and luminal sides, allows imaging of the organ using compound or inverted microscopes and fluorescence. The dynamic environmental control that this model offers allows the study of both facultative anaerobes (e.g., uropathogenic *Escherichia coli* and *Salmonella enterica*) and strict anaerobes (e.g., the protozoan parasite *Entamoeba histolytica*) in real time for more than 8 h.

4. IN VIVO ANIMAL MODELS

Numerous animal models have been used to study the dynamic, ecologically diverse community of microorganisms that inhabit in the gastrointestinal tract and provide an understanding of the biological complexities of the processes that govern host-microbiota symbiosis. These models provide a powerful tool to study microorganism(s) in order to establish unique roles for key members of the gut microbiota in the context of different health and disease perspectives (Sekirov et al. 2010). Furthermore, animal models provide a means of studying non-cultivable commensal bacteria, an important factor that is not possible using the in vitro methods reviewed above.

Conventional animal species have been widely used for studying various aspects of the human gut microbiota, including stabilization, colonization and colonization resistance, as well as effects of various antimicrobial agents and dietary interventions (Burr et al. 1982, Gorbach et al. 1988, Van der Waaij & Van der Waaij 1990, Nielsen & Schlundt 1992, Mysore & Duhamel 1994, Pazzaglia et al. 1994, Berends et al. 1996). The rodent is the most common animal model followed by the guinea pig, pig, chicken, Japanese monkey, Mongolian gerbil, ferret, and quail (Boureau et al. 2000). Conventional animals hold many advantages over intestinal model systems, e.g., (*a*) much fewer ethical restrictions than human trials; (*b*) complete environmental control (diet, stress, etc.); (*c*) genetic control of subject population; and (*d*) accessibility of intestinal contents, tissues, and organs at autopsy (Boureau et al. 2000). However, the additional layer of complexity introduced in animal models can impede interpretation of data, especially when modeling diseases of the gastrointestinal tract. Even the most widely used animal model (i.e., the mouse) differs substantially from that of humans in terms of physiology (see review by Gibbons & Spencer 2011). For example, Paneth cells in the mouse small intestine produce more than 10 times more defensins (cryptdins) than human cells, which likely impacts microbial colonization and survival (Mestas & Hughes 2004).

Gnotobiotic animal models offer a simplified approach for studying host-microbe interactions in vivo (Xu & Gordon 2003, Phillips 2009). Tlaskalová-Hogenová et al. (2011) review the different gnotobiotic and germ-free animal models available for studying the role of the gut microbiome and the mucosal barrier in cancer, and inflammatory and autoimmune diseases. These animal models provide useful information on how bacteria affect normal development, establishment, and maintenance of the immune system and epithelial cell functions. To establish such models, animals are raised in germ-free environments to avoid exposure to microbes. Subsequently, these animals may be experimentally colonized at different life stages. This allows complete control over microflora parameters, permitting colonization by one or more organisms of choice. Such models therefore offer an appealing system for studying pathogen challenge and dietary interventions,

for example, the effects of probiotics on *Salmonella* spp. (Maia et al. 2001) in vivo. Simplification of the intestinal microbiota not only facilitates interpretation of data, but also leads to reduced realism due to fewer inter- and intraspecies interactions (Boureau et al. 2000).

To overcome this issue, human fecal microbiota animal models have been developed. These have stemmed from the work of Raibaud et al. (1980) and Hazenberg et al. (1981), whereby germ-free mice inoculated with suspensions of human feces had a similar composition of total intestinal bacteria as that of humans and were distinct from the indigenous murine microbiota. These humanized systems allow incorporation of complex metagenomic analyses into gut model studies for analysis of temporal, spatial, and intergenerational patterns of microbial colonization. Turnbaugh et al. (2009) used these tools to assess the effect of switching from a low-fat, plant polysaccharide–rich diet to a high-fat, high-sugar "Western" diet on the human-derived colonic microbiota of the gnotobiotic C57BL/6J mouse model. This study found that switching to the Western diet shifted the structure of the microbiota within a single day, resulting in changes in metabolic pathways and gene expression of the microbiome as well as increased adiposity, thus providing an effective tool for studying obesity.

5. SAMPLING GUT MODELS

Once established, there are several tools available to monitor bacterial responses to pathogen/inhibitor challenges, disease states, and probiotic/prebiotic intervention studies during gut model experiments. These include culture-dependent and independent approaches, including microscopic, metabolomic, and molecular-based techniques.

5.1. Enumerating Bacterial Populations

Traditional culture-dependent methods involve plating out samples of interest onto selective agars and incubation under physiologically relevant conditions (see review by Temmerman & Swings 2004). Such methods offer a means of distinguishing between viable and nonviable cells but are, however, biased to those bacterial species that are physiologically and metabolically able to grow under these in vitro conditions. Indeed, 20–80% of gut microbes are predicted to be unculturable using current methods; hence, a substantial degree of microbial diversity is lost using these approaches. Microscopic enumeration of bacteria using fluorescence in-situ hybridization (FISH) offers an alternative culture-independent approach. This involves labeling bacteria with 16S rRNA-targeted oligonucleotide fluorescent probes, which label key bacterial groups present within the intestinal tract (Franks et al. 1998). Fixing of samples is required for fluorescent labeling; as such, viable and nonviable cells cannot be distinguished. A further limitation is that only group-specific probes are usually employed; therefore, species-level differentiation is lost. FISH is routinely applied to enumerating bacterial populations in a three-stage continuous culture colonic model system to study probiotic and prebiotic interventions (Hobden et al. 2013).

Molecular-based approaches, including dot blot hybridization of 16S rRNA oligonucleotide probes, enable group, genus, or species-level identification of fecal microbiota (Marteau et al. 2001). Similarly, temperature or denaturing gradient gel electrophoresis (TGGE or DGGE) provide rapid analysis of microbial communities based on sequence-specific separation of 16S DNA amplicons (Zoetendal et al. 1998, Satokari et al. 2001). DGGE has been combined with human intestinal tract chip (HITChip; Rajilić-Stojanović et al. 2009) microarray to characterize the microbial composition in the TwinSHIME (Van den Abbeele et al. 2010) and TIM-2 (Rajilić-Stojanović et al. 2010) gut model systems. However, these methods can mainly generate qualitative information on species diversity, meaning that changes in relative proportions of bacterial groups over time are not possible. Real-time PCR (qPCR), however, provides a rapid

means of quantifying bacterial DNA as a number of target 16S rDNA gene copies from a range of environments, with probes currently available for more than 300 bacterial species (Rinttilä et al. 2004). qPCR has been applied to quantify bacterial proportions in the three-stage colonic model system (Maccaferri et al. 2010). Frequently, combinations of culture-based, microscopic, and/or molecular-based approaches are employed in gut model analyses to obtain a complete picture of microbial community dynamics over time.

5.2. Biochemical Measurements

Routine monitoring of chemical end products produced by the microbiota in gut model systems mainly involves analysis of SCFA, of which butyric, acetic, and propionic acids are the key components. Stabilization in the production of relative proportions of SCFAs by the microbial community is an important indicator of colonization of the steady state within gut model systems (usually after eight full volume turnovers; see Macfarlane et al. 1998). Gas chromatography is employed to determine SCFA production according to Macfarlane et al. (1992). However, higher levels of SCFAs are usually observed in vitro due to a lack of absorptive capabilities. Other components of interest, e.g., residual carbohydrates from prebiotic intervention trials, may be determined by high-performance liquid chromatography according to Quigley & Englyst (1992). ^{13}C-labeling of carbohydrate substrates, e.g., glucose, starch, inulin, lactose, and galactooligosaccharide, has helped unravel an additional degree of metabolic complexity in the TIM-2 colonic model system, by allowing the study of species-specific substrate utilization and subsequent cross-feeding of metabolic end products (de Graaf & Venema 2008, Kovatcheva-Datchary et al. 2009, de Graaf et al. 2010, Maathuis et al. 2012).

6. MATHEMATICAL AND COMPUTATIONAL MODELS

Early papers dating back to the 1980s recognized the potential benefit of mathematical modeling for the research of human gastrointestinal microbial ecosystems. The total number of publications in this area is, however, very small, implying that this field remains in its infancy. Despite this, in theory, some of the aforementioned difficulties associated with in vivo trials and in vitro experiments can be overcome by mathematical/computational models. Using computational simulation based on appropriate mathematical models, one can take into account the physiological interactions and metabolic processes that are difficult to implement through in vitro experiments. This also negates issues such as high cost and questionable repeatability.

Coleman et al. (1996) presented the first comprehensive piece of work on computational modeling of gut microbial ecosystems. In this work, a chemostat model was adopted, and a C program was coded to simulate the growth of six microbial groups competing for five nutrients (glucose, lactose, starch, sorbose, and serine). Rather than being a conclusive study, this work demonstrated the feasibility of computational modeling in the investigation of human colonic ecosystems. Wilkinson (2002a,b,c) reported on a second series of notable computational studies. The gut was modeled as a rigid axisymmetric tube, and microbial metabolism was based on the basic Monod model, with a series of ad hoc extensions to take into account, e.g., toxin inactivation, symbiotic food interactions, and binding site competition. This study marked a notable effort toward building a comprehensive computer simulator for human gut microbial ecosystems. Notably, this model has some fundamental differences in both hydraulic transport and microbial metabolism. The gastrointestinal track is elastic, not rigid, and the diffusion motion is negligible due to the high viscosity of normal gut medium. Thus, despite various additional terms, the growth model adopted by Wilkinson is essentially similar to an early study by Hansen & Hubbell (1980). Indeed, steady-state coexistence of multiple

bacteria is a rare event and is unstable following this metabolic model. The Wilkinson (2002a,b,c) approach also contains several parameters that are very difficult, if not impossible, to determine experimentally. Hence, de Jong et al. (2007) reverted back to the simple chemostat model and designed a relatively simple simulation framework incorporating both small and large intestines. The authors demonstrated how to interpret in vivo experimental data using simulation-based approximation.

More recently, Muñoz-Tamayo et al. (2010, 2011) simulated the human large intestine using three pairs of chemostat models sequentially connected, representing the ascending, transverse, and descending colon. Each section of the gastrointestinal tract was represented by two chemostats, simulating the lumen and the mucus layer. Modeling the mucus layer in the same way as the lumen space is fundamentally different from the previous wall attachment models (e.g., Freter 1983), where the mucus layer is assumed static. A further contribution made by these authors was the classification of functional groups of gut microbiota and associated fermentation pathways. Lawson et al. (2011) present a statistical estimation method to help determine the parameters of their gut model. Similar to Muñoz-Tamayo et al. (2010, 2011), these authors modeled the gastrointestinal tract as a chemostat.

This research field has continuously attracted researchers from both biological and mathematical backgrounds. However, due to the high complexity of the gut microbial ecosystem and the advanced mathematical/computational skills required, limited research is currently available in this area. **Table 2** provides a summary of the different model parameters used in the current literature.

7. APPLICATIONS OF GUT MODEL SYSTEMS IN DIETARY INTERVENTION TRIALS

The composition and functionality of the gut microbiota are host-dependent and reliant on several factors including host genetics, environment, inflammatory state, and diet. Hence, there

Table 2 An overview of study parameters employed in mathematical modeling studies

	Freter et al. (1983)	Smith and coworkers[a]	Coleman et al. (1996)	Wilkinson (2002a,b,c)	de Jong et al. (2007)	Muñoz–Tamayo et al. (2010, 2011)	Lawson et al. (2011)
Theoretical analysis	Y	Y	N	N	N	N	N
Computational simulation	N	N	Y	Y	Y	Y	Y
Chemostat	Y	Y	Y	N	Y	Y	Y
Plug flow	N	Y	N	Y	N	N	N
Monod growth model	Y	Y	Y	Y	Y	Y	Y
Wall attachment/ mucus layer	Y	Y	N	Y	N	Y	N
Multiple species (>2)	N	N	Y	Y	Y	Y	Y
Multiple substrates (>1)	N	N	Y	Y	Y	Y	Y

[a]Includes Ballyk et al. 1998; Ballyk & Smith 1999, 2001; Jones & Smith 2000, 2002; Stemmons & Smith 2000.

is a substantial degree of inter- and intra-individual variation in relative abundance of core phylotypes, with an estimated 12- to 2,200-fold difference between individuals (Qin et al. 2010); thus, the composition of a "normal" microbiota has yet to be defined. Despite this, the role of microbial dysbiosis has been recognized in numerous human diseases, e.g., obesity, diabetes, IBD, and IBS (see review by Gerritsen et al. 2011). The concept of modulating the human microbiome, particularly that of the gut, is therefore an important avenue of research. To this end, probiotics, prebiotics, and synbiotics (combinations of the latter) are of particular importance. **Table 3** provides examples of in vitro, animal, and human trials involving synbiotics.

Table 3 Evaluation of the efficacy of various synbiotics in in vitro, animal (rodent), and clinical studies

Study type	Parameters	Synbiotic combination	Effect	Reference
In vitro	Anaerobic, pH-controlled fecal batch cultures from healthy donors (each combination in triplicate), pH 6.8 (distal colon)	*L. fermentum* ME-3, *L. plantarum* WCFS1, *L. paracasei* 8700:2, and *B. longum* 46 *L .plantarum* WCFS1 (10^7–10^8 CFU/ml) + FOS 1% (w/v)	SYN and PRE increased bifidobacteria and the *Eubacterium rectale–Clostridium coccoides* group. Lower levels of *E. coli* were retrieved with these combinations after 5 and 10 h of fermentation. No effect of PRO alone.	Saulnier et al. 2008
	TIM-1 model (artificial meal used as the inoculum)	*L. amylovorus* DSM 16698 and *B. animalis* subsp. *lactis* Bb-12 + GOS 1% (w/v)	SYN increased survival of *L. amylovorus*. *L. amylovorus* and *B. animalis* subsp. *lactis* degraded GOS 2 h after initiation of TIM-1.	Martinez et al. 2011
	SHIME model using a fecal inoculum	*L. acidophius* 74–2 (10^8–10^9 CFU) + FOS 1% (w/v)	SYN increased lactobacilli (ascending colon), bifidobacteria (all colon vessels), SCFAs (butyric and propionic acid), and β-galactosidase activity.	Gmeiner et al. 2000
Rodent models	Adult male Wistar rats	*B. longum* KN29.1 (7.2×10^9), *B. longum* KNA1 (4.7×10^9), *B. animalis* KSp4 (3.3×10^9) + oligofructose 5% (w/w)	1.4-log CFU/g increase in fecal bifidobacteria. SYN was more effective than PRO but was slightly less effective than administering OF alone (1.6-log increase)	Bielecka et al. 2002
	Male Wistar rats (3–4 weeks)	*B. lactis* Bb-12 (1×10^{10}), *L. salivarius* UCC500 (1×10^9), *L. rhamnosus* GG (1×10^{10}) + chicory root inulin 8% (w/w) + streptomycin sulfate (4 mg/ml)	SYN increased caecal pool of butyric and propionic acids as compared to PRE only.	Nilsson & Nyman 2007
	7-week-old male BALB/c mice	*B. breve* strain Yakult (10^8 CFU/mouse) + transgalactosylated oligosaccharides (2–50 mg/mouse)	SYN enhanced the anti-infective properties of *B. breve* under *Salmonella enterica* serovar Typhimurium LT-2 as compared to PRO alone. 2-log increase in *B. breve* numbers, which continued 2 weeks after discontinuing administration.	Asahara et al. 2001
Clinical trials	18 elderly, DB, PC, randomized	*B. bifidum* Bb02, *B. lactis* Bb01 (3.5×10^{10} CFU/d) + FOS/inulin (6 g/d)	Increase of *B. bifidum*, *B. lactis* and bifidobacteria with SYN. Detectable levels of probiotics 3 weeks post-treatment with SYN.	Bartosch et al. 2005
	12 healthy volunteers, PC	*Bifidobacterium* spp. and *B. lactis* Bb12 (3×10^{10} CFU/d) + inulin (18 g/d,) GOS (8 g/d)	Inulin did not enhance increase of bifidobacteria with SYN. Higher number of bifidobacteria 2 weeks post-treatment with SYN.	Bouhnik et al. 1996
	30 healthy volunteers, randomized	Bb12 (3×10^{10} CFU/d) + GOS (8 g/d)	Increase in LAB with all combinations. Increase of Bb12 with PRO and SYN. GOS did not enhance survival/persistence of Bb12. Bb12 may transiently replace *B. longum*.	Malinen et al. 2002

Abbreviations: DB, double blind; FOS, fructooligosaccharide; GOS, galactooligosaccharide; OF, oligofructose; PC, placebo controlled; PRE, prebiotic; PRO, probiotic; SCFA, short-chain fatty acid; SHIME, simulator of the human intestinal microbial ecosystem; SYN, synbiotic; TIM, TNO intestinal model.

8. CONCLUSIONS

No in vitro, ex vivo, or in vivo (animal) studies can offer a complete replacement for human studies. Instead randomized placebo-controlled, double-blinded human trials will always be the preferred ultimate research strategy for the most reliable investigation of human gastrointestinal microbial ecosystems. Despite this, such trials present some important limitations, specifically related to inaccessibility of sampling along the intestinal tract, ethical restrictions, lack of complete environmental control, as well as low compliance and high dropout rates. In vivo experiments using animals provide an alternative route. However, it is well-documented that the gastrointestinal tract of animals has very different microbial population structures and physiological mechanisms. Hence, care must be taken when interpreting such data. As a result, in vitro and ex vivo experimental models have become a very popular approach for studying the microbiota of the human gastrointestinal tract. The different gut models discussed in this review vary in complexity and physiological relevance, but provide a useful starting point for translational studies in humans, some of which have been shown to have real parallels to in vivo situations. Understanding the role of the gut microbiome in health and disease has renewed importance now that we are approaching the predicted post-antibiotic era. Thus, application of gut model systems to study new dietary interventions and discovery of novel drugs are important avenues of research.

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