Overview and Comparison of Intestinal Organotypic Models, Intestinal Cells, and Intestinal Explants Used for Toxicity Studies

Marc Maresca, Philippe Pinton, El Hassan Ajandouz, Sandrine Ménard, Laurent Ferrier, Isabelle P. Oswald

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Marc MARESCA[1], Philippe PINTON[2], El Hassan AJANDOUZ[1], Sandrine MENARD[2], Laurent FERRIER[2], Isabelle P. OSWALD[2,*]

[1] Aix Marseille Univ, CNRS, Centrale Marseille, UMR 7313, iSm2, Marseille, France.

[2] Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse, France.

* corresponding author: Isabelle.Oswald@inra.fr

Abstract

The intestine is a complex organ formed of different types of cell distributed in different layers of tissue. To minimize animal experiments, for decades, researchers have been trying to develop *in vitro/ex vivo* systems able to mimic the cellular diversity naturally found in the gut. Such models not only help our understanding of the gut physiology but also of intestinal toxicity. This review describes the different systems used to evaluate the effects of drugs/contaminants on intestinal functions and compares their advantages and limitations. The comparison showed that the organotypic model is the best available model to perform intestinal toxicity studies, including on human tissues.

Introduction:

Humans are exposed to ingested contaminants, such as heavy metals, pesticides, fungal toxins (mycotoxins) and other natural or human-made toxins, every day. Although virtually all organs and tissues are exposed once the ingested toxins cross the intestinal wall, the gut is the first organ exposed and to the highest concentrations/doses of toxins. This is well documented in the case of mycotoxins and many of these toxins are able to affect intestinal functions in both animals and humans (Grenier and Applegate 2013). For these reasons, toxicological studies focusing on the intestine, including toxicodynamics (effects/impact of toxins on the human gut) and toxicokinetics (metabolization and handling of the toxins by the human gut) aspects, are of primary importance.

Although some morphological variations can be observed in the general structure of the gastrointestinal tract, all parts of the human gut are formed by four functional layers: i) the mucosa or epithelium layer that forms the most inner layer facing the gut lumen, ii) the

submucosa containing small blood vessels and immune cells, iii) the muscularis propria responsible for intestinal peristaltism and containing the enteric nervous plexus and iv) the adventitia, the outer layer containing the main blood vessels and nerves. Although all intestinal layers can be exposed to and affected by ingested toxins, the mucosal / epithelial layer is in direct contact with the intestinal lumen, and is thus the most exposed.

The intestinal epithelium is formed by distinct cell types distributed along the crypt-villus axis. Although they all derive from a common stem cell progenitor located in the crypts, their morphology and roles differ. The main cell type (89-95 % of total cells) comprises absorbent cells (*i.e.* enterocytes and colonocytes) that are mainly responsible for the terminal digestion of food constituents and the absorption/secretion of ions, nutrients and water. These cells also have an immune function since they form the intestinal barrier and can produce pro-inflammatory cytokines and antimicrobial molecules such as nitric oxide and antimicrobial peptides. In addition, absorbent cells express enzymes and transporters that enable the metabolization and excretion of xenobiotics (chemical substance not naturally produced or expected to be present within the human or animal). These cells are thus the first line of defense against ingested toxins. Microfold cells (or M cells) lie on the gut-associated lymphoid tissue (GALT) of the Peyer's patches in the small intestine and represent a facilitating way for transcytosis. Goblet cells are the second most common type of cell and are responsible for the production/secretion of highly hydrated glycoproteins called mucins that form the mucus. The latter displays a mechanical and a chemical protective role in addition to providing board and lodging and a substrate for the commensal microbiota. Although they represent only 0.2-0.6 % of epithelial cells, enteroendocrine cells play an important role as they release hormones that regulate intestinal function and the functions of other organs including the liver and pancreas. In the small intestine, Paneth cells are located close to stem cells, and the immune role they play is related to their ability to secrete antimicrobial agents such as antimicrobial peptides and lysozyme. Finally, tuft cells, identified in 2011 (Gerbe et al. 2011), are the main source of endogenous intestinal opioids and provide defense against intestinal parasitic infections (Gerbe et al. 2016).

Although the cells that form the mucosal / epithelial layers play major roles in gut physiology and could be major targets of ingested toxins, it is now recognize that other cells are also important. For example, immune cells (lymphocytes, dendritic cells, macrophages) present in the mucosa and the sub-mucosa are involved in normal and pathological inflammatory reactions of the gut. Similarly, the enteric nervous system, considered as the second brain due to the number of neurons located in the gut wall, controls gut motility and other functions; enteric glial cells are able, for example, to secrete molecules that act on intestinal barrier and prevent inflammation. Finally, the intestinal microbiota are now consider as a real organ since they play critical roles in almost all intestinal functions, including the degradation/

metabolization of ingested toxins (Robert et al. 2017). The role of microbiota and the immune/nervous systems in maintaining the gut homeostasis is particularly well illustrated by the fact that perturbations of one or more of these systems are associated with functional (e.g. irritable bowel syndrome) and organic gastrointestinal disorders (e.g. Crohn's disease).
This complex organ consisting of epithelial cells, immune cells, enteric nervous cells, microbiota each with its own function and role, is rendered even more complex by interconnections between the cells.
Because of the difficulty in accessing human tissue and to reduce animal experimentation, *in vitro/ex vivo* models aim to respond to the need to study the huge numbers of contaminants for intestinal toxicology (either toxicodynamic or toxicokinetic mechanisms). In this review, we describe the advantages and limitations of existing *in vitro* and *ex vivo* models used to mimic the human/animal gut, including primary cells or cell lines, intestinal explants and organoids. Our focus is on organoids as a promising emerging model.

1. Cell models

Despite their inherent limitations, isolated cells have been extensively used in the field of toxicology. With respect to the gut, several cell models of both animal and human origin, from different parts of the gut, have provided scientists with useful tools to assess parameters of importance in toxicology, *e.g.* barrier function, metabolism, proliferation, etc…
The main challenge using isolated cells is to maintain the organization of the epithelial lining *in vitro*. Classical approaches are represented by primary cell cultures, cell derived from healthy intestine and cell lines from tumor origin.

1.1. Primary cell cultures

Primary cells are isolated directly from animal or human intestinal tissue using mechanical and/or enzymatic dissociation. Thus, they closely represent a "real" tissue, with a low risk of mutation. However, their short lifespan limits their use to short-term toxicity studies. In 1984, Walton, Acton and Stich (Walton et al. 1984) evaluated the toxicity of various molecules, including the mycotoxin aflatoxin B1, on freshly isolated cells from rainbow trout intestine, and observed a 10% mortality of untreated cells reached after 6 hours. In 1997, Branka et al. (1997) described a promising technique, based on a new culture medium designed to maintain primary human normal colonocytes for 48 hours. In 1998, Kaeffer and Briollais (1998) reported the use of a rotating bioreactor seeded with rat colon epithelial cells that allows consistent tissue reorganization up to 20 days with proliferative compartments and cells organized as monolayers. With this model, these authors confirmed the cytotoxic properties of seaweed-derived polysaccharides (Kaeffer et al. 1999), but the use of such systems is hard to standardize and they have rarely been used for toxicity studies.

1.2 Cells derived from healthy intestine

Quaroni et al. (1979) established an intestinal cell line, derived from rat small intestine able to maintain an epithelioid morphology, establish tight junction, and exhibited a normal karyotype. The non-malignant of the cells was confirmed by their inability to induce tumors when injected in a naïve animal. These cells, referred to as IEC-6 cells, and other IEC-x cells described later have been used for toxicology studies. For example, Del Regno et al. (2015) showed that the mycotoxins, nivalenol and deoxynivalenol exert pro-oxidant toxicity, an effect that involves NADPH oxidase and NFκB and NRf2-dependent pathways. IEC-6 have also been used to demonstrate that deoxynivalenol exacerbates the genotoxicity of gut microbiota (Payros et al. 2017). Earlier, Duizer et al. (Duizer et al. 1999) determined on IEC-18 cell line that cadmium alters epithelial barrier by modifying tight junction proteins such as zonula-occludens-1 and occludin. These studies demonstrated the advantage of using intestinal epithelial cell lines in toxicity studies as they make it possible to specifically investigate certain parameters such as epithelial barrier function, oxidative stress….

A few normal lines derived from human intestine are also commercially available, but mainly show a fibroblast phenotype, as CCD-112CoN cells. A normal epithelial cell line was described by Thompson et al (1985) and is referred to as CCD-841CoN. However, these cells lack typical epithelial markers like cytokeratin, and have not been used for toxicology studies so far. FHC cells isolated from the fetal human colon were described by Siddiqui and Chopra (1984), but these cells displayed karyotypic alterations and seemed to be tumorigenic rather than normal (Soucek et al. 2010).

In addition, two intestinal epithelial cell lines have been isolated from piglets. IPEC-J2 cells were isolated from the jejunum and IPEC-1 cells from a mixture of ileal and jejunal tissue of newborn piglets. These cell lines are able to form polarized monolayers with high transepithelial electrical resistance when cultured on pore-size filters. They have been used to investigate the toxic effect of metals such as zinc and cadmium (Zhou et al. 2017, Razzuoli et al. 2018), mycotoxins such as fumonisin, deoxynivalenol and fusarenone-X (Loiseau et al. 2007, Pinton et al 2012, Alassane-Kpembi et al. 2017a), as well as the ability of mycotoxin detoxifying agents to interact with drugs (Devreese et al. 2013).

1.3. Tumor cell models

Three main human intestinal epithelial cell lines have been used in laboratories: Caco-2, T84 and HT29, including many sub-clones. HT29 was the first to be established, in 1964, followed by Caco-2 in 1974, and T84 in 1984.

Cell lines obtained from human tumors exhibit variable degrees of differentiation that may be due to their origin. HT29 was obtained from a human colorectal adenocarcinoma of a 44-year-old female (Fogh and Trempe 1975), Caco-2 from a colorectal adenocarcinoma (Fogh et al. 1977) in a 72-year-old male and T84 from colorectal carcinoma derived from metastatic lung of a 72-year-old male (Dharmsathaphorn et al. 1984).

These human cell lines can be grown on culture inserts to polarize and differentiate. Their tight junctions are functional and their transepithelial electrical resistance can be measured. Furthermore, they have apical microvilli, express brush border enzymes and both apical and basolateral specific transporter (Hidalgo et al. 1989). Caco-2 cells and T84 cells differentiate with the characteristics of mature absorptive cells *i.e.* a polarized monolayer with an apical brush border, tight junctions and brush border-associated hydrolases and/or transporters. The HT29 cell line presents two main subtypes: absorptive and mucus secreting cells (HT29-MTX and HT29-16E clones) (Augeron and Laboisse 1984). They are less differentiated and behave more like a pluripotent cell line with sparse microvilli and low or no brush border-enzyme activities (Rousset 1986; Bolte et al. 1997). Even though both Caco-2 and T84 appeared to be well differentiated models of the intestinal epithelial monolayer, a recent study has highlighted their differences. They present distinct morphological, biochemical and functional characteristics, since T84 cells maintain their original colonic characteristics throughout differentiation whereas Caco-2 cells acquire a mature small intestinal enterocytes signature (Devriese et al. 2017).

These human intestinal epithelial cell lines have been extensively used in toxicology studies. For example, Caco-2 cells have been used to investigate the cocktail effect of food contaminants such as mycotoxin and heavy metal. When these cells were expose to deoxynivalenol and the cadmium the interaction between these two contaminants ranged from nearly additive to antagonistic depending on their concentration (Le et al. 2017). This cell line was also used to compare the toxicity of the mycotoxin deoxynivalenol and several modified forms of this toxin (Pierron et al 20016a, b) or to explain the mechanism of action of polychlorinated biphenyls on intestinal integrity (Choi et al 2010). In the same way, human goblet cells (HT29-16E cells) were used to understand the mechanism of action of deoxynivalenol on mucus production. Deoxynivalenol was shown to activate the protein kinase R and the mitogen-activated protein kinase p38 ultimately leading to the inhibition of the expression of resistin-like molecule beta, a known positive regulator of mucin expression (Pinton et al. 2015).

2D cell culture on culture inserts has been extensively used as a screening tool to investigate and predict intestinal absorption of drugs, nutrients and environmental toxics (Hidalgo et al. 1989; Artursson et al. 2001; Kobayashi et al. 2013; Sessa et al. 2014; Vázquez et al. 2015) as well as their potential deleterious effects on the intestine (Jensen-Jarolim et al. 1998;

Okada et al. 2000; Hurley and McCormick 2003). Recently, combined analyses of the three cell lines (Caco-2, HT29 and T84) in monolayer were performed and showed that they were sensitive enough to distinguish between hazardous and non-hazardous proteins (Hurley et al. 2016). Testing compound toxicity on the three cell lines in parallel was shown to be more effective than testing it on only one cell.

Colon cancer cell lines have well-recognized limitations including numerous undefined DNA mutations, derive of the cell lines that sometime prevent reproducible results from one laboratory to another and the lack of epithelial cell diversity (for a review on Caco-2 see Sun et al. 2008).

Nevertheless, 2D monolayer of intestinal epithelial cells (either primary cultures or cell lines) is a useful tool as it maintains epithelial cell polarity and allows access to the apical and basolateral compartments. Furthermore, compared to animal experimentation, this technique is less expensive, easy to replicate, and allows a first set of screening trials, thus reducing the need for animal testing. Moreover, these models are excellent tools to decipher the direct interaction between toxics contaminants and intestinal epithelial cells, thus avoiding the complex interactions between epithelial and neighboring cells in one hand, and between epithelial cells and microbes on the other. Tumoral cell lines therefore represent a widely used model for toxicity studies, and the literature shows some adaptations of these models. First, a recent study of co-culture that include intestinal, hepatic and immune cells has nicely evidenced strong differences in the toxicity of two mycotoxins zearalenone and deoxynivalenol in individual cell lines compared with bi- or tri-culture system (Smith et al 2018), highlighting the importance of interactions to determine toxicity of compounds in more complex systems. Second, methodological developments have allowed maintaining these models valuable to monitor deleterious effects of these contaminants on transepithelial resistance. As an example, Akbari et al. (2014) have observed that upon exposure to deoxynivalenol, intestinal cells show an initial drop in transepithelial resistance, followed by increase permeability to macromolecules. More recently, Flynn and Vohra have described an elegant coupling of permeability determination in Caco-2 cells with mass spectrometry to determine interactions of molecules in complex mixtures (Flynn and Vohra 2018). Thus, the authors have determined permeability of alkaloids from serpentine wood (*Rauwolfia serpentina*) across Caco-2 cells monolayers, and have simultaneously measure metabolites, which may exhibit bioactivity and toxicity, in the basolateral compartment.

2. Explant model

The explant (or organ culture) *ex vivo* model originally derives from the inverted intestinal sacs and ring models developed in the early 1950s to measure the absorption of nutrients (Wilson and Wiseman 1954). Organ culture was almost totally limited to embryonic tissues,

until in 1959, Trowell cultured mature tissues for the first time (Trowell 1959). Later, using a modified version of Trowell's technique, Browning and Trier were able to successfully maintain adult human small intestinal mucosa in culture for up to 24 h (Browning and Trier 1969). This technique then expanded within the scientific and medical communities and the concept was extended to other tissues, particularly bronchi.

The explant model was developed as an intermediate tool between animals and humans to study the homeostasis of the targeted organ and its behavior in physiological and pathological conditions. It is also an alternative to the use of isolated primary cells developed concomitantly (Kaminski 1953), which has its own limitations and advantages, as explained in the section dealing with cell models above.

Technically speaking, human intestinal explants are prepared from gut segments (either stomach, small intestine or colon). Adventitia, muscularis and submucosa may be removed, leaving only the epithelial layer, or not. Next, intestinal explants are cut-off from the cleaned resections using (for example) surgical punches with different diameters. The human intestinal explants are then maintained in culture using different approaches including culture at the air-liquid interface, or immersion culture either in wells or after mounting in a Ussing chamber, a plastic device that allows specific access to either the apical/luminal or the basolateral/serosal compartments of the tissue (for details see Randall et al. 2011).

Intestinal explants have the advantage of being a closer model to the human gut than cell models, since they harbor all the epithelial cell types normally present in the mucosa in the correct proportions. In addition to epithelial cells, intestinal explants also contain other cells such as immune cells, myofibroblasts and enteric nervous cells that play important roles in gut physiology. The main limitation of this technique is the fact that the tissue undergoes progressive and rapid degeneration (within 12-48 h) after collection from patients, although improvements in culture conditions make it possible to maintain the tissue for up to 14 days in some cases (Randall et al. 2011).

Nowadays, although several thousands papers can be found in PubMed dealing with the use of animal or human intestinal explants, in-depth analysis of this literature revealed that only a limited number of articles deals with the use of intestinal explants in toxicology. A few studies have been performed on animal intestinal explants, mainly from rodents or pigs. Rat intestinal explants have been used for example to study the toxicity and metabolism of carcinogenic toxins, including the mycotoxin aflatoxin B1 (Autrup et al. 1978; Kolars et al. 1994). More recently, pig intestinal explants were used to study the toxicity of food-associated mycotoxins, especially type B trichothecenes. Upon exposure to these toxins, intestinal explants produced an inflammatory response (Pierron et al. 2016a; Alassane-Kpembi et al. 2017a) and activation of MAPKinases (Pinton et al. 2012; Garcia et al. 2018). A synergistic inflammatory response was observed between Deoxynivalenol (DON) and

Nivalenol, particularly at low doses (Alassane-Kpembi et al. 2017b). Similarly, when tested on pig jejunal mucosal explants, the surfactant lauroyl-L-carnitine, 1-decanoyl-rac-glycerol and nonaethylene glycol monododecyl ether at 2 mM caused profound change in the permeability and organization of the intestinal barrier (Danielsen & Hansen, 2017). Lastly, it is worth noting that pigs fed DON contaminated feed and pig jejunal explants exposed to DON showed similar histological and signaling changes (Lucioli et al. 2013). In both models, DON led to histological lesions and an increase in the phosphorylation of ERK 1/2 and p38 but not in the phosphorylation of JNK.

Most studies dealing with the use of human intestinal explants in toxicology, were conducted in the 1970s and 1980s and provided information on the toxicity and metabolic behavior of some natural and neoformed carcinogens in humans. Using radiolabeled molecules, Autrup et al. (1979) incubated aflatoxin B1 with colon explants and identified two DNA adducts derived from aflatoxin B1 (AFB1). The DNA binding level of AFB1 was lower than that of benzo(a)pyrene (BP), which was also tested in the study. Similarly, Autrup et al. (1980a, b) reported that the metabolic profiles of AFB1, BP, and 1,2-dimethylhydrazine (DMH) in colon explants from human and ratwere similar, although the binding level of AFB and DMH to colonic DNA was higher in rat than in human. These authors also found a correlation between the DNA binding potential of BP and DMH in 42 individuals, suggesting that similar enzyme systems are involved in the metabolism of BP and DMH. Using human fetal explants, the same group found that the DNA binding level of AFB, BP and certain N-nitrosamines is higher in the stomach than in the liver and the esophagus (Autrup et al. 1984). The profiles of the DNA adducts of fetal liver explants were identical to those observed in adult human tissues, suggesting that these carcinogens are metabolized at an early stages of age.

The usefulness of explants for toxicology is also illlustrated by the study of Cohen et al. (1983) dealing with the oxidative metabolism of BP and the conjugative metabolism of 1-naphthol by healthy and cancereous colon explants. Several BP metabolites were identified, including monools, diols, triol, tetrols and quinones, whereas in normal tissue 1-naphthol (20 µM) was mainly conjugated to sulfate. Interestingly, the ratio of sulfate ester conjugates to glucuronic acid conjugates was significantly higher in the normal tissue than in the tumor tissue (Cohen et al. 1983).

Except for these studies, to our knowledge, human explants had not been used to investigate the toxicity or metabolism of ingested toxins until a recent study investigating the deacetylation of acetylated mycotoxins (3- and 15-acetyldeoxynivalenol) by human gut and liver explants (Ajandouz et al. 2016). This study identified the small intestine and the liver as major deacetylation sites and showed that 3-AcDON is more subjected to deacetylation than 15-AcDON, in agreement with the data obtained on cells and in animals.

In conclusion, it appears that the *ex vivo* explant model was underused in the last decades for the topic of the present review, since it early proved to be highly relevant to humans with respect to metabolism patterns of aflatoxin B1 and benzo(a)pyrene. One should argue that the model is fully functional only for a short time, it requires access to human tissues and that it is not really suitable for large-scale screening trials. Explants are undoubtedly a robust *ex vivo* model, ideally if used together with other *in vitro* models in integrative approaches, for the risk assessment of toxic contaminants in human food.

3. Organoids model

Preserving tissue architecture and obtaining cellular differentiation is difficult in culture. However, maintaining highly organized structures, joining adjacent cells with asymmetrical distribution of membrane proteins and lipids in the two plasma membrane domains and tight junctions is particularly important for the study of intestinal absorption, metabolism and toxicity (Rodriguez-Boulan and Nelson 1989). The development of intestinal organoid models has benefitted from the progress made in the characterization and culture of stem cells originating from the gut.

To perform long-term culture and provide the basic crypt–villus physiology in absence of mesenchymal niche, Sato et al (2009) combined the growth factor requirements of the intestinal epithelium observed *in vivo*. Gradients of specific factors were used for the culture including Wnt3a signaling, required for crypt proliferation, the Wnt agonist R-spondin that induces marked crypt hyperplasia *in vivo*, epidermal growth factor associated with intestinal proliferation and the transgenic expression of Noggin that induces an expansion of crypt numbers. To facilitate the growth and maintenance of intestinal organoids, cell lines can be engineered to secrete Wnt3a, R-spondin and Noggin as shown with mouse fibroblast producing conditioned media used to grow both mouse and human intestinal crypt organoids (Powell and Behnke, 2017). Organoids display the 3D architecture required to obtain the *in vivo* functional differentiation (Simian and Bissell 2017). Organoids rely on artificial extracellular matrices to facilitate their self-organization into 3D structures that resemble native tissue architecture. For this purpose, a semi-solid, laminin/collagen-rich material was used to support intestinal epithelial growth.

In this pioneer development of gut organoids, mouse crypt preparations as well as single-sorted $Lgr5^+$ cells were expanded for long-term culture. Single crypts underwent multiple crypt fission events and simultaneously generated villus-like epithelial domains. Single sorted $Lgr5^+$cells also initiated these crypt–villus organoids. In these structures, the $Lgr5^+$ stem cells were able to divide and generate a self-renewing stem cell population and cells able to differentiate into enterocytes, enteroendocrine cells and goblet cells. All these cells could exfoliate from the villus tip into the lumen, thus mimicking the physiological turnover of the

adult intestinal epithelium. Since the seminal study of Sato et al. (2009), organoids have been produced from primary tissue, and from embryonic or induced pluripotent stem cells. Despite their diversity, they are used for the common goal of capturing complex biological interactions by re-creating physiologically relevant mechanical organ functions, cellular diversity and spatial architecture (Hynds and Giangreco 2013).

Although intestinal organoids have no enteric nerve cells or immune cells, which limits their use in modeling inflammatory responses to infection or drugs (Fatehullah et al. 2016), the development of consistently stable cell culture conditions led to a broad array of applications. Various proof-of-concept investigations have shown that organoids grown from intestinal crypts can be used for controlled gene expression, used in various analytical procedures (microarrays, sequencing, immunohistochemistry, mass spectrometry, etc) or cryopreserved for long-term storage. The 3D organoid model is a powerful tool for the study of a range of *in vivo* biological processes including stem cell functions and maintenance, candidate gene function in tissue physiology and carcinogenesis or tissue responses to drugs, virus or bacterial infections.

Healthy organoids represent a closed system with a clean, light appearance with clear epithelial integrity. When the number of dead or dying cells increases, the outer surface is disrupted and takes on an overall darker and less uniform appearance. To quantify cell death, it is possible to use traditional cell culture systems like MTT, which is converted to formazan through the NAD(P)H-dependent cellular oxido-reductase enzymes, the CellTiter-Glo® assay, which uses the luciferase reaction to quantify ATP levels, or the PrestoBlue® assay, which becomes fluorescent in the presence of the reducing cytosol associated with living cells. Nevertheless, for these assays, some modifications are needed to solubilize the semi solid structure of the organoids (Young, Reed, 2016). Damage to the epithelial cells can affect the intestinal barrier function. Recently, microinjection of fluorescent-labeled dextran and microscopy imaging were performed to assess the barrier permeability of organoids (Hill et al, 2017).

Large-scale proteome variations can be measured in organoids as demonstrated by (Gonneaud et al, 2016). These authors used a stable isotope labeling by amino acids in cell culture (SILAC)-based quantitative proteomic approach to measure changes in protein expression changes in intestinal organoids under conditions inhibiting their growth and development. Organoids have been used to understand the role of the intercalating Paneth cells in crypt cell maintenance (Yin et al. 2014). They have also been used to demonstrate the role of the Toll-like Receptor 4 (TLR4) in intestinal cell proliferation (Neal et al. 2012) as well as in intestine-microbe/virus interactions (Leslie et al. 2015, Karve et al. 2017, Sigal et al. 2015, Foulke-Abel et al. 2014).

Regarding toxicology researches, the use of organoids is in its infancy and very few papers have been published to date. The impact of different potent inducers of intestinal epithelial cells apoptosis (TNF-α, X-ray irradiation and the chemotherapeutic drug, 5-Fluoro-Uracile) was assessed on intestinal organoids. The results concerning cell death and cell survival using microscopic and colorimetric approaches showed that organoids are useful and physiologically relevant models to test *in vitro* the damage caused by drugs and toxins *in vitro* (Grabinger et al. 2014).

As the intestine is exposed to various aryl hydrocarbon receptors (AhR) ligands (dioxins and dioxins-like, dibenzofurans and biphenyls) and polycyclic aromatic hydrocarbon through contaminated food, organoids have been used to investigate whether AhR can affect their development and modulates signaling pathways associated with intestinal epithelial cell development. Thus, 2,3,7,8-tetrachlorodibenzo-p-dioxin and 6-formylindolo[3,2-b] carbazole, a tryptophan metabolite (FICZ) inhibited the development of organoids and FICZ also reduced the level of active β-catenin, suggesting that this metabolite interferes with the canonical Wnt signaling pathway (Park et al. 2016).

Similarly, the intestinal transport and toxicity of ricin was assessed using organoids. Results showed that ricin crosses the intestinal wall without apparent damage up to 24 h of exposure, whereas at 48 h, damage was visible and most cells had become rounded and detached from the scaffold. Importantly, *in vivo* results confirmed that no apparent histological damage was apparent in mice up to 16 h after ricin ingestion, thereby confirming the predictive value of organoids (Flora et al. 2013).

Recently, the effect of dietary nutrients on the growth of murine intestinal organoids was evaluated by measuring the surface area of organoid culture. Among these nutrients, caffeic acid inhibited organoid growth in a concentration- dependent manner, curcumin exhibited variable effectiveness, and vitamin C had no effect on organoids growth (Cai et al. 2018).

Organoids have also been shown to be an appropriate tool to study the function of different genes and enzymes involved in intestinal drug metabolism and toxicity. Crypt cultures were treated with different prototypical agonists of xenobiotic nuclear receptors (XNR) leading a notable induction of the representative downstream target genes, including cytochrome p450. To demonstrate that this induction was a consequence of ligand–receptor binding, organoids obtained from XNR-null mice were tested and failed to induce the specific Cyp450 genes (Lu et al. 2017).

Beyond studies of specific signaling pathways, organoids can be used to study the cellular mechanisms behind intestinal diseases through studies of gene expression or manipulation (Jung et al. 2011). Recent studies have shown that CRISPR/Cas9-mediated gene editing of healthy organoids is a potential tool to evaluate candidate gene function following exposure to drugs or toxic compounds (Drost et al. 2015). However, up to now, despite abundant

knowledge and evidence for the successful implementation of 3D culture in fundamental biological research, these models for toxicity assessment has rarely been used (Astashkina and Grainger 2014).

Recently, Stappenbeck' group has adapted the intestinal epithelial organoids obtained from biopsies to established human primary epithelial cell monolayers from multiple regions of gastrointestinal tract from healthy subjects and inflammatory bowel disease patients (VanDussen et al. 2015). With this reproducible technique, organoids were grown for 2 to 3 weeks and then disrupted and seeded on culture inserts for 2 to 3 days to obtain monolayers. Like organoid culture, this technique makes it possible to establish and store cells from specific patients. This model is of particular interest to study pathogen adherence (VanDussen et al. 2015; Wang et al. 2017) and to access to apical compartment which is rather difficult with organoids (McCracken et al. 2014; Leslie et al. 2015). However, to our knowledge, the toxicological relevance of a toxic contaminant remains to be tested in this promising model.

Conclusion

As described in this chapter, different intestinal models, cell culture, explant and organoids are used for toxicity studies. These models are increasingly complex, ranging from simple 2D models to 3D structures, including the structural and functional aspects of the mammalian intestinal epithelium. Intestinal cell cultures are easy to use and could be maintain virtually with no time limit, thereby enabling studies of long-term exposure. Although their survival time is limited, intestinal explants have the advantage of conserving the microenvironment of the gut mucosa. As they are currently not commercially available, they require access to human tissues obtained from surgical units. Organoids are promising models for intestinal toxicology. They represent an emerging system with high potential that recapitulate the lumen and crypt-villus structure of the intestine with cell types found in the intestinal epithelium. They can be obtained from stem cells pooled from different donors and are now available from different companies. This suggests that organoids are the more promising models for intestinal toxicology. One should nevertheless bear in mind the physiological complexity and the compensatory mechanisms of the intestinal mucosal barrier and also that the intestinal barrier is itself connected to other organs in well-controlled way. In addition, the recent development of organ-on-a-chip technologies that combined cell or organ culture with microfluidics, allows a better mimicking of physiological regulations induced by shear stress or peristaltism. Given the tremendous development of intestinal *in vitro/ex vivo* models, especially organoids, significant advances in the ability of these models to approach animals and humans for toxicology studies one may expect in the near future.

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Model	Advantages	Limitations
Primary cells	- Close to reality - Simple model enable easier identification of molecular events involved in toxicity	- Isolation of primary human cells requires access to human tissue - Short lifespan limits their use to very short-term studies - Lack of standardization
Immortalized cells	- Can be maintained for a long time in culture (allowing long term exposure experiments) - Simple model enabling easier identification of molecular events involved in toxic mechanisms - Low variability of results obtained with this model	- Not all cell types present in the epithelium can be isolated and cultured - Immortalized cells are transformed
Tumor cell lines	- Commercially available - Can be maintained for a long time in culture (allowing long term exposure experiments) - Enable separate evaluation of the effects of toxins on major cell types present in the epithelium when co-cultured (e.g. enterocytes and goblet cells)	- Display genetic alterations - Far from the physiological state
Explants	- Contain all cell types normally present in the epithelium, in the correct proportions - May also contain other cells types such as immune cells, myofibroblasts and cells from the enteric nerve system (ENS) (neurons and enteric glial cells)	- Limited survival time (48h) - Access to apical compartment only when mounted in a Ussing chamber - Require access to human intestinal tissue - Higher variability due to inter-individual differences in response to toxins
Organoids	- Contain most of the cell types normally present in the epithelium (with but no guarantee of them being in the correct proportion) - Can be maintained for a long time in culture, allowing long term exposure to toxins	- Contain only epithelial cells (absence of immune or ENS cells except if co-cultured with organoids)

	- Commercially available (low variability) -Derived from healthy or diseased individuals	

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