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Minireview

MINIREVIEW – Food Microbiology

Intestinal fermentation *in vitro* models to study food-induced gut microbiota shift: an updated review

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One sentence summary: Intestinal *in vitro* models to study changes in the composition and metabolism of the gut microbiota in response to the diet.

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ABSTRACT

In vitro gut fermentation models were firstly introduced in nutrition and applied microbiology research back in the 1990s. These models have improved greatly during time, mainly over the resemblance to the complexity of digestion stages, the replication of experimental conditions, the multitude of ecological parameters to assay. The state of the science is that the most competitive models shall include a complex gut microbiota, small working volumes, distinct interconnected compartments and rigorous bio-chemical and ecological settings, controlled by a computer, as well as a free-hands accessibility, not to contaminate the mock microbiota. These models are a useful tool to study the impact of a given diet compound, e.g. prebiotics, on the human gut microbiota. The principal application is to focus on the shift of the core microbial groups and selected species together with their metabolites, assaying their diversity, richness and abundance in the community over time. Besides, it is possible to study how a compound is digested, which metabolic pathways are triggered, and the type and quantity of microbial metabolites produced. Further prospective should focus on challenges with pathogens as well as on ecology of gut syndromes. In this minireview an updated presentation of the most used intestinal models is presented, basing on their concept, technical features, as well as on research applications.

Keywords: *in vitro* gut model; gut microbiota; diet

INTRODUCTION

It is renown that a large proportion of the elements of any diet, such as polyols or phenolic compounds are not metabolized, nor absorbed by the host prior transformation driven by colon microbiota (Possemiers *et al.* 2011). In fact, the beneficial microbes of the thousands of species inhabiting the large intestine are deputed for this action (Hooper, Littman and Macpherson 2012). In particular, an equilibrium in the ratio of beneficial, commensals and even opportunistic species is fundamental. The deployment of this milieu primes detrimental pathologies, such as metabolic syndromes (Qin *et al.* 2012), malabsorption, (Kau *et al.* 2011), or inflammatory bowel disease (IBD) (Frank *et al.* 2007). Diet is the principal factor influencing the eubiosis of the gut microbiota, including its long-term metabolism (Wu *et al.* 2011; David *et al.* 2014), thus plays a fundamental role in the comprehension of the microbial health benefits towards the host. Notwithstanding, this goal is hard to be addressed, because *in vivo* research on the human gut is restricted for ethical grounds and mainly limited to pathological conditions or to pharmacological trials. Consequently, microbiologists and nutritionists over the last twenty years have established several *in vitro* gut fermentation models to mime human digestion of foods or their single constituents

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and study the shifts and roles of the associated microbiota (Guerra *et al.* 2012; Venema and Van den Abbeele 2013; Koutsos *et al.* 2017).

Relationship between diet and human gut microbiota

Overall, the human intestine guests more than one hundred trillion of microbial cells per gram of almost two thousand different species (Rajilic-Stojanovic, Smidt and De Vos 2007; Chong *et al.* 2019). From a relative low number of cells presents in the gastric tissue (thousand cells per gram) the abundance rises exponentially descending the gastro-intestinal tract (GIT), passing from a mean of ten thousand cells per gram in the duodenum, to a million of cells per gram of the jejunum and ten millions of the ileum. Nevertheless, this abundance is still little due to the inhibition of growth mainly derived from: (i) bile salts, (ii) exocrine secretion from pancreas and (iii) frequent peristalsis. Finally, in the colon, where the ecological niches are plenty of substrates, the pH is neutral and the redox potential optimal for microbes, the microbial population extend top richness accounting for trillion cells per gram in the ascending tract and 10 trillions in the sigmoid tract or alternatively up to 2 kg of host weight (Gibson and Roberfroid 1995). The core microbiota of the GIT is made by four bacterial phyla, namely: *Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria* (Dethlefsen *et al.* 2006; Dethlefsen, McFall-Ngai and Relman 2007). *Firmicutes* is the richest phylum, comprising 46–58% of the total bacteria (Harmsen *et al.* 2002; Lay *et al.* 2005). Other key residents are the *Bacteroides-Prevotella* group (10–30%), *Bifidobacterium* (4.4–4.8%), *Enterobacteriaceae* (0.1–0.2%), *Lactobacillus* and *Enterococcus* (both 0.1–1, 8%, Lay *et al.* 2005; Zoetendal, Vaughan and De Vos 2006; Payne *et al.* 2012). It has been acknowledged that the type of diet and the dietary habits are the factors most impacting on the cline of gut microbiota (GM, Rinninella *et al.* 2019). Otherwise, in previous reports the efficacy of diets on variations of core phyla (*Bacteroidetes* and *Firmicutes*) of GM was debated. The efficacy of different diet types seems more influencing the GM at lower phylogenetic levels. For example, a regime of altered carbohydrate intake could impact on specific bacterial groups, as was observed that a low carbohydrates diet was effective on the reduction of *Bifidobacterium* spp., *Roseburia* spp. and *Eubacterium rectale*, and on the content of short chain fatty acids (SCFA), mainly by a drastic loss of butyric acid (Duncan *et al.* 2007). An important contribute to the shifts of GM populations is derived even by the nature of carbohydrates, likewise resistance to digestion. A study of Walker *et al.* (2011) has evidenced that the consumption of resistant starch (RS) can augment the amount of *Ruminococcus bromii*, *Roseburia* and *E. rectale*, while RS combined in a low carbohydrate and high protein (WL) diet can augment *Oscillibacter valericigenes*, but can diminish the abundances of *Roseburia* and *E. rectale*. Similarly, a high fiber diet can foster the growth of *Bifidobacterium, Ruminococcus* and *Lactobacillus-Enterococcus* group (Shen, Zhao and Tuohy 2011; Schroeder *et al.* 2018). To obtain such results over microbes, the main techniques employed so far ranged from culture-dependent microbiology to FISH (Fluorescent *in situ* Hybridization) and qPCR (quantitative PCR) up to NGS (Next Generation Sequencing) technologies. Additionally, to investigate over metabolites most of the studies achieved robust results through the aid of chromatographic techniques and more recently NMR (Nuclear Magnetic Resonance) analysis.

IN VITRO FERMENTATION MODELS

As said above, the restriction to study *in vivo* the behave of GM during a diet intervention has led researcher to develop *in vitro* gut fermentation models. This approach permits to explore over the influence on GM of a vast variety of factors such as dietary compounds, microbial pathogens, bioactives, pharmaceuticals and toxic substances. The rationale of *in vitro* models is to cultivate to the more representative way the human GM under regulated environmental conditions and study its metabolism and shifts over time. In this view, many models apply, for example, fecal inoculum from healthy or diseased donors, in order to better mime the desired colon-related ecosystem of investigation. However, the most of these models lack host feedback. In fact, epithelial cells and immune cells are missing, which are important components in host-microbe interactions. Looking to highlight some applications of the *in vitro* gut fermentation models covered in this Minireview, in Table 1 are reported some studies applied to the described systems.

Batch fermentation models

Mimicking GM ecosystem with batch fermentation models (BFM) is the simplest, most versatile and accessible approach because it is characterized by a closed anaerobic environment and a short time simulation. These static models imply the termination of testing when the substrate is exhausted and consequently the accumulation of toxic microbial metabolites disrupt the initial microbial balance. BFM can be arranged by varying degrees of complexity, from closed flasks inoculated with defined microbial species to controlled reactors working with fecal suspensions.

Typically, BFM are used to study the interplay of a given compound with the GM. The ecological conditions in the controlled reactors are mastered by heating plate for temperature, alkali or acid dosing for a stable pH and N_2 bubbling on a chemical basal medium for anaerobiosis and nutrition. As shown in Table 1, batch fermentation studies demonstrated the prebiotic effect of trans-galactooligosaccharides (B-GOS) and inulin (Liu, Gibson and Walton 2016) and proved the selective effect of dextrin on gut microbiota, SCFA and gas production (Sarbini *et al.* 2011). This kind of systems has the cons to have a limited resemblance to *in vivo* condition, but the pros to be cost accessible, logistically flexible and operator friendly.

Dynamic fermentation models

Higher resemblance to *in vivo* occurrence could be obtained with dynamic fermentation models (DFM). These models permit to evaluate GM response to food compounds over a prolonged time and spatially over a more complex ecosystem of the different GIT niches. In the DFM the GM does not comply with starvation, because the system is constantly fed by essential nutrients via peristaltic pumps, and the ecological milieu is kept rigorously. Most human DFM are multi-stages set up likewise the Reading Model made by three connecting vessels, that was originally build up at the University of Reading (Fig. 1) (Gibson, Cummings and Macfarlane 1988). These vessels are aligned in series in order to mimic the ecological niches of the proximal, transverse and distal colon. The microaerophilic and anoxic atmosphere are maintained at desired levels sparging doses of N_2 or O_2 driven by dissolved oxygen (DO) sensors, or by mechanical stirrers. N_2 dosing is preferred than CO_2, because the latter can influence the pH. The first vessel is characterized by a mild acid

Table 1. Recent application of *in vitro* intestinal models in food science.

Model	Applied studies	References
Batch fermentation model	Effect of trans-galactooligosaccharides (B-GOS) and inulin on gut microbiota.	Liu, Gibson and Walton (2016)
	Effect of dextran on gut microbiota, SCFA and gas production.	Sarbini *et al.* (2011)
Reading model	Impact of wheat dextrin on the composition and metabolic activity of the gut microbiota.	Hobden *et al.* (2013)
	Effect of orange juice formulations on prebiotic activity.	Costabile *et al.* (2015)
TIM-2	Effect of galactoligosaccharides, lactulose, apple fiber and sugar beet pectin on human colonic microbiota of lean and obese healthy subjects.	Aguirre *et al.* (2014)
	Effect of iron preparations on microbial growth and metabolism.	Kortman *et al.* (2016)
	Study of the prebiotic potential of mango peel.	Sáyago-Ayerdi, Zamora-Gasga and Venema (2019)
SHIME®	Effect of arabinogalactan (AG) and FOS on the composition and activity of the microbial community.	Terpend *et al.* (2013)
	Prebiotic potential of native chicory inulin.	Van de Wiele *et al.* (2004)
	Effects of Olive and Pomegranate By-Products on Human Microbiota.	Giuliani *et al.* (2019)
M-SHIME	Effects of an extract of *Vitis vinifera*, containing t-resveratrol and ε-viniferin, on the intestinal microbiota.	Giuliani *et al.* (2016)
	Modulatory effects of long-chain arabinoxylans (LC-AX) towards the luminal and mucous microbiota.	Truchado *et al.* (2017)
SIMGI	Relationship between apparent viscosity of chia mucilage and human intestinal microbiota.	Tamargo *et al.* (2018)
	Study of the impact of red wine on colonic metabolism.	Cueva *et al.* (2015)
PolyFermS	Evaluation of the modulating effect of fermentable dietary fibers (DFs) on two distinct microbiotas of the adult human proximal colon, independently of the host.	Poeker *et al.* (2018)
	Effect of nucleotides and nucleosides on the infant gut microbiota.	Doo *et al.* (2017)
MiniBio	Development of a highly bio-relevant but generic *in vitro* digestion system that simulates the aged intestine.	Levi and Lesmes (2014)
TSI	Development of a small volume *in vitro* model with increased throughput focusing on simulating passage through the stomach and small intestine (SI).	Cieplak *et al.* (2018)

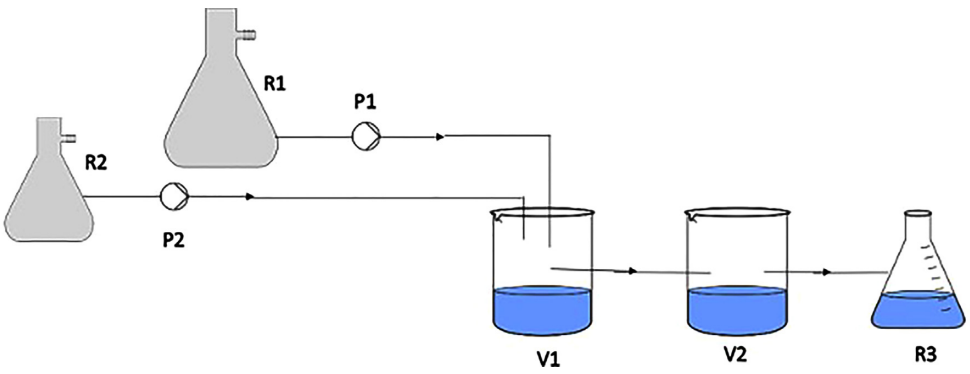

Figure 1. Three-stage continuous culture system (adapted from Gibson *et al.* 1988).

environment nutritional plenty, inducing a quick GM growth akin to the proximal colon. Instead, the other vessels have a neutral pH and lack of substrate, causing a lazy GM growth analogous to the colon distal regions. Along the whole system the viability of GM populations, derived by a fecal sample, is kept by a stable temperature and the use of a basal nutritional media. In Table 1 are reported some explanatory examples of these models. Hobden *et al.* (2013) tested the effect of wheat dextrin on the gut microbiota using a three-stage model while Costabile *et al.* (2015) used this dynamic model to study different orange juice formulations on prebiotic functionality. This latter study had demonstrated effective microbiota shift related to those juices with prebiotic supplements, such as the increase in abundance of *Bifidobacterium* spp. and the reduction of *Clostridium hystolycum* group, as the production of higher SCFA yields. These outputs were obtained by FISH techniques and Gas Chromatographic (GC) analyses. This more complex system has a limited reproducibility due to possible fluctuations on parameters settings, but the advantage to be cost accessible, customizable and operator friendly.

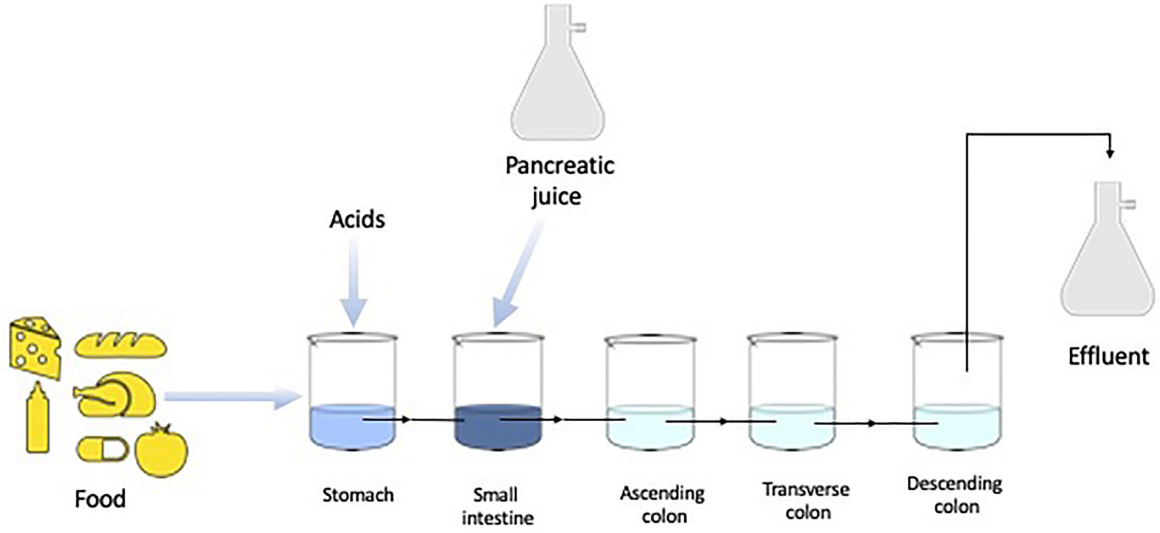

Figure 2. Schematic representation of the SHIME (adapted from https://www.prodigest.eu/en/technology/shime-and-m-shime).

The TNO *in vitro* model of the colon (TIM-2)

The TIM-2 is a patented *in vitro* model of the colon developed by the Netherlands Organization for Applied Research located in The Hague. It can work along with the TIM-1, that simulate the ecological niches of the stomach and the small intestine. The combo model has been accepted by the most important regulatory agencies worldwide and could include some adds-on, such as intestinal segment and human cell cultures. So far, TIM-1 along with TIM-2 can simulate the whole human GIT. Focusing just on the TIM-2, the system is a DFM which mime the proximal part of the large intestine divided in four working compartments. Most of the parameters that run the system are managed by a computer. In addition to the standard ecological features, a software controls the absorption of water and microbial metabolites by a semipermeable membrane and a dialysis system, with the intent to prevent a pile-up of toxic metabolites. Successively, the software can analyze the release, the bio-accessibility and the interaction profile of the assayed substance in each single compartment. The TIM-2 is characterized also by a flow of heated water within the four glass containers and a flexible membrane squeezing similarly to peristalsis, promoting also the mix and move of intestinal liquids. The GM is derived by inoculum of human fecal suspensions (Maathuis *et al.* 2009; Reimer *et al.* 2014) and is fed with a special medium, named SIEM (simulated ileal efflux medium), made of complex sugars, indigestible proteins, residual bile along with selected minerals and vitamins (Venema *et al.* 2000; van Nuenen, Meyer and Venema 2003). The GM growth in combination with N_2 bubbling maintains the redox potential around − 300 mV, likely that recorded in the human colon. The acids resulting from the microbial metabolism are neutralized through the release of NaOH, thus allowing to maintain the pH of the system at 5.8, the value found in the proximal colon. Prior to begin the experiment, the GM needs to acclimate for almost 16 h, afterwards the experimental period lasts 72 h. Among the many different applications in Table 1 are reported TIM-2 studies demonstrating how the fermentation of different non-digestible carbohydrates proceeds differently for the GM of lean and obese subjects (Aguirre *et al.* 2014), how iron preparations modify the GM (Kortman *et al.* 2016) and demonstrated the prebiotic potential of mango peel (Sáyago-Ayerdi, Zamora-Gasga and Venema 2019).

The study of Aguirre *et al.* (2014), applying NGS Roche apparatus, found differences in *Bacteroidetes/Firmicutes* ratio, as well as different proportions of *Actinobacteria* and *Proteobacteria,* when different prebiotics were supplemented. Moreover, sequencing data were coupled with SCFA, BCFA (branched chain fatty acids), as well as ammonia and lactate evaluation. This kind of systems has the cons to be time and effort consuming and not cost accessible, but the pros to have an elevated *in vivo* resemblance, to be logistically fitting on small lab rooms and experimentally high reproducible.

The simulator of the human intestinal microbial ecosystem (SHIME®)

The Stimulator of the Human Intestinal Microbial Ecosyatem (SHIME) is a system patented under the aegis of ProDigest and the University of Ghent. It is made of five connected reactors built in series to reproduce the whole GIT, from the gastric tract to the distal colon (Van den Abbeele *et al.* 2010). Reactors are double-jacket glass vessels interconnected by peristaltic pumps (Fig. 2). The initial two of them simulate the stomach and the small bowel respectively and thanks to a filling and drawing technique, at precise intervals, a nutritional medium is pumped to the first, while pancreatic and bile juices to the second (Venema and Van den Abbeele 2013). The three sections of the colon are made up of compartments with different working capacity, i.e. 500 mL, 600 mL and 800 mL, and *in vivo* different resembling pH values, subjected to uninterrupted impellers agitation (Van den Abbeele *et al.* 2010). The microbial inoculum of the large bowel is a stool specimen from healthy donors, which need at least 14 days of acclimation with the reactors ecological regime, so that the fecal microbes can advance to a representative colon GM (Van den Abbeele *et al.* 2010; Venema and Van den Abbeele 2013). The SHIME system is a useful *in vitro* tool to preliminarily investigate interactions between complex microbial ecosystems, as demonstrated by Giuliani *et al.* (2019), who investigated the effect of vegetable by-products on human microbiota. Terpend *et al.* (2013) and Van de Wiele *et al.* (2004) used the SHIME system to investigate the effect on the GM of AG and FOS and native chicory inulin, respectively (Table 1). This kind of systems is time and effort consuming and not cost accessible, nor

suitable for small labs, but they have maybe the highest *in vivo* resemblance among other models.

M-SHIME

The M-SHIME is a recent DFM updated from the former version, which additionally combines a distinct segment for mucous microbes (Fig. 3) (Van den Abbeele *et al.* 2011b). Indeed, a more realistic simulation of intestinal microbiology envisages the inclusion of a representative microbiota associated with the mucin layer set on the epithelium (Swidsinski *et al.* 2008; Van den Abbeele *et al.* 2011c; Belzer and de Vos 2012). *In vivo*, this latter microbial population interact more effectively with the epithelium than microbiota associate to the intestinal lumen, resulting crucial to generate immunomodulatory effects (Lievin-Le Moal and Servin 2006). One of the principal capabilities of the microbiota of this niche includes the degradation of host mucus glycans (Derrien *et al.* 2004). The specific metabolites produced serve successively as substrates for the lumen GM, directly influencing its composition (Schroeder 2019). The mucus layer is mainly inhabited by methanogenic Archaea, sulphate-reducing bacteria and acetogenic bacteria (Nava *et al.* 2012; Nkamga, Henrissat and Drancourt 2017; Singh *et al.* 2019). In the M-SHIME, a microcosm, set up with a microbial consortium representative of human intestinal mucous, is immersed in mucin agar and diffused in a polyethylene net. In each vessel reproducing the large bowel, M-SHIME harbors a GM made combining the mucus microcosm to a human fecal suspension, successively inoculated. As showed in Table 1, Giuliani *et al.* (2016) studied the effects of an extract from *Vitis vinifera* on intestinal microbiota using the M-SHIME. This model was also used to study the modulatory effects of long-chain arabinoxylans (LC-AX) towards the luminal and mucous microbiota (Truchado *et al.* 2017) (Table 1). Results from 16S microbiota sequencing with Miseq (Illumina, Hayward, CA) and GC assays of volatile fatty acids suggested potential prebiotic benefit of LC-AX as it was effective in the enrichment of *Bifidobacterium*, *Akkermansia*, *Roseburia* and *Enterococcus* (Truchado *et al.* 2017).

The SIMulator gastro-intestinal: SIMGI

The SIMGI model is a multi-chamber system capable of replicating the entire human GIT developed in Spain at the Food Science Research Institute (CIAL-CSIC-UAM, Madrid, Spain) (Barroso *et al.* 2015). The model is composed by five reactors controlled remotely by a PC with a dedicated software and connected by peristaltic pumps. The first two units represent the gastric sector and the small bowel, while the other three represent the three main sections of the large bowel (Fig. 4). In the first unit two methacrylate modules are set above a tank and separated by an in-between jacket. While the tank is used as reservoir for gastric juices, the mobile jacket serves to mix its content with the aid of water flushes. The other downstream four units are instead glass reactors where the media is kept in agitation employing magnetic stirrers. Similar to other DMF models, the ecological conditions are distinct in each compartment, mainly setting specific pH and atmosphere parameters. The experimental periods vary up to 6 days, depending on the desired simulation study (Barroso *et al.* 2015). SIMGI studies demonstrated the effect of chia seed mucilage (Tamargo *et al.* 2018) and red wine (Cueva *et al.* 2015) on the human gut microbiota (Table 1). The results of Tamargo *et al.* (2018) were obtained with culture dependent microbiology and with SPME-GCMS analysis of SCFAs, and demonstrated that no significant changes affected the microbial groups of colonic microbiota even though significant increases were scored for SCFAs production. SIMGI has the cons to be time consuming and less realistic and reproducible due to less strict environmental parameters, but the pros to be cost accessible, logistically flexible and operator friendly.

Polyfermentor intestinal model (PolyFermS)

The intestinal DFM described so far reproduces the GM using a fecal inoculum. However, this versatile method, although advantageous, has been questioned, mainly because does not take into account the different skills and strategies used by microorganisms to colonize and resist in the host intestinal epithelia e.g. adhesion to binding sites. The mock community is unstable and those microbial groups able to overcome others mainly by resistance to starvation, ecological displacement and/or rapid growth impose their will. Thus, essential populations so far less aggressive of the struggle for the intestinal substrate become uncompetitive and limit the model resemblance to reality. Another example enlarging the fault, is the lack in stool specimens of microbial populations associated in a biofilm (Macfarlane and Macfarlane 2007; Macfarlane and Dillon 2007).

To solve this problem and keep a certain level of species diversity and abundance during long-term experiments, an immobilization process was developed, which consists on trapping the fecal microbiota via micro-encapsulation technique (Le Blay *et al.* 2010; Zihler *et al.* 2011; Payne *et al.* 2012; Dostal *et al.* 2013). The first system to apply this technology was the PolyFermS model, that is a DFM established at the ETH of Zurich by a jointly European mandate (Zhiler-Berner *et al.* 2013). Each of the five reactors of the model has independent microbial inoculum, and the model can keep a stable microbial community standing a stress test of 38 days. Besides, the artificial microbiota resembles the stool microbiota of healthy donor, preserving major taxonomical bacterial groups all along the experiments both in diversity and in abundance (Zihler-Berner *et al.* 2013).

The model (Fig. 5) is composed of an inoculum reactor for the upper proximal colon made of micro-encapsulated GM. The content of this section is used to continuously supply downstream reactors arranged in parallel, including second-stage reactors and experimental reactors, that run accordingly with the proximal colon environmental condition. Second-stage reactors serve as control to compare the response to experimental features (Zihler-Berner *et al.* 2013; Poeker *et al.* 2018). PolyFermS was used to evaluate the gut microbiota-modulating effects of fermentable dietary fibers (Poeker *et al.* 2018) and to study the impact on infant gut microbiota of some components of infant formula which imitate the content of breast milk (Doo *et al.* 2017) (Table 1). Poeker *et al.* (2018), coupling HPLC and 16S MiSeq Sequencing (Illumina, San Diego, CA, USA) technologies, demonstrated positive correlations of *Prevotellaceae* with proprionate and *Lachnospiraceae* and *Ruminococcaceae* with butyrate after prebiotic supplementation. PolyfermS is time and effort consuming, but it seems highly stable over time improving the *in vivo* parallels.

Small scale reactors

The Mini-Bio *in vitro* model is a patented system from Applikon Biotechnology (Delft, The Netherlands) recently introduced in applied research. Mini-Bio represent a flexible *in vitro* system, that can operate as BFM or DFM generating high speed and massive data, controlled, analyzed and stored by a dedicated

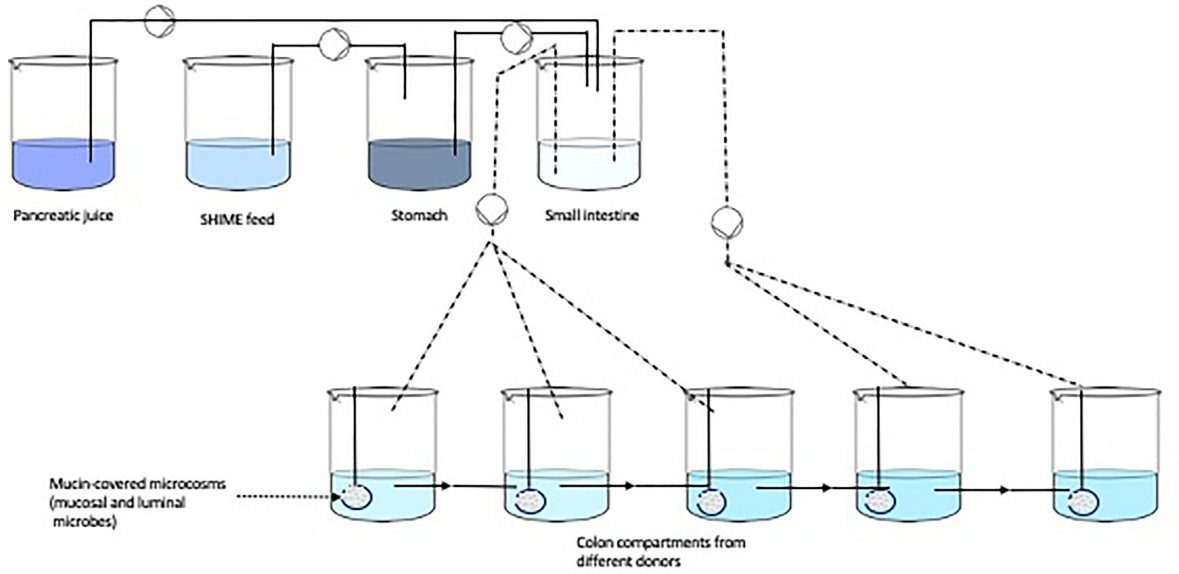

Figure 3. Schematic representation of M-SHIME (adapted from Van den Abbeele *et al.* 2013).

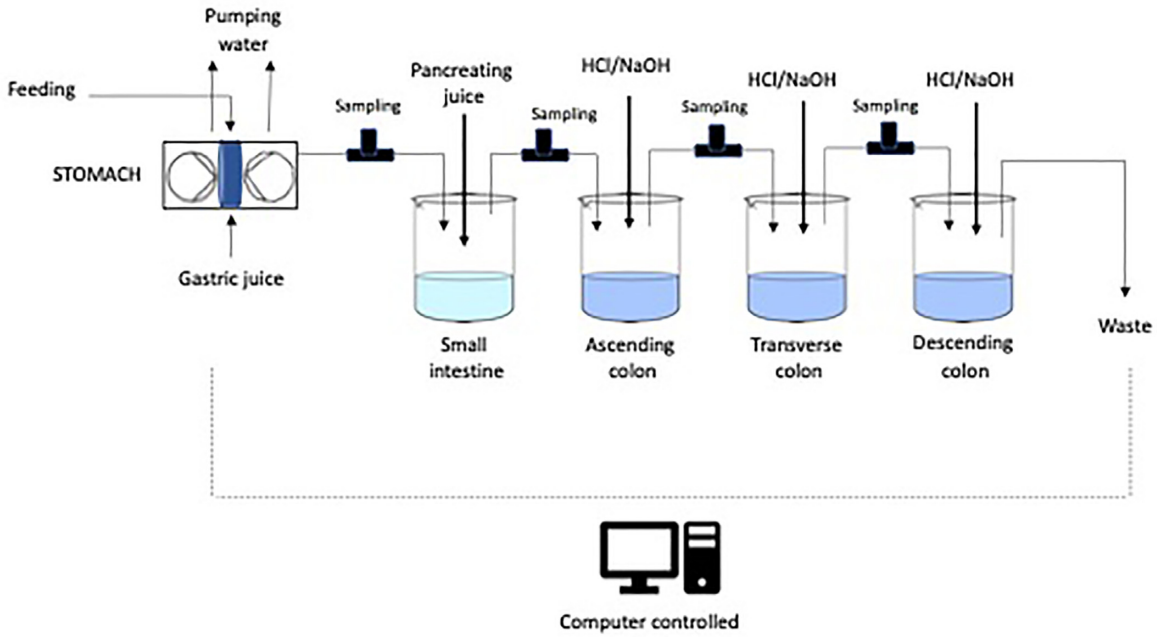

Figure 4. Schematic diagram of the SIMGI (adapted from Barroso *et al.* 2015).

powerful software. The parameters for the various culture conditions are saved in a database and loaded directly onto the system in order to replicate exactly the experiments already ran (O'Donnell *et al.* 2018). The principal novelty is the ability of the software to remotely supervise the fermentation and to control parallel experiments managing up to 32 bioreactors. The working volume of each glass bioreactor can be adjusted to desired conditions, up to a minimum of 50 mL. Besides, different devices permit superior performance, likewise: (i) a set of mechanical impellers moving up to 2000 rpm, that allow to cultivate high density cell cultures or viscous media; (ii) an antifoam system integrated with level sensors; (iii) gas spargers regulated by four mass flow controllers; (iv) six digital adjustable speed pumps or micro addition valves (microliters); (v) selectable autotuning adaptive PID control that regulate the process conditions change during fermentation; (vi) a liquid-free perfused peltier device to control and quickly adjust temperature; (vii) an electrical condenser able to maintain a pure atmosphere; (viii) an optical micro camera to monitor confluency of cell culture (Lattermann and Büchs 2015). As reported in Table 1, Levi and Lesmes (2014) used Mini-Bio systems to develop a model that mimics the conditions of the elderly alimentary canal, useful for studying foods and oral formulations developed to meet geriatric needs. Mini-Bio systems have the cons to be less cost accessible, but the pros to be flexible, logistically fitting under a lab hood and a high reproducibility due to rigorous parameters setting and control.

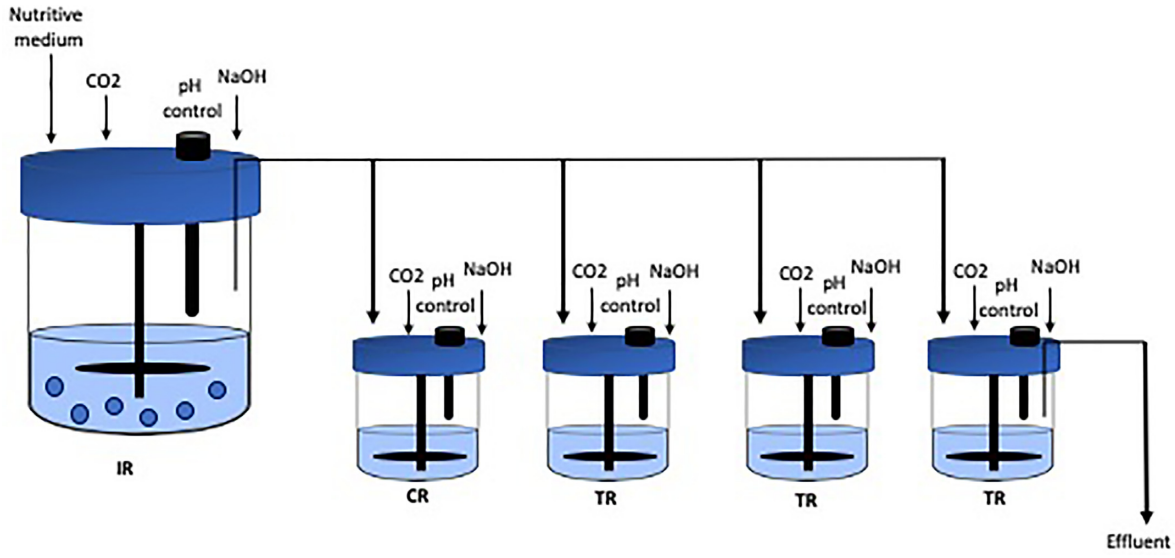

Figure 5. Design of the Polyfermentor Intestinal Model (PolyFermS): effluents of the inoculum reactor (IR) were used to feed a set of second-stage control (CR) and test (TR) reactors (adapted from Zihler Berner *et al.* 2013).

The smallest intestine *in vitro* model (TSI)

The TSI model is a DFM made of five reactors running with a minimum volume of 12 mL each, that simulate the transit through the small bowel (Cieplak *et al.* 2018). Each reactor is sealed in a PVC chambers and together are comprised in a box where the temperature is constantly set by a liquid stream from a circulating water tank heated by a thermostat serpentine. Atmosphere is established and kept stable by constant N_2 bubbling or traditional anaerobiosis catalyst. Agitation of the media is done by a plate magnetic stirrer divided in five-unit. A dialysis chamber simulates the absorption of nutrients. Reactors are isolated with a septic cover through which the pH probe and the needle for the addition of pancreatic juices can be introduced, along with sampling and input and output for the dialysis chamber (Cieplak *et al.* 2018). The main parameters are controlled by a computer, for example pH regime is monitor and alkali and acids are dosed automatically through a specific script coded in Matlab software (The MathWorks, MA). Distinct artificial gastric and intestinal fluids are used to replicate the electrolytic composition and osmotic pressure likewise that occur *in vivo* (Minekus *et al.* 2014). As shown in Table 1, Cieplak *et al.* (2018) tested in the TSI model the behavior of three putative probiotic *Lactobacillus* strains, and by means of culture-dependent microbiology demonstrated the strains' survival passing through the upper GIT. TSI has the cons to be less realistic and reproducible due to less strict environmental settings, but the pros to be logistically flexible and operator friendly.

DEFINED MICROBIAL COMMUNITIES FOR *IN VITRO* INTESTINAL MODEL

Most *in vitro* intestinal models use a fecal suspension as an inoculum, therefore the possibility to create a complex reproducible microbial community is limited (Fehlbaum *et al.* 2015). Furthermore, the main obstacle is the complexity of GM. In fact, more than 2000 bacterial species reside in the gut (Chong *et al.* 2019), combined with interpersonal diversity generating a multifaceted entity (Lederberg 2000). To improve GM resemblance, defined microbial populations have been suggested as models to study intestinal ecology (De Roy *et al.* 2014). Schäpe *et al.* (2019) proposed an extended basic human GM (SIHUMIx) for in *in vitro* model applications. This mock microbial consortium is composed of eight bacterial species (*Anaerostipes caccae*, *Bacteroides thetaiotaomicron*, *Bifidobacterium longum*, *Blautia producta*, *Clostridium butyricum*, *Clostridium ramosum*, *Escherichia coli* K-12 and *Lactobacillus plantarum*) that drive the main metabolic events achieved *in vivo* in the gut (Becker *et al.* 2011). The use of a basic model microbiota, in comparison to fecal microbiota, conveys clearer the assessment of the influence of ecological inputs on the composition and function of the reproduced GM (Guzman-Rodriguez *et al.* 2018). The main limitation of simplified microbial communities is the minor stability over long-time procedure than complex ones (Vandeputte *et al.* 2016).

CONCLUSION

Intestinal *in vitro* models are a useful tool to study the impact of the diet on the microbiota of distinct gut regions. The different models may represent a flexible arsenal to study the role of the different component of the diet to the microbiota shift of distinct gut regions. However, it is necessary to know characteristics, advantages and limits of each model to choose the most suitable for the study to perform. The different gut models discussed in this minireview vary in complexity, reproducibility and versatility, providing a showcase of options to conduct *in vitro* nutrition and microbial ecology research. The resemblance to reality is the major feature for a successful simulation and could be based both on the complexity of the mock microbiota or the environmental parameters of the reactors. Some of the approaches presented have been shown to have strict analogies to *in vivo* situations. Notwithstanding, there are several aspects that should be reinforced as the stability of the whole microbial ecosystem over mid- and long-term experiments or the technology to include host cells. Even if this research approach was born for understanding prebiotic effect of fibers, studies focusing gut microbiota interactions with prebiotic compounds within complex food matrices should be encouraged. This Minireview wanted to describe concisely the main features, the pros and cons of the most famous *in vitro* models, so could facilitate the choice to

take on which model fits better to the desired research aims and capabilities.

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