

# Epithelial Stem Cells and Tissue Engineered Intestine

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**Abstract:** The intestinal mucosa has an amazing regenerative capacity, enabling rapid restoration of its physiological functions following injury. The ability to do this resides with the epithelial stem cells located within glandular invaginations in the mucosal surface. Recent advances toward the isolation and characterization of epithelial stem cells has paved the way for exploring novel therapeutic approaches for gastrointestinal disease. Possible stem cell-based therapy of gastrointestinal disorders range from the repair of damaged mucosa through to tissue engineering of artificial intestinal constructs for patients with short bowel syndrome. Before these benefits are realized further information is required on the biological characteristics of intestinal stem cells, their interactions with surrounding cells, and the environment in which they reside. This includes discovering markers to assist in the identification and purification of stem cell populations and techniques to manipulate the cells both *in vivo* and *in vitro*. Because intestinal transplantation for patients still represents a significant challenge, it is hoped that one day a tissue-engineered intestine will provide a feasible option for patients with short bowel syndrome. This review aims to introduce the reader to the main characteristics of epithelial stem cells and provide an overview of the current status of intestinal tissue engineering and the problems still being faced.

**Keywords:** Epithelial stem cells, intestine, tissue engineering.

## INTRODUCTION

Short bowel syndrome is a condition in which there is insufficient intestinal mucosal surface area to allow adequate absorption of nutrients to maintain body weight and support life. Extensive small bowel resection resulting in short bowel syndrome can be the result of many conditions including inflammatory bowel disease, neonatal necrotizing enterocolitis, volvulus, or trauma. Although the small intestine has a large functional reserve capacity, resection of 70-80% of the small intestine invariably results in short bowel syndrome, with increased severity associated with simultaneous resection of the ileocecal region or colon. A remnant of small intestine <50 cm long is significantly associated with a decreased survival rate [1,2]. Intestinal adaptation of the remaining intestine is a complex physiological response aimed at compensating the loss of absorptive surface area. It involves a number of morphological and cellular changes to intestinal mucosa, including increases in mucosal wet weight, protein and DNA content, villus height and crypt depth, and absorptive surface area [3]. Short bowel syndrome occurs if the intestine cannot adapt sufficiently to increase the absorptive surface area to maintain nutritional equilibrium [4]. Current therapeutic options available for patients with short bowel syndrome are aimed at ensuring an adequate supply of nutrients, water, electrolytes, trace elements and vitamins. This can be achieved via parenteral nutrition (where nutrition is maintained by intravenous infusion) [5,6], or surgical intervention aimed at increasing the mucosal surface area or slowing gastrointestinal transit time [7]. Whilst transplantation of the small intestine is an option for young

patients with non-tolerable complications of long-term parenteral nutrition, it still represents a significant challenge with potential problems including donor organ shortage and the need for long-term immunosuppression [8]. The first attempts at human intestinal transplantation were conducted in the 1960s but it was not until the introduction of cyclosporine in the early 1980s that successful transplants were performed. Now there are over 60 centres worldwide that conduct intestinal transplantation. Between 1985 and 2003, 989 transplants were performed on 923 patients, with 484 current survivors at the end of the data collection period (The Intestinal Transplant Registry; [www.intestinaltransplant.org](http://www.intestinaltransplant.org)). Although improved anti-rejection drugs such as tacrolimus now exist and the short-term survival rate is comparable to that of lung transplant results, the immune system of the intestine appears to contribute to many of the difficulties associated with transplantation.

The epithelium of the intestine is a confluent cell layer consisting of a number of cell subtypes each with specialized functions that help it to perform its two major physiological functions: (1) to provide an effective barrier between the luminal contents of the intestinal tract and underlying sterile mucosa, and (2) to efficiently absorb nutrients and water from ingested food. Loss of either of these functions is, at best, associated with varying degrees of morbidity, but if left unchecked, can be fatal. Therefore the body has evolved an efficient mechanism of maintaining homeostasis within the epithelium by ensuring a steady supply of cells are available to repopulate the mucosa. Mucosal epithelium undergoes a continuous rapid turnover throughout life, with the entire epithelial lining being replaced every 3-5 days. However the potential capacity of this mechanism is only demonstrated during pathological conditions when major damage has occurred to the intestine, for example following trauma or disease, resulting in increased epithelial regeneration. The ability of the multipotent intestinal epithelial stem cells to repopulate insufficient areas of

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gastrointestinal epithelium is now recognised as a potential therapeutic strategy for conditions where the absorptive and/or barrier function of the intestine are insufficient. An increased understanding of intestinal epithelial stem cells over the past decade has led to attempts to develop tissue-engineered small intestine that could alleviate the symptoms of short bowel syndrome by increasing the mucosal surface area. The current review will introduce to the reader the main characteristics of intestinal epithelial stem cells and provide an overview of the current status of intestinal tissue engineering using epithelial stem cells.

## EPITHELIAL CELL LINEAGES OF THE SMALL INTESTINE AND COLON

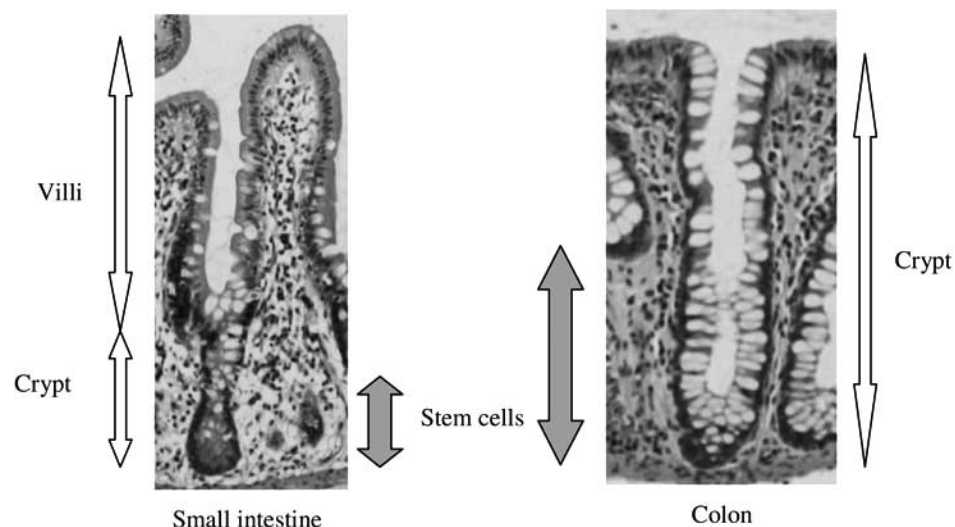
Intestinal epithelium consists of five cell lineages. Enterocytes, also called columnar or absorptive cells, are the most plentiful cell type. This set of epithelial cells participates in membrane digestion and absorption of nutrients within the lumen of the gastrointestinal tract, facilitated by the presence of microvilli at their apical surface that increase the absorptive surface area. Goblet cells are found scattered between enterocytes and can be easily identified by the large mucin-filled vacuole in their cytoplasm (Fig. 1). Enteroendocrine cells secrete peptide hormones and are found scattered throughout the epithelium. M (membranous or microfold) cells are antigen-sampling cells and are found near lymphoid follicles. Paneth cells are found at the base of crypts in the small intestine and ascending colon and are packed with strongly eosinophilic granules, which contain lysozyme and antibacterial defensins. Enterocytes, goblet cells and enteroendocrine cells become more differentiated as they mature and migrate upward from the base of the crypt towards the villous tip. Paneth cells differ by completing their terminal differentiation as they migrate down towards the crypt base [9-11]. Once the epithelial cells reach the tip of the villi they undergo spontaneous apoptosis and are shed into the lumen or are phagocytosed, a process that controls the number of epithelial cells in the normal healthy gastrointestinal tract. In the healthy gastrointestinal tract, the

net amount of apoptosis equals the influx of epithelial cells migrating onto the villi per day, maintaining homeostasis of the epithelial cell population [12].

## INTESTINAL EPITHELIAL STEM CELLS

Intestinal epithelial stem cells reside in specific niches within glandular invaginations, called crypts, on the mucosal surface of the small intestine and colon (Fig. 1). Despite the lack of well-characterized molecular stem cell markers, the likely location and identity of the intestinal stem cells has been determined using both cell morphological and functional criteria. Morphologically, stem cells appear undifferentiated and may exhibit embryonic cell-like features [13,14]. Functional characteristics include the ability to proliferate to replicate themselves and to regenerate the lineage precursors that differentiate and produce mature cell populations that migrate upwards from the crypt base. The use of microcolony assays, originally described by Withers and Elkind, has demonstrated the regenerative capacity of intestinal stem cells following pathological damage, for example with cytotoxic radiation [15].

Small intestinal stem cells are located in the base of the crypts just above the Paneth cells [16,17]. Stem cells from several crypts provide the epithelium for each of the villi that project into the intestinal lumen. In the colon stem cells are situated more towards the mid-crypt region of the ascending colon and the crypt base in the descending colon [18] (Fig. 1). The number of stem cells per crypt has not been definitively established and estimates vary widely from 0.4% to 60% of the crypt cell population. The variation in estimates appear to depend on differences in opinion regarding what defines a stem cell and/or which species and stage of development is being studied [11, 19]. Estimates of the number of stem cells per crypt have been achieved by applying increasing doses of irradiation to successively reduce the number of crypt stem cells. A hierarchal crypt structure consisting of three tiers of stem cells has been proposed, with 4-6 actual stem cells residing in the first tier



**Fig. (1).** Histological sections of adult human small intestine and colon indicating the regions that contain epithelial stem cells. Goblet cells, with their mucin-filled vacuoles, are easily identifiable amongst enterocytes in the epithelium.

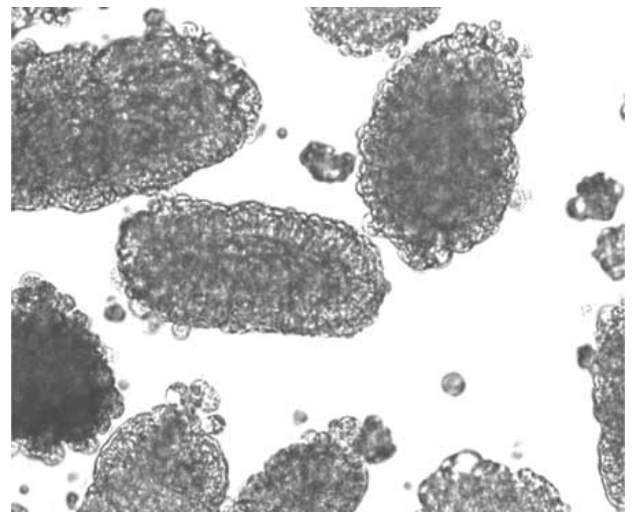
of each crypt. The next two tiers contain potential or clonogenic stem cells derived from division of the actual stem cells that can either act as stem cells or retain their clonal expansion capacity to regenerate the crypt if required, providing a total population of approximately 30-40 clonogenic cells. The cells continue to divide as they move upwards through the tiers but gradually lose their stem cell properties. Transit epithelial cells that continue to migrate upwards above the three tiers retain no clonogenic properties [19-23].

It was not until recently that a putative molecular marker for intestinal epithelial stem cells was identified that potentially enables the identification of stem cells *in situ* within intact tissue sections. *Musashi (d-msi)* is a neural RNA-binding protein that controls *Drosophila* external sensory organ development by regulating target genes post-transcriptionally and controlling asymmetric cell divisions [24]. Mouse-Musashi-1 (m-Msi-1) was the first vertebrate member of the Musashi-related subfamily of neural RNA-binding proteins to be identified and characterized, and is suggested to be a mammalian neural stem cell marker [25]. Musashi-1 is expressed in undifferentiated, proliferative central nervous system stem-like cells, localized to the perikarya, but not in cells committed to the oligodendroglial lineage [26]. The expression pattern of Msi-1 has been examined in the mouse small intestine using immunohistochemistry and *in situ* hybridization. Cytoplasmic Msi-1 staining was observed in a few cells immediately above the Paneth cell compartment but not in villus structures or the upper part of the crypt – a location that coincides with the theoretical position of intestinal stem cells based on previous morphological and clonogenic studies. Whilst Paneth cells were negative for Msi-1 expression, the intercalated cells within the Paneth cell compartment were positive for Msi-1, suggesting that crypt base columnar cells also have stem cell characteristics. However, labeling for Msi-1 has also been observed above the theoretical location of stem cells, which was suggested to arise from immediate daughter cells of the stem cells retaining Msi-1 expression [27,28]. The transcription factor HES-1 (Hairy enhancer of split) is also co-expressed by crypt cells that are positive for Msi-1 and in lower crypt cells just above the Paneth cells, and like Msi-1 localization, Paneth cells are negative [28]. The Msi-1+/HES-1+ cells were also positive for Ki-67, indicating that these cells have high proliferative activity. To maintain the number of cells in each crypt the ‘ultimate lineage ancestral stem cells’ are not thought to be highly proliferative, producing on average one stem cell and one daughter cell that provide the transit dividing cells [19]. Thus the true identity of the highly proliferative Msi-1+/HES-1+ cells might be that of dividing transit progenitor cells rather than stem cells. The findings with Msi-1 should also be interpreted with some caution since they are derived from murine intestine, which may differ, for example, in the number and behavior of stem cells compared with humans. However, a study by Nishimura and colleagues studied the expression of Msi-1 in human colonic crypts that had been microdissected from biopsy specimens. Msi-1 expression was primarily located within the lower part of the crypt, mainly between cell positions 1 and 10, which would correspond with the proposed location of stem cells in the colon [29].

## INTESTINAL TISSUE ENGINEERING

Intestinal tissue engineering is an attractive therapeutic alternative to intestinal transplantation, avoiding the risks of immune system rejection, transfection of microbiological hazards, and potentially overcoming the shortage of donor organs. The principal of tissue engineering involves using a scaffold that provides both the initial mechanical structure and template for the engineered tissue. Cells are seeded onto the scaffold, which organize into the desired tissue either before or after implantation. The scaffold may be a permanent implant or tailored to degrade after extracellular matrix has been deposited and the tissue has become mechanically self-supporting. Tissue engineering is a term widely used, but in this review it will be limited to approaches involving the implantation of scaffolds seeded with harvested intestinal cells. However, it should be noted that alternative approaches involving guided tissue regeneration, where an acellular scaffold is implanted and infiltrated by cells from adjacent native tissue, have also successfully generated gastrointestinal tissues [30-34].

A major advancement for small intestinal tissue engineering resulted from the development of a reproducible method suitable for isolating and growing small intestinal epithelium in primary cultures. One of the most commonly used techniques involves the gentle enzymatic digestion of tissue fragments of intestine into multi-cellular aggregates, termed ‘organoid units’ that contain polarized epithelium including the epithelial stem cells, surrounding a core of mesenchymal derived stromal cells [35] (Fig. 2). Many of the intestinal tissue engineering studies to date have isolated organoid units from neonatal rat intestine, which contains small blunt villi but no well-developed crypts. As a result of this the stem cells are present on the inter-villus plateau regions or small buds at the base of the villi [35]. The success of this technique for isolating functional epithelial stem cells may be due to the presence of the mesenchymal derived stromal cells maintaining the niche in which the stem cells reside *in vivo*. The stromal cells in the organoid unit may enable signalling between the stem cells and the surrounding host environment that stimulates organoid unit



**Fig. (2).** Intestinal organoid units isolated from a neonatal rat using the procedure described by Evans *et al.* [35].

engraftment and the formation of an organized neomucosa. The organoid units derived from isolated intact crypts are not enriched in stem cells compared with crypts found in intact mucosa. The proliferative cells within the organoid units are either stem cells or transit amplifying cells, and each individual organoid unit may consist of a mixture or one type of the proliferative cell populations, or non-proliferative differentiated cells [36].

Tait and colleagues successfully used the technique for isolating organoid units to generate grafts of neomucosa *in vivo* [37]. Epithelial organoids isolated from postnatal rat small intestine were seeded under dorsal subcutaneous skin flaps of adult recipients. The cells were seeded onto a silicone sheet that acted as a scaffold/barrier to prevent adhesion between the skin flap and thoracic wall. The grafts regenerated into tubular structures consisting of a central lumen surrounded by a layer of epithelium resembling the small intestine with well-formed crypt-villus structures. Proliferative cells were confined to the crypt region and absorptive enterocytes, goblet cells, Paneth cells, and enteroendocrine cells were identified in the neomucosa. Sucrase and lactase were expressed in the regenerated neomucosa, along with apical alkaline phosphatase in the villus enterocytes. Layers of smooth muscle-like stromal cells were found adjacent to crypt and villus epithelium. The authors suggested at the time that the smooth muscle-like cells may have originated from contaminating lamina propria isolated with the organoid units or that these cells may have been recruited from the host. Results from more recent studies suggest that the cells surrounding the crypts in the neomucosa could have been subepithelial myofibroblasts, possibly derived from recipient bone marrow [38]. In native intestine crypts are surrounded by a sheath of subepithelial myofibroblasts that provide an interface between the epithelium and the underlying lamina propria. Myofibroblasts secrete an array of cytokines, growth factors and inflammatory mediators, some of which are likely to regulate stem cell differentiation [39].

Using a similar technique Tait and colleagues generated a 'colonic' neomucosa by grafting isolated small intestinal organoid units on to denuded colonic muscle following mucosectomy. In this model the denuded colonic muscle acted as the scaffold. The neomucosa that subsequently developed exhibited a small intestinal phenotype, containing crypts and villi lined by epithelium containing goblet cells, enteroendocrine cells, Paneth cells and absorptive enterocytes [40]. Although the authors suggest that the technique may be of value in the surgical management of colonic mucosal disorders, such as ulcerative colitis and familial adenomatous polyposis, the small intestinal phenotype of the generated neomucosa suggests this technique could also be applicable to patients with short bowel syndrome. This study also indicates that small intestine organoid units retain the phenotype of the tissue from which they have been isolated from despite being transplanted into a different host environment, such as the denuded colon, suggesting that the signals regulating their phenotype may reside in the mesenchymal derived stromal cells present in the organoid units.

To be of functional value the tissue engineered neomucosa would need to possess an absorptive capacity similar to native small intestine, which will depend on the

expression of specific digestive enzymes and transporter proteins expressed by the absorptive enterocytes. Tait and colleagues went on to characterize the digestive and absorptive capacity of the neomucosa formed subcutaneously in the dorsal region of recipient rats, demonstrating that neomucosal digestive enzyme activity and D-glucose transport at 25 days after stem cell transplantation were similar to that of age-matched control rodent small intestine [41].

Characterization of tissue-engineered small intestine has progressed further using neomucosa engineered in rats created by seeding organoid units onto a polymer scaffold that is wrapped in the omentum [42]. The polymer scaffolds containing the organoid units develop into small cysts that are lined with intestinal neomucosa. The neomucosa shows the formation of villi and crypts lined by columnar epithelium with goblet cells. Electrophysiology studies of the neomucosa reveal similar transepithelial resistance values to adult rat ileal mucosa, but decreased active transepithelial ion-transport. The brush border enzyme sucrase and basement membrane protein laminin also have a similar distribution to that found in adult rat ileum [43].

The studies using organoid units have also demonstrated that the responsiveness to physiological stimuli of the intestinal epithelial stem cells in the tissue-engineered intestine appears to remain intact. Significant trophic effects on neomucosal morphogenesis occur following anastomosis between the native small bowel and the tissue-engineered neointestine [44]. As with native intestine, massive small bowel resection causes intestinal adaptation, producing trophic stimuli for the tissue-engineered intestine that results in a significantly greater number of villi, villus height and area, and mucosal surface length [45]. When tissue-engineered small intestine was anastomosed to the native intestine at the time of an 85% enterectomy in rats it reduced the overall period of weight loss by half and improved postoperative weight gain compared with animals that received small bowel resection alone [46].

Anastomosis to the native small bowel results in the exposure of the neomucosa to luminal contents, including bacteria, gastrointestinal secretions and nutrients, which coincides with the development of a mucosal immune system that contains a similar population of immune cells to that of native jejunum [47]. Lymphoid aggregates similar to Peyer's patches or isolated lymphoid follicles were identified in the tissue engineered cysts, but the phenotype of the follicle associated epithelium overlying the gut associated lymphoid tissue or presence of M cells were not reported [47].

The ability of the stem cells within the seeded organoid units to populate the neointestine has been demonstrated by labeling organoid units with green fluorescent protein prior to seeding. The presence of labelled organoid units in the neomucosa confirmed that the donor organoid units provided the epithelium rather than infiltration of native epithelium from adjacent small intestine [46].

The technique involving the seeding of polymer scaffolds with organoid units has been modified further by Grikscheit and colleagues, who have tissue engineered colon in rats from colonic organoid units derived from both adult rats and from tissue engineered colon itself [48]. Histology of the

tissue-engineered colon was indistinguishable from native colon raising the possibility of a novel therapy for alleviating morbidity associated with colectomy and potentially avoiding the complications associated with ileal pouch formation. Moreover, the ability to use organoid units derived from adult rat colon is of great value, as this enables the harvesting of a far greater number of organoid units per adult animal (approximately 800,000 per resected specimen) compared with the number derived from neonatal rat colon (approximately 60,000 per resected specimen). This overcomes one of the main obstacles for translating this technique to adult humans meaning it might be feasible to harvest autologous organoid units from adults, thus avoiding the difficulties associated with sourcing neonatal organoid units and rejection of non-autologous tissues. In addition, the modified technique will also reduce the number of animals required for this model and may also allow the generation of engineered experimental intestine from different adult phenotypes.

## THE FUTURE OF INTESTINAL TISSUE ENGINEERING

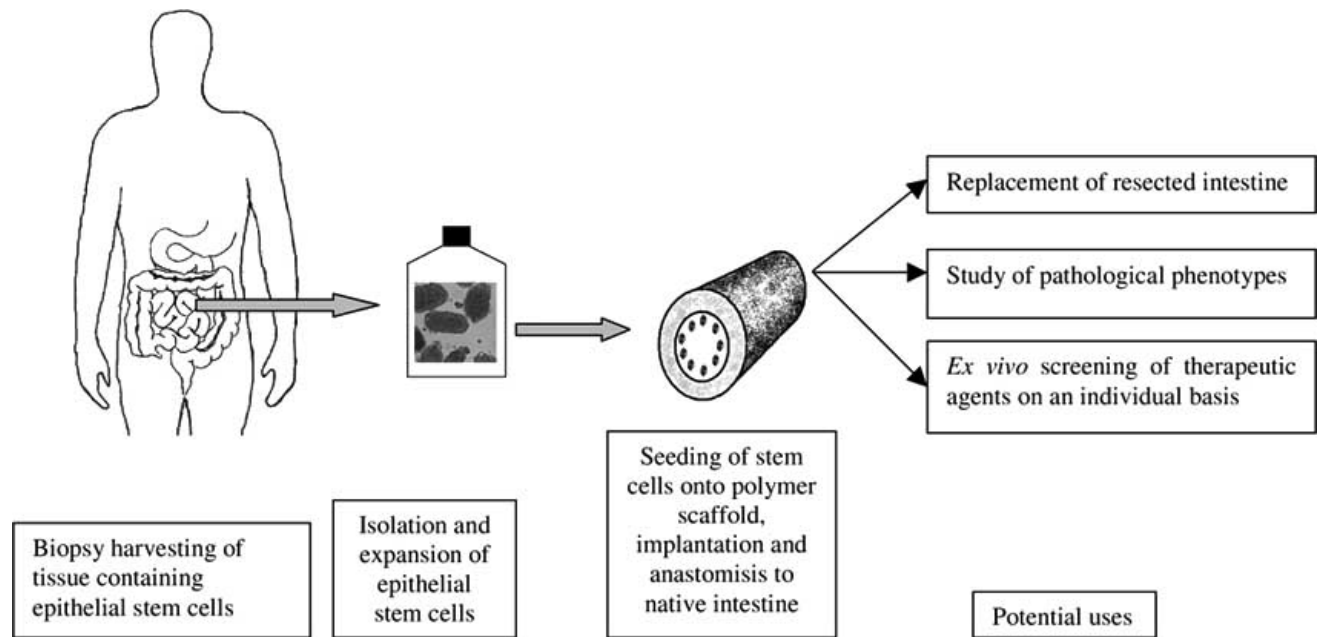
### Current Problems

There are many problems yet to be resolved before small intestinal tissue-engineered constructs become a reality for treating patients with intestinal insufficiency. Unless sources of autologous epithelium can be harvested from the patient, long-term postoperative immunosuppression will be necessary to prevent rejection between the host and donor tissue. Harvesting sufficient numbers of viable stem cells before surgery is likely to be difficult to achieve in patients with diseased or damaged intestine that has led to the need for massive resection. It might be possible to harvest small quantities of organoid units from the remnant small bowel over a period of time that could then be expanded *in vitro*. However to date, none of the studies have demonstrated the ability to tissue engineer a length of intestine equivalent to the amount resected to supply the organoid units and the amount of intestine that is engineered is not likely to markedly increase the absorptive capacity of the mucosa. One and a half full lengths of small bowel from neonatal rats are required to obtain a sufficient number of organoid units to fabricate a 1 cm length of anastomosed neointestine [49]. Therefore the methodology used to date to engineer neointestine will not be applicable for use with harvesting autologous organoid units unless ways can be found to expand either the number of organoid units or isolated intestinal stem cells. With the latter approach the isolated stem cells will not benefit from the presence of the mesenchymal derived stromal cells maintaining the stem cell niche. There may also be difficulties associated with expanding organoid units *in vitro*, with their fate appearing to depend upon the proliferation and differentiation capacity of each organoid unit and the environment in which they are growing. The study by Slorach and colleagues revealed that the majority of organoid units cultured in uncoated tissue culture flasks failed to adhere, but those that did adhere could be categorized into three groups: differentiated colonies with little or no proliferation that expressed alkaline phosphatase (expressed in differentiated epithelium) but were

negative for cytokeratin 18 (expressed in undifferentiated crypt epithelium); colonies that proliferated rapidly for 2-3 days that were positive for alkaline phosphatase and negative for cytokeratin 18; and colonies that proliferated for up to two weeks that were negative for alkaline phosphatase and positive for cytokeratin 18 [36]. Three-dimensional cultures of the organoid units suspended in Matrigel supplemented with hepatocyte growth factor resulted in the formation of cyst structures composed of an epithelial layer surrounding a central lumen but did not increase the number of organoid units [36]. The fate of organoid units grown in culture is also dependent on the quality of the basal cell culture medium. Using optimised medium Evans and colleagues demonstrated that cells derived from organoid units continued to proliferate *in vitro* for at least 28 days after their isolation [35]. With non-optimized medium the period of viability was much shorter. The plethora of factors needed to regulate the proliferation of stem cells within the organoid units may come from a variety of cell types, including intestinal myofibroblasts [39] and macrophages [50] that are known to secrete growth regulatory peptides, which have yet to be characterized.

### Maximising the Functional Capacity of Tissue Engineered Intestine

A possible solution to increasing the absorptive surface area of the neointestine might exist with the administration of trophic peptides to the tissue-engineered intestine. Glucagon-like peptide-2 (GLP-2) is a peptide hormone secreted by endocrine L-cells and controls proliferation rates, the size of crypts and villi, and intestinal mass [51]. The trophic effects of GLP-2 on the intestine are now well documented [52]. The study by Ramsanahie and colleagues in 2003 demonstrated that stimulation of neomucosa with GLP-2 resulted in an increase in villus height, crypt depth, and crypt cell proliferation index, together with a reduction in apoptosis in the neomucosa epithelium - results similar to the effects of GLP-2 on native small intestine [53]. It is possible that GLP-2 may also stimulate higher efficiency of organoid unit seeding through environmental signalling or increasing the proliferative status of the stem cells, similar to that proposed for the protection offered by GLP-2 in the irradiated intestine [54]. Other approaches to increasing the functional capacity of the tissue-engineered intestine might include gene therapy, for example by increasing the expression of brush border enzymes and membrane transporter proteins. Intestinal epithelial stem cells within the organoid units prior to seeding would be an ideal target for gene therapy due to their relative abundance and accessible location. After stable transfection the stem cells would theoretically provide a steady supply of epithelial cells that expressed the recombinant gene. The study by Kawaguchi and colleagues in 1998 reported the transplantation of genetically altered intestinal 'stem' cells. This study actually used the IEC6 intestinal epithelial cell line, derived from intestinal crypts from Sprague Dawley rats, which was transfected with the reporter gene *lac Z* and seeded onto membranes that were implanted into the intestine of Sprague Dawley rats [55]. Since the IEC6 cells used were only a model for intestinal stem cells it cannot be guaranteed that true intestinal stem cells would function in



**Fig. (3).** Schematic illustration of the processes involved in intestinal tissue engineering and some of the possible for uses for the engineered

the same manner. Also, further studies involving gene therapy in general are necessary to allay the fear surrounding the use of gene transfer and the associated potential risks, such as oncogenic transformation.

To enable continued growth and viability of the neointestine, a sufficient blood supply will be necessary to ensure the delivery of oxygen and nutrients and removal of waste products. A study by Gardner-Thorpe and colleagues in 2003 assessed angiogenesis in the tissue-engineered small intestine and showed that the engineered intestine has lower levels of the angiogenic growth factors, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), compared with the juvenile intestine from which the stem cells were harvested [56]. Neovascularization in the engineered tissue will be required to support any increase in tissue mass, which may in turn provide an additional stimulus for the growth and improved function of the neointestine. This might be achieved by using tissue engineering scaffolds that release steady quantities of angiogenic growth factors [57,58] or that stimulate the endogenous release of growth factors [59].

Functional peristalsis of the luminal contents in tissue-engineered intestine will require a neuromuscular layer. Histology of tissue-engineered intestine has revealed actin in the muscularis mucosa and ganglion cells present in the Auerbach and Meissner's plexi, indicating the presence of nerve and muscle in the neomucosa [46]. However, to date no tissue engineering studies have successfully provided enough muscle tissue to offer efficient peristalsis. Attempts to seed autologous canine mesenchymal stem cells onto a collagen sponge scaffold that was used to reconstruct a segment of resected jejunum failed to provide long-term muscle layer regeneration [60]. The authors suggested that failure may have resulted from an insufficient number of cells seeded on the scaffold, or that the mesenchymal stem cells used require a stimulus for differentiating into muscle cells.

### Alternative Sources of Stem Cells

The pluripotency and availability of other non-epithelial stem cells might provide an alternative source of epithelium for the tissue-engineered intestine. For example, haematopoietic stem cells can be readily harvested and transplanted to reconstitute the haematopoietic system in a myeloablated host. Under certain conditions they exhibit a remarkable degree of plasticity, and it has been suggested that they can differentiate into cells outside of the haematopoietic lineage. Transdifferentiation of Y-chromosome-positive male donor bone marrow cells transplanted into female recipients has been suggested for a variety of tissue types, as reviewed by Poulsom and colleagues [61]. In 2002 Okamoto and colleagues suggested that adult bone marrow cells have the potential to repopulate damaged areas of the gastrointestinal epithelium. Biopsy specimens collected from various parts of the gastrointestinal tract (oesophagus, stomach, small intestine, colon) taken from females who had received a bone marrow transplant from male donors revealed epithelial cells that contained a Y chromosome. The 'transdifferentiated' epithelial cells were detected in tissue samples eight years after the bone marrow transplantation, indicating the longevity of their production from the bone marrow [62]. The ability of haematopoietic stem cells to transdifferentiate into non-haematopoietic tissue is, however, controversial. Some studies have found no evidence to support transdifferentiation [63], whilst others have demonstrated that without a rigorous analytical method and appropriate control staining for donor derived epithelial cells and CD45+ haematopoietic cells, some intraepithelial lymphocytes may be misinterpreted as donor derived epithelial cells [64]. There are also arguments as to whether donor cells fuse with recipient cells and give the appearance of transdifferentiation. Without looking at the karyotype of cells this can be difficult to ascertain in recipients of bone marrow from sex-mismatched donors

[65,66]. If transdifferentiation does occur, the molecular cues required for transdifferentiation of haematopoietic stem cells into intestinal epithelium will need to be identified before the process can be applied to intestinal tissue engineering. Okamoto and Wantanabe have suggested that bone-marrow cells enter the epithelium as differentiated 'epithelial cells' but at a low frequency. Alternatively, they suggest the bone marrow cells become integrated as stem-cell-like progenitor cells but their proliferation and differentiation is suppressed by residing competitive epithelial stem cells. Regeneration of the mucosa stimulates an increase in the number of bone-marrow derived cells within the intestinal epithelium as a result of either an increase in the frequency of bone-marrow-derived stem cell like progenitor cells entering the epithelial surface, or the reduced suppression of proliferation of the resident bone-marrow cells already in the epithelium [67]. Further studies will be needed to determine whether either of these processes occurs with tissue-engineered intestine.

### Long-Term Outcome and Alternative Applications of Tissue Engineered Intestine

Other as yet unanswered questions relate to the long-term stability of the tissue-engineered intestine. For example, will it be able to regulate cell turnover and retain the protective mechanisms against genetic damage thought to exist in the native small intestine? If the patient has an underlying idiopathic or genetic disease, such as Crohn's disease, will the tissue-engineered intestine be susceptible to the disease like the original intestine? Will the stem cells retain their functional competence to divide and provide transit progenitor cells? It will be important to find the answers to these questions before the tissue-engineered intestine can be used in patients who have undergone massive small bowel resection.

In addition to the direct replacement of resected intestine, a number of other applications for tissue-engineered intestine have been proposed. Rocha and Whang have envisaged tissue-engineered intestine being derived from organoid units harvested from patients with inflammatory bowel disease or cancer and used to create models for studying the disease pathogenesis. An array of individual patient's engineered intestine could also be used for high-throughput screening of therapeutic agents [68]. Considering the problems yet to be resolved before tissue-engineered intestine can be used to replace resected bowel, these *ex vivo* applications may be of more immediate value to the patient population.

### CONCLUSION

Despite the current limitations, the attempts at tissue engineering intestine to date have provided the first important steps towards creating a replacement for this complex organ. Further studies and alternative approaches are clearly needed to provide the composite intestinal tissue consisting of vascularized mucosal tissue, epithelium and neuromuscular tissue before it will be of therapeutic value to patients with short bowel syndrome or other forms intestinal failure. With the increased interest intestinal tissue engineering is generating from a variety of different scientific disciplines, including surgery, cell and molecular biology, and materials science, a functional tissue-engineered intestine

similar to the native intestine should be a realistic possibility in the not too distant future.

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