2 Intestinal Physiology and Immunology

2

Intestinal Morphology, Intestinal Regeneration and the Promise of Tissue Engineering

David A.J. Lloyd and Simon M. Gabe

<div style="border:1px solid">

Key points

- Intestinal morphology reflects its role as an absorptive surface and a barrier against luminal contents.
- Small intestinal surface area is increased about 600-fold by a combination of micro- and macroscopic features.
- Both small and large intestinal mucosal surfaces are rapidly replaced as a result of the activity of stem cells found in the intestinal crypts.
- Understanding of stem cell dynamics and signaling has increased rapidly in recent years.
- Tissue engineering of intestinal mucosa has been performed in animal models and offers the possibility of a novel therapy for patients with short bowel syndrome.

</div>

Introduction

The morphology of the intestine reflects its two principle roles: the digestion and absorption of nutrients, and the maintenance of a barrier against the external environment. This chapter will briefly describe the basic macroscopic and microscopic structure of the small and large intestine and will review current understanding of the role of stem cells in intestinal regeneration. An improved knowledge of stem cell biology has led to the

production of tissue engineered intestine and progress in this exciting field will be explored.

Morphology of the intestine

Macroscopic structure

The gross anatomy of the small and large intestine is well characterized, well understood, and is the topic of numerous texts. As such, a detailed description is not warranted here. However, there are a number of points that are relevant in the context of intestinal failure, in particular the length of normal small intestine and the vascular supply of the small and large intestine.

The combined length of the jejunum and ileum ranges from 3 to $8^1/_2$ m [1]. The variability of small intestinal length between individuals is difficult to predict and therefore it is vitally important that the length of remaining intestine rather than the length of resected intestine is recorded at surgery. The absorptive area of the small intestine is increased about threefold by the presence of circular infoldings known as plicae circulares. These are most marked in the jejunum, becoming smaller and less frequent as the colon is approached. The length of the large intestine is less variable, being about 1–1.5 m long. It is separated from the small intestine by the ileocaecal valve; the importance of the presence or absence of the ileocaecal valve postoperatively will be discussed in further chapters.

Other than the upper half of the duodenum, which receives blood from the pancreaticoduodenal artery, a branch of the gastroduodenal artery, the small intestine receives its arterial blood supply from branches of the superior mesenteric artery. Blood supply to the
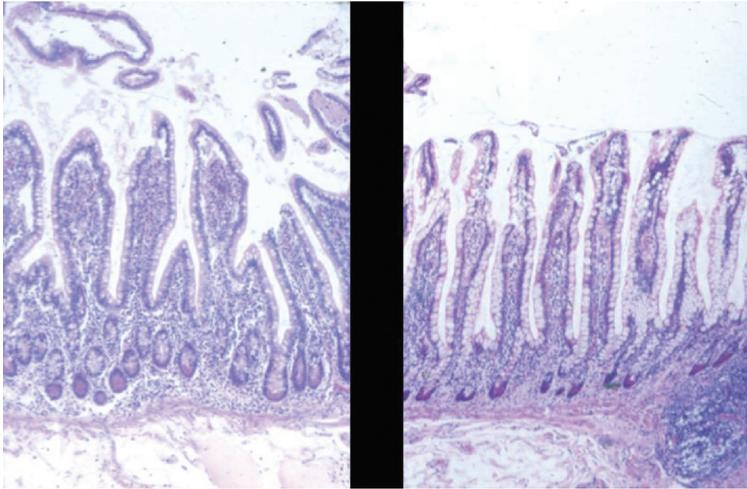
Figure 2.1 Cross-section through jejunum (left) and ileum (right) showing crypt-villus structure. (Images kindly supplied by P. Domizio.)

proximal large intestine, up to the distal third of the transverse colon, is also from branches of the superior mesenteric artery. However, the distal large intestine receives its blood supply via branches of the inferior mesenteric artery. Corresponding veins drain blood from both the small and large intestine into the portal circulation. It can be seen that the superior mesenteric artery and vein are of paramount importance to blood supply to and from the intestine and is the reason why infarction and thrombosis are so devastating.

Microscopic structure

Both the small and large intestine are composed of four distinct layers: the mucosa, the submucosa, the muscularis externa, and the serosa. The mucosa consists of a layer of columnar epithelium below which lies the lamina propria, a loose connective tissue layer containing blood vessels, lymphatics, and some lymphoid tissue. In the small intestine, the mucosa forms fingerlike projections known as villi. These are most pronounced in the ileum and serve to increase small intestinal surface area about tenfold. Each villus contains a dense capillary network, which lies just below the epithelium, and blind-ending lymphatic vessels, lacteals, which drain into lymphatics forming a plexus in the lamina propria. In between and in continuity with the villi are the intestinal crypts (Figure 2.1). These crypts contain intestinal epithelial stem cells which allow repopulation and repair of the small intestinal mucosa. Although the mucosa of the colon does not form villi, stem cells remain located in crypts.

Scattered throughout the lamina propria and submucosa of the small intestine, predominantly the ileum, are visible aggregates of lymphoid tissue known as Peyer's patches. The mucosa overlying the Peyer's patches is flattened and contains numerous antigen-sampling M cells. In the colon, the lamina propria contains larger numbers of smaller lymphoid nodules. The lamina propria is separated from the submucosa by a thin inner circular layer and an outer longitudinal layer of smooth muscle known as the muscularis mucosae. The submucosa contains a network of blood vessels and lymphatics as well as groups of neurons, which make up Meissner's plexus.

The muscularis externa, like the muscularis mucosae, consists of an inner circular layer and an outer longitudinal layer of smooth muscle. In the large intestine, the outer smooth muscle layer forms three bands known as teniae coli. Between the inner and outer smooth muscle layers are blood vessels and lymphatics, and neurons which make up Auerbach's plexus. The outer serosa is a thin layer of loose connective tissue containing vasculature and lymphatics, covered on the outer surface by mesothelium.

Mucosal cell types

There are five main epithelial cell lineages, all of which are derived from stem cells in the intestinal crypts. The most plentiful cells in the mucosal epithelium are columnar enterocytes, which make up the absorptive surface of the intestine. The apical surface of these cells

consists of a layer of densely packed microvilli, visible with electron microscopy. Each enterocyte has about 3,000 microvilli on its apical surface and their presence increases small intestinal surface area about 20-fold. Intestinal enterocytes contain numerous transport proteins in their apical and basal membranes, which allow active and passive transport of nutrients from the gut. In addition, several digestive enzymes, such as disaccharidases, are bound to the enterocyte microvilli.

Located among the enterocytes are mucin-containing goblet cells. These cells produce mucus and other glycoproteins, which lubricate and protect the mucosal surface. Scattered enteroendocrine cells secrete peptide hormones, which exert endocrine and paracrine actions; the exact peptides secreted vary throughout the intestine. Paneth cells, unlike the other epithelial cell lines, are found at the crypt bases. They are characterised by granules containing lysozyme, tumour necrosis factor-α and defensins, and are believed to play an antibacterial role. Finally, M cells are believed to be involved in antigen sampling and transportation.

The lamina propria contains a wide range of cell types including smooth muscle cells, vascular endothelial cells and fibroblasts. Of particular interest are the intestinal subepithelial myofibroblasts. These cells are located in proximity to the mucosal epithelium and are believed to produce growth factors, including hepatocyte growth factor, which promote proliferation of the intestinal epithelial stem cells [2].

Regeneration of the intestinal mucosal epithelium

The intestinal mucosa has an impressive capacity for replication and regeneration both under normal physiological conditions and following injury. The entire small intestinal epithelium is replaced every 3–5 days. As mentioned previously, this regenerative capacity is dependent on the activity of intestinal epithelial stem cells found toward the base of the epithelial crypts.

Stem cell proliferation and epithelial cell differentiation

The majority of stem cell divisions are believed to result in a single daughter cell and a single stem cell, which retains the original template DNA. These daughter cells then undergo further divisions to produce a population of transit-amplifying (TA) cells. These TA cells are rapidly proliferating and divide and further differentiate to produce the different epithelial cell lines. Stem cell daughter cells and TA cells retain their clonogenicity and are able to revert back to stem cells if the crypt is damaged and existing stem cells are lost. However, as the TA cells divide further, they lose their capacity for clonal expansion. Enterocytes, goblet cells, and enteroendocrine cells undergo further differentiation as they migrate upward toward the tip of the villus. They then are either shed into the intestinal lumen or undergo apoptosis. Each crypt contributes to the epithelium of a number of villi. Unlike the other cell lines, Paneth cells undergo differentiation as they migrate to the base of the crypts. The concept that a single intestinal stem cell can give rise to all intestinal epithelial cell lines is known as the Unitarian hypothesis and is supported by a significant body of evidence [3].

Although the hierarchical pattern of cell proliferation and differentiation from crypt to villus is firmly established, the exact number of the stems cells in each crypt is less clear. Historically, progress in this area has been hampered by a lack of reliable molecular stem cell markers, although the discovery of Musashi-1 and Hes1 as putative stem cell markers is encouraging [4]. It is believed that there are four to six stem cells in each crypt, which are located in a specific stem cell compartment also known as the "stem cell niche" (Figure 2.2). This niche comprises the intestinal epithelial stem cell, neighboring proliferating cells, and adjacent mesenchymal cells such as the pericryptal fibroblasts and intestinal subepithelial myofibroblasts [5]. These mesenchymal cells are believed to play an important role in the maintenance of the stem cell population and the control and regulation of proliferation via the secretion of various peptides including hepatocyte growth factor, keratinocyte growth factor, and tissue growth factor-β. There are complex signaling pathways between the different components of the stem cell niche, including the Wnt, hedgehog, and notch pathways. Our understanding of this signaling is increasing rapidly but is beyond the scope of this chapter and has been reviewed recently [3,6].

Although the majority of stem cell divisions result in one stem cell and one daughter cell as described
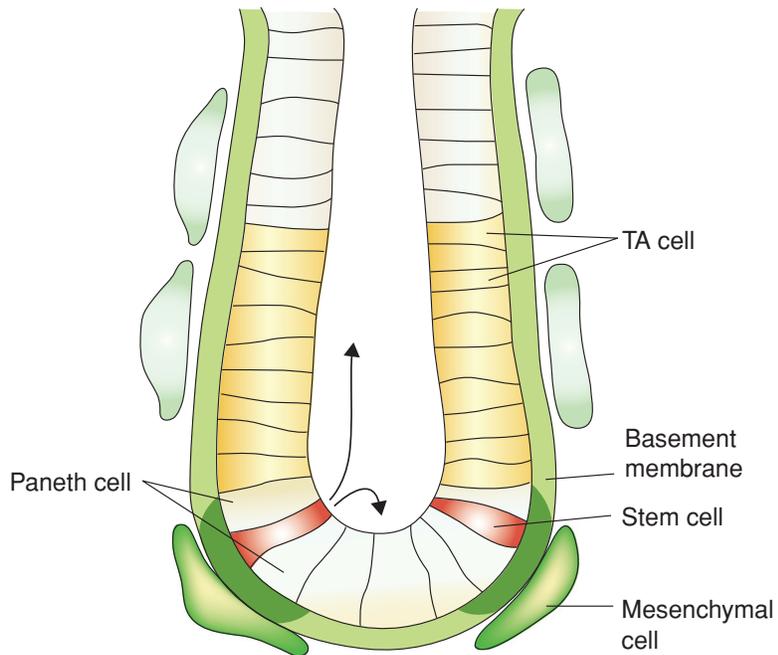
Figure 2.2 Intestinal crypt. Stem cells (red) are found at the crypt bases. Stem cell progeny (yellow) known as transit amplifying (TA) cells migrate up the crypt undergoing further replication and differentiation. Basement membrane and mesenchymal cells (green) surround the stem cell niche. (Reprinted from Spradling et al. [5], with permission from Macmillan Publishers Ltd.)

earlier, division may be symmetrical, resulting in either two daughter cells or two stem cells. If the former occurs, then that stem cell will be lost from the crypt. This ultimately may lead to all cells in a crypt being descendants of a single stem cell. Conversely, if division results in two stem cells then the total number of stem cells in the crypt will increase. It has been proposed that increases in stem cell number may be triggers for crypt fission, a process vital to intestinal growth and repair after injury.

Stem cell plasticity

Stem cell plasticity refers to the ability of organ-specific stem cells from one tissue to produce cells of a different lineage and tissue. For example, adult bone marrow cells may be able to engraft into other tissues and differentiate into cell types specific to that organ, such as hepatocytes or skeletal myocytes. There is increasing evidence to support this hypothesis, especially from observations that Y-chromosome containing cells can be seen in the tissues of female recipients of bone marrow from male donors, although it has been argued that this may represent cell fusion rather than stem cell plasticity. In the small and large intestine, bone marrow

engraftment and differentiation into mesenchymal cells have been demonstrated in humans and mice [7]. The possibility that bone marrow derived cells could be used to populate and regenerate intestinal mucosa is clearly exciting and of particular relevance to the field of tissue engineering.

Tissue engineering of intestinal mucosa

Tissue engineering offers a novel approach to the treatment of short bowel syndrome. The basic concept is to seed intestinal tissue onto artificial, biodegradable scaffolds which will support the growth and development of organized intestinal "neomucosa." The use of autologous cells to create biocompatible tissue-engineered intestinal constructs has several benefits, including the stimulation of natural biological mechanisms for repair and remodeling, complete biocompatibility, the potential for further growth, and, of course, the avoidance of problems associated with transplantation. Potentially, the engineering of small intestine as a functional absorptive area could render patients with short bowel syndrome independent of parenteral nutrition.

Culturing intestinal epithelium

A necessary initial step in the creation of intestinal neomucosa has been the development of techniques to produce primary cultures of intestinal epithelium. In the early 1990s, Evans and colleagues described a method by which "organoid units" were derived from neonatal rat small intestine by partial digestion using a mixture of collagenase and dispase [8]. These organoid units are cellular aggregates consisting of polarised intestinal epithelium surrounding a core of mesenchymal cells. As digestion is incomplete, it is believed that the stem cell niche is maintained. Experiments have shown that the organoid units can be successfully cultured and maintained *in vitro* [8].

Generation of neomucosa

Tait and colleagues demonstrated that suspensions of organoid units transplanted into subcutaneous pockets in adult rodents could develop into small tubular structures, which consisted of a central lumen surrounded by a circumferential epithelial layer [9]. As early as 2 weeks after organoid unit transplantation, this epithelial layer had formed crypts and villi and was histologically similar to small intestinal mucosa. This neomucosa was shown to contain all epithelial cell lineages [9]. Although the majority of subsequent studies have used rodent tissue exclusively, it is noteworthy that Sattar and colleagues demonstrated that organoid units produced from human foetal small intestine could be successfully implanted subcutaneously into SCID mice to produce cysts of neomucosa similar to that described in rat models [10].

Growth of neomucosa on artificial scaffolds

A major challenge in the development of tissue engineered intestine is the production of a three-dimensional tubular structure with the correct orientation of mucosal, submucosal, and muscular layers. Artificial biodegradable scaffolds have been employed by several groups in an attempt to produce tubular structures lined with neomucosa [11,12] (see Figure 2.3). These scaffolds need to be flexible but also must maintain their structural integrity *in vivo,* and must be porous enough to allow cell migration and neovascularization to occur through the scaffold.
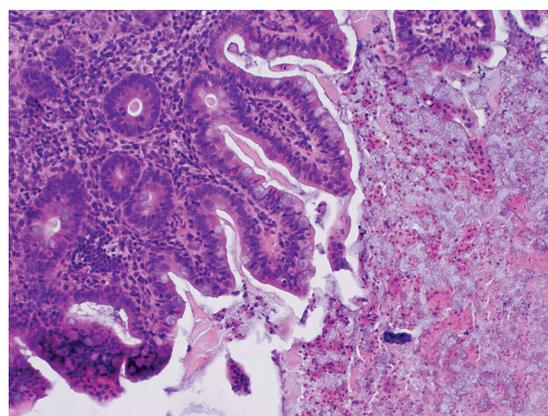
Figure 2.3 H&E staining of artificial scaffold 4 weeks after implantation of organoid units. There is evidence of mucosa and submucosa lining the entire circumference of the luminal surface of the scaffold. At high magnification, the appearance of the mucosa and submucosa lining the luminal surface of the scaffold is similar to that of normal small intestinal tissue. There is columnar epithelium containing mucin producing goblet cells, which has developed into crypts and villi. The submucosa contains macrophages, fibroblasts, and other inflammatory cells.

Vacanti and colleagues developed a model for the tissue engineering of intestinal neomucosa in which organoid units were seeded onto collagen coated polymer scaffolds, which were then implanted into the peritoneum of a rat model. By 2 weeks, the luminal surface of the tubular scaffold was covered with a layer of epithelium and between 2 and 6 weeks there was development of crypts and villi and evidence of epithelial maturation and cellular differentiation. Below the epithelium, smooth muscle-like cells and,

possibly, early neural tissue were identified. Electrophysiological studies demonstrated the generation of transepithelial resistance, indicative of an active barrier, and evidence of active transport across the neomucosal membrane [13]. These scaffolds have been successfully reimplanted into native rat small intestine. When reassessed 5 months after reimplantation, the neomucosa had well-developed crypt-villus architecture similar to native small intestine; rates of proliferation and apoptosis were also similar [14]. Reimplanted neomucosa was significantly more developed than nonreimplanted tissue, suggesting a trophic effect of luminal bowel contents, and further studies have demonstrated that this can be amplified further by injection of GLP-2 [15]. This group has applied the same principles to other intestinal tissue and has produced tissue-engineered esophagus, stomach, and colon [16–18].

Functional capacity of neomucosa

Neomucosa produced by subcutaneous transplantation of organoid units has been shown to express brush border digestive enzymes [9, 19] and quantitative functional studies have demonstrated that the activity of these digestive enzymes was similar to that of age-matched control rat small intestine [19]. More recently, Grikscheit and colleagues implanted scaffolds containing tissue-engineered neomucosa into rats that had undergone 85% enterectomy and demonstrated that postoperative weight loss was reduced and subsequent weight gain enhanced [20]. Although this effect was attributed to the potential absorptive capacity of the implanted neointestine; it is possible that it may be, in part, mediated by the effects of the nonperistaltic neointestine on gut transit time.

Conclusions

Knowledge of intestinal regeneration and repair and, especially, of stem cell behaviour and function is increasing rapidly. This improved understanding has made it possible to produce experimental models of tissue-engineered intestine. Although the initial work is very encouraging, there are still significant limitations. One major problem is that the methodology employed is very inefficient with large quantities of neonatal tissue being required to produce a small section of neointestine. Clearly, this significantly limits clinical application at present and future research will need to address the issues of yield and explore possible alternative sources of tissue with which to seed scaffolds. However, the concept of stem cell plasticity raises the possibility that one day bone marrow–derived stem cells might be used as substrates to produce significant quantities of artificially engineered intestine.

References

1 Nightingale J, Spiller R. *Normal Intestinal Anatomy and Physiology*. In: Nightingale J, ed. *Intestinal Failure*. London: Grenwich Medical Media, 2001:17–36.

2 Powell DW, Mifflin RC, Valentich JD, et al. Myofibroblasts. II. Intestinal subepithelial myofibroblasts. *Am J Physiol* 1999;277:C183–C201.

3 Leedham SJ, Brittan M, McDonald SA, Wright NA. Intestinal stem cells. *J Cell Mol Med* 2005;9:11–24.

4 Kayahara T, Sawada M, Takaishi S, et al. Candidate markers for stem and early progenitor cells, Musashi-1 and Hes1, are expressed in crypt base columnar cells of mouse small intestine. *FEBS Lett* 2003;535:131–135.

5 Spradling A, Drummond-Barbosa D, Kai T. Stem cells find their niche. *Nature* 2001;414:98–104.

6 Rizvi AZ, Hunter JG, Wong MH. Gut-derived stem cells. *Surgery* 2005;137:585–590.

7 Brittan M, Hunt T, Jeffery R, et al. Bone marrow derivation of pericryptal myofibroblasts in the mouse and human small intestine and colon. *Gut* 2002;50:752–757.

8 Evans GS, Flint N, Somers AS, Eyden B, Potten CS. The development of a method for the preparation of rat intestinal epithelial cell primary cultures. *J Cell Sci* 1992;101(Pt 1):219–231.

9 Tait IS, Flint N, Campbell FC, Evans GS. Generation of neomucosa in vivo by transplantation of dissociated rat postnatal small intestinal epithelium. *Differentiation* 1994;56:91–100.

10 Sattar A, Robson SC, Patel HR, Angus B, Campbell FC. Expression of growth regulatory genes in a SCID mouse-human model of intestinal epithelial regeneration. *J Pathol* 1999;187:229–236.

11 Choi RS, Vacanti JP. Preliminary studies of tissue-engineered intestine using isolated epithelial organoid units on tubular synthetic biodegradable scaffolds. *Transplant Proc* 1997;29:848–851.

12 Lloyd DA, Ansari TI, Gundabolu P, et al. A pilot study investigating a novel subcutaneously implanted pre-cellularised scaffold for tissue engineering of intestinal mucosa. *Eur Cell Mater* 2006;11:27–33.

13 Choi RS, Riegler M, Pothoulakis C, et al. Studies of brush border enzymes, basement membrane components, and electrophysiology of tissue-engineered neointestine. *J Pediatr Surg* 1998;33:991–996.

14 Tavakkolizadeh A, Berger UV, Stephen AE, et al. Tissue-engineered neomucosa: morphology, enterocyte dynamics, and SGLT1 expression topography. *Transplantation* 2003;75:181–185.

15 Ramsanahie A, Duxbury MS, Grikscheit TC, et al. Effect of GLP-2 on mucosal morphology and SGLT1 expression in tissue-engineered neointestine. *Am J Physiol Gastrointest Liver Physiol* 2003;285:G1345–G1352.

16 Grikscheit T, Ochoa ER, Srinivasan A, et al. Tissue-engineered esophagus: experimental substitution by onlay patch or interposition. *J Thorac Cardiovasc Surg* 2003;126:537–544.

17 Maemura T, Ogawa K, Shin M, et al. Assessment of tissue-engineered stomach derived from isolated epithelium organoid units. *Transplant Proc* 2004;36:1595–1599.

18 Grikscheit TC, Ogilvie JB, Ochoa ER, et al. Tissue-engineered colon exhibits function in vivo. *Surgery* 2002;132:200–204.

19 Tait IS, Penny JI, Campbell FC. Does neomucosa induced by small bowel stem cell transplantation have adequate function? *Am J Surg* 1995;169:120–125.

20 Grikscheit TC, Siddique A, Ochoa ER, et al. Tissue-engineered small intestine improves recovery after massive small bowel resection. *Ann Surg* 2004;240:748–754.