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Animal models for intestinal tissue engineering

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Abstract

Although total parenteral nutrition prevents patients with short bowel syndrome from dying of starvation, having short bowel remains a severely debilitating condition. The best current treatment for inadequate absorptive surface area is through intestinal transplantation. However, this therapy is associated with significant morbidity and patients suffer from consequences of long-term immunosuppression. Additionally, the numbers of organs are limited. A new frontier in medicine is the field of tissue engineering. We will review the progress of intestinal bioengineering with a focus on the use of animal models. Investigators initially used autologous tissue as a patch to study intestinal regeneration. Subsequent studies focused on the use of absorbable biomaterials as a patch for tissue ingrowth. The most novel methodology consists of seeding a resorbable scaffold and implanting this construct to observe the regeneration of neointestine. Successful creation of esophagus, stomach, small bowel and colon has been demonstrated. Although these studies are preliminary, the results suggest that tissue-engineered intestine will become a real therapeutic option in the not too distant future for patients with inadequate intestinal tissue.

Keywords: Animal model; Intestine; Scaffold; Regeneration; Tissue engineering; Biomaterials

1. Introduction

Short bowel syndrome is the condition in which there is inadequate intestine to support normal somatic growth and development. The loss of small bowel absorptive mass results in malabsorption of nutrients, fluids and electrolytes. The need for new intestinal tissues arises from many disorders including intestinal ischemia, tumors, and inflammatory bowel disease. The absolute length of small bowel that is required is impossible to define and is dependent upon the patient's age, the length of the small and large bowel present, and the presence or absence of the ileocecal valve. Infants and children generally have better adaptive responses and can survive on much shorter length of small intestine than adults.

Other than the small bowel, many patients might benefit if other parts of the gastrointestinal tract could be tissue engineered. Patients who require total gastrectomy would benefit from improved physiologic function if gastric substitutes are available. Currently, these patients simply live without a stomach. Esophageal replacement would be ideal for many patients who have a variety of disorders including congenital esophageal atresia, caustic injury, tumors, and reflux esophagitis. The ability to replace colonic loss with novel colonic tissue would also be an improvement over the current management strategies.

The current best solution for replacing inadequate small bowel length and absorptive surface is intestinal transplantation [1–3]. Because the results of intestinal transplantation are so morbid, no one has consider transplanting other less vital parts of the gastrointestinal tract such as the esophagus, stomach or colon. An alternative and potentially better therapy for all of these needs can be created through intestinal tissue engineering. The number of investigations in this arena is relatively sparse but the momentum is increasing as more investigators pursue the creation of novel organs.

In 1967, Stanley Dudrick and his colleagues [4] at the University of Pennsylvania provided the first report that complete nutritional support could be provided intravenously and inferred that the intestine may not be a vital organ. Their work has allowed countless patients formerly thought to be unsalvageable because of insufficient bowel length to survive and not die from starvation. However, we have learned that chronic dependence on hyperalimentation is associated with significant morbidity and mortality [5]. All will have

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problems with diminishing venous access sites, and most will have infectious complications from a chronic indwelling catheter. Additionally, many patients develop chronic liver failure from long-term hyperalimentation with an eventual need for liver transplantation. This supportive therapy is a tremendous financial burden, and the quality of life is significantly diminished for patients.

Surgeons have tried to circumvent the problems of inadequate bowel length through creative surgical procedures [6–11]. Some have tried to slow transit time by placing a reversed segment of bowel to alter the motility. Others have constructed valves to diminish forward flow. Intestinal pouches and recirculating loops have also been tried. Operations aimed at increasing absorptive area of the gut also have been reported. None of these procedures have been uniformly successful. Complete bowel replacement through transplantation may offer relief, but it remains a highly morbid therapeutic option and has inherent limitations due to the need for long-term immunosuppression, inadequate number of organs, and recurrent episodes of rejection.

Although the study of intestinal regeneration and the attempt to tissue engineer neointestine are relatively novel fields of study, investigators have developed some creative animal models in this pursuit. Although the only truly vital part of the gastrointestinal tract is the small bowel because of its absorptive capacity, the ability to bioengineer other parts of the intestinal tract would also represent improved therapy for many patients. In this review, we will explore the various animal models that exist for investigating tissue engineering of the gastrointestinal tract.

2. Autologous patch model

In the 1963, Kobold and Thal [12] reported on the feasibility of repairing traumatic duodenal wounds with jejunal serosa. They created sizable duodenal openings in mongrel dogs and patched these defects with jejunal serosa. By 8 weeks, the entire serosal patch was covered by relatively normal duodenal epithelium. In 1973, Binnington and his colleagues [13] at Washington University in St. Louis used this observation to perform studies in a dog model in an attempt to create novel jejunal mucosa. Their experimental model consisted of incising the jejunum on the antimesenteric border and patching this opening using the serosa of the adjacent descending colon. They observed that new mucosa grew from the margins to cover the colonic serosa and presented the first report of creating novel intestinal surface using an animal model.

Using the same model, Binnington [14] demonstrated that the new jejunal mucosa had active transport of amino acids. Activities of aminopeptidase, maltase, and lactase were similar in the neomucosa when compared to normal jejunum, but neomucosa samples contained less activity of alkaline phosphatase, sucrase, and trehalase. However, some of the dogs had persistent bare serosal areas that remained uncovered by neomucosa despite waiting up to 36 weeks. In general, the neomucosa adjacent to the original mucosa had a villous surface pattern, but the architecture was less uniform and more immature in the central areas. Using a short gut model in miniature swine, they demonstrated that patching the jejunum led to increased weight gain despite lack of evidence of larger absorptive surface area [15].

In a New Zealand white rabbit model, Lillemoe and his colleagues [16] used a vascularized abdominal wall pedicle flap to patch a defect created in the terminal ileum. A $2 \times 6\,cm^2$ rectus muscle flap was fashioned with vascular supply from the inferior epigastric artery. A 5 cm defect was created on the terminal ileum and the pedicle flap was sutured to the bowel. Seventy percent of the 27 rabbits survived the duration of the experiment. The pedicle flap was covered entirely by neomucosa by 4 weeks, and the circumference of the neointestine was 70% larger than the adjacent normal ileum. Light and scanning electron microscopy showed the presence of blunted mucosa and thickened muscle layer that contained both smooth and skeletal muscle. In comparison to normal ileal mucosa, the neomucosa had similar electrophysiologic parameters, response to administration of glucose and bile salt absorption capacity.

Jon Thompson and his colleagues have performed multiple studies evaluating the process of intestinal regeneration using a New Zealand white rabbit model. Using a patch methodology, they compared the technique of patching with colonic serosa and abdominal wall muscle [17]. Initial ingrowth of mucosa occurred along the margins, and 85% of the patches were completely covered by mature villi and muscularis mucosae by 8 weeks. The ileal diameter at the patched site in both groups was approximately 35% larger than the adjacent ileum. Activities of sucrase and maltase were significantly less than controls in the muscle wall patch group. The serosal patch group had similar brush border enzyme activity of sucrase, maltase, and lactase. The authors concluded that both colon serosa and abdominal muscle wall are useful for support of the growth of functional neomucosa.

Using the same rabbit model, Thompson and Bragg [18] have also evaluated the influence of patch size on the ingrowth of neomucosa over colonic serosa and found that multiple smaller patches are covered more completely and rapidly than a single large patch. They also evaluated the feasibility of interposing a 5 cm tubular segment of colon serosa in various configurations to evaluate longitudinal ingrowth of neomucosa [19]. The only group that had survivors beyond 7 days had a stent placed at the time the serosal tube was

created. Only 40% of the tube was covered by mucosa at 6 weeks, and the 5 cm tubes had contracted to 3 cm.

In an innovative study, Saday and Mir [20] combined an intestinal lengthening technique with neomucosal growth to create neointestine. They opened the bowel longitudinally on the antimesenteric side and formed two tubes out of the bowel halves. Part of the mucosa in one lumen is regenerated neomucosa that had formed on the serosal surface of the common wall. The histology of the neomucosa resembled that or normal intestine with no significant differences in villus height and width, villi number and crypt depth.

A vital aspect of intestinal tissue engineering is the ability for intestine to regenerate. Although the exact regulation for intestinal growth and regeneration remains unclear, growth factors and cytokines appear to have significant effects. Numerous enterocyte mitogens have been investigated including epidermal growth factor, hepatocyte growth factor, fibroblast growth factor, neurotensin, growth hormone, interleukin-11 (IL-11), glucagon-like peptide-2, and glutamine.

Thompson and his colleagues [21] have attempted to improve the rate of neomucosal growth by infusion of systemic urogastrone in rabbits. Urogastrone and epidermal growth factor (EGF) have similar biological activities and a high degree of homology of amino acid sequence. They observed that the areas that are patched by serosa are covered more rapidly and completely when urogastrone is given. Additionally, there is significantly less contraction of the patched area. This effect was dose dependent and the lowest dose increased epithelialization without stimulating proliferative activity. Enhanced cell migration may be the earliest effect of urogastrone [22]. They also demonstrated that long-term administration results in increased quantity of neomucosa [23].

Because intestinal resection is thought to stimulate proliferation of the intestinal remnant, Thompson [24] performed 50% enterectomy in rabbits and found that neomucosal coverage of the serosal patch was significantly increased at 1 and 2 weeks but this advantage dissipated by 3 and 4 weeks. Because of contraction of the patched area, the total amount of neomucosa was not higher in rabbits that had bowel resection. Since luminal contents may affect intestinal regeneration, Thompson [25] also compared the regeneration of neomucosa in patched ileum left in continuity with the rest of the bowel versus a patched and bypassed ileal segment. All patched areas were similarly covered by neomucosa at 4 and 8 weeks. Histologically, the neomucosa was less developed in the bypassed group with significantly diminished villous height and significantly less disaccharidase activity. Interestingly, in vitro uptake of H^3 thymidine was significantly higher in neomucosa in bypassed segments. The authors speculated that the bypassed segment may have greater portion of dividing immature cells to account for the increased uptake of H^3 thymidine. Thompson [26] reported another example of luminal content influence on intestinal regeneration when they observed that neomucosal growth was better in the patched ileum when compared to the jejunum. This may represent differences in the luminal content, local humoral differences, or intrinsic growth and adaptive capacity between the jejunum and ileum.

Investigators have also performed studies in rats by using the peritoneum as a base for regeneration of neomucosa. An entire tube of functional neomucosa was generated in one study using the peritoneum [27]. Erez [28] performed an enteroperitoneal anastomosis and demonstrated that the peritoneum of a pig was also a capable site for generating neomucosa. They advocated the use of peritoneum because fluids and electrolytes can be absorbed through the peritoneum while waiting for the neomucosa to complete coverage of the patched area.

3. Prosthetic and biomaterials

Biomaterials may be an essential component for successful growth of new organs. Biomaterials serve as a cell delivery vehicle and provide a structure for tissue ingrowth. As a scaffold, biomaterials guide tissue growth and provide a transient base for cellular generation. They mimic natural extracellular matrix function by eliciting cellular responses that promote tissue growth and may aid in tissue organization. The three-dimensional structure may be engineered to guide tissue growth to fit a particular need. Biomaterials also may be used as a delivery vehicle for cells and/or bioactive factors. Direct injection of cell suspensions without biomaterials is difficult to control. Localization and organization of the transplanted cells are unpredictable. Additionally, many mammalian cells are anchorage-dependent and will not survive or proliferate without a cell-adhesion substrate. Bioactive growth factors as yet undefined may be present in biomaterials. Biomaterials can also be engineered to serve as a depot for the local release of growth factors.

The biomaterials selected must possess the proper mechanical and physical properties. They should be biocompatible, nontoxic and biodegradable. The material must support the growth of the tissue and yet be readily absorbed so that minimal inflammation and foreign-body response is elicited. Degradation products should not be toxic and should be readily removed by normal metabolic mechanisms. Ideally, the biomaterial should promote cellular interaction, growth, and tissue development. Cellular interactions with various biomaterials have not been characterized well. Preliminary investigations have shown that cell-adhesion ligands and soluble growth factors are present in this

microenvironment [29]. The ultimate success of tissue engineering may rest on the selection of the appropriate type of biomaterials.

The initial reports on the use of prosthetic materials as a patch for neomucosal growth were felt to be unsuccessful. In 1979, Cogan and his colleagues [30] used Dacron as a patch to repair defects created in the distal ileum of rabbits. They noted that the material acted as a bridge for ingrowth of intestinal tissue. Once the bowel wall regenerated, the prosthetic material was extruded into the lumen of the bowel. Similarly, Watson and his colleagues [31] at the Letterman Army Institute of Research reported on the use of polytetrafluoroethylene (PTFE) prosthetic tube in a dog model. They placed a tubular segment in continuity in the jejunum but found only minimal ingrowth of mucosa.

Fukushiwa and his colleagues [32] also experimented with a Dacron tube as a replacement for the esophagus in a dog model. They fabricated a silicon rubber tube surrounded with a Dacron mesh. The tubes measured 5–7 cm in length and 1.5–2 cm in diameter with a 1.5 mm thick wall. This tubular construct was placed as an esophageal replacement in 16 dogs. Seven of 16 dogs (44%) survived more than 12 months and four survived more than 6 yr. Interestingly, the tubes were extruded by 6 months in 6 of the 7 survivors, but it provided an adequate base in these dogs to allow thin layer of squamous epithelium to form. The submucosa near the anastomoses appeared similar to native tissue but the submucosa at the central portion of the prosthetic tube only had fibrous connective tissue without muscle or mucous glands. Nonabsorbable material appears to be a poor choice for tissue ingrowth. The regenerated tissue has architectural disarray and the best result is a stenotic tubular structure that appears to be useful only as a conduit.

Thompson [33] also investigated the use of prosthetic materials for generating neomucosa. He evaluated the feasibility of patching with Dacron, polyglycolic acid (PGA) mesh, and PTFE. By 8 weeks, both Dacron and PTFE grafts were extruded, but the PGA meshes were noted to have dissolved. The residual neomucosa only occupied 15% of the original defect. When Dacron tubes were interposed between two ends of small bowel, there was no evidence of neomucosal growth identified. Although the author did not make note of the significance, this was the first report on the use of an absorbable biomaterial (PGA) as a scaffold for ingrowth of neomucosa. However, the authors felt that prosthetic materials were not likely to be useful in clinical settings because the residual neointestine had contracted significantly when compared to the original patch. A criticism of this conclusion is that there was no physiologic reason for the intestine to remain dilated at the patched site. The patched bowel contracted because a dilated segment of bowel was physiologically unsound and inefficient. Persistence of dilated bowel results in dysmotility and stasis. If the authors wanted the patched area to remain enlarged, partially obstructing the bowel distal to the patched area would have provide a stimulus for such an effect.

Joseph Vacanti and his colleagues [34–37] in Boston have been pioneers in tissue engineering neointestine using an absorbable biomaterial. Their methodology consists of seeding intestinal organoids onto a scaffold made of polyglycolic acid and polylactic acid. The constructs are implanted into a rat's omentum where they develop into vascularized cystic structures resembling neointestine and the biomaterials are completely absorbed. Histologically, the neomucosa is characterized by a columnar epithelium containing goblet and Paneth cells. Crypt-like invaginations that resemble crypt-villus structures are also evident. This neointestine has the histologic appearance of small bowel and has been anastomosed to the native bowel without causing feeding problems. Brush-border enzymes, basement membrane components, and electrophysiologic properties similar to those of normal small bowel have been demonstrated to be present.

Vacanti's use of intestinal organoids is based on the work of Evans and Tait [38–40]. Evans and his colleagues have shown that disaggregated intestinal tissue (intestinal organoids) can be used to regenerate a neomucosa. The presence of progenitor cells, epithelium, and mesenchymal stroma in the disaggregated intestinal tissue allows for the formation of a three-dimensional organized neomucosa with the presence of all cell types. Intestinal organoids appear to be superior to intestinal cells for seeding the biomaterial because the organoids contain stromal elements in addition to crypt cells that appear to facilitate the generation of neointestine. Additionally, intestinal stem cells may also be present in this preparation. Vacanti has used intestinal organoids to develop a consistent and reproducible technique for creating neointestine. Vacanti's group [41,42] also has shown that seeding the polyglycolic acid/polylactic acid scaffold with processed stomach and colon organoids will result in the formation of tissues resembling these organs.

Our laboratory [43,44] has reported on the use of small intestinal submucosa (SIS) as a scaffold for creating neointestine. SIS is an extracellular matrix harvested from porcine small intestine and has been used in tissue engineering experiments in a variety of other sites [45–48]. Matsumoto [45] first described the use of inverted small intestine to replace large veins in dogs in 1966. Improved processing of this biomaterial has made it readily available for tissue engineering studies. Extensive in situ tissue remodeling has been demonstrated in both rat and canine models. It has been used as a scaffold for regeneration of the abdominal wall, urinary bladder, tendons, blood vessels, and dura

mater. As a biomaterial for visceral organ bioengineering, SIS has been most extensively studied as a scaffold for creating new bladder. It is commercially marketed as a scaffold for body wall repair and to assist poor wound healing. Human uses have included repair of abdominal wall defects, diaphragmatic hernias, tendon repairs, and as a coverage graft for compromised wounds.

We reported on preliminary studies using SIS as a patch for small bowel and esophageal regeneration in an in situ xenograft model [43,44]. Defects on the wall of the esophagus and small bowel in dogs were patched with SIS. After several weeks, the SIS scaffold dissolved as new growth of esophagus and small bowel were demonstrated. The defects that had been created were substantial and would have healed with a significant stricture if allowed to heal primarily without a patch. The SIS patch allowed the bowel to grow over the scaffold and maintain normal diameter. The necropsy specimens showed that the patched bowel had no narrowing and the histology of the neointestine resembled the native bowel with minimal architectural disarray. The use of SIS as a scaffold for intestinal engineering makes intuitive sense because it is an acellular matrix harvested from a gastrointestinal source.

Other studies using SIS, fibrin glue and polyglycolic acid as scaffold for tissue engineering neointestine have also been done in our laboratory. Fibrin glue is a naturally occurring biomaterial that is commercially available as a tissue adhesive (Tisseel) and has been most commonly used for controlling hemorrhage. The components are natural biological factors that include fibrinogen, thrombin, calcium chloride, and fibrinolysis inhibitor (aprotinin). For our experimental model, we used the three biomaterials alone and in combination to determine which would serve as better scaffold for intestinal regeneration. We seeded the constructs with intestinal organoids and implanted them into the omentum of a rat. The biomaterials served as cell-delivery vehicles in this model and we were successful in creating cystic structures that resemble native small bowel.

Others have also evaluated the use of resorbable material as scaffold for regeneration of neointestine. Hori and his colleagues [49,50] used a collagen sponge enzymatically processed from porcine skin as a scaffold for regeneration of stomach and small bowel. They placed the collagen sponge as a patch for gastric tissue regeneration in an in situ beagle model. They covered the luminal side of the sponge with a sheet of silicone to prevent digestion from the acidic fluid. At 16 weeks, the tissue-engineered stomach wall was highly organized with presence of the proton pump and a thin muscle layer. They have also shown that small bowel may regenerate over this absorbable scaffold. They resected a 5 cm-long segment from the jejunum from beagle dogs and reconstructed it by wrapping an acellular collagen material over a silicone tube stent. The stent was removed endoscopically at 1 month. By 4 months, the collagen sponge was resorbed and the luminal surface was covered by regenerated intestine.

Isch and his colleagues [51] have also performed esophagoplasty by patching defects in the cervical esophagus in a dog with AlloDerm. AlloDerm is human skin that has been processed to remove all epidermal and dermal cells while preserving the remaining biological dermal matrix. All the dogs survived without any leak or stricture formation. There is partial reepithelialization of the patch with neovascularization at 1 month. By 2 months, the mucosa has completely regenerated and the collagen framework has been absorbed.

4. Conclusion

Patients with short bowel syndrome have an extremely difficult lifestyle. They are confined to hyperalimentation with its attendant complications. Intestinal transplantation is becoming a less morbid therapy, but it remains complicated and is limited by donor availability. Although intestinal tissue engineering is a relatively novel field of study, the momentum is rapidly increasing as more investigators seek to find the ideal methodology for creating neointestine. This review summarizes much that has been done but important work remains to be done regarding the motility, absorption, innervation, and immune function of the bioengineered bowel. Animal models represent the best way to achieve an understanding of how we may create novel tissues. The use of resorbable biomaterials as cell delivery vehicles and as scaffolds to guide tissue regeneration is a vital component. As our understanding of how intestinal tissues regenerate improve, we can better select the ideal cell types to seed the construct. The studies reviewed in this report represent important advances in the field of intestinal tissue engineering and will lead to the eventual creation of tissue-engineered neointestine.

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