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A new era: endoscopic tissue transplantation

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Abstract

Purpose of review—To describe basic principles of tissue engineering with emphasis on the potential role of gastrointestinal endoscopy in regenerative medicine.

Recent findings—Stricture associated with endoscopic submucosal resection and circumferential endoscopic mucosal resection can be prevented through transplantation of autologous epidermal cell sheets or seeded decellularized biological scaffolds. Lower esophageal sphincter augmentation through injection of muscle-derived cells is a novel potential treatment for gastroesophageal reflux disease. Stem cell derived tissue has been used to repair injured colon in a mouse model of colitis. A bioengineered internal anal sphincter has been successfully implanted in mice and showed preserved functionality.

Summary—The immediate foreseeable application of tissue engineering in gastrointestinal endoscopy is in the field of mucosal repair after acute injury. Tissue regeneration can be achieved through expansion of autologous somatic cells or by induction of multipotent or pluripotent stem cells. Advances in cellular scaffolding have made bioengineering of complex tissues a reality. Tissue engineering in endoscopy is also being pioneered by studies looking at enteral sphincter augmentation and regeneration. The availability of engineered tissue for endoscopic application will increase with advances in cell-culturing techniques.

Keywords

cellular scaffolds; gastrointestinal endoscopy; regenerative medicine; stem cells; tissue engineering

INTRODUCTION

Recent advances in the field of tissue engineering and regenerative medicine make the possibility of replacing complex tissue and whole organs a likely reality in the future. In gastroenterology, seminal studies have shown successful efforts in liver tissue transplantation and functional reconstruction of the esophagus [1,2]. Luminal endoscopy is well suited for the repair of damaged gastrointestinal epithelium. The feasibility of endoscopic tissue transplantation to patch mucosal defects associated with submucosal dissection has been shown in preliminary studies [3]. The field of tissue engineering in endoscopy is also being pioneered by studies on enteral sphincter augmentation through injection of smooth muscle cells for treatment of gastroesophageal reflux disease [4].

Advances in three-dimensional cellular culture techniques show promise in tissue regeneration and transplantation of intestinal epithelium in mice with potential clinical application in humans [5]. This review will describe basic principles of tissue engineering with emphasis on the potential application of regenerative medicine in gastrointestinal endoscopy.

THE INTESTINAL EPITHELIUM

The intestinal epithelium has a complex organotypic architecture composed of a heterogeneous cell population. Its function within the digestive system varies depending on its cellular composition and organization. Under physiological conditions, the integrity of the intestinal epithelium depends on mucosal proliferation and renewal, made possible by a small population of multipotent stem cells. The small intestine mucosa is structurally complex and has a cellular high turnover rate. The location of cells within the crypts and villi in the small intestine defines their function. Epithelial cells produced in the lower part of the crypt migrate up to the crypt before being shed into the intestinal lumen. In this process, cells differentiate into various cell types with unique functions [6]. Colonic epithelium is similar in architecture to the small bowel but differs in cellular contents and kinetics. Unlike intestinal epithelium, the esophagus is lined with stratified squamous mucosa that is much simpler in terms of cellular organization. It is apparent that the intricacy of the tissue organization in the gastrointestinal tract is related to the complexity of its function.

PRINCIPLES OF TISSUE ENGINEERING

The basic principle of tissue engineering is to generate tissue in an environment that physiologically mimics that of the organ of origin. Tissue regeneration can be achieved through expansion of autologous somatic cells or by induction of multipotent or pluripotent stem cells to differentiate into a desired lineage [7]. Viable tissue is associated with an extracellular matrix that provides the three-dimensional support necessary for mechanical and biological cellular interactions. In bioengineering of tissue, a three-dimensional cellular scaffold is used to replace the extracellular matrix and then seeded with cells in enriched media. Several cell types are often cultured in conjunction to represent the natural cell population.

Stem cells

Stem cells are unique in their capacity to differentiate into various cell lineages while self-maintaining their own population [7]. Initial studies in tissue engineering used human embryonic stem cells; however, their application has fallen out of favor due to safety and ethical concerns. An alternative approach is to obtain adult stem cells from tissue for in-vivo expansion and differentiation. This approach, however, faces several challenges including identification of stem cells in tissue and adequate culturing techniques to ensure appropriate differentiation and organization. The discovery of the stem cell marker Lgr5 (leucine-rich-repeat-containing G-protein-coupled receptor 5) has helped to identify the location of adult quiescent stem cells in the gastrointestinal tract that have been shown to be precursors for intestinal gland cells [8]. It is now widely accepted that stem cells are located close to the crypt base in the small intestine and at the crypt base in the colon [6]. Sato *et al.* have shown that single Lgr5 positive stem cells from small intestine, stomach and colon of mice can be harvested and expanded *in vitro* into organoids that self-organize into architecture reminiscent of in-vivo histology although continuing to self-replicate [9,10]. Yet another alternative is to use a pluripotent stem cell to direct differentiation into intestinal tissue. This has been successfully achieved by Spence *et al.* [11] who describe the generation of human

intestinal tissue with a three-dimensional architecture and cellular composition similar to fetal intestine.

Cellular scaffolds

A cellular scaffold provides a three-dimensional structure for the growth of cells that mimics physiological cellular interactions and allows diffusion of oxygen and nutrients [7]. Several natural and synthetic materials have been used to produce cellular scaffolds. Biological materials such as collagen, fibrin and various types of glycosaminoglycans are commonly used but carry the risk of immunogenicity and infection. Synthetic materials include polyethylene glycol, polyglycolic acid and polylactic acid. The advantage of these biocompatible materials is that they can be molded into a desired shape and can be industrially produced. Another source of cellular scaffolds is decellularized natural tissue. Tissue explants are rinsed in detergent to remove cells and cellular byproducts creating a residual matrix that is then seeded with cultured cells [12,13].

Initial studies using cellular scaffolds consisted of simply seeding the material with enterocytes [14]. Although appropriate cellular growth was observed, the cells did not appear to form villous structures. More recent studies have relied on concomitantly establishing multiple cell lines. In one study, Human Caco-2 cells (colonic adenocarcinoma) cocultured with human microvascular endothelial cells on decellularized porcine intestine produced enterocytes with epithelial properties that model native enterocytes [15]. Another study used human colonic fibroblasts cocultured with human umbilical vein endothelial cells in an enriched collagen matrix that produced villus-like structures with brush borders. It appears that there is substantial influence of various cells types originating from the same tissue that facilitates structural growth in the correct polarity required for proper anatomic organization. The growth of esophageal cell lines has also been tested on various scaffolding materials including a human esophageal matrix, porcine matrix and human dermal matrix. A study that compared these three cellular scaffolds concluded that human esophageal squamous cells seeded onto a porcine esophageal matrix closely resembled human esophageal epithelium [16].

THE ROLE OF TISSUE ENGINEERING IN ENDOSCOPY

The immediate foreseeable application of tissue engineering in gastrointestinal endoscopy is in the field of mucosal repair. Endoscopic therapy of early gastrointestinal cancers of the esophagus with wide spread circumferential mucosal resection and submucosal dissection can leave large mucosal defects that on healing can form fibrosis and stricturing [17]. Recent studies show that postresection strictures can be prevented with endoscopic transplantation of autologous epidermal cell sheets. Ohki and coworkers [3, 18] describe a technique in which epithelial cells extracted from oral buccal mucosa were seeded onto temperature-responsive culture inserts and transplanted as cell sheets into esophageal ulcerations immediately following submucosal dissection (Fig. 1). The epidermal cell sheets were endoscopically delivered inside an endoscopic resection cap and positioned over the site of ulceration using endoscopic forceps without the use of a tissue adhesive. The investigators report complete re-epithelization within a median time of 3–5 weeks with no associated stricturing. The use of a tubular scaffold made of decellularized porcine urinary bladder has also been reported following circumferential mucosal resection in a canine model [19]. The scaffold was delivered over a 30-mm achalasia balloon under endoscopic guidance and secured in place with surgical adhesive (Fig. 2). The investigators report adequate remodeling of esophageal tissue without stricture formation at 8 weeks.

Cell sheets and scaffolds are useful in repairing localized epithelial damage but have a limited role in repairing injury involving large surface areas such as the mucosal damage

associated with inflammatory bowel disease. The organotypic complexity of small bowel and colon epithelium also make tissue transplantation challenging. In a remarkable study, Yui *et al.* [5] demonstrated how single stem cells from the mouse colon can be expanded *in vitro* to form three-dimensional structures termed organoids that resemble in-vivo histology. Using a mouse model of colitis, they further demonstrate that transplanted organoids colonize areas of injured colon tissue and form functional epithelium. Organoid engraftment remained stable for more than 6 months and showed evidence of mucosal proliferation and renewal.

Tissue engineering is also evolving in the treatment of enteral sphincter dysfunction. Gastroesophageal reflux can be associated with an incompetent lower esophageal sphincter. Sphincter augmentation techniques have been reported with variable degrees of clinical success in the treatment of reflux. A study examining the endoscopic injection of skeletal muscle-derived cells into the lower esophageal sphincter of a canine model reported significant augmentation of baseline sphincter pressure [4]. The investigators extracted myocytes from muscle biopsies from hind limb muscles of dogs and cultured them in collagen-coated flasks. Cells suspended in a saline solution were then injected into the lower esophageal sphincter using a variceal sclerotherapy needle. The muscle-derived cells appeared to integrate into smooth muscle and enhance sphincter contractility. The reconstruction of an internal anal sphincter has also been described for treatment of fecal incontinence. Somara *et al.* [20] describe a technique in which human internal anal sphincter cells were cultured in a fibrin three-dimensional matrix. The bioengineered anal sphincter exhibited ex-vivo physiologic functionality with spontaneous myogenic basal tone. In a subsequent proof of principle study, the investigators successfully implanted the bioengineered sphincter in mice and demonstrate preserved functionality [21].

FUTURE DIRECTIONS

As skills in creating multilayered tissue cultures improve, it may be best to transplant multiple cell types in the transplant to allow repopulation of the entire wall that theoretically could allow resection of deeply invading cancers. Endoscopic therapy could regenerate the muscle as well as the mucosal epithelium. This type of therapy could also be used to treat patients who have functional problems. Therapy could allow regeneration of tissues in fistula tracts and areas of chronic inflammation.

The clinical application of bioengineered tissue is limited in part by the need to produce large numbers of epithelial cells, the time required to process tissue, and the need for immediate availability of tissue at the time of endoscopy. Tissue acquisition through endoscopy is relatively simple and inexpensive. Primary cell culturing of harvested tissue, on the contrary, can be costly and time consuming. Precise timing of cell sheet implantation is important in ensuring tissue viability. This requires coordination between a tissue engineering laboratory and endoscopy unit, a factor that can be challenging in clinical practice. The use of pluripotent stem cells could circumvent the need for autologous tissue harvesting and could help create a tissue bank for endoscopic transplantation. Further animal studies have to be performed before stem cell derived tissue is used in human beings. The endoscopic delivery of bioengineered tissue will also need improvement. The size of a cell sheet for transplantation is currently designed to fit in an endoscopic mucosal resection cap. Another approach is the use of a tubular scaffold; however, its use limits versatility when it comes to implantation. Advances in cellular scaffolding may allow for flexible materials to be engineered that could potentially enhance the ease of endoscopic delivery of bioengineered tissue. Lastly, it is still unclear on how we can assure consistent tissue uptake at the injured mucosal site. This may be an important factor with regards to gut motility and management of luminal contents such as bacteria, food and secretions. Regardless of the

present limitations in this field, the current strides in tissue engineering will one day make it possible to apply localized therapy to the gastrointestinal tract using engineered or regenerative therapy instead of the current strategies of surgical closure of defects and obliteration of diseased cavities.

CONCLUSION

The field of tissue engineering is rapidly evolving and in the near future will become clinically available to the gastrointestinal endoscopist. Tissue repair with autologous or stem cell derived tissues is perhaps the immediate foreseeable application of this field. Exciting frontiers are also being explored in sphincter augmentation and reconstruction. Tissue availability and culture yield currently limit wider use of tissue engineering in endoscopy but advances in three-dimensional cellular culture techniques show promise in tissue regeneration and transplantation.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

of special interest

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KEY POINTS

- Autologous harvesting of epidermal mucosa with in-vitro growth is feasible.
- Advances in cellular scaffolding have made bioengineering of complex tissues a reality.
- Bioengineered tissue can be successfully implanted with successful integration with the native mucosa.

FIGURE 1.

Treatment of ulceration after esophageal endoscopic submucosal dissection by transplantation of autologous oral mucosal epithelial cell sheets. (1) Biopsy specimens obtained from oral buccal mucosal tissue. (2) Epithelial cells seeded onto temperature-responsive culture inserts. (3) Oral mucosal epithelial cell sheets harvested by reducing the culture temperature to 20° C. (4) Autologous oral mucosal epithelial cell sheets on a support membrane transplanted with endoscopic forceps onto the bed of the esophageal ulceration. Adapted with permission from [3].

FIGURE 2.

Schematic of the surgical deployment of the porcine urinary bladder extracellular matrix device with an achalasia balloon and delivery of surgical adhesive. (Adapted with permission from [19]).