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BRIEF REVIEW

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Tissue Engineering in the Gut: Developments in Neuromusculature

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The complexity of the gastrointestinal (GI) tract lies in its anatomy as well as in its physiology. Several different cell types populate the GI tract, adding to the complexity of cell sourcing for regenerative medicine. Each cell layer has a specialized function in mediating digestion, absorption, secretion, motility, and excretion. Tissue engineering and regenerative medicine aim to regenerate the specific layers mimicking architecture and recapitulating function. Gastrointestinal motility is the underlying program that mediates the diverse functions of the intestines, as an organ. Hence, the first logical step in GI regenerative medicine is the reconstruction of the tubular smooth musculature along with the drivers of their input, the enteric nervous system. Recent advances in the field of GI tissue engineering have focused on the use of scaffolding biomaterials in combination with cells and bioactive factors. The ability to innervate the bioengineered muscle is a critical step to ensure proper functionality. Finally, in vivo studies are essential to evaluate implant integration with host tissue, survival, and functionality. In this review, we focus on the tubular structure of the GI tract, tools for innervation, and, finally, evaluation of in vivo strategies for GI replacements.

Keywords: Intestinal Tissue Engineering; Neoinnervation; Enteric Nervous System; Smooth Muscle.

The gastrointestinal (GI) tract is a continuous tubular organ responsible for the transport and digestion of food, absorption of nutrients, and excretion of waste. The activity of the GI tract is a summation of several complex cell types that include smooth muscle cells, neurons, glia, interstitial cells, and different kinds of intestinal epithelial cells. The outer layer of the GI tract is composed of 2 types of smooth muscle tissues: circular and longitudinal smooth muscle. The sphincters of the GI tract allow unidirectional and directed flow of luminal contents. Apart from the smooth musculature, the GI tract contains several kinds of intestinal epithelial cells that mediate absorption and secretion within the gut. Smooth muscle tissues are the primary effectors of motility in the gut, mediating the movement of luminal content. The function of the muscle tissue is dictated by the enteric nervous system (ENS), which is the intrinsic innervation of the gut. Several classes of functional neurons (sensory, motor, secretory, and so forth) and glia are present in the ENS, with a diversity paralleled only by the central nervous system.[1] The ENS is responsible for the variety of gastrointestinal motor patterns produced in different parts of the gut, as well as the coordination of function between various segments of the gut. The interstitial cells of Cajal also additionally are implicated in pacemaking function within the gut,[2] rounding out the primary players responsible for gastrointestinal motility.

Gastrointestinal motility can be altered postnatally as a result of disease, damage, surgical or obstetric trauma, and age. Congenital defects of GI motility include but are not limited to Hirschsprung disease, intestinal pseudoobstruction, and achalasia.[3] Although the therapeutic mainstay for motility disorders has remained pharmacologic, surgical correction also does not provide a long-lasting solution. Regenerative medicine seeks to replace GI segments, preferably using the patient's own cells while using the optimal route of delivery. Advances in biomaterials and tissue engineering have catapulted regenerative medicine strategies forward, bringing them closer to the bedside.

This review focuses on regenerative medicine strategies aimed at the restoration of the neuromuscular anatomy and/or function of the neuromusculature of the GI tract. This review highlights both biomaterial-based and cell transplantation–based methods. Finally, a future perspective is provided indicating the complexities of sourcing and maintaining phenotypes of many constituent cells, neoinnervation, and neovascularization.

Tissue Engineering of GI Tubular Organs: Where Do We Start?

Anatomy and Function

Tissue engineering the GI tract has fundamental challenges that one would encounter when faced with most biological systems—anatomic and physiological complexity. The complexity of the GI tract lies in the different cell layers that exist within the tract. These cells work in coordination

Abbreviations used in this paper: **CNS, central nervous system; ENS, enteric nervous system; GI, gastrointestinal; IAS, internal anal sphincter; ICC, interstitial cells of Cajal; LES, lower esophageal sphincter; VIP, vasoactive intestinal peptide.**

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to respond appropriately to different stimuli. In GI tissue engineering, each of the different cell types must be considered. The first question that arises in any tissue engineering application is the appropriate source of cells. Can the several cell types required to duplicate physiological complexity be sourced? If yes, can they be sourced in adequate numbers from a biopsy specimen, which is preferably minimally invasive?

Musculature. The GI tract is a complex, highly regulated, multilayered system. Although the muscularis propria is divided into several different layers, its complexity is determined by its anatomy. For example, different muscle types exist along the length of the esophagus. The first one third is composed of skeletal muscle and the lower one third is composed of both circular and longitudinal smooth muscle. The middle part of the esophagus is a mixture of both skeletal and smooth muscle. The variety of muscle types is essential to ensure swallowing and propulsion of food into the stomach. In addition to the circular and longitudinal smooth muscle layers that make up the gastric musculature, another oblique smooth muscle layer exists.[4]

Taking into account the different muscle types and their orientation is critical when engineering GI segments. In the small and large intestine, the muscular layer is divided again into longitudinal and circular muscle layers. The basic unit of intestinal musculature is the smooth muscle. It receives regulatory inputs from different levels to perform its contractile functions. Smooth muscle contraction is initiated by membrane depolarization, which activates voltage-gated calcium channels and leads to calcium (Ca^{2+}) influx into the cell. Entry of Ca^{2+} stimulates complex signaling cascades within the smooth muscle cell, leading to contraction. Different intracellular proteins are involved in mediating the contractile response via a series of phosphorylation and dephosphorylation (G proteins, phospholipases, calmodulin, myosin light chain kinase, Rho kinases, and phosphatases). Although similar pathways exist for both circular and longitudinal smooth muscle layers, mechanisms for Ca^{2+} mobilization differ between the two. Depolarization of smooth muscle is controlled at different cellular levels in the GI tract. Contractile response is divided into 2 phases: initial and sustained contractions. A balance between phosphorylation and dephosphorylation events is responsible for the occurrence of the 2 phases. A complete regeneration of the musculature of the GI tract requires the maintenance of all the intracellular pathways. Smooth muscle cells also can be classified as phasic or tonic smooth muscle based on the contents of contractile protein isoforms and their levels of expression. A higher level of contractile protein expression is observed in smooth muscle cells present in the high-pressure zones of the GI tract, namely the sphincters of the GI tract. For example, circular smooth muscle of the lower esophageal sphincter (LES) or the internal anal sphincter (IAS) differ significantly from the smooth muscle of the esophageal body or the colorectum, respectively.[5,6]

Epithelium and defense functions. The epithelium of the gut performs various critical functions such as enzyme secretion, nutrient absorption, and acts as a physical barrier to perform a highly sophisticated defense function. Secretion and absorption functions require a large surface area, which is provided by finger-like villi structures that face the luminal side of the gut. The epithelial monolayer is characterized by apicobasolateral polarity and is divided into several specialized cell types: enteroabsorptive cells, goblet cells, Paneth cells, and neuroendocrine cells.[7]

Polarity of the epithelium is essential for function, it allows cells to sense and respond to stimuli.[8] The epithelium differs in structure and function along different parts of the GI tract.[9] Apart from nutrient absorption, the epithelium also provides a defense function. The gut has to have an appropriate defense system capable of protecting itself from commensal bacteria as well as foreign antigens in its luminal content. A single layer of epithelial cells makes up the epithelial barrier as a primary defense wall. A coordinated interaction between the different epithelial cells contributes to the defense function of the epithelium. The integrity of the epithelial barrier is maintained by tight junctions. In addition to tight junctions, adherens junctions and gap junctions also are involved in cell–cell interaction. What makes the epithelial barrier more complex is the fact that it is a dynamic structure that is renewed continuously in the context of epithelial cell shedding and proliferation.[10,11] Thus, the gut is vulnerable and is a potential site for infection, inflammatory diseases, and loss of the epithelial barrier integrity. Part of the regeneration process must involve generating a polarized epithelium with tight junction integrity to reinstate mucosal function.

Regulatory apparatus. The regulatory apparatus of the GI tract is multilayered—with input arising from the intramural innervation, integrated inputs from the central and autonomic nervous system, as well as input from interstitial cells of Cajal (ICCs). Ultimately, GI physiology is a complex addition and interpretation of several signals that lead to smooth muscle motor activity, mucosal secretion/transport, local blood flow/vasodilation, and intestinal immune and endocrine function. The ENS is contained entirely within the musculature of the GI tract and is arranged within ganglionated plexuses. The myenteric plexus is located anatomically between the circular and longitudinal muscle layers, and extends the full length of the digestive tract from the esophagus to the rectum. The submucosal plexus lies between the mucosa and the inner circular muscle layer. Together, the neurons of the ENS regulate diverse functions such as control muscle activity, secretory activity of intestinal glands, motility of the blood vessels, and sensory functions that include reflex pathways. Depending on the region of the gut, the size and compositional diversity of the ganglia within the plexuses vary. For example, the submucosal plexus is far more obvious in the small and large intestines than in the stomach, and the number and neuron density of myenteric ganglia are higher in the colon near the mesenteric attachment.[12–14] Different classes of enteric neurons exist with diverse neurochemical coding.

Neurotransmitters in the intestine are similar to those of the central nervous system (CNS), and include acetylcholine, tachykinins, serotonin, nitric oxide, purines, and several neuropeptides.[15] Motor neurons are responsible for