Trends in Food Science & Technology 112 (2021) 808–822

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Bioengineering approaches to simulate human colon microbiome ecosystem

Dalila Roupar [a], Paulo Berni [a], Joana T. Martins [a], Ana C. Caetano [b], José A. Teixeira [a], Clarisse Nobre [a,*]

[a] CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal
[b] ICVS- Life and Health Sciences Research Institute, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal

ARTICLE INFO

Keywords:
Colon
Bioreactors
In vitro intestinal models
Microbiota-host interaction
Prebiotics
Probiotics

ABSTRACT

Background: Several diseases associated to colon microbial imbalance (dysbiosis), such as obesity, diabetes, inflammatory bowel disease, cardiovascular disease and cancer, are being reverted by modulation of gut microbiota composition through treatment with prebiotics and probiotics. Multiple *in vitro* models have been developed over the past three decades, with several experimental configurations, as they provide a quick, easy, and cost-effective approach to study the gut microbiome, as compared to troublesome and time-consuming *in vivo* studies.

Scope and approach: This review aims to provide an overview of the most relevant available *in vitro* models used to mimic the human colon microbiome dynamics, including macro-scale and microfluidic-based models. Main characteristics, functionalities, current applications and advantages or disadvantages of the models are discussed in order to provide useful information for end users (namely food and pharmaceutical researchers), when selecting the most appropriated model for assessing health claims and safety of novel functional food and drugs. Finally, the use of these colon models as a tool to study prebiotic and probiotic response in host-microbiota interaction is reviewed.

Key findings and conclusions: A wide range of *in vitro* models representing specific colon parts have been developed. However, none of these models can simultaneously cover all the key conditions found in the human colon (namely anatomical, physical, biochemical, and biological characteristics), as well as the complex microbiome-host interaction. Thus, there is a significant opportunity for further improvement of the models' experimental setups towards more realistic operating systems, including mucosal surfaces, intestinal cells and tissues allowing microbiome–host crosstalk representation.

1. Introduction

The human gastrointestinal (GI) tract harbours the highest microorganisms' diversity of the human body ecosystem. Numerous studies have pointed out the significance of the microbial community structure in the GI tract (Liang, Leung, Guan, & Au, 2018; Proctor et al., 2019), as well as its contribution to homeostatic processes, including protein and amino acid synthesis (Neis, Dejong, & Rensen, 2015), vitamin biosynthesis (Magnúsdóttir, Ravcheev, De Crécy-Lagard, & Thiele, 2015), carbohydrate metabolism, short chain fatty acids (SCFA) fermentation (Bach Knudsen, 2015) and host immune system development (Azad, Sarker, & Wan, 2018; Rajput & Li, 2012). Intestinal microorganisms closely interact with the host and play a key role on its health (von Martels et al., 2017). Moreover, changes on gut microbiota structure and function have a direct influence on therapeutic intervention (Vázquez-Baeza et al., 2018). Therefore, these complex microbial communities have become a major research focus, since evidence increasingly suggests their pivotal role in host's health and disease.

In vivo trials have been conducted to detect changes in human gut microbiota growth and metabolism response to diseases, dietary interventions and drug treatments. However, these human trials predominantly rely on endpoint data alone, usually derived from faecal samples, since there are many constraints related to biopsies in different gut regions. This scenario prevents the dynamic follow-up of the gut microbiota effects along the GI tract (May, Evans, & Parry, 2017; Williams et al., 2015). Human and animal models are often impaired by inter-individual differences related to an endless list factors, such as age,

* Corresponding author.
E-mail addresses: clarissenobre@deb.uminho.pt, clarissenobre@gmail.com (C. Nobre).

https://doi.org/10.1016/j.tifs.2021.04.035
Received 4 September 2020; Received in revised form 6 April 2021; Accepted 17 April 2021
Available online 27 April 2021

Abbreviations

CSSC	Continuous single-stage culture models
TVSC	Twin-vessel single-stage chemostat system
SIEM	Simulated ileal environment medium
GI	Gastrointestinal
SCFA	Short Chain Fatty Acids
MBRAs	MiniBioReactor Arrays
ECSIM	Proximal-Environment Control System for Intestinal Microbiota
TIM-TNO	Gastro-Intestinal Model
ARCOL	Artificial Colon
M-ARCOL	Mucosal Artificial Colon
PolyFermS	Polyfermentor Intestinal Model
SHIME	Simulator of the Human Intestinal Microbial Ecosystem
M-SHIME	Mucosal Simulator of the Human Intestinal Microbial Ecosystem
SIMGI	Computer-Controlled Dynamic SIMulator of the Gastro-Intestinal Tract
HMI™	Host Microbiota Interaction
TLRs	Toll-Like Receptors
ILs	Interleukins
TNFs	Tumor Necrosis Factors
IFNs	Interferons
TGF	Transforming Growth Factor
DCs	Dendritic Cells
FOS	Fructooligosaccharides
XOS	Xyloogliosaccharides
GOS	Galactooligosaccharides
α-GOS	α-Galactooligosaccharides
HMOs	Human Milk Oligosaccharides
AG	Arabinogalactan
PDMS	Polydimethylsiloxane
TEER	Transepithelial electrical resistance
LGG	*Lactobacillus rhamnosus* GG

sex, diet, geography, genetic background, and use of antibiotics (May et al., 2017; Venema & Van Den Abbeele, 2013; Vermeiren, Possemiers, Marzorati, & de Wiele, 2011). Thus, more effective alternatives are required to study GI microbial community dynamics.

In vitro models are extremely useful tools, especially to the food industry, to investigate the effects of food, novel food and functional food in the human microbiota, allowing to validate health claims and evaluate food safety. They provide a quick, easy, and cost-effective means of studying the gut microbiome dynamics, in one or more gut zones (May et al., 2017; Venema & Van Den Abbeele, 2013; Vermeiren et al., 2011). A wide range of setups are available, including pure cultures, over-simplified single vessels batch cultures or more complex single- or multi-stage pH-regulated continuous cultures (Williams et al., 2015). These *in vitro* models enable monitoring changes in microbiota and its metabolism. Various relevant physiological conditions can be simulated, such as gut peristaltic movement, mucus layer and intestine cells, bringing these models one step closer to mimic *in vivo* conditions. Nevertheless, these *in vitro* models do not always provide accurate simulation of *in vivo* conditions, as they lack the real-time interaction with host intestinal mucosa, immune system, and endocrine functions (Boureau, Hartmann, Karjalainen, Rowland, & Wilkinson, 2000). The main advantages of the *in vitro* models are: a) being tightly controlled under reproducible conditions; b) allowing detailed mechanistic analysis; c) having limited ethical restrains; and d) requiring no expensive time-consuming procedures (as required for animal and human clinical trials) (von Martels et al., 2017).

The main objective of this review is to provide significant information regarding the most relevant *in vitro* models used to simulate the colon, including macro-scale and microfluidic-based models (so-called organ-on-a-chip). A summary of the human colon fundamental morphology is provided. Also, a critical analysis of the models' design, setup (*e.g.* flow rates, mixing and feeding conditions), characteristics, applications, and advantages or disadvantages are discussed. Finally, the use of these colon models as a tool to study prebiotic and probiotic response in host-microbiota interaction is reviewed.

2. Brief overview on human colon morphology

The GI system consists of a series of hollow organs, from the mouth to the anus, associated with several accessory glands that add secretions to these organs. The GI tract is responsible for food digestion and absorption – after ingestion, food is digested, nutrients are released and absorbed, and the residues are expelled as faeces. The GI system is divided into four concentric layers: mucosa, submucosa, *muscularis* extern and serosa, from the inner to the outer layer. The myenteric plexus situated between the circular and longitudinal muscle layers, forms the enteric nervous system along with the submucosal plexus. The enteric nervous system is responsible for the motor and secretory activity. It can be modulated by the autonomic nervous system (sympathetic branch and parasympathetic branch). The motor activity includes peristalsis (coordinated contraction of the muscle layers), sphincters (high pressure muscles) and segmental contractions. The muscle activity is initiated by pacemaker cells named cells of Cajal. Additionally, the GI tract contributes to immune function of the human body (Hounnou, Destrieux, Desmé, Bertrand, & Velut, 2002; Stranding, 2016).

The large intestine is a part of the lower GI tract and it lies between the small intestine and the anus. It is subdivided into cecum, ascending colon, transverse colon, descending colon and sigmoid colon (Fig. 1). The large intestine reabsorbs fluids and electrolytes and stores the faecal matter before its expulsion from the body. The proximal colon has two types of motor activity: non-propulsive segmentation and mass peristalsis. The segmental contractions promote a mixing phase and give to the colon its typical appearance of haustra. During this motor activity, material is retained in the proximal colon for long periods, which allows fluids and electrolytes absorption. Mass peristalsis occurs one to three times a day, frequently stimulated by eating – the haustra disappear and a portion of the colonic content is propelled distally more than 20 cm (Boron & Boulpaep, 2016).

Regarding the histology, the large intestine has a specialized epithelial structure that correlates well with its transport function. The cells lining the large intestine are surface epithelial cells, and interspersed over the colonic surface, are numerous apertures of colonic crypts. The surface epithelial cells of the large intestine are the primary cells responsible for colonic electrolyte absorption, whereas colonic crypt cells are generally believed to mediate ion secretion. Some intestinal cells secrete mucus that forms a double mucus layer, which reinforces the intestinal barrier and promotes favourable niches for bacteria colonization (Labarthe et al., 2019). Gut fluid absorption is usually defined as 1.9 L/day and it is calculated as the difference between ileocecal flow (approximately 2.0 L/day) and stool water (approximately 0.1 L/day) (Cremer, Arnoldini, & Hwa, 2017). The information on the physical parameters of the different colon segments is essential to understand the bacterial growth dynamics. Fig. 1 summarizes the most important features regarding gut parameters (Boron & Boulpaep, 2016; Cremer et al., 2017; Moore, Dalley, & Agur, 2014).

Gut bacteria represent around 70% of the human microbiota. Their primary location in the colon relates to their main function – soluble fibres not completely broken down by human digestive enzymes are

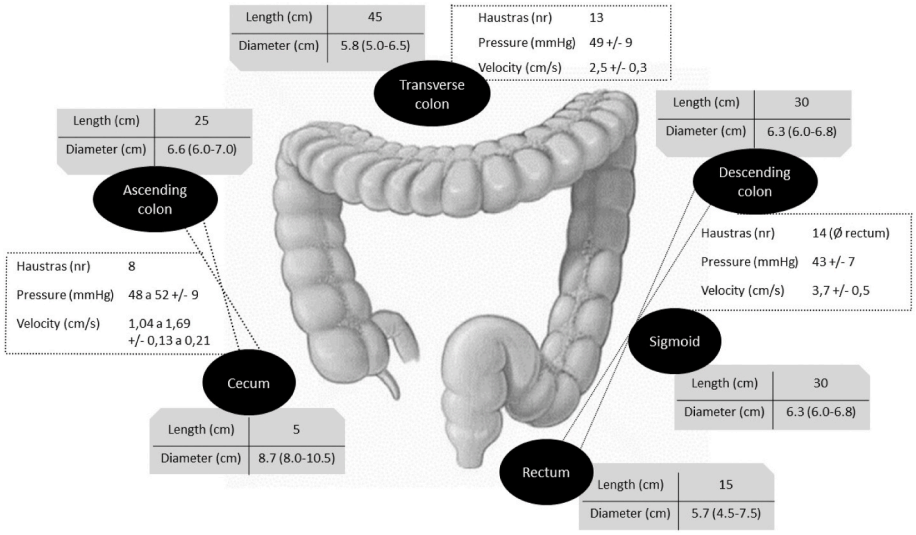

Fig. 1. Main physical colonic parameters (Data collected from Cremer et al., 2017; Moore et al., 2014; Boron & Boulpaep, 2016).

fermented in the colon by gut bacteria. The colon microbiome can be described regarding its taxonomic composition, *i.e.,* the relative abundance of microorganisms classified, for example in phyla, genera or species. Taxonomically, the colon microbiota of healthy humans is dominated by the bacterial phyla Firmicutes followed by Bacteroidetes (both accomplish 90% of the colon microbiome), Actinobacteria and Proteobacteria (Das & Nair, 2019). The Firmicutes/Bacteroidetes ratio (around 5/20 in healthy adults) represents an important marker of the microbiome homeostasis, since it is related to age and to the aetiology of some diseases like obesity and autism (Das & Nair, 2019; Mariat et al., 2009).

The predominant genus in the colon is *Bacteroides*. Metagenomics analysis of microbiota from United Kingdom and United States of America donors identified that 8 of the 20 most prevalent bacteria were from the same *Bacteroides* genus (*Bacteroides vulgatus*, *Bacteroides uniformis*, *Bacteroides cellulosilyticus*, *Bacteroides ovatus*, *Bacteroides xylanisolvens*, *Bacteroides thetaiotaomicron*, *Bacteroides caccae* and *Bacteroides dorei*) (Forster et al., 2019). There are also some minority genera like *Lactobacillus* and *Enteroccocus* that are important markers of health/-disease (Mariat et al., 2009). For example, the minor abundant but much relevant species from the phyla Verrucomicrobia (such as *Akkermansia muciniphila*) prevents high fat diet induced overweight (Das & Nair, 2019; Mariat et al., 2009).

The individual human colon microbiota is related with various factors such as age, diet, ethnicity, and geography (Liang et al., 2018; Priya & Blekhman, 2019). Furthermore, even at lower levels of the taxonomic classification, microbiota composition changes from person to person (Priya & Blekhman, 2019). The evolutionary forces shape the microbiota, which affect its adaptation within the microbial community. This adaptive behaviour impacts their long-term persistence in the gut, thereby influencing host health (Liang et al., 2018; Priya & Blekhman, 2019).

The complex gut microbiota dynamics encompass all its fundamental factors, such as fluid mechanic physics, dietary fibre degradation, epithelial motility and peristalsis connected to the bacteria population dynamics and the spatiotemporal digestion mechanisms. However, as identified by Labarthe et al. (2019), the key drivers of the colon microbiota biogeography can be comprehended as: a) viscosity gradients that allow the creation of favourable niches in the vicinity of the mucus layer; b) epithelial motility, preponderant for the colonization of the ascending colon; c) in the transverse colon, fibre levels and chemotaxis have the strongest impact on the microbial communities distribution; and d) residual dietary fibres that are the main driver of the microbiota spatial structure in the descending bowel.

The microbiome can be described regarding its functionality, since most microorganisms have carbohydrate and amino-acid metabolism that plays an important role for the host's nutrition, along with some functions that are restricted to a species or strain, including pathogenicity, vitamin and drug catabolism, motility and nutrient transporters (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012). The microbiome has a core set of genes that sustain its physiology and the healthy microbiome–host interactions despite the many observed variations related with *e.g.* age, ethnicity and diet. However, microbiome functions are determined more by this core set of genes than its taxonomic profile. In fact, some functions are related with low-abundant bacteria *i.e.* some functions derived from proteins expressed by genes that are specific from certain species or strains (Arumugam et al., 2011). In this sense, the human microbiome can be classified in bacteria clusters regrouped by functions (so-called enterotypes) that include different genera: a) Enterotype I ferments mainly carbohydrates through glycolysis and pentose phosphate pathways, holding high *Bacteriodes* proportion; b) Enterotype II is represented mostly by *Prevotella*, that degrades mucin glycoproteins; and c) Enterotype III includes other bacteria that efficiently bind and hydrolyse the mucus layer, being mostly represented by *Ruminococcus*. Therefore, enterotypes are mainly defined by diet (Arumugam et al., 2011; Rinninella et al., 2019).

In summary, the gut's anatomy and morphology create an environment that influences the microbiota biogeography throughout physical forces, while the substrates (*e.g.* dietary fibres and bioactive compounds) are the main factors driving its composition and function. Since the microbiota-host crosstalk is essential for maintaining a health-disease balance, and its mechanisms and intricacies are not well understood, there is a need to overcome methodological challenges to better understand microbiome complexity. Therefore, *in vitro* models that properly simulate human microbiome environment, its functions and interactions with human cells and tissues are required.

3. *In vitro* colon models as a tool to study microbial communities

In vitro colon models have been developed to simulate microbiota activity. These models range from simple single vessels to complex series of tailor-made bioreactors able to mimic one or several colon zones. These models allow researchers to observe and measure real-time effects on the gut microbiome. Current models can be broadly grouped according to: 1) system dynamics (batch cultures *vs* continuous cultures); 2) the number of represented colon regions (single *vs* multi-stage); and

3) the inclusion of human cells to study microbiota–host interactions (*e.g.* intestine epithelium and immune system).

Most relevant *in vitro* human colon models available are summarized in Fig. 2. They will be discussed in the following sections, regarding its usefulness, versatility, applications, and unique insights into the microbial ecosystem.

Table 1 provides an overview on the experimental setup, culture protocols and growth media that have been used in the different *in vitro* colon systems, while highlighting the main advantages and disadvantages of each model.

3.1. Batch cultures

Batch cultures are the simplest *in vitro* models used to study the human gut microbiota. They are mainly applied to assess specific substrates metabolization by selected strains or complex faecal microbial communities (Venema & Van Den Abbeele, 2013). Usually, batch cultures are run in single bioreactor vessels, containing basal media supplemented with a specific substrate or a growth inhibitor. Operational conditions such as constant physiological temperature (37 °C) and anoxic atmosphere (generally by flushing N_2) are applied. Typically, assays are run for short periods of time (24 h–48 h and a maximum of 72 h) (El Oufir et al., 2000; Rumney & Rowland, 1992) (Table 1). Microbial communities, pure cultures or faecal material suspensions, are inoculated into the sterile media to initiate fermentation, with or without pH control and without additional supply of nutrients.

These models are used to study the effect of different substrates on the gut microorganisms' physiology and biodiversity. The impact on the microbiota is evaluated by quantitative and qualitative molecular techniques, and the impact on metabolic activity is assessed by analysis of the SCFA released, as well as other metabolites. Batch models are appropriate to check inter-individual variability in response to a particular bioactive compound or agent, and to compare consequences of exposure to different compound sources or doses. In addition, they provide a first assessment on microbial metabolites formed and help to elucidate metabolic pathways involved (Verhoeckx et al., 2015).

The advantages of batch cultures include quick assembling, inexpensive running, easy operation and reproducibility, as a result of overall setup design simplicity. The high throughput and potential automation features make them almost an initial mandatory method to investigate gut microbiota composition, metabolism, and modulation by probiotics and diet compounds. Since these models are easily downscaled, the required media volume and test compounds concentration may be reduced. Batch cultures are usually employed before conducting more complex multi-vessel continuous fermentation experiments (Williams et al., 2015).

The main disadvantages of the batch cultures are the rapid substrate depletion, the high accumulation of microbial metabolites and the medium acidification, which prevents further microbial activity and operation under longer operation times (in average, fermentations are run up to 48 h) (Payne, Zihler, Chassard, & Lacroix, 2012; Venema & Van Den Abbeele, 2013). For this reason, the distal colon is usually modelled on these systems at pH 6.8. The simulation of more complex GI functionalities, such as peristalsis, is not possible in these models. Lastly, short term fermentations may prevent the establishment of trophic chain, where metabolites produced by some species become the substrate of other species (Pham & Mohajeri, 2018).

3.2. Continuous single-stage culture models

Continuous Single-Stage Culture models (CSSC) are based on original *in vitro* semi-continuous cultures developed to investigate rumen microbial communities by Rufener, Nelson, and Wolin (1963) and Slyter, Nelson, and Wolin (1964), as well as Pirt's fed batch cultures (Pirt, 1974). These models have been used to culture complex microbial communities, such as those found in faeces (McDonald et al., 2013, 2015), and more simple cultures, such as defined bacterial communities (Drake & Brogden, 2002; Newton, MacFarlane, & MacFarlane, 2013). CSSC are often designed to simulate the proximal colon conditions, reproducing its metabolic activity.

CSSC systems are physiologically more relevant than batch cultures. They provide a constant nutrients influx and waste products efflux over a defined retention time, which enables continuous operation. In fact, these models allow to run experiments for several days or months, due to the continuous monitoring and correction of several parameters including pH, temperature, nutrient input, and waste removal (Drake & Brogden, 2002). The stable environment enables growing complex bacterial communities up to a steady state. In addition, the chemostat model provides tightly controlled atmospheric conditions and reproduces the GI tract anaerobic environment, offering ideal conditions for fastidious anaerobic organism culture. CSSC systems can be programmed to monitor atmospheric oxygen levels, and continuously flush N_2 into each vessel to create the desired anaerobic environment (Drake & Brogden, 2002).

The main disadvantage of the CSSC models is that, generally, only a single colon zone is simulated, lacking information on the microbial dynamics behaviour along the GI tract. This prevents the identification of relevant patterns and mechanisms taking place in the different colon zones, which may hinder a comprehensive understanding of the phenomena under study.

Most relevant examples of CSSC are briefly described in the following sub-sections.

3.2.1. Twin-vessel single-stage chemostats system

The Twin-Vessel Single-Stage Chemostats system (TVSC) was developed to simulate the distal colon. Two similar vessels run simultaneously, allowing to test different conditions on the same inoculated sample (McDonald et al., 2013). One vessel is usually used as a test

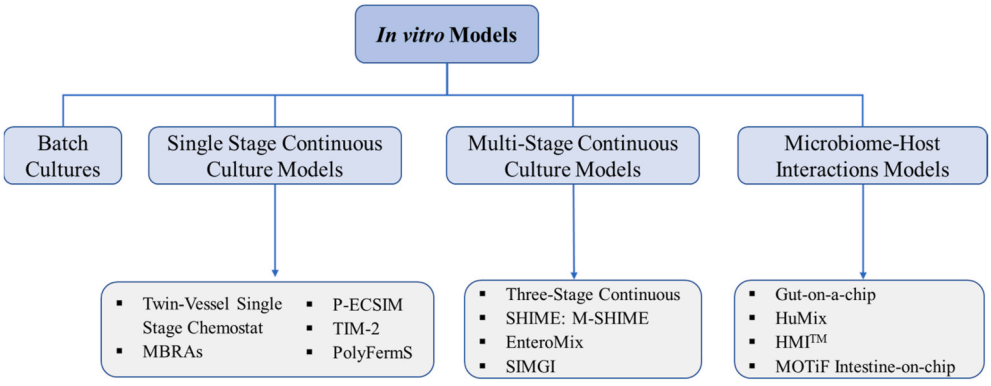

Fig. 2. Overview of relevant *in vitro* colon models currently used to study the gut microbiota.

Table 1
Overview of the operational conditions, growth media, advantages and disadvantages of *in vitro* colon systems.

Gut Model	Operational Conditions	Growth Media	Advantages	Disadvantages
Batch Cultures (Takagi et al., 2016)	**GI Simulated Section:** Colon **Inoculum:** Human faecal samples **Gas Mixture:** N_2 and CO_2 (80:20) **Temperature:** 37 °C **Stirring:** 300 rpm **pH:** 6.5 **Working Volume:** 100 mL	Gifu anaerobic broth pH 6.5 supplemented with fructooligosaccharides, galactooligosaccharides, isomaltooligosaccharides, xylooligosaccharides, raffinose, lactulose or lactosucrose	- Fast, cheap, easy to operate, and reproducible - Small quantities of testing compounds - Reduced cost - Closed systems - Less risk of contamination	- Short culture periods - Nutrients decrease over time - Limited amount of growth media - Build-up of waste products - Lack of environment control
Twin-Vessel Single Stage Chemostats (McDonald et al., 2013, 2015)	**GI Simulated Section:** Distal colon **Inoculum:** Human faecal samples **Gas Mixture:** N_2 **Temperature:** 37 °C **pH:** 6.9–7.0 **Working Volume:** 400 mL **Feed flow rate (medium):** 400 mL/d -Vessels were allowed to run for 48 d post-inoculation	Peptone water, yeast extract, $NaHCO_3$, $CaCl_2$, pectin, xylan, arabinogalactan, starch, casein, inulin, $NaCl_2$, KH_2PO_4, $MgSO_4$, hemin, menadione, bile salts, L-cysteine, porcine gastric and mucin	- Reproducibility - Long period of culture - Perturbation studies - Simultaneous control can be monitored	- Risk of contamination due to daily operation - Anatomy of intestine not mimicked - Long operation increases the cost per run - No host cells
MBRAs (Auchtung et al., 2015; Robinson et al., 2014)	**GI Simulated Section:** Distal colon **Inoculum:** Human faecal samples **Gas Mixture:** 5% H_2 – 5% CO_2,90% N_2 **Temperature:** 37 °C **Stirring:** magnetic stir plates **pH:** 6.8 **Working Volume:** 15 mL **Waste removal flow rate:** 1.875 mL/h	Tryptone, proteose peptone, yeast extract, arabinogalactan, maltose, D-cellobiose, NaCl, hemin, $MgSO_4$, $CaCl_2$, KH_2PO_4, phosphate dibasic, tween 80 pH = 6.8, bovine bile, D-glucose, inulin, sodium bicarbonate and vitamin K3	- Small processing volumes - Reduced cost per run - Up to 48 systems operating simultaneously - Reaches steady state faster	- Only simulates proximal region of the colon - Inhibition of growth by excess of end products - No host simulation
P-ECSIM (Brugere et al., 2011; Feria-Gervasio et al., 2011)	**GI Simulated Section:** Proximal colon **Inoculum:** Glycerol stocks of human faecal samples **Gas Mixture:** N/A, anaerobic conditions maintained by microbial metabolism **Temperature:** 37 °C **Stirring:** 400 rpm **pH:** 5.75 **Working Volume:** 2 L **Retention Time:** Short one = 12.5 h and Longer one = 25 h	Mucin, starch, pectin, guar gum, xylan, arabinogalactan, inulin, L-cystein, HCl, casein, peptone, tryptone, yeast extract, bile salts, tween 80, $FeSO_4$, NaCl, KCl, KH_2PO_4, $MgSO_4$, $CaCl_2$, $NaHCO_3$, hemin, $MnSO_4$, $FeSO_4$, $CoSO_4$, $ZnSO_4$, $CuSO_4$, $AlK(SO_4)$, H_3BO_3, Na_2MoO_4, $NiCl_2$, Na_2SeO_3, menadione, D-biotin, pantothenate, nicotinamide, vitamin B12, thiamine and p-aminobenzoic acid	- Self-maintenance of the anaerobic conditions	- No simulation of digestion and absorption of nutrients - No peristaltic movement simulation - No host simulation
TIM-2 (Minekus, 2015; Minekus et al., 1999; Smeets-Peeters et al., 1999)	**GI Simulated Section:** Proximal colon **Inoculum:** Human faecal samples; 11% microbiota (w/w) **Gas Mixture:** N_2 **Temperature:** 37 °C **Stirring:** Contraction of the flexible walls by water pressure **pH:** 5.8 **Working Volume:** 200 mL **Feed flow rate (medium):** 4 mL/h **Chyme removal flow rate:** 2 mL/h **Dialysis flow rate:** 6 mL/min **Retention Time:** 80 h 12 h microbial adaptation 2–4 h starvation period **Running Time:** 3 d	Pectin, xylan, arabinogalactan, amylopectin, starch, tween 80, bactopeptone, casein, hemin, cystein, menadione, D-biotin, vitamin B12, pantothenate, nicotinamide, p-aminobenzoic acid, thiamine, NaCl, K_2HPO_4, $CaCl_2$, $MgSO_4$, $FeSO_4$ and bile salts	- Peristaltic movements changed the water pressure on the flexible walls - Dialysis system - Parallel control and treatment	- No host simulation
Three-Stage Colonic Model System (Gibson et al., 1988; Macfarlane et al., 1998)	**GI Simulated Section:** Ascending (V1), transverse (V2) and descending (V3) colon **Inoculum:** Human faecal samples **Gas Mixture:** O_2-free N_2 **Temperature:** 37 °C **Stirring:** magnetically stirred **pH:** V1 = 6.0; V2 = 6.5; V3 = 7.0 **Working Volume:** V1 = 220 mL; V2 = 320 mL; V3 = 320 mL **Dilution rates:** V1 = 0.14 h^{-1}	Starch, pectin, guar gum, mucin (porcine gastric type III), xylan, arabinogalactan, inulin, casein, peptone water, tryptone, bile salts, yeast extract, $FeSO_4$, NaCl, KCl, KH_2PO_4, $MgSO_4$, $CaCl_2$, $NaHCO_3$, cysteine, hemin and tween 80	- Simulates proximal, transversal and distal colon - Allows sampling at different zones of the colon	- No simulation of digestion or absorption of nutrients - Risk of contamination - No host cells

(*continued on next page*)

Table 1 (*continued*)

Gut Model	Operational Conditions	Growth Media	Advantages	Disadvantages
	(Retention Time = 7 h); V2 = 0.10 h^{-1} (Retention Time = 10 h); V3 = 0.10 h^{-1} (Retention Time = 10 h) **Running Time:** 16 d			
SHIME (Molly et al, 1993, 1994; Van de Wiele et al., 2015)	**GI Simulated Section:** Stomach (V1), small intestine (V2), ascending (V3), transverse (V4) and descending (V5) colon **Inoculum:** Human faecal samples; 20% faecal suspension **Gas Mixture:** O_2-free N_2 or 90/10% N_2/CO_2 **Temperature:** 37 °C **Stirring:** 150 rpm **pH:** V3 = 5.6–5.9; V4 = 6.15–6.4; V5 = 6.6–6.9 **Working Volume:** V3 = 500 mL; V4 = 800 mL; V5 = 600 mL **Retention Time:** V3 = 20 h; V4 = 32 h; V5 = 24 h **Feed Flow Rate** = 25 mL/h **Four stages:** stabilization (2 weeks); basal (2 weeks); treatment (2–4 weeks); washout (2 weeks) **Running Time:** typically, 30 d per cycle	Arabinogalctan, pectin, xylan, dextrins, starch, glucose, yeast extract, proteose-peptone, mucin, cysteine, $NaHCO_3$, NaCl, K_2HPO_4, KH_2PO_4, $CaCl_2$, $MgSO_4$, hemin, tween 80, menadione, biotin, pantothenate, nicotinamide, vitamin B12, thiamin and p-aminobenzoic acid	- Integrates the entire GI tract - Allows sampling at different regions of the GI tract - Maintains microbiome stability over a long timeframe - Differentiation between mucosal and luminal microbiome in M-SHIME setup	- No host simulation
EnteroMix (Mäkivuokko et al., 2006; Mäkivuokko, Nurmi, Nurminen, Stowell, & Rautonen, 2005)	**GI Simulated Section:** Ascending (V1), Transverse (V2) and Descending Colon (V3), Sigmoid-Colon (V4) **Inoculum:** Human faecal samples **Gas Mixture:** O_2-free N_2 **Temperature:** 37 °C **pH:** V1 = 5.5; V2 = 6.0; V3 = 6.5; V4 = 7.0 **Working Volume:** V1 = 3 mL; V2 = 5 mL; V3 = 7 mL; V4 = 9 mL **Running Time:** 48 h of experience	Starch, pectin, guar gum, mucin (porcine gastric type III), xylan, arabinogalactan, inulin, casein, peptone water, tryptone, bile salts, yeast extract, $FeSO_4$, NaCl, KCl, KH_2PO_4, $MgSO_4$, $CaCl_2$, $NaHCO_3$, cysteine, hemin and tween 80	- Ability to run four parallel experiments using the same faecal sample as inoculum	- Short-term experiments can be performed - No simulation of digestion and absorption of nutrients - No host cells
SIMGI (Barroso et al., 2015a, 2015b)	**GI Simulated Section:** Stomach, Small Intestine (SI), Large Intestine: Ascending (AC), Transverse (TC) and Descending Colon (DC) **Inoculum:** Human faecal samples **Gas Mixture:** N_2 **Temperature:** 37 °C **Stirring:** 150 rpm **pH:** AC = 5.6 ± 0.2; TC = 6.3 ± 0.2; DC = 6.8 ± 0.2 **Working Volume:** AC = 250 mL; TC = 400 mL; DC = 300 mL **RT:** 76 h **Feed Flow Rate** = 5 mL/min **Stabilization period:** 14 days	Arabinogalactan, pectin from apple, xylan, potato starch, glucose, yeast extract, peptone, mucin and L-cysteine. The media for the small intestine is supplemented with pancreatic juice, $NaHCO_3$, oxgall (dehydrate fresh bile) and porcine pancreatine	- Digestion is simulated - Peristaltic movements are mimicked - Long period of culture	- No host-interactions - Risk of contamination due to daily operation

vessel, while the other is the control. Specific parameters including pH, temperature and gas injection are controlled by a computer software (McDonald et al., 2013, 2015). Each vessel is operated at a feed rate of 400 mL/day (100 mL of *inocula* in 300 mL of culture media) to mimic 24 h transit time. A 36-day stabilization period is needed to reach steady-state, *i.e.* without experimental manipulation. Cultures are gently mixed and flushed with N_2, and pH is set at 6.9–7.0. Fermentation runs for 48 days' post-inoculation. Once fermentation starts, media is continuously fed to the vessels, while the excess volume is transferred to the waste containers. The pH is regulated by automatic addition of acid or alkali solutions, and the temperature is controlled by a steel water jacket. Stirrers, at identical speed, maintain homogeneous conditions inside the vessels (McDonald et al., 2013, 2015). The TVSC is an adequate model to conduct perturbation studies.

The TVSC has been applied to investigate the significance and efficacy of functional food ingredients, providing important information on their bioactivity, stability, bioaccessibility and bioavailability. For example, the stability and biological activity of wild blueberry polyphenols during GI digestion have been studied (Correa-Betanzo et al., 2014). The TVSC has been also applied to evaluate food safety. The potential hazardous effects of the ingested food additive titanium dioxide nanoparticles, which are used as an additive (E171 or INS171) in *e.g.* gums, candies and puddings, have been evaluated within the TVSC (Dudefoi, Moniz, Allen-Vercoe, Ropers, & Walker, 2017).

3.2.2. *MiniBioReactor arrays*

The MiniBioReactor Arrays (MBRAs) is a simple continuous-flow system, which works at small volumes and simulates the distal colon. It was developed to evaluate microbial community dynamics (Auchtung, Robinson, & Britton, 2015). The MBRAs was designed by a computer

assisted design software and manufactured by stereolithography with DSM Somos® Watershed XC 11122 (Robinson, Auchtung, Collins, & Britton, 2014). Each MBRA consists of six reactors with an internal volume of 25 mL and a working volume of 15 mL. The system operates inside a heated anaerobic chamber (37 °C), under 5% CO_2 – 5% H_2 - 90% N_2 atmosphere. Prior to use, MBRAs and media are sterilized by autoclaving. Then, the system is equilibrated for 72 h to reach an anaerobic environment. Typically, media is fed at a continuous-flow of 1.875 mL/h, controlled by two 24-channel peristaltic pumps. The reactor contents are continuously stirred during the experiments. Due to their small size and simplistic design, it is possible to run up to 48 reactors simultaneously in a single anaerobic chamber, assessing multiple environmental disturbances on the microbiota. The MBRAs have the advantage of the short time required to reach the steady state, while the low volume used in the system reduces running costs.

The MBRAs were used to evaluate the effects of antibiotic clindamycin and polyphenol extracts from pomegranate and blueberries on faecal microbial communities' modulation. Microbiota modulated with polyphenols showed decreased colonization resistance against *Clostridioides difficile* but could neutralize cytotoxicity, showing its potential as a non-antibiotic agent for the alleviation of *C. difficile* infection (Spinler et al., 2017).

3.2.3. Environment control system for intestinal microbiota

The Environment Control System for Intestinal Microbiota (ECSIM) is a modular system consisting of three reactors that can be used in several configurations to mimic different zones and functions of the human colon. For example, P-ECSIM simulates nutritional and physicochemical relevant conditions of the gut microbiota in the proximal zone, and 3S-ECSIM simulates the proximal, transverse and distal colon zones (Feria-Gervasio, Denis, Alric, & Brugère, 2011). The ECSIM differs from other systems, since the anaerobic atmosphere is guaranteed through the gases released from the microbiota metabolism and no N_2 or any other gas/gases mix are flushed (Feria-Gervasio et al., 2011).

The composition of the culture medium used in the P-ECSIM mimics the terminal ileal chyme of an individual consuming a common western diet, as applied in other models (Macfarlane, Macfarlane, & Gibson, 1998; Molly, Woestyne, & Verstraete, 1993). Each bioreactor has its own controlling terminal connected to a computer and four peristaltic pumps that feed nutrients, test solutions, and neutralizing solutions (Brugere, Feria-Gervasio, Popse, Tottey, & Alric, 2011). It consists of a 2 L stirred tank run with a fixed 1 L working volume, which is continuously adjusted (Brugere et al., 2011). A water jacket ensures bioreactors' temperature (37 °C). Experiments are run at 400 rpm and pH is maintained at 5.75 by the automatic addition of 2 M NaOH. Two different retention times are typically used, namely 12.5 h and 25 h, simulating physiological or slow transit time (48 h or 96 h, respectively).

The P-ESCIM has been used to unveil the link between colonic transit time and gut microbiota composition and metabolism (Tottey et al., 2017). It provides additional insight towards the design of functional food solutions counter-acting modifications seen in the gut microbiota of the elderly, as well as patients with slow transit time.

3.2.4. TNO gastro-intestinal model

The TNO Gastro-Intestinal Model (TIM) is a system with different variations (TIM-1, TIM-2, tinyTIM, TIM-age). The proximal colon is simulated by the TIM-2, which consists of four glass compartments holding flexible membranes. When the membranes are compressed by water (set at 37 °C for humans, 39 °C for pigs, 41 °C for birds, etc.), the luminal content is mixed and moved through the system by a peristaltic wave (Minekus, 2015). The mixing allows working with high density microbiota, viscous "meals" and insoluble components. The system is equipped with a dialysis system which allows water absorption and regulation of the metabolites concentration, such as the SCFA that are taken up by the epithelial cells, avoiding microorganism inhibition or death, and keeping physiological concentrations (Minekus et al., 1999).

The TIM-2 has been inoculated with pooled or individual faecal samples from healthy human volunteers or patients with GI disorders and obesity (Martinez et al., 2013). Pig's (Martinez et al., 2013) and dog's microbiota (Smeets-Peeters, Minekus, Havenaar, Schaafsma, & Verstegen, 1999) have also been tested. An adaptation period between 12 h and 16 h is applied to the microbiota before the experiment starts, which normally lasts for 72 h. After this adaptation stage, a 2–4 h starvation period is applied to allow fermentation of all available carbohydrates prior to feed the tested substrate. Anaerobic conditions are kept by flushing with N_2. In the first TIM-2 setup, feeding flow rate was set at 4 mL/h, chyme was removed at 2 mL/h, and the dialysis fluid flow was set at 6 mL/min. Samples were taken from lumen and dialysate to assess microbial metabolites production and to study changes in microbiota composition (Minekus et al., 1999).

The TIM-2 takes advantage of peristalsis and nutrient absorption, which are key differentiating factors to provide a more realistic simulated environment for the study of gut microbiota dynamics. Hence, this *in vitro* system is a landmark in the field. It has been widely used to assess fermentation patterns and the effects of prebiotic and probiotic supplementation in the microbial composition (Kortman et al., 2016; Kovatcheva-Datchary et al., 2009; Minekus et al., 1999; Van Nuenen, Meyer, & Venema, 2003). Recently, new prebiotics obtained from food wastes, such as mango peel (Sáyago-Ayerdi, Zamora-Gasga, & Venema, 2019) and mixtures of pectin-derived oligosaccharides from lemon peel (Miguez, Vila, Venema, Parajo, & Alonso, 2020) have been tested with gut microbiota from elderly donors. TIM-2 model has been also used to study the effect of food plant sterols enrichment dose on gut microbial profile, cholesterol metabolites and SCFA production of lean and obese populations (Cuevas-Tena, Alegria, Lagarda, & Venema, 2019).

The Artificial Colon (ARCOL) is another example of a bioreactor system that simulates the colon. It was firstly used when coupled to the TIM-1 model and it is a one-stage, semi-continuous fermentation system. Several probes control the essential parameters, such as pH 6.0 and temperature at 37 °C, that are identical to the parameters observed in the human large intestine. Passive absorption is simulated using hollow fibre membranes (cut-off 30,000 Da). This dialysis step keeps electrolyte and metabolite balance, as well as the operating volume (450 mL). Ileal effluents, usually from TIM-1, are transferred to the ARCOL bioreactor in presence of a metabolically active human microbiota. Microbiota is maintained in anaerobic conditions only by the activity of intestinal microbiota (Blanquet-Diot et al., 2012). The ARCOL model has been applied to study the antagonistic effect of probiotic strain *Saccharomyces cerevisiae* against enterohemorrhagic *E. coli* O157:H7 and to understand some mechanisms of its pathogenesis (Cordonnier, 2015).

Recently, the ARCOL was upgraded to the M-ARCOL, which includes mucin beads that represent the colon mucus microenvironment. Mucin beads are produced by dropping porcine stomach type II mucin/sodium alginate solution into 0.2 M $CaCl_2$ solution, under agitation. The beads are inserted in an airtight glass compartment, where the microbial community circulates. Mucin beads accounted for a total surface area of 556 cm^2 for each fermentation, reproducing the mucus-associated microbiota. After the experiments, mucin/alginate beads are washed with phosphate buffered saline (PBS) and stored at − 80 °C for further analyses (Deschamps et al., 2020).

3.2.5. Polyfermentor intestinal model

The Polyfermentor Intestinal Model (PolyFermS) system mimics the proximal colon zone. It overcomes biological reproducibility problems associated to gut microbiota modulation, since it allows the study of multiple effects of different treatments, such as environmental parameters, dietary compounds and drugs on microbiota (Poeker et al., 2018). The PolyFermS uses immobilized faecal inoculum allowing continuous and prolonged culture of the microbiome. Fresh faecal microbiota is trapped in gel beads, growing to high concentration. Effluents of this first-stage inoculum reactor are then used to continuously feed several second-stage control and experimental reactors operating in parallel.

Fermented effluents are equally distributed on the reactors (working volumes of 300 mL), for a mean retention time of 7.5 h (Berner et al., 2013). The PolyFermS significantly improved bacterial density, biodiversity and long-term microbiota stability, which is reflected on operation times up to 80 days (Dostal, Fehlbaum, Chassard, Zimmermann, & Lacroix, 2013; Fehlbaum et al., 2015). The microbiota immobilization prevents washout of less competitive bacteria and ensures the repeated exposure of a single microbiota, for example, to different dietary fibres (Berner et al., 2013). The PolyFermS has been used to evaluate the potential of different type of bacteria and dietary fibres as probiotic or prebiotic, *e.g.* β-glucans, α-galactooligosaccharides (α-GOS) and xylooligosaccharides (XOS) (Fehlbaum et al., 2019; Poeker et al., 2018). Changes observed in the SCFA profile were correlated with changes in the abundance of specific bacteria upon prebiotic supplementation. These results highlight the importance of understanding the inter-individual response to a prebiotic treatment.

3.3. Multi-stage continuous culture models

Multi-stage continuous models represent a more reliable approach to replicate the environment 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 nutrients are depleted towards the transverse (middle) colon and reaches near pH-neutral in the distal (descending) colon. Therefore, these multi-stage systems usually use three fermentation vessels connected sequentially to mimic specific environment physicochemical conditions over the three major anatomic colon regions (Williams et al., 2015). The majority of these *in vitro* gut models are based on the three-stage colonic model design developed by Gibson, Cummings, and Macfarlane (1988). Influx of gastric juice, pancreatic juice and bile salts, peristaltic motility, absorption capacity and high shear forces (Williams et al., 2015) allow to model the gut dynamic environment. Parameters such as temperature, flow rate, pH, retention time, and anaerobiosis of the medium are strictly controlled to mimic the *in vivo* human colon, which contributes to the establishment of steady-state conditions for microbial composition and metabolic activity (Bajury, Nashri, King Jie Hung, & Sarbini, 2018).

The most relevant examples of multi-stage continuous culture models are briefly described in the next sub-sections.

3.3.1. Three-stage colonic model system

Macfarlane et al. (1998) validated one of the first multi-stage continuous culture models - proposed by Gibson et al. (1988) in their 80's work - to simulate ascending, transverse and descending colon environmental and nutritional conditions. This *in vitro* model comprises three fermentation vessels (V1, V2 and V3) connected sequentially to simulate the microbial activities in the ascending, transverse, and descending colon. Each of these fermentation vessels takes into account the following characteristics: 1) the ascending colon is a nutrient-rich environment comprising microbial growth at low pH; and 2) the other two colon subparts are nutrient limited and present slow microbial growth at an increasing pH whereas oxygen-free conditions, pH, transit time and stirring conditions are controlled (Gibson et al., 1988; Macfarlane, Cummings, Macfarlane, & Gibson, 1989).

A typical experimental setup of this model consists in the inoculation of each vessel with human faecal slurry. Microbiota is then grown overnight, in batch, to increase initial biomass. Operating volumes of 220 mL, 320 mL and 320 mL, are used for V1, V2 and V3 (vessels), respectively. Individual dilution rates of 0.14 h^{-1}, 0.10 h^{-1}, and 0.10 h^{-1} are used, yielding a total retention time of ~27 h (Macfarlane et al., 1998). The vessels are held at 37 °C and at pH 6.0, 6.5 and 7.0, respectively. Medium, continuously flushed with N_2, is feed by a peristaltic pump up to V1, at a rate correspondent 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 (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 microbial communities reach steady state, which typically takes 8 turnovers, or 16 days. Steady state is confirmed after obtaining consistent SCFA profiles, during three consecutive days (Macfarlane et al., 1998).

The representativeness of the true *in vivo* conditions reached by 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 shown to be similar (Macfarlane et al., 1998). Although the system does not incorporate host factors such as intestinal immunology, secretions, or absorption – hence not offering a fully accurate human gut representation of chemical and physiological conditions – it does provide an inexpensive and reliable tool for modelling colon microbial ecology and activity. Consequently, it has become a standard reference in the field and the basis to all multi-stage continuous culture models. Recently, this model was used to validate health benefits from a commercial wheat dextrin soluble fibre, which have shown significant butyrogenic effect on gut microbiota ecology (Hobden et al., 2013). It has been also used to evaluate the prebiotic activity of orange juice supplemented with a GOS mixture with and without Bimuno (B-GOS). The combination of orange juice and B-GOS resulted in an increase of *Roseburia* subcluster and consequently, butyrate production increased, which have been associated with many benefits to host health (Costabile et al., 2015).

3.3.2. Simulator of the human intestinal microbial ecosystem

The Simulator of the Human Intestinal Microbial Ecosystem (SHIME) is one of the few gut models that mimics the entire GI tract. It consists of five double-jacketed glass vessels that are connected through peristaltic pumps, simulating stomach, small intestine and three colon regions – ascending (pH = 5.6–5.9), transverse (pH = 6.15–6.4) and descending (pH = 6.6–6.9) (Molly et al., 1993). The first two reactors mimic the enzymatic and physicochemical environment by controlling pH, residence time, and culture medium volume, including enzymes and bile salts (Molly, Woestyne, Smet, & Verstraete, 1994). These two reactors have a fill-and-draw system with a dialysis filter used to simulate absorptive processes occurring in the stomach and small intestine (Vermeiren et al., 2011). Upon digestion on the gastric and intestinal compartments, the slurry is pumped into the ascending colon vessel, where fermentation is initiated. The last three-stage reactors, which simulate the large intestine, are continuously stirred vessels inoculated with fresh faecal samples, corresponding to the *in vivo* conditions in terms of metabolic activity and community composition based on the aforementioned Gibson et al. (1988) model. The vessels are held at 37 °C and the entire SHIME system is kept under anaerobic conditions by daily flushing the respective compartments headspace with N_2 or a 90/10% N_2/CO_2 gas mixture (Van de Wiele, Van den Abbeele, Ossieur, Possemiers, & Marzorati, 2015). The digestive slurry is mixed with magnetic stir bars. Since the stirring is magnetic, no peristalsis mimicking mechanisms are available in the SHIME, which represents a potential limitation of this specific approach. Three times per day, 140 mL of nutritional medium and 60 mL of pancreatic juice are added to the stomach and small intestine compartments, respectively (Molly et al., 1993). The operating volumes and respective residence times for each colon vessels are: ascending 500 mL and 20 h; transverse 800 mL and 32 h; and descending 600 mL and 24 h (Williams et al., 2015).

A typical SHIME experiment consists of four stages: 1) a stabilization period (2 weeks) to allow microbial community adaptation to the environmental conditions of each colon zone; 2) a basal period (2 weeks), operating under nominal conditions to measure baseline parameters; 3) a treatment period (2–4 weeks) to test the effect of a specific treatment on the GI microbial community; and 4) a washout period (2 weeks) to determine how long the changes induced by the tested substance remain after finishing the treatment (Van de Wiele et al., 2015). This approach has been applied to investigate probiotics and prebiotics

activity and stability along the GI tract; microbial conversion of bioactive food components; metabolism of pharmaceutical compounds; efficacy of colonic targeted delivery systems; and conversion and biological (in)activation of food and/or ingested environmental contaminants (Van de Wiele et al., 2015). The SHIME model has been used to study the effects of fructooligosaccharides (FOS) on the microbiota fermentation pattern (Sivieri et al., 2014), highlighting not only its bifidogenic effect but also the increase of SCFA production. Recently, the SHIME model was used to compare the bifidogenic effects of goat and cow milk-based infant formulas of human breast milk on 3-month-old infants (Gallier, Van den Abbeele, & Prosser, 2020). Results showed that the naturally present oligosaccharides stimulate gut microbial activity and community composition in a way comparable to human milk, despite the absence of specific supplementation with human milk oligosaccharides (HMOs).

To achieve a representative mucosal environment, like that observed in the human colon, a mucous compartment was incorporated in the SHIME, containing hundreds of mucin-covered microcosms (M-SHIME) (Van den Abbeele et al., 2012). These microcosms are cylindrical high-density polyethylene ring shaped carriers (length: 7 mm; diameter: 9 mm; total surface area: 800 m^2 m^{-3}). The microcosms are coated by submersion into mucin agar. The M-SHIME has been used under the same operation parameters as the ones used in the SHIME, in experiments run up to 28 days. Results shown that probiotic bacteria - *L. rhamnosus GG* (LGG) *and L. mucosae* - selectively colonize the microcosms (Van den Abbeele et al., 2012). The M-SHIME was used also to study the benefits associated with the intake of a commercial probiotic formulation (MegaDuo™) containing *Bacillus coagulans* SC208 and *Bacillus subtilis* HU58 (Marzorati et al., 2020). This approach is relevant for long-term studies comprising microbial colonization of the mucus layer, being particularly useful for demonstrating prebiotic, probiotic, and antibiotic effects.

3.3.3. EnteroMix

The EnteroMix model is a modified, semi-continuous culture colon simulator with four parallel units (Mäkivuokko, Saarinen, Ouwehand, & Rautonen, 2006). Each unit comprises four glass vessels representing the ascending, transverse, descending, and sigmoid-colon/rectum, working with small volumes (3, 5, 7, and 9 mL, respectively) and controlled pH (5.0, 6.0, 6.5, and 7.0, respectively). The simulator unit is kept in a thermostatic room at 37 °C and anoxic N_2 is gassed in all vessels to keep anaerobic conditions. Three hours after inoculation of the faecal inoculum the test substance is pumped to the first vessel - 3 mL of fresh medium with (three test channels) or without (one control channel). The content is fermented for 3 h before being transferred to the second vessel with simultaneous addition of 3 mL fresh medium into the first vessel. The same process is repeated for the third and fourth vessel, the system setup is completed within 15 h post-inoculation. The fermentation lasts for 48 h with substrate feeding every 3 h, so that the microbiota can adapt to the substrate, and bacteria profile and its metabolites can be analysed (Mäkivuokko et al., 2006).

The EnteroMix model was designed specifically to study the carbohydrate fermentation by colonic microbiota (Mäkeläinen et al., 2010; Mäkeläinen, Mäkivuokko, Salminen, Rautonen, & Ouwehand, 2007; Mäkivuokko et al., 2006). An advantage of this model is the ability to run four parallel experiments using the same faecal inoculum. However, only short-term experiments can be performed due to the small working volumes and semi-continuous nature of the system, thus reducing correlation with *in vivo* studies (Williams et al., 2015). Among the food-related applications of the EnteroMix, the comparison between prebiotic effect of FOS and XOS is of the utmost relevance (Mäkeläinen et al., 2010). The EnteroMix has shown that *B. lactis* and XOS mixture form a successful, symbiotic combination. More recently, the EnteroMix was used to demonstrate the positive effects of HMOs (Salli et al., 2019).

3.3.4. Computer-controlled dynamic simulator of the gastro-intestinal tract

The computer-controlled dynamic SIMulator of the GastroIntestinal tract (SIMGI) is a fully automated GI multi-chamber simulator. It comprises five units, simulating the stomach, small intestine and three stages of the large intestine (ascending, transverse and descending), which are interconnected by pipes and peristaltic valve pumps that transfer the content between the successive units (Barroso, Cueva, Peláez, Martínez-Cuesta, & Requena, 2015a, 2015b). The stomach unit is comprised of two transparent and rigid methacrylate plastic modules covering a reservoir of flexible silicone walls where the gastric content is mixed by peristaltic movements. The gastric peristalsis is achieved by pumping thermostatized water in the jacket between the plastic modules and the flexible reservoir, which keeps gastric temperature content at 37 °C (Barroso, Cueva, Peláez, Martínez-Cuesta, & Requena, 2015b). The stomach compartment has different ports for input of experimental food components, gastric juice, and HCl. The meal received by the stomach compartment is mixed with gastric electrolytes and enzymes. The decrease of pH is regulated by computer-controlled addition of 0.5 M HCl to follow the curve resulting from a linear fit of data representing experimental *in vivo* conditions (Barroso et al., 2015b).

The small intestine consists of a double jacket glass reactor vessel, magnetic stirred at 150 rpm that receives the gastric content which is mixed with pancreatic juice and bile. The intestinal content is digested during 2 h at 37 °C and kept at pH 6.8 (Barroso et al., 2015b).

Lastly, large intestine stages are simulated in three double jacket glass reactors and the colon content is kept at 37 °C by pumping water into the space between the glass jackets. The glass reactors are inoculated with faecal samples, filled and pre-conditioned with the nutritive medium that will fed the system (*i.e.* 250, 400 and 300 mL for the ascending, transverse and descending colon compartments, respectively). The pH is controlled by addition of 0.5 M NaOH and/or 0.5 M HCl to maintain pH values at 5.6 ± 0.2 in the ascending, 6.3 ± 0.2 in the transverse and 6.8 ± 0.2 in the descending compartments. The colonic volume units and the transit of colonic content between compartments, that occurs three times a day at a flow rate of 5 mL/min, are intended to give an overall residence time of 76 h. The colon microbiota stabilization process in the three colonic units is usually reached after 14 days under the aforementioned conditions (Barroso et al., 2015a, 2015b).

The SIMGI is designed to operates with five units simulating the dynamics of whole GI process. In addition, the SIMGI software allows the operation of the stomach and the small intestine modules in a continuous mode to study food digestion together with the microbial community development in the colonic vessels. The system is flexible and can be adapted according to the envisaged experimental approach (Barroso et al., 2015a). SIMGI has been extensively used in the last few years in multiple food-related applications to assess digestibility and prebiotic effect, *e.g.* citrus pectin (Ferreira-Lazarte, Moreno, Cueva, Gil-Sánchez, & Villamiel, 2019) and chia seed mucilage (Tamargo, Cueva, Laguna, Moreno-Arribas, & Muñoz, 2018). Besides serving as a platform for functional food evaluation, SIMGI-related research has been revealing its use as an *in vitro* tool to evaluate silver nanoparticles digestion (Cueva et al., 2019), prior to human studies, and therefore, minimizing animal testing.

3.4. Microbiome-host interaction models

Human colon microbiome plays a major role in the health-disease process as a result of interactions between microbiome, intestinal epithelium and immune system (Proctor et al., 2019). It has been a challenge to study microbiome-host interaction since: 1) it is not possible to reach microbiota specific sites at different digestion times in human trials, which unable control of all variables required for elucidating mechanistic and testing hypothesis (Kostic, Howitt, & Garrett, 2013); 2) despite animal models advances (*e.g.* using mice microbiome), they are still not reliable to represent the human physiology and microbiome due to differences on biological organization and structure (Kostic et al., 2013); and 3) human and animal

studies involve major ethical issues, are time consuming and costly (Wilmes, Calatayud, & Van de Wiele, 2018). On the other hand, *in vitro* models have significantly evolved in the last decade, from simple static to complex dynamic systems (Berni, Chitchumroonchokchai, Canniatti-Brazaca, De Moura, & Failla, 2015; Kortman et al., 2016; Silva et al., 2018). Reliable and convenient microbiome-host simulators are critical for research advances in this field. However, at present, no available system offers a holistic approach comprising peristalsis simulation-microbiome-enterocytes or micro biome-epithelium-immune system.

The main challenge to be addressed by microbiome-host models is how to emulate all the interactions features on a single model, including physiological biochemistry (*e.g.* digestive enzymes and anaerobic/aerobic microenvironment), biological organization (*e.g. villi*, microbial adhesion and epithelial barrier) and physical forces (*e.g.* peristalsis and shear stress) (May et al., 2017). Each feature requires the establishment of detailed and precise models that need to be integrated in an overall setup. For example, it is necessary to confirm the cell monolayers integrity and barrier formation, usually measured by transepithelial electrical resistance (TEER) and microscopy methods that assess tight junctions and epithelium morphology (Shah et al., 2016). Also, it is important to establish how long cellular cultures survive with bacteria while running the system (May et al., 2017) and to test whether the intestinal barrier permeability is at physiologic level (Kim & Ingber, 2013). Finally, it is of utmost importance to choose relevant biomarkers to assess if the involved biochemistry is as close as possible to human physiology (Eain et al., 2017).

At the moment, four microfluidics-based microbiome-host interface models have been described: the pioneer Gut-on-a-Chip (Kim, Huh, Hamilton, & Ingber, 2012); the HuMiX (Shah, Fritz, Estes, Zenhausern, & Wilmes, 2014); the HMI™ module for the SHIME (Marzorati et al., 2014); and the MOTiF (multi-organ-tissue-flow) intestine-on-chip (Maurer et al., 2019). Their structures, operational parameters, cellular and microbial cultures are presented in Table 2 and discussed in the next sub-sections.

3.4.1. *Microfluidic-based microbiome-host models: design, operational parameters and co-cultures*

Microfluidics and bioengineering have been combined to mimic the human intestinal functions in a small-scale fluid flow system, similar to a chip. Current models evolved from static co-cultures cultivated on Transwell® (Bein et al., 2018) to co-cultures in continuous flow similar to *in vivo* physiologic shear-stress and eventually, under mechanic forces (peristalsis) (Kim et al., 2012). These models need to define the intestinal part and/or health/disease state to be modelled since the intestinal epithelium morphology and microenvironment may be quite different on various intestinal sections (Donaldson, Lee, & Mazmanian, 2015) and under specific health/disease conditions (von Martels et al., 2017). Microbiome-host models need at least two compartments simulating microaerophilic intestinal lumen environment and aerobic conditions to human enterocytes, *i.e.* host's colon epithelium, growth and differentiation (May et al., 2017). It is also required a barrier where metabolites, biomarkers, oxygen, nutrients, and other compounds of interest can

Table 2
Overview of microbiome-host interaction models' structure, operational parameters, cellular and microbial cultures.

Gut-on-a-Chip (Kim et al., 2012, 2015, 2016)	**Human cell types**	Enterocytes (Caco-2), capillary endothelial, lymphatic microvascular endothelial, immune system (monocytes, lymphocytes and granulocytes)
	Microbial cultures	*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Escherichia coli* (GFP-EC and EIEC), *Bifidobacterium adolescentis* and *Eubacterium hallii*
	Media flowing in the system	DMEM, 20% FBS, 1% antibiotics – for co-culture is used antibiotics free media, for anaerobic conditions is used anoxic media. Flow: 30 μL h^{-1}
	Device structure	Upper and lower microchannels (1 mm width × 10 mm length × 0.15 mm height) confectioned in PDMS polymer, bilateral vacuum chambers (1.68 mm × 9.09 mm × 0.15 mm)
	Barrier and permeability	30 μm thick PDMS membrane containing 10 μm diameter circular pores with 25 μm spacing; apparent permeability was ≈4 × 10^{-8} cm s^{-1} at 30 μL h^{-1} flow and 4 times higher under strain (peristalsis)
	Peristalsis	0.02 dyne cm^2 shear stress by flow, mechanical deformations (10% in cell strain, 0.15 Hz in frequency) by vacuum pumps (≈15 kPa of suction pressure)
HuMiX (Shah et al., 2016)	**Human cell types**	Epithelial colorectal cells (Caco-2 and CCD-18Co) and immune cells (CD4+T)
	Microbiota	*Lactobacillus rhamnosus GG* and *Bacteroides caccae*
	Media flowing in the system	DMEM, 20% FBS, 1% antibiotics – for co-culture is used antibiotics free media, for anaerobic conditions is used anoxic media; Flow: 25 μL min^{-1}
	Device structure	Three spiral channels (4 mm width × 200 mm length × 0.5 mm height; surface area ~8 cm^2 each channel) confectioned in silicon rubber gasket (65 mm × 65 mm), covered by two sides of polycarbonate enclosure
	Barrier and permeability	Polycarbonate membranes, 50 nm pore sizes among microbial and epithelial chambers, coated with mucin, and 1 μm pore size among epithelial and perfusion chambers, coated with collagen. Permeability from microbial microchamber to the perfusion microchamber was 5.41 × 10^{-6} cm s^{-1}
	Peristalsis	There is no peristalsis system beyond the shear stress caused by the flow
HMI™ (Marzorati et al., 2016)	**Human cell types**	Epithelial colorectal cells (Caco-2)
	Microbiota	*Lactobacillus rhamnosus GG, Saccharomyces cerevisiae* and complex microbial community from human fecal sample
	Media flowing in the system	DMEM, 10% FBS, 1% NEAA, 2% antibiotics and fungizone; for experiment was used media free of antibiotics and fungizone in the lower compartment. Upper compartment was feed with ascendant colon stage of SHIME. Flow: 6.5 mL min^{-1} upper compartment, 2 mL min^{-1} in the lower compartment
	Device structure	Module connected to SHIME, two compartments (60 mm width × 100 mm length), area for culture was a glass cover ~ 25 mm wide x 75 mm long[a]
	Barrier and permeability	Polyamide membrane (0.2 μm pore size, 115 μm of thickness) with a mucus layer (mucin + agar, 200–250 μm of thickness), permeability ranging 2.4 × 10^{-6} to 7.1 × 10^{-9} cm s^{-1}
	Peristalsis	3 dyne cm^2 shear stress by flow. There is no additional peristalsis system
MOTiF intestine-on-chip (Maurer et al., 2019)	**Human cell types**	Human umbilical cord vein endothelial cells (HUVECs), epithelial colorectal cells (Caco-2), peripheral blood mononuclear cells and primary macrophages (PBMCs)
	Microbiota	*Lactobacillus rhamnosus GG* and *Candida albicans*
	Media flowing in the system	The endothelial layer was cyclically perfused with a flow rate of 50 μl min^{-1} while the luminal chamber was linearly perfused with 25 μl min^{-1}
	Device structure	Upper and lower chambers with 700 and 400 μm height, respectively, feed by two microchannel. The available area for cell culture was 1.1 cm^2. Constructed in polystyrol
	Barrier and permeability	12 μm thin polyethylene terephthalate (PET) membrane (8 μm pore size). Endothelial and Caco-2 cells were cultured in the opposite sides of the same membrane. Permeability ≈5 μg mL^{-1} of FITC dextran after 30 min perfusion
	Peristalsis	0.07 Pa of shear stress at endothelial side and 0.03 Pa at epithelial side. There is no additional peristalsis system

[a] Approximation based on Marzorati et al., 2016, since specific details are not reported.

permeate (Wilmes et al., 2018).

The Gut-on-a-Chip (Kim et al., 2012) and the HMI™ (Marzorati et al., 2014) are both structured as two channels aligned on top of each other, while the HuMiX (Shah et al., 2016) have three channels aligned in spiral format. Regarding the MOTiF intestine-on-chip structure, epithelial cells are grown on one side of the membrane and bacteria on the top chamber, while endothelial cells are cultured on the opposite side of the membrane with immune cells in the bottom chamber making it a 4-stage system (Maurer et al., 2019). Although these devices seek to model the microbiome-host interaction, each structural design provides a different approach. The Gut-on-a-Chip has the narrower channel (0.15 mm high x 1 mm wide) and therefore, the smaller culture area, followed by the MOTiF intestine-on-chip model (1.1 cm^2 culture area) (Table 2). These narrow channels may be a limitation for applications including complex microbiome-host experiments. For example, many compounds firstly need to be metabolized by microbial species in a cross-feeding mode (Marzorati et al., 2014), and many prebiotics, like soluble fibres, increase faeces viscosity causing channel clogging (McRorie & McKeown, 2017). Moreover, localized bacteria overgrowth induced epithelial cells death within 48 h, despite being counterbalanced by continuous media flow and peristalsis-like motions (Kim et al., 2012). Eain et al. (2017) claimed that the Nutri-Humix system works for extended periods of time with bacteria and host cells co-cultures. However, only two probiotic strains, LGG and *Bacteroides caccae* in media supplemented with a soy prebiotic were used and co-cultured with human Caco-2 cells in the epithelial chamber for 24 h (Shah et al., 2016). On the other hand, the three membrane-separated channels of the HuMiX succeed to create a microbial-immune system interaction, when co-culturing anaerobic LGG, epithelial colorectal cells (Caco-2 and CCD-18Co) and immune CD4+T cells (Shah et al., 2016). Thus, the modular three channels design is particularly interesting, due to its flexibility and dynamics, for simulation of different health/disease conditions.

The HMI™ model has a completely different approach - it was designed to work as an external module coupled to the SHIME. It introduces one more step in the system: indirect assessment of the microbiome-host epithelium interaction. The HMI™ module provides the possibility to work in parallel with control samples, or with more than one treatment at same time (Marzorati et al., 2014). Furthermore, the HMI™ could be adapted to other models similar to the SHIME. This device presents superior surface area for cellular and bacteria cultures growth requiring higher media volumes; hence, it works at higher shear stress level compared to the Gut-on-a-Chip and the HuMiX models (Table 2). The great difference is that the top chamber is fed by complex microbiota from the SHIME. It has a membrane covered with artificial mucus layer, similar to the human intestinal mucus, where bacteria adhere and grow. Caco-2 cells are cultured on the lower chamber until monolayers are differentiated. This model does not have a basal side, *i.e.* the HMI™ only simulates the epithelium's surface (Marzorati et al., 2014).

Caco-2 cells are largely used to simulate intestinal epithelium (Jochems, Garssen, Van Keulen, Masereeuw, & Jeurink, 2018) in several models for studying nutrient absorption and metabolism (Berni et al., 2015) and bacteria-host interactions (Barnett, Roy, Cookson, & McNabb, 2018), which is the case of the four microfluidic-based approaches presented previously (Table 2). However, there are two main limitations in culturing Caco-2 cells in human gut models despite their extended use in several scientific fields. Caco-2 are undifferentiated cells that spontaneously differentiate in small intestine phenotype (Jochems et al., 2018). Differentiation can be accelerated by continuous media flow, mechanical peristalsis-like deformation and shear stress from the Gut-on-a-Chip device – presenting *villi*, crypt and mucin-2 in shorter culture times (Kim & Ingber, 2013). However, there is no *villi* occurring at the colon section of the human intestine (May et al., 2017). It is worth pointing out that the gut microbiota is mainly located in the colon (Labarthe et al., 2019). Thereby, the colon microbiome-enterocytes-like Caco-2 cells crosstalk has to be analysed with caution when extrapolating *in vitro* results to the context of human subjects. Additionally, the Caco-2 cell is a colon cancer-derived cell line; thus, these cells can behave differently from normal epithelial cells (May et al., 2017). For example, Caco-2 cells can overexpress peptide transporters or even express transporters that do not occur in the human intestine (Jochems et al., 2018). Thus, Caco-2 cells may not be the most suitable cell to be used on these models but, for the moment are still the most feasible.

Regarding flow and peristalsis simulation in microfluidic devices, it is fundamental to recall that these physical forces inside human colon will drive not only the microbiota location (*i.e.* mainly in the descending colon due to faeces straightforward motility and increasing viscosity) but also their inner ecology, prevalence, biogeographic distribution, bacterial motility *vs* mucus adhesion, and microbiome-host interaction (Donaldson et al., 2015; Labarthe et al., 2019). Some of these forces have already been partially simulated (Table 2). For example, mechanical deformation was performed by vacuum pumps mimicking peristalsis motion (Kim et al., 2012). Media flow has been precisely controlled reaching shear stresses similar to the human intestine epithelium surface (Kim et al., 2012; Marzorati et al., 2014). The artificial mucus layer, placed in microfluidic devices, allowed selective adhesion of bacteria (Marzorati et al., 2014). However, some complex events and interactions were not yet implemented, like bacteria chemotaxis that is dependent on mucosal morphology, faeces viscosity and differential carbon source along the gut.

The probiotic effect of LGG has been tested in the Gut-on-a-Chip model (Kim et al., 2012). Main results shown that LGG co-cultured in the lumen of the intestinal epithelial channel increased the intestinal barrier function. In another study, the VSL#3® probiotic formulation (*i.e.* a mix of 8 strains of lactic acid–producing bacteria) suppressed villus blunting and loss of barrier function induced by pathogenic *E. coli* (Kim, Li, Collins, & Ingber, 2016).

Controlling the peristalsis and flow of microfluidic devices has additional and important effects, such as cellular morphogenesis modulation (Shin, Hinojosa, Ingber, & Kim, 2019), and selective adhesion and growth regulation of the bacterial community (Kim et al., 2016). Moreover, when continuous flow media are applied to Caco-2 cells using the Gut-on-a-Chip device, they exhibit a robust 3D small intestine morphology presenting differentiated absorptive, goblet, enteroendocrine, and paneth cells localized in basal crypts (Kim & Ingber, 2013). Also, high media flow increased Caco-2 cells height and polarization and accelerated epithelial monolayers development usually from 21 to 3 days, generating a specialized apical brush border, augmented barrier function, and mucus production (Kim & Ingber, 2013). It has been shown that the transepithelial Wnt antagonist Dickkopf-1 gradient and flow-induced Frizzled-9 receptor regulation mediate this morphogenesis (Shin et al., 2019). It is important to highlight that this morphology is typical from small intestine and cannot be applied for colonic microbiota-host studies. Therefore, it is essential to induce cellular culture differentiation to colon-like morphology to study colon microbiome-host interactions.

4. *In vitro* models' as a tool to predict host-microbiota response to prebiotics and probiotics

The use of probiotics and prebiotics could be an affordable strategy for microbiota modulation, and it represents a promising option for human health promotion (Valdés et al., 2016). Probiotics are non-pathogenic bacteria or yeasts that have an impact on host health or physiology. The probiotics are known to be implicated in intestinal defence against pathogens, improving intestine epithelial layer and enhancing the immune response (Cordonnier et al., 2015). The main mechanism of action comprises epithelial cells stimulus and dendritic cells by toll-like receptors (TLRs) activation which produce cytokines (Rajput & Li, 2012). Probiotics immunomodulatory effects are ascribed to cytokines release, including interleukins (ILs), tumour necrosis factors

(TNFs), interferons (IFNs), transforming growth factor (TGF), and chemokines from immune cells, *i.e.* lymphocytes, granulocytes, macrophages, mast cells, epithelial cells, and dendritic cells (DCs), that control the innate and adaptive immune system (Azad et al., 2018). On the other hand, prebiotics such as inulin, FOS and GOS, which are mainly obtained from plants by direct extraction, natural polysaccharides hydrolysis or enzymatic synthesis, have the capacity to increase probiotic population and/or activities in the human large intestine (de la Rosa et al., 2019; Nobre, Gonçalves, Teixeira, & Rodrigues, 2018). Several positive effects of prebiotics on numerous diseases are already well established, *e.g.* diabetes, Alzheimer's disease, obesity, cancer, irritable bowel disease, osteoporosis, mood alterations, cardiovascular and immune functions (Iramaia Angelica Neri-Numa, 2020; Nobre, Cerqueira, Rodrigues, Vicente, & Teixeira, 2015). However, due to the arrival of new prebiotic candidates, especially non-digestible oligosaccharides, it is fundamental to understand their main mechanisms of action (Gibson et al., 2017).

In vitro models are an excellent approach to assess host-microbiota interaction with probiotics and prebiotics. However, most of the research studies report the use of less complex *in vitro* colon models (*i.e. in vitro* fermentation systems without immobilized human cells, such as TIM-2 model) compared to multipart, continuous *in vitro* host-microbiota models (*e.g.* the Gut-on-a-Chip and the HuMiX). Some examples of *in vitro* fermentation models and cell culture models, used independently, to study the effect of probiotics and prebiotics were reported. For instance, an *in vitro* wheat aleurone fermentation (using a batch culture model) by faecal bacteria combined with probiotic strains of LGG and *Bifidobacterium lactis* Bb12 was evaluated to identify the effects of aleurone on HT-29 and LT97 human colon cell lines (Borowicki et al., 2010). In this study, the generated fermentation supernatants were applied to cell cultures. The cellular growth, apoptosis, and differentiation, as well as the expression of genes involved in pathways of cell cycle regulation (*p21*), apoptosis (*DR5*), or both events (*WNT2B*) was investigated (Borowicki et al., 2010). It was observed a considerable increase in apoptosis and an up-regulation of genes involved in cell growth and apoptosis (*i.e. p21* and *WNT2B,* respectively) when treated with all fermentation metabolites. In another study, a single bioreactor system was used to evaluate the survival, recovery, and dynamics of multi-strain probiotics composed of bifidobacteria and lactobacilli combined with mixed prebiotics (GOS, FOS, XOS or soluble starch) under GI tract changing environmental conditions (Adamberg et al., 2014). The authors observed that *Bifidobacterium breve* 46, *Lactobacillus plantarum* F44, and *Lactobacillus paracasei* F8 were capable to grow synergistically in mixed culture and they were the most resistant strains under acid and bile exposure conditions. Other authors studied the effect of the daily administration of *Kluyveromyces marxianus* B0399 on the human intestinal microbiota composition and metabolic activity was investigated in a 3-stage continuous-culture system human colon simulator (Maccaferri, Klinder, Brigidi, Cavina, & Costabile, 2012). The effects of the lactic yeast *K. marxianus* B0399 on adhesion and immune mediator's production (*i.e.* cytokines, chemokines and growth factors) on Caco-2 cells and peripheral blood mononuclear cells (PBMCs) were also studied. The results showed an increase of bifidobacteria concentration in the colonic model system when in contact with *K. marxianus* B0399, together with an increase in the SCFA acetate and propionate concentrations. *K. marxianus* adhesion on Caco-2 cells increased and *K. marxianus* B0399 provoked an increase in the levels of production of proinflammatory cytokines in PBMCs.

The *in vitro* models effectiveness on the study of metabolic and immunological responses induced by host-probiotic interactions and prebiotic fermentation, depends on *in vitro* cultures of human intestinal epithelial cell lines jointly with complex bacterial communities (Marzorati et al., 2011). These models can offer valuable data on host responses to colon fermentation, bacteria adhesion and immune modulatory activity of prebiotics and probiotics (Moon, Li, Bang, & Han, 2016). Although, research studies are limited in this field, some have assessed the host response factors through the combination of *in vitro* colon microbiota cultures and human intestinal cell models (*i.e.* microbiome-host interaction models). For example, Arboleya et al. (2015) examined the immune response modulation by two probiotic Bifidobacterium species (*Bifidobacterium bifidum* IPLA 20015 and *Bifidobacterium breve* IPLA 20005) in a single-stage continuous-culture system inoculated with infant faeces combined with HT-29 epithelial cells. This study showed that *B. breve* promoted higher levels of cytokine formation by HT-29 cells than *B. bifidum*. The authors considered that fermentation combined with HT-29 cells could be a tool to probiotic potential screening of various bacterial species (Arboleya et al., 2015). On another study, the effects of prebiotics arabinogalactan (AG) and FOS were assessed in the SHIME, inoculated with faecal material from a patient with inflammatory bowel disease, coupled with co-cultures of Caco-2 cells and macrophages (THP1) (Daguet, Pinheiro, Verhelst, Possemiers, & Marzorati, 2016). FOS and AG showed a different fermentation profile in the proximal and distal colon, respectively, and both prebiotics had a positive effect on gut barrier and inflammation. Moreover, AG results showed a significantly higher TEER of Caco-2 cells monolayers, AG decreased the nuclear factor (NF)-κB activity, and increased the IL-10 production. Recently, Greenhalgh et al. (2019) used the HuMiX model to assess metabolic and immune responses in human colorectal cancer cells (Caco-2) after co-culturing with a probiotic (LGG) under a simulated high-fibre diet (prebiotic regimen). These authors demonstrated that this symbiotic regimen led to a downregulation of Caco-2-associated signalling pathways and oncogenes expression, and attenuated Caco-2 self-renewal ability.

As can be seen from the research outcomes mentioned above, combined and continuous *in vitro* host-microbiota models are a viable platform for evaluating host-microbiota responses to prebiotic and probiotic fermentation processes.

5. Conclusions

Current existing models can be distinguished considering two main approaches: 1) the continuous bioreactors targeted to complex human microbiota cultivation; and 2) microfluidic devices operating under continuous medium flow and co-culture of representative bacteria and human cells. Existing models have been designed according to questions intended to address, *e.g.* assess the biochemical profile of diet-microbiota relationship or the biological profile of microbiota-host interface. Although these models present different degrees of complexity, none of them is able to simultaneously cover all the key conditions found within the human colon. Thus, there is still a significant opportunity to further improve the experimental *in vitro* setups accuracy and realism. New setups are required to provide comprehensive simulation of anatomical, physical, biochemical and biological conditions found within the human gut to better study its microbiota. The development of sequential modules that simulate all microbiome-host interactions levels – *i.e.* food digestion, microbiota fermentation, biochemical cross-talk, intestine cells behaviour and host response – is of utmost importance.

New solutions that cover the drawbacks mentioned are needed to bring new insights into the interplay between gut microbiota and host health. These novel approaches will improve the development of new functional foods and nutraceuticals, introduce new therapeutic strategies for colon diseases, and help to consolidate the pivotal role of microbiome on human health.

CRediT authorship contribution statement

Dalila Roupar: is responsible for writing the chapters 1 and 3, as well as collecting the data for, table 1 and Fig. 2, Writing – original draft, Data curation. **Paulo Berni:** is responsible for writing chapters 2 and 3 and collecting the data for table 2, Writing – original draft. **Joana T. Martins:** is responsible for writing chapter 4, Writing – original draft.

Ana C. Caetano: is responsible for writing chapter 2 and drawing Fig. 1, Writing – original draft. **José A. Teixeira:** is responsible for revising the manuscript, Supervision, and, Funding acquisition, Writing – review & editing. **Clarisse Nobre:** is responsible for writing chapters 3 and 4, revising and editing the manuscript, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgment

Dalila Roupar acknowledges the grant SFRH/BD/139884/2018 from the Portuguese Foundation for Science and Technology (FCT). This study was supported by the FCT under the scope of the strategic funding of UIDB/04469/2020 unit and the Project ColOsH PTDC/BTM–SAL/30071/2017 (POCI–01–0145–FEDER–030071).

References

Adamberg, S., Sumeri, I., Uusna, R., Ambalam, P., Kondepudi, K. K., Adamberg, K., … Ljungh, A. (2014). Survival and synergistic growth of mixed cultures of *Bifidobacteria* and *Lactobacilli* combined with prebiotic oligosaccharides in a gastrointestinal tract simulator. *Microbial Ecology in Health and Disease, 25*, 1–9. https://doi.org/10.3402/mehd.v25.23062

Arboleya, S., Bahrami, B., Macfarlane, S., Gueimonde, M., Macfarlane, G. T., & de los Reyes-Gavilán, C. G. (2015). Production of immune response mediators by HT-29 intestinal cell-lines in the presence of *Bifidobacterium*-treated infant microbiota. *Beneficial Microbes, 6*(4), 543–552. https://doi.org/10.3920/bm2014.0111

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., … Bork, P. (2011). Enterotypes of the human gut microbiome. *Nature, 473*(7346), 174–180. https://doi.org/10.1038/nature09944

Auchtung, J. M., Robinson, C. D., & Britton, R. A. (2015). Cultivation of stable, reproducible microbial communities from different fecal donors using minibioreactor arrays (MBRAs). *Microbiome, 3*(1), 1–15. https://doi.org/10.1186/s40168-015-0106-5

Azad, M. A. K., Sarker, M., & Wan, D. (2018). Immunomodulatory effects of probiotics on cytokine profiles. *BioMed Research International*, 1–10. https://doi.org/10.1155/2018/8063647, 2018.

Bach Knudsen, K. E. (2015). Microbial degradation of whole-grain complex carbohydrates and impact on short-chain fatty acids and health. *Advances in Nutrition, 6*(2), 206–213. https://doi.org/10.3945/an.114.007450

Bajury, D. M., Nashri, S. M., King Jie Hung, P., & Sarbini, S. R. (2018). Evaluation of potential prebiotics: A review. *Food Reviews International, 34*(7), 639–664. https://doi.org/10.1080/87559129.2017.1373287

Barnett, A. M., Roy, N. C., Cookson, A. L., & McNabb, W. C. (2018). Metabolism of caprine milk carbohydrates by probiotic bacteria and Caco-2:HT29–MTX epithelial co-cultures and their impact on intestinal barrier integrity. *Nutrients, 10*(7), 1–21. https://doi.org/10.3390/nu10070949

Barroso, E., Cueva, C., Peláez, C., Martínez-Cuesta, M. C., & Requena, T. (2015a). The computer-controlled multicompartmental dynamic model of the gastrointestinal system SIMGI. In K. Verhoeckx, et al. (Eds.), *The impact of food bioactives on health: In vitro and ex vivo models* (pp. 319–327). Springer Open.

Barroso, E., Cueva, C., Peláez, C., Martínez-Cuesta, M. C., & Requena, T. (2015b). Development of human colonic microbiota in the computer-controlled dynamic SIMulator of the GastroIntestinal tract SIMGI. *LWT - Food Science and Technology, 61*(2), 283–289. https://doi.org/10.1016/j.lwt.2014.12.014

Bein, A., Shin, W., Jalili-Firoozinezhad, S., Park, M. H., Sontheimer-Phelps, A., Tovaglieri, A., … Ingber, D. E. (2018). Microfluidic organ-on-a-chip models of human intestine. *Cellular and Molecular Gastroenterology and Hepatology, 5*(4), 659–668. https://doi.org/10.1016/j.jcmgh.2017.12.010

Berner, A. Z., Fuentes, S., Dostal, A., Payne, A. N., Gutierrez, P. V., Chassard, C., … Lacroix, C. (2013). Novel polyfermentor intestinal model (PolyFermS) for controlled ecological studies: Validation and effect of pH. *PloS One, 8*(10), Article e77772. https://doi.org/10.1371/journal.pone.0077772

Berni, P., Chitchumroonchokchai, C., Canniatti-Brazaca, S. G., De Moura, F. F., & Failla, M. L. (2015). Comparison of content and *in vitro* bioaccessibility of provitamin A carotenoids in home cooked and commercially processed orange fleshed sweet potato (ipomea batatas lam). *Plant Foods for Human Nutrition, 70*, 1–8. https://doi.org/10.1007/s11130-014-0458-1

Blanquet-Diot, S., Denis, S., Chalancon, S., Chaira, F., Cardot, J.-M., & Alric, M. (2012). Use of artificial digestive systems to investigate the biopharmaceutical factors influencing the survival of probiotic yeast during gastrointestinal transit in humans. *Pharmaceutical Research, 29*(6), 1444–1453. https://doi.org/10.1007/s11095-011-0620-5

Boron, W. F., & Boulpaep, E. L. (2016). *Medical physiology E-book* (3rd ed.). Elsevier Health Sciences.

Borowicki, A., Michelmann, A., Stein, K., Scharlau, D., Scheu, K., Obst, U., et al. (2010). Fermented wheat aleurone enriched with probiotic strains LGG and Bb12 modulates markers of tumor progression in human colon cells. *Nutrition and Cancer, 63*(1), 151–160. https://doi.org/10.1080/01635581.2010.516874

Boureau, H., Hartmann, L., Karjalainen, T., Rowland, I., & Wilkinson, M. H. F. (2000). Models to study colonisation and colonisation resistance. *Microbial Ecology in Health and Disease, 12*, 247–258. https://doi.org/10.1080/089106000750060503

Brugere, J.-F., Feria-Gervasio, D., Popse, Z., Tottey, W., & Alric, M. (2011). The ECSIM concept (Environmental Control System for Intestinal Microbiota) and its derivative versions to help better understand human gut biology. *Applied Biomedical Engineering, 4*, 63–82. https://doi.org/10.5772/21602

Cordonnier, C. (2015). Enterohemorrhagic *Escherichia coli* infection has donor-dependent effect on human gut microbiota and may be antagonized by probiotic yeast during interaction with Peyer's patches. *Applied Microbiology and Biotechnology, 99*(21), 9097–9110. https://doi.org/10.1007/s00253-015-6704-0

Cordonnier, C., Thévenot, J., Etienne-Mesmin, L., Denis, S., Alric, M., Livrelli, V., et al. (2015). Dynamic *in vitro* models of the human gastrointestinal tract as relevant tools to assess the survival of probiotic strains and their interactions with gut microbiota. *Microorganisms, 3*(4), 725–745. https://doi.org/10.3390/microorganisms3040725

Correa-Betanzo, J., Allen-Vercoe, E., McDonald, J., Schroeter, K., Corredig, M., & Paliyath, G. (2014). Stability and biological activity of wild blueberry *(Vaccinium angustifolium)* polyphenols during simulated *in vitro* gastrointestinal digestion. *Food Chemistry, 165*, 522–531. https://doi.org/10.1016/j.foodchem.2014.05.135

Costabile, A., Walton, G. E., Tzortzis, G., Vulevic, J., Charalampopoulos, D., & Gibson, G. R. (2015). Effects of orange juice formulation on prebiotic functionality using an *in vitro* colonic model system. *PloS One, 10*(3), 1–12. https://doi.org/10.1371/journal.pone.0121955

Cremer, J., Arnoldini, M., & Hwa, T. (2017). Effect of water flow and chemical environment on microbiota growth and composition in the human colon. *Proceedings of the National Academy of Sciences of the United States of America, 114*(25), 6438–6443. https://doi.org/10.1073/pnas.1619598114

Cueva, C., Gil-Sánchez, I., Tamargo, A., Miralles, B., Crespo, J., Bartolomé, B., et al. (2019). Gastrointestinal digestion of food-use silver nanoparticles in the dynamic SIMulator of the GastroIntestinal tract (simgi®). Impact on human gut microbiota. *Food and Chemical Toxicology, 132*, 110657. https://doi.org/10.1016/j.fct.2019.110657

Cuevas-Tena, M., Alegria, A., Lagarda, M. J., & Venema, K. (2019). Impact of plant sterols enrichment dose on gut microbiota from lean and obese subjects using TIM-2 *in vitro* fermentation model. *Journal of Functional Foods, 54*, 164–174. https://doi.org/10.1016/J.JFF.2019.01.005

Daguet, D., Pinheiro, I., Verhelst, A., Possemiers, S., & Marzorati, M. (2016). Arabinogalactan and fructooligosaccharides improve the gut barrier function in distinct areas of the colon in the Simulator of the Human Intestinal Microbial Ecosystem. *Journal of Functional Foods, 20*, 369–379. https://doi.org/10.1016/j.jff.2015.11.005

Das, B., & Nair, G. B. (2019). Homeostasis and dysbiosis of the gut microbiome in health and disease. *Journal of Biosciences, 44*(5), 1–8. https://doi.org/10.1007/s12038-019-9926-y

Deschamps, C., Fournier, E., Uriot, O., Lajoie, F., Verdier, C., Comtet-Marre, S., … Blanquet-Diot, S. (2020). Comparative methods for fecal sample storage to preserve gut microbial structure and function in an *in vitro* model of the human colon. *Applied Microbiology and Biotechnology, 104*(23), 10233–10247. https://doi.org/10.1007/s00253-020-10959-4

Donaldson, G. P., Lee, S. M., & Mazmanian, S. K. (2015). Gut biogeography of the bacterial microbiota. *Nature Reviews Microbiology, 14*(1), 20–32. https://doi.org/10.1038/nrmicro3552

Dostal, A., Fehlbaum, S., Chassard, C., Zimmermann, M. B., & Lacroix, C. (2013). Low iron availability in continuous *in vitro* colonic fermentations induces strong dysbiosis of the child gut microbial consortium and a decrease in main metabolites. *FEMS Microbiology Ecology, 83*(1), 161–175. https://doi.org/10.1111/j.1574-6941.2012.01461.x

Drake, D. R., & Brogden, K. A. (2002). Continuous-culture chemostat systems and flowcells as methods to investigate microbial interactions. In K. A. Brogden, & J. M. Guthmiller (Eds.), *Polymicrobial diseases* (pp. 21–30). Washington (DC): ASM.

Dudefoi, W., Moniz, K., Allen-Vercoe, E., Ropers, M. H., & Walker, V. K. (2017). Impact of food grade and nano-TiO2 particles on a human intestinal community. *Food and Chemical Toxicology, 106*, 242–249. https://doi.org/10.1016/j.fct.2017.05.050

Eain, M. M. G., Baginska, J., Greenhalgh, K., Fritz, J. V., Zenhausern, F., & Wilmes, P. (2017). Engineering solutions for representative models of the gastrointestinal human-microbe interface. *Engineering, 3*(1), 60–65. https://doi.org/10.1016/J.ENG.2017.01.011

El Oufir, L., Barry, J. L., Flourié, B., Cherbut, C., Cloarec, D., Bornet, F., et al. (2000). Relationships between transit time in man and *in vitro* fermentation of dietary fiber by fecal bacteria. *European Journal of Clinical Nutrition, 54*(8), 603–609. https://doi.org/10.1038/sj.ejcn.1600687

Fehlbaum, S., Chassard, C., Haug, M. C., Fourmestraux, C., Derrien, M., & Lacroix, C. (2015). Design and investigation of PolyFermS *in vitro* continuous fermentation models inoculated with immobilized fecal microbiota mimicking the elderly colon. *PloS One, 10*(11), 1–22. https://doi.org/10.1371/journal.pone.0142793

Fehlbaum, S., Chassard, C., Schwab, C., Voolaid, M., Fourmestraux, C., Derrien, M., et al. (2019). *In vitro* study of *Lactobacillus paracasei* CNCM I-1518 in healthy and *Clostridioides difficile* colonized elderly gut microbiota. *Frontiers in Nutrition, 6*, 1–16. https://doi.org/10.3389/fnut.2019.00184

Feria-Gervasio, D., Denis, S., Alric, M., & Brugère, J. F. (2011). *In vitro* maintenance of a human proximal colon microbiota using the continuous fermentation system P-ECSIM. *Applied Microbiology and Biotechnology, 91*(5), 1425–1433. https://doi.org/10.1007/s00253-011-3462-5

Ferreira-Lazarte, A., Moreno, F. J., Cueva, C., Gil-Sánchez, I., & Villamiel, M. (2019). Behaviour of citrus pectin during its gastrointestinal digestion and fermentation in a dynamic simulator (simgi®). *Carbohydrate Polymers, 207*, 382–390. https://doi.org/10.1016/j.carbpol.2018.11.088

Forster, S. C., Kumar, N., Anonye, B. O., Almeida, A., Viciani, E., Stares, M. D., … Lawley, T. D. (2019). A human gut bacterial genome and culture collection for improved metagenomic analyses. *Nature Biotechnology, 37*(2), 186–192. https://doi.org/10.1038/s41587-018-0009-7

Gallier, S., Van den Abbeele, P., & Prosser, C. (2020). Comparison of the bifidogenic effects of goat and cow milk-based infant formulas to human breast milk in an *in vitro* gut model for 3-month-old infants. *Frontiers in Nutrition, 7*, 1–11. https://doi.org/10.3389/fnut.2020.608495

Gibson, G. R., Cummings, J. H., & Macfarlane, G. T. (1988). Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Applied and Environmental Microbiology, 54*(11), 2750–2755. https://doi.org/10.1128/aem.54.11.2750-2755.1988

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., … Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology, 14*(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75

Greenhalgh, K., Ramiro-Garcia, J., Heinken, A., Ullmann, P., Bintener, T., Pacheco, M. P., … Wilmes, P. (2019). Integrated *in vitro* and in silico modeling delineates the molecular effects of a synbiotic regimen on colorectal-cancer-derived cells. *Cell Reports, 27*(5), 1621–1632. https://doi.org/10.1016/j.celrep.2019.04.001

Hobden, M. R., Martin-Morales, A., Guérin-Deremaux, L., Wils, D., Costabile, A., Walton, G. E., … Gibson, G. R. (2013). *In vitro* fermentation of NUTRIOSE® FB06, a wheat dextrin soluble fibre, in a continuous culture human colonic model system. *PloS One, 8*(10), 1–7. https://doi.org/10.1371/journal.pone.0077128

Hounnou, G., Destrieux, C., Desmé, J., Bertrand, P., & Velut, S. (2002). Anatomical study of the length of the human intestine. *Surgical and Radiologic Anatomy, 24*(5), 290–294. https://doi.org/10.1007/s00276-002-0057-y

Iramaia Angelica Neri-Numa, G. M. P. (2020). Novel insights into prebiotic properties on human health: A review. *Food Research International, 131*, 108973. https://doi.org/10.1016/j.foodres.2019.108973

Jochems, P. G. M., Garssen, J., Van Keulen, A. M., Masereeuw, R., & Jeurink, P. V. (2018). Evaluating human intestinal cell lines for studying dietary protein absorption. *Nutrients, 10*(3), 1–15. https://doi.org/10.3390/nu10030322

Kim, H. J., Huh, D., Hamilton, G., & Ingber, D. E. (2012). Human Gut-on-a-Chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab on a Chip, 12*(12), 2165–2174. https://doi.org/10.1039/c2lc40074j

Kim, H. J., & Ingber, D. E. (2013). Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation. *Integrative Biology (United Kingdom), 5*(9), 1130–1140. https://doi.org/10.1039/c3ib40126j

Kim, H. J., Li, H., Collins, J. J., & Ingber, D. E. (2016). Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human Gut-on-a-Chip. *Proceedings of the National Academy of Sciences of the United States of America, 113*(1), E7–E15. https://doi.org/10.1073/pnas.1522193112

Kortman, G. A. M., Dutilh, B. E., Maathuis, A. J. H., Engelke, U. F., Boekhorst, J., Keegan, K. P., … Tjalsma, H. (2016). Microbial metabolism shifts towards an adverse profile with supplementary iron in the TIM-2 *in vitro* model of the human colon. *Frontiers in Microbiology, 6*, 1–18. https://doi.org/10.3389/fmicb.2015.01481

Kostic, A. D., Howitt, M. R., & Garrett, W. S. (2013). Exploring host-microbiota interactions in animal models and humans. *Genes & Development, 27*(7), 701–718. https://doi.org/10.1101/gad.212522.112

Kovatcheva-Datchary, P., Egert, M., Maathuis, A., Rajilić-Stojanović, M., De Graaf, A. A., Smidt, H., … Venema, K. (2009). Linking phylogenetic identities of bacteria to starch fermentation in an *in vitro* model of the large intestine by RNA-based stable isotope probing. *Environmental Microbiology, 11*(4), 914–926. https://doi.org/10.1111/j.1462-2920.2008.01815.x

Labarthe, S., Polizzi, B., Phan, T., Goudon, T., Ribot, M., & Laroche, B. (2019). A mathematical model to investigate the key drivers of the biogeography of the colon microbiota. *Journal of Theoretical Biology, 462*, 552–581. https://doi.org/10.1016/j.jtbi.2018.12.009

Liang, D., Leung, R. K. K., Guan, W., & Au, W. W. (2018). Involvement of gut microbiome in human health and disease: Brief overview, knowledge gaps and research opportunities. *Gut Pathogens, 10*(1), 1–9. https://doi.org/10.1186/s13099-018-0230-4

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature, 489*(7415), 220–230. https://doi.org/10.1038/nature11550

Maccaferri, S., Klinder, A., Brigidi, P., Cavina, P., & Costabile, A. (2012). Potential probiotic *Kluyveromyces marxianus* B0399 modulates the immune response in Caco-2 cells and peripheral blood mononuclear cells and impacts the human gut microbiota in an *in vitro* colonic model system. *Applied and Environmental Microbiology, 78*(4), 956–964. https://doi.org/10.1128/aem.06385-11

Macfarlane, G. T., Cummings, J. H., Macfarlane, S., & Gibson, G. R. (1989). Influence of retention time on degradation of pancreatic enzymes by human colonic bacteria grown in a 3-stage continuous culture system. *Journal of Applied Bacteriology, 67*(5), 521–527. https://doi.org/10.1111/j.1365-2672.1989.tb02524.x

Macfarlane, G. T., Macfarlane, S., & Gibson, G. R. (1998). Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. *Microbial Ecology, 35*(2), 180–187. https://doi.org/10.1007/s002489900072

Magnúsdóttir, S., Ravcheev, D., De Crécy-Lagard, V., & Thiele, I. (2015). Systematic genome assessment of B-vitamin biosynthesis suggests cooperation among gut microbes. *Frontiers in Genetics, 6*, 148. https://doi.org/10.3389/fgene.2015.00148

Mäkeläinen, H., Forssten, S., Saarinen, M., Stowell, J., Rautonen, N., & Ouwehand, A. C. (2010). Xylo-oligosaccharides enhance the growth of *Bifidobacteria* and *Bifidobacterium lactis* in a simulated colon model. *Beneficial Microbes, 1*(1), 81–91. https://doi.org/10.3920/BM2009.0025

Mäkeläinen, H. S., Mäkivuokko, H. A., Salminen, S. J., Rautonen, N. E., & Ouwehand, A. C. (2007). The effects of polydextrose and xylitol on microbial community and activity in a 4-stage colon simulator. *Journal of Food Science, 72*(5), 153–159. https://doi.org/10.1111/j.1750-3841.2007.00350.x

Mäkivuokko, H., Nurmi, J., Nurminen, P., Stowell, J., & Rautonen, N. (2005). *In vitro* effects on polydextrose by colonic bacteria and Caco-2 cell cyclooxygenase gene expression. *Nutrition and Cancer, 52*(1), 94–104. https://doi.org/10.1207/s15327914nc5201_12

Mäkivuokko, H. A., Saarinen, M. T., Ouwehand, A. C., & Rautonen, N. E. (2006). Effects of lactose on colon microbial community structure and function in a four-stage semi-continuous culture system. *Bioscience, Biotechnology and Biochemistry, 70*(9), 2056–2063. https://doi.org/10.1271/bbb.60022

Mariat, D., Firmesse, O., Levenez, F., Guimarães, V. D., Sokol, H., Doré, J., … Furet, J. P. (2009). The *Firmicutes/Bacteroidetes* ratio of the human microbiota changes with age. *BMC Microbiology, 9*, 1–6. https://doi.org/10.1186/1471-2180-9-123

von Martels, J. Z. H., Sadaghian Sadabad, M., Bourgonje, A. R., Blokzijl, T., Dijkstra, G., Faber, K. N., et al. (2017). The role of gut microbiota in health and disease: *In vitro* modeling of host-microbe interactions at the aerobe-anaerobe interphase of the human gut. *Anaerobe, 44*, 3–12. https://doi.org/10.1016/j.anaerobe.2017.01.001

Martinez, R. C. R., Cardarelli, H. R., Borst, W., Albrecht, S., Schols, H., Gutiérrez, O. P., … Smidt, H. (2013). Effect of galactooligosaccharides and *Bifidobacterium animalis* Bb-12 on growth of *Lactobacillus amylovorus* DSM 16698, microbial community structure, and metabolite production in an *in vitro* colonic model set up with human or pig microbiota. *FEMS Microbiology Ecology, 84*(1), 110–123. https://doi.org/10.1111/1574-6941.12041

Marzorati, M., Van den Abbeele, P., Bubeck, S. S., Bayne, T., Krishnan, K., Young, A., … DeSouza, A. (2020). *Bacillus subtilis* HU58 and *Bacillus coagulans* SC208 probiotics reduced the effects of antibiotic-induced gut microbiome dysbiosis in an M-SHIME® model. *Microorganisms, 8*(7), 1028. https://doi.org/10.3390/microorganisms8071028

Marzorati, M., Van den Abbeele, P., Possemiers, S., Benner, J., Verstraete, W., & Van de Wiele, T. (2011). Studying the host-microbiota interaction in the human gastrointestinal tract: Basic concepts and *in vitro* approaches. *Annals of Microbiology, 61*(4), 709–715. https://doi.org/10.1007/s13213-011-0242-5

Marzorati, M., Vanhoecke, B., De Ryck, T., Sadaghian Sadabad, M., Pinheiro, I., Possemiers, S., … Van De Wiele, T. (2014). The HMI™ module: A new tool to study the host-microbiota interaction in the human gastrointestinal tract *in vitro*. *BMC Microbiology, 14*(1), 1–14. https://doi.org/10.1186/1471-2180-14-133

Maurer, M., Gresnigt, M. S., Last, A., Wollny, T., Berlinghof, F., Pospich, R., … Mosig, A. S. (2019). A three-dimensional immunocompetent intestine-on-chip model as *in vitro* platform for functional and microbial interaction studies. *Biomaterials, 220*, 119396. https://doi.org/10.1016/j.biomaterials.2019.119396

May, S., Evans, S., & Parry, L. (2017). Organoids, organs-on-chips and other systems, and microbiota. *Emerging Topics in Life Sciences, 1*(4), 385–400. https://doi.org/10.1042/ETLS20170047

McDonald, J. A. K., Fuentes, S., Schroeter, K., Heikamp-deJong, I., Khursigara, C. M., de Vos, W. M., & Allen-Vercoe, E. (2015). Simulating distal gut mucosal and luminal communities using packed-column biofilm reactors and an *in vitro* chemostat model. *Journal of Microbiological Methods, 108*, 36–44. https://doi.org/10.1016/j.mimet.2014.11.007

McDonald, J. A. K., Schroeter, K., Fuentes, S., Heikamp-deJong, I., Khursigara, C. M., de Vos, W. M., et al. (2013). Evaluation of microbial community reproducibility, stability and composition in a human distal gut chemostat model. *Journal of Microbiological Methods, 95*(2), 167–174. https://doi.org/10.1016/j.mimet.2013.08.008

McRorie, J. W., & McKeown, N. M. (2017). Understanding the physics of functional fibers in the gastrointestinal tract: An evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber. *Journal of the Academy of Nutrition and Dietetics, 117*(2), 251–264. https://doi.org/10.1016/j.jand.2016.09.021

Miguez, B., Vila, C., Venema, K., Parajo, J. C., & Alonso, J. L. (2020). Prebiotic effects of pectooligosaccharides obtained from lemon peel on the microbiota from elderly donors using an: *In vitro* continuous colon model (TIM-2). *Food and Function, 11*(11), 9984–9999. https://doi.org/10.1039/d0fo01848a

Minekus, M. (2015). The TNO gastro-intestinal model (TIM). In K. Verhoeckx, et al. (Eds.), *The impact of food bioactives on health: In vitro and ex vivo models* (pp. 37–46). Springer Open.

Minekus, M., Smeets-Peeters, M., Havenaar, R., Bernalier, A., Fonty, G., Marol-Bonnin, S., … Huis In't Veld, J. H. J. (1999). A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. *Applied Microbiology and Biotechnology, 53*(1), 108–114. https://doi.org/10.1007/s002530051622

Molly, K., Woestyne, M. V., Smet, I. D., & Verstraete, W. (1994). Validation of the simulator of the human intestinal microbial ecosystem (SHIME) reactor using microorganism-associated activities. *Microbial Ecology in Health and Disease, 7*(4), 191–200. https://doi.org/10.3109/08910609409141354

Molly, K., Woestyne, M. V., & Verstraete, W. (1993). Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. *Applied Microbiology and Biotechnology, 39*(2), 254–258. https://doi.org/10.1007/BF00228615

Moon, J. S., Li, L., Bang, J., & Han, N. S. (2016). Application of *in vitro* gut fermentation models to food components: A review. *Food Science and Biotechnology, 25*, 1–7. https://doi.org/10.1007/s10068-016-0091-x

Moore, K. L., Dalley, A. F., & Agur, A. M. R. (2014). *Clinically oriented anatomy* (7th ed.). Wolters Kluwer Health.

Neis, E. P. J. G., Dejong, C. H. C., & Rensen, S. S. (2015). The role of microbial amino acid metabolism in host metabolism. *Nutrients, 7*(4), 2930–2946. https://doi.org/10.3390/nu7042930

Newton, D. F., MacFarlane, S., & MacFarlane, G. T. (2013). Effects of antibiotics on bacterial species composition and metabolic activities in chemostats containing defined populations of human gut microorganisms. *Antimicrobial Agents and Chemotherapy, 57*(5), 2016–2025. https://doi.org/10.1128/AAC.00079-13

Nobre, C., Cerqueira, M.Â., Rodrigues, L. R., Vicente, A. A., & Teixeira, J. A. (2015). Chapter 19 – production and extraction of polysaccharides and oligosaccharides and their use as New Food Additives. In Ashok Pandey, et al. (Eds.), *Industrial biorefineries & white biotechnology* (pp. 653–679). Elsevier B.V.. https://doi.org/10.1016/B978-0-444-63453-5.00021-5

Nobre, C., Gonçalves, D. A., Teixeira, J. A., & Rodrigues, L. R. (2018). One-step co-culture fermentation strategy to produce high-content fructo-oligosaccharides. *Carbohydrate Polymers, 201*, 31–38. https://doi.org/10.1016/j.carbpol.2018.08.051

Payne, A. N., Zihler, A., Chassard, C., & Lacroix, C. (2012). Advances and perspectives in *in vitro* human gut fermentation modeling. *Trends in Biotechnology, 30*(1), 17–25. https://doi.org/10.1016/j.tibtech.2011.06.011

Pham, V. T., & Mohajeri, M. H. (2018). The application of *in vitro* human intestinal models on the screening and development of pre-and probiotics. *Beneficial Microbes, 9*(5), 725–742. https://doi.org/10.3920/BM2017.0164

Pirt, S. J. (1974). The theory of fed batch culture with reference to the penicillin fermentation. *Journal of Applied Chemistry and Biotechnology, 24*(7), 415–424. https://doi.org/10.1002/jctb.5020240706

Poeker, S. A., Geirnaert, A., Berchtold, L., Greppi, A., Krych, L., Steinert, R. E., … Lacroix, C. (2018). Understanding the prebiotic potential of different dietary fibers using an *in vitro* continuous adult fermentation model (PolyFermS). *Scientific Reports, 8*(1), 1–12. https://doi.org/10.1038/s41598-018-22438-y

Priya, S., & Blekhman, R. (2019). Population dynamics of the human gut microbiome: Change is the only constant. *Genome Biology, 20*(1), 1–4. https://doi.org/10.1186/s13059-019-1775-3

Proctor, L., LoTempio, J., Marquitz, A., Daschner, P., Xi, D., Flores, R., … Team, N. I. H. (2019). A review of 10 years of human microbiome research activities at the US National Institutes of Health, fiscal years 2007-2016. *Microbiome, 7*(1), 1–19. https://doi.org/10.1186/s40168-019-0620-y

Rajput, I. R., & Li, W. F. (2012). Potential role of probiotics in mechanism of intestinal immunity. *Pakistan Veterinary Journal, 32*(3), 303–308.

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., et al. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms, 7*(1), 1–22. https://doi.org/10.3390/microorganisms7010014

Robinson, C. D., Auchtung, J. M., Collins, J., & Britton, R. A. (2014). Epidemic *Clostridium difficile* strains demonstrate increased competitive fitness compared to nonepidemic isolates. *Infection and Immunity, 82*(7), 2815–2825. https://doi.org/10.1128/IAI.01524-14

de la Rosa, O., Flores-Gallegos, A. C., Muñíz-Marquez, D., Nobre, C., Contreras-Esquivel, J. C., & Aguilar, C. N. (2019). Fructooligosaccharides production from agro-wastes as alternative low-cost source. *Trends in Food Science & Technology, 91*, 139–146. https://doi.org/10.1016/j.tifs.2019.06.013

Rufener, W. H., Nelson, W. O., & Wolin, M. J. (1963). Maintenance of the rumen microbial population in continuous culture. *Applied Microbiology, 11*, 196–201. https://doi.org/10.1128/aem.11.3.196-201.1963

Rumney, C. J., & Rowland, I. R. (1992). *In vivo* and *in vitro* models of the human colonic flora. *Critical Reviews in Food Science and Nutrition, 31*(4), 299–331. https://doi.org/10.1080/10408399209527575

Salli, K., Anglenius, H., Hirvonen, J., Hibberd, A. A., Ahonen, I., Saarinen, M. T., … Ouwehand, A. C. (2019). The effect of 2′-fucosyllactose on simulated infant gut microbiome and metabolites; a pilot study in comparison to GOS and lactose. *Scientific Reports, 9*(1), 1–15. https://doi.org/10.1038/s41598-019-49497-z

Sáyago-Ayerdi, S. G., Zamora-Gasga, V. M., & Venema, K. (2019). Prebiotic effect of predigested mango peel on gut microbiota assessed in a dynamic *in vitro* model of the human colon (TIM-2). *Food Research International, 118*, 89–95. https://doi.org/10.1016/j.foodres.2017.12.024

Shah, P., Fritz, J., Estes, M., Zenhausern, F., & Wilmes, P. (2014). HuMiX: A microfluidics-based in vitro co-culture device for investigating host-microbe molecular interactions. In *18th international conference on miniaturized systems for chemistry and life sciences*. San Antonio, United States: MicroTAS 2014.

Shah, P., Fritz, J. V., Glaab, E., Desai, M. S., Greenhalgh, K., Frachet, A., … Wilmes, P. (2016). A microfluidics-based *in vitro* model of the gastrointestinal human-microbe interface. *Nature Communications, 7*, 1–15. https://doi.org/10.1038/ncomms11535

Shin, W., Hinojosa, C. D., Ingber, D. E., & Kim, H. J. (2019). Human intestinal morphogenesis controlled by transepithelial morphogen gradient and flow-dependent physical cues in a microengineered Gut-on-a-Chip. *iScience, 15*, 391–406. https://doi.org/10.1016/J.ISCI.2019.04.037

Silva, H. D., Poejo, J., Pinheiro, A. C., Donsì, F., Serra, A. T., Duarte, C. M. M., … Vicente, A. A. (2018). Evaluating the behaviour of curcumin nanoemulsions and multilayer nanoemulsions during dynamic *in vitro* digestion. *Journal of Functional Foods, 48*, 605–613. https://doi.org/10.1016/J.JFF.2018.08.002

Sivieri, K., Morales, M. L. V., Saad, S. M. I., Adorno, M. A. T., Sakamoto, I. K., & Rossi, E. A. (2014). Prebiotic effect of fructooligosaccharide in the simulator of the human intestinal microbial ecosystem (SHIME® model). *Journal of Medicinal Food, 17*(8), 894–901. https://doi.org/10.1089/jmf.2013.0092

Slyter, L. L., Nelson, W. O., & Wolin, M. J. (1964). Modifications of a device for maintenance of the rumen microbial. *Applied Microbiology, 12*(4), 374–377.

Smeets-Peeters, M. J. E., Minekus, M., Havenaar, R., Schaafsma, G., & Verstegen, M. W. A. (1999). Description of a dynamic *in vitro* model of the dog gastrointestinal tract and an evaluation of various transit times for protein and calcium. *Alternatives to Laboratory Animals, 27*(6), 935–949. https://doi.org/10.1177/026119299902700608

Spinler, J. K., Auchtung, J., Brown, A., Boonma, P., Oezguen, N., Ross, C. L., … Savidge, T. C. (2017). Next-generation probiotics targeting *Clostridium difficile* through precursor-directed antimicrobial biosynthesis. *Infection and Immunity, 85*(10), 1–16. https://doi.org/10.1128/IAI.00303-17

Stranding, S. (2016). *Gray's anatomy* (41st ed.). Elsevier Health Sciences.

Takagi, R., Sasaki, K., Sasaki, D., Fukuda, I., Tanaka, K., Yoshida, K., … Osawa, R. (2016). A single-batch fermentation system to simulate human colonic microbiota for high-throughput evaluation of prebiotics. *PloS One, 11*(8), 1–16. https://doi.org/10.1371/journal.pone.0160533

Tamargo, A., Cueva, C., Laguna, L., Moreno-Arribas, M. V., & Muñoz, L. A. (2018). Understanding the impact of chia seed mucilage on human gut microbiota by using the dynamic gastrointestinal model simgi®. *Journal of Functional Foods, 50*, 104–111. https://doi.org/10.1016/j.jff.2018.09.028

Tottey, W., Feria-Gervasio, D., Gaci, N., Laillet, B., Pujos, E., Martin, J. F., … Brugère, J. F. (2017). Colonic transit time is a driven force of the gut microbiota composition and metabolism: *In vitro* evidence. *Journal of Neurogastroenterology and Motility, 23*(1), 124–134. https://doi.org/10.5056/jnm16042

Valdés, L., Salazar, N., González, S., Arboleya, S., Ríos-Covián, D., Genovés, S., … Gueimonde, M. (2016). Selection of potential probiotic *Bifidobacteria* and prebiotics for elderly by using *in vitro* faecal batch cultures. *European Food Research and Technology, 243*(1), 157–165. https://doi.org/10.1007/s00217-016-2732-y

Van Nuenen, M. H. M. C., Meyer, P. D., & Venema, K. (2003). The effect of various inulins and *Clostridium difficile* on the metabolic activity of the human colonic microbiota *in vitro*. *Microbial Ecology in Health and Disease, 15*, 137–144. https://doi.org/10.1080/08910600310018959

Van den Abbeele, P., Roos, S., Eeckhaut, V., Mackenzie, D. A., Derde, M., Verstraete, W., … Van de Wiele, T. (2012). Incorporating a mucosal environment in a dynamic gut model results in a more representative colonization by *Lactobacilli*. *Microbial Biotechnology, 5*(1), 106–115. https://doi.org/10.1111/j.1751-7915.2011.00308.x

Van de Wiele, T., Van den Abbeele, P., Ossieur, W., Possemiers, S., & Marzorati, M. (2015). The simulator of the human intestinal microbial ecosystem (SHIME®). In K. Verhoeckx, et al. (Eds.), *The impact of food bioactives on health: In vitro and ex vivo models* (pp. 305–317). Springer Open.

Vázquez-Baeza, Y., Callewaert, C., Debelius, J., Hyde, E., Marotz, C., Morton, J. T., … Knight, R. (2018). Impacts of the human gut microbiome on therapeutics. *Annual Review of Pharmacology and Toxicology, 58*(1), 253–270. https://doi.org/10.1146/annurev-pharmtox-042017-031849

Venema, K., & Van Den Abbeele, P. (2013). Experimental models of the gut microbiome. *Best Practice & Research Clinical Gastroenterology, 27*(1), 115–126. https://doi.org/10.1016/j.bpg.2013.03.002

Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., … Wichers, H. (2015). In vitro fermentation models: General introduction. In K. Verhoeckx, et al. (Eds.), *The impact of food bioactives on health: In vitro and ex vivo models* (pp. 275–279). Springer Open.

Vermeiren, J., Possemiers, S., Marzorati, M., & de Wiele, T. V. (2011). The gut microbiota as target for innovative drug development: Perspectives and a case study of inflammatory bowel diseases. *Drug development: A Case Study Based Insight Into Modern Strategies*, 437–468. https://doi.org/10.5772/27905

Williams, C. F., Walton, G. E., Jiang, L., Plummer, S., Garaiova, I., & Gibson, G. R. (2015). Comparative analysis of intestinal tract models. *Annual Review of Food Science and Technology, 6*(1), 329–350. https://doi.org/10.1146/annurev-food-022814-015429

Wilmes, P., Calatayud, M., & Van de Wiele, T. (2018). Resolving host–microbe interactions in the gut: The promise of *in vitro* models to complement *in vivo* research. *Current Opinion in Microbiology, 44*, 28–33. https://doi.org/10.1016/J.MIB.2018.07.001