Regenerative medicine for childhood gastrointestinal diseases

Brendan C. Jones[1,2,*], Soichi Shibuya[1,*], Natalie Durkin[1,2], Paolo De Coppi[1,2,#]

[1]Stem Cell and Regenerative Medicine Section, Developmental Biology and Cancer Research and Teaching Department, Great Ormond Street Institute of Child Health, University College London, London, United Kingdom.
[2]Specialist Neonatal and Paediatric Surgery Unit, Great Ormond Street Hospital, London, United Kingdom.

* These authors contributed equally to this work
# Correspondence: p.decoppi@ucl.ac.uk

Competing interests
The authors declare no competing interests.

Keywords
Tissue engineering; regenerative medicine; bioengineering; congenital disorders; clinical translation

Abstract

Several paediatric gastrointestinal diseases result in life-shortening organ failure. For many of these conditions, current therapeutic options are suboptimal and may not offer a cure. Regenerative medicine is an inter-disciplinary field involving biologists, engineers, and clinicians that aims to produce cell and tissue-based therapies to overcome organ failure. Exciting advances in stem cell biology, materials science, and bioengineering bring engineered gastrointestinal cell and tissue therapies to the verge of clinical trial. In this review, we summarise the requirements for bioengineered therapies, the possible sources of the various cellular and non-cellular components, and the progress towards clinical translation of oesophageal and intestinal tissue engineering to date.

Introduction

Regenerative medicine and bioengineering aim to rescue organ failure by the restoration of function through the generation of cells, tissues, or organs *de novo*. This field combines insights from developmental and stem cell biology with expertise in biomaterials science and engineering to develop personalised therapy for specific needs. Currently, several congenital malformations and diseases acquired in early childhood result in life-limiting organ failure due to sub-optimal treatment options. In particular, this applies to gastrointestinal diseases including, oesophageal atresia, intestinal failure, and intestinal neuropathies. In this regard, there is an unmet clinical need in these children that regenerative medicine aims to address.

Oesophageal atresia (OA) is a congenital disorder of the oesophagus resulting in a lack of continuity from the mouth to the stomach, commonly associated with an abnormal connection between the oesophagus and trachea (tracheoesophageal fistula, TOF). Approximately 10% of OA patients have no communication with the trachea. This is associated with a large tissue deficit between the proximal and distal oesophagus making primary anastomosis unfeasible, necessitating oesophageal replacement [1]. Even patients undergoing primary anastomosis may require an oesophageal substitute following anastomotic leak, recurrent fistula, or severe strictures refractory to endoscopic intervention. Currently, the options for oesophageal replacement are interposition grafts using stomach, jejunum, or colon. However, each technique is associated with substantial disadvantages; gastric interpositions tend to cause reflux and microaspiration, jejunal grafts are associated with a significant risk of anastomotic leak and ischaemia, and colonic grafts have a high incidence of redundancy, delayed emptying, and a lifelong malignancy risk [2]. Long-term feeding problems are common in OA patients and can be severe, such that extra surgical interventions may be required, such as anti-reflux or feeding ostomy formation.

Intestinal failure (IF) is a group of conditions defined by the lack of sufficient functional intestine to maintain hydration, nutrition, and growth through enteral nutrition alone. Currently, there is no cure for IF, with available treatments including parental nutrition, intestinal lengthening procedures, and intestinal transplantation [3-5]. However, complications of IF and its current treatments are many, including liver failure, bacterial overgrowth, sepsis, eventual loss of central venous access, metabolic bone disease, transplant-associated immunosuppression, and ultimately death [4]. In the most severe

cases, 5-year survival may be as low as 20%, with small intestinal transplantation carrying a 5-year survival of only 60% [6-8].

In addition to the anatomical absence of gut, disorders of the enteric nervous system (ENS) may cause significant morbidity, including IF, and have limited treatment options. Hirschsprung disease (HSCR) is congenital disorder of the digestive tract due to absence of ganglion cells in the myenteric and submucosal plexuses. While the exact aetiology of HSCR is unclear, failure of rostro-caudal migration of sacral neural crest ENS progenitor cells has been hypothesised as the main cause. Currently, the definitive treatment is surgical resection of the aganglionic gut and pull-through of ganglionated gut to the anal canal. Although surgery is lifesaving by relief of the functional bowel obstruction, there is a significant proportion of patients with long-term morbidity and decreased health-related quality of life [9, 10]. Multiple factors are speculated to contribute to persisting bowel dysfunction, including the suggestion that patients with HSCR have inherent dysmotility, even in their ganglionic bowel.

It is clear that in conditions such as OA, IF, and HSCR, regenerative medicine holds tremendous potential to fulfil an unmet clinical need; that is, the durable restoration of organ function where no ideal alternative therapy exists. In this review, we discuss the aims, strategies, and progress of regenerative medicine for congenital and acquired childhood disorders of the gastrointestinal tract. We also address the remaining challenges and barriers to clinical translation of engineered therapies in the future.

What are we trying to achieve?

Goals and aims of bioengineering for gastrointestinal disorders

The current treatment options for congenital and childhood-acquired GI disorders are suboptimal. Therefore, the goal of bioengineering for these conditions is to generate cells or tissues *de novo* to ameliorate, cure, or perhaps even prevent these diseases [11, 12]. Just as the causes of congenital and childhood-acquired disorders are diverse, so too then must be the regenerative medicine strategies to treat them (Figure 1). Bioengineered therapies must aim to faithfully recapitulate normal anatomy and function in order to achieve tissue reconstruction. In the case of the gastrointestinal tract, the multi-layered structure of mucosa, submucosa, muscularis externa and adventitia must be restored.

Mucosa and Submucosa

Crucial to intestinal function is the self-renewing simple columnar epithelium with a diversity of absorptive (enterocytes) and secretory (enteroendocrine, goblet, and Paneth) cell lineages arranged in well-defined repeating crypt-villus units as an intact epithelial barrier [13]. This diversity of cells arises from somatic intestinal stem cells (ISCs), which express leucine rich repeat-containing G protein coupled receptor 5 (Lgr5) and are located at the base of the crypt [13, 14]. Under normal homeostatic conditions, this is achieved through the complex interplay of signalling gradients along the crypt villus axis including Wnt/β-catenin, Notch, transforming growth factor-β (TGF-β), bone morphogenic protein, and hedgehog signalling pathways [13, 15-17]. However, it is worth noting that following injury, more mature cell types can 'de-differentiate' to replenish the ISC compartment in a pattern that has remarkable resemblance to the foetal development program [18, 19].

The oesophageal epithelium consists of a highly proliferative basal layer with a stratified layer of increasingly flattened superficial cells. The stratified squamous epithelium provides a physical barrier from chemical and mechanical injury during the passage of food and refluxing gastric acid. Evidence from lineage tracing and cell cycle tracing data in mice suggest oesophageal epithelial homeostasis is supported by a single stem population of progenitor cells stochastically producing one daughter for stem maintenance and one for subsequent differentiation [20]. When injury occurs, cells close to the wound transiently increase the production of progenitor cells, allowing for rapid replacement of the superficial layer as part of a co-ordinated set of responses involving molecular signals including, EGF, HGF/c-MET and NGF/TrkA [21, 22].

The epithelium is supported by the lamina propria comprised of mesenchymal cells, including fibroblasts and myofibroblasts, and a lymphovascular network. In the intestine, the mesenchymal cells are a crucial component of the ISC niche, supplying growth factors that act through the signalling pathways mentioned above [23-25]. The lymphovascular network, beginning in the villus as capillaries and lacteals, is responsible for absorption of digested nutrients and fluid, as well as immune surveillance. The oesophageal submucosa is composed of a loose connective tissue layer rich in collagen and elastin. The orientation of these fibres allows for high degrees of circumferential distensibility which is essential for swallowing without compromising longitudinal strength [26]. The vascular and neural networks regulate

secretion of mucus from submucosa glands directly into the oesophageal lumen and co-ordinate contraction of the muscularis mucosae.

Muscularis externa

The mucosa and submucosa of the gut tube are wrapped in a two-layered smooth muscle tube with orthogonally orientated smooth muscle fibres, except for the proximal oesophagus which contains skeletal muscle in continuity with the pharynx. Together with an organised and complex enteric nervous system (ENS) comprising both submucosal and myenteric nerve plexuses, the neuromuscular coat is responsible for coordinated peristalsis [27]. Furthermore, the ENS has been shown to have additional functional importance including the regulation of intestinal hormone secretion, maintenance of epithelial growth and barrier function, and communication of host-microbe interactions [28-30]. Understanding of the developmental biology of the neuromusculature of the gut has been crucial to the development of protocols for isolation and culture of cells that can act as muscle and neural precursors.

Supply and defence: vasculature and immune tissue

Beyond the mucosal lymphovascular network, the large and medium sized lymphatic and vascular tree is clearly essential to normal gut tube oxygenation, nutrition, waste removal, and immune surveillance. Recapitulating an intact non-thrombogenic lymphovascular tree will be crucial to engineering anything more than cell-based therapies, for example, multi-layered engineered tissues. For the intestine, this could be achieved by using the existing vascular tree, in the form of a decellularised scaffold (see below) or as a pattern for bioengineering, combined with vascular progenitor cells [31]. While for the oesophagus, this is more complex as there is no single major vessel providing vascular supply.

As one of the largest interfaces with the external environment, the intestinal immune system, including myeloid and lymphoid cells encounters a vast antigenic load. However, it is clear that normal function of the immune system and its interaction with the microbiome are essential to intestinal homeostasis, while dysregulation of these components contributes infectious and inflammatory diseases of the intestine, which may lead to intestinal failure [32, 33].

Orientation and organisation: the extracellular matrix

Finally, these various cell types must be organised into an appropriately compartmentalised multi-layered organ, and this is achieved through a finely micro-patterned extra-cellular matrix (ECM). Apart from micro-structural organisation and mechanical properties provided by 'structural' proteins, the ECM also contains a vast array of ECM-associated proteins, including matrix modifying enzymes, ECM-bound growth factors, and ligands for cell surface receptors [34, 35]. Furthermore, the ECM exists in a state of dynamic reciprocity with its resident cells; that is, the ECM instructs cellular polarity and phenotype, and the cells digest and secrete ECM in response to chemical and mechanical signals in the microenvironment [34, 36, 37].

Sourcing the Building Blocks: Cells and Scaffolds

Having considered the cell and tissue types required to bioengineer the gut, we now discuss efforts to source these cell types for translational purposes. Ideally, cells to be used for regenerative medicine applications should be able to proliferate to clinically relevant numbers but be available from minimal source tissue, while retaining the capacity to differentiate into mature cell subtypes and not give rise to unwanted tissues, including neoplasia, in the process.

Epithelial cells

Bioengineering of the epithelium with or without supporting mesenchyme is the most advanced aspect of bioengineering for the gut. Initial work on intestinal tissue engineering (ITE) utilised intestinal "organoid units" derived from enzymatically digested minced intestine [38-42]. These organoid units comprised epithelium and supporting mesenchyme but have limited *in vitro* expansion potential relative to the amount of source tissue required. Establishment of stroma-free ISC-derived intestinal organoid culture was first published by the Clevers group in 2009 [13, 14, 43]. These organoids give rise to a full diversity of epithelial cell types (i.e., they retain tissue-specific multipotency), morphologically resemble crypt-villus architecture, are genetically stable in long-term culture, and can have near unlimited potential to be expanded exponentially [14, 43]. Another strategy to generate intestinal organoids is from pluripotent stem cells (PSCs), either induced-PSCs (iPSCs) or embryonic stem cells (ESCs) [44-49]. Clinical translation of ESC-based therapies is still limited by

immunological and ethical concerns. However, iPSC-derived human intestinal organoids (HIOs) contain all mature epithelial cell subtypes, like ISC-derived organoids, and additionally smooth muscle and mesenchymal cells, with HIOs exhibiting a level of maturity similar to foetal intestine [45, 50]. Potential problems with iPSC intestinal organoids include variability across iPSC lines, the risk of persistence of tumorigenic pluripotent cells, and possible genetic and epigenetic instability [51-54]. Clearly these are significant issues to be addressed prior to clinical trial. However, both organoid systems can be cultured in a 3D ECM-based hydrogel using chemically defined media, which can be manufactured according to good manufacturing protocols (GMP), making organoids an attractive option for the mucosal components of intestinal bioengineering.

The stratified squamous epithelium of the oesophagus is not readily cultured in 3D organoid-like conditions, although it has been reported [55, 56]. Epithelial cells for oesophageal bioengineering may be sourced from either buccal mucosal biopsy or endoscopic oesophageal biopsy. Cells delivery to mucosal defects can be by direct injection as trialled in animal models [57] or more recently as a cell sheets cultured on feeder layers, usually mouse fibroblasts (J2-3T3 cells), or in 2D on basement membrane coated tissue culture flasks [56, 58]. Culture of epithelial cells on thermo-responsive polymers allows for detachment of the epithelial cell layer without compromise to cell morphology or function by decreasing the temperature which converts the polymer from a hydrophobic to hydrophilic state [59]. This technique is now extensively used for clinical delivery of epithelial cells for treatment of mucosal defects, discussed later.

Smooth and skeletal muscle

In comparison, engineering of organised smooth and skeletal muscle layers is much earlier in development. The primary problems to be overcome include the reliable isolation, expansion, and differentiation of visceral smooth muscle progenitors from the intestine, although these have recently been characterised in mouse [60]. Alternatively, several groups have investigated non-intestinal mesenchymal stem cell (MSC) sources of smooth muscle progenitors. For example, visceral smooth muscle cells (viSMCs) can be derived from mesoangioblasts (MABs) in the blood vessels of skeletal muscle [58, 61-63], from autologous amniotic fluid stem cells (AFSCs) via culture in media containing TGF-β1 and PDGF-BB [64], or

from bone marrow mesenchymal stem cells via inhibition of the ERK/MAP kinase signalling pathway [65].

Successful bioengineering of muscle for the oesophagus is essential as this layer must provide the graft strength, elasticity and ultimately, peristalsis. Cells at different stages of maturity, from stem to mature muscle cells, have been used as the source of muscle tissue for oesophageal engineering. The use of mature smooth muscle cells in tissue engineering is limited due to volume of source tissue required and relative inaccessibility. In addition, they demonstrate slower expansion than other cell lines used for muscular replacement. Alternative and more viable options include using muscle progenitor cells which can subsequently differentiate into smooth muscle. Mesoangioblasts are a subset of pericytes which are well suited to this application as they have both smooth and skeletal muscle differentiation potential [66, 67], can be reliably cultured and have already been used in clinical trials for the treatment of muscular dystrophies [68]. MSCs derived from bone marrow, adipose tissue and amniotic fluid have all been used in oesophageal animal models with relative success and continue to be an attractive option for muscle tissue engineering due to their multipotency, ease of harvesting and positive immunomodulatory effects [69-72]. Another possible cell source is skeletal muscle progenitors, such as myoblasts, which are readily available from skeletal muscle biopsies and have potential to give rise to multinucleated skeletal muscle fibres in scaffolds [73]. However, it is unclear whether skeletal muscle offers the ideal mechanical properties given that the dominant muscle type of the oesophagus is smooth muscle, apart from in the proximal segment.

Enteric neurons and neural precursors

Development of techniques to reconstitute the ENS have made substantial progress recently. Enteric neural stem cells (ENSCs) represent a multipotent stem cell population with the capacity to differentiate into neurons, glial cells, and myofibroblasts and are considered the most likely cell source for cell and tissue engineered therapies [74-77]. ENSCs were initially isolated from embryonic gut tissue and later from postnatal gut [74, 78]. Three-dimensional organotypic cultures called neurosphere-like bodies (NLBs) have become the preferred mode of *in vitro* propagation of ENSCs with the ability to recapitulate biological behaviour of ENS cells and colonise the bowel following transplantation [76].

Full-thickness segments of gut containing the smooth muscle and myenteric plexus were the major source of ENSCs, but the large amount of source tissue required has been prohibitive. The discovery of the existence of ENSCs within the mucosal layer has made it possible to derive NLBs even from endoscopic biopsies, allowing the possibility of autologous cell therapy [79]. However, a disparity between ENSCs sourced from the submucosal plexus versus the myenteric plexus has been observed in terms of proliferation and differentiation potential [80]. By contrast and of great relevance to the treatment of intestinal neuropathies, NLBs generated from HSCR biopsy specimens have been reported to be equivalent to those from healthy gut [81]. Finally, tissue processing to purify ENSCs is crucial, using fluorescence-activated cell sorting (FACS) or magnetically activated cell sorting (MACS) isolation based on p75 or RET positivity, as contamination with mesenchymal cells may have a detrimental effect on proliferation and differentiation [82].

Neural crest cells (NCCs) have been isolated from intestinal tissue and derived from PSCs [79, 83, 84]. NCCs are ENS progenitors and can form neurospheres in culture exhibiting both differentiated neuronal and glial cell types [74, 78, 81]. Neurosphere cultured NCCs have been combined with HIOs and have been shown to generate myenteric and submucosal plexus-like structures which demonstrate contractility both *in vitro* and *in vivo* [83-86]. Interestingly, in one study, non-enteric pre-migratory NCCs were successfully combined with HIOs, suggesting cell candidates for ENS reconstruction may be found beyond enteric origins [86].

Endothelial cells

Existing tissue engineering strategies have relied on *in vivo* vascularisation of implanted constructs [40, 58, 87, 88]. However, pre-vascularisation *in vitro* will likely be key to engineering organs of clinically significant size, given the increased complexity of the gut compared to previously engineered organs. Endothelial progenitor cells can be found circulating in the blood [89] or derived from the bone marrow or blood vessels [90-92]. Furthermore, the isolation and culture of endothelial cells derived from human umbilical vein (HUVECs) has been well described and offers the potential for autologous therapy. However, senescence of endothelial cells in culture has proved problematic for clinical translation. A potential strategy to overcome this has been described recently through the partial re-programming of vascular endothelial cells, including HUVECs, using ETS variant transcription

factor 2 (ETV2). ETV2-expressing endothelial cells exhibit a more plastic and vasculogenic phenotype *in vitro* and *in vivo* [31].

Scaffolds

Scaffolds for digestive tract bioengineering must meet several criteria:

1. Allow for cellular attachment and proliferation while maintaining cytocompatibility.
2. Provide mechanical cues to facilitate formation of normal microanatomy.
3. Exhibit similar mechanical and biochemical properties to native tissue to mimic native organ microenvironment, whilst being robust enough to be transplanted.
4. GMP-grade processes should be available for manufacture of the scaffolds.

Bioscaffolds meeting these criteria can be grouped according to their origin. Synthetic scaffolds include polyglycolic acid (PGA) and poly-L-lactic acid (PLLA), both of which have been successfully used in tissue engineered small intestine (TESI) and oesophageal engineering already [69, 85, 93-95]. Natural scaffolds include single component mammalian ECM proteins, such as collagen I, chitosan, alginates, and scaffolds derived from decellularisation of native tissues (Figure 2) [96-99].

Various features of the scaffold are important. Scaffold topography has been shown to affect spatial distribution of intestinal epithelial cell subtypes [100], while stiffness has an impact on epithelial organisation and ISC maintenance [101, 102]. Biochemical cues from the ECM are vitally important; these are maintained in ECM-based scaffolds [103-105] but generally lacking from synthetic scaffolds. The main advantage of synthetic scaffolds is the mechanical properties and size can be customised to the patient and they are available 'off the shelf.' However, it has been demonstrated that decellularised scaffolds can be cryopreserved which would also allow them to be stored and readily available at the point of use [106].

Excitingly, it has been shown that decellularisation of native intestine is possible in small and large animals, as well as humans, using a variety of detergent and enzymatic-based protocols delivered via the lumen, vasculature, and/or immersion [88, 98, 104, 107]. Decellularised intestine maintains native tissue microarchitecture and a critical diversity of ECM proteins and growth factors [98, 104]. Similar decellularisation techniques have been applied to oesophageal tissue engineering in rodent and porcine models [58, 108]. Porcine

oesophagus is likely the preferred source for clinical translation, as it has similar anatomical dimensions to the human oesophagus and acellular porcine products are widely used in clinical surgical practice already. While this is by no means the only approach to digestive bioengineering, scaffolds from decellularised organs could well provide the most physiological scaffold, provided its xenogeneic or allogenic origin are not barriers to transplantation.

Bioengineering for Oesophageal Atresia

Having considered the necessary building blocks, we will now review the progress made towards translation of engineered cell and whole-organ therapies. Clearly, any bioengineered product requires rigorous evaluation prior to clinical use, particularly with regards to implant integration, immune response, durability, long-term functionality, and neoplastic risk. This is especially vital when considering tissue engineered therapies for children.

Mucosal sheets

Whilst full thickness replacement of the oesophagus is still far from human translation, attempts at mucosal replacement alone have shown some successful results in both large animal and human studies. Techniques for epithelial cell delivery include use of organoid units or epithelial cell sheets [59, 109, 110]. Where autologous epithelial sheets were transplanted in canine and porcine models immediately after endoscopic submucosal dissection (ESD), those treated with epithelial sheets had significantly lower stricture rate and degree of fibrosis compared to controls [111-113]. The hypothesised mechanisms for reduced stricture include the protective effect of an epithelial barrier from further mechanical damage and secretion of growth factors and cytokines to recruit host epithelial cells to repair the wound. These successful animal studies paved the way for similar approaches to be used in humans. The first transplantation of autologous epithelial sheets in humans demonstrated reduction in wound healing times and subsequent studies have demonstrated decreased stricture rates with no significant complications or adverse reactions [114, 115].

Full-thickness patch oesophagoplasty

Patch oesophagoplasty models, whereby a full-thickness defect in the oesophagus is replaced with a bioengineered patch have shown promising results in animal studies. The use of acellular small intestine submucosa (SIS) for patch repair has shown good survival rates with decreased incidence of stricture and leak in rat and canine models [116-118]. ECM scaffolds seeded with smooth muscle and bone marrow derived MSC also have demonstrated earlier epithelialisation and improved muscular regeneration compared to controls [119]. Mucosal regeneration on the patch and tissue remodelling at the transplantation sight has been identified [117]. Epithelial cell seeding on SIS has been reported to promote re-epithelialisation and skeletal muscle regeneration in a canine model [120].

By contrast, patch models using biodegradable synthetic scaffolds have had varied results, with relatively low survival rates and complications, including pseudodiverticula formation and anastomotic leak [121, 122]. Cell-seeded synthetic patches showed better epithelial and smooth muscle regeneration compared to unseeded controls in a rabbit model [123]. However, regardless of scaffold type or pre-seeding of epithelial cells, ingrowth of endogenous epithelium appears to occur by three months [120]. Therefore, it remains unclear if pre-seeding of epithelial cells is necessary before transplantation.

Full-thickness circumferential oesophageal replacement

Although replacements of part-circumference oesophageal defects have shown promising results, circumferential, full-thickness repair is even more complex. An in vitro model replicating full thickness native oesophagus has been developed by seeding MABs, fibroblasts, neural precursors, and epithelial cells on decellularised rat oesophagi and cultured them in a custom-made bioreactor. This resulted in an organised oesophageal construct with a multi-stratified epithelium and an innervated smooth muscle layer [58]. In this context, efficiency of cell repopulation is highly dependent on culture conditions; culture of the seeded constructs in bioreactors was shown to improve the morphology of engineered oesophagus, especially in the muscle layers (Figure 3) [58].

Initial attempts using acellular natural scaffolds to repair cervical oesophageal defects in vivo in canine models appeared positive [124]. However, subsequent adaptation of the technique to an intra-thoracic model was complicated by high occurrence of stenosis [125].

Other circumferential reconstruction techniques without cell seeding have led to high rates of stricture formation [70, 95, 126]. What is clear from this early experience, is that both the presence of a stent and its duration in situ appear to be critical to reduce stricture if acellular scaffolds alone are to be used and has led to the use of pre-seeded scaffolds instead.

Nakase et al. performed an intra-thoracic interposition of un-stented PGA scaffolds seeded with epithelial cells and fibroblasts after three weeks of maturation and vascularisation in the omentum. Within three weeks, stratified epithelialisation was complete with polarised smooth muscle-like regeneration [95]. This suggests that cell seeding of scaffolds accelerates both epithelial and muscle regeneration *in vivo*. The effect of stenting in cell-seeded constructs also appears to be positive with less incidence of stricture and anastomotic leak [73].

An alternative approach is the use of synthetic scaffolds as temporary templates to guide endogenous tissue regrowth. La Francesca et al. used polyurethane electro-spun scaffolds seeded with autologous adipose-derived MSCs to replace the thoracic oesophagus in pigs. Although multiple stent replacements were required, epithelialisation and organised smooth muscle were reported with symptom-free survival of two pigs at 18 and 19 months [127]. The same model was used to determine whether seeding with epithelial or mesenchymal cells resulted in better tissue regeneration. Scaffolds seeded with amniotic fluid derived MSCs appeared to have improved muscular regeneration in the scaffolds compared to the animal seeded with oesophageal epithelium only, however numbers were very small (n=4) [69]. The presence of MSCs has been reported to enhance both epithelialisation and muscularisation. They also appear to support angiogenesis and healing, likely by salutary paracrine signalling [69-72].

There has been a single case report of a full thickness circumferential replacement in human on compassionate grounds after extensive oesophageal injury failed all conventional treatment. The 5-cm defect in the cervical oesophagus in a 24-year-old patient was repaired using a self-expanding metal stent covered with an acellular dermal matrix, coated with autologous platelet-rich plasma adhesive gel. The stent was left in place for three years with no evidence of stricture or fistula one year after removal [128]. Although it is difficult to judge to what extent the tissue remodelling processes contributed to this clinically successful outcome, these results suggest that full coverage with exogenous cells may not be essential.

Bioengineering for Intestinal Failure and Intestinal Neuropathies

Cell therapies for intestinal failure

It is likely that initial engineered therapies for intestinal failure will be cell-based, rather than full thickness tissue, and in fact may be able to prevent the development of intestinal failure in the first place. A series of studies have examined AFSCs as a therapy for necrotising enterocolitis (NEC), a severe multi-factorial inflammatory disease of the gut that is common in premature infants and a leading cause of acquired intestinal failure in children [129-131]. In a rat model of NEC, those treated with intraperitoneal injection of AFSC had improved survival, intestinal morphology, increased enterocyte proliferation and decreased apoptosis, and improved barrier function and gut motility, compared to controls [130, 131]. Interestingly, the beneficial effects occurred within hours after injection and before histological evidence of engraftment of AFSCs in the bowel wall. Paracrine signalling acting via a COX-2 dependent mechanism was shown to be responsible for the clinical effect [130, 131]. Besner and colleagues examined the efficacy of different sources of MSCs in the treatment of NEC. Again, in a rat model, AFSC, BM-MSC, AF-derived neural SC (AF-NSC), and enteric neural SC (E-NSC) administered by intra-peritoneal injection all reduced the incidence and severity of NEC and preserved intestinal barrier function [132, 133]. Each cell line produced a similar magnitude of benefit, but interestingly E-NSC were shown to ameliorate the enteric nervous system damage caused by NEC and may be useful as a rescue therapy for post-NEC dysmotility [134]. Given the likely paracrine effect of MSC-based therapies, further studies have utilised exosomes derived from MSCs to treat NEC in animal models and have found similar beneficial effects to the administration of MSCs themselves. Mechanistically, this may occur through the induction of a protective endoplasmic reticulum stress response [133, 135-137].

Another cause of intestinal failure in children is gastroschisis, a congenital abdominal wall defect characterised by herniation of the intestine into the amniotic cavity and is associated with chemical, ischaemic, and mechanical damage to the intestine. In parallel to the NEC studies described above, AFSC injected into the amniotic cavity of rat foetuses with surgically created gastroschisis were able to engraft in the exposed bowel and placenta, while exerting positive effects on bowel wall thickness and mucosal health [138-140].

ISC organoids have been shown in several studies to be capable of treating colonic mucosal defects in rodent models of colonic ulceration (induced via EDTA or dextran sulphate

sodium and mechanical abrasion) [141-144]. In an initial study by Yui et al [144], Lgr5+ mouse colon organoids infused trans-anally engrafted and contributed to improved healing of the colonic mucosa. Subsequent studies have utilised organoids from mouse small intestine, which either take on a colonic phenotype (foetal mouse intestinal organoids) [141] or retain a small intestinal phenotype [142] following engraftment in the colon. More recently, Sugimoto et al were able to replicate these results, but this time utilising human colon organoids infused into the lumen of immunodeficient mice [143]. Delivery of epithelial organoids into injured mucosa, perhaps in combination with CRISPR-Cas9 gene editing techniques, is a promising methodology for treatment of mucosal disorders such as inflammatory bowel disease, chemoradiotherapy induced mucosal injury, or microvillus inclusion disease [143, 145].

When planning clinical translation of intestinal cell therapies, one must consider the delivery mechanism. All studies mentioned above utilise either cells suspended in isotonic solutions, cell culture media, or ECM-based hydrogels. In general, results favour the use of ECM-based hydrogels. However, the most common ECM-based hydrogel in use is Matrigel®, which is a laminin-rich mixture of ECM proteins secreted by the Engelbreth-Holm-Swarm mouse sarcoma cell line, excluding it from direct clinical translation [96, 146]. However, recent work from our group has shown that a hydrogel derived from porcine small intestine mucosa and submucosa (pSIS) can support a diverse range of endodermal organoid cultures with comparable performance to Matrigel® for up to four passages [104]. From a translational perspective, pSIS-based products are already in widespread use in surgical practice, all reagents used in generating the hydrogel are already commercially available at GMP-grade, and we demonstrated that the highly antigenic galactose-α1,3-galactose (α-Gal) was absent in the gel [104].

Cell therapies for intestinal neuropathies

Transplantation of neural stem or progenitor cells into neuropathic gut is expected to be a promising strategy to restore the ENS dysfunction. Hirschsprung disease (HSCR) is congenital disorder of the digestive tract due to absence of ganglion cells in the myenteric and submucosal plexuses leading to a functional bowel obstruction. Several mouse models have been established, including *Ret*$^{-/-}$, endothelin receptor type B deficiency (*Ednrb*$^{-/-}$), and

nNOS$^{-/-}$ mice. Furthermore, these models have been combined with immunodeficient murine phenotypes to enable the study of transplanted human neural cells [75, 147].

Several reports have demonstrated that ENSCs are able to colonize the muscle layers when transplanted into the mouse colon. The process has yet to be fully understood and optimised, to ensure the entire aganglionic lesion is covered following transplantation [77, 148, 149]. However, it is promising that long-term survival and widespread functional integration with the endogenous ENS of transplanted ENSCs has been reported in mouse, suggesting the potential of ENSCs not only to form neurons but also give rise to various neuronal subtypes *in vivo* [75]. In addition to engraftment and differentiation, McCann et al demonstrated functional improvement in contractility in *nNOS*$^{-/-}$ mice after transplantation of ENSC neurospheres [148]. Improved survival of *Ednrb*$^{-/-}$ mice after injection of ESC-derived ENC precursor cells has also been reported [147]. These findings have yet to be reproduced in large animal models, but ENSC cell therapy may prove a therapeutic option for enteric neuropathies in the future.

Partial and full thickness tissue engineered small intestine (TESI)

As discussed above, initial attempts at TESI utilised rat intestinal "organoid units" seeded onto synthetic scaffolds. Adaptation of the technique to human organoid units was soon achieved, developing TESI with crypt-villus like structures that was capable of rescuing weight loss in a rat model of intestinal failure [40, 41]. Moving on from organoid units, Lgr5+ organoids have been seeded on PGA scaffolds to generate TESI [93]. The constructs were successfully transplanted into the peritoneal cavity of recipient mice and showed preservation of the ISC compartment, mature epithelial cell lineages, and formation of primitive crypt-villus structures, along with mesenchymal and smooth muscle cells recruited from the host animal [93, 150]. HIOs have also been combined with synthetic scaffolds to produce TESI, surviving 12 weeks *in vivo* [85]. Excitingly, TESI produced from HIOs and ENS progenitors have been shown to form neuroepithelial connections and produce nerve-mediated contractile activity *in vitro* and *in vivo* [83, 84]. However, concerns about genetic stability, tumorgenicity, and immunoreactivity of iPSCs and the prohibitive cost of generating autologous iPSC lines in GMP-compliant conditions, remain barriers to clinical translation of iPSC-based tissues [51-54].

TESI has thus far relied on *in vivo* vascularisation. However, two recent important works have helped to move forward the field of pre-vascularisation. The Ott group were able to repopulate a decellularised rat intestine scaffold with HIOs via the intestinal lumen and pre-vascularise the TESI via infusion of HUVECs via the superior mesenteric artery and vein. Endothelial cells were seen in the lamina propria, and the graft was able to survive and absorb glucose following heterotopic transplantation [88]. In another study, ETV2-HUVECs were also able vascularise ISC-derived organoids *in* vitro and repopulate a decellularised rat intestine scaffold down to the capillary level and could be perfused with human blood [31]. Similar to the oesophagus, dynamic culture conditions using perfusion bioreactors have shown positive effects in preparing TESI for transplant, including improved maturation of epithelial and vascular components [58, 88, 107].

While the development of full thickness TESI continues, two recent studies of partial thickness TESI are worth mentioning for their capacity for translation in near future. In 2020, the generation of partial thickness TESI using patient derived organoids, fibroblasts, and scaffolds was reported [107]. Organoids and fibroblasts were derived from duodenum, jejunum, and ileum of patients with short bowel syndrome, thereby showing this strategy capable of producing autologous TESI. Following exponential expansion, the organoids retained regional identity, including disaccharidase and protease activity, whether they were seeded on decellularised human small intestinal or colonic scaffolds, highlighting the ability of this approach to be region-specific based on the required epithelial cell identity. This mucosal TESI could be transplanted into immunodeficient mice and survive for two weeks but was enterocyte dominant and had immature crypt-villus morphology, highlighting the areas requiring optimization for clinical trial [107].

This year, Sugimoto and colleagues reported a technique to repurpose the colon for intestinal failure treatment [151]. In a rat model, the authors were able to remove the colonic epithelium by a combination of EDTA and mechanical scraping while leaving the neuromuscular coat intact. Seeding of rat ileal organoids into the denuded colon resulted in reconstitution of an ileal epithelium with crypt-villus structures, LYVE-1 positive lacteals, and evidence of glucose, peptide, and fat absorption, while maintaining function of the colonic neuromuscular coat. When introducing this treatment into a rat model of intestinal failure, there was a marked improvement in survival. However, the authors did not repeat the experiments using human organoids [151]. If results from these two studies can be

recapitulated with human intestinal organoids in large animal models, the repurposing of residual colon to small intestine and/or the use of engineered mucosal sheets will be a major step closer to clinical trial.

Challenges and unanswered questions

There are several significant challenges which will require further study to overcome prior to clinical translation. Stricture at the sites of anastomosis and in the engineered construct itself, which may be worsened by an inadequate blood supply, exposure to gastric acid, or an incomplete epithelial barrier [116, 125]. Another hurdle for clinical translation is vascularisation. Various methods have been demonstrated to be useful for the oesophagus, including in vivo maturation in the greater omentum, latissimus dorsi, or thyroid gland flaps [152]. For the intestine, the presence of a single feeding arterial supply is advantageous for vascular anastomosis at transplantation, but much work remains to be done to achieve complete coverage of the vascular scaffold [31, 88]. Furthermore, key to normal intestinal physiology is a functioning lymphatic network. Lymphatic endothelial cells can be formed from pluripotent stem cells and self-organising lymphatic networks have been observed *in vitro* when cultured in ECM hydrogels [153, 154]. However, combining these lymphatic cells in to existing TESI models is still in its infancy.

Nervous innervation and coordinated peristalsis do not appear to be essential for replacement of a small segments of oesophagus but will be vital for large defects [69, 73, 127]. In the case of long-segment TESI and intestinal neuropathies, achievement of near-normal motility will be crucial to therapy success. While the ENS replacement strategies discussed above show promise, understanding of the behaviour of implanted neural precursors remains incomplete [27, 148]. Finally, the effect of pre-transplantation bioreactor maturation still needs to be addressed, because prolonged maturation protocols may compromise mechanical strength and cell survival, especially for epithelial cells and the lymphovascular tree [31, 70, 73, 88, 95].

Future Perspectives

The ultimate goal of bioengineering for congenital digestive disorders is the generation of a full thickness segment of fully functional organ. The engineered organ must be of a clinically relevant size, exhibit regionalised absorptive, endocrine, barrier, and/or

immune functions, be vascularised and have coordinated peristalsis, all whilst the cells in the construct remain genetically stable and capable of homeostasis over time [11, 12]. While this goal is some way from being met, engineering of individual components, particularly epithelium, is much more advanced. Therefore, it is likely that initial clinical translation will focus on cell-based therapies or partial thickness reconstruction [11], or perhaps, as discussed above, even utilising these approaches to prevent progression to intestinal failure [133, 136, 137]. The feasibility of cell-based approaches is exemplified by the ability to generate region-specific patient-derived intestinal organoids that can be loaded onto scaffolds to generate mucosal sheets [107], the use of CRISPR-Cas9 based gene editing technology in organoids [145], and the successful treatment of colonic mucosal defects or repurposing of the colon to small intestine-like function using organoids [142, 144, 151]. Furthermore, cell-based therapies offer promise in the treatment of intestinal neuropathies [27, 148].

Much work remains to move beyond cell and mucosal sheet therapies to full thickness engineered organs. Examples of major issues still to be addressed include improvement in the *in vitro* derivation of organised neuromusculature, defining the best approach to pre-vascularisation of constructs, upscaling of engineered organs to clinically relevant proportions, understanding how best to combine the separate cell and tissue types onto a suitable scaffold *in vitro*, and optimisation of transplantation strategies in animal models prior to first-in-human clinical trials. It has been shown previously that presence of at least 10% of neonatal intestinal length (~20cm residual) can allow patients to wean from parenteral nutrition [6, 155], and it is likely that an even shorter segment of engineered oesophagus would be required. From a regulatory perspective, bioengineered organs must be developed using good manufacturing practice (GMP) compliant protocols, produced in a reasonable timeframe, and in a sustainable fashion. Finally, the implanted engineered organs should be durable over a lifetime, with lifelong follow-up surveillance of initial trial participants to ensure the promise of bioengineering for congenital disorders is fulfilled [11, 12].

Practice Points

- Congenital disorders of the digestive tract cause substantial morbidity and mortality, with long-gap oesophageal atresia, intestinal failure, and long-segment Hirschsprung's disease all having sub-optimal treatments at present.
- Pre-clinical research is advancing towards the possibility of cell therapies and transplantable engineered organs that could provide durable solutions to congenital digestive defects, possibly using autologous cell sources.
- Various tissue engineering techniques have been employed with a focus on individualisation and disease-specific strategies, which have yielded promising results in small and large animal pre-clinical studies.
- Cell therapies hold promise to treat dysfunction of specific tissue subtypes within an organ (e.g. neural stem cell therapy for intestinal neuropathies), while major tissue loss or absence (e.g. long gap oesophageal atresia, short bowel syndrome) may require full thickness multi-layer organ regeneration.

Research Agenda

- Optimisation of methods to vascularise and innervate engineered gut.
- Optimisation of scaffold selection and cell source for full-thickness oesophageal engineering.
- Derivation of neural stem/progenitor cells and improvement of integration, migration and differentiation after transplantation.
- Pre-clinical studies in large mammals for engineered intestinal epithelium/mucosa and full thickness oesophagus, prior to first-in-human clinical trial.
- Maintaining rigorous ethical standards throughout the development and execution of clinical trials. Regulators and patient groups should be involved in the research process as early as possible. Furthermore, this resource intense and potentially costly group of therapies must be developed with environmental impact and equity of access issues in mind.

Acknowledgements

This review is a snapshot of the current state of bioengineering for congenital digestive disorders and we apologize to the many colleagues whose work could not be cited here due to space limitations. The authors and this work were funded by Horizon 2020 grant INTENS (668294) on the project 'Intestinal Tissue Engineering Solution for Children with Short Bowel Syndrome'. P.D.C. is supported by an NIHR Professorship, NIHR UCL BRC-GOSH, the Great Ormond Street Hospital Children's Charity and the Oak Foundation. B.C.J. is supported by the General Sir John Monash Foundation, Australia, and University College London. S.S. is supported by the Japan Society for the Promotion of Science Overseas Research Fellowship (310072). N.D. is supported by the Lewis Spitz Great Ormond Street Children's Charity Surgical Scientist Fellowship.

Figure Legends

Figure 1: Schematic overview of proposed autologous regenerative medicine strategies for childhood gastrointestinal diseases. Created with BioRender.com.

Figure 2: Perfusion detergent-enzymatic treatment (DET) decellularisation of a segment of porcine intestine. Such a scaffold is of appropriate dimensions to act as the basis for TESI of a size relevant for transplantation into a human infant.

Figure 3: Oesophageal bioreactors allowing for dynamic culture of various cell types seeding onto decellularlised oesophageal scaffolds.

References

[1] van der Zee DC, Bagolan P, Faure C, Gottrand F, Jennings R, Laberge JM, et al. Position Paper of INoEA Working Group on Long-Gap Esophageal Atresia: For Better Care. Front Pediatr. 2017;5:63.

[2] Gallo G, Zwaveling S, Groen H, Van Der Zee D, Hulscher J. Long-gap esophageal atresia: A meta-analysis of jejunal interposition, colon interposition, and gastric pull-up. Eur J Pediatr Surg; 2012. p. 420-5.

[3] Kim HB, Fauza D, Garza J, Oh J-T, Nurko S, Jaksic T. Serial transverse enteroplasty (STEP): A novel bowel lengthening procedure. Journal of Pediatric Surgery. 2003;38:425-9.

[4] Mutanen A, Wales PW. Etiology and prognosis of pediatric short bowel syndrome. Semin Pediatr Surg. 2018;27:209-17.

[5] Ramos-Gonzalez G, Kim HB. Autologous intestinal reconstruction surgery. Semin Pediatr Surg. 2018;27:261-6.

[6] Spencer AU, Neaga A, West B, Safran J, Brown P, Btaiche I, et al. Pediatric short bowel syndrome: redefining predictors of success. Ann Surg. 2005;242:403-9; discussion 9-12.

[7] Kesseli S, Sudan D. Small Bowel Transplantation. Surg Clin North Am. 2019;99:103-16.

[8] Martinez Rivera A, Wales PW. Intestinal transplantation in children: current status. Pediatr Surg Int. 2016;32:529-40.

[9] Davidson JR, Kyrklund K, Eaton S, Pakarinen MP, Thompson DS, Cross K, et al. Long-term surgical and patient-reported outcomes of Hirschsprung's Disease. J Pediatr Surg. 2021.

[10] Rintala RJ, Pakarinen MP. Long-term outcomes of Hirschsprung's disease. Semin Pediatr Surg. 2012;21:336-43.

[11] Clevers H, Conder RK, Li VSW, Lutolf MP, Vallier L, Chan S, et al. Tissue-Engineering the Intestine: The Trials before the Trials. Cell Stem Cell. 2019;24:855-9.

[12] Cossu G, Birchall M, Brown T, De Coppi P, Culme-Seymour E, Gibbon S, et al. Lancet Commission: Stem cells and regenerative medicine. The Lancet. 2018;391:883-910.

[13] Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature. 2007;449:1003-7.

[14] Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009;459:262-5.

[15] Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. Cell. 2012;149:1192-205.
[16] VanDussen KL, Carulli AJ, Keeley TM, Patel SR, Puthoff BJ, Magness ST, et al. Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. Development. 2012;139:488-97.
[17] van den Brink GR. Hedgehog signaling in development and homeostasis of the gastrointestinal tract. Physiol Rev. 2007;87:1343-75.
[18] Guiu J, Hannezo E, Yui S, Demharter S, Ulyanchenko S, Maimets M, et al. Tracing the origin of adult intestinal stem cells. Nature. 2019;570:107-11.
[19] van Es JH, Sato T, van de Wetering M, Lyubimova A, Yee Nee AN, Gregorieff A, et al. Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. Nature cell biology. 2012;14:1099-104.
[20] Piedrafita G, Kostiou V, Wabik A, Colom B, Fernandez-Antoran D, Herms A, et al. A single-progenitor model as the unifying paradigm of epidermal and esophageal epithelial maintenance in mice. Nat Commun. 2020;11:1429.
[21] Juhl CO, Jensen LS, Steiniche T, Moussa E. Recombinant human epidermal growth factor prevents sclerotherapy-induced esophageal ulcer and stricture formations in pigs. Dig Dis Sci. 1994;39:393-401.
[22] Gundogdu G, Tosun M, Morhardt D, Gheinani AH, Algarrahi K, Yang X, et al. Molecular mechanisms of esophageal epithelial regeneration following repair of surgical defects with acellular silk fibroin grafts. Sci Rep. 2021;11:7086.
[23] Meran L, Baulies A, Li VSW. Intestinal Stem Cell Niche: The Extracellular Matrix and Cellular Components. Stem Cells Int. 2017;2017:7970385.
[24] Powell DW, Pinchuk IV, Saada JI, Chen X, Mifflin RC. Mesenchymal cells of the intestinal lamina propria. Annu Rev Physiol. 2011;73:213-37.
[25] Yen TH, Wright NA. The gastrointestinal tract stem cell niche. Stem cell reviews. 2006;2:203-12.
[26] Bonavina L, Segalin A, Pavanello M, Faranda C, Cioffi U, Peracchia A. Surgical treatment of esophageal stenosis caused by reflux. Annali italiani di chirurgia. 1995;66:621-4.
[27] McCann CJ, Thapar N. Enteric neural stem cell therapies for enteric neuropathies. Neurogastroenterol Motil. 2018;30:e13369.
[28] Bjerknes M, Cheng H. Modulation of specific intestinal epithelial progenitors by enteric neurons. Proc Natl Acad Sci U S A. 2001;98:12497-502.

[29] Obata Y, Castano A, Boeing S, Bon-Frauches AC, Fung C, Fallesen T, et al. Neuronal programming by microbiota regulates intestinal physiology. Nature. 2020;578:284-9.
[30] Puzan M, Hosic S, Ghio C, Koppes A. Enteric Nervous System Regulation of Intestinal Stem Cell Differentiation and Epithelial Monolayer Function. Sci Rep. 2018;8:6313.
[31] Palikuqi B, Nguyen DT, Li G, Schreiner R, Pellegata AF, Liu Y, et al. Adaptable haemodynamic endothelial cells for organogenesis and tumorigenesis. Nature. 2020;585:426-32.
[32] Saha S, Aranda E, Hayakawa Y, Bhanja P, Atay S, Brodin NP, et al. Macrophage-derived extracellular vesicle-packaged WNTs rescue intestinal stem cells and enhance survival after radiation injury. Nat Commun. 2016;7:13096.
[33] Biton M, Haber AL, Rogel N, Burgin G, Beyaz S, Schnell A, et al. T Helper Cell Cytokines Modulate Intestinal Stem Cell Renewal and Differentiation. Cell. 2018;175:1307-20 e22.
[34] Hussey GS, Cramer MC, Badylak SF. Extracellular Matrix Bioscaffolds for Building Gastrointestinal Tissue. Cell Mol Gastroenterol Hepatol. 2018;5:1-13.
[35] Nelson CM, Bissell MJ. Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. Annu Rev Cell Dev Biol. 2006;22:287-309.
[36] Hynes RO. The extracellular matrix: not just pretty fibrils. Science. 2009;326:1216-9.
[37] Hynes RO, Naba A. Overview of the matrisome--an inventory of extracellular matrix constituents and functions. Cold Spring Harb Perspect Biol. 2012;4:a004903.
[38] Choi RS, Vacanti JP. Preliminary studies of tissue-engineered intestine using isolated epithelial organoid units on tubular synthetic biodegradable scaffolds. Transplantation proceedings. 1997;29:848-51.
[39] 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. Journal of cell science. 1992;101 (Pt 1):219-31.
[40] Grant CN, Mojica SG, Sala FG, Hill JR, Levin DE, Speer AL, et al. Human and mouse tissue-engineered small intestine both demonstrate digestive and absorptive function. American journal of physiology Gastrointestinal and liver physiology. 2015;308:G664-77.
[41] Grikscheit TC, Siddique A, Ochoa ER, Srinivasan A, Alsberg E, Hodin RA, et al. Tissue-engineered small intestine improves recovery after massive small bowel resection. Annals of surgery. 2004;240:748-54.

[42] Organ GM, Mooney DJ, Hansen LK, Schloo B, Vacanti JP. Transplantation of enterocytes utilizing polymer-cell constructs to produce a neointestine. Transplantation proceedings. 1992;24:3009-11.
[43] Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011;141:1762-72.
[44] Cao L, Gibson JD, Miyamoto S, Sail V, Verma R, Rosenberg DW, et al. Intestinal lineage commitment of embryonic stem cells. Differentiation. 2011;81:1-10.
[45] Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature. 2010;470:105.
[46] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861-72.
[47] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663-76.
[48] Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282:1145-7.
[49] Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007;318:1917-20.
[50] Watson CL, Mahe MM, Munera J, Howell JC, Sundaram N, Poling HM, et al. An in vivo model of human small intestine using pluripotent stem cells. Nature medicine. 2014;20:1310-4.
[51] Doss MX, Sachinidis A. Current Challenges of iPSC-Based Disease Modeling and Therapeutic Implications. Cells. 2019;8.
[52] Churko JM, Lee J, Ameen M, Gu M, Venkatasubramanian M, Diecke S, et al. Transcriptomic and epigenomic differences in human induced pluripotent stem cells generated from six reprogramming methods. Nat Biomed Eng. 2017;1:826-37.
[53] Hussein SM, Batada NN, Vuoristo S, Ching RW, Autio R, Narva E, et al. Copy number variation and selection during reprogramming to pluripotency. Nature. 2011;471:58-62.
[54] Gore A, Li Z, Fung HL, Young JE, Agarwal S, Antosiewicz-Bourget J, et al. Somatic coding mutations in human induced pluripotent stem cells. Nature. 2011;471:63-7.

[55] Kasagi Y, Chandramouleeswaran PM, Whelan KA, Tanaka K, Giroux V, Sharma M, et al. The Esophageal Organoid System Reveals Functional Interplay Between Notch and Cytokines in Reactive Epithelial Changes. Cellular and Molecular Gastroenterology and Hepatology. 2018;5:333-52.
[56] Trisno SL, Philo KED, Mccracken KW, Catá EM, Ruiz-Torres S, Rankin SA, et al. Esophageal Organoids from Human Pluripotent Stem Cells Delineate Sox2 Functions during Esophageal Specification. Cell Stem Cell. 2018;23:501-15.e7.
[57] Sakurai Y, Masui T, Yoshida I, Tonomura S, Shoji M, Nakamura Y, et al. Randomized clinical trial of the effects of perioperative use of immune-enhancing enteral formula on metabolic and immunological status in patients undergoing esophagectomy. World J Surg. 2007;31:2150-7; discussion 8-9.
[58] Urbani L, Camilli C, Phylactopoulos DE, Crowley C, Natarajan D, Scottoni F, et al. Multi-stage bioengineering of a layered oesophagus with in vitro expanded muscle and epithelial adult progenitors. Nat Commun. 2018;9:4286.
[59] Yamato M, Utsumi M, Kushida A, Konno C, Kikuchi A, Okano T. Thermo-responsive culture dishes allow the intact harvest of multilayered keratinocyte sheets without dispase by reducing temperature. Tissue Eng. 2001;7:473-80.
[60] Perin S, McCann CJ, De Coppi P, Thapar N. Isolation and characterisation of mouse intestinal mesoangioblasts. Pediatric surgery international. 2019;35:29-34.
[61] Dellavalle A, Sampaolesi M, Tonlorenzi R, Tagliafico E, Sacchetti B, Perani L, et al. Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. Nat Cell Biol. 2007;9:255-67.
[62] Minasi MG, Riminucci M, De Angelis L, Borello U, Berarducci B, Innocenzi A, et al. The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. Development. 2002;129:2773-83.
[63] Thurner M, Deutsch M, Janke K, Messner F, Kreutzer C, Beyl S, et al. Generation of myogenic progenitor cell-derived smooth muscle cells for sphincter regeneration. Stem Cell Res Ther. 2020;11:233.
[64] Ghionzoli M, Repele A, Sartiani L, Costanzi G, Parenti A, Spinelli V, et al. Human amniotic fluid stem cell differentiation along smooth muscle lineage. FASEB J. 2013;27:4853-65.

[65] Tamama K, Sen CK, Wells A. Differentiation of bone marrow mesenchymal stem cells into the smooth muscle lineage by blocking ERK/MAPK signaling pathway. Stem cells and development. 2008;17:897-908.
[66] Cossu G, Bianco P. Mesoangioblasts--vascular progenitors for extravascular mesodermal tissues. Curr Opin Genet Dev. 2003;13:537-42.
[67] Sampaolesi M, Torrente Y, Innocenzi A, Tonlorenzi R, D'Antona G, Pellegrino MA, et al. Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. Science. 2003;301:487-92.
[68] Cossu G, Previtali SC, Napolitano S, Cicalese MP, Tedesco FS, Nicastro F, et al. Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne muscular dystrophy. EMBO Mol Med. 2015;7:1513-28.
[69] Jensen T, Wanczyk H, Sharma I, Mitchell A, Sayej WN, Finck C. Polyurethane scaffolds seeded with autologous cells can regenerate long esophageal gaps: An esophageal atresia treatment model. J Pediatr Surg. 2019;54:1744-54.
[70] Luc G, Charles G, Gronnier C, Cabau M, Kalisky C, Meulle M, et al. Decellularized and matured esophageal scaffold for circumferential esophagus replacement: Proof of concept in a pig model. Biomaterials. 2018;175:1-18.
[71] Tan B, Wei RQ, Tan MY, Luo JC, Deng L, Chen XH, et al. Tissue engineered esophagus by mesenchymal stem cell seeding for esophageal repair in a canine model. J Surg Res. 2013;182:40-8.
[72] Wang F, Maeda Y, Zachar V, Ansari T, Emmersen J. Regeneration of the oesophageal muscle layer from oesophagus acellular matrix scaffold using adipose-derived stem cells. Biochem Biophys Res Commun. 2018;503:271-7.
[73] Poghosyan T, Sfeir R, Michaud L, Bruneval P, Domet T, Vanneaux V, et al. Circumferential esophageal replacement using a tube-shaped tissue-engineered substitute: An experimental study in minipigs. Surgery. 2015;158:266-77.
[74] Almond S, Lindley RM, Kenny SE, Connell MG, Edgar DH. Characterisation and transplantation of enteric nervous system progenitor cells. Gut. 2007;56:489-96.
[75] Cooper JE, Natarajan D, McCann CJ, Choudhury S, Godwin H, Burns AJ, et al. In vivo transplantation of fetal human gut-derived enteric neural crest cells. Neurogastroenterology and Motility. 2017;29.

[76] Hetz S, Acikgoez A, Voss U, Nieber K, Holland H, Hegewald C, et al. In Vivo transplantation of neurosphere-like bodies derived from the human postnatal and adult enteric nervous system: A pilot study. PLoS ONE. 2014;9:e93605-e.
[77] Hotta R, Stamp LA, Foong JPP, McConnell SN, Bergner AJ, Anderson RB, et al. Transplanted progenitors generate functional enteric neurons in the postnatal colon. Journal of Clinical Investigation. 2013;123:1182-91.
[78] Bondurand N, Natarajan D, Thapar N, Atkins C, Pachnis V. Neuron and glia generating progenitors of the mammalian enteric nervous system isolated from foetal and postnatal gut cultures. Development. 2003;130:6387-400.
[79] Metzger M, Caldwell C, Barlow AJ, Burns AJ, Thapar N. Enteric nervous system stem cells derived from human gut mucosa for the treatment of aganglionic gut disorders. Gastroenterology. 2009;136:2214-25 e1-3.
[80] Becker L, Kulkarni S, Tiwari G, Micci MA, Pasricha PJ. Divergent fate and origin of neurosphere-like bodies from different layers of the gut. American Journal of Physiology - Gastrointestinal and Liver Physiology. 2012;302.
[81] Metzger M, Bareiss PM, Danker T, Wagner S, Hennenlotter J, Guenther E, et al. Expansion and differentiation of neural progenitors derived from the human adult enteric nervous system. Gastroenterology. 2009;137:2063-73 e4.
[82] Binder E, Natarajan D, Cooper J, Kronfli R, Cananzi M, Delalande JM, et al. Enteric neurospheres are not specific to neural crest cultures: implications for neural stem cell therapies. PLoS One. 2015;10:e0119467.
[83] Schlieve CR, Fowler KL, Thornton M, Huang S, Hajjali I, Hou X, et al. Neural Crest Cell Implantation Restores Enteric Nervous System Function and Alters the Gastrointestinal Transcriptome in Human Tissue-Engineered Small Intestine. Stem cell reports. 2017;9:883-96.
[84] Workman MJ, Mahe MM, Trisno S, Poling HM, Watson CL, Sundaram N, et al. Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. Nature medicine. 2017;23:49-59.
[85] Finkbeiner SR, Freeman JJ, Wieck MM, El-Nachef W, Altheim CH, Tsai YH, et al. Generation of tissue-engineered small intestine using embryonic stem cell-derived human intestinal organoids. Biology Open. 2015;4:1462-72.

[86] Yuan H, Hu H, Chen R, Mu W, Wang L, Li Y, et al. Premigratory neural crest stem cells generate enteric neurons populating the mouse colon and regulating peristalsis in tissue-engineered intestine. Stem Cells Transl Med. 2021;10:922-38.
[87] Elliott MJ, De Coppi P, Speggiorin S, Roebuck D, Butler CR, Samuel E, et al. Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study. The Lancet. 2012;380:994-1000.
[88] Kitano K, Schwartz DM, Zhou H, Gilpin SE, Wojtkiewicz GR, Ren X, et al. Bioengineering of functional human induced pluripotent stem cell-derived intestinal grafts. Nature communications. 2017;8:765.
[89] Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest. 2000;105:71-7.
[90] Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275:964-7.
[91] Shi Q, Rafii S, Wu MH-D, Wijelath ES, Yu C, Ishida A, et al. Evidence for Circulating Bone Marrow-Derived Endothelial Cells. Blood. 1998;92:362-7.
[92] Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat Med. 2003;9:702-12.
[93] Cromeens BP, Liu Y, Stathopoulos J, Wang Y, Johnson J, Besner GE. Production of tissue-engineered intestine from expanded enteroids. The Journal of surgical research. 2016;204:164-75.
[94] Zakhem E, Tamburrini R, Orlando G, Koch KL, Bitar KN. Transplantation of a Human Tissue-Engineered Bowel in an Athymic Rat Model. Tissue engineering Part C, Methods. 2017;23:652-60.
[95] Nakase Y, Nakamura T, Kin S, Nakashima S, Yoshikawa T, Kuriu Y, et al. Intrathoracic esophageal replacement by in situ tissue-engineered esophagus. J Thorac Cardiovasc Surg. 2008;136:850-9.
[96] Blondel D, Lutolf MP. Bioinspired Hydrogels for 3D Organoid Culture. Chimia (Aarau). 2019;73:81-5.
[97] Feksa LR, Troian EA, Muller CD, Viegas F, Machado AB, Rech VC. Hydrogels for biomedical applications. Nanostructures for the Engineering of Cells, Tissues and Organs. 2018. p. 403-38.

[98] Totonelli G, Maghsoudlou P, Garriboli M, Riegler J, Orlando G, Burns AJ, et al. A rat decellularized small bowel scaffold that preserves villus-crypt architecture for intestinal regeneration. Biomaterials. 2012;33:3401-10.
[99] Catry J, Luong-Nguyen M, Arakelian L, Poghosyan T, Bruneval P, Domet T, et al. Circumferential Esophageal Replacement by a Tissue-engineered Substitute Using Mesenchymal Stem Cells: An Experimental Study in Mini Pigs. Cell transplantation. 2017;26:1831-9.
[100] Nikolaev M, Mitrofanova O, Broguiere N, Geraldo S, Dutta D, Tabata Y, et al. Homeostatic mini-intestines through scaffold-guided organoid morphogenesis. Nature. 2020;585:574-8.
[101] Gjorevski N, Sachs N, Manfrin A, Giger S, Bragina ME, Ordonez-Moran P, et al. Designer matrices for intestinal stem cell and organoid culture. Nature. 2016;539:560-4.
[102] Perez-Gonzalez C, Ceada G, Greco F, Matejcic M, Gomez-Gonzalez M, Castro N, et al. Mechanical compartmentalization of the intestinal organoid enables crypt folding and collective cell migration. Nat Cell Biol. 2021;23:745-57.
[103] Capeling MM, Czerwinski M, Huang S, Tsai YH, Wu A, Nagy MS, et al. Nonadhesive Alginate Hydrogels Support Growth of Pluripotent Stem Cell-Derived Intestinal Organoids. Stem Cell Reports. 2019;12:381-94.
[104] Giobbe GG, Crowley C, Luni C, Campinoti S, Khedr M, Kretzschmar K, et al. Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture. Nature Communications. 2019;10.
[105] Holloway EM, Capeling MM, Spence JR. Biologically inspired approaches to enhance human organoid complexity. Development. 2019;146.
[106] Urbani L, Maghsoudlou P, Milan A, Menikou M, Hagen CK, Totonelli G, et al. Long-term cryopreservation of decellularised oesophagi for tissue engineering clinical application. PLoS One. 2017;12:e0179341.
[107] Meran L, Massie I, Campinoti S, Weston AE, Gaifulina R, Tullie L, et al. Engineering transplantable jejunal mucosal grafts using patient-derived organoids from children with intestinal failure. Nat Med. 2020;26:1593-601.
[108] Totonelli G, Maghsoudlou P, Georgiades F, Garriboli M, Koshy K, Turmaine M, et al. Detergent enzymatic treatment for the development of a natural acellular matrix for oesophageal regeneration. Pediatr Surg Int. 2013;29:87-95.

[109] Grikscheit T, Ochoa ER, Srinivasan A, Gaissert H, Vacanti JP. Tissue-engineered esophagus: Experimental substitution by onlay patch or interposition. Journal of Thoracic and Cardiovascular Surgery. 2003;126:537-44.
[110] Spurrier RG, Speer AL, Hou X, El-Nachef WN, Grikscheit TC. Murine and human tissue-engineered esophagus form from sufficient stem/progenitor cells and do not require microdesigned biomaterials. Tissue Eng Part A. 2015;21:906-15.
[111] Perrod G, Rahmi G, Pidial L, Camilleri S, Bellucci A, Casanova A, et al. Cell Sheet Transplantation for Esophageal Stricture Prevention after Endoscopic Submucosal Dissection in a Porcine Model. PLoS One. 2016;11:e0148249.
[112] Ohki T, Yamato M, Murakami D, Takagi R, Yang J, Namiki H, et al. Treatment of oesophageal ulcerations using endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets in a canine model. Gut. 2006;55:1704-10.
[113] Kanai N, Yamato M, Ohki T, Yamamoto M, Okano T. Fabricated autologous epidermal cell sheets for the prevention of esophageal stricture after circumferential ESD in a porcine model. Gastrointest Endosc. 2012;76:873-81.
[114] Jonas E, Sjoqvist S, Elbe P, Kanai N, Enger J, Haas SL, et al. Transplantation of tissue-engineered cell sheets for stricture prevention after endoscopic submucosal dissection of the oesophagus. United European Gastroenterol J. 2016;4:741-53.
[115] Yamaguchi N, Isomoto H, Kobayashi S, Kanai N, Kanetaka K, Sakai Y, et al. Oral epithelial cell sheets engraftment for esophageal strictures after endoscopic submucosal dissection of squamous cell carcinoma and airplane transportation. Sci Rep. 2017;7:17460.
[116] Badylak S, Meurling S, Chen M, Spievack A, Simmons-Byrd A. Resorbable bioscaffold for esophageal repair in a dog model. Journal of Pediatric Surgery. 2000;35:1097-103.
[117] Urita Y, Komuro H, Chen G, Shinya M, Kaneko S, Kaneko M, et al. Regeneration of the esophagus using gastric acellular matrix: an experimental study in a rat model. Pediatr Surg Int. 2007;23:21-6.
[118] Lopes MF, Cabrita A, Ilharco J, Pessa P, Paiva-Carvalho J, Pires A, et al. Esophageal replacement in rat using porcine intestinal submucosa as a patch or a tube-shaped graft. Dis Esophagus. 2006;19:254-9.
[119] Marzaro M, Vigolo S, Oselladore B, Conconi MT, Ribatti D, Giuliani S, et al. In vitro and in vivo proposal of an artificial esophagus. J Biomed Mater Res A. 2006;77:795-801.

[120] Wei RQ, Tan B, Tan MY, Luo JC, Deng L, Chen XH, et al. Grafts of porcine small intestinal submucosa with cultured autologous oral mucosal epithelial cells for esophageal repair in a canine model. Exp Biol Med (Maywood). 2009;234:453-61.
[121] Diemer P, Markoew S, Le DQS, Qvist N. Poly-ε-caprolactone mesh as a scaffold for in vivo tissue engineering in rabbit esophagus. Diseases of the Esophagus. 2015;28:240-5.
[122] Lynen Jansen P, Klinge U, Anurov M, Titkova S, Mertens PR, Jansen M. Surgical mesh as a scaffold for tissue regeneration in the esophagus. Eur Surg Res. 2004;36:104-11.
[123] Park SY, Choi JW, Park JK, Song EH, Park SA, Kim YS, et al. Tissue-engineered artificial oesophagus patch using three-dimensionally printed polycaprolactone with mesenchymal stem cells: a preliminary report. Interact Cardiovasc Thorac Surg. 2016;22:712-7.
[124] Takimoto Y, Nakamura T, Yamamoto Y, Kiyotani T, Teramachi M, Shimizu Y. The experimental replacement of a cervical esophageal segment with an artificial prosthesis with the use of collagen matrix and a silicone stent. The Journal of Thoracic and Cardiovascular Surgery. 1998;116:98-106.
[125] Yamamoto Y, Nakamura T, Shimizu Y, Matsumoto K, Takimoto Y, Kiyotani T, et al. Intrathoracic esophageal replacement in the dog with the use of an artificial esophagus composed of a collagen sponge with a double-layered silicone tube. The Journal of Thoracic and Cardiovascular Surgery. 1999;118:276-86.
[126] Saito M, Sakamoto T, Fujimaki M, Tsukada K, Honda T, Nozaki M. Experimental study of an artificial esophagus using a collagen sponge, a latissimus dorsi muscle flap, and split-thickness skin. Surg Today. 2000;30:606-13.
[127] La Francesca S, Aho JM, Barron MR, Blanco EW, Soliman S, Kalenjian L, et al. Long-term regeneration and remodeling of the pig esophagus after circumferential resection using a retrievable synthetic scaffold carrying autologous cells. Sci Rep. 2018;8:4123.
[128] Dua KS, Hogan WJ, Aadam AA, Gasparri M. In-vivo oesophageal regeneration in a human being by use of a non-biological scaffold and extracellular matrix. The Lancet. 2016;388:55-61.
[129] Hackam D, Caplan M. Necrotizing enterocolitis: Pathophysiology from a historical context. Seminars in Pediatric Surgery. 2018;27:11-8.
[130] Zani A, Cananzi M, Fascetti-Leon F, Lauriti G, Smith VV, Bollini S, et al. Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotising enterocolitis via a COX-2 dependent mechanism. Gut. 2014;63:300-9.

[131] Zani A, Cananzi M, Lauriti G, Fascetti-Leon F, Wells J, Siow B, et al. Amniotic fluid stem cells prevent development of ascites in a neonatal rat model of necrotizing enterocolitis. Eur J Pediatr Surg. 2014;24:57-60.
[132] McCulloh CJ, Olson JK, Wang Y, Vu J, Gartner S, Besner GE. Evaluating the efficacy of different types of stem cells in preserving gut barrier function in necrotizing enterocolitis. J Surg Res. 2017;214:278-85.
[133] McCulloh CJ, Olson JK, Wang Y, Zhou Y, Tengberg NH, Deshpande S, et al. Treatment of experimental necrotizing enterocolitis with stem cell-derived exosomes. J Pediatr Surg. 2018;53:1215-20.
[134] Zhou Y, Besner G. Transplantation of amniotic fluid-derived neural stem cells as a potential novel therapy for Hirschsprung's disease. J Pediatr Surg. 2016;51:87-91.
[135] Li B, Lee C, Chuslip S, Lee D, Biouss G, Wu R, et al. Intestinal epithelial tight junctions and permeability can be rescued through the regulation of endoplasmic reticulum stress by amniotic fluid stem cells during necrotizing enterocolitis. FASEB J. 2021;35:e21265.
[136] O'Connell JS, Lee C, Farhat N, Antounians L, Zani A, Li B, et al. Administration of extracellular vesicles derived from human amniotic fluid stem cells: a new treatment for necrotizing enterocolitis. Pediatr Surg Int. 2021;37:301-9.
[137] Rager TM, Olson JK, Zhou Y, Wang Y, Besner GE. Exosomes secreted from bone marrow-derived mesenchymal stem cells protect the intestines from experimental necrotizing enterocolitis. J Pediatr Surg. 2016;51:942-7.
[138] Chalphin AV, Tracy SA, Kycia I, Chan C, Finkelstein A, Zurakowski D, et al. Donor mesenchymal stem cell kinetics after transamniotic stem cell therapy (TRASCET) in a rodent model of gastroschisis. J Pediatr Surg. 2020;55:482-5.
[139] Chalphin AV, Tracy SA, Lazow SP, Kycia I, Zurakowski D, Fauza DO. A comparison between placental and amniotic mesenchymal stem cells in transamniotic stem cell therapy for experimental gastroschisis. J Pediatr Surg. 2020;55:49-53.
[140] Feng C, Graham CD, Connors JP, Brazzo J, 3rd, Pan AH, Hamilton JR, et al. Transamniotic stem cell therapy (TRASCET) mitigates bowel damage in a model of gastroschisis. J Pediatr Surg. 2016;51:56-61.
[141] Fordham RP, Yui S, Hannan NRF, Soendergaard C, Madgwick A, Schweiger PJ, et al. Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury. Cell stem cell. 2013;13:734-44.

[142] Fukuda M, Mizutani T, Mochizuki W, Matsumoto T, Nozaki K, Sakamaki Y, et al. Small intestinal stem cell identity is maintained with functional Paneth cells in heterotopically grafted epithelium onto the colon. Genes & development. 2014;28:1752-7.
[143] Sugimoto S, Ohta Y, Fujii M, Matano M, Shimokawa M, Nanki K, et al. Reconstruction of the Human Colon Epithelium In Vivo. Cell Stem Cell. 2018;22:171-6 e5.
[144] Yui S, Nakamura T, Sato T, Nemoto Y, Mizutani T, Zheng X, et al. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5(+) stem cell. Nat Med. 2012;18:618-23.
[145] Schwank G, Koo B-K, Sasselli V, Dekkers JF, Heo I, Demircan T, et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell stem cell. 2013;13:653-8.
[146] Cruz-Acuna R, Quiros M, Farkas AE, Dedhia PH, Huang S, Siuda D, et al. Synthetic hydrogels for human intestinal organoid generation and colonic wound repair. Nat Cell Biol. 2017;19:1326-35.
[147] Fattahi F, Steinbeck JA, Kriks S, Tchieu J, Zimmer B, Kishinevsky S, et al. Deriving human ENS lineages for cell therapy and drug discovery in Hirschsprung disease. Nature. 2016;531:105-9.
[148] McCann CJ, Cooper JE, Natarajan D, Jevans B, Burnett LE, Burns AJ, et al. Transplantation of enteric nervous system stem cells rescues nitric oxide synthase deficient mouse colon. Nature Communications. 2017;8:15937.
[149] Stavely R, Bhave S, Ho WLN, Ahmed M, Pan W, Rahman AA, et al. Enteric mesenchymal cells support the growth of postnatal enteric neural stem cells. Stem Cells. 2021.
[150] Cromeens BP, Wang Y, Liu Y, Johnson J, Besner GE. Critical intestinal cells originate from the host in enteroid-derived tissue-engineered intestine. Journal of Surgical Research. 2018;223:155-64.
[151] Sugimoto S, Kobayashi E, Fujii M, Ohta Y, Arai K, Matano M, et al. An organoid-based organ-repurposing approach to treat short bowel syndrome. Nature. 2021.
[152] Kim IG, Wu Y, Park SA, Cho H, Choi JJ, Kwon SK, et al. Tissue-Engineered Esophagus via Bioreactor Cultivation for Circumferential Esophageal Reconstruction. Tissue Eng Part A. 2019;25:1478-92.

[153] Lee SJ, Park C, Lee JY, Kim S, Kwon PJ, Kim W, et al. Generation of pure lymphatic endothelial cells from human pluripotent stem cells and their therapeutic effects on wound repair. Sci Rep. 2015;5:11019.
[154] Gibot L, Galbraith T, Kloos B, Das S, Lacroix DA, Auger FA, et al. Cell-based approach for 3D reconstruction of lymphatic capillaries in vitro reveals distinct functions of HGF and VEGF-C in lymphangiogenesis. Biomaterials. 2016;78:129-39.
[155] Quiros-Tejeira RE, Ament ME, Reyen L, Herzog F, Merjanian M, Olivares-Serrano N, et al. Long-term parenteral nutritional support and intestinal adaptation in children with short bowel syndrome: a 25-year experience. J Pediatr. 2004;145:157-63.