Cell Biol Toxicol (2011) 27:267–284
DOI 10.1007/s10565-011-9187-5

Explant culture of gastrointestinal tissue: a review of methods and applications

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Received: 19 October 2010 / Accepted: 25 February 2011 / Published online: 8 March 2011

Abstract The gastrointestinal (GI) tract is an important target organ for the toxicity of xenobiotics. The toxic effects of xenobiotics on this complex, heterogeneous structure have been difficult to model in vitro and have traditionally been assessed in vivo. The explant culture of GI tissue offers an alternative approach. Historically, the organotypic culture of the GI tract proved far more challenging than the culture of other tissues, and it was not until the late 1960s that Browning and Trier described the means by which intestinal tissues could be successfully cultured. This breakthrough provided a tool researchers could utilise, and adapt, to investigate topics such as the pathogenesis of inflammatory intestinal diseases, the effect of growth factors and cytokines on intestinal proliferation and differentiation, and the testing of novel xenobiotics for efficacy and safety. This review considers that intestinal explant culture shows much potential for the application of a relatively under-used procedure in future biomedical research. Furthermore, there appear to be many instances where the technique may provide experimental solutions where both cell culture and in vivo models have been unable to deliver conclusive and convincing findings.

Keywords Explant · Gastrointestinal · Organ culture · Toxicology

Abbreviations

ALI	Air–liquid interface
BrdU	Bromo-2′-deoxyuridine
EGF	Epidermal growth factor
EGPx	Extracellular glutathione peroxidase
EHC	Enzyme histochemistry
ELISA	Enzyme-linked immunosorbent assay
ENS	Enteric nervous system
GI	Gastrointestinal
IHC	Immunohistochemistry
IBD	Inflammatory bowel disease
LPS	Lipopolysaccharide
NSAID	Non-steroidal anti-inflammatory drug
SEA	Staphylococcal enterotoxin A
SEB	Staphylococcal enterotoxin B
TGF	Transforming growth factor
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labelling

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Introduction

The gastrointestinal tract

The gastrointestinal (GI) tract is, simplistically, a muscular tube, contiguous with the external environ-

ment, which is responsible for breaking down and absorbing food. Although there are considerable differences in gross anatomy between mammalian species (Kararli 1995), the basic structure is conserved; the tract comprises four distinct, concentric functional layers: the mucosa, submucosa, muscularis propria and adventitia (Young and Heath 2000). The innermost layer, the mucosa, which lines the gut lumen, comprises the epithelium, a supporting lamina propria and a thin smooth muscle layer. The submucosa is a loose collagenous layer that supports the mucosa and contains larger blood vessels, lymphatics and nerves. The inner circular and outer longitudinal smooth muscle layers of the muscularis propria provide the peristaltic basis of propulsion. The adventitia is the outer layer of loose supporting tissue conducting the major vessels and nerves (Fig. 1).

Morphological variations in this general structure have been described along the length of the GI tract. In the small intestine, the villi vary in shape from broad and ridge-like (proximal duodenum) to spatulate or leaf-like (distal duodenum and proximal jejunum) to finger-like (distal jejunum and ileum). The height of these villi decreases caudally, although the density remains constant. The number of intervening glands, the crypts, decreases caudally, reducing the crypt to villus ratio (Brennan et al. 1999). The large intestine lacks villi and has an entirely glandular epithelium.

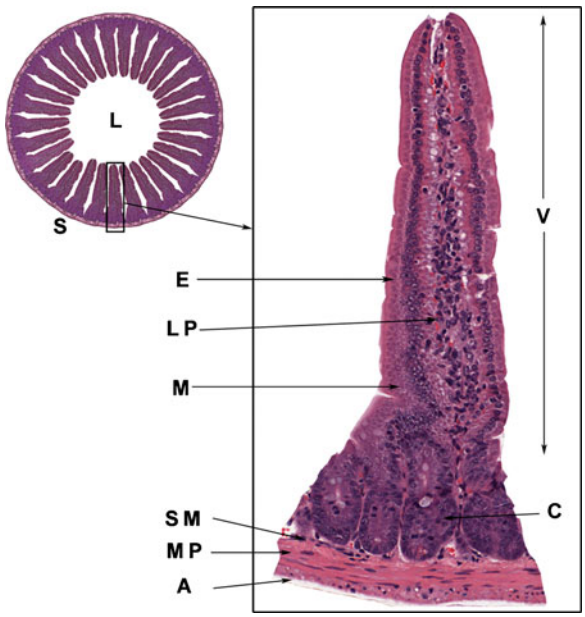

Fig. 1 The small intestine of the mouse is a tubular structure of concentric layers. The mucosa (*M*) lines the lumen (*L*) and consists of epithelium (*E*) supported by the lamina propria (*LP*). Beneath the mucosa lies the submucosa (*SM*) and the muscularis propria (*MP*). The adventitia (*A*) is the outer layer and is referred to as the serosa (*S*) in those parts of the intestine covered by mesothelium. The mucosa is formed into villi (*V*) and intervening crypts (*C*)

The mucosal epithelium comprises four distinct cell types, all arising from a common stem cell precursor traditionally thought to be located, in a ring, four cells up from the base of the crypts, although recent work has suggested the stems cells may be distributed throughout the bottom of the crypts (Snippert et al. 2010). The enterocytes, which are tall columnar cells, fringed by a brush border on their luminal side, are the absorptive cells of the intestine and represent 89% to 95% of the epithelial cells in the small intestinal mucosa (Cheng and Leblond 1974). Goblet cells, producing mucins for lubrication and protection, are scattered in the epithelium amongst the enterocytes. The entero-endocrine cells release hormones to coordinate functions of the gut, liver and pancreas and, although concentrated in the crypts, are distributed throughout the epithelium where they form a small minority (0.2% to 0.6%) of cells (Cheng and Leblond 1974). These three cell types all migrate upwards from the crypts towards the tips of the villi where they are sloughed off into the lumen. This migration takes between 54 and 60 h in the mouse (Cheng and Bjerknes 1982). The Paneth cells, by contrast, migrate into the base of the crypt where they secrete antimicrobial agents such as cryptdins, defensins and lysozyme to control the microbial content of the intestine (Porter et al. 2002; Ouellette 2010). Paneth cells are absent from the large intestine.

This simplistic view is complicated by other functions and structures of the GI tract. For instance, the enteric nervous system (ENS) has, since the early 1900s, been referred to as a second brain since it was discovered that the ENS control of intestinal motility and secretion was largely independent of the central nervous system (CNS). The ENS, in common with the spinal cord, contains approximately 80 to 100 million neurons, and the functional and chemical diversity of the ENS closely resembles that of the CNS (Goyal and Hirano 1996). The GI tract also possesses complex, integrated hormonal networks, and the gut-associated immune system is essential in maintaining homeostasis with potentially harmful intestinal microflora (Young and Heath 2000).

The GI tract is, evidently, a complex organ consisting of different layers and cell types, each

with their own function. It is difficult, therefore, for any researcher wishing to investigate the morphogenesis, physiology, pathogenesis of disease or reaction to xenobiotics to replicate this complexity of the GI tract in vitro using a cell culture-type approach. The culture of explants of GI tissue, though, offers a solution to this problem.

The gastrointestinal tract as a target for toxicity

The GI tract is an important target organ for the toxicity of xenobiotics. The intestine is the major route of pharmaceutical administration and, as such, is exposed to these agents at a far higher concentration than other organs.

Irritation of the GI mucosa caused by oral therapeutic agents can occur by direct contact, by interference with protective factors, or via the systemic route (Fara et al. 1988). Compounds, such as non-steroidal anti-inflammatory drugs (NSAIDS) and corticosteroids, have been shown to cause irritation in a large proportion of patients (Borsch and Schmidt 1985). Other than traditional in vivo assessment of GI irritancy, by administering compounds orally, the only useful model for specific evaluation of potential damage to the small intestinal or colonic mucosa was described by Fara et al. (1988). These authors used an in situ rabbit colon model as a sensitive and reproducible test to evaluate the topical effect of up to three substances applied to the colonic mucosa, and found that, of the chemicals applied, doxycycline hyclate and propranolol hydrochloride produced the most macro- and microscopic damage.

Intestinal injury induced by cytotoxic drugs, such as cyclophosphamide, 5-fluorouracil and chlorambucil, is characterised by the loss of intestinal mucosal integrity that results from widespread apoptosis and death of proliferating cells (Manzano et al. 2007). The damage includes perturbed brush-border hydrolase activity, reduced mucosal DNA, RNA and protein contents, blunted villus height and crypt depth, and decreased proliferation and increased apoptosis of crypt cells. Prolonged exposure may ultimately lead to the death of regenerative stem cells. Predictions of the GI toxicity of this class of compounds have traditionally been made using in vivo animal systems, such as the pig model proposed by Manzano et al. (2007). Cytotoxicity and metabolic studies have also often been performed in vitro using isolated cells or microsomal preparations. In recent years, however, new in vitro models employing cells in culture have been proposed both for the screening of the absorption of drugs and, more in general, for the study of toxicity and metabolism of xenobiotics (Sambruy et al. 2001).

The impact of xenobiotics on GI microfloral homeostasis is assessed, in the main, in vivo (Zak and O'Reilly 1991). The gut flora of an individual is susceptible to transient or more permanent modification by several exogenous factors, such as diet, pathogenic microorganisms and antimicrobial drugs and agents affecting gastrointestinal physiology and secretions, which alter the gastrointestinal environment. Such modification can influence the metabolism of many xenobiotic and naturally occurring compounds, and, in turn, have important consequences for the toxicity of these substances in the host (Rowland and Mallett 1985).

A history of tissue culture

The in vitro culture of explanted tissues has its origins in the morphological characterisation of foetal and embryological tissue development, and the comparison of this with tumour growth, early in the twentieth century. The initial work showed that the growth of vertebrate embryonic tissue in culture proceeded either in an uncontrolled manner, eventually leading to a culture consisting of undifferentiated cells (Thomson 1914; Fischer 1922), or the culture would develop in an 'organotypic' fashion in which the whole fragment behaved in a controlled way and demonstrated some degree of progressive differentiation (Maximow 1925; Strangeways and Fell 1926). Whilst the exact reasons for these significant differences were unknown at the time, it was the organotypic growth that offered the greatest promise to the study of embryology and morphogenesis.

Despite ongoing refinement to the explant culture methods, their application was almost totally limited to embryonic, rather than mature organs, until Trowell's seminal paper of 1959. Trowell (1959) believed that the previous restriction of successful culture to immature/foetal organs was that, because of their small size, embryonic organs could be cultured intact. Additionally, embryonic tissues also appeared to tolerate the hypoxic/anoxic culture conditions far better than mature organs. Trowell also considered the paucity of publications detailing the culture of mature

tissue to be a product of the focus of researcher interest, which had previously been concentrated on the study of morphogenesis in vitro.

Trowell developed the apparatus, media and methodology to maintain an array of adult rat tissues, in a satisfactory morphological/histological state, for up to 9 days (Trowell 1959). He considered, though, that the culture of gastrointestinal tissue would prove too problematic, because of the real possibility of infection, and did not report attempting its culture.

Another 10 years would pass before Browning and Trier (1969) detailed a modified version of Trowell's technique that enabled the successful culture of adult human small intestinal mucosa, maintaining the morphology and function of the tissue, for 24 h. The system of Browning and Trier (1969) opened up the possibility of using explanted mature intestinal fragments to investigate a number of areas including the pathogenesis of disease, the maintenance of homeostasis in the gut, the production of enzymes and hormones, the identification of potential carcinogens and toxins, and, potentially, as a model of drug efficacy and a screening tool for drug toxicity. The explant system offered all of the advantages of an in vitro system, whilst retaining the relationships of the tissues, and potentially maintaining the complex patterns of differentiation seen in vivo. In particular, the explant system offered a more controlled environment for experimental manipulation, compared with in vivo models, the ability to perform interspecies studies under reproducible conditions, and the possibility of harvesting multiple explants from a single donor, thus increasing the statistical power of any investigation. The ability to sensitively control and manipulate the immediate environment of the explant also lends itself perfectly to a detailed exploration of the factors controlling cell migration and differentiation in the various compartments of the intestine.

Culture techniques

The method of Browning and Trier (1969)

The now classic method of Browning and Trier (1969) has been employed, in one form or another, by many investigators (Danielsen et al. 1982; Tham et al. 1998; Fletcher et al. 2006). In the original report, biopsies were taken from the duodeno-jejunal junction in young, adult male volunteers. The biopsies, approximately 3 mm in diameter, were rapidly transferred to the organ culture system and placed, mucosal-side up, on to a stainless steel wire mesh. The mesh was suspended over the central well of a sterile plastic culture dish. The central well contained sufficient culture medium to bathe the serosal aspect of the biopsy, but not immerse the villous side. A thin layer of medium was drawn across the villous surface by capillary action. An outer well containing a cotton pad saturated with saline was used to maintain humidity (Fig. 2a). After the lids were replaced, the dishes were placed in a McIntosh jar, gassed with 95% O_2 and 5% CO_2, and maintained at 37°C. The explant medium was renewed and the jar re-gassed at 6 hourly intervals. It was thought that the success of this technique, compared with previous methods, was primarily due to the prevention of anoxia within the tissue, the constitution of the medium and the lack of

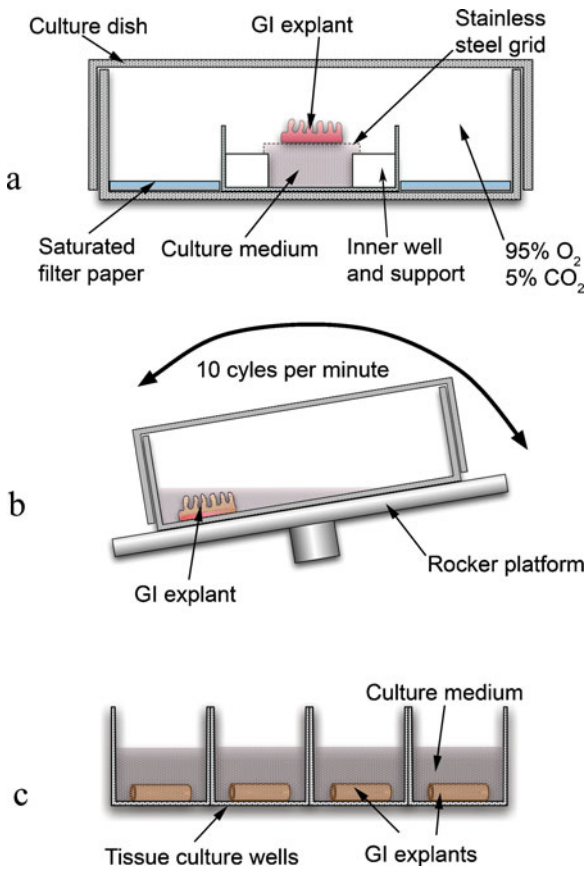

Fig. 2 A diagrammatic representation of the main methods of gastrointestinal explant culture: air–liquid interface (ALI) culture (**a**), rocker culture (**b**) and immersion culture (**c**)

agitation. Agitation was a common method used in other tissue explant systems, but which Browning and Trier thought too traumatic for organ survival.

Variations on the technique of culturing tissue at the air–liquid interface (ALI) have been used frequently not only in the culture of mature GI explants but also for the culture of skin (Jacobs et al. 2002; Lehé et al. 2006), bronchus (Zimmermann et al. 2009), cornea (Seeber et al. 2008), nasal epithelium (Yeh et al. 2007), liver (Clayton et al. 2005) and in the regeneration of nervous tissue from stem cells (Stoppini et al. 1991; Preynat-Seauve et al. 2009). It has been shown that culture at the ALI promoted the differentiation of gastric mucus cells in a reconstruction culture of the gastric surface epithelium using a gastric surface mucous cell line GSM06 grown on a collagen gel (Ootani et al. 2000). This was interpreted as indicating that the culture at the ALI had more importance than one of merely preventing anoxia.

Adaptations of the Browning and Trier's method have included the use of collagen-coated plates (Tham et al. 1998), membrane inserts in plates (Bareiss et al. 2008), gelfoam rafts (Fletcher et al. 2006) or fibrin foam cubes (Schiff 1975) in place of the stainless steel grid, but the overall principle remains the same.

Static versus moving cultures

Some workers have eschewed a static culture system in favour of a more dynamic system, with movement of the medium relative to the explant. Explants of human jejunum have been successfully cultured for up to 48 h using a roller-based system (Mitchell et al. 1974). Here the intestinal explants were placed in culture tubes with a small amount of medium and subjected to 12 revolutions per hour using a drum roller. An alternative system with the explant being placed on one edge of a culture dish, and incubated on a rocker platform, set at 10 cycles per minute, with the medium flowing intermittently over the mucosal surface (Autrup et al. 1978a, b; Shamsuddin et al. 1978), has been used successfully (Fig. 2b). In both these set-ups, the atmospheric conditions stipulated by Browning and Trier, i.e. 95% O_2, 5% CO_2 at 37°C, were mirrored. The constant (static system) or intermittent (dynamic system) exposure of the tissue to an oxygen-rich atmosphere appeared to be necessary to prevent hypoxia-induced damage to the explant.

Using these systems Autrup et al. (1978a, b), Shamsuddin et al. (1978) and Finney et al. (1986) were able to keep explants of colon viable for up to 9 weeks. These dynamic explant techniques offer a system that may serve to prevent the accumulation of mucus, digestive enzymes, secreted hormones and growth factors within the mucosa and on the villous surface. The obverse of this is that the dilution of intrinsic hormones and growth factors, by agitation within large volumes of medium, may inhibit the role that biochemical gradients have in differentiation, organisation and survival of the gut explants. A further advantage may present itself if one wished to measure biomarkers excreted or released into the medium, or nutrients and growth factors absorbed from the medium. The interchange of macromolecules between the explant and culture medium would potentially be inhibited in a static system compared to a dynamic arrangement.

Conventional tissue culture

Recently, a more conventional, cell culture-type method has become favoured. Here the explant is maintained, immersed in medium, within tissue culture wells (Abud et al. 2005; Simon-Assmann et al. 2007) (Fig. 2c), or within culture rings on fibronectin-coated coverslips (Quinlan et al. 2006). This approach is favoured by those using embryological material (Abud et al. 2005; Quinlan et al. 2006. Intact lengths of embryonic mouse gut have been cultured suspended in medium (Hearn et al. 1999) or embedded in collagen gel matrix (Natarajan et al. 1999). Natarajan and co-workers also achieved the same results when the guts were cultured as free-floating organs in medium.

The method of culture technique would be expected to have a major influence on the outcome of any experimental investigation. Robertson et al. (2000) demonstrated that, when studying the adhesion of pathogenic bacteria to rat ileal explants, significantly different results were obtained, from the same starting material, with ALI culture compared to static total immersion systems. It was found that in vivo, the pathogen, *Salmonella*, has to migrate through the liquid luminal contents to close proximity with the gut epithelium. Here it binds and interacts with the mucus and epithelium to initiate colonisation. The immersion culture method more closely models the liquid phase

of infection. The ALI culture model avoids this phase as the inoculum is applied directly to the tissue, resulting in higher bacterial counts. The ALI culture does have the advantage, though, of permitting the modulation of the mucus layers, opening avenues of investigation of the factors influencing infection by *Salmonella*.

Culture media

The choice of medium varies with investigators and laboratory. Schiff (1975) investigated the effect of various media, and atmospheric composition, on the long-term culture of rat and hamster colon. It was found that explants cultured Leibovitz's L-15 medium, buffered with HEPES, remained for 24 days when cultured in 95% air/5% CO_2, whereas medium 199, also buffered with HEPES, provided the most successful combination, maintaining viability for 14 days, in 95%O_2/5% CO_2. Utilising HEPES as the buffering agent conferred up to six extra days of viable culture, compared with sodium bicarbonate.

Other workers have reported success with Trowell's T-8 medium (Browning and Trier 1969; Danielsen et al. 1982), CMRL-1066 (Autrup et al. 1978a, b; Shamsuddin et al. 1978; Robertson et al. 2000) and Eagle's or Dulbecco's modification (DME) (Mitchell et al. 1974; Tham et al. 1998; Abud et al. 2005; Fletcher et al. 2006). A summary of some of the conditions employed is given in Table 1. A common feature of these media is the requirement for the addition of a combination of antibiotics, especially in the period immediately after harvest, unless the purpose of the experiment was to investigate the behaviour of bacteria (Robertson et al. 2000). This comes as no surprise; the culture environment would provide optimised conditions for bacterial growth, either endogenous or exogenous. Overgrowth of bacteria or fungi within the medium would rapidly overwhelm the explant, destroying it, and result in failure of the system.

Origin of explant material

Site of explant

Although often thought of as a single entity, the GI tract exhibits significant variation in morphology, physiology and function along its length. Explant cultures have been maintained from most areas of the gastrointestinal tract, from the stomach (Losonsky et al. 1999) to the rectum (Fletcher et al. 2006). Looking at the frequency of culture of the various regions, there appears to be a roughly even split between those investigating small intestine and those studying the large intestine. This presumably reflects the areas of interest of the researchers, using the explant culture technique as a tool, rather than any apparent variability in the difficulty in culturing either region. A summary of the some of the origins of explant material is given in Table 2.

Period of culture

There does, however, appear to be evidence suggesting significant variability in the length of time that explants from different regions of the intestine, irrespective of species, can be maintained as viable cultures. Browning and Trier's (1969) initial experiments resulted in the successful culture of adult small intestinal biopsies for only 24 h, a period matched by most others (Danielsen et al. 1982; Wheeler and Challacombe 1997; Tham et al. 1998). After 48 h, the morphology of the explant was shown to deteriorate rapidly, along with a reduced production of enzymes (Mitchell et al. 1974; Zachrisson et al. 2001). Losonsky et al. (1999) reported maintenance of gastric antral and duodenal cultures for up to 7 days, but these were accompanied with a progressive degradation of morphology.

The large intestinal explants, by comparison, seem more tolerant of culture conditions. Autrup et al. (1978b) successfully cultured adult rat colon, with preservation of a relatively normal morphology, for up to 14 days. The epithelial cells gradually changed in appearance from the normal columnar shape to a less differentiated cuboidal form with goblet cells diminishing in frequency and the colonic glands becoming progressively shorter. After 63 days in culture, the glands had disappeared, leaving a single layer of cuboidal epithelial cells with rare goblet cells. Although the morphology had significantly diverged from that present in vivo, the epithelial cells still possessed a microvillus brush border and were capable of the metabolism of various classes of procarcinogens into reactive species. With some modification of Autrup's method, it has been possible to preserve cultures of adult rat colon for up to

Table 1 A summary of some of the conditions employed in explant culture

Reference	Medium	Antimicrobial	Serum	Supplements	Buffer	Atmosphere
Abud et al. 2005	DME	P, S	FCS	Glut	n/s	95% Air+5% CO_2
Autrup et al. 1978a, b	CMRL-1066	P, S, G, A	FCS	G, HC, BRA, Glut	Tricine	95% O_2+5% CO_2
Bareiss	DME	n/s	HS	ITS, A, HC, Gluc, TT, AP, LA, E, KGF	HEPES	95% Air+5% CO_2
Browning and Trier 1969	Trowell T-8	P, S	FCS	n/s	n/s	95% O_2+5% CO_2
Challacombe and Wheeler 1991	CMRL-1066	P, S, F	None	I, HC, AA	HEPES	95% O_2+5% CO_2
Danielsen et al. 1982	Trowell T-8	P, S	FCS	n/s	n/s	95% O_2+5% CO_2
Dionne et al. 1998, 2003	CMRL-1066	P, S, G, A	FCS	n/s	Tris	95% O_2+5% CO_2
Finney et al. 1986	Waymouth MB 752/1	P, S, M	FCS	AA, HC, FS	n/s	95% O_2+5% CO_2
Fletcher et al. 2006	DME	P, S, G	n/s	Glut	n/s	95% Air+5% CO_2
Jarry et al. 2008	RPMI 1640	P, S, F	BSA	n/s	n/s	95% O_2+5% CO_2
Kik et al. 1991	Trowell T-8	P, S	FCS	n/s	n/s	95% O_2+5% CO_2
Losonsky et al. 1999	RPMI 1640	P, S, G	FCS	n/s	HEPES	95% O_2+5% CO_2
Metzger et al. 2007	DME	n/s	HS	ITS, A, HC, Gluc, TT, AP, LA, E, KGF	n/s	95% Air+5% CO_2
Mitchell et al. 1974	Eagle's	P, S	FCS	n/s	SB	95% O_2+5% CO_2
Natarajan et al. 1999	optiMEM	P, S, A	n/s	Glut	n/s	95% Air+5% CO_2
Quinlan et al. 2006	Eagle's	G	FCS	Glut	n/s	95% Air+5% CO_2
Robertson et al. 2000	CMRL-1066	None	FCS	G, M, HC, BRA, Glut	Tricine	95% Air+5% CO_2
Schiff 1975	DME/m199/L-15	P, S, A	BSA	Glut	HEPES/SB	95% O_2/Air+5% CO_2
Shamsuddin et al. 1978	CMRL-1066	P, S, A	FCS	G, Glut, I, HC	n/s	95% O_2+5% CO_2
Tham et al. 1998	DME	n/s	FCS	Glut, SS	n/s	92.5% O_2+7.5% CO_2
Wheeler and Challacombe 1997	CMRL-1066	P, S, F	None	G, Glut, HC, AA	n/s	95% O_2+5% CO_2
Zachrisson et al. 2001	CMRL-1066	P, S, F	BSA	ITS, Glut, AA, G	n/s	95% O_2+5% CO_2

DME Dulbecco's modified Eagle's, *P* penicillin, *S* streptomycin, *G* gentamicin, *A* amphotericin B, *F* fungizone, *M* mycostatin, *FCS* fetal calf serum, *BSA* bovine serum albumin, *HS* horse serum, *A* albumax, *AA* ascorbic acid, *AP* ascorbate-2 phosphate, *BRA* β-retinyl acetate, *E* estradiol, *G* glucose, *Gluc* glucagons, *Glut* glutamine, *HC* hydrocortisone, *I* insulin, *ITS* insulin/transferrin/selenite mix, *KGF* keratinocyte growth factor, *LA* linolic acid, *TT* triiodo-L-thyronine, *SS* sodium selenite, *FS* ferrous sulphate, *n/s* none stated

91 days (Shamsuddin et al. 1978), although most researchers report shorter experiments of 2 days (Dionne et al. 2003; Jarry et al. 2008) to 2 weeks (Fletcher et al. 2006; Bareiss et al. 2008).

The disparity in the periods of apparent morphological viability, between the small and large intestine, may be due, in part, to the lower rate of cell turnover in the latter. In mouse, at least, it has been shown that the average life span of an enterocyte in the jejunum is less than 54 h, whereas in the colon the life span is over 60 h (Cheng and Bjerknes 1983). The greater rate of cell turnover and a defined structure such as a small intestinal villus means that any perturbation of the balance of cell replacement or differentiation has a more profound impact on morphology than that seen in the flattened structure of the large intestine.

The difference in survival times also suggests that the large intestine explants may be inherently more resistant to the anoxia and associated oxidative stress present in the culture conditions; the explants are solely reliant on the gaseous diffusion of oxygen, rather than vascular perfusion present in vivo, leading to problems of ischaemia and necrosis when large explants are cultured (Shamsuddin et al. 1978). Rat colon is able to metabolise glucose to lactate, anaerobically, so swiftly that Autrup et al. (1978b) found it necessary to add additional tricine buffer to the CMRL-1066 culture medium to prevent the rapid accumulation of acid.

Table 2 A summary of some of the variations in explant culture methods

Reference	Origin	Incubation	Species	Age of donor	Size of explant	Layers
Abud et al. 2005	SI/LI	Immersion	Mouse	Embryo	n/s	Full thickness
Autrup et al. 1978a, b	LI	Rocker	Human/rat	Adult	5×5 mm	Less ME
Bareiss	Colon	Immersion	Mouse	Adult	2×2 mm	Full thickness
Browning and Trier 1969	Duodenum/jejunum	ALI	Human	Adult	n/s	Biopsy
Challacombe and Wheeler 1991	Duodenum	Rocker	Human	Adult	3×3 mm	Biopsy
Danielsen et al. 1982	SI	ALI	Pig	Adult	1.5–2 mg	Full thickness
Dionne et al. 1998, 2003	Colon	Rocker	Human	Adult	n/s	Biopsy
Finney et al. 1986	Colon	Rocker	Rat	Adult	2×2 mm	Less ME
Fletcher et al. 2006	Colorectal	ALI	Human	Adult	2–3 mm^3	Biopsy/less ME
Jarry et al. 2008	Colon	Rocker	Human	Adult	20–30 mg	Less ME
Kik et al. 1991	Jejunum	ALI	Pig	Adult	9 mm^2	Mucosa
Losonsky et al. 1999	Gastric antrum, duodenum	Rocker	Human	Adult	6.3–19.5 mg	Biopsy
Metzger et al. 2007	Colon	ALI	Mouse	Embryo	Slice	Full thickness
Mitchell et al. 1974	Jejunum	Roller	Human	Adult	1 mm^2	Biopsy
Natarajan et al. 1999	GI tract	Immersion	Mouse	Embryo	Whole gut	Full thickness
Quinlan et al. 2006	SI	Immersion	Mouse	Embryo	Segment of gut	Full thickness
Robertson et al. 2000	Ileum	ALI/immersion	Rat	Adult	1 cm^2	Full thickness
Schiff 1975	Colon	ALI	Rat/hamster	Adult	2×2mm	Full thickness
Shamsuddin et al. 1978	Colon	Rocker	Rat	Adult	0.3–1 cm^2	Full thickness
Tham et al. 1998	LI	ALI	Human/mouse	Adult	5×5 mm	Full thickness
Wheeler and Challacombe 1997	Duodenum	Rocker	Human	Adult	2 mm^2	Biopsy
Zachrisson et al. 2001	Duodenum	Rocker	Human	Adult	2 mm^2	Biopsy

SI small intestine, *LI* large intestine, *ALI* air–liquid interface, *ME* muscularis externa, *n/s* none stated

Age of donor

The stage of development of the tissue donor appears to have a significant influence on the survival time of explants. Explants harvested from embryos appear to be much more tolerant of culturing. This mirrors the experience of the early pioneers of tissue culture prior to Trowell's work in the 1950s. The smaller size of the foetal tissues, their apparent resistance to anoxia and the fact that they are in the process of morphogenesis when sampled, compared to the homeostatic mature state in tissue taken from adults, render embryologic intestine more amenable to culture even when fully immersed in medium. Foetal explants have been cultured, maintaining the full repertoire of intestinal epithelial cell types (enterocytes, goblet cells, Paneth cells and enteroendocrine cells), in a cell-culture system, for 2 weeks (Abud et al. 2005; Quinlan et al. 2006). Clearly the harvesting of embryological tissues presents more technical challenges than harvesting the intestines from adults but, for the longer term culture of small intestine, foetal animals appear to provide a more successful source. No investigations using neo-natal GI explants appear to have been reported, but these may offer a compromise source of material.

Species differences

Explant culture has been demonstrated with adult intestine from human, rodent and porcine origins. Being able to use biopsy material from living human donors has benefited research into a number of diseases, including celiac disease and inflammatory bowel disease (Howdle 1984; Dionne et al. 1998). Researchers have tended to modify the culture

conditions to suit the species that they were working on at the time, and some, for example, found it necessary to remove the muscularis externa when using material from larger species, such as human (Autrup et al. 1978a; Fletcher et al. 2006; Jarry et al. 2008), to prevent excessive anoxia occurring within the very thick samples. Most human material has been gathered via biopsy and, therefore, comprised mainly mucosa with some sub-mucosa. One of the drawbacks of biopsy material is, however, the limited amount of material available for subsequent analysis. When examining some aspects of human physiology or pathophysiology of the mucosa using explant cultures, it seems important to remove the other layers (muscularis mucosa/submucosa and muscularis propria) since these compartments behave differently in terms of initiation of a post-ischaemic inflammation by resident cells (anergy of the mucosa) (Jarry et al. 2006). A summary of some of the variations in culture method, age of donor, etc. is illustrated in Table 2.

Method of assessment of viability

Preservation of morphology

Questions that all researchers using intestinal explants have faced are 'what endpoints are available for assessment' and 'how is one able to extract information from this culture system'? The simplest, and most widely employed measure of explant integrity, is a comparison of the morphology of the cultured samples with equivalent uncultured samples taken from the same individual or group of individuals. The premise behind the use of the morphology of the explant lies in the basis that morphological integrity will mirror functional integrity and that it is generally considered a simple assessment to perform. These comparisons have taken place at both the light microscopic and ultra-structural levels.

Browning and Trier (1969) employed both light and EM techniques in their original assessments. They recorded that although there was evidence, by light microscopy, of a shortening and widening of the small intestinal villi after 24 h culture, a reduction of the number of cells in the lamina propria, accumulation of mucus and cellular debris on the epithelial surface and foci of partial necrosis, the majority of the epithelium appeared intact and well differentiated. The impression that a near normal epithelial cell morphology was preserved after 24 h culture was confirmed by transmission electron microscopy. The enterocytes, although appearing slightly more cuboidal near the tips of the villi, possessed normal appearing, and correctly distributed, cytoplasmic organelles. Goblet cells also appeared normal, and some were identified actively secreting mucus. The Paneth cells, enteroendocrine cells and other cellular components of the lamina propria all exhibited ultrastructural morphology virtually identical to that seen in biopsies fixed immediately after excision.

The qualitative assessment of histological integrity has been used extensively throughout the history of GI explant study and provides an accessible indication of the health of the explant. The successful processing and embedding of these materials, however, has not been without technical challenges. The explants have been, usually, less than 3 mm in maximum extent and are both easy to lose during processing and difficult to correctly orientate when embedding. A detailed description of how this has been achieved is lacking in the literature. In this laboratory, we pre-embed the fixed explants in 3% low melting point agarose, which allows correct orientation of small samples, before processing the tissues (Randall and Foster 2007).

Functional measures

Cell replication

However, any alterations in morphology are only reflections of other changes taking place in proliferation and differentiation, cell migration, RNA and protein synthesis, absorption and secretion, metabolism, and other functional and structural processes. Browning and Trier (1969) were well aware of this and they additionally measured the uptake and incorporation of tritiated thymidine by autoradiography to study cell proliferation and, in time course experiments, cell migration between the crypt and villus compartments. A continuation of both proliferation and migration was observed during the course of culturing the explants. Other investigators have employed DNA synthesis or cell cycling as a marker of explant integrity, although there have been differences in methodology. Hence, Schiff (1975) and Autrup et al. (1978a, b) used scintillation counting of tritiated thymidine whilst

Challacombe and Wheeler (1991) and Zachrisson et al. (2001) counted Feulgen stained metaphases, and Finney et al. (1986) counted mitotic figures in haematoxylin and eosin stained sections. Abud et al. (2005) and Bareiss et al. (2008) used the immunohistochemical localisation of incorporated bromo-2′-deoxyuridine (BrdU) to study proliferation and migration. It has generally been shown that, while there is a tendency towards a more de-differentiated morphology with time, the proliferation and migration of epithelial cells is generally maintained when culture conditions are optimised.

Apoptosis

Apoptosis plays a key role in the maintenance of morphology and function in the GI tract. In the small intestine, cells migrate from the proliferative zone in the crypts up the shafts of the villi until they are shed, usually from an area close to the tip. Apoptosis is closely associated with this homeostatic process, although it remains unclear whether apoptosis initiates shedding or results as a consequence (anoikis) (Bullen et al. 2006).

The frequency of apoptosis may be determined by morphological evaluation alone, although immunohistochemical (IHC) demonstration of apoptotic biomarkers can greatly improve the ease and accuracy of this assessment. Abud et al. (2005) used an IHC method to detect cleaved caspase 3, a key intracellular mediator of apoptosis, in their investigations of the role of the epidermal growth factor (EGF) signalling pathway during the early stages of intestinal morphogenesis and showed that the patterns of apoptosis observed in the explant model were extremely similar to those observed in normal development in vivo. Jarry et al. (2008), in work to investigate the role of mucosal interleukin-10 (IL-10) and transforming growth factor (TGF)-β in the maintenance of human colonic mucosa integrity, utilised cleaved caspase 3 staining alongside IHC for M30, an antibody against a cytokeratin-18 neoepitope resulting from an early caspase cleavage event and considered to be an early marker of epithelial apoptosis. The TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling) method, which identifies DNA strand breaks and is considered to be a key marker for apoptosis, was also used. The depletion of IL-10 caused high levels of interferon (IFN)-γ, which were responsible for surface epithelium damage and crypt loss, mainly via apoptosis.

Protein expression

In functional measures of viability assessment, different approaches were needed to show that the enterocytes were functioning normally. Browning and Trier (1969) found, microscopically, that the absorptive cells in culture continued to be able to absorb micellar fat. Continued protein synthesis has been shown, using radio-labelled amino acids and scintillation counting, and revealed continued uptake of labelled amino acids, and secretion of proteins, containing either tritiated leucine (Autrup et al. 1978a) or [^{35}S] methionine (Danielsen et al. 1982).

Rather than only look at protein synthesis as a whole, other investigators have attempted to identify the continued expression of key proteins by measuring the activity of enteric enzymes in cultured explants. For example, Mitchell et al. (1974) found that the activity of three brush border enzymes (alkaline phosphatase, α-glucosidase and leucyl-β-naphthylamidase) and one lysosomal enzyme (*N*-acetyl-β-glucosaminidase) showed a progressive decrease in the explant with time using a fluorimetric micro-assay of tissue homogenates (Fig. 3). Kik et al. (1991) used the activity of the brush border enzyme sucrase–isomaltase as a biomarker of the damage caused to cultured pig jejunum by *Phaseolus vulgaris* isolectins.

Enzyme histochemistry (EHC) is a useful tool for localising enzyme activity in frozen tissue sections. Bareiss et al. (2008) used alkaline phosphatase histochemistry to demonstrate the polarity of colon enterocytes in explant cultures, and, more recently, studies have been carried out investigating the usefulness of EHC in the localisation of alkaline phosphatase activity in frozen and formalin-fixed paraffin-embedded tissue sections of mouse small and large intestine explants (Randall et al. 2010; unpublished findings) (Fig. 4). Quinlan et al. (2006) used sucrase–isomaltase as a biomarker, but identified the enzyme protein, rather than a demonstration of enzyme activity, using IHC and reported a preservation of enterocyte polarity and absorptive phenotype.

In situ hybridisation has been utilised to compare the influence of dexamethasone on the gene expression of the serum- and glucocorticoid-inducible kinase, in explant cultures of adult mouse colon, with its respective expression in vivo (Bareiss et al. 2008). Tham et al. (1998) employed a combination of immunoprecipitation, northern blotting, in situ

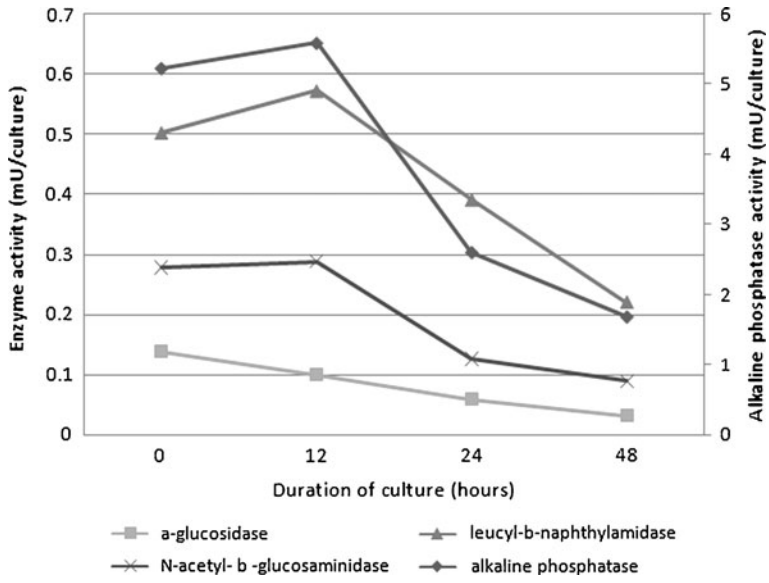

Fig. 3 Enzyme activities of cultured human jejunal biopsies over time (adapted from Mitchell et al. 1974)

hybridisation, western blot analysis and IHC to monitor changes in the expression of extracellular glutathione peroxidase (EGPx) in human and mouse gut during explant culture and found that, although the level of EGPx mRNA expression, relative to α-tubulin, was similar throughout the mouse GI tract, the highest protein levels were found in the caecum.

Immunohistochemistry has been used to track alterations in epithelial structural and organisational proteins such as cytokeratin, laminin, smooth muscle actin and β-tubulin (Metzger et al. 2007; Bareiss et al. 2008). However, Bareiss et al. (2008) went further and challenged the integrity of the epithelial barrier in infection experiments with the fungal pathogen, *Candida albicans*, demonstrating the potential usefulness of this intestinal in vitro model for the study of microbiological infections.

Metabolism in explants

To evaluate whether the metabolic activity of colonic explants was preserved, Autrup et al. (1978a, b)

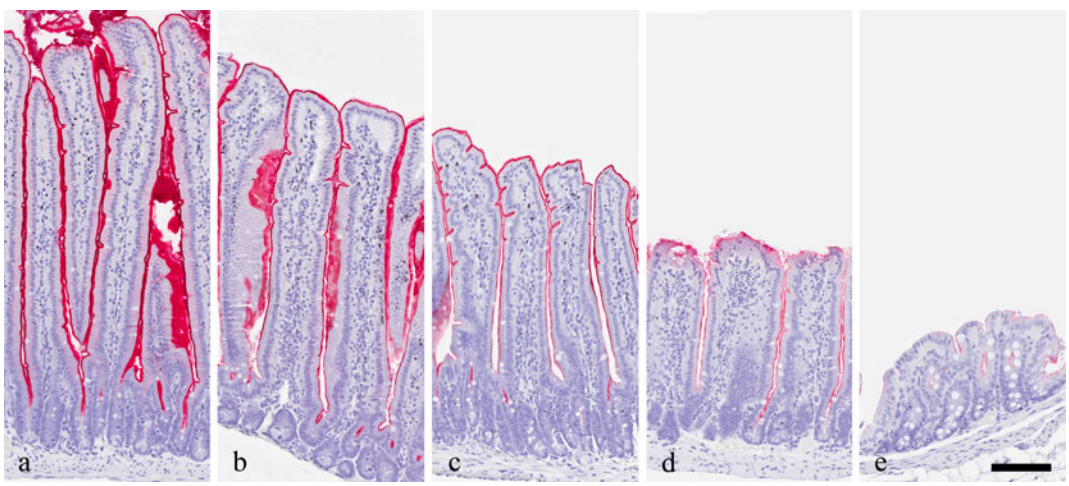

Fig. 4 A histochemical demonstration of alkaline phosphatase activity in the mouse gastrointestinal tract. The relative intensity of staining, and the height of the villi, decreases caudally from the proximal (**a**) and distal (**b**) duodenum, through the jejunum (**c**) to the ileum (**d**). The colon (**e**) has little alkaline phosphatase activity and is without villi. *Bar*=100 μm

measured lactate and glucose levels in the culture medium and found a rapid increase in lactate production with a concomitant decrease in glucose (more than 50% was consumed within 48 h). It was also found that the rat colon, cultured for 4 days before challenge, was able to metabolize benzo[*a*] pyrene, 7,12-dimethylbenz[*a*]anthracene, aflatoxin B_1, dimethylnitrosamine, 1,2-dimethylhydrazine and methylazoxymethanol acetate into chemical species that bind to cellular DNA and protein, after incubations of between 8 and 24 h.

A non-destructive way of interrogating an explant culture system is to measure the absorption and release of biological markers in the medium, this having the advantage of enabling internally controlled time course experiments. An early example of this approach was used by Autrup et al. (1978a) where glucose and lactate levels were measured in the medium, as an indication of explant functional metabolic activity. Rouet-Benzineb et al. (2004) measured the leakage of LDH as a viability biomarker when exploring the pro-apoptotic role of orexins in human colonic mucosa explants. Fletcher et al. (2006), investigating the efficacy of microbicides in human colorectal explants, assessed the viral load, by an HIV-1 p24 ELISA and an HIV-1-RNA copy number assay, of the culture supernatant rather than the tissue itself. Similarly, in their work studying colon homeostasis, and the modulation of inflammation, Jarry et al. (2008) determined, by ELISA, the release by the tissue of the inflammatory cytokines IL-10, IFN-γ, TNF-α and IL-17 into the medium.

Utilisation of explant technology

Inflammatory intestinal diseases

Up to the present day, the in vitro culture of intestine explants has found diverse applications, including studies of patho-physiological and developmental mechanisms with respect to the function of the epithelial barrier, cell proliferation, differentiation or regeneration. The exploration of the pathogenesis of intestinal disease has been one focus. Early work to successfully develop an in vitro model of coeliac disease, to search for the toxic moiety of gluten, was described in a review by Howdle (1984). More recently Mazzarella et al. (2008) cultured jejunal biopsies from celiac disease patients with a wheat gliadin, pA2, and showed that these peptides are able to activate, via a T-cell receptor/HLA class I interaction, a $CD8^+$ T-cell-mediated response resulting in enterocyte apoptosis.

Dionne et al. (1998) used colonic explants to explore the hypothesis that patients with inflammatory bowel disease (IBD) exhibited an imbalance between IL-1β and IL-1 receptor antagonist (IL-1Ra). IL-1β had been implicated in mediating a sustained inflammatory response whilst IL-1Ra, a naturally occurring inhibitor of IL-1β, had been shown to have beneficial effects in experimental models of IBD. The levels of IL-1βand IL-1Ra were measured, by ELISA, in homogenised biopsies and in the explant culture supernatant. It was found that the IL-1Ra/IL-1β ratios, in both the homogenised biopsies and the culture supernatant, were significantly decreased in involved IBD tissue, as compared with non-inflammatory controls.

Later, Dionne et al. (2003) investigated if the pro-inflammatory cytokine release, from cultured colonic explants, could be modulated by bacterial antigens. Biopsies from patients suffering from ulcerative colitis or Crohn's disease were cultured in the presence of bacterial super-antigens such as the staphylococcal enterotoxins A (SEA) and B (SEB), which were known to induce T-cell proliferation and cytokine secretion. Release of the pro-inflammatory cytokines TNF-α and IL-1β, as well as that of the anti-inflammatory cytokine IL-1Ra, was shown to be increased by incubation of colonic tissue with SEA and SEB. The authors asserted that their findings supported the concept that bacterial luminal products amplified the immune response in IBD tissue, in part by inducing TNF-α and IL-1 release. Their data were considered to have further substantiated the suggested role of bacterial products in IBD relapse or initiation.

In experiments investigating the possible immunosuppressive role of IL-10 in intestinal homeostasis, and using cultures of human colon, Jarry et al. (2008) were able to add neutralising antibodies against IL-10, IFNγ and transforming growth factor (TGF)-βRII to the culture medium and study their effects. It was shown that IL-10 was constitutively expressed and secreted by the human normal colonic mucosa, including epithelial cells, and that depletion of IL-10 in mucosal explants induced both down-regulation of the IL-10-inducible, immunosuppressive gene BCL3,

and up-regulation of IFN-γ, TNF-α and IL-17. Jarry et al. (2008) demonstrated that adding a commensal bacteria strain to mucosa explant cultures depleted of both IL-10 and lipopolysaccharide (LPS) reproduced the ability of endogenous LPS to induce IFN-γ secretion. It was also shown that IL-10 ablation led to an endogenous IFN-γ-mediated inflammatory response via LPS from commensal bacteria in the human colonic mucosa; both IL-10 and TGF-β were also demonstrated to play crucial roles in maintaining human colonic mucosa homeostasis. Di Sabatino et al. (2008) cultured endoscopic biopsies of ileum and/or colon from patients suffering from functional diarrhoea, in the presence of a TGF-β neutralising antibody, to support the hypothesis that TGF-β has a crucial role in maintaining homeostasis by dampening T-cell-mediated tissue damaging inflammation.

Morphogenesis and differentiation

The complex nature of the GI tract has made detailed dissection of the morphogenesis, proliferation, integration and differentiation of its component parts, and their combination into the functioning organ, problematic in vivo and hampered by a lack of suitable culture systems in vitro. Hearn et al. (1999) successfully developed a suspension 'caternary culture' system for embryonic mouse gut segments, which allowed the assessment of growth, morphology, cell differentiation, ability to support neural precursor migration and contractile activity. Cell differentiation from the endoderm, mesoderm and ectoderm all continued throughout the 5 to 7 days of culture.

The control of the proliferation and differentiation of human enteric nervous system progenitor spheroids has been investigated by transplantation of these cells into aganglionic hindgut explants from mice (Lindley et al. 2008; Metzger et al. 2009). Both groups found that spheroid derived cells could be differentiated into many neuronal subtypes and glial cells with characteristics of the enteric nervous system, offering new strategies for the stem cell-based treatment of conditions such as Hirschsprung's disease.

The role of Wnt growth factor in the positive regulation of intestinal proliferation has been studied in explant cultures of mouse neonatal small and large intestine (Ootani et al. 2009). It was found that culture at the air–liquid interface within a collagen matrix gave rise to spherical 'organoids'. The results indicated that long-term intestinal culture within a microenvironment, accurately modelling the Wnt- and Notch-dependent intestinal stem cell niche, is possible. It has been possible to isolate intestinal crypts from adult mice and generate organoids by culturing the crypts in laminin-rich Matrigen (Sato et al. 2009). The upper opening of the cultured crypts sealed over but the crypt region continued to proliferate, generating buds which expanded to create organoids. The organoids comprised more than 40 crypt domains surrounding a central lumen, lined by villus-like epithelium. It was possible to trace the stem cell hierarchy and conclude that intestinal crypt–villus units are self-organising structures, which can be built from a single stem cell.

Manipulation of explant culture conditions

The ability to accurately alter the conditions in which the explant is cultured is just one of the advantages that this in vitro system holds over in vivo experiments. For instance, Challacombe and Wheeler (1991) were able to demonstrate the trophic action of EGF on human duodenal mucosa. The addition of 400 ng/ml of EGF to one of paired explants caused a five-fold increase in duodenal crypt cell proliferation in their experiment. In later work, Wheeler and Challacombe (1997) generated a dose–response curve when characterising the effect of recombinant human growth hormone and insulin-like growth factor I on crypt epithelial cell proliferation (Fig. 5).

Similarly, Zachrisson et al. (2001) examined whether TNF-α and interleukin affected the mitotic activity in explants of human duodenal mucosa, and estimated the release of cytokines, by incubating the explants with precise, measured doses of TNF-α, IL-8 and anti-IL-8. They discovered that TNF-α and IL-8 increased the mitotic activity in the small intestinal crypts of duodenal mucosal explants. The explants spontaneously released IL-8, and to a lesser extent IL-6, which were markedly increased following incubation with TNF-α. The mitogenic action of TNF-α was primarily a direct effect of this cytokine and, to a minor extent, mediated by the secondary production of IL-8. These findings indicated that the cytokines studied may have been important regulators of epithelial cell proliferation in human duodenal mucosa (Zachrisson et al. 2001).

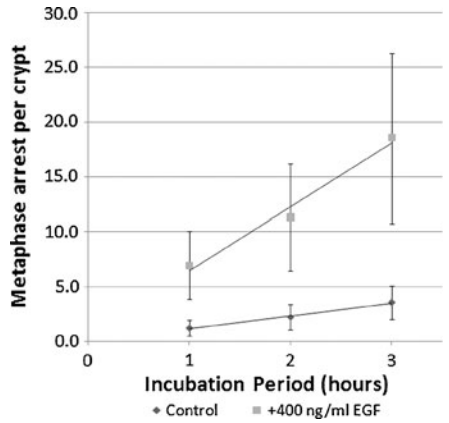

Fig. 5 The trophic effect of epidermal growth factor (*EGF*) on the proliferation of crypt cells in cultures of human duodenal mucosa (adapted from Challacombe and Wheeler 1991). *Bars* indicate standard deviation from the mean

The above examples demonstrate how much more controllable explant experimental systems can be, compared with equivalent in vivo systems. The addition, to the culture system, of precise, reproducible doses of cytokines, antibodies, xenobiotics etc., and the ability to take accurately timed samples, either from the explant itself or from the culture medium, over the length of the experiment, has proved to be powerful research technique. Explant systems may also reduce animal usage by allowing multiple explants to be prepared from a single animal, or human donor, not only improving the legitimacy of controls but also working towards the concept of the 3Rs (reduction, refinement, replacement) in animal experimentation (Broadhead and Bottrill 1997).

Explants in toxicology

Toxicology is constantly striving to establish more relevant assays for risk assessment purposes. While whole animal studies have been the mainstay, and gold standard, for testing for many years, the incentives given by the 3Rs initiative has made it imperative that where possible the number of animals used and their use per se needs to be justified with an earnest investigation as to any possible non-animal alternative (Buck 2007). While in vitro assays have been introduced in large numbers their restrictions have been identified and acknowledged (Brusko et al. 2007; Nigsch et al. 2007), and they represent at best a proportion of the information/biological systems present in the whole animal (Ito et al. 1998). They do permit precise experimentation of specific aspects of any particular pathway and are excellent tools for hypothesis testing. However, as a screen they lack the overall components of the in vivo system and as a result their predictivity is limited to certain well-defined endpoints such as DNA damage and mutation.

Precision cut slices of tissues have evolved to become very useful for the short-term evaluation of processes and intestinal slices have been utilised to study xenobiotic metabolism up to a 24-h period for the small intestine (De Kanter et al. 2004). The advantage of the precision cut systems over the in vitro cell systems are that all the relevant cell types, metabolic enzymes, transporters and co-factors are present (De Kanter et al. 2002a) and hence should, at least in theory, more closely represent the function in vivo. Intestinal slices have also been used successfully to compare the metabolic capabilities in the intestine of a number of different animal species including human (De Kanter et al. 2002b).

An ex vivo system has been used by Takemoto et al. (2003) and Emoto et al. (2000) whereby everted rat or mouse intestinal sacs were used to compare the metabolic profiles of a number of chemicals including testosterone, ethoxyresorufin and 7-ethoxycoumarin with isolated intestinal microsomes. In this system, incubations were typically 30–180 min in duration and longer term incubation was not attempted but the metabolic capacity of the sacs was nevertheless identified to be at least equivalent to that seen in isolated intestinal microsomes and in some cases was found to be appreciably more, dependent upon the specific enzyme system studied (Emoto et al. 2002).

However, in order for any new bioassay to be adopted in toxicology, a number of basic criteria have to be met by the assay (OECD 1996, 2002), namely:

- Assessment of the relevance and reliability of the test method (test optimisation).
- Full assessment of the relevance and reliability of the test method (characterising in full the performance of the test).
- Overall evaluation of the validation study (independent peer review).
- Publication of the data validation exercise including the methodology in the peer-reviewed scientific literature.

None of the applications of GI explant culture in toxicology have been subjected to this level of

scrutiny. It is, therefore, unsurprising that GI explant technology has yet to be accepted as part of an in vitro screening assay. The relative paucity of published data in this arena may be indicative of the difficulty of routinely culturing GI explants with the level of consistency demanded by validated toxicological bioassays.

Explants in therapeutic drug development

GI tract explants can be generated from healthy and diseased human donors enabling the testing of novel xenobiotics for efficacy and safety. The effects of potential drugs/chemicals on explants from laboratory animals could also be directly compared to changes observed in human explants in drug discovery, and employ considerably smaller amounts of chemicals than would be used in conventional in vivo studies. Explant culture could also perform an important link role in translational medicine where they could be incorporated into the discovery process in the intervening period between preclinical and clinical trials in man.

Fletcher et al. (2006) used colorectal explants derived from endoscopic biopsies and surgical resections to evaluate the efficacy of candidate microbicides in protecting against HIV infection. Explants were treated with the microbicides diluted in the culture medium for 1 h before being challenged by the addition of HIV-1 stock solution for 2 h. The explants were then washed and cultured for up to a further 12 days. Fletcher et al. (2006) were able to measure the activity of the different concentrations of the antiviral agents directly by sampling the culture medium and assessing the viral load by an HIV-1 p24 ELISA, and an HIV-1-RNA copy number assay. The proviral DNA content of the tissue was analysed by a multiplex quantitative real-time polymerase chain reaction for HIV-1 DNA. Using these techniques, it was possible to differentiate the efficacies of a range of candidate microbicides in a model considered to closely mimic the proposed rectal application of these compounds. In addition, the possible toxicity of the compounds could be assessed by studying tissue morphology, by evaluating the cell phenotype and activation status of leucocytes isolated from the tissue, and by using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] viability assay.

Summary

The present review sets out some of the uses, advantages and potential benefits of intestinal explant culture in investigations into the basic science of explants, and considers their possible use in evaluating the activity and toxicity of xenobiotics. It is considered that the culture of explants demonstrates the following advantages in comparison with more conventional in vivo studies:

- Administration of defined doses of test substances directly to the tissue under investigation.
- Comparison of samples from the same donor under both test and control conditions across the time course of the experiment, greatly improving the statistical power of the study.
- Generation of multiple explants from a single donor, reducing animal usage.
- Time course sampling of biomarkers.
- Comparison of activity and toxicity on the GI tract across species.
- Evaluation of novel compounds, with unknown toxic liability, without risk to the donor.
- Investigation of the metabolism of xenobiotics by the GI tract.
- Validation of the in vivo toxicology findings and vice versa.
- Potential to test the GI toxicity of a xenobiotic with minute amounts of compound.

In conclusion, it is considered that intestinal explant culture shows much potential for the application of a relatively under-used procedure in future biomedical research. Furthermore, there appear to be many instances where the technique may provide experimental solutions where both cell culture and in vivo models have been unable to deliver conclusive and convincing findings.

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