

## Review

# Recent advance of *in vitro* models in natural phytochemicals absorption and metabolism

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## ABSTRACT

Natural phytochemicals absorption and metabolic process are mainly in the human gut. Simulating the absorption and metabolism of natural phytochemicals *in vitro* to predict the rate and degree of absorption of natural phytochemicals provides convenience for many researchers. However, in this process, many physiological factors *in vitro* are affected, such as stomach and intestinal juice composition, pH, intestinal transmission rate and so on. In recent years, the research methods have gradually improved to make these models more suitable for the natural phytochemicals absorption process, *in vitro* simulation models have become an essential means to study natural phytochemicals absorption. Therefore, this paper introduces the advantages and disadvantages of commonly used *in vitro* simulation models of natural phytochemicals absorption and metabolism, as well as briefly introduces the working principle of each model. To provide a theoretical basis for simulating natural phytochemicals absorption *in vitro* and development and utilization of natural phytochemicals.

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## 1. INTRODUCTION

Natural phytochemicals, often referred to as phytonutrients, are natural bioactive components rich in foods like vegetables, fruits, whole grain products, nuts, and seeds [1]. The most common type of food plant chemicals include polyphenols, carotenoids, flavonoids, coumarin, indole, isoflavone, lignans, organic sulfur, catechins, phenolic acid, styrene, isothiocyanates, saponins, procyanidins, styrene acrylic element, anthraquinone, ginseng saponin and so on [2–4]. The research shows that natural phytochemicals have cancer prevention, oxidation antiviral ability [5, 6]. Natural phytochemicals provide unique and renewable resources for the discovery of potential new functional foods and new biological activities [7–9]. It changes in the mouth, stomach and intestine, and is finally absorbed by the lymphatic circulation or blood through the intestinal epithelial cells, some that are difficult to digest can reach the colon and be broken down by bacteria [10] (Figure 1). It is necessary to understand the structure of the *in vitro* gastrointestinal model, as well as absorption characteristics, to improve the theoretical basis for the development of new resource foods.

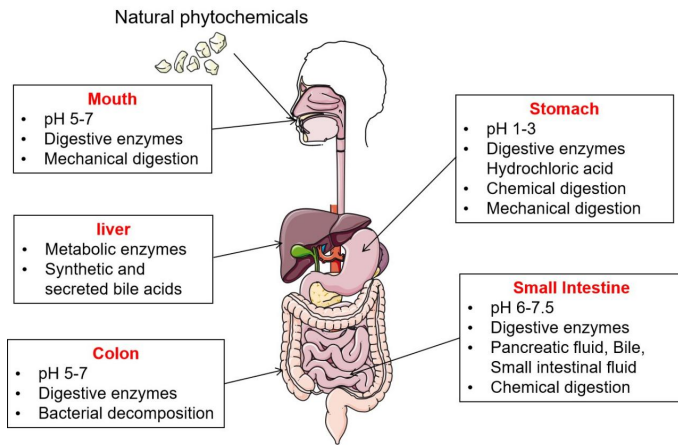
*In vitro* system is a research system developed according to the physiological function of human gastrointestinal tract [11], which can simulate the digestion and absorption of natural phytochemicals *in vivo*. In the current research, it is usually

characterized by substitution, simplification and accuracy [12]. In the early stage of natural phytochemicals development, *in vitro* gastrointestinal absorption is an ideal method to simulate natural phytochemicals absorption and metabolism, which can accurately predict the reaction process of natural phytochemicals *in vivo*, thus improving the success rate of natural phytochemicals developers and natural phytochemicals absorption scholars [13–16]. At present, most of the methods used are cell models, everted gut sac, *in situ* intestinal segments, and Ussing chamber [17–19]. The liver is one of the metabolic organs of human body, and most natural phytochemicals must be removed by liver metabolism to study metabolism [20, 21]. To research liver metabolism and liver toxicity *in vitro*, including primary hepatocytes, immortalized cell lines (Hep3B, HuH7, HepG2, and HepG), sperm liver slices and so on [22–24]. With further development, many dynamic models have been developed [25], such as the dynamic gastrointestinal model, different atrioventricular models are used to simulate different states of intestinal absorption, and the influence of intestinal velocity on natural phytochemicals absorption and metabolism is further simulated based on the applied force [26, 27]. The latest 3D model enables researchers to observe the absorption of intestinal epithelial cells more intuitively [28, 29]. This paper introduces and discusses the research progress of natural phytochemicals absorption and metabolism models *in vitro*, and evaluates their advantages and disadvantages.

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**Figure 1** Absorption and metabolism of natural phytochemicals in human body.

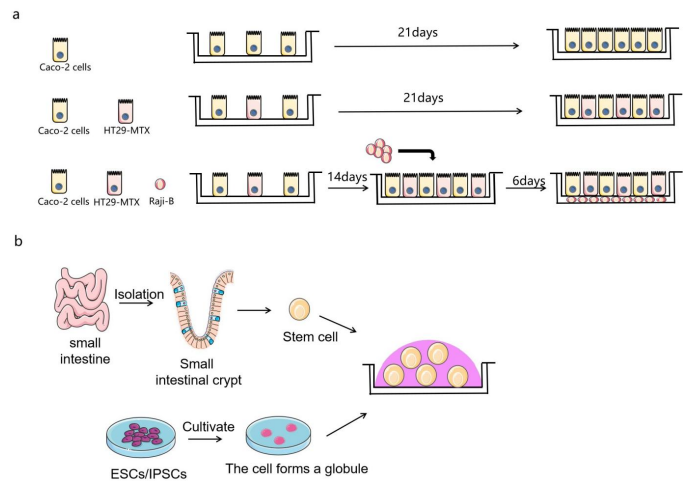
## 2. THE *IN VITRO* MODELS FOR NATURAL PHYTOCHEMICALS ABSORPTION

### 2.1. Cell model

The intestinal epithelial cells are the absorption cells of intestinal epithelium, which constitute most of the intestinal epithelium [30, 31]. Water, inorganic salts, vitamins and other nutrients are absorbed through intestinal epithelial cells [32, 33]. There are many cell models to study natural phytochemicals absorption, including (Caco-2, HT29-MTX, MDCK, TC-7 Cell models and so on ) strike a balance between accuracy (simulating a natural reaction environment) and efficiency [34] (Table 1). And the Caco-2 cell line is the most commonly used model of intestinal epithelial detection system *in vitro* [35–37]. It derived from human epithelial colorectal adenocarcinoma cells, when Caco-2 cells differentiate and polarize, their structure and biochemical function are similar to intestinal cells, very close to normal human intestinal epithelium [38]. Therefore, Caco-2 cell model is a reliable *in vitro* model to study the intestinal absorption and metabolism of natural phytochemicals, and is also a powerful tool to clarify the mechanism of natural phytochemicals absorption [39, 40]. Boyer et al. [41] used Caco-2 cells to test the uptake of quercetin 3-glucoside and quercetin 3-glucoside as purified compounds and extracts from whole onion and apple skins. It concluded that Caco-2 cells absorbed a small amount of quercetin 3-glucoside from extracts from apple skins, but the absorption of quercetin 3-glucoside was not detected. Chen et al. [42] evaluated the absorption rate and mechanism of Avenanthramides (AVNs) by using the Caco-2 cell model, AVNs transported the Caco-2 monolayer by paracellular diffusion and were affected by monoamine oxidase and efflux transporters (P-gp, MRP2) during absorption. In conclusion, transported by passive diffusion (especially with high permeability), the permeability measured by cell model is consistent with that of human body.

The primary cells obtained through purification and isolation will also lose their original functions and characteristics after 2D culture *in vitro*. The emergence of 3D co-culture solves these problems well [43–46] (Figure 2 a). The co-culture of Caco-2 cells and HT29-MTX cells, compared with Caco-2 cells, the connections

between HT29-MTX cells are not so tight, so this advanced model is considered to be a more suitable model than Caco-2 cell monolayer model [47, 48]. In addition, A triple co-culture model was proposed, including Caco-2, Raji-B and HT29-MTX cell, is closer to intestinal epithelium because Raji-B can induce the phenotype of M cells, and HT29-MTX cells are similar to goblet cell [49]. These three kinds of cells are the same as the human intestine, and they are simulated closer to the human body [50]. Selby-Pham et al. [51] use co-cultures of Caco-2 and HT29-MTX-E12 cells were used to determine the apparent permeability (Papp) of dietary phytochemicals extracts in the single layer of co-cultured cells. Organoids culture is another type of 3D culture, embryonic or adult stem cells are cultured *in vitro* to proliferate and differentiate into organ-like 3D cell clusters with certain morphological structure and functions [34]. The small intestine organoid, in particular, is currently an advanced technique *in vitro* model studies of physiological and pathological mechanisms (Figure 2b). It can replicate tissue morphology and physiological functions *in vivo* by maintaining key physiological conditions and functions of the gut over a long period of time (e.g. crypt and villi formation, cytochrome P-450 metabolic activity, mucus secretion) [52]. Today, several 3D organ-type cultures have been developed to replicate various organs involved in GIT digestive tract, such as pancreas [53], stomach [54], and intestine [55]. Recently, remarkable progress in developing 3D cell culture platforms have been achieved by mimicking fundamental physiological cues present in the *in vivo* native tissue.



**Figure 2** Schematic illustration of 3D co-culture (a); Schematic illustration of organoid culture of small intestine (b).

### 2.2. Ex vivo model

*Ex vivo* model of excising intestinal segments provides a theoretical means for human intestinal absorption [71]. They include everted intestinal sac [72] and the Ussing chamber [73]. The main advantages of using human intestinal tissue are to maintain the shape and structure, as well as the expression of enzymes in natural phytochemicals metabolism and transporters reflecting the state of the body [74]. The U.S. FDA recommended that excised human intestinal tissue be used in osmotic research *in vitro* [75].

Table 1 Characteristics of cell models.

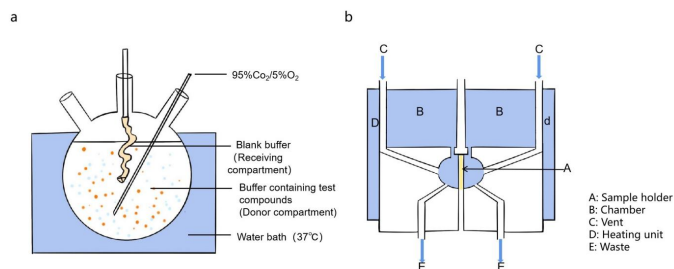
Cell model	Cell Source	Morphology	Advantage	Dis-advantage	Ref.
<b>Caco-2</b>	Human colon adenocarcinoma cell line	Polarized monolayer with strong tight junctions, apical brush borders and microvilli	<ul style="list-style-type: none"> <li>High accuracy in replicating the enterocytic phenotype</li> <li>Long-term cell viability</li> <li>Good reproduction of the active pathways</li> </ul>	<ul style="list-style-type: none"> <li>Long differentiation time (3 weeks)</li> <li>Lack of physiological factors (mucus, bile salts, cholesterol)</li> <li>Underestimation of paracellular transport</li> </ul>	[44] [56] [57] [58]
<b>TC-7</b>	Caco-2 subclone	Polarized monolayer with tight junctions, apical brush borders and microvilli	<ul style="list-style-type: none"> <li>Fast cellular growth</li> <li>Great homogeneity ensuring consistent results</li> <li>Good reproduction of the passive pathways</li> <li>Good correlation with Caco-2 for passively</li> </ul>	<ul style="list-style-type: none"> <li>Tighter junction than Caco-2</li> </ul>	[44] [59] [60]
<b>MDCK</b>	Canine distal renal tissue -derived line	permeability for drugs with low passive transcellular transport is higher than Caco-2 but still underpredicted	<ul style="list-style-type: none"> <li>Short culture time (3-5 days)</li> <li>Low costs</li> <li>Possibility to obtain several sub-clones</li> <li>Good reproduction of the passive pathways</li> </ul>	<ul style="list-style-type: none"> <li>Non-human origin</li> <li>Non-intestinal origin</li> <li>High variability</li> <li>Only intestinal epithelial cell transport studies can be performed</li> </ul>	[44] [61] [58] [62]
<b>HT-29</b>	Human colon adenocarcinoma cell line	Polarized monolayer with tight junctions, apical brush borders with sparse and short microvilli or a mucus layer	<ul style="list-style-type: none"> <li>No expression of P-gps</li> <li>Possibility to obtain oblet-like cells</li> <li>Possibility to study the mucus layer</li> </ul>	<ul style="list-style-type: none"> <li>Non-small intestinal origin</li> <li>The differentiation process occurs only under specific culture conditions and require a long time</li> <li>Absence of the major metabolizing proteins</li> </ul>	[44] [63] [64]
<b>IEC</b>	Small intestinal crypt- derived rat cell line	Polarized monolayer with poorly developed junctions, brush border, apical microvilli and an amorphous substance similar to the basement membrane	<ul style="list-style-type: none"> <li>Intestinal origin</li> <li>Good reproduction of the paracellular path</li> <li>Useful model for studying cholesterol synthesis and the role of growth factors in epithelial layers</li> </ul>	<ul style="list-style-type: none"> <li>Crypt origin</li> <li>Long differentiation time (~3 weeks)</li> <li>Instability (rapid loss of differentiation markers)</li> <li>No transporting proteins</li> </ul>	[44] [65]
<b>HIEC</b>	Small intestinal crypt- derived human cell line	Polarized monolayer with poorly organized tight junctions, apical brush borders and dense microvilli	<ul style="list-style-type: none"> <li>Pore sizes and distribution similar to the human small intestine</li> <li>Long-term proliferation capability</li> <li>Ability to differentiate into several cell types</li> <li>Great accuracy in predicting the human paracellular uptake</li> </ul>	<ul style="list-style-type: none"> <li>Interindividual variations</li> <li>Ethical issues prohibiting their use in several states</li> </ul>	[44] [66] [67]
<b>3D cell model</b>	Human colon adenocarcinoma cell lines	Polarized monolayer with tight junctions, apical brush borders, microvilli and mucus layer	<ul style="list-style-type: none"> <li>Mucus production</li> <li>High permeability for passively absorbed drugs</li> <li>Possibility of modifying the permeability barrier of the cell monolayer (flexibility)</li> <li>Good reproducibility</li> </ul>	<ul style="list-style-type: none"> <li>High costs</li> <li>High variability (source, size, shape)</li> <li>Relatively complex fabrication process</li> </ul>	[44] [68–70]

### 2.2.1. Everted intestinal sac

Wilson and Wiseman put forward the model of everted intestinal sac, the basic steps to take a certain length of intestine (e.g. intestines of rat, dog) end ligation, layer and flip the intestine mucosa and serosa layer, ligation on the other side, the formation of intestinal sac, to the blank buffer into my gut sac (receptor side), and then puts intestinal sac containing drug buffer (donor), access to the entire unit carbon gas put in 37 °C constant temperature water bath, Periodic sampling from the intestinal sac for examination [76] (Figure 3a). It can use to explore the effects of absorption, metabolism or transformation in gastrointestinal tract, efflux transport, interaction between natural plant compounds and absorption of efflux transport regulators [77]. In the latest research, Wang et al. [78] discussed the absorption of helicid in different intestinal segments based on the rat model of turning intestinal sac *in vitro*, and concluded that duodenal segment was the main part of absorption and metabolism of helicid in different intestinal segments. Liu et al. [79] studied the intestinal absorption properties of Polygonum orientale extract in normal rats and myocardial ischemia rats by using intestinal capsule eversion model, the results showed that all components in polygonum orientale extract could be absorbed by the intestinal capsule. Therefore this method is that because of the small size of the serosa layer in the intestinal sac, the drugs in the mucosal layer can be concentrated on the serosa side after being transported through the intestinal segment, which is easy to detect [80], the disadvantage is that the residual mucosa muscle layer may lead to drug adhesion, which makes the determination result low, intestinal fluid stagnation, and morphological damage of everted intestinal tissue, etc [81, 82].

### 2.2.2. Ussing chamber system

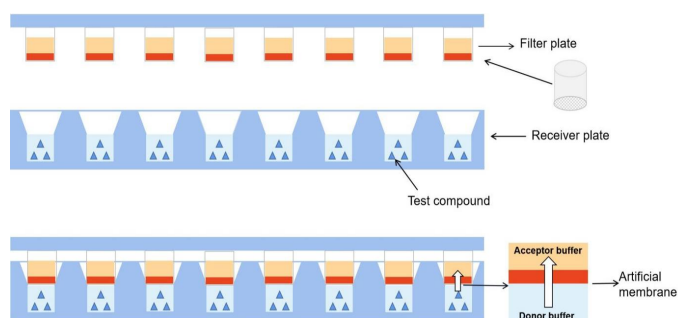
The Ussing chamber system was made up of a chamber and a perfusion system [83]. The operation procedure is to take the target isolated intestine segment and cut it into an appropriate intestine segment, which is fixed in the sample holder (a) and installed between two diffusion pools (b). One side of the two diffusion pools was filled with drug-containing buffer (donor side), and the other side was filled with blank buffer (recipient side), and carbon gas was passed through the vent (C) to maintain intestinal tissue activity [84, 85] (Figure 3b). Cardinali et al. [86] used the Ussing chamber system technique to study the intestinal absorption of *verbascoside triosaponin*. Rtibi et al. [87] used this system to predict the absorption of *ncarob pod* aqueous extracts on gastrointestinal transit (GIT) and intestinal epithelium permeability. This method can simulate the mucosal layer, one of the main absorption barriers, and make up for the deficiency that the everted intestinal sac method is easy to change the cell morphology and affect the permeability [88], the experimental period is short, simple operation and strong controllability, and is more suitable for rapid determination of natural phytochemicals intestinal permeability [89]. However, the sample processing process is complicated, which reduces the activity of enzymes in intestinal tract and may affect the permeability of some natural phytochemicals [90]. Despite these defects, *Ex vivo* methods are simple and widely used in the design and testing of potential natural phytochemicals.



**Figure 3** Schematic illustration of everted intestinal sac (a); Schematic illustration of Ussing chamber (b).

### 2.3. Artificial membrane model

PAMPA model (Parallel Artificial Membrane Assay) was established by Kansy et al. [91]. It is usually used to simulate different biomembranes by diffusing different lipid solutions on sandwich structures, and then to predict transmembrane absorption by measuring permeability [92] (Figure 4). This is another method for determining gastrointestinal permeability in the screening of new natural phytochemicals, which has the advantages of high throughput, low cost, convenient and flexible detection means and so on [93]. Petit et al. [94] used hexadecane membrane parallel artificial membrane (HDM-PAMPA) to measure the passive intestinal absorption of *Angelica archangelica*, *Waltheria indica*, *Pueraria montana* var. *lobata*. The effective passive permeability NPs obtained by parallel artificial membrane is also worthy of giving priority to the separation of bioactive compounds in the early stage of drug discovery, so as to verify whether it is known that the biological activity principle of medicinal plants has good potential for passive absorption [95]. However, there are some limitations, such as only a single passive diffusion mechanism, unable to accurately predict natural phytochemicals permeability through active transport mechanism, and inability to simulate complex *in vivo* environment, which leads to inaccurate results [96]. In recent years, researchers have optimized the reference drug, phospholipid ratio, buffer, pH value, concentration and permeation time on the basis of traditional PAMPA to get a more accurate model [97, 98] (Table 2). It can also be combined with the Caco-2 cell model and MDCK cell model, which is also an effective solution to avoid the above problems [99].



**Figure 4** Schematic illustration of parallel artificial membrane permeability assay (PAMPA).

**Table 2** Characteristics of PAMPA models.

Assay	Main lipid constituents (w/v% dissolved in solvent)	Solvent	Donor–acceptor buffer	Buffer additive	Ref.
Egg-PAMPA	10% egg lecithin, cholesterol	n-Dodecane	Same	Bile salt in donor	[90]
HDM-PAMPA	100% n-hexadecane	n-Hexadecane	Same	None	[99]
DOPC-PAMPA	2% dioleoylphosphatidylcholine (synthetic)	n-Dodecane	Same	None	[100]
BM-PAMPA	0.8% phosphatidylcholine, 0.8% phosphatidylethanolamine, 0.2% phosphatidylserine, 0.2% phosphatidylinositol, 1% cholesterol	1,7-Octadiene	Same	None	[101]
DS-PAMPA	0.8% phosphatidylcholine, 0.8% phosphatidylethanolamine, 0.2% phosphatidylserine, 0.2% phosphatidylinositol, 1% cholesterol	1,7-Octadiene	Same	None	[101]

## 2.4. Dynamic gastrointestinal model

*In vitro* gastrointestinal simulation system is a biological research system that simulates food digestion behaviour based on physiological functions of the human gastrointestinal tract [102]. It is often used for the substitute test research of living organisms. It has the advantages of simple operation, convenience, safety, rapidity and so on [103]. Most gastrointestinal simulation systems add amylase, mucin, inorganic salts, pepsin, organic acids, bile, trypsin and simulated oral cavity (optional simulation items) and gastrointestinal physiological conditions in order in the container, based on the physiological conditions of human body, a gastrointestinal simulation system is constructed [104]. The dynamic gastrointestinal simulation system fully considers the influence of gastrointestinal micro-ecosystem on food digestion in the application research of simulating human gastrointestinal digestion behaviour [105]. The simulation is higher, and it is close to the real gastrointestinal digestion of the human body; it can be well used for *in vitro* gastrointestinal simulation of food digestion [103]. In recent years, commonly used *in vitro* gastrointestinal models are The TIM Model (TNO Gastro-Intestinal Model) [106] and SHIME (The Simulator of the Human Intestinal Microbial Ecosystem) [107]. People have explored many valuable studies on natural plant nutrients by using them.

TIM model is a multi-chamber dynamic model, Tim-1 is the most frequently used configuration of the Tim platform [108] (Figure 5a). It aims to simulate the dynamic conditions in gastrointestinal tract, such as time, pH change, real secretion, digestive juice composition and other physiological parameters. It can also stimulate the flow of human body temperature, saliva, gastric juice and pancreas, and simulate specific conditions, such as age, diet type and human health or disease state [109]. TIM-1 model was used to evaluate the dynamic bioavailability of curcumin in Pickering emulsion with and without encapsulation. Combined with two *in vitro* models, the influence of Pickering emulsion with stable starch particles on the bioavailability of curcumin was fully revealed [110].

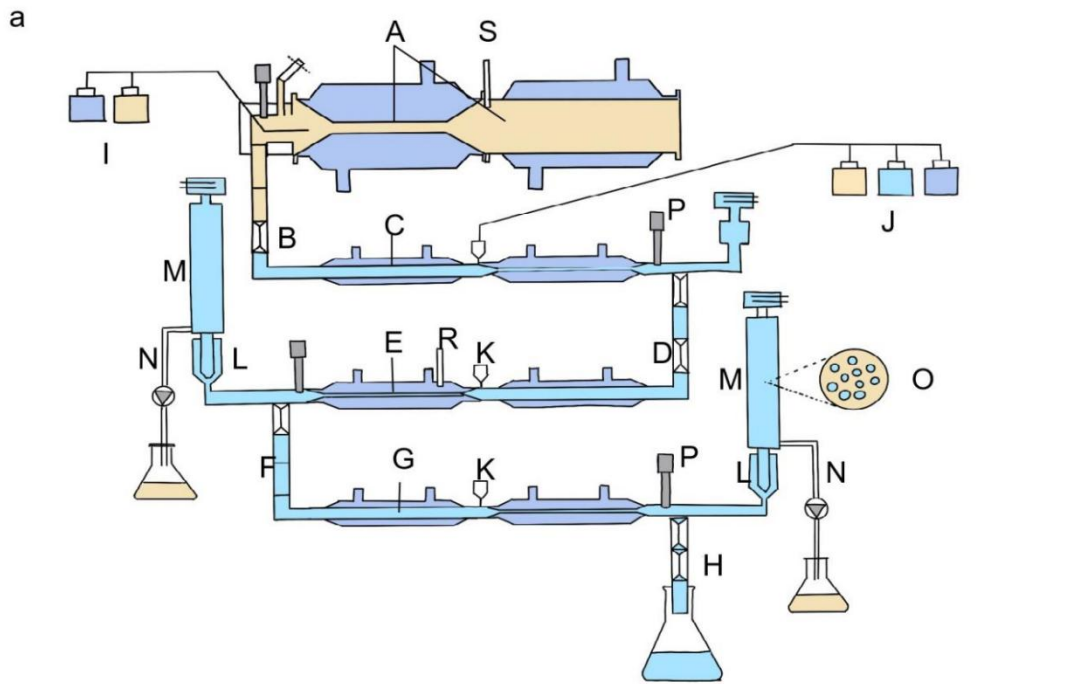
SHIME model is one of the few intestinal models that simulate the whole gastrointestinal tract, including stomach, small intestine

and different colon areas [111]. It combines the conditions of the upper digestive tract, resulting in five continuous chambers that simulate the upper digestive tract (stomach, small intestine) and the lower digestive tract (ascending, transverse and descending colon) [107] (Figure 5b). Van den Abbeele et al. [112] used these two models SHIME and TIM, how long-chain arabinosyl (LC-AX) and inulin (IN) regulate the production of short-chain fatty acids and the composition of Bifidobacterium were compared. Truchado et al. [113] used Mucus-SHIME model to study the regulatory effect of long-chain arabinosyl (LC-AX) on intestinal and mucosal microflora. LC-AX may be potentially beneficial to host health by stimulating the abundance and metabolic activity of Bifidobacterium.

At present, there are still many shortcomings and limitations in domestic *in vitro* gastrointestinal simulation systems, and the change of a single factor (e.g. temperature, pH, and fermentation time) will have a great influence on the research results. Therefore, it will be a great challenge to establish a complete and satisfactory *in vitro* human gastrointestinal simulation system to study the uptake of phytochemicals.

## 2.5. Intestinal 3D *in vitro* modelling

Organotypic intestinal models are an attractive middle ground between *in vitro* and *in vivo* systems because they include the three-dimensional architecture of the gut wall while still providing easily controllable experimental parameters [114] (Figure 6). Microfluidic tissue-on-a-chip devices provide powerful alternatives for modelling physiological systems. Such devices show promise for use in GI research [115]. The combination of fluid flow and 3D reconstruction of intestinal microstructure induced intestinal epithelial cells to behave more like native tissue [116]. Based on the static Transwell model, gut on a chip microfluidic devices have been developed that allow a continuous infusion of media on opposite sides of the cellular porous membrane that represents the intestinal epithelial barrier [117]. The addition of microfluidics to these devices improves cell viability and longevity, continuously removes toxic cellular waste, and allows control of nutrient delivery [118]. More recently, microorganisms have been incorporated into a



A. gastric compartment; B. pyloric sphincter; C. duodenal compartment; D. peristaltic valve; E. jejunal compartment; F. peristaltic valve; G. ileal compartment; H. ileal-cecal valve; I.gastric secretion; J. duodenal secretion;K. bicarbonate secretion; L. pre-filter; M.filtration system;N.filtrate with bio-accessible fraction; O. hollowfiber system (cross section); P. pH electrodes; Q.level sensors; R. temperature sensors; S. pressure sensor

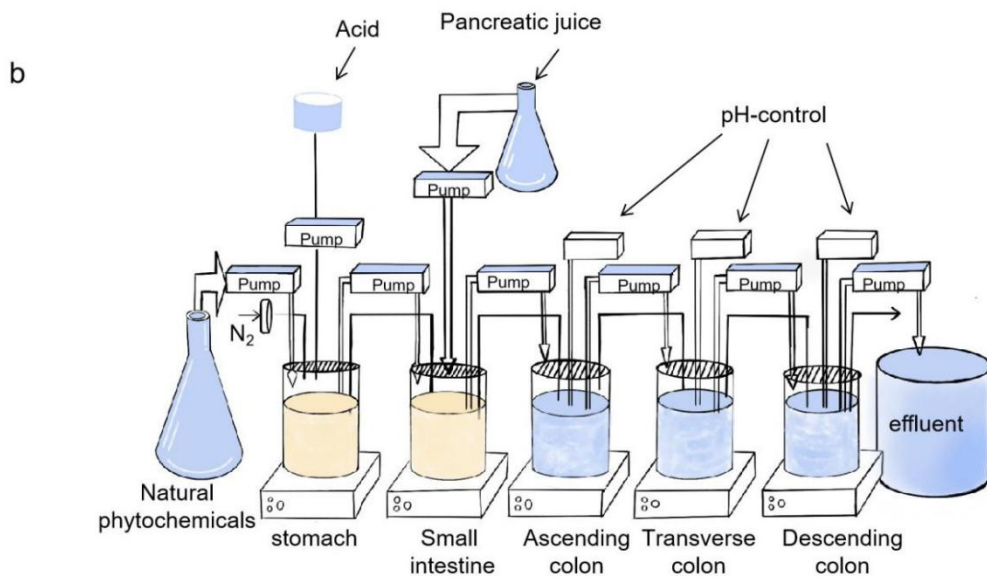
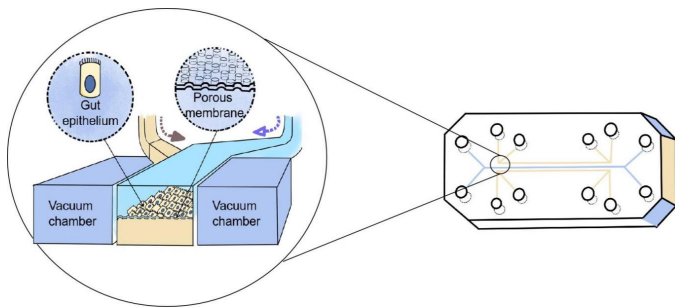


Figure 5 Schematic illustration of TIM-1(a); Schematic illustration of SHIME model.

number of *in vitro* microfluidic gut models by generating oxygen gradients between microfluidic channels [119]. In fact, intestinal 3D *in vitro* modelling has been tested with solid biopsies of the intestine. Yissachar et al. [120] adjusted the gas-liquid interface culture model to a microfluidic format to prevent the loss of mucosal structure at 40 h. Dawson et al. [121] reported microfluidic culture of perforated human intestinal segments, in which the luminal and serosal sides were infused with medium for 72 h. Richardson et al. [115] used a microfluidic organotypic device (MOD) that enables media flow with differential oxygen concentrations across luminal and muscular surfaces of gut tissue *ex vivo*. Tissue was shown to be viable for 72 h and lowering oxygen concentration to a more physiologic level impacted bacterial populations. Magnetic alginate microspheres (MAM) and chitosan as magnetic alginate microspheres (CAM) were studied by droplet microfluidic device, under the condition of moderate control release, this microfluidic technology for micro/nano particles of controllable synthesis provides a convenient and efficient fluid design [122], for natural phytochemicals controlled release, and slow-release provides a potential choice. Although the organotypic intestinal culture model lacks the nerve, immune and muscle components of the intestinal wall, it can simulate the dual flow of the intestinal lumen and vascular system, and more closely reproduce the *in vivo* physiology of the intestinal wall than other *in vitro* models [123]. Organotypic intestinal culture models can clearly demonstrate a new technology paradigm that can open applications in the field of food technology, or for the analysis of some types of natural plant materials.



**Figure 6** | Schematic illustration of microfluidic tissue-on-a-chip devices.

### 3. THE *IN VITRO* MODELS FOR NATURAL PHYTOCHEMICALS METABOLISM

#### 3.1. Immortalized cell lines

Human hepatocytes are considered the gold standard of the human liver model in toxicology research [124]. There are also frozen the human hepatocytes on the market. However, these cells have a high cost, limited availability and significant differences in CYP activities among individuals [125]. Most of the available liver-derived immortalized cell lines do not possess phenotypic characteristics of the liver tissue [126]. Common immortalized liver-derived cell lines in use are Fa2N-4, HepG2, Hep3B, PLC/PRFs Huh7, HBG, and HepaRG [127]. HepG2 cell is a representative established hepatoma cell line [128]. The expression of cytochrome P450 (P450) enzymes, transporter proteins, and transcription factors

were stable in differentiated HepaRG cells over a period of 6 weeks when cultured with DMSO [129]. In the identification of plant active components with protective effects on the liver, Thabrew et al. [130] incubated various hepatic toxins with HepG2 cells in 96-well microtitre plates, and used the crude extract of a known protective liver plant *Osbeckia aspera* and two pure established protective liver plant compounds-catechin and silymarin, tested the protective effect of these drugs on a toxic injury. Mohammed et al. [131] have analyzed the antiproliferative effect of ethyl acetate fraction of *Anethum graveolens* L. (dill) on the HepG2 cell line. HepG2 continues to find application in the evaluation of a range of nutritional factors, including spice constituents, soybean derivatives and tuber essential oils, as well as providing a means of evaluating medicinal materials from natural sources. Still, the expression of liver-specific functions in HepG2 cell is still much lower on average than that of primary hepatocytes, and they represent a phenotype from a single donor, thereby reducing their predictive value for the human population [132].

#### 3.2. Precisely cut the liver slice model

PCLS (Precise cut liver slices) for humans or other animals (e.g. mice) retain the structure and cellular components of the natural liver, and it involves the cutting of viable, ultrathin (around 100-250  $\mu\text{m}$  thick) liver slices. Representing an improved system for studying liver fibrosis compared with two-dimensional single culture or co-culture [133]. As this may be an interesting tool not only for the investigation of hepatotoxic and protective effects but also for bio guided fractionations schemes, the usefulness of PCLS was compared with an *in vivo* test of liver function. [134]. However, the PCLS model has some limitations. For example, there is a lack of infiltrating immune cells to regulate the disease process [135]. In addition, liver slices are unsuitable for rapid screening applications [136]. A study successfully used isolated liver slices treated with bile acid to simulate cholestatic liver injury, and finally evaluated the mechanism of liver fibrosis [137]. In addition, PCLSs culture has opened up a new way for high-throughput experiments and provided a new strategy for studying the specific molecular characteristics of metabolic genotypes [138].

### 4. CONCLUSION AND PROSPECT

The properties of natural phytochemicals, their effects on the body, and the study of their entry into the body are crucial. To date, poor oral bioavailability and inefficient intestinal absorption have been a hindrance to the development of natural substances. Therefore, in the early stages of the discovery and development of natural phytochemicals, several *in vitro* culture systems simulating intestinal epithelium have been widely used to predict intestinal permeability more quickly, while reducing animal testing and thus accelerating the development of natural phytochemical foods [139]. 2D models are currently the most standardized platforms because they allow for cost-effectiveness and high-throughput screening. In particular, Caco-2 cells have been accepted as the gold standard because of their ability to approximate the intestinal cell phenotype. These conventional cell cultures have a good quantitative correlation with the absorption fraction of natural phytochemicals transported through cells in humans, but the results

were not accurate enough due to colonic origin and changes in the expression of basic metabolic and transport proteins. The correlation between co-cultured cells and *in vivo* data has improved. With increasing evidence that 3D multi-cell models *in vitro* can better reproduce the *in vivo* environment, models combined with fluid dynamics systems have made great progress in GIT research in recent years. However, there are many challenges to obtaining repeatable tests using these platforms. Therefore, the future realization of these aspects, as well as more vision-like models that include the entire microbiome, mucus layer, and other cell types (immune cells), will increase the robustness and predictive potential of these systems.

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