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Integrated multi-scale strategies to investigate nutritional compounds and their effect on the gut microbiota

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A complex relationship occurs in the intestine between the gut microbiota, diet, and host. The modulation of the gut microbiota composition and activity is a target for health-promoting strategies and possible novel therapeutic approaches. Different *in vitro* and *in vivo* strategies have been applied to elucidate mechanisms or functions of dietary compounds on the gut microbiota, health, and physiology of human. Recent research has shown the potential of combining *in vitro* models and *in vivo* investigations within a coherent strategy. This review highlights recent developments and limits of *in vitro* gut fermentation and cellular models, gnotobiotic animals, and human trials. Combination of experimental scales is illustrated for resolving the complex mechanisms of dietary iron on gut microbiota, health, and infections.

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Introduction

The human digestive tract is populated by a large number of microbes, whose cell and gene numbers have been estimated to exceed that of human cells and human genome by ten-fold and hundred-fold, respectively [1]. The composition and functionality of the gut microbiota reciprocally affect each other, and subsequently impact host physiology, shaping a complex interaction network directing gut health. Inter-individual differences in the gut microbiota and functions are dictated by many modulating factors including diet, host genotype, microbe–microbe and host–microbe interactions [2]. Identification of the wide range of metabolic activities mediated by the commensal microbiota has advocated modulation of the composition and metabolic activity of the microbiota as a potential health-promoting therapeutic target. Homeostatic maintenance of both microbe–microbe and host–microbe interactions is essential for a healthy microbiome and host. Perturbations in gut microbiota composition, referred to as dysbiosis, may represent an important mechanism of disease. It is, therefore, crucial to better understand the relationships between host, diet and the gut microbiota, starting by investigating the specific effects of the main components of the diet. Different strategies can be followed to elucidate the mechanisms or functions of dietary compounds on the gut microbiota, gut health, and the physiology of the human host. Recent research has shown the potential of combining different levels of complexity, *in vitro* and *in vivo* within a coherent strategy, to decipher functions and mechanisms [3–5].

With focus on recent findings, this review aims to present relevant strategies combining *in vitro* modeling and *in vivo* investigation, and combination thereof which are used to study complex effects of dietary compounds on the gut microbiota and gut health, and on host physiology at different scales.

In vitro models

In vitro gut modeling has gained momentum in recent years as a powerful strategy to isolate and investigate factors of the gut microbiota and evaluate specific effects on host cells. *In vitro* models allow the application of a highly controlled environment and the study of mechanistic effects of dietary, microbial, drug and physiological factors on gut microbiota at levels that cannot be reached in *in vivo* setup. *In vitro* models are particularly well suited for screening, for example, prebiotics or probiotics for special functions in the gut, before moving to *in vivo* investigation of effective conditions. In addition *in vitro* models reduce *in vivo* animal and human testing, owing to societal and ethical considerations, and this trend is likely to continue.

A range of systems has been developed to model fermentation of the gastro intestinal tract, from simple anaerobic batch culture systems in flasks to multistage continuous flow models (reviewed by [6,7]). These mainly target the colon which harbors the highest density of microbes, while models for the small intestine have been much

less studied, although this compartment has profound effects on various aspects of the host's physiology (El Aidy *et al.*, 2015, in this issue). Fermentation models have been combined with cellular models that are widely accepted for evaluating host microbiota interaction such as cytokines production, responses to pathogen, absorption and transport [8,9,10•,11]. However, it should be emphasized that all models, fermentation and cellular, are different with respect to conditions and output, and selection of the most suitable model should be done carefully, considering their features and limits in relation with the scientific question addressed. Lately a number of new or improvements of existing models, for example, adding modularity and enhancing target and accuracy, highlights the potential to design innovative, reliable and accurate models for gut research. A few highlights of such improvements is given below.

An important pre-requisite for *in vitro* study is the rational selection of models and conditions, while keeping in mind that models are only representations of reality. A fermentor, for example, will likely develop a complex ecosystem even when inoculated with damaged microbiota. However, this may not be representative of the host situation due to the loss of some important phylogenetic clades or functions, or the artificial niche might not favor the same microbes as the host does. Therefore, a model should always be confronted with reliable *in vivo* data for composition, but also for functionality and dynamic response to perturbations (e.g. with antibiotic treatments for infection model) [12]. In many instances models are mainly selected based on the experience with or availability of particular technology, which may not be optimal. Additionally, special attention should be paid to selection of the fecal donor, application of protective conditions, and handling of fecal material from donor to reactor, as well as technique used for inoculation, and reactivation and cultivation of the gut microbiota. Significant longitudinal, along the gastro-intestinal tract, and latitudinal variations, between mucosal and luminal bacteria, have been reported in various studies [13,14]. Inoculation of most *in vitro* continuous models is done with a liquid fecal suspension. The use of fecal inocula only reflects the composition of luminal distal colon microbiota which is easily accessible. To date there is no reliable methodology for long term preservation of the overall viability and activity of microbiota needed to inoculate a model, only a fresh inoculum of highest quality derived from a single donor should be used to inoculate valid models [6,15]. A rapid washout of less competitive or slow growing bacteria occurs during continuous fermentation which may partly explain the slow stabilization lasting up to 36 days [16], and the lack of stability and low cell density of continuous gut fermentation models [17]. Inoculation of such systems also requires large amount of fecal material which is the most important limit to infant colon modeling [18]. In most models it is difficult to reproduce both the planktonic (free-cell) and sessile (biofilm-associated) bacterial populations in the colon. Two solutions based on immobilization have been developed to address these problems: a process of immobilization of fecal microbiota in gel beads mimicking cell density and competition of *in vivo* gut microbiota [6,19], and incorporation of a mucosal environment, either using mucin gel in a classical continuous model [20] or mucin-covered microcosms in the SHIME model [21]. One drawback of the latter systems is the rapid digestion of the mucus structure leading to limited operation time of models while soluble mucin can be supplied continuously in the intestinal fermentation medium. Novel models, based on the new PolyFermS platform, were recently validated with immobilized human and swine gut microbiota [17,22•]. These models are composed of a first-stage inoculum reactor seeded with immobilized fecal microbiota and used to constantly inoculate (5–10%) parallel operated systems and set to mimic different colon sections. PolyFermS models can be expanded to various configurations, allowing the comparison of different treatment effects and a control inoculated with the same microbiota [23,24]. This model is particularly well suited for parallel screening and mechanistic investigations of gut microbiota factors.

Future model development should aim for work towards down-scaling and miniaturization; interfacing gut dynamic simulator with cell cultures; and improving control by automation. The host–microbiota interaction (HMI) module [11] and biomimetic 'human gut-on-a-chip' microfluidic device [25] are recent examples of co-culturing a gut-representative microbial community, respectively a strain of *Lactobacillus rhamnosus* GG, with enterocyte-like cells and may therefore contribute to the mechanistic understanding of host–microbiome. This type of construct would facilitate more precise investigations of the effects of specific treatments at the level of the luminal microbial community and of the host surface colonization and signaling, which is complicated by physical and ethical limitations relating to sampling in human studies.

Animal models

Investigations of gut microbiota/host interactions have extensively been performed in animal studies. Animal facilities usually provide a highly controlled environment for experiments while diet intake and composition can be also effectively controlled and recorded. Therefore, animal models represent a robust tool to study the impact of diet and specific food components on the gut microbiota. Most of the current knowledge of how diet influences the gut microbiota has been obtained in conventional rodents (mainly mice and rats). They offer the possibility of relatively accessible and repeatable experiments ensuring a sufficient number of animals for further valuable statistical analyses. Genetically modified animals and