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Reviews

Tissue Engineering of Small Intestine—Current Status

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Short bowel syndrome (SBS) has always posed a great threat to patients and has been one of the biggest challenges for doctors due to its high morbidity and mortality. So far, parenteral nutrition (PN) and small bowel transplantation remain the only viable therapeutic options. However, sepsis and liver failure associated with PN and limited availability of the donor organs and high graft rejection rates associated with transplantation have limited their use to a nonpermanent solution. Clearly, there is a need for an alternative therapy whereby increasing the absorptive surface area would help neonates and adults suffering from permanent intestinal failure. Techniques such as sequential intestinal lengthening are being explored in animal models with little success. Attempts to engineer small intestine since the late 1980s have achieved varying degrees of success in animal models with evolving refinements in biotechnology. The most encouraging results so far have been the generation of intestinal neomucosa in the form of cysts when intestinal epithelial organoid units isolated from neonatal rats were seeded onto biodegradable polymers before implantation in syngeneic adult rats' omentum. Although still experimental, continued attempts worldwide using cultured stem cells and improved polymer technology offer promise to provide an off-the-shelf artificial intestine as a novel therapy for patients with SBS. This article reviews the current status of progress in the field of small intestinal tissue engineering and addresses various types of cell sources and scaffold material having potential to be used in this field.

Introduction

Short Bowel Syndrome (SBS). The Need for Small Intestine Tissue Engineering. Loss of more than 70% small intestine causes SBS, which is characterized by diarrhea, steatorrhea, severe weight loss, malnutrition, and eventually failure to thrive resulting in a high incidence of mortality both in children and in adults. It is a condition of nutritional malabsorption related to the surgical removal or disease of a large portion of the small intestine. In neonates, small intestine measures around 250 cm in length, and it grows to around 750 cm in adults. In adult SBS patients, small bowel length of <100 cm is highly predictive of permanent intestinal failure.[1]

The most common cause for SBS remains extensive surgical resection of the small intestine due to necrotizing enterocolitis in neonates and Crohn's disease in adults.

Improving the nutritional status of the patients by enteral (EN) or parenteral (PN) feeding has been used as a life-saving treatment for patients with intestinal failure.

Parenteral Nutrition. Approximately 600 patients in the U.K. are treated with home parenteral nutrition (HPN) per year.[2] The estimated annual cost in the U.K (1995) for HPN is about £55 000 per patient/year.[3] In most patients with remaining small intestine length of <100 cm, dependence on PN is highly likely. The goal of treatment remains to wean off from this dependence. Most of the neonates with SBS die of sepsis or liver failure secondary to PN. Other complications include electrolyte imbalances, bone metabolic disorders, catheter occlusion,

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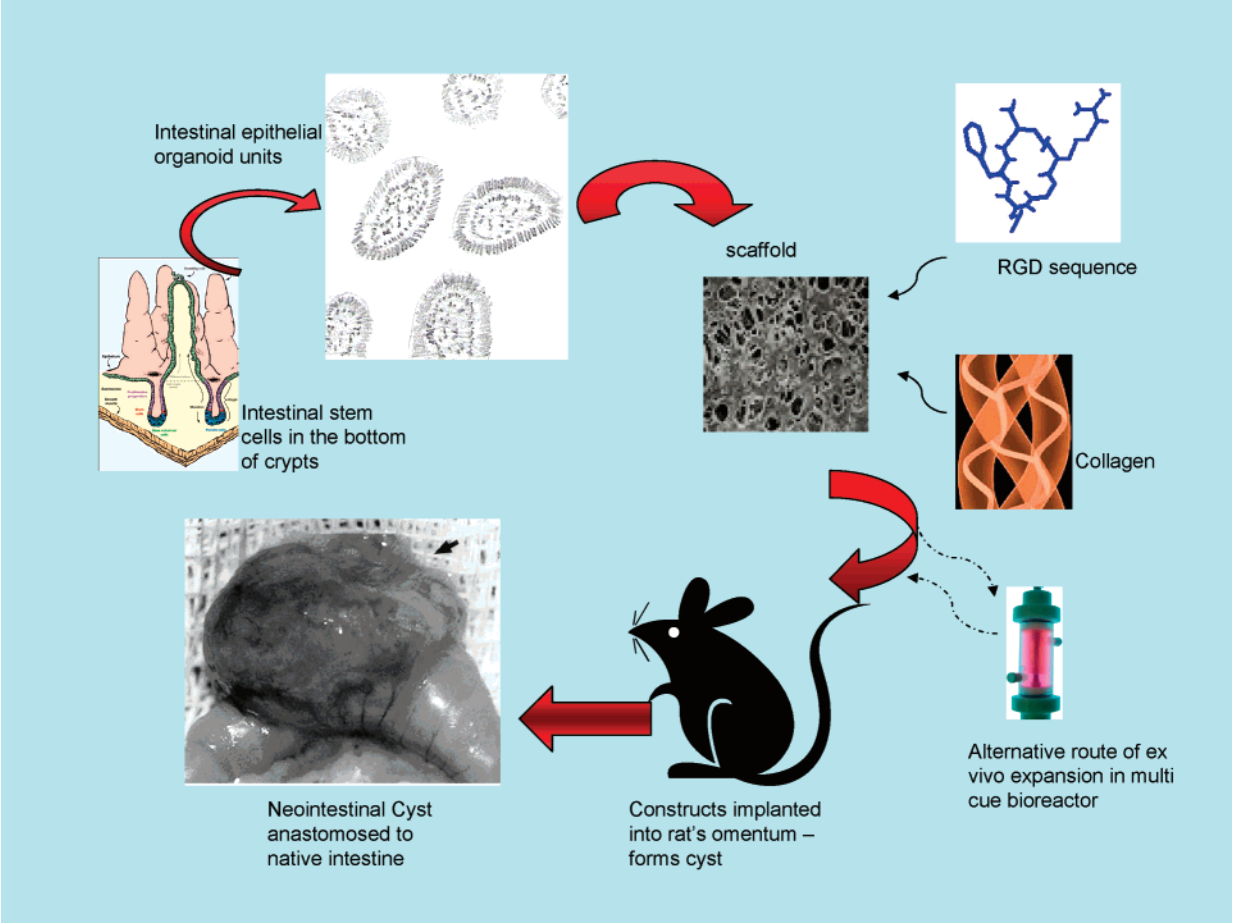

Figure 1. Schematic diagram of overview of small intestinal tissue engineering (partly adapted with permission from ref 25 and 69).

catheter-induced central vein thrombosis, and pulmonary embolism. In a recent cohort study, mortality rates were as high as 37.5% in neonates with SBS who were on PN,[4] and a recent study in Spain showed mortality figures in patients using HPN as high as 50%.[5]

Other methods to treat SBS include surgical procedures such as sequential intestinal loop lengthening. For patients dependent on PN, intestinal transplantation is the last hope in the present medical field.

Sequential Intestinal Lengthening Procedures for SBS. Bianchi's technique has been employed in the past with little success where a small bowel nipple valve is constructed distally to provide temporary partial obstruction and thereby induce dilatation and lengthening of the proximal small intestine.[6] A recent publication by the Safford group describes the first successful application of tension to induce length and growth in rat intestine.[7] A similar procedure was described in 1997, but it lacked measurement of all the parameters of intestinal growth.[8] With the use of an intestinal lengthening device to apply longitudinal mechanical tension, they have demonstrated growth of intestine in the form of increased length by 149%, total weight by 218%, mucosal weight by 122%, and protein mass by 164% as compared to the controlled limb of the bowel. This experimental study has shown promising results but yet needs to be evaluated clinically.

Small Bowel Transplantation. The most promising treatment for SBS, to date, remains intestinal transplantation. As of 1999, 474 cases including isolated intestinal transplant, liver/intestinal transplant, and multivisceral transplant have been performed worldwide, with 1-year graft and patient survival rates of 66% and 54%, respectively.[9] High incidence of rejection, despite the introduction of immunosuppressant Tacrolimus (FK506) in 1989, and limited availability of donor organs are the major restrictions. Overall worldwide survival for isolated small bowel transplantation is around 50% at 5 years and for combined small bowel and liver transplantation 40%.[10] However, another study on 43 patients who received intestinal transplantation showed better long-term results in those who had intestinal and multivisceral transplantation rather than intestinal transplantation alone.[11]

Many patients die while waiting for the intestinal transplant. Another problem is the size of the donor. A donor graft size smaller than the recipient is preferable especially if the recipient has undergone previous abdominal surgery for resection resulting in the contraction of the abdominal cavity.

Tissue Engineering of Small Intestine

Initial attempts to engineer small intestine dates back in 1988, when Vacanti and co-workers[12] used enterocytes to grow intestinal tissue on biodegradable polymers with little success. Tissue engineering principles are based on the utilization of three primary components, namely, the cell, the biomaterial (whether biological or synthetic), and the biomolecules, which serve to integrate and to functionally regulate the behavior of the first two (Figure 1). Cells can be differentiated as progenitor cells, adult or embryonic stem cells, or tissue- or organ-specific cells. They can be autologous, allogenic, or xenogenic.

Cell Source. *From Bone Marrow—Multipotent (Mesenchymal) Stem Cells.* Adult bone marrow contains mesenchymal stem cells (MSCs), which essentially contribute to the regeneration of mesenchymal tissues such as bone, cartilage, muscle,

ligament, tendon, adipose, and stroma.[13] MSCs also have the potential to differentiate into mature cells of various organs such as hepatic oval cells, hepatocytes, cholangiocytes,[14−16] skeletal muscle cells,[17] astrocytes, neurons,[18] and even renal tubular epithelial cells.[19] To determine whether circulating stem cells have a similar potential, Korbling et al. concluded from a study on the biopsy specimen from liver, skin, and gastrointestinal tract from patients with leukemia who had undergone transplantation of hematopoietic stem cells from peripheral blood or bone marrow that circulating stem cells can differentiate into mature hepatocytes and epithelial cells of the skin and gastrointestinal tract.[20] Krause et al.[21] demonstrated a quantitative analysis of the donor engraftment of nonhematopoietic tissues eleven months post-transplant of single bone marrow stem cell in mice. In addition to engraftment of columnar epithelial cells in the small bowel, donor-derived epithelial cells were identified throughout much of the gastrointestinal (GI) tract, including the lining of the esophagus, stomach, and large bowel.

In the human bone marrow, the sialomucin CD34 is a hematopoietic cell surface antigen that has been extensively exploited for the selection of long-term repopulating cells with multilineage potential, though not all HSCs express this marker. It has been demonstrated that bone marrow cells could differentiate into human GI tract epithelia and that the increase of these bone marrow derived cells was closely related to the recovery from epithelial damage.[22] They were the first to conclude that cells derived from transplanted bone marrow could "repopulate" every part of the human GI tract epithelia and contribute to the regeneration of damaged epithelial tissues.

A study on rodent bone marrow has indicated that certain mesenchymal stem cells—termed multipotent adult progenitor cells (MAPCs)—differentiates, at the single cell level, not only into mesenchymal cells but also cells with visceral mesoderm, neuroectoderm, and endoderm characteristics in vitro.[23] When injected into an early blastocyst, single MAPCs contribute to most, if not all, somatic cell types. On transplantation into a nonirradiated host, MAPCs engraft and differentiate to the haematopoietic lineage, in addition to the epithelium of liver, lung, and gut, depending on the environmental cues provided by different organs. This technique of expansion of MAPCs in vitro and, when transplanted, differentiation in vivo into cells of different lineages can have the potential to be used in tissue engineering of small intestine in the future.

From Peripheral Blood. Zhao Y et al. have identified, cultured, characterized, and propagated adult pluripotent stem cells (PSCs) in vitro from a subset of human peripheral blood monocytes. They induced these cells to differentiate into mature macrophages by lipopolysaccharide, T lymphocytes by IL-2, epithelial cells by epidermal growth factor, endothelial cells by vascular endothelial cell growth factor, neuronal cells by nerve growth factor, and liver cells by hepatocyte growth factor. The pluripotent nature of these individual PSCs was further confirmed by a clonal analysis.[24]

Gastrointestinal Stem Cells (Tissue-Specific Cells). Intestinal stem cells are believed to reside in the base of the crypts of Lieberkuhn in the small intestine. Putative stem cells reside immediately above the Paneth cells near the crypt bottom. Proliferating progenitor cells occupy the remainder of the crypt. Differentiated cells populate the villus and include goblet cells, enterocytes, and entero-endocrine cells[27] (Figure 2). Little is known of the location and fate of the stem cells within the gastrointestinal tract, due to the lack of distinct stem cell markers, but they are usually said to appear histologically primitive and can be identified functionally by their ability to repopulate crypts and glands after damage.[26] There are no reliable specific means to identify these stem cells. Musashi 1, a neural stem cell marker, is expressed in immature cells in the lower crypt and may prove useful as an intestinal stem cell marker.[27]

Another priority should be the development of robust in vitro culture systems for the intestinal epithelium.[28] A reproducible method for growing small intestinal epithelium (from suckling rat intestine) in short-term cultures has been established.[29] Isolation of the epithelia and, significantly, preservation of its three-dimensional (3D) integrity was achieved using a collagenase/Dispase digestion technique. Weiser described his method where he dissociated cells using citrate and obtained sequential fractions of epithelial cells in a villus-to-crypt gradient by a series of incubations and washings of gut loops avoiding overmanipulation.[30]

Intestinal Epithelial Organoid Units. After the attempted use of enterocytes, Vacanti and group moved on to use intestinal epithelial organoid units as the cell source to be seeded onto synthetic biodegradable polymer scaffolds and achieved considerable success in regenerating neointestinal tissue. Their laboratory was the first to report in 1997 making tissue-engineered small intestine by the transplantation of organoid units on a polymer scaffold into the omentum of the Lewis rat.[31] These intestinal epithelial organoid units were isolated from neonatal rats as per the method first developed in 1992.[29] Intestinal epithelial organoid units are multicellular units derived from neonatal rat intestine, containing a mesenchymal core surrounded by a polarized intestinal epithelium, and contain all of the cells of a full-thickness intestinal section.[29,30] A majority of researchers have adopted this methodology to isolate intestinal stem cells.

Scaffold (Matrix) Used for Tissue Engineering Intestine. The material may be natural, like collagen or fibrin, or synthetic, like polyglycolic acid (PGA). The matrix can be fibrous, foam, capsules, gels, or highly complex structures. An ideal scaffold should be 3D, highly porous, and biocompatible with a controlled degradation rate; should have an appropriate surface for cell adhesion, proliferation, and differentiation; and should maintain proper mechanical properties. An ideal tissue replacement would be designed to replace a specific tissue. This tissue-engineered scaffold would have the same mechanical properties as the natural tissue, and it would serve as a scaffold for tissue regeneration. The optimal scaffold/tissue response for a biodegradable scaffold is shown in Figure 3. Initially, the scaffold withstands the majority of the stress. As the scaffold begins to degrade and tissue ingrowth occurs, the newly regenerating cells are gradually loaded with physiological stress, further simulating tissue regeneration. Eventually, the scaffold completely degrades, and the regenerated tissue bears the stress.

Natural Scaffold. Naturally occurring scaffold materials include small intestinal submucosa, acellular dermis, amniotic membrane tissue, cadaveric fascia, and the bladder acellular matrix graft. These naturally occurring scaffolds can be processed in such a way as to retain growth factors, such as basic fibroblast growth factor (FGF-2) and transforming growth factor-β (TGF-β),[32] glycosaminoglycans, such as heparin and dermatan sulfate, and structural elements, such as fibronectin, elastin, and collagen.[33] These materials prevent many of the complications associated with foreign material implants, because they provide a natural environment onto which cells can attach and migrate and within which they can proliferate and differentiate.[33]

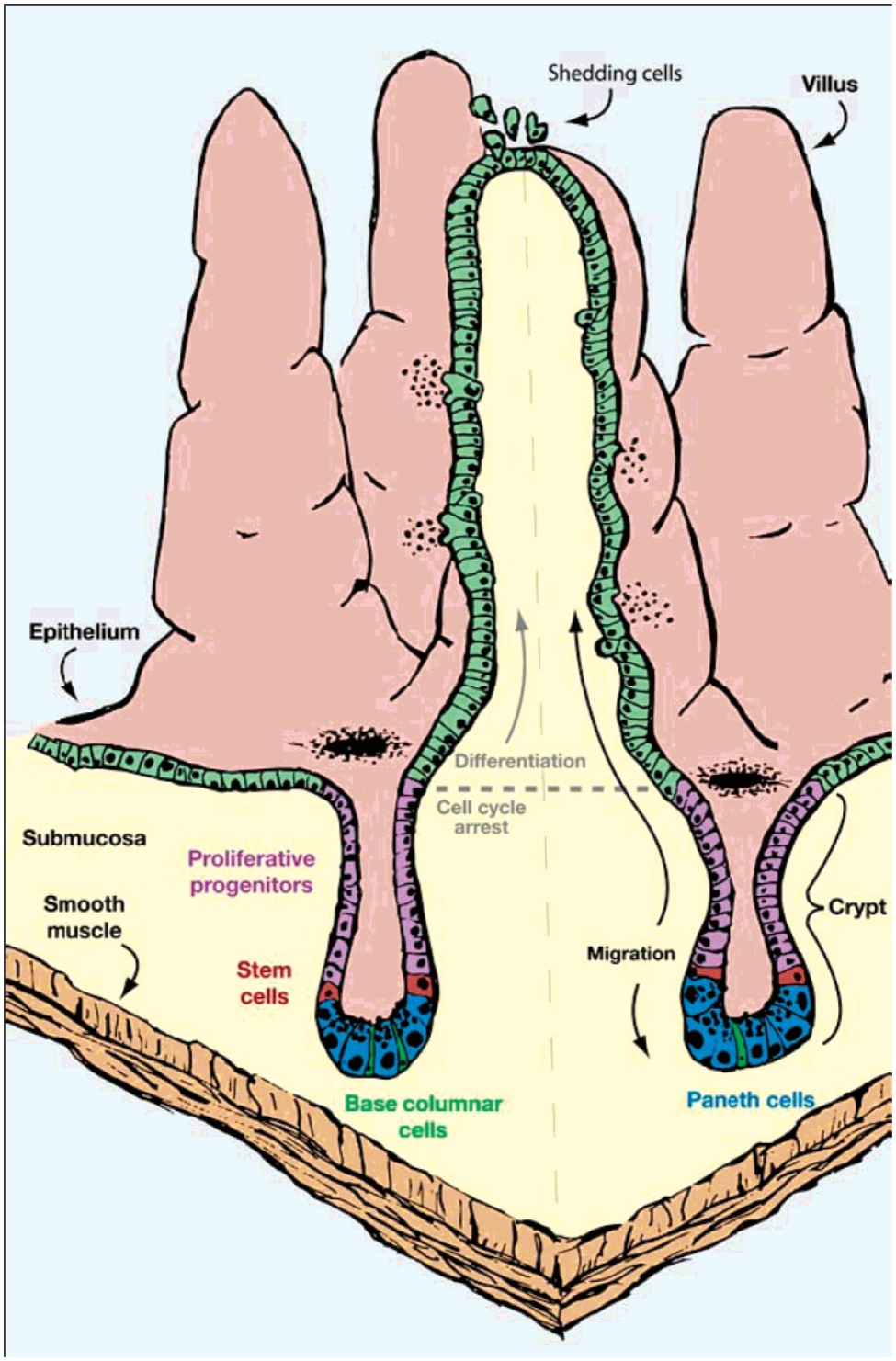

Figure 2. Putative stem cells reside immediately above the Paneth cells near the crypt bottom. Proliferating progenitor cells occupy the remainder of the crypt. Differentiated cells populate the villus and include goblet cells, enterocytes, and entero-endocrine cells (reprinted, with permission, from the Annual Review of Cell and Developmental Biology, Volume 20, copyright 2004 by Annual Reviews www.annualreviews.org[25]).

Small Intestinal Submucosa (SIS). Small intestinal submucosa (SIS) is a naturally occurring, acellular xenogenic biomaterial that has been used in many studies as a scaffold for small bowel tissue engineering.[34−36] It is mainly derived from porcine small intestine, but other mammals such as rats and dogs have also been used. When SIS is implanted as a naturally occurring biopolymer scaffold, it stimulates angiogenesis, connective and epithelial tissue growth and differentiation, as well as deposition, organization, and maturation of ECM components that are functionally and histologically appropriate to the site of implantation.[37] SIS comprises primarily fibrillar collagens and adhesive glycoproteins which serve as a scaffold onto which cells can migrate and multiply.

Collagen Scaffold. There has been only one study reported on tissue engineering of small intestine on dogs when they used "acellular" collagen sponge scaffold grafting with a silicon tube stent.[38] The length of the graft they used was 5 cm long. We cannot however say with confidence whether similar migration of the adjacent cells would occur to regenerate tissue if much longer segments of the small intestine are resected and replaced

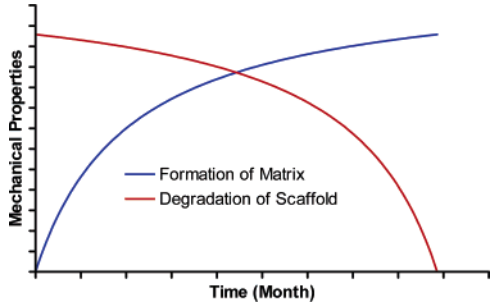

Figure 3. Optimal relationship between matrix formation and scaffold degradation.

with acellular sponge scaffolds. Also, a proper muscle layer essential for functional peristalsis was not observed.

Synthetic scaffold. Vacanti and colleagues have pioneered the arena of small intestinal tissue engineering by using a highly porous, synthetic, biodegradable polymer tubes fabricated from nonwoven sheets of polyglycolic acid (PGA) fibers with a fiber diameter of 5 μm, mesh thickness of 2 mm, bulk density of 60 mg/cm^3, porosity of 95%, and mean pore size of 250 μm. Mooney et al. in their study on biomaterials have emphasized the role of poly(L-lactic acid) (PLLA) and a 50/50 copolymer poly(D,L-lactic-*co*-glycolic acid) (PLGA) in stabilizing PGA meshes to form 3D structures such as tubes when sprayed over PGA meshes.[39] They showed that PGA meshes when sprayed with PLLA or PLGA were capable of withstanding large compressive forces in vitro (50−200 mN) and maintained their structure in vivo when implanted into the rat omentum.

In majority, biodegradable polymer has been the choice of scaffold. Although biodegradable polymer has been generally accepted as the most useful scaffold material, the use of non-biodegradable scaffold in organ building like small intestine may prove vital for its stability.

Organoid Unit Polymer Constructs Implantation. Many studies by Vacanti laboratory in Boston, MA, have shown successful formation of cysts between 1 and 8 weeks postimplantation of the organoid unit polymer constructs.

Vacanti and his group have studied parameters such as angiogenesis, lyphangiogenesis, immunology, mucosal morphology, and enterocyte dynamics. They have also studied the effects of anastomosis of tissue-engineered small intestine (TESI) to the native bowel with and without massive small bowel resection on neointestinal regeneration and weight gain. They have demonstrated that massive small bowel resection contributes significant regenerative stimuli for the heterotropically transplanted tissue-engineered intestine.[41,42] They have also demonstrated that anastomosis between the TESI and native small bowel contributes significant trophic effects on neomucosal morphogenesis (Table 1).

Need for Trophic Factors after Massive Bowel Resection. Ray et al. have studied the enterocyte nutrient transport after massive bowel resection when the intestine is in adaptation. They concluded from their study that parenteral growth hormone (GH) and epidermal growth factor (EGF), when given in combination for 2 weeks immediately after massive enterectomy, synergistically enhance Na^+-dependent glutamine (GLN) uptake. This study emphasizes the importance of the need of the growth factors to be considered after implantation of tissue-engineered small intestine.[43]

Surface Modification of Scaffolds. Most of the biodegradable synthetic polymers (like PGA and PLA) are hydrophobic and may require modifications on their surface for better cell attachment. This can be achieved by surface coating, chemical coating, plasma treatment, or modifying the fabrication of scaffold itself.[44] A rough, rather than smooth, fiber surface is favorable for cell attachment. Nanopatterning of biomaterial surfaces has been used as a surface modification strategy to enhance protein activities, cellular functions, and tissue responses. Coating the surface with extracellular matrix (ECM) proteins such as fibronectin, vitronectin, and collagen provides an adhesive interface between the polymer scaffold surface and cells that resemble the native cellular milieu. It is one of the simplest surface modification methods. The cell-binding domain of fibronectin, vetronectin, and collagen contains the tripeptide RGD (Arg-Gly-Asp).[44,45] Stem cells from adipose tissue are found to attach better to scaffolds coated with peptide sequences derived from ECM laminin.[46,47]

Examples of other coating methods include chitosan nanoscaffolds modified with a sugar unit,[48] electrostatic coating of hyaluronic acid and chitosan onto polymeric scaffolds making them protein-resistant,[49,50] and coating of IGF 1 (insulin-like growth factor) to collagen scaffolds showing increased osteoblast proliferation.[51]

In one of the studies investigating tissue engineering of the esophagus, a biodegradable and flexible poly(L-lactide-*co*-caprolactone) (PLLC) copolymer was surface modified using aminolysis by 1,6-hexanediamine to introduce free amino groups. With these amino groups used as bridges, fibronectin and collagen were subsequently bonded with glutaraldehyde as a coupling agent. The protein-bonded surface presented as being more hydrophilic and homogeneous. In vitro long-term (12 d) culture of porcine esophageal cells proved that the fibronectin- and collagen-modified PLLC surfaces could more effectively support the growth of smooth muscle cells and epithelial cells[51,52]

Self-assembling peptide hydrogel scaffolds have been shown in many studies to effectively promote cell−material and cell−cell reactions enabling significant improvements in generating bone,[53] cartilage,[54] cardiovascular,[55] and neural tissues.[56] These are basically amphiphilic peptides that have alternating repeat units of positively charged lysine or arginine and negatively charged aspartate and glutamate residues. These peptides contain 50% charged residues and are characterized by their periodic repeats of alternating ionic hydrophilic and hydrophobic amino acids; thus, the interaction between the distinct polar and nonpolar surfaces facilitates self-assembly of the material into a nanofiber hydrogel scaffold which can coat surfaces or encapsulate cells as a 3D weak gel.[54,57,58]

In the field of small intestinal tissue engineering, most studies have used synthetic polymers, commonly PGA,[41,42,59−62] and some have used SIS[34−36] or collagen sponge.[38] In the majority of studies using PGA, the polymer was coated with 0.1−1% collagen type I. As shown in one of the studies, a significant improvement was seen in cell engraftment, and larger cysts were formed when collagen was coated (92.9% vs 63.6%).[63] SIS due to its richness in collagen has obviously an advantage over polymers to be used as a scaffold as far as cell attachment is concerned. All studies investigating small intestinal tissue engineering have used in vivo implantation of cell−polymer constructs. How intestinal epithelial units can be expanded ex vivo prior to their implantation is not clear and not published so far. All studies incorporating the use of intestinal epithelial units as the cell source have used only PGA polymers with or without PLLA. It is also not clear whether the use of other synthetic polymers or natural polymers in a similar setup would have resulted in different results. Regulation of epithelial cell proliferation, migration, and differentiation under physiological conditions is poorly understood. A better understanding of how

Table 1. Summary of in Vivo Assessment of Small Bowel Tissue Engineering[a]

first author, year	cell source	scaffold	animal model	methodology	outcome	pitfalls
Vacant, 1988[12]	isolated enterocytes	Polyglactin 910, polyanhydrides, polyorthoester	rats	cell preparations cultured on biodegradable polymers prior to implantation into rat omentum and mesentery	3/23 implantations successful (max 6 mm cyst)	preliminary study
Organ, 1992[70]	EC	PGA	rats	EC-polymer constructs in omentum, mesentery, or subcutaneously for 2 weeks	engraftment in omental and mesenteric but not subcutaneous; stratified endothelium seen in one only.	preliminary study using EC; only mucosal cells regenerated
Organ, 1993[71]	isolated enterocytes	PGA	rats	as above compared with animals who had 75% SBR	engraftment lower in SBR than non-resected group; constructs vascularized and viable over a 1-month period	lower engraftment in SBR group
Choi, 1998[63]	organoid units	PGA with PLLA +/− collagen	rats	studied brush border enzymes, basement membrane components, and electrophysiology of TESI	maturation of the neointestine (2−6 weeks); increased villi and crypts, more mature columnar epithelium and epithelial cells with collagen	good results but requires long-term studies
Kim, 1999[42]	organoid units	PLLA-coated PGA	rats	small bowel resection, partial hepatectomy, and portacaval shunt compared	neointestinal cyst size was significantly greater in SBR group	absorption and anastomosis to native bowel not tested
Kim, 1999[69]	organoid units	PLLA-coated PGA	rats	effects of anastomosis of TESI to native small bowel	anastomosis contributed significant regenerative stimuli for morphogenesis and differentiation of TESI	good results but require long-term studies
Kaihara et al.[72]	organoid units	PLLA-coated PGA	rats	end-to-end anastomosis of TESI and native small bowel	patency rate of 78% with a trophic effect on cyst size and neomucosa	lower patency than side-to-side (90%) with higher mortality
Chen, 2001[35]	none	porcine SIS	dogs	defect on small bowel repaired with a SIS patch, or resection interposed with tubular SIS	patch regenerated mucosal epithelial layer, some smooth muscle; tubular group died due to leakage	layers not well-organized and not feasible in longer resected segments
Perez, 2002[73]	prganoid units	PLLA-coated PGA	rats	studied the immune system of TESI	TESI developed an immune cells; dependent on exposure to the luminal stimuli and duration	no evidence for source of cells (donor or host)
Hori, 2002[38]	autologous MSCs	collagen sponge	dogs	MSC seeded scaffold around silicone stent	mucosal layer was created	failed to generate muscle layer
Demirbilek, 2003[34]	none	porcine SIS	rabbits	SIS patch on 6 cm jejunal defect	well-organized mucosa and submucosa in 6 weeks	some graft contraction; anastomosis not tested
Gardner-Thorpe, 2003[59]	organoid units	PLLA-coated PGA	rats	angiogenesis in TESI	VEGF and bFGF delivery may prove useful for TESI	further studies required to elucidate mechanism
Ramasanahie, 2003[61]	organoid units	PLLA-coated PGA	rats	effects of GLP-2 on mucosal morphology and SGLT1 expression	GLP-2 increased villus height and crypt depth and enhanced SGLT1 expression	further studies required to elucidate mechanism
Tavakkolizadeh, 2003[62]	organoid units	PLLA-coated PGA	rats	impact of luminal contents on epithelium, morphology, cellular dynamics and nutrient transporter tested	anastomosed neomucosa regenerated the SGLT1 mRNA expression topography of the native jejunum and epithelial proliferation higher	mechanism not studied
Wang, 2003[36]		rat SIS	rats	tubular SIS (2-cm) with bilateral anastomosis in isolated ileal loop	mucosa showed smooth muscle and serosa (24 weeks); SIS biocompatible, resistant to infection, induced neovascularisation, and remodeled	small length of the tubular segment; no re-ennervation
Grikscheit, 2004[60]	organoid units	PLLA-coated PGA	rats	studied the effect of TESI anastomosis on recovery after MSBR	TESI anastomosis (side-to-side) improved postoperative weight and B12 absorption after MSBR	Villin mRNA and IAP levels suggestive of differentiation were found to be in contrast
Duxbury, 2004[74]	organoid units	PLLA-coated PGA	rats	studied lymphangiogenesis in TESI	lymphangiogenesis in submucosa and lamina propria by 8 weeks, stain positive for VEGFR-3	further studies required to elucidate mechanism

[a] Key: MSC, mesenchymal stem cell; EC, enterocytes; PGA, poly(glycolic acid); PLLA, poly(L-lactic acid); SIS, small intestine submucosa; SBR, small bowel resection; MSBR, massive SBR; TESI, tissue-engineered small intestine; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; GLP-2, glucagon-like peptide; SGLT1-Na^+, glucose cotransporter; IAP, intestinal alkaline phosphatise; VEGFR, vascular endothelial growth factor receptor.

the intestinal epithelial cells interact with their underlying basement membrane is required. A likely mechanism of this interaction as suggested by Beaulieu is through integrins, a specific subset of cell surface-binding proteins.[64] The epithelial basement membrane (BM) of the human intestine contains all major components specific to most BMs such as type IV collagens, laminins, and proteoglycans. However, direct cause−effect relationships between particular integrins and specific cell functions and the signaling molecules specifically involved need to be ascertained.[65]

Discussion

Stem cells have played a pivotal role in tissue engineering of small intestine. Unlike the MSCs and HSCs, intestinal stem cells almost guarantee the regeneration of all types of intestinal cells (absorptive-columnar, goblet, Paneth, and autoendocrine cells), including muscle cells. Although HSCs from the peripheral blood have been shown to engraft in the gut lining when leukemia patients received transplantation of hematopoietic stem cells from peripheral blood, the exact mechanism of origin and physiological role of these cells is unknown.[66] A low-level engraftment was seen as individual scattered cells. These randomly inserted single cells may not be fully functional, since they did not appear to proliferate. Although little is known about how these cells obtain the degree of differentiative potential, tissue injury, local environmental signals, and recombinant human granulocyte colony-stimulating factor may have a significant role in the above process.[20,21]

Adult bone marrow stem cells have many characteristics which make them suitable for their potential use in tissue engineering of small intestine; for example, they can replicate as undifferentiated cells that have the potential to differentiate into lineages of mesenchymal tissues, they display a stable phenotype, they are immunocompatible, and they have no ethical issues. However, there are a number of technical obstacles, such as how to isolate stem cell preparations without contamination by other cells, how to control the permanent differentiation to the desired cell types, and how to increase the production of the large number of cells needed to create tissues.[67]

Hori et al. in 2001 did succeed in regenerating intestinal tissue on the luminal surface of the collagen scaffold seeded by autologous MSCs which they applied to replace a 5 cm defect in the dog jejunum. However, they failed to generate a muscle layer, which is essential for functional peristalsis. Presence of mucosal layer in their study appears to come from migration of epithelial cells from the surrounding healthy intestinal mucosa. However, the mechanism of cell migration to generate a mucosal layer seems highly impractical where a much longer intestinal scaffold is required to replace small intestine with an aim to treat short bowel syndrome. As suggested by them, the failure of muscle layer regeneration might be due to various reasons. First, the number of seeded cells could be inadequate or expansion of MSCs on the scaffold ex vivo might be required before implantation; a second possibility is that preconditioning of MSCs by some additional directional stimulus for differentiation into muscle might be necessary before implantation. Hence, as suggested by them, advancement in the use of trophic factors with the MSCs such as basic fibroblast growth factor or vascular endothelial growth factor may be effective in regenerating highly differentiated and organized intestinal tissue including muscle layers.

It has been successfully demonstrated that fibroblasts such as macrophages (f-Mφ) derived from monocyte-rich samples of peripheral blood to show their ability to mature into epithelial cells when treated with epidermal growth factor (EGF) and cells of other lineages when exposed to the respective growth factors.[24] These cells have a potential for tissue repair and regeneration, but their use in small intestinal tissue engineering has not been attempted so far. Can these PSCs be used in vitro to seed the polymer*;* will it generate all the layers of bowel wall; are there enough and appropriate growth factors available? These are some of the questions which remain unanswered. More knowledge about growth factors, cell markers, optimum culture medium, and their exact function is required before they can be evaluated in tissue engineering of small intestine.

At present, intestinal stem cells remain the only valid option as the cell source because of their ability to differentiate in all types of intestinal cells. Isolation of pure stem cells from intestinal crypts poses a great difficulty due to the lack of specific stem cell markers and also difficulty in physically retrieving them from their niche where they are interspersed between their numerous differentiated Paneth cell daughters.[68] The laser capture microdissection technique has been used with little success to harvest these cells.

Use of intestinal epithelial organoid units by Vacanti and his group since 1997 has proven to be a successful method in providing the cell source. These units of <1 mm^3 pieces of minced neonatal rat intestine are prepared by enzymatic digestion (as described earlier in this article) containing all of the cells of a full-thickness intestinal section. About 40 000 organoid units are harvested from one complete small intestine of a neonatal rat. The number of organoid units required to form a cell−polymer construct is also in the range 30 000−50 000 organoid units. Although technically promising, the practicality of acquiring cells from one complete small intestine, sufficient enough to form only one construct, seems daunting. A method whereby these cells can be expanded in vitro before implantation would be a real asset to the research.

The polymer used in all studies by Vacanti and his group has been the biodegradable polymer poly(glycolic acid) (PGA). This has been coated with 0.1−1% collagen type I, facilitating better cell engraftment and larger cyst size. Surface coating with other agents such as fibronectin, and so forth, still remains to be tested. Others have used porcine small intestine submucosa (SIS) as a scaffold. No one has evaluated intestinal tissue generation on non-biodegradable polymers so far. Biodegradable polymers are believed to give room to the growing intestine beyond its original size when implanted and should have less likelihood of foreign body reaction and extrusion in the long term. On the other hand, non-biodegradable polymers should give better stability, but the exact mechanism by which it would allow the growing cells to orient themselves in a layered fashion remains unpredictable.

Regulation of epithelial cell proliferation, migration, and differentiation under physiological conditions is poorly understood. A better understanding of how the intestinal epithelial cells interact with their underlying basement membrane is required. A likely mechanism of this interaction as suggested by Beaulieu is through integrins, a specific subset of cell surface-binding proteins.[64] Peptides when coated with the polymers can be helpful in cell attachment.

Apparently, much work is needed in the field of small intestine tissue engineering. A better understanding of cell−cell and cell−ECM interactions, specific stem cell markers, and trophic growth factors is required. Advancements in developing larger absorptive surface area of the TESI with formation of a better muscular layer would prove to be a very successful step forward.

The Future

The tissue engineering of small bowel remains at an early stage. The absorptive capacity of TESI is yet to be adequately tested, and current methods for cell isolation would provide insufficient cells for clinical applications. The use of organoid units suggests that mucosal stem cells are useful in the generation of mature mucosa in TESI. Studies on rats have shown that large numbers of such units are needed to produce short lengths of TE construct. The isolation of the stem cells from these units or the use of stem cells from other sources, such as the MSC from the bone marrow, may provide a clinical

alternative to the organoid units. In vitro culture of these cells is likely to be necessary to obtain clinically useful numbers of such cells.

The use of cultured stem cells and improved polymer technology provide the most likely future for TESI. Peptides other than collagen like fibronectin or self-assembling hydrogels bound to the polymers have been shown to be useful in bone, cartlage, and cardiovascular tissue engineering and provide further hope for TESI.

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