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Generating intestinal tissue from stem cells: potential for research and therapy

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Abstract

Intestinal resection and malformations in adult and pediatric patients result in devastating consequences. Unfortunately, allogeneic transplantation of intestinal tissue into patients has not been met with the same measure of success as the transplantation of other organs. Attempts to engineer intestinal tissue *in vitro* include disaggregation of adult rat intestine into subunits called organoids, harvesting native adult stem cells from mouse intestine and spontaneous generation of intestinal tissue from embryoid bodies. Recently, by utilizing principles gained from the study of developmental biology, human pluripotent stem cells have been demonstrated to be capable of directed differentiation into intestinal tissue *in vitro*. Pluripotent stem cells offer a unique and promising means to generate intestinal tissue for the purposes of modeling intestinal disease, understanding embryonic development and providing a source of material for therapeutic transplantation.

Keywords

development; differentiation; embryoid bodies; human; intestine; organoids; pluripotent; regeneration; stem cell; tissue engineering; transplantation

Tissue engineering is a broad and emerging field that includes a range of research areas from developmental biology and disease to regeneration and generation of tissues and organs for transplantation. The underlying principle of tissue engineering involves manipulating cells in combination with biologic or synthetic materials in order to cultivate new tissues and organs [1]. This can be done fully *in vitro* to derive engineered tissue, or scaffolds can be transplanted *in vivo* and then seeded by endogenous cell types. Recent emphasis has been placed on personalized medicine, where a patient's own cells are used to generate autologous tissue, which eliminates the need for immunosuppressive therapy following transplantation in order to prevent tissue rejection. Human clinical trials using tissue engineering include the transplantation of skin, cartilage, bone, blood vessels, corneas, urinary structures and lung bronchi [2–15]. As of 2007, the commercial market for tissue engineered products comprised 50 firms or businesses employing 3000 people and generating revenues of US $1.3 billion annual [16].

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Since the emergence of tissue engineering biology in the 1980s, initially for the growth and development of liver tissue *in vitro* [17–22], various strategies have been employed including *ex vivo* manipulation of organs or organoid units, fabrication of organs by seeding cells on extracellular scaffolds, isolation and manipulation of adult stem cells *in vivo* and *in vitro*, and, more recently, the directed differentiation of pluripotent stem cells (PSCs) into organ cell types and tissues [23–25]. There are two types of human PSC (hPSC): human embryonic stem cells (hESCs), derived from the inner cell mass of the blastocyst of an embryo, and human induced pluripotent stem (hiPS) cells [26–28]. hiPS cells are generated by nuclear reprogramming of somatic cell types into hESC-like cells through introduction of molecular factors that control pluripotency [29–31]. In the last several years, hPSCs have been used *in vitro* in unprecedented studies of human developmental biology to identify molecular pathways that regulate the differentiation of progenitor cell types into organ-specific cells [32–36]. In this article, we will discuss how basic studies of intestinal developmental biology and adult intestinal stem cells have led to recent progress in engineering intestinal tissue.

Intestine structure & function

One major challenge of tissue engineering is generating tissues that have the full complement of organ function *in vitro*. An example is the intestine, which is comprised of two hierarchal structures: the small and large bowel (**Figure 1A)**. The bowel's chief functions are to process and absorb nutrients from food and eliminate enteral waste from the body. Along the length of the intestine, distinct regionalization exists, and defined functions are performed in each segment. For example, the small bowel contains the subdivisions of duodenum (most proximal), jejunum and ileum (most distal) and participates in the neutralization of stomach acid during digestion, enzymatic processing of food products within the lumen, and both passive and active absorption of nutrients [37,38]. The large bowel is composed of cecum (most proximal); ascending, transverse, descending and sigmoid colon; and rectum (most distal) [39]. The main purpose of the colon is to resorb water and sodium while concentrating fecal matter for eventual excretion from the body. In cross-section, the small and large intestine are radially symmetrical and both are composed of outer layers of serosa and musculature which participate in peristalsis, middle layers of submucosa and muscularis mucosa, and innermost layers of lamina propria and a simple columnar epithelium, which lines the luminal surface (**Figure 1A)** [37,40]. In the colon, large longitudinal muscular bundles, called taenia coli, are present to aid in peristalsis. During digestion, a coordinated interplay occurs between the intestinal layers, which is mediated by hormone signals, an enteric nervous system and vasculature to allow for the controlled movement and efficient processing of food, nutrient absorption and endocrine-mediated communication with other systems to control numerous processes, including glucose homeostasis and satiety. Within the lumen, bacterial symbiosis also allows for breakdown of molecules that the body cannot handle [41].

The intestinal epithelium is a complex environment composed of active cell proliferation and differentiation of many cell types (**Figure 1B)**. In the small intestine, villus and microvillus projections into the lumen exponentially increase the surface area available for nutrient absorption, whereas these projections are absent in the colon. Intestinal stem cells reside near the bottom of epithelial crypts and give rise to progenitor cells, which rapidly divide and differentiate into subtypes of cells that comprise the intestinal epithelium (**Figure 1B)** [37]. The most common intestinal epithelial cells are enterocytes which are mainly responsible for absorption of nutrients and water via active and passive transport. Goblet cells, which are more abundant in the colon than the small intestine, secrete mucins and other proteins that are used for lubrication and as a barrier defense against pathogens, and Paneth cells secrete lysozyme to prevent bacterial infection. Paneth cells also play a key role

in providing a niche for the stem cells in the crypts (described in more detail later) [42,43]. Enteroendocrine cells are the rarest of gut epithelial cells (comprising 1% of the epithelium) and include approximately ten different subtypes, which secrete a variety of hormones that participate in glucose homeostasis (glucose insulinotropic peptide and glucagon-like peptide 1), satiety (ghrelin), pH balance (secretin), gall bladder contraction (cholecystokinin), neuromuscular contraction and gut motility (neurotensin and motilin) and the regulation of pancreatic and pituitary hormone secretion (somatostatin) [44–47]. Subtypes of enteroendocrine cells are often confined to specific segments of the gut. Given that different proximal–distal segments of the gut have different cellular compositions and functions, the gut represents an unusual challenge for tissue engineering efforts.

Intestinal disorders & transplantation

Worldwide, only approximately 200 allogeneic intestinal transplants occur each year due to both donor shortage and the technical challenges inherent in the procedure [48]. Among adults, the most common reasons for transplant include ischemic bowel, inflammatory bowel disease (such as Crohn's disease or ulcerative colitis) and traumatic injury complicated by bowel resection [48]. In the pediatric population, especially neonates, the primary reasons for a bowel transplant are congenital malformations, such as Hirschsprung's disease or intestinal atresias, acquired dysfunction or short gut syndrome as a result of intestinal resection due to necrotizing enterocolitis, volvulus or gastroschisis. Bowel transplantation as part of a multivisceral procedure involving the liver, intestine, stomach and pancreas occurs in approximately a quarter of all pediatric transplant cases [48]. Transplants are also required in some cases of severe gastroparesis and in complications of resection caused by meconium ileus and cystic fibrosis, among others. In children aged 1–18 years, 5-year survival rates from intestinal transplants are significantly lower (46–76%) when compared with renal (95–96%), liver (77–86%) and heart (72–77%) transplants [49,50]. This higher morbidity and mortality is believed to be caused by a greater need for post-transplant immunosuppression, since a large part of the immune system resides in the intestine, rendering it highly immunogenic [51]. Undersuppression can lead to graft rejection and elimination of intestinal barrier function, while oversuppression can yield local or systemic infection in the recipient. It is the hope of physicians, scientists and patients that intestinal engineering efforts will improve the outcome of intestinal transplants through generating autologous tissue or by devising methods for stimulating tissue regeneration *in situ*.

Ex vivo manipulation of intestinal organoids for tissue engineering

In the 1980s, studies demonstrated that a rat duodenal cell line (IEC-17), when cultured long term, formed organized structures composed of closed central lumens and stratified layers of polarized tissue [52]. These observations raised the possibility of *ex vivo* manipulation of intestinal tissue as a means to engineer intestine. Subsequent studies demonstrated that disaggregated small intestinal tissue from rat harvested by enzymatic digestion formed self-contained intestinal units when transplanted subcutaneously into donor rats [53]. Grafts contained tissue with a circumferential epithelium surrounding a central lumen and exhibited expression of markers of absorptive enterocytes, goblet cells, Paneth cells and enteroendocrine cells. Work in fetal intestine clarified the necessity of mesenchymal–epithelial interactions for the survival and ultimate differentiation potential of *ex vivo* intestinal tissue [54,55]. The Vacanti laboratory demonstrated that disaggregated fetal intestinal units, which they termed organoids, could be seeded onto sheets of nonwoven polyglycolic acid and incubated for 7 days *in vitro* prior to transplantation into the peritoneal cavity of recipient Lewis rats [56,57]. After harvest, these implants, also called tissue engineered neointestine (TENI), showed crypt structures and mature intestinal histology.

Other laboratories attempted to dissociate the small intestine of adult rats into organoids for culture and transplantation into recipients with only modest recovery of mature intestinal tissue [1,58,59]. As the polyester scaffolds commonly used to grow intestine *in vitro* and *in vivo* have become more refined, addition of collagen and poly-L-lactic acid to scaffolds of polyglycolic acid improved engraftment rate of organoids in rats, and increased the size and number of villus structures present in the resulting tissue [60,61]. Further work demonstrated that anastomosis of TENI to native bowel of rats significantly improved weight loss and malabsorption commonly associated with massive bowel resection [62–65].

Attempts to isolate organoid units from regions of the gut other than the small intestine have also met with success. Grikscheit *et al.* derived tissue engineered colon by disaggregation of rat colon similar to previous methods used to isolate small intestine organoids [66,67]. When cultured with polymer scaffolds and transplanted into Lewis rats with ileal anastomoses, they contributed to neocolon tissue *in vivo*, and treated rats had decreased stool transit time, lower stool moisture content and higher total serum bile acids compared with rats with end-ileostomies alone, suggesting some functional competency of the transplanted colon. The same group had similar results following dissociation of esophagus and stomach organoid units and showed functional contribution of these tissues in respective, transplant recipient rats [68,69]. Interestingly, the functional capacity of region-specific intestinal organoid segments from adult animals seems to be preserved after harvesting, *ex vivo* expansion and transplantation into alternate intestinal sites. For example, organoids derived from rat ileum retained the ability to express ileal-specific bile acid transporter protein when transplanted into rat jejunum [70]. This would suggest that adult intestinal tissue and perhaps the stem cells therein are limited in their capacity to adopt a new regional cell fate.

Neovasculaturization, lymphangiogenesis and immune cell contribution have all been observed in engrafted organoid units that contribute to the intestine [71–73]. Recent work has demonstrated that anal sphincter musculature can be harvested from humans and engineered *ex vivo* into an innervated construct by coculture with fetal enteric neurons [74,75]. Subcutaneous implantation of this tissue in immunocompromised recipient mice yielded grafts with functional properties of anal sphincter musculature including the ability to respond to neurotransmitters that relaxed or contracted the tissue appropriately. More recently, TENI derived from mouse intestine has been isolated and transplanted into recipients with contribution to functional small intestine, which now allows for the use of mouse genetics in future TENI studies [76]. In addition, disaggregated small intestinal and stomach organoids have also been derived from large animals, including pigs, and autologous transplantation has been performed following generation of TENI [77].

Isolation & culture of intestinal stem cells

Intestinal stem cells are known to exist in the base of crypts, where they are maintained in a unique microenvironmental niche capable of preserving multipotency and allowing differentiation of progenitor cells into all epithelial cell lineages. Several trophic factors are provided by adjacent cell types, including mesenchyme and Paneth cells, to promote active WNT signaling in the stem cell [78–80]. WNT antagonist studies have demonstrated disrupted epithelial architecture in the small intestine and reduced proliferation of epithelial subtypes, suggesting that WNT signaling supports the stem cell niche and allows for proliferation of stem cells [81–85]. Studies have demonstrated that BMP is expressed in villi, where it opposes cell proliferation and promotes cell differentiation [86,87]. Use of BrdU, LacZ and Cre-lox reporter systems have demonstrated that the lineage of all the cells of several adjacent villi can be traced to stem cells within crypts [88,89].

Ootani *et al.* studied intestinal stem cells within disaggregated organoid units derived from rat small intestine in long-term *ex vivo* cultures and found that growth was slowed in the presence of the WNT inhibitor DKK1 and stimulated by the presence of Rspondin-1, a WNT agonist [90]. Treatment with the γ-secretase inhibitor, dibenzapine, as well as overexpression of neurogenin-3, a transcription factor essential for enteroendocrine cell differentiation, induced goblet and enteroendocrine cell formation within organoid cultures. These results showed that *ex vivo* manipulated intestinal tissue has the ability to respond to normal stem cell signaling to direct proliferation and differentiation of intestinal epithelium. However, to date, no comprehensive study has been undertaken to characterize similarities and differences in the functional properties and differentiation potential of intestinal stem cells located in different region-specific segments of the intestine.

Multiple intestinal stem cell markers have been identified in mice, including Lgr-5, Bmi-1, Msi-1, Ephb-2 and Dcamkl-1 [91–95]. Lgr-5, a G-protein coupled receptor closely related to the thyroid-stimulating hormone receptor, is regulated by the WNT/APC/β-catenin pathway and is perhaps the most well-studied marker [88,96,97]. It is expressed in a limited number of columnar epithelial cells located at the crypt base suggesting that its location is restricted to the stem cell compartment. Sato *et al.* demonstrated that Lgr-5^+ cells can be isolated from the crypts of postnatal mice, purified by FACS, and cultured as single cells in the presence of EGF, Noggin and R-spondin-1 to give rise to spherically shaped organoid units with an inner luminal structure, villus domains and with outward projecting crypt-like structures containing Lgr-5^+ stem cells [98]. Further ana lysis showed the presence of goblet, Paneth and enteroendocrine cell subtypes within each organoid. The fact that each organoid structure was derived from single Lgr-5^+ cells demonstrates the extraordinary capability of these cells to contribute to all known intestinal epithelial cells.

Sato *et al.* also demonstrated that coculture of Lgr-5^+ cells with FACS sorted $CD24^+$ Paneth cells significantly improved *in vitro* organoid formation, and genetic removal of Paneth cells *in vivo* is associated with loss of Lgr-5^+ cells [99]. This work suggests that the Paneth cells are part of the intestinal stem cell niche and provide important microenvironmental cues. Along with WNT signaling, Notch, a cell surface receptor that interacts with its ligand (Delta) via cell-to-cell contact, is known to play a role in intestinal stem cell homeostasis, which is probably facilitated by intimate interaction between cells within the intestinal crypt [100]. Inhibition of Notch signaling provides instruction to stem cells to undergo differentiation into secretory cell lineages; although the timing, duration and coordination of these signals are not well studied [101,102].

Intestinal embryonic development

One approach to tissue engineering intestine is to differentiate PSCs into intestinal cell types and tissues. Historically, differentiation of embryonic and induced pluripotent stem cells into specific organ cell types such as liver and pancreas has required an intimate knowledge of the molecular pathways that regulate embryonic development of that organ. Similar to the liver and pancreas, the intestines are derived from the endoderm primary germ layer that arises following gastrulation of the developing embryo (**Figure 2)** [103–106]. Nodal, a member of the TGF-β superfamily, is required for specification of endoderm tissue in all vertebrate species [107,108]. A complex series of morphogenetic events occurs to transform a simple sheet of definitive endoderm (DE) into a 3D gut tube, starting with initial closure of the tube at the anterior and posterior intestinal portals, which then proceeds toward the middle of the embryo resulting in a primitive gut tube that spans the length of the embryo (**Figure 2)**. During its formation, the gut tube epithelium undergoes elongation and patterning along the anterior–posterior axis to form specific subdomains: the foregut, midgut and hindgut. The foregut gives rise to the thyroid, esophagus, lungs, stomach, pancreas,

liver, gall bladder and duodenum; and the mid and hindgut give rise to the small and large intestine, respectively. Although relatively little is known about the mechanisms that control the early stages of gut tube morphogenesis [109], patterning is known to involve WNT, FGF and BMP signaling **Figure 2)** [110–117]. At this developmental stage, WNT and FGF signaling each repress foregut fate and promote development of mid and hindgut lineages [109,117,118]. Refining the regional identity of the gut tube into duodenum, jejunum, ileum and colon is then established through interplay between region-specific factors in the mesenchym and epithelium. This involves signaling molecules such as BMP, hedgehog, WNT, PDGF and EGF [119–123] and transcription factors including CDX2 and various HOX proteins [119,120,124–128] that function in regionalization and formation of villus structures, which proceeds in an anterior to posterior wave along the entire length of the intestine. Final maturation of the intestinal epithelium occurs during the prenatal and neonatal period and involves the formation of crypts, the establishment of flora and the activation of digestive programs in response to feeding.

Differentiation of PSCs into intestinal tissue

Mouse intestinal differentiation

The molecular pathways that control embryonic intestinal development have proven central to recent successes in the directed differentiation of PSCs into intestinal cell types and tissues. Mouse embryonic stem cells (mESCs) are derived from the inner cell mass of the murine blastocyst and have the ability differentiate into all cell types of the developing embryo [129–131]. Efforts to generate specific cell and tissue types *in vitro* from mESCs have broadly taken two approaches. The first involves the formation of spherical, multicellular aggregates known as embryoid bodies (EBs), which favors cell–cell interactions, but is stochastic in nature and results in less control over differentiation. The second approach is to direct the differentiation of monolayer cultures with addition and removal of soluble factors to mimic embryonic intestinal development. This approach allows for better directed differentiation into specific cell types, but loses the advantage of the normal 3D environment of the embryo and the resulting cell–cell contact.

Spontaneous differentiation of murine EBs has resulted in formation of gut-like tissue *in vitro* [132–135]. These reports described both physiologic and molecular evidence for the formation of intestinal tissue, including rhythmic beating reminiscent of intestinal peristalsis and cells that histologically resembled intestinal cell types such as goblet cells. Expression of molecular markers that are present during embryonic intestinal development, including Foxa2/HNF3b, Sox17, Id2 and Gata4 were detected in *in vitro* gut-like structures by PCR and *in situ* hybridization. Formation of gut-like structures from spontaneous differentiation of EBs was also observed with murine induced pluripotent stem (miPS) cells. These reports all benefited from the strength of 3D EB cultures, but also suffered from the primary weakness of EB-based approaches: that spontaneous differentiation results in the formation of many organ tissues, the result being relatively inefficient formation of intestinal tissue.

Engraftment and subsequent growth of EBs *in vivo* resulted in more differentiated intestinal tissues [136,137]. Markers of hindgut formation, including Cdx2 and 5-hydroxytryptamine (serotonin) were present, but the stomach-specific marker H^+/K^+ ATPase was absent. The intestinal stem cell marker, Bmi-1, was also present in transplanted tissues. Host organisms contributed blood vessels and neuronal innervation. In another functional study of mESC contribution to intestinal differentiation *in vivo*, Kudo *et al.* directly injected male mESCs into irradiated intestinal mucosa of female recipients and demonstrated that Y-chromosome-positive cells were present in the mouse intestine at 14 days [138].

A recent report improved upon the low efficiency of spontaneous differentiation of EBs by incorporating growth factor manipulations that were designed to direct EB differentiation into intestine [139]. Mouse EBs were generated and then first exposed to activin A, a Nodal analogue, for 6 days to enrich for the formation of DE. EBs were then exposed to Wnt3a and cell-conditioned media, which elevated the expression of intestinal markers including Cdx2, Fabp2 and Id2. Within the EB-derived tissues, there were regions of epithelium and some of these expressed markers including Ephb-2 and Lgr-5. Impressively, injection of DE derived from green fluorescent protein-expressing mESCs into the colonic mucosa of mice resulted in contribution of green fluorescent protein-positive cells to endogenous intestinal tissue. As to whether or not these cells acquired molecular and functional properties of intestine *in vivo* will undoubtedly be the focus of future studies.

Human intestinal differentiation

As in mice, hPSCs come from embryos (hESC [140]) or can come from nuclear reprogramming of somatic cells (hiPS cells [30,31]). Both have the capacity for differentiation into organ cell types derived from the three primary germ layers. One unique advantage of hiPS cells is that they can be derived from specific patients and, therefore, tissue derived from these hiPS cells can be transplanted autologously. hiPS cells are derived from adult somatic cells, such as fibroblasts or keratinocytes, and are induced toward a pluripo-tent state by several different methods including expression of transcription factors including Nanog, Oct4, Sox2, Klf4 and c-Myc [29,30,141], or more recently through expression of siRNAs [142]. hiPS cell lines can be maintained indefinitely in culture in the same manner as hESCs; however molecular comparison of hESCs and hiPS cells demonstrate some subtle differences, and some evidence suggests that hiPS cells retain a certain degree of epigenetic memory of the tissue from which they are derived [143]. In addition, recent data suggest that pluripotent miPS cells are unable to efficiently form teratomas when transplanted into genetically identical recipient mice and induce a T-cell infiltration at the site of transplantation, suggesting that these cells are rejected by the host immune system [144]. The molecular basis of this rejection is not known, and it could be caused by the method or efficiency of reprogramming. In addition, it is unclear whether differentiated tissues derived from induced pluripotent stem cells will be similarly immunogenic. Since tissue derivatives from hiPS cells have only been tested in immunocompromised animal models, it remains to be seen if they are capable of generating an immune response in autologous transplants as well. Nevertheless, both hESCs and hiPS cells represent powerful tools for the *in vitro* generation of human tissue types and for the study of developmental biology and organo-genesis and the potential use of PSC derivatives in animal models of disease will undoubtedly be an area of intense study in the future.

A recent approach has been described to efficiently direct hESCs and hiPS cells into intestinal tissue *in vitro* that utilized both 3D growth **Figure 3)** and directed differentiation, based on the signaling pathways that control the different stages of vertebrate embryonic intestinal development [145]. In this protocol, monolayers of hPSCs were first directed into the DE lineage using a highly efficient protocol involving low serum and activin A exposure for 3 days, resulting in cultures of 90% DE. Subsequent exposure to WNT3A and FGF4 efficiently directed the formation of posteriorized, CDX2-expressing hindgut endoderm. Specification of hindgut endoderm resulted in the spontaneous induction of 3D, gut tube-like morphogenesis and the formation gut tube spheroids. When grown in 3D conditions that are known to favor intestinal growth **Figure 3A)** spheroids undergo intestinal morphogenesis and eventually yield intestinal organoids that contain an intestinal epithelium that is almost entirely $CDX2^+$, from which $LGR\text{-}5^+$ crypt-like domains, intervillus regions, villus-like projections and a microvillus brush border develop. Intestinal organoids also contain a

laminated intestinal mesenchyme with layers of smooth muscle actin expressing cells and intestinal subepithelial myofibroblasts.

Human intestinal organoids contained all known intestinal epithelial cell types including absorptive enterocytes, mucin-secreting goblet cells, lysozyme secreting Paneth cells and chromogranin A-positive enteroendocrine cells **Figure 3B)** and had both absorptive and secretory function. The utility of this culture system for molecular studies of human development and disease were demonstrated using the transcription factor neurogenin-3, in which mutations in humans cause loss of enteroendocrine cells and enteric anendocrinosis. In intestinal organoids, endogenous neurogenin-3 function was knocked down by introduction of shRNA and resulted in the loss of enteroendocrine cells. By contrast, overexpression of neurogenin-3 resulted in a five-fold increase in the number of enteroendocrine cells [145]. Together, these data suggest that the *in vitro* derived intestinal organoid system should allow both basic studies of human intestinal development and disease.

Future perspective

With increasing frequency, stem cells are being utilized in the study of human disease and basic developmental biology. With the advent of induced pluripotent stem cells and their demonstrated capacity for directed tissue differentiation, it is now theoretically possible to harvest cells from patients for the purpose of reprogramming them into hiPS cells, which can be used to generate intestine for autologous transplantation. Furthermore, when harvested from patients with genetic syndromes or mutations, hiPS cells are a powerful tool with which to study the developmental origin and pathophysiology of the disease. The immunogenicity of pluripotent miPS cells has recently been described in genetically identical recipient mice [144]; however, no detailed evidence of the immunogenic potential of differentiated tissue derived from induced pluripotent stem cells exist, although this will no doubt be the subject of further evaluation. Nevertheless, careful evaluation of the safety of tissues derived from human pluripotent sources is needed prior to initiation of transplantation into allogeneic or autologous recipients.

Directed differentiation of PSCs into human intestine represents a leap forward in human developmental biology, since the direct investigation and observation of human embryonic development is limited. However, critical gaps in our understanding of intestinal development remain, which limit the utility of PSC-derived intestinal tissue. For example, it is not well understood how regional segments of the intestine are established during embryonic development and this information would facilitate generation of region-specific segments of intestine from PSCs. *In vitro* derivation of specific segments of human intestinal tissue would facilitate pharmacological study of drug effects and toxins, reveal the impact of specific nutrients on enteroendocrine cell behavior and allow for studies of regional stem cell properties. In addition, testing of medical devices, surgical techniques, tissue injury and wound healing, and studies of nutrient and environmental exposure could potentially be conducted on human intestinal tissue that is grown outside the body. Studies of the functional contribution of *in vitro* hPSC-derived intestinal organoids to the native intestines of injured animal transplant recipients are ongoing. Future advances are needed, such as the use of bioengineered tissue scaffolding, which could allow for the massive expansion of human intestinal organoids from current sizes (a few millimeters in diameter) to that which would be adequate for both *in vitro* studies and for transplantation into human patients.

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■ of interest

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study to demonstrate that intestinal tissue can be derived *in vitro* by robust, efficient differentiation of human pluripotent cells by the manipulation of exogenous growth factors.]

Executive summary

Need for intestinal tissue for transplantation

- Intestinal disorders and resection caused by congenital or acquired anomalies result in the need for intestinal tissue for transplantation in both adults and children.
- Data indicate that allogeneic intestinal transplantation has a poor 5-year graft survival and patient mortality compared with transplantation of other solid organs including kidney, liver and heart.
- Tissue engineering offers a promising means to generate nascent intestinal tissue *in vitro* utilizing stem cells and developmental biology principles to provide tissue capable of autologous transplantation.

Intestinal disaggregation & use of polyester scaffolding to generate in vitro intestinal tissue

- Several research laboratories have demonstrated that disaggregated intestinal tissue from rats is able to generate sustainable units of intestinal tissue, termed organoids, when cultured *in vitro*.
- Transplantation of organoids into recipient animals generates donor-derived intestinal tissue capable of functional properties of the region in which they are transplanted.
- Polyester scaffolding, comprised of polyglycolic acid or poly-L-lactic acid, has been demonstrated to increase the size of intestinal grafts and has been successfully used in intestinal transplantation into recipient animals.

Intestinal stem cell identification & characterization

- Recent advances have demonstrated that single LGR-5^+ cells harvested from adult mouse intestine are capable of regenerating all cell types of the intestinal epithelium *ex vivo*.
- The intestinal stem cell is supported in intestinal crypts by Paneth cells and is maintained in a microenvironmental niche through signaling via WNT and Notch.
- The signaling events that instruct the differentiation of intestinal stem and progenitor cells into the various mature epithelial cell types are not well understood.

Spontaneous generation of intestinal tissue with embryonic stem cells

- Embryoid bodies derived from mouse embryonic stem cells have been demonstrated to spontaneously give rise to cells expressing intestinal-specific markers.
- Transplantation of embryoid body-derived intestinal cells can contribute to formation of new intestinal tissue following surgical injury or radiation.
- Few published attempts have been made to direct the fate of embryonic stem cells or embryoid bodies into intestinal cells with specific culture conditions and soluble factors utilizing developmental biology principles.

Directed differentiation of human pluripotent stem cells into intestinal tissue

- A recent study has demonstrated the ability of human pluripotent stem cells to undergo directed differentiation into intestinal tissue through stages that recapitulate embryonic development.

■ Definitive endoderm derived *in vitro* is capable of intestinal organoid formation by exposure to high levels of WNT and FGF.

■ 3D culture conditions support the growth of organoids into mature intestinal tissue containing a laminated mesenchyme and an epithelium containing all known intestinal cell types and capable of functional peptide transport.

■ Use of induced pluripotent stem cells to generate human intestinal tissue offers the possibility to model disease in the intestine, test pharmaceuticals in an *in vitro* environment and provide material for autologous transplantation of intestine.

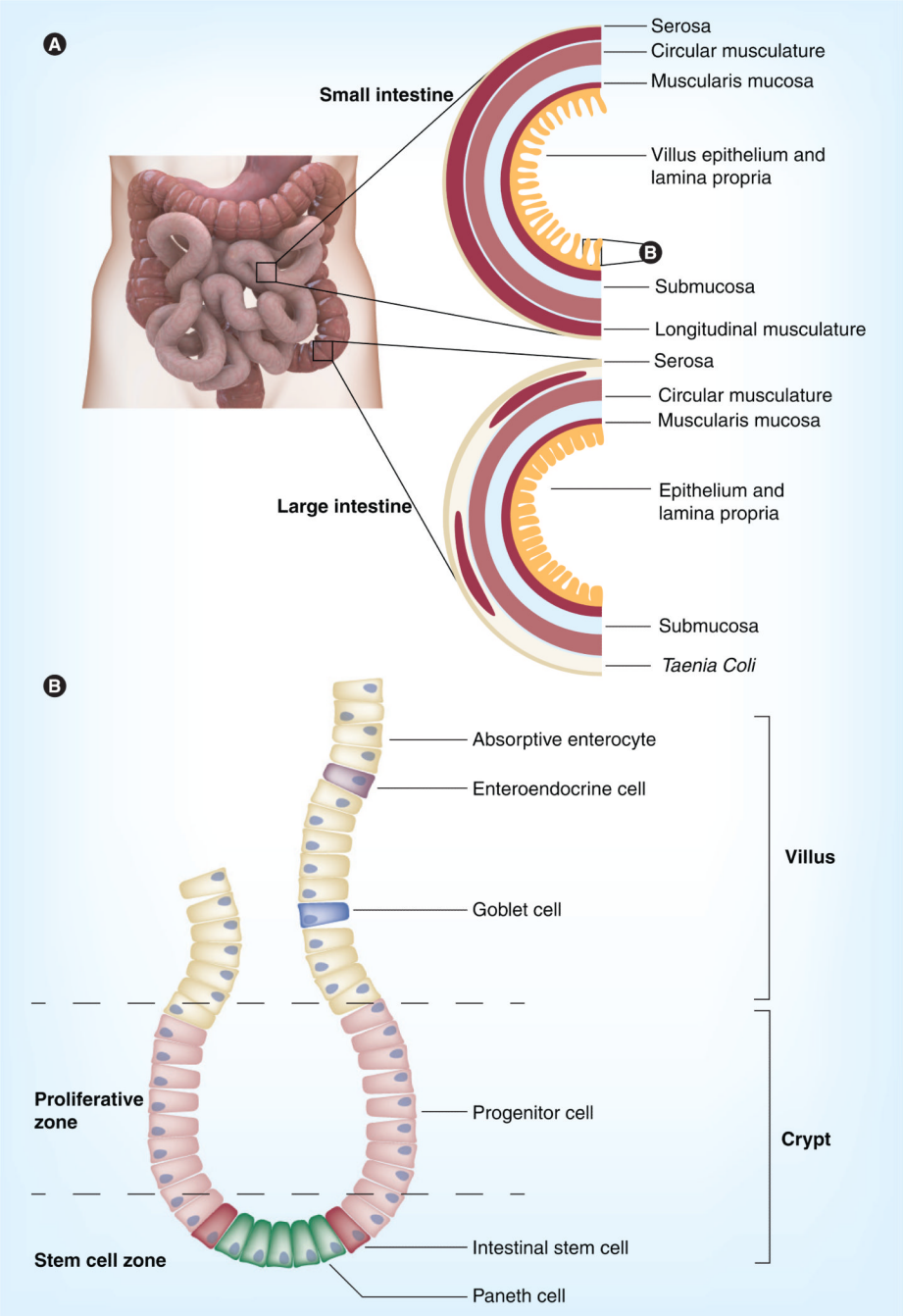

Figure 1. Histology of intestinal layers and crypts

(A) In cross-section, both small and large intestine contain outer layers of serosa and both longitudinal and circular musculature. The large intestine has large muscular ribbons, called taenia coli, to aid in contraction and peristalsis. Middle layers include submucosa and muscularis mucosa. The innermost layers are the lamina propria and epithelium. In the small intestine, villi project into the intestinal lumen, which expands the surface area available for absorption. Villi are absent in the large intestine. **(B)** Intestinal crypts contain resident stem cells capable of generating all cell types of the mature epithelium (small intestinal crypt and villus). In the small intestine, Paneth cells provide a crucial microenvironmental niche for stem cells. Paneth cells are largely absent in the large intestine. Following asymmetrical cell

division, stem cells give rise to progenitor cells that differentiate into enterocytes (capable of absorption of nutrients and water) hormone secreting enteroendocrine cells, mucin secreting goblet cells and the aforementioned Paneth cells.

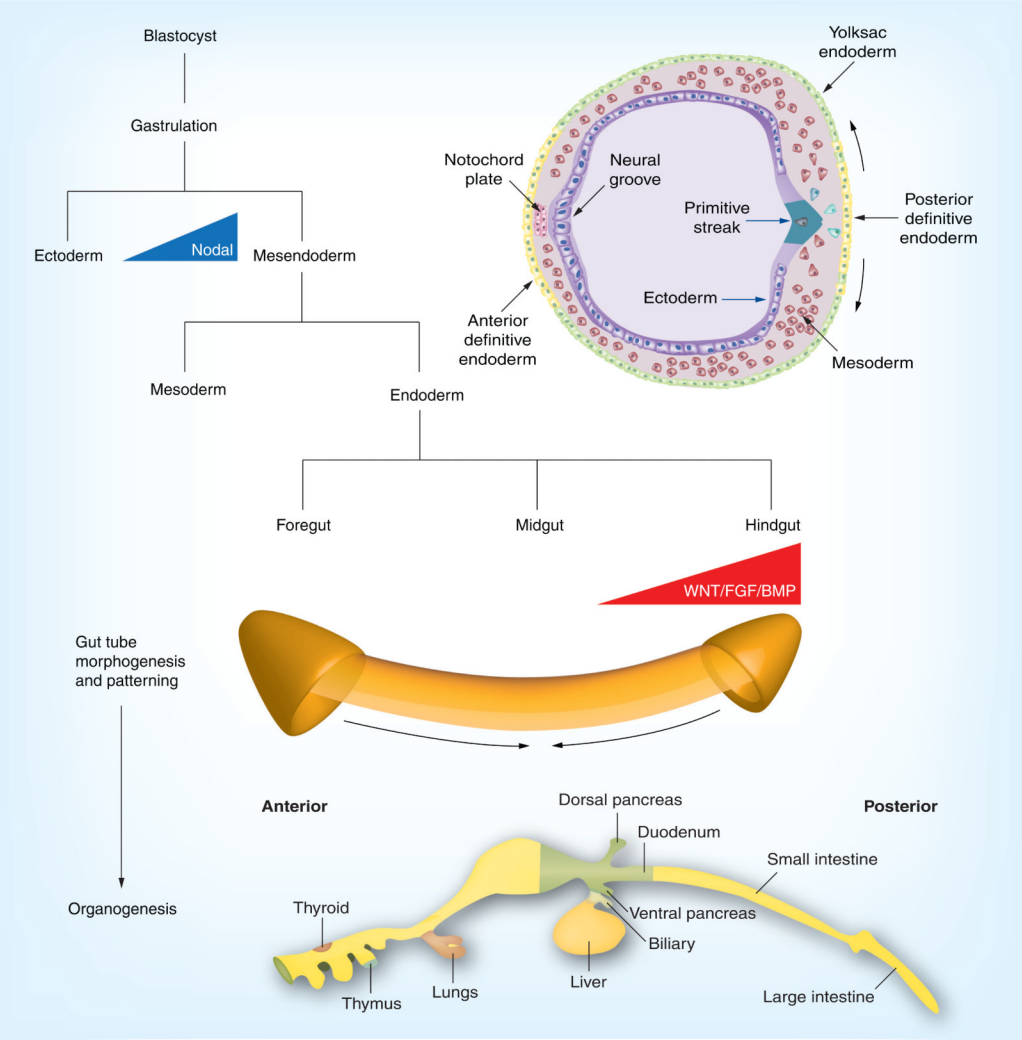

Figure 2. Embryonic development of vertebrate endoderm and intestine

Following gastrulation of the blastocyst and migration of cells through the primitive streak, the formation of definitive endoderm is induced by exposure to the TGF-b superfamily member Nodal. The endoderm layer eventually forms a primitive gut tube along the anterior–posterior axis that defines proximal and distal gut structures. During gut tube morphogenesis, the influence of various factors establishes patterning of tissue along the anterior–posterior axis such that different domains are able to undergo region-specific organogenesis. During this stage, WNT, FGF and BMP signaling induces hindgut specification and represses the development of foregut tissue. Initial closure of the gut tube occurs at the anterior and posterior ends and proceeds toward the middle until complete. Foregut develops into anterior structures including thyroid, lung, esophagus, stomach, liver, gall bladder and pancreas. Midgut domains develop into small intestine and the proximal large intestine, while hindgut develops into the remainder of the colon.
Adapted with permission from [103,115].

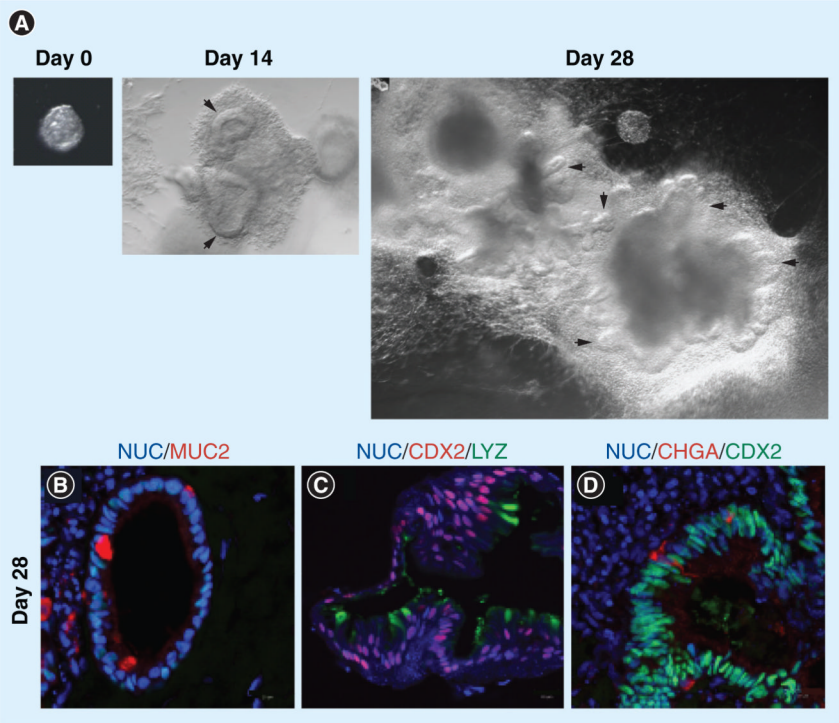

Figure 3. Human pluripotent cells are capable of directed differentiation into mature human intestine

(A) Intestinal organoids can be generated by directed differentiation of human pluripotent cells first into definitive endoderm by low serum and exposure to activin A *in vitro*. Following culture in WNT3a and FGF4, floating intestinal progenitor spheroids form. When collected and plated at day 0 in the semisolid matrix (Matrigel™; BD Biosciences, Franklin Lakes, NJ, USA), they grow in size and complexity by 28 days to form epithelium with crypt and villus-like structures (arrowheads). Day 28 organoid epithelium contains all known mature cell types including **(B)** MUC2-expressing goblet cells, **(C)** LYZ-expressing Paneth cells and **(D)** CHGA-expressing enteroendocrine cells.
CHGA: Chromogranin A; LYZ: Lysozyme; MUC: Mucin.