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Tissue engineering and regenerative medicine as applied to the gastrointestinal tract

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Abstract

The gastrointestinal (GI) tract is a complex system characterized by multiple cell types with a determined architectural arrangement. Tissue engineering of the GI tract aims to reinstate the architecture and function of all structural layers. The key point for successful tissue regeneration includes the use of cells/biomaterials that elucidate minimal immune response after implantation. Different biomaterial choices and cell sources have been proposed to engineer the GI tract. This review summarizes the recent advances in bioengineering the GI tract with emphasis on cell sources and scaffolding biomaterials.

1- Introduction

The gastrointestinal tract (GI) is a continuous tubular system that extends from the mouth to the anus. The GI tract is composed of the following main components: mouth, esophagus, stomach, small intestine and large intestine. Alternating contractions and relaxations, a process defined as peristalsis, help propelling and digesting the food along the tract. Digested materials are absorbed into the blood through the epithelium while the indigestible and unabsorbed substances get excreted from the body as waste through the anus. Several diseases alter motility throughout the gastrointestinal tract. Surgical intervention does not provide a long-lasting solution. Patients become overwhelmed by psychological and socio-economic issues [1-6].

The GI tract is a hollow organ populated by multiple cell types. The smooth muscle component is divided into 2 layers: the outer longitudinal smooth muscle cells that run parallel to the tract and the inner circular smooth muscle cells that are aligned circumferentially around the lumen of the tract. The mucosal layer of the GI tract is composed of heterogeneous cell types that serve for protection, secretion and absorption. Intramural innervation of the gut is provided through 2 ganglionated plexi: the myenteric plexus between the 2 muscle layers and the submucosal plexus between the circular muscle and mucosal layers. Interstitial cells of Cajal (ICCs) form a network that extends along the

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GI tract. ICCs provide pacemaker activity and regulate smooth muscle function. Tissue engineering is an evolving field that provides an elegant approach to duplicate the architecture and function of the tract.

2- Biomaterials, cells and extracellular matrix components

In GI tissue engineering, cell choice is a major challenge due to the limited availability of autologous cell sources. An important characteristic is the ability to isolate enough cells from a small biopsy and to rapidly expand them *in vitro*. Several cell types have been tested for their ability to regenerate the innervated muscularis layer as well as the epithelial component. Recently, alternative sources of cells are being tested for their use in tissue engineering, these include mesenchymal stem cells from various sources, induced pluripotent stem cells and intestinal crypts [7-10].

Tissue engineering of the GI tract often employs the use of porous scaffolds. Degree of porosity mediates smooth muscle cell alignment, contraction and relaxation. Additionally, the scaffolds must support neuronal differentiation which is key to regulate peristalsis. Tissue remodeling and host tissue integration are also important factors to consider while designing 3D scaffolds. Studies are being conducted to test different biomaterials that are suitable in tissue engineering [11,12]. Biomaterials include natural polymers, synthetic polymers or a combination of both, depending on the application [13,14]. The use of composite scaffolds takes into account the mechanical properties of synthetic polymers and biological activity of natural polymers [15,16]. Despite the advances in scaffolds fabrication, their mechanical properties and degradation kinetics are limiting steps in their design [17-22].

Extracellular matrix (ECM) components mediate cell adhesion, proliferation, differentiation and migration. In GI tissue engineering, ECM components facilitate epithelial cell differentiation along the gut. Examples of ECM include collagens, laminin and other proteoglycans. During the process of designing scaffolds, it is important to take into account the addition of ECM components to facilitate cell-cell and cell-scaffold interaction. In many applications, glycosaminoglycans were crosslinked to scaffolds to improve cell attachment, proliferation, differentiation and to test cell migration [23-25]. The use of decellularized matrices as scaffolds is advantageous due to the preservation of the extracellular matrix components.

Recent studies in GI tissue engineering have focused on seeding organoid units onto synthetic scaffolds. The organoid units were successful in regenerating the muscularis and epithelial layers. However, organoid units are hard to grow *in vitro*. Smooth muscle cells require scaffolds that allow them to attach, survive and align themselves. To date, there is evidence that synthetic scaffolds are more suitable to guide smooth muscle regeneration. Nevertheless, natural scaffolds can be also modified using bioactive components to enhance their ability to regenerate the muscle layer. Epithelial cells are effectively used as cell source to reconstruct the mucosal layer of the GI tract. Both synthetic and natural (decellularized) scaffolds have demonstrated promising results for regenerating the mucosal layer. For complete regeneration of GI tissues, all cellular components must be included in the design of the tissue. A combination of smooth muscle cells and neural stem cells provides the route for developing functional innervated muscularis layer. Epithelial cells have shown the ability to proliferate and differentiate into different epithelial cell types when seeded onto synthetic or natural scaffolds. A 'sandwich' porous scaffold that allows dual seeding of smooth muscle cells on the outer layer and epithelial cells on the inner layer can be a potential candidate for GI tissue engineering.

Neo-vascularization of the engineered tissues imposes a great challenge to the survival and functionality of the tissues *in vivo*. Vascularization promotes survival of the implanted

tissues by ensuring oxygen and nutrient exchange. Pre-vascularization and delivery of angiogenic growth factors are common methods to induce vascularization of the engineered tissues [26-28]. Table 1 outlines the recent achievements in mechanical and biochemical functions of engineered GI tissues.

3- Tissue engineering of the GI tract: Esophagus, Stomach, Small Intestine and Large Intestine

i- Esophagus

The esophagus is a long muscular tube extending from the pharynx to the stomach. It mediates the transport of food to the stomach via a series of coordinated rhythmic peristaltic waves. Impaired peristaltic function in cases such as esophageal atresia and tracheoesophageal fistula results in esophageal stricture and gastroesophageal reflux disease (GERD) [29]. Other forms of disorders include esophageal cancer which is associated with high impact on the quality of life. Treatment of these conditions requires restoration of the gravitational characteristic and the coordinated rhythmic activity of the esophagus.

To tissue-engineer the esophagus, several factors must be taken into account. First, the engineered replacement must have a hollow tubular structure to mimic the native esophagus. Second, the function of the esophagus is dependent on different cell types. The lumen along the entire esophagus contains epithelial cells. The muscle component is divided into 2 parts, the upper esophagus is formed of striated muscle and the lower part is formed of smooth muscle. In order to ensure proper swallowing and peristalsis, the two types of muscles are regulated by intrinsic and extrinsic neural pathways. Third, the extracellular matrix component of the esophagus is an essential mediator required for the growth and differentiation of these cell types. Evaluation of the composition of the esophagus basement membrane provides a starting point in designing scaffolds for tissue engineering [30]. Any disorder affecting one or more of these components will affect food transit down through the esophagus.

The use of natural scaffolds has been studied in esophageal tissue engineering. Smooth muscle tissue was successfully engineered using OptiMaix collagen scaffolds. Those scaffolds were characterized by unidirectional porous structures which allowed directional smooth muscle growth [31]. Decellularized matrices have also gained attention. Different sources of tissues have been decellularized and used in esophageal reconstruction such as esophageal matrix, urinary bladder matrix, gastric matrix and small intestinal submucosa. Ovine esophagi were decellularized and resulted in matrices with rough surface and preserved extracellular matrix. These decellularized tissues can be suitable for esophageal tissue engineering [32].

Other studies developed extracellular matrices from porcine urinary bladder and were implanted in dog models to reconstruct the esophagus. The implanted bioscaffolds that were in direct contact with a part of the native muscle tissue, resulted in a functional esophagus with mechanical properties similar to native tissue. Histological analysis demonstrated the presence of organized layers that constitute the esophagus [33]. Bo Tan et. al. isolated bone marrow mesenchymal stem cells (BMSCs) from dogs and seeded them onto small intestine submucosa (SIS). A section of the esophagus was removed from the same dogs and replaced with the SIS seeded with autologous cells. The seeded scaffolds facilitated re-epithelization, re-vascularization and muscular regeneration verified by histological and immunohistochemical evaluation [34].

Synthetic biomaterials have been evaluated for their application in esophageal reconstruction. The use of synthetic composite tubes composed of nitinol and silicon rubber

to replace the esophagus resulted in anastomosis and shedding problems. The addition of polyester connecting ring to these tubes decreased anastomotic leakage and improved the shedding time [35]. Porcine esophageal epithelial cells were seeded on polycaprolactone (PCL) membranes coupled to type IV collagen. The PCL-collagen composite accelerated the regeneration of the epithelium, cell spreading and interconnection between cells. Immunostaining studies confirmed the epithelial phenotype of the cells [36].

Modifying the chemical nature of the scaffolds to closely mimic that of the native tissue can change the behavior of the seeded cells. Electrospun elastic biodegradable poly(L-lactide-co-caprolactone) (PLLC) scaffolds immobilized with fibronectin immobilization supported epithelial cell attachment and growth. Cells grown on PLLC-Fibronectin scaffolds displayed morphological characteristics of epithelial origin. These cells were also able to synthesize collagen IV, an important structure protein of the basement membrane. Cells grown on PLLC without fibronectin grew in clusters and at a slow rate [37]. Other approaches have seeded adipose sourced smooth muscle cells onto biodegradable PGA/PLGA-based scaffold. These scaffolds were implanted in rat models and histological studies showed re-epithelialization of the lumen and regeneration of the muscularis layers [38].

ii- Stomach

Food mixing and grinding occur in the stomach through cycles of well-coordinated rhythmic contractile activities. The stomach content is then emptied into the small intestine. Loss of mechanical or neuronal signals results in gastric dysmotility. Gastroparesis is characterized by delayed gastric emptying due to autonomic neuropathy [39,40]. Gastric electrical stimulation offers a solution which requires surgical intervention to implant a stimulator in the patient's body [41,42]. Complications have been associated with infection at the implant site, pain, and device relocation.

Gastric cancer is a common form of cancer worldwide with gastrectomy remaining the only potential therapy. However, people undergoing gastrectomy face significant morbidities such as malnutrition, anemia and weight loss. Several approaches to reduce the complications of surgical resection have resulted in poor life improvement. Tissue engineering provides a solution to restore the appropriate physiology and reservoir characteristics of the native stomach.

Bioengineered polymer scaffolds composed of polyglycolic acid (PGA), coated with poly-L-lactic acid and type I collagen were used for seeding organoid units. These organoid units were isolated from the stomach and they consisted of epithelium and mesenchyme. The seeded scaffolds were implanted in the abdominal cavity. Histological studies demonstrated a fully differentiated epithelium similar to native stomach and a muscularis layer [43]. However, the regenerated muscularis did not mimic the native architecture of the stomach. Maemura *et. al.* have used a gastric patch composed of epithelial organoid units seeded onto the lumen of biodegradable PGA scaffolds. These scaffolds were implanted in the anterior wall of a defective stomach. The patch was integrated with the host tissue and appeared to have a continuous epithelium at the luminal side. The cells stained positive for the proton pump -subunit indicating the presence of parietal cells, adjacent cells stained positive for -actin smooth muscle indicating the regeneration of a muscle layer [44]. Other groups have also implanted PGA tubes seeded with stomach-derived organoid units in an attempt to regenerate the stomach [45]. All of these reports have successfully regenerated the epithelium of the stomach. However, the regeneration of the muscularis layer, in terms of architecture and function, remains a challenge.

iii- Small intestine

The small intestine is the major site of nutrient absorption. Absorption is facilitated by the microvilli structures lining the epithelium of the intestine. Smooth muscle contraction and relaxation help in food transit and increase the surface area to promote nutrient absorption. Short bowel syndrome is the outcome of massive truncation of regions of the intestine due to cancer or inflammation. This results in malnutrition, malabsorption and motility dysfunction. Those symptoms are characterized by weight loss, vitamin deficiency and potential infections [2]. Current therapies for intestinal disorders extend from chronic parenteral nutrition to intestinal transplantation. Transplantation poses challenges on the patients in terms of costs, availability of donors and immunosuppression of the graft [1-3].

Tissue engineering of the bowel requires the use of tubular scaffolds with tunable mechanical properties. Additionally, regeneration of the muscularis layer with the specific orientation is of paramount importance. Innervation of this muscle layer by the myenteric plexus ensures coordinated motility. The submucosal plexus lies between the circular muscle layer and the mucosal layer. It plays a role in regulating absorption and secretion. Therefore, the tissue-engineered intestine should take into account this complex architecture for proper functionality.

Early reports have employed mesenchymal stem cells to study the possibility of muscle regeneration [46]. The limitations included the inability to regenerate the smooth muscle layer. Recent advances in intestinal tissue engineering were successful in differentiating mice induced pluripotent stem cells (iPSCs) *in vitro* into smooth muscle with peristaltic features [47]. The smooth muscle sheets demonstrated coordinated contraction which could be used to tissue engineer physiologically functional intestine.

The use of organoid units isolated from intestinal biopsies has gained special attention. Those organoid units were seeded onto polymer scaffolds and tested for their ability to regenerate the intestine post implantation. Muscle and nerves were observed in those newly formed intestines and the architecture resembled the native tissue [48,49]. Recently, Sala et. al. have derived organoid units from mice and seeded them onto PGA scaffolds that were coated with PLLA and type I collagen. The seeded scaffolds were immediately implanted into the omentum of mice. Histological analysis showed epithelial regeneration with villi structures. The muscularis layer stained positive for alpha smooth muscle actin and for smooth muscle myosin heavy chain [50]. However, the transversal and circular organization was not seen in the tissue engineered intestine.

Decellularized scaffolds have been also investigated for their potential use in intestinal tissue engineering. Small intestinal submucosa (SIS) sheets were seeded with intestinal smooth muscle cells and implanted in rat jejunal interposition model. Eight weeks post-implantation, partial epithelialization has occurred and neovascularization was seen at the site of implantation. A small number of cells stained positive for α -smooth muscle actin, while a larger number expressed calponin and smooth muscle myosin heavy chain [51]. The seeded smooth muscle cells failed to regenerate the muscularis layer due to low cell survival post-implantation. Totonelli et. al. have successfully decellularized rat small bowel in an attempt to use them as scaffolds in intestinal regeneration. An optimized protocol of employing 1 cycle of detergent and enzymatic treatment has led to complete removal of cellular component. The decellularized matrices maintained the native architecture of an intestine and they retained their mechanical properties [52].

iv- Large intestine

The colon represents the last segment of the gastrointestinal tract. It is a continuity of the muscular and neuronal layers of the small intestine. It is the site of water absorption and

waste excretion. As in the small intestine, peristalsis in the colon is mediated by the innervated longitudinal and circular smooth muscle layers. Inflammation of the colon (Crohn's disease) and loss of colonic innervation (Hirschsprung's disease) result in colonic dysmotility which is characterized by either constipation or diarrhea. This increases the urge to tissue engineer colonic segments that could serve as functional replacements.

Attempts to bioengineer the large intestine included the use of organoid units isolated directly from the colon and seeded onto polymer scaffolds. The tissue engineered large intestine had an architecture identical to the native colon with the regeneration of the muscularis layer [49]. However, peristalsis was not measured in these constructs. Recently, colons of rats were denervated by treatment with benzalkonium chloride. Isolated neural crest progenitors were delivered to the denervated colon where they differentiated into neurons and glia and restored motility [53]. Our group has recently tissue-engineered a functional colonic segment using a combination of cells and biomaterials. We have bioengineered circular smooth muscle tissue constructs that resemble the architecture of the circular muscle layer of the colon. These tissue constructs were placed around tubular chitosan/collagen scaffolds. These constructs were then taken off the scaffold and their physiological functionality was assessed by real time force generation. The constructs exhibited contractile and relaxant responses indicating the maintenance of their functionality [54]. We were also successful in bioengineering the longitudinal muscle layer of the bowel using laminin gel. Smooth muscle cells were seeded onto molds with longitudinal grooves where they aligned themselves transversally. The bioengineered longitudinal tissues responded to contractile and relaxant reagents [55].

The internal anal sphincter (IAS) mediates the excretion of the luminal content from the rectum to the outside of the body. It is made of tonically contracted circular smooth muscle cells that keep the sphincter closed. The sphincter remains closed until a signal induces its relaxation to allow the passage of the fecal material out of the body. Aging causes weakening of the IAS with loss of its intrinsic innervation. Our lab has successfully bioengineered and implanted innervated IAS tissue constructs. Those constructs maintained their myogenic and neuronal functionality [56].

4- Conclusion

Defects in parts of the GI tract result in devastating consequences whether for pediatrics or adult patients. Different approaches to tissue engineer segments of the GI tract attempt to reverse life-threatening problems. Due to the multiple functions performed by the GI tract, bioengineered tissues should mimic the native organ architecturally and functionally for successful long term results. Selection of cells and biomaterials is essential to ensure the viability of cells after implantation and the integration of the scaffolds with the host tissue. Finally, the scaffold/cell design must take into consideration the possibility of innervation and vascularization of the engineered tissue, which are crucial for proper functionality.

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- of special interest
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Highlights

- Scaffolds in tissue engineering are used as temporary matrix for the cells.
- In vitro and in vivo studies were conducted to test cell-biomaterial interaction.
- The proposed bioengineered tissues focused on muscle and epithelial regeneration.
- Scaffolds seeded with cells enhanced in vivo tissue reconstruction.

Table 1

Summary of different approaches to regenerative medicine of the GI tract, and their mechanical and biochemical outcomes and their importance in GI tissue engineering.

	Approach	Mechanical Outcome	Biochemical Outcome	Importance
<i>Esophagus</i>	Autologous BMSCs on SIS implanted in canine [34]	Muscular regeneration	Re-epithelialization Re-vascularization	Epithelialization enhances healing. BMSCs produce angiogenic factors.
	Nitinol composite artificial esophagus implanted in pigs [35]	No peristaltic function	Esophageal mucosa formed	Stratified squamous epithelium covers scar and long term survival of pigs.
	Epithelial cells seeded onto PCL scaffolds grafted with collagen IV [36]	Not assessed	Epithelial regeneration	Collagen IV promotes epithelial cell migration/epithelium regeneration. Interconnection between cells.
	Epithelial cells seeded on PLLC scaffolds grafted with Fibronectin [37]	Not assessed	Epithelial regeneration	Accelerated epithelial regeneration. Good spreading and interconnection. Epithelial phenotype confirmed.
	Adipose-derived smooth muscle cells seeded onto PGA/PLGA scaffolds and implanted in rats [38]	Muscularis layer regeneration	Re-epithelialization observed	Formation of mucosa, muscularis and serosa.
<i>Stomach</i>	PGA/PLLA scaffolds seeded with organoid units and implanted in rats omentum [43]	Muscularis development but did not reproduce circular or longitudinal alignment	Regeneration of a differentiated epithelium	Regeneration of an epithelium with the ability to proliferate and differentiate into the 4 cell types.
	PGA/PLLA scaffolds seeded with organoid units and implanted in rats [44]	Presence of smooth muscle layer	Continuous neomucosa formation	Successful transplantation of gastric patch as a pre-requisite for transplantation of engineered stomach
<i>Intestine</i>	PGA/PLLA scaffolds seeded with organoid units and implanted intraperitoneally in pigs [45]	Innervated muscularis mucosae	Intestinal epithelium formed including all differentiated cell types	Full thickness tissue reproduces native intestine using autologous cells.
	PGA/PLLA scaffolds seeded with organoid units and implanted in the omentum of mice [50]	Regeneration of innervated muscularis layer. Alignment was not seen	Re-epithelialization including all differentiated epithelial cell types	Differentiated epithelium next to an innervated muscularis, mesenchymal-epithelial interaction preserved.
	SIS seeded with intestinal smooth muscle cells and implanted in rats [48]	Contractile protein expression decreased by week 4. Lack of orientation and organization	Epithelialization by week 8 Neo-vascularization by week 2	Loss of contractile phenotype, biomechanically less favorable, minimal epithelial regeneration.

(BMSCs: bone marrow mesenchymal stem cells, SIS: small intestine submucosa, PCL: polycaprolactone, PLLC: poly(L-lactide-co-caprolactone), PGA: polyglycolic acid, PLGA: poly (lactide-co-glycolide), PLLA: poly-L-Lactic acid)