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Omental Incubation of Tissue-Engineered Small Intestine with Tubularized PLGA in a Rabbit Model

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ABSTRACT

Introduction and Objective: This study aimed to incubate the tissue-engineered small intestine (TESI) organoids in rabbit omentum with tubularized PLGA and to compare them with a control group.
Method: Twelve adult male white rabbits were equally divided into a study and control group. PLGA-formed 1-cm tubes were coated with 1:100 collagen type 1. Organoid units were isolated with full-thickness biopsies from the small intestine and were seeded onto PLGA polymer. Laparotomy was made by a midline incision and the omentum was prepared for implantation. A cell-seeded polymer tube was stented to secure the tubular structure, wrapped by the omentum, and fixed with unabsorbable sutures as the control group.
Results: Five animals (83.3%) survived both in the study and control groups. Examination of cell suspension on an inverted microscope revealed that the mesenchyme cell was surrounded by epithelial cells, cuboid cells with a centrally located nucleus and large cytoplasm, forming intestinal organoid units. The ratio of viable cells was 95% before seeding on polymers. The gross appearance of the tissue showed the formation of a sufficient vascular supply and an intact tubular structure. Examination of sections obtained from the study group, stained with H+E and Masson showed polymer fibers surrounding intestinal cells, epithelial cells located on the luminal surface, and enterocytes embedded in the connective tissue. Control group sections contained fibrotic tissue around intact polymer fibers.
Conclusion: TESI with orthotopic transplantation of the intestinal organoids provides a reasonable model to obtain intestinal function for the future of the procedure being investigated.

Keywords: Tissue-Engineered Small Intestine, Intestinal Organoids, Short Bowel Syndrome.

INTRODUCTION

Short bowel syndrome (SBS) may develop as a result of a decrease in the functional surface area of the intestine following congenital or acquired diseases and includes a wide variety of metabolic and physiologic disturbances; including fluid, nutrient, and weight loss seconder to insufficient length of the bowel. SBS is accompanied by excessive fluid and electrolyte losses and results in significant malabsorption of macronutrients, vitamins, and minerals. This affects the capacity to gain weight, grow, and develop normally and causes metabolic acidosis as well as biliary and renal calculi formation, liver disease, as well as other morbidities and mortalities (1-4).

The purpose of SBS management is to achieve intestinal autonomy, reduce long-term dependence on parenteral nutrition, and increase the functional capacity of the remnant bowel. Therapeutic approaches follow a sequential strategy and parenteral nutrition, the promotion of enteral feeding, and dietary supplementation, in conjunction with other medical treatments as first-line therapies (4, 5). Unresponsive patients require surgical management to palliate motility or malabsorptive symptoms, and to lengthen the remaining bowel (1, 3, 6). To provide an increased functional segment and a functional surface area for absorption and in order to decrease segmental dilatation, two successful options are longitudinal intestinal lengthening and tailoring (LILT) and serial transverse enteroplasty (STEP) (7, 8). Intestinal transplantation is an advanced management option for patients with refractory disease,

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complications, or contraindications to all other medical and surgical options (3, 9). Advancements in intestinal transplantation were noted due to significant refinements in surgical techniques, monitorization of the transplanted segment, medical management, and immunosuppression, so long-term outcomes have increased (10).

The regeneration of intestinal cells from pluripotent stem cells has recently led to a new therapeutic application for short bowel syndrome. Three-dimensional intestinal units are derived from stem cells and include different cell types such as epithelial cells, mesenchymal tissue, or other combinations called organoids. Intestinal organoids are able to develop macroscopic tubal structures with an intact epithelium lining on the inside, and cryptlike forms on the outer diameter and were studied both in vitro and in vivo (11-13). The complex architecture of the intestine and issues concerning transplantation, implantation, vascularization, or tissue scaffolds and the construction of enteral autonomy, peristaltic motion, endocrine, and immunologic functions remain limiting problems for the therapeutic potential of intestinal organoid units, with experimental research continuing apace on these issues (9, 14-16).

This experimental study aimed to incubate tissue-engineered small intestine organoids in rabbit omentum with tubularized PLGA and to compare them with a control group.

METHOD

All experiments in this study were approved and reviewed by the Animal Research Committee of Istanbul University (2010/41). Care and handling of the animals were in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Surgical Procedure

In this study, we used 12 adult male New Zealand white rabbits, equally divided between a study and control group. Surgical operations were performed under intramuscular ketamine (25mg/kg) and xylazine (5mg/kg) anesthesia with prophylactic intramuscular ceftriaxone (50 mg/kg) also administered.

Polymer Scaffold

Polymers were comprised of nonwoven PLGA (Biomedical Structures, Warwick, RI), which have a mesh thickness of 2 mm and a bulk density of 60 mg/cm3, and formed 1-cm tubes with outer and inner diameters of 6 mm and 2 mm, respectively. Polymer tubes were coated with 1:100 collagen type 1 (Vitrogen)/ Phosphate Buffered Saline (PBS) solution for 30 minutes at 4°C and washed with 500 mL of PBS, then sterilized with ethylene oxide.

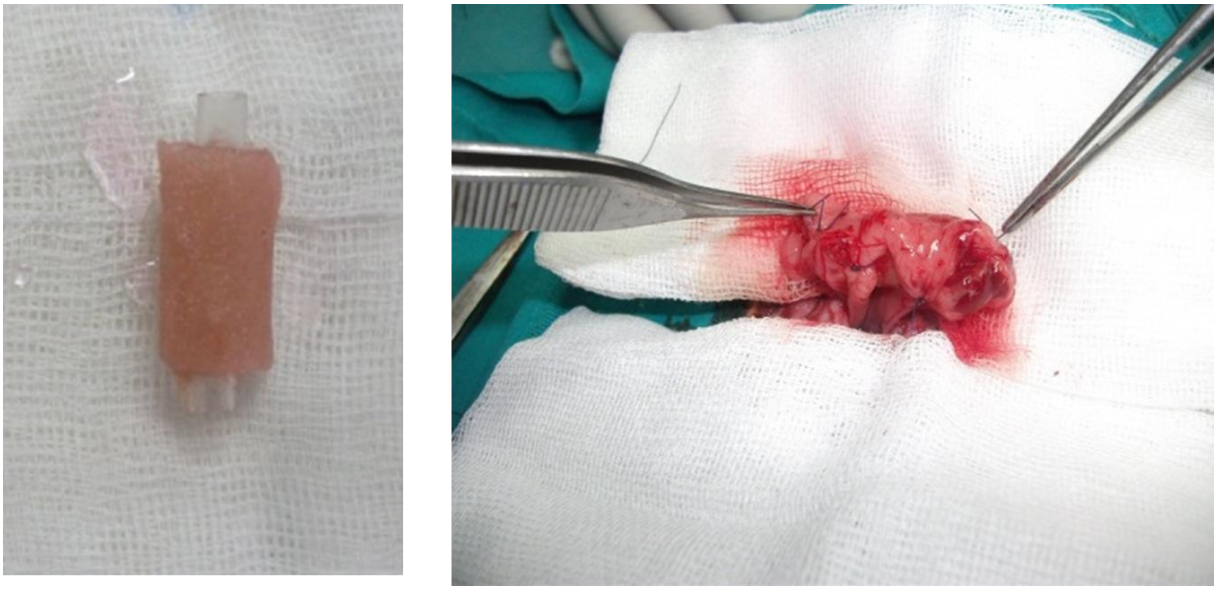
Organoid Unit Isolation And Seeding Onto Polymer

Full-thickness biopsies were obtained from the small intestine (10x5 mm in size) and washed with Hank's solution three times. Tissues were mechanically disrupted with the scalpel in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco BRL, Gaithersburg, MD) and then transferred into the enzyme solution containing 500 U/ml collagenase type I (Sigma Chemical Co., St. Louis, MO) and 0.1 mg/mL of dispase (Boehringer Mannheim, Indianapolis, IN) and placed on a shaker for 30 minutes at 37°C. Intestinal cell solutions were further purified by centrifugation in DMEM with a 2.5% fetal bovine serum. Organoid units were kept in BrdU, containing a solution for one hour at 40C in the incubator. BrdU-labelled cells were seeded onto the polymer and allowed to attach for 1 hour at 4°C before implantation.

Implantation

Laparotomy was made by a midline incision and the omentum was prepared for implantation. A cell-seeded polymer tube was stented to secure the tubular structure, wrapped by the omentum, and fixed with unabsorbable sutures (Figure 1). Acellular tubes were implanted and served as the control group.

Histological examination: After four weeks, the animals were sacrificed by intravenous sodium pentobarbital administration (200 mg/kg) and implants were harvested and prepared for histological examination. Cross sections of tubular tissues fixed in 10% formalin were routinely processed. 4-micrometer fine tissue sections were stained with Hematoxylin-Eosin and Masson and examined under a light microscope. Immunohistochemical detection of the BrdU-labelled cells was performed using anti-BrdU antibodies.



A **B**

Figure 1. A: Isolated and seeded organoid units onto the polymer, B: TESI during implanting in the omental surface.

RESULTS

Five of the animals (83.3%) survived both in the study and control groups. The rest of the animals were healthy at the time of the harvesting of the implants. Healing biopsy side of intestine was seen to be uneventful on second laparotomy. After mechanical disruption, intestinal cells were successfully isolated by enzymatic treatment. Examination of cell suspension on an inverted microscope revealed that the mesenchyme cell was surrounded by epithelial cells; cuboid cells with a centrally located nucleus and large cytoplasm, forming intestinal organoid units. Cell suspension was examined by trypan blue staining to assess cell viability. The ratio of viable cells was 95% before seeding on the polymers.

All implants in the study groups were patent at the time of harvesting. The gross appearance of the tissue showed formation of a sufficient vascular supply and an intact tubular structure.

Examination of sections obtained from the study group, stained with H+E and Masson, showed polymer fibers surroundING intestinal cells, with epithelial cells located on the luminal surface and enterocytes imbedded in connective tissue (Figure 2). Histologic analyses confirmed immature mesenchyme with subepithelial elements and muscular layers, and the intestinal epithelium with the presence of enterocytes, enteroendocrine cells, goblet cells, and Paneth cells (Figure 2). It was identified that implants were stained positive with anti-BrdU antibodies (Figure 3). Control group sections contained fibrotic tissue around intact polymer fibers.

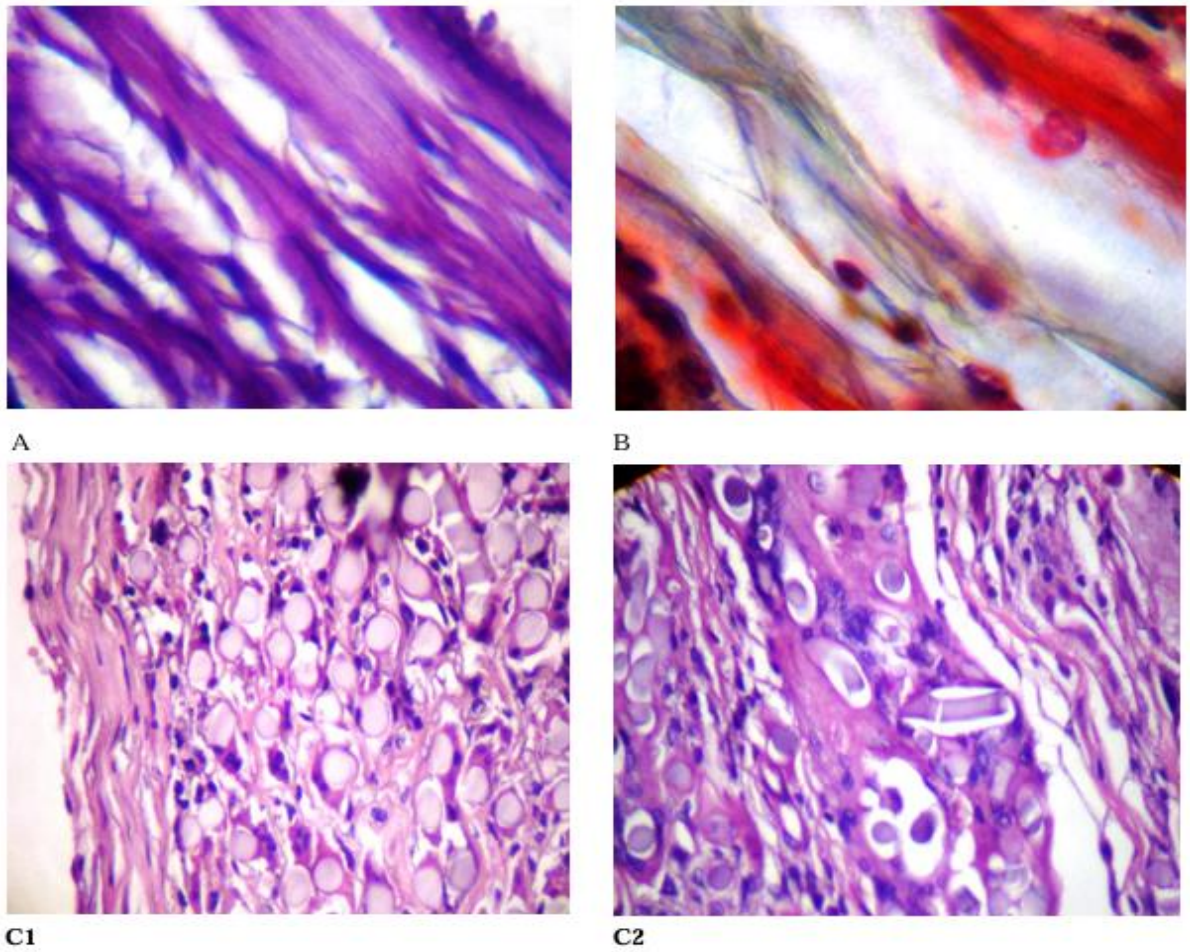
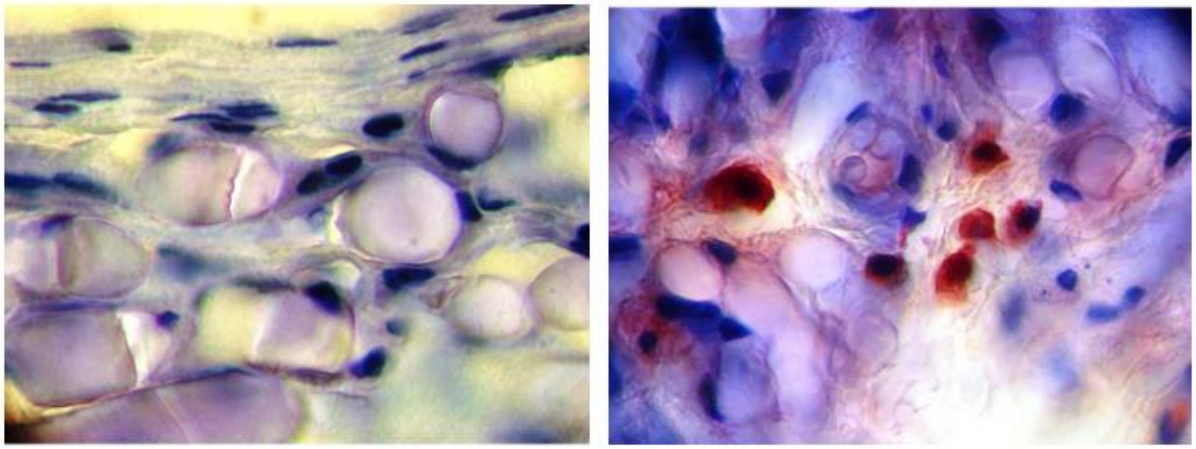


Figure 2. A: Control group; connective tissue cells around the omentum. B: Epithelial cells between abundant connective tissue fibers and connective tissue cells by Mason staining, C: Connective tissue fibers close to tubular lumen, epithelial cells between flattened regular tight connective tissue cells.



A: Control Group B: Study Group

Figure 3. A: Connective tissue cells with blue nuclei (BrdU -), B: Proliferating epithelial cells with red nuclei (BrdU +).

DISCUSSION

The results of this study demonstrated the feasibility of the isolation of intestinal organoid units and of creating a neo-intestinal structure using tissue engineering techniques. Organoid units reorganized on

the tubular scaffold and the omentum provided excellent vascularization and mobilization of the implant. In the field of intestinal tissue engineering, the complexity of the organ structure necessitates t that whole cell types of the tissue be obtained and propagated instead of selective cell isolation. The ideal tissue-engineered intestinal substitute would be anatomically and microbiologically constructed to mimic the structure and function of the native intestine. In addition, the tissue-engineered intestine would grow with the patient and require less maintenance or replacement, as a self-repairing and self-proliferating tissue replacement.

Currently, studies in the field of intestinal tissue engineering have focused on evaluating the potential of these alternative approaches including isolation, proliferation, tissue scaffolds, implantation, and transplantation as a neo-intestinal segment (15, 17). The first study on intestinal tissue engineering in the literature was reported by Vacanti et al. (18). They seeded fetal intestinal tissue samples on a non-woven flat polymer surface and implanted them into adult animals, then the activity of the intestinal cells was shown to be intact (18). Organoids are a cluster of diverse cells and originated from stem cells, and are capable of maintaining the functionality, molecular and cellular heterogeneity of the originating organ, such as the brain, retina, liver or intestine after specific processes and self-organization. Currently, improvements in intestinal organoid culture systems has pioneered in vitro studies of the small intestine epithelium, than followed by experimental animal studies. Additionally, it has been argued that developments in Tissue-Engineered Small Intestine (TESI) may prove promising and lead to the emergence of a new field in regenerative and personalized medicine (9, 14, 15, 19-21).

TESI technologies allow for organoid maintenance in long- term cultures and permit in vitro reorganization of basic crypt-villus physiology, so serve a comparable physiology with in vivo implantation. Initially, organoid systems were cultured on animal-derived extracellular matrices [mainly Matrigel and basement membrane extract (BME)], but, these are limited for the purposes of manipulation and clinical utilization (20). Natural and synthetic reproducible 2D and 3D biomaterials as well as tissue scaffolds [polyglycolic acid (PGA), polylactic acid (PLA), poly-ε-caprolactone (PCL), polylactic-co-glycolic acid (PLGA)] for in vitro architectural models for intestinal organoids were investigated for the hierarchical development of targeted form to organ (20-22). Biomaterials and scaffolds ideally promote cell reorganization for the architecture of functional tissue during the spreading and migration of implanted cells through the surrounding material, and facilitate adhesion, proliferation and differentiation similar to that in the in vivo microenvironment. In addition, while these biomaterials allow both the development of artificial organs with the help of in vitro organoid cultures and the in vivo implantation of these organoids, there are also experimental studies in which both types of research were carried out together (9, 20, 22, 23). The broad consensus within the literature with respect to the therapeutic usage of organoids is that heterotopic and orthotopic transplantation systems of intestinal organoids via synthetic or natural scaffolds are fast and inexpensive, with the models reasonable (15, 20, 24, 25).

Cortez et al. (26) presented a practicable and successful transplantation model for human intestinal organoids in mice mesentery as the host. They transplanted the human intestinal organoids into the mesentery of immunosuppressed mice after in vitro differentiation. After 10 weeks growing in mice mesentery, they were able to perform an organoid-to-intestine anastomosis in 6 of 20 mice (30%) and 50% of mice with anastomosis survived to 21 days at the time of harvest. In this study, the conveniences and difficulties of transplanted intestinal organoids are discussed by making comparisons with the kidney model developed in the retroperitoneum by the same study team (26). Liu et al. (27) demonstrated that 5-day-old newborn rats as cell donors were the best for producing TESI. They showed that TESI produced from fetal donors reached the biggest size, had an irregular surface, and had a mucosa consisting of multiple neomucosal cysts that occupied the lumen and protruded around the edge of the TESI (27). They also reported that the intestinal organoids developed the longest pedicle length with wrapping with the uterine horn membrane, and the shortest pedicle length with intestinal mesentery (23). The same study team also compared different scaffolds including polyglycolic acid (PGA), polycaprolactone (PCL), and collagen as scaffolds for TESI production. They found that tubular PGA

scaffolds had convenient pores, a mechanical structure, and an absorption time leading to the production of TESI with an architecture similar to that of native rat intestines (28).

The results of this study confirmed the creation of neo-intestinal structures using tissue engineering techniques in an orthotopic transplantation model. Organoid units reorganized on the PGA scaffold and omentum provided excellent vascularization.

Strengths and Limitations

The most important limitation of this study is that it cannot show any output for the functions of the TESI structure produced, as in the literature. In addition, other cell and system structures required for autonomous intestinal activity, such as the neurovascular components of the TESI structure, are not included in the histological analysis. Furthermore, the orthotopic transplantation of intestinal organoids is not new, and merely supports findings in the existing literature. Future studies should include the production and transfer of a functional TESI in an SBS model and should look to determine its effect on the disease.

CONCLUSION

TESI provides a model for dealing with intestinal function. Although there are some practical limitations in the application of this strategy in humans, continuous progress in this field may see this treatment emerge as an alternative approach in the near future. Currently, numerous research groups have been working on defining the physiology of this neo-intestine and on achieving various improvements in order to make this a clinically viable option. We speculate that this technique could represent a successful approach to replacing the small intestine when structural and functional deficiencies present.

DESCRIPTIONS

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