Press Release

Keio University

February 25, 2021
Keio University School of Medicine

Development of Transplant Therapy Gives the Large Intestine Digestive and Absorptive Functions Unique to the Small Intestine

Understanding and developing treatments for short bowel syndrome and other small bowel diseases

A joint research team at Keio University has developed a technology for creating a small intestinalized colon (SIC)—a large intestine with absorption unique to the small intestine and peristaltic functions. This was achieved by replacing the native colonic epithelium with ileum-derived organoids. (Note 1) The team was led by Professor Toshiro Sato and Assistant Professor Shinya Sugimoto at the Department of Organoid Medicine, Sakaguchi Laboratory, Keio University School of Medicine, and Project Professor Eiji Kobayashi at the Department of Organ Fabrication, Keio University School of Medicine.

The small intestine has unique hair-like projections called villi (Fig. 1), which play an important role in the digestion and absorption of food. In contrast, the large intestine does not have these kinds of protruding structures and can hardly digest or absorb nutrients. It has long been a mystery why these projection structures are created only in the small intestine. In this study, the team found that the epithelium inside the small intestine senses streams of intestinal juice and creates villous structures, and they were successful in culturing a villous small intestinal epithelial organoid. This discovery led to the development of a technology that enables the team to generate a “small intestinalized” colon (SIC) by replacing colon epithelium with small intestinal epithelium using organoid transplantation. By transplanting this SIC into a rat short bowel syndrome model, the team has shown, for the first time, that the SIC has a therapeutic effect on rats with short bowel syndrome.

Patients who have had a large portion of their small intestine removed due to Crohn's disease, intestinal torsion, or severe enteritis in the neonatal period develop short bowel syndrome, and the inability to adequately digest and absorb proteins, sugars, and fats leads to short bowel syndrome with poor outcomes. Intestinal transplantation currently remains the only treatment option for severe short bowel syndrome, but due to the shortage of donors and comparatively strong rejection response, the number of transplants remains low when compared to other organs. And so there has been a need for the development of regenerative medicine as an alternative treatment for small intestinal transplantation, but until now it has been impossible to create complex organs including blood vessels and lymph vessels that carry absorbed nutrients throughout the body. The SIC technology developed in this study transforms a different organ into a needed one and takes us one step closer to rejection-free organ transplantation through regenerative medicine. This study has also elucidated part of the origin of the projection structure of small intestinal villi, which is expected to lead to a better understanding of the pathogenesis of various small bowel diseases.

The results of this research were published in digital edition of the British journal *Nature* on February 24 (GMT), 2021.

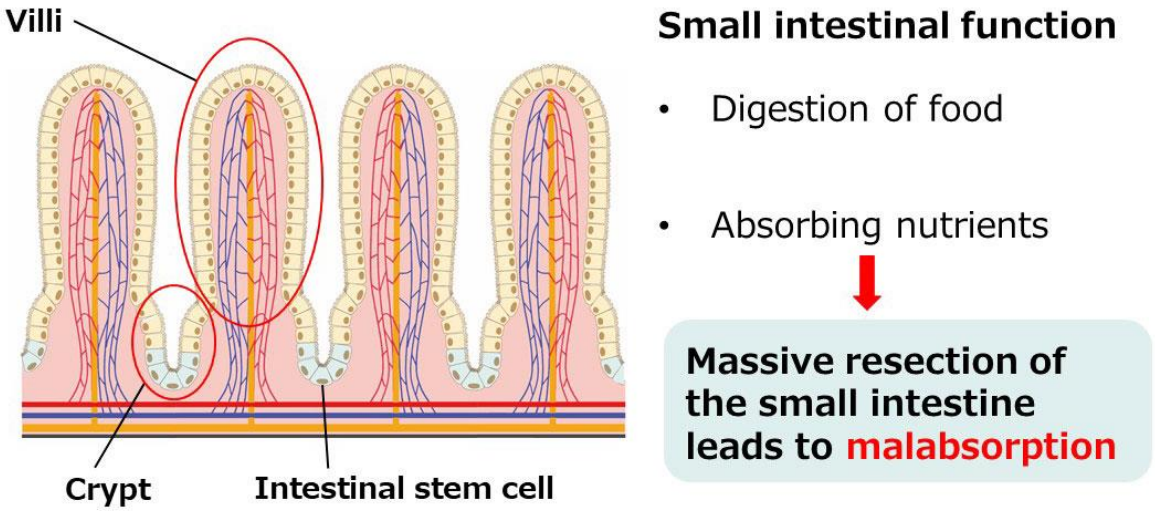
1. Research Background

A Long-Awaited New Approach to Small Intestinal Transplantation

The mucosa of the small intestine has the essential function of absorbing nutrients and water in order to sustain life. The surface of the small intestine is covered with epithelial cells, and for efficient digestion and absorption, there are numerous projections about 0.5 mm long called villi. Between the villi are indentations known as crypts. (See Fig. 1) The base of the crypt contains intestinal epithelial stem cells, which are responsible for the regeneration mechanism of the small intestinal epithelium and enable the fastest epithelial cell turnover in the body. Intestinal

epithelial stem cells continually replicate themselves throughout life (self-renewal), differentiate into all types of intestinal tissue cells (multipotency), and migrate from the crypt to the villi.

Fig. 1 Small intestinal structure and fuction



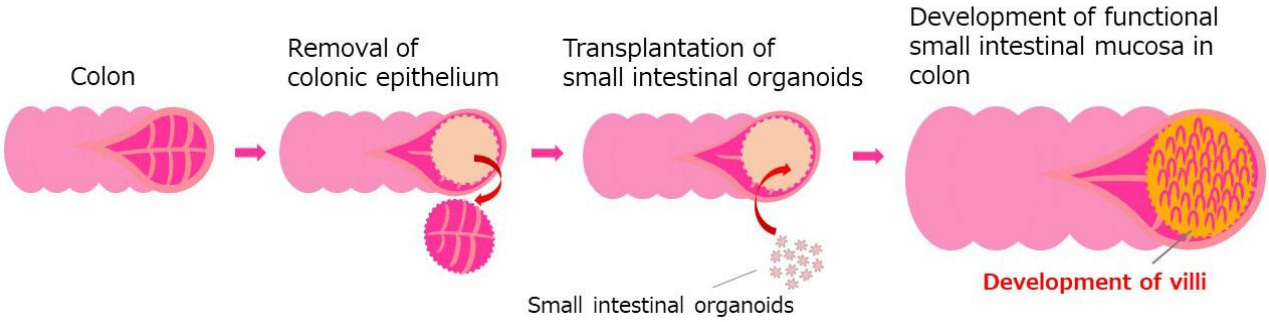
When a large portion of the small intestine is removed for diseases such as Crohn's disease, patients may develop short bowel syndrome, a serious and chronic malabsorption disorder. In severe cases, patients experience oral feeding difficulties, with intravenous nutrition given through a central venous catheter, which involves injecting nutrients into the veins through a tube. As a result, serious complications such as catheter-associated infection and liver dysfunction can lead to significantly reduced quality of life or even loss of life. Treatments for such cases include surgery to extend the length of the small intestine, and outside of Japan, treatment with GLP-2, a drug that stimulates the growth of intestinal villi. (Not yet approved for use in Japan) However, all of these treatments are inadequate and currently the only viable treatment is a bowel transplant, in which a healthy small bowel is transplanted from a donor. However, because the small intestine is more susceptible to rejection than other organs, the graft survival rate is low, and in Japan, only 32 small intestinal transplants (13 living and 19 brain-dead), in a limited number of facilities, were performed on 28 patients in the 20-plus years between the first operation in 1996 and December 2019. And even if a transplant is successful, there are still concerns about rejection, infections associated with the use of immunosuppressive drugs, and the development of malignant tumors, among other issues. For this reason, the number of small bowel transplants has remained limited, contrary to the increase in the number of transplants for other organs.

Currently, there is a great need for innovative new treatments for intractable small intestinal diseases for which there is no definitive cure, and expectations for regenerative medicine are high. However, since the small intestine is an extremely complex structure that includes blood vessels, nerves, muscle layers, and lymphatic vessels involved in the absorption of lipids—in addition to the structure of the crypt and villi—it is difficult to construct all of those pieces in vitro, and until now the idea of using regenerative medicine to treat small intestinal diseases has not been realistic.

Replacing the colonic epithelium with intestinal epithelium tissue for a functional small intestinalized colon

A research team led by Professor Toshiro Sato and Assistant Professor Shinya Sugimoto has previously established a technology that allows for the long-term culturing of intestinal epithelial stem cells prepared from intestinal epithelium as a three-dimensional mass of cultured cells known as an organoid (Sato T, et al. *Nature* 2009, Fujii M, et al. *Cell Stem Cell* 2018). Organoids are expected to have potential not only as a research tool for human epithelial cells in vitro, but also as potential transplantation cells, since they are epithelial stem cells cultured directly from human intestinal cells. However, as mentioned above, it has not been possible to create the human small intestine itself from a collection of human small intestinal epithelial cells, and it was technically difficult to transplant normal human intestinal epithelial cells. Recently, a research team has succeeded in developing a technique to construct healthy human colon epithelium in mice by completely removing the colonic epithelium of immunodeficient mice and replacing it with human colonic epithelium (Sugimoto S, et al. *Cell Stem Cell* 2018). We wondered if we could use this technology for regenerative purposes to replace the colonic epithelium with cultured small intestinal epithelium in patients with short bowel syndrome. (See Fig. 2)

Fig. 2 Generation of small intestinalized colon

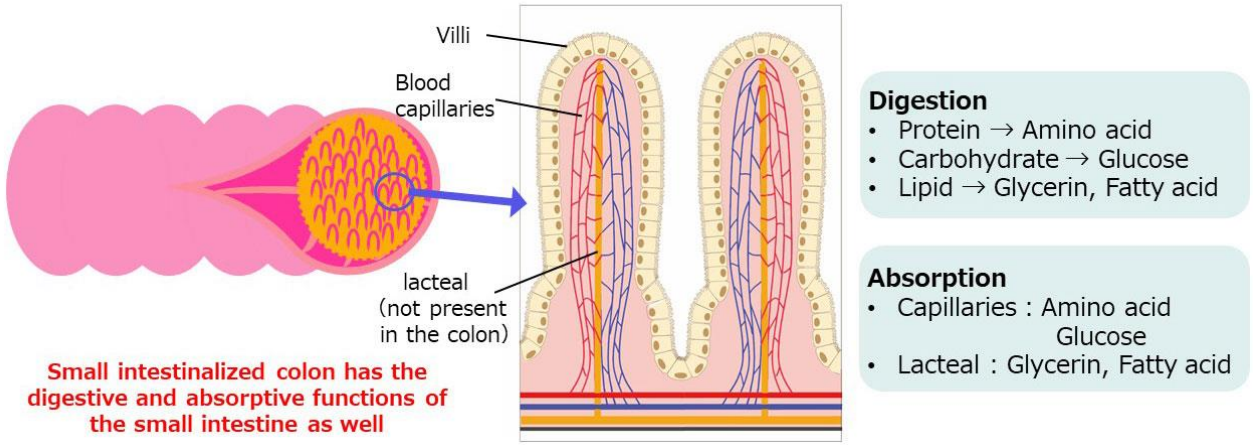


2. Research Significance and Future Development

Xenotransplanted human small intestinal epithelium re-builds villus structures in mouse colon

In this study, the research team first removed the colon epithelium of mice and attempted to transplant human small intestinal epithelium. As a result, the transplanted small intestinal epithelium formed a different villi structure from that of the transplanted colonic epithelium and showed expression of proteins related to digestion and absorption that are only found in the small intestine. The formation of microvilli, which is important for absorption, was also observed in transmission electron microscope imaging. Furthermore, we confirmed that small intestine-specific lymphatic vessels, known as lacteal, which are important for fat absorption, are also formed in the large intestine through the transplantation of small intestinal epithelium. This showed that the small intestinalized colon, with replaced epithelium, can digest and absorb nutrients and transport them into the body through the lacteal.
(See Fig. 3)

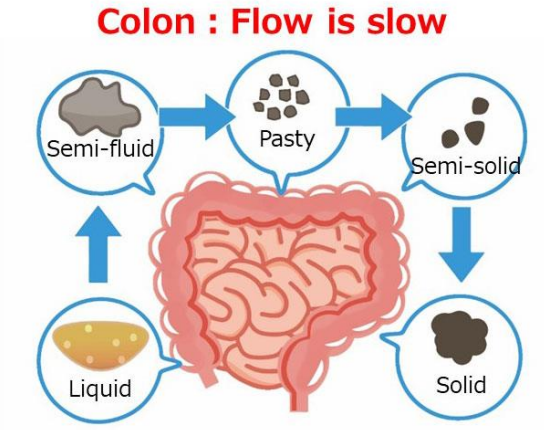
Fig. 3 Villus structure of the small intestinalized colon and its absorptive function



Luminal flow instructs villus formation

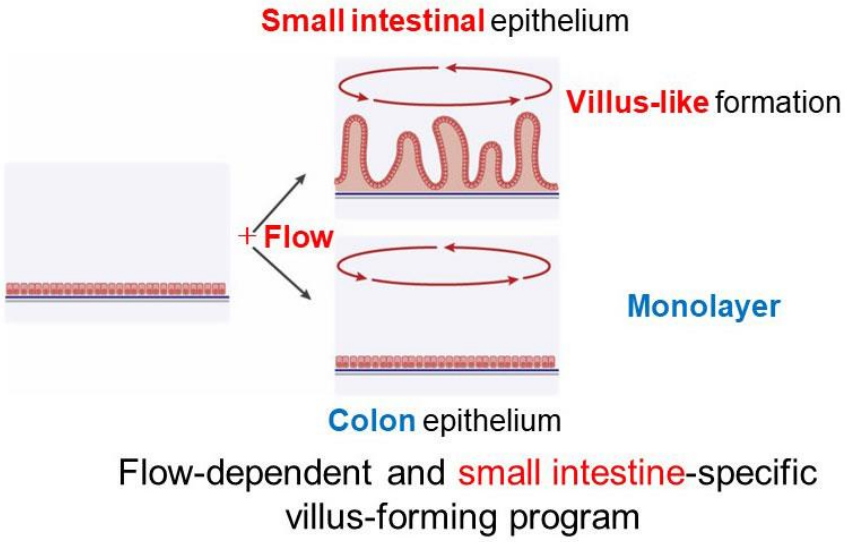
On one hand, the villous structure of the human small intestinal epithelium transplanted onto the colorectal surface near the anus of mice was immature and inadequate compared to the original. Luminal contents of the human intestine passes through the small intestine in a liquid state, and as it moves through the large intestine, it absorbs water and salts and solidifies into feces. (See Fig. 4) For this reason, we thought that the formation of villous structures may not be promoted near the rectum at the end of the large intestine due to insufficient “flow” of intestinal juice.

Fig. 4 Solidification of the luminal contents in the colon



The research team then cultured the organoids of the large intestine and the small intestine as a monolayer, artificially agitated the culture overlay medium to create a “flow,” and further cultured the organoids in this environment. As a result, the small intestinal organoids facilitated the formation of villus-like structures that protruded toward the lumen. On the other hand, colon organoids showed no such changes and remained flat, indicating the existence of a unique mechanism of villus formation in the small intestinal epithelium that is dependent on this digestive “flow.” (See Fig. 5)

Fig. 5 Villus formation of the small intestinal epithelium in 2D culture



Demonstrating the use of small intestinal epithelial organoids to treat short bowel syndrome in rats

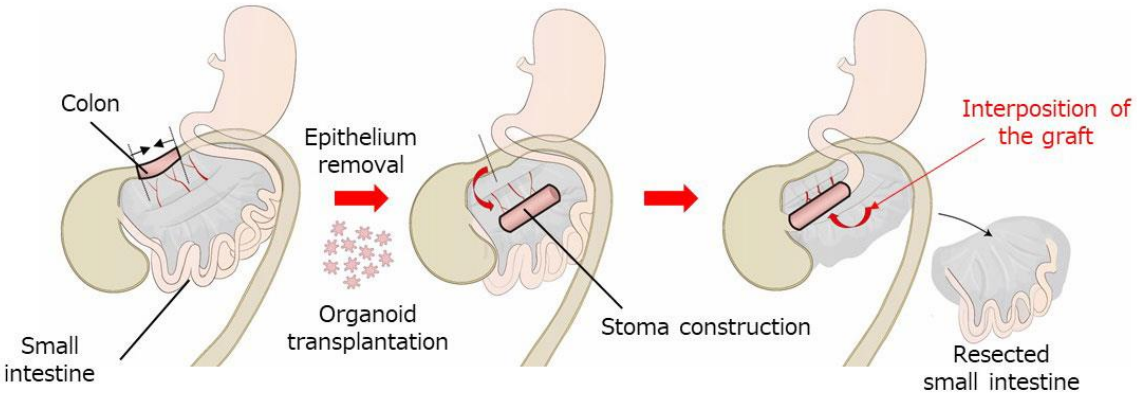
Based on these findings, the research team then conducted an experiment in rats to demonstrate that short bowel syndrome can be treated using small intestinal organoids. In collaboration with Project Professor Eiji Kobayashi of Keio University School of Medicine and Professor Yoji Hakamata of Nippon Veterinary and Life Science University, we sought to create a small intestinalized colon transplanted using small intestinal organoids established from transgenic rat small intestinal cells that express a luminescent substance called luciferase and then conduct transplantation experiments in healthy rats.

First, a part of the large intestine was cut away while preserving blood flow in the nutrient vessels. (See Fig. 6 left) During the surgery, the epithelium of the detached colon was removed and a small intestinal organoid was transplanted to the site. The grafts were then sewn to the abdominal wall and kept in the rat's body while maintaining blood flow until the epithelium began to grow and attach. (See Fig. 6 center) This technique allows the transplanted small intestinal epithelial cells to remain free of fecal passage, allowing them to wait for the transplanted small intestinal epithelial cells to become engrafted in the colonic epithelium without being shed, making it possible to transplant small intestinal organoids over a wide area.

However, when fixed to the abdominal wall, there was no “flow” on the surface of the small intestinal epithelium following transplantation, so sufficiently matured villi did not form. Therefore, in rats in which the whole small intestine has been resected to reproduce short bowel syndrome, the transplantation was performed in the terminal

ileum, which is adjacent to the small intestine and is where “flow” exists in the intestinal tract. The structure and function of the graft formed by ileum-derived organoid that differentiated and matured in a “flowing” environment following transplantation, and the effect of transplantation on short bowel syndrome, were compared between cases using small intestinal epithelial organoids and cases using colonic organoids. (See Fig. 6 right)

Fig. 6 Demonstration of therapeutic effects on the small intestinalized colon in rats



As a result, survival time was prolonged in ileum organoid-transplanted rats, and we observed extensive engraftment of transplanted cells and formation of villi in survivors. In contrast, we did not observe any prolongation of survival in the colon organoid transplant group. Similar to the experiments in mice, the grafts in the small intestinal organoid transplantation group showed formation of lacteal-like structures and blood vessels, as well as intestinal peristalsis with neurotransmission, and we confirmed them to be functional small intestinal grafts with the ability to absorb lipids, glucose, and peptides.

These results indicate that the small intestinal epithelium has the ability to change and redesign the structure of the transplanted tissue. This strategy of transplanting a small intestinalized colon, in which small intestinal organoids replace the colonic epithelium, is expected to be applicable in humans, who are larger than mice or rats, and is expected to pave the way for the development of new therapies using organoids that do not require immunosuppressive drugs. This research method can also provide new insights into the formation of villous structures of the small intestinal epithelium, an important structure for digestion and absorption, and is expected to provide clues to understanding the pathogenesis of various small intestinal diseases.

3. Notes

This research is an Advanced Research & Development Program for Medical Innovation (AMED-CREST) of the Japan Agency for Medical Research and Development (AMED): “Understanding of Pathophysiological Processes and Discovery of Medical Technology Seeds through Spatiotemporal Research of Tissue Adaptation and Repair Mechanisms,” Research and Development Objectives: ”Dissecting intestinal fibrogenic diseases by a newly developed 4D disease model system” (Principle investigator: Professor Toshiro Sato, Keio University School of Medicine) as well as the following programs:

- Japan Agency for Medical Research and Development (AMED)’s Research Center Network for Realization of Regenerative Medicine: “Center for development of mucosal regenerative therapies for inflammatory bowel diseases using cultured intestinal epithelial stem cells”
- JSPS Grants-in-Aid for Scientific Research (KAKENHI) JP20H03746 and JP17H06176
- The Mochida Memorial Foundation for Medical and Pharmaceutical Research
- Keio University Academic Development Funds

4. Research Paper

Title: An organoid-based organ-repurposing approach to treat short bowel syndrome

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Publication: *Nature* (online)

DOI：https://dx.doi.org/10.1038/s41586-021-03247-2

Glossary

Note 1: **Organoid**

Cultivated in the form of sheets using conventional cell culture technology. The term organoid refers to cultured cells grown as a three-dimensional structure using hydrogels as scaffolding and nutrients known as growth factors. From a single stem cell, it is possible to create a tissue-like structure in vivo and infinitely increase the number of healthy cells found in various tissues such as the stomach, small intestine, large intestine, pancreas and liver. In a previous study, the research team succeeded in culturing human intestinal epithelial organoids using a novel culture method that reproduced most of the differentiated cells found in living tissues. This method was also used in this study. It is also possible to culture organoids in sheet form, as shown in Fig. 5.

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