Original

Alternative non-oral nutrition in a rat model: a novel modified gastrostomy technique

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Abstract: The gastrostomy technique is essential for esophageal reconstruction using a scaffold. To date, there are no established methods to supply nutrients through a gastrostomy tube in rats. The purpose of this study was to analyze the feasibility of a newly modified gastrostomy technique for non-oral nutrition in an adult rat model. We modified the gastrostomy technique for adult rats in a few different ways. (1) The external opening for food injection was made at the midpoint between the ears to prevent damage due to self-harm behaviour. (2) An imbedded subcutaneous tunnel was created between the internal and external openings of the gastrostomy. We compared the efficacy and safety between groups with a T-tube for biliary drainage (TT group, n=14) and a conventional silicone Foley catheter (FC group, n=7) as optimal gastrostomy tubes for in a rat model. We also evaluated the feasibility of the heparin cap connector at the end of gastrostomy tube to control food supply in the TT group (with a cap, n=7; without a cap, n=7). No mortality was observed in the TT group with a cap, whereas most rats in the FC group died within 2 weeks after the procedure. Weight loss decreased significantly in the TT group with a cap compared with all the other groups. The appearance and attitude scores were significantly better in the TT group with a cap. In addition, histologic analysis showed that the TT group a cap showed a marked decrease over time in tissue fibrosis and macrophages compared with the other experimental groups. Therefore, gastrostomy using a silicone T-tube plugged with a cap proved to be a stable and effective option for non-oral feeding in an adult rat model.
Key words: esophageal reconstruction, gastrostomy, rat model, upper digestive tract cancer

Introduction

There is certainly a need for gastrostomy in a variety of animal models for human disease [1]. Pharyngo-esophageal transplantation is emerging as an important therapeutic approach in the field of tissue engineering, due to the increased incidence of upper digestive tract cancer [2–4]. Anastomosis site leakage and necrosis of the implanted substitutes inevitably causes mortality because the mediastinum and neck compartments become contaminated [5, 6]. Therefore, it is extremely important to prevent ingestion of food or saliva into the wound, and a nasogastric tube should be inserted for 1 to 2 weeks after surgery. However, a method of performing nasogastric intubation in such cases has not been established in rat models. Diverse intragastric infusion techniques, including the Janeway technique and percutaneous endoscopic gastrostomy (PEG), have been widely used over the past 20 to 30 years in human patients with upper digestive tract cancer [7–9]. However, the application of these techniques to rodent (mouse and rat) models has met with comprehensive complications caused by damage to devices as result of spontaneous self-biting. Several studies have investigated the effects of devices in the gastrointestinal tract with respect to safety and long-lasting effects [10–13]. Ueno *et al.* re-

ported a mouse intragastric infusion model with a flow-through swivel system for physiological and metabolic studies [10]. However, they did not examine the possibility of its application to a rat model. To date, most gastrostomy studies have been performed on neonatal rats or mice, and none have been successful in adult rats [14, 15]. This is because the rats were so active and uncontrollable that they could not maintain the feeding tube in the stomach for a long time. Therefore, there is a great demand for new gastrostomy models in the rat to explore the application of bioengineering of the pharyngoesophagus. The purpose of this study was to analyze the feasibility of a newly modified gastrostomy technique for non-oral nutrition on two fronts: 1) as a gastric delivery route that allows for the provision of various nutrients through the rat stomach for a long time and 2) as a rat gastrostomy method in which intragastric devices can be maintained in adults rats that are extremely active and have uncontrollable personalities. In this study, we chose two types of intragastric tracts (the Foley catheter and T-type tube) for the determination of an appropriate rat gastrostomy method (Fig. 1A). We also evaluated the efficacy of these devices after tube insertion into the rat stomach to determine whether the newly modified gastrostomy technique is feasible for rat survival without oral feeding.

Materials and Methods

Materials and animals

We modified the gastrostomy technique for adult rats in a few different ways. (1) The external opening for food injection was made at the midpoint between the ears (the blind spot for self- harm behavior) (2) An imbedded subcutaneous tunnel was created between the internal and external openings of the gastrostomy. We compared the efficacy and safety between a T-tube for biliary drainage and a conventional silicone Foley balloon catheter as optimal gastrostomy tubes for a rat model. We also evaluated the feasibility of a heparin cap connector at the end of the gastrostomy tube to control food supply in the T-tube insertion group. Because the Foley catheter has a large 2-way port, it was easy for the rats to damage it through self-harm behaviour. Therefore, we could not apply a cap in the Foley catheter group to prevent food backflow.

Foley balloon catheters were purchased from Sewon Medical Co., Ltd. (Cheonan, Korea). A T-type gastrostomy tube was created from a two-way silicone tube (Sewoon Medical Co., Ltd.). A rubber ring was mounted onto the end of the T-tube (Fig. 1B). Injection port caps (Heparin caps) were purchased from BD (Seoul, Korea). Sprague Dawley (SD) rats weighing 400–420 g were chosen as the animal model to evaluate the intragastric infusion techniques. The SD rats were supplied by Orient Bio, Inc. (Seongnam, Korea).

Establishment of the animal model (surgical procedure)

All surgical procedures were performed according to the appropriate guidelines. This study was carried out in strict accordance with the guidelines of the Animal Research Committee, Seoul National University Hospital. All protocols and experimental design parameters were reviewed and approved by the Institutional Animal Care and Use Committee of the Seoul National University Hospital (IACUC No. 17-0164-S1A0). SD rats were randomly assigned to one of three groups: a Foley cath-
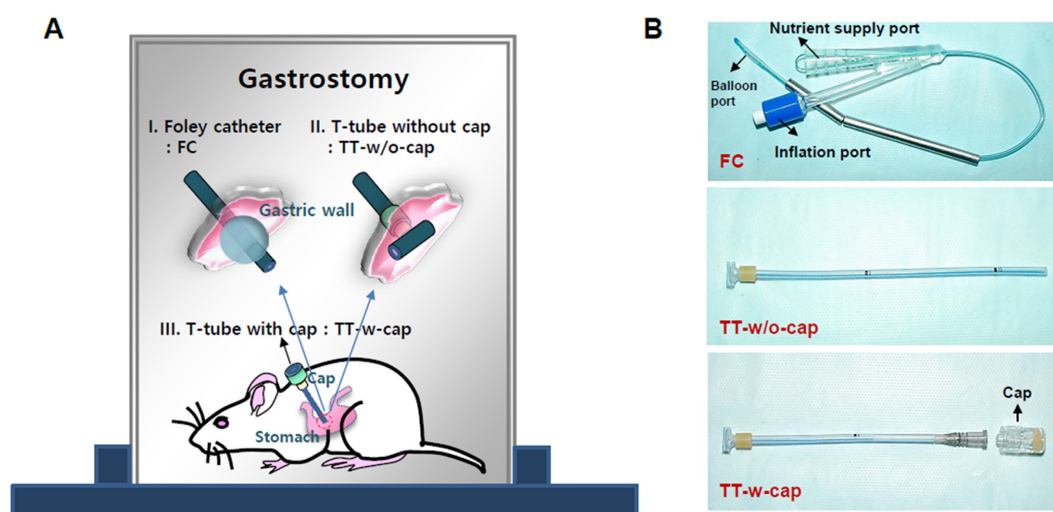

Fig. 1. Gastrostomy techniques. (A) Schematic diagram showing three types of gastrostomy techniques in the rat model. (B) Tube gastrostomy apparatus having different components.

eter insertion group (FC group, n=7), and two T-type tube insertion groups, one with a heparin cap connector at the end of the gastrostomy tube to control food supply (TT-w-cap group, n=7) and one without it (TT-w/o-cap group, n=7). The animals were allowed free access to standard chow and water. Twenty-four hours prior to surgery, they were restricted from food. Surgical procedures were performed under anaesthesia induced by an intramuscular injection of Zoletil (20 mg/kg dose; Virbac Laboratories, Carros, France) and 2% xylazine hydrochloride (5 mg/kg; Rompun, Bayer Korea, Seoul, Korea). A small midline incision was made, and the stomach was exposed. A fixed suture was placed for the gastrostomy using a Foley catheter, and the anterior gastric wall was lifted using forceps. An orifice into the stomach was then made with a 19G needle. The Foley catheter was then placed into the stomach, and air was blown into the catheter using a syringe. A 4/0 Vicryl purse string suture was then immediately performed (Fig. 2). One millilitre of saline was injected through the catheter into the stomach to check for any leakage. The funnel part of the catheter was gently pulled from the dorsal side. The peritoneal cavity and the abdominal skin were closed using 4/0 Vicryl in an interrupted suture and a continuous suture, respectively. Finally, the dorsal skin was closed with 2/0 Vicryl in an interrupted suture. The Foley catheter tubing was covered with a stainless steel sleeve to prevent damage due to self-harm behavior.

For gastrostomy using the T-tube with or without a cap, surgery was performed under anaesthesia, and a midline incision was made to expose the stomach. A 3-mm incision was made in the gastric wall and opening of the stomach. The tip of the T-tube was then inserted into the stomach. Next, 4/0 Vicryl was used to suture around the orifice in the gastric wall and tubing (Fig. 2 and Supplementary Video 1). Bleeding was flushed away with sterile saline. The tube was pulled back to the dorsal side through the tunnel under the skin and fixed to the dorsal skin with a 2/0 Vicryl suture. The abdominal wall incision and the skin were closed with 4/0 Vicryl. A heparin cap was used to control food injection and prevent the gastric contents from flowing backward (Fig. 2). An angiocatheter (Angiocath, BD, Franklin Lakes, NJ, USA) was used to connect the heparin cap and tube. Perioperative antibiotic gentamicin (20 mg/kg; Septopal®, Biomet Orthopedics, Warsaw, IN, USA) was administered subcutaneously at the end of the procedure.

All rats were housed individually in cages obtained from our animal facility. Liquid feed was supplied through the gastrostomy site 3 times/day at regular time intervals. Detailed nutritional information is provided in Supplementary Table 4. Body weight, feeding, and general physical status (appearance and attitude) of the animals were monitored daily. Both experimental and control rats were evaluated by one of the authors for appearance and attitude using a 5-point scale (Table 1) [16]. Tissue flaps at the gastrostomy site were harvested for histological analysis at the time of death. We set up humane endpoints in the planning stage of the animal studies [17, 18]. If severe pain, abnormal posture, respiratory disorders, or vocalization problems were observed during animal testing, chemical relief agents, such as
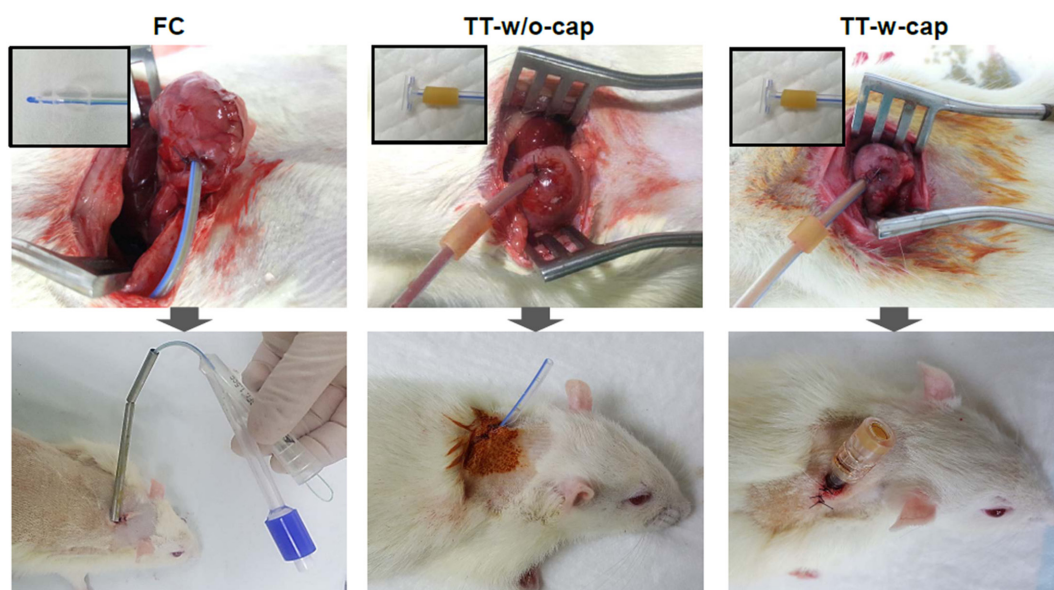

Fig. 2. Surgical procedure for implantation of each gastric catheter. A puncture hole was made in the middle of the forestomach, and the catheter tip was inserted into the forestomach. The inlet part of the catheter was positioned along the dorsal midline incision.

Table 1. Appearance and attitude scales

Score	Appearance	Attitude
5	Normal; normal skin tent and posture	Normal; active in cage prior to and during handing
4	Skin tent present on dorsum	Decreased activity, but alert, responsive to handling
3	Hunched posture, piloerection present, moderate skin tent	Lethargic, decreased resistance to handing
2	Eyes sunken in, piloerection and skin tent severe	Nonresponsive mouse only moves when touched
1	Failure to right itself	Failure to flee when hand is presented in cage

analgesics and sedatives, were provided. If an animal's weight decreased sharply by more than 20% within several days, the animal was euthanized after discussion with a veterinarian. At 4 weeks post-operation, all rats in the TT-w-cap group in good condition were euthanized by an overdose of CO_2 gas.

Blood test

On the 7th and 28th days after surgery, rats were anaesthetized, and blood samples were collected into ethylenediamine tetraacetic acid (EDTA) anticoagulant bottles (Vacutainer, BD) for the evaluation of various blood parameters: blood urea nitrogen (BUN), white blood cell (WBC), hemoglobin (HGB), and albumin (ALB). Blood tests were performed by 2 methods, as follows: biochemical test for BUN and ALB and a complete blood count (CBC) analysis for WBC and HGB. Biochemical tests were performed using a Hitachi 7180i Clinical analyzer (Hitachi High-Tech Corp., Tokyo, Japan). Complete blood count was determined using an ADVIA 2120i haematology autoanalyser (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). All samples were analysed within 30 min of collection. Each analysis was done according to the manufacturer's operational guidelines.

Urinalysis

Urine samples collected in the cages were transferred into urine transport vials. Next, the various contents of the urine samples were determined using a Urine Chemistry Analyzer (Clinitek Advantus, Siemens Healthcare Diagnostics).

Histopathological assessment of fibrous tissue and inflammation

Tissue flaps at the gastrostomy site were rapidly isolated and fixed in 4% paraformaldehyde. The sample tissues were processed routinely and embedded in paraffin wax. The embedded specimens were sectioned (5 mm) along the longitudinal axis of tube insertion. The sections were deparaffinized in xylene and rehydrated using an alcohol gradient. To observe fibrotic tissue formation around the intubation site, tissue sections were stained with Masson's trichrome stain. Histological images were obtained in triplicate for each group using a light microscope (Olympus, Tokyo, Japan). Three sites were randomly chosen from each specimen. Thicknesses of fibrotic scar tissue and collagen-positive area were then quantified using the ImageJ software (U.S. National Institutes of Health, Bethesda, MA, USA; n=5 per group).

To examine the inflammation response at the gastrostomy tube placement site, the section slides were deparaffinized, rehydrated, and then microwaved to retrieve the antigen. Tissue samples were treated with 3% hydrogen peroxidase to block endogenous peroxidase and then exposed for 1 h at room temperature to 10% bovine serum albumin (BSA) to block nonspecific binding. Samples were subsequently incubated with a mouse anti-F4/80 monoclonal antibody (1:200; Santa Cruz Biotechnology, Dallas, TX, USA) overnight at 4°C. The antigen-antibody interaction was conjugated with an avidin-biotin-peroxidase complex staining kit (ABC kit, Vector Laboratories, Burlingame, CA, USA) and then visualized via a DAB kit (Vector Laboratories). Cell nuclei were counterstained with Mayer's haematoxylin (violet colour). The number of F4/80-positive cells was counted from five different fields (×400 magnification; n=5 per group).

Statistical analysis

Statistical analysis was performed with the Tukey-Kramer post hoc test using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). The data obtained from each study group were averaged and expressed as the mean ± SD. Differences were considered statistically significant at $P<0.05$ or $P<0.01$.

Results

Weight loss studies after insertion of tubes

All experimental procedures were complete by the 28th of the experiment. The clinical conditions of the experimental rats were monitored daily, and humane endpoints were discussed with a veterinarian based on the general conditions of the rats (Supplementary Tables 1–3). In the FC group, the physical condition of the rats rapidly deteriorated beginning 5 days after surgery on

average. At this point, the majority of the animals showed rapid weight loss due to damage to the gastrostomy device as a result of self-harm behaviour. On the other hand, the animals in the TT-w/o-cap group rapidly deteriorated beginning 11 days after surgery. We euthanized the FC and the TT-w/o-cap groups at days 7 and 14, respectively, on average based on humane endpoint guidelines [17, 18]. On the other hand, no mortality was observed in the TT-w-cap group. The FC group was autopsied immediately to confirm the cause of death, which was primarily found to be peritonitis due to a deflated balloon in the gastric wall and the peritoneal leakage of gastric contents from the gastrostomy site. The Foley catheter of one rat also fell out of the incision site. The health of the rats in the TT-w/o-cap group began to deteriorate within 2 weeks, and these rats presented with various complications due to tube regurgitation of gastric contents. Weight loss was determined as the absolute change from the initial weight (Fig. 3A). There was no significant difference between the FC and TT-w/o-cap groups. Weight loss in the TT-w-cap group was significantly different from that in all the other groups at 1 week after the procedure. All animals in the TT-w-cap group gradually began to maintain their weight at 2 weeks after the procedure.

Nutritional grading system of the animal model

At 1 week post-surgery, the physical conditions of the animals were examined by direct observation for appearance and attitude. Scores were blindly evaluated according to the appearance and attitude scales, as shown in Table 1. The appearance score was significantly better in the TT-w-cap group compared with that of the FC group at 1 week after gastrostomy tube placement (Fig. 3B). The attitude score also demonstrated a similar tendency (Fig. 3C). In particular, the TT-w/o-cap group showed a sharp decline in both appearance and attitude scores compared with the TT-w-cap group at 2 weeks after gastrostomy (Supplementary Tables 2 and 3). These results suggest that the physical activity of the rat model can be maintained via a stable supply of nutrients using TT-w-cap gastrostomy.

Blood parameters over 28 days after gastric intubation

The values obtained for the BUN, WBC, HGB, and ALB blood parameters at 0, 7, and 28 days after the procedure are shown in Fig. 4. Both the FC and TT-w/o-cap groups showed statistically significant increases in BUN and WBC ($P<0.01$) compared with the levels in the normal group at 7 days. This indicates that both the TT-w/o-cap and FC groups might have experienced serious dehydration due to the inflammation in the abdominal region. In addition, HGB was significantly lower ($P<0.05$) in the FC group than in the normal group, but only at 7 days. However, the TT-w-cap and TT-w/o-cap groups did not show a change in HGB level with time. Total ALB was significantly lower in all experimental groups than in the normal group at 7 days. These results demonstrated that initial fasting after tube gastric intubation resulted in initial hypoalimentation in the animal model.

Urinalysis for assessment of the nutritional status

A small amount of bilirubin was detected in the FC group on day 7 (Table 2). A trace amount of ketone was
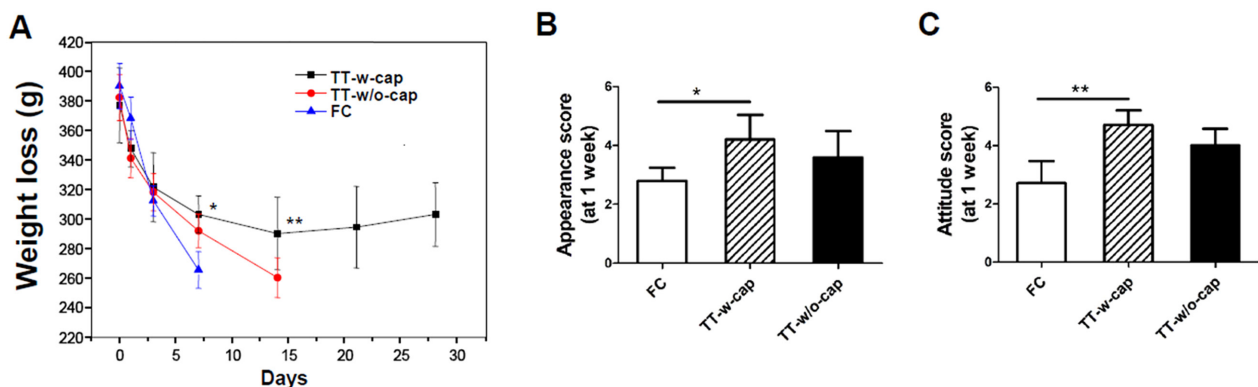

Fig. 3. Weight loss and physical condition after insertion of tubes. (A) Weight loss was determined as the absolute change from the initial weight. The FC group showed rapid weight loss after 3 days post-operation. The FC group was subsequently euthanized at an average of 1 week after the procedure based on humane endpoint guidelines. Similarly, the TT-w/o-cap group was euthanized at an average of 2 weeks after the procedure. Weight loss in the TT-w-cap group was significantly different from that in the other groups at 1 week after the procedure, with the rats gradually beginning to maintain their weight at 2 weeks after the procedure (*$P<0.05$). (B) Appearance score at 1 week after gastrostomy tube placement. The appearance score was significantly better in the TT-w-cap group than in the FC group. (C) Attitude score at 1 week after gastrostomy tube placement. The TT-w-cap group also had a significantly better attitude score than the FC group (**$P<0.01$).
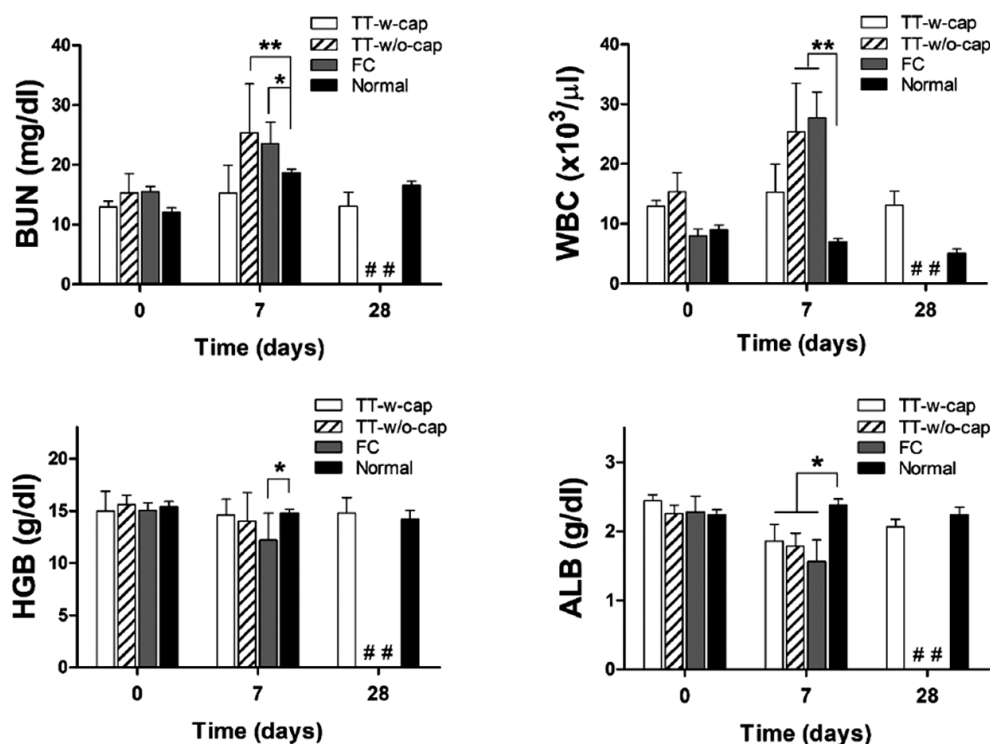
Fig. 4. Blood parameters over 28 days after each gastrostomy. BUN was higher in the TT-w-cap and TT-w/o-cap groups than in the normal group after 7 days. The WBC count was also significantly higher in the TT-w/o-cap and FC groups than in the normal group. Only the FC group showed a significantly lower HGB level than the normal group at day 7. Of note, total ALB was significantly lower in all experimental groups than in the normal group after 7 days (*P<0.05; **P<0.01).

Table 2. Urinalysis results

Test parameters	Sensitivity					
	Foley catheter		T-tube (w/o cap)		T-tube (w cap)	
	1 day	7 days	1 day	7 days	1 day	7 days
Bilirubin (mg/dl)	Neg	Small	Neg	Neg	Neg	Neg
Ketone (mg/dl)	Trace	Neg	Neg	Trace	Neg	Neg
Blood (Ery/µl)	Large	Trace	Large	Trace	Large	Neg
Protein (mg/dl)	100	≥300	100	≥100	100	Trace
Nitrite (mg/dl)	Pos	Neg	Pos	Neg	Pos	Neg
Glucose (mg/dl)	≥1000	≤250	≥1000	≤250	≥1000	Neg
Urobilinogen (E.U./dl)	≥1.0	≤0.2	≥1.0	≤0.2	≥1.0	≤0.2
Specific gravity	≥1.030	≤1.010	≥1.030	≤1.010	≥1.030	≤1.010
pH	6.25 ± 0.25	7.5 ± 0.5	6.25 ± 0.25	8.25 ± 0.25	6.5 ± 0.25	7.5 ± 0.5

detected in both TT-w/o-cap and FC groups during the initial experimental period, whereas no ketones were found in the TT-w-cap group. Urinary blood also decreased in all groups over time. A large quantity of protein was found in the urine samples of the FC group. The nitrite, glucose, and urobilinogen levels showed an overall decline with time. Although the specific gravity and PH levels were abnormal in all experimental groups shortly after tube gastrostomy, they quickly recovered. The results of the TT-w-cap group for most of the urine parameters were negative at 7 days. These results demonstrate that nutrition supply was most effective with gastrostomy using a T-tube with a cap.

Representative gross appearance and histological evaluation

Examination of the groups at 4 weeks after the gastrostomy procedure revealed that all groups had been successfully intubated via the gastric wall. However, serious peritonitis was observed in both the FC and TT-w/o-cap groups, as confirmed by microscopic examination (Fig. 5). On the other hand, no granulomatous inflammation in TT-w-cap group was present around the intubation site. To investigate the histological stability

of the intubation site, we performed Masson's trichrome staining for tissue sections at 1, 2, and 4 weeks, respectively (Fig. 6A). The FC group demonstrated the formation of thick fibrotic scar tissue around the outer region of gastric wall at 1 week. A quantitative analysis revealed that there was significantly more scar tissue in the FC group than in the TT-w/o-cap group (Fig. 6C). In the FC group, leakage might have occurred due to the rupture of the balloon fixed on the stomach wall. This was likely caused by the rats biting the insertion port at the end of the Foley catheter. Furthermore, collagen deposition associated with fibrosis (blue) was significantly higher the FC and TT-w/o-cap groups than in the TT-w-cap group (Fig. 6D). During the entire period, the TT-
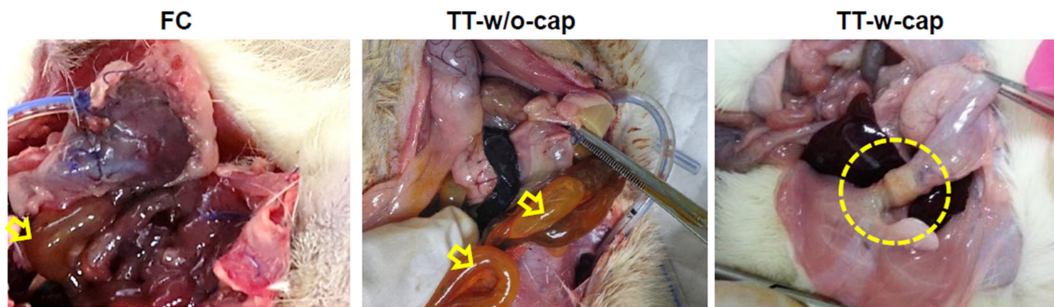

Fig. 5. Gross appearance around the gastrostomy site. Representative gross appearances of the entire gastrostomy site for each type of gastrostomy tube after 1, 2, and 4 weeks. Serious peritonitis (arrows) was observed in both the FC and TT-w/o-cap groups, as confirmed by microscopic examination. The catheter tip is indicated by a yellow circle in the TT-w-cap group.
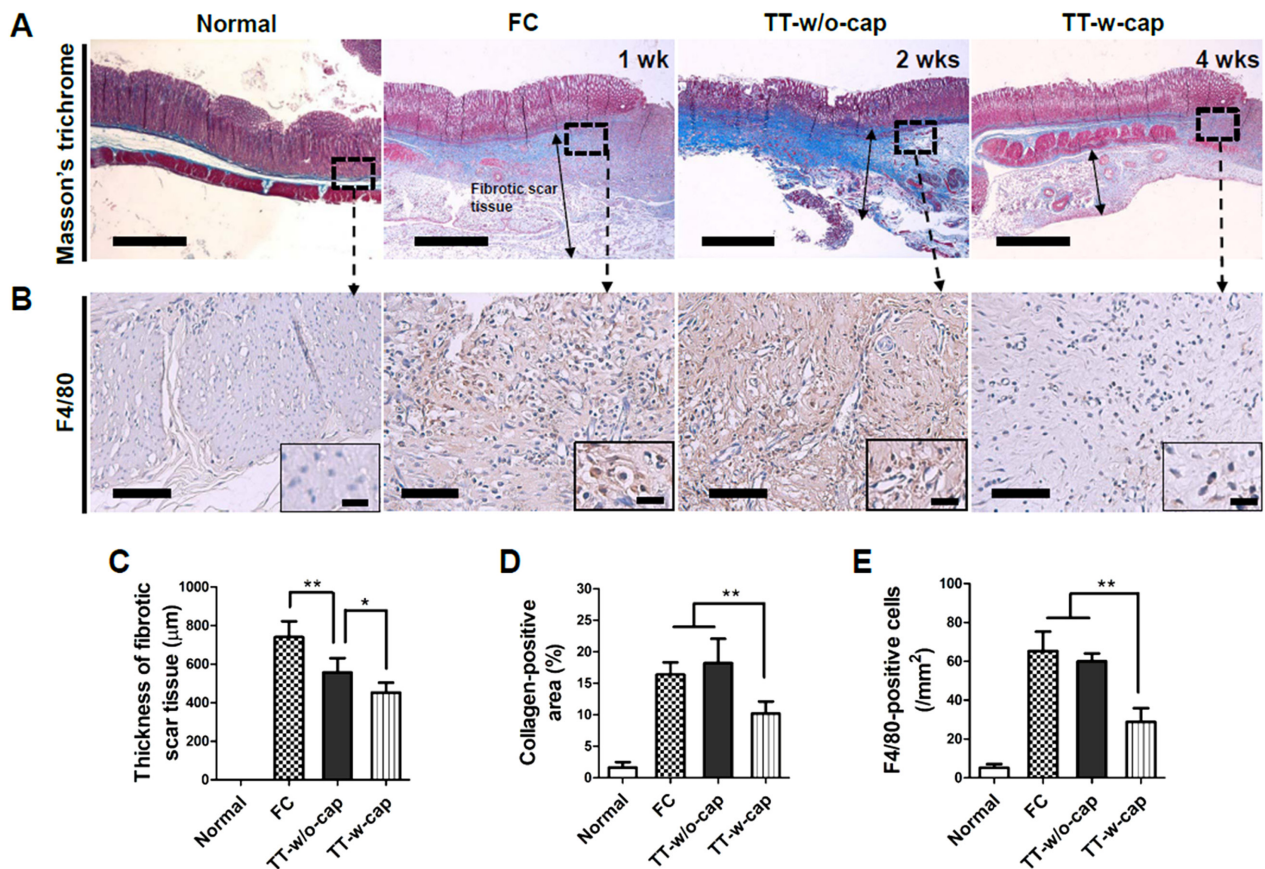

Fig. 6. Histological evaluation around the gastrostomy site. Collagen deposition (A) and macrophage expression (B) at the graft site were analyzed by Masson's trichrome staining (scale bar, 500 μm) and F4/80 immunostaining (scale bar, 200 μm; inset scale bar, 50 μm), respectively. (C) The thickness of scar tissue was significantly increased in the FC and TT-w/o-cap groups than in the TT-w-cap group. (D) A statistically significant increase in collagen deposition was observed in the FC and TT-w/o-cap group than in the TG-w-cap group. In addition, (E) F4/80-positive cells decreased significantly in the TT-w-cap group compared with the other two groups.

w-cap group consistently showed decreased tissue fibrosis when compared with the other experimental groups. To characterize the extent of host inflammatory cell infiltration within and around tube inserted-mucous layers, we stained tissue sections with the macrophage marker F4/80 (Fig. 6B). Macrophages increased significantly in the FC group compared with the other two groups (Fig. 6E). Interestingly, they were markedly decreased at 4 weeks in the TT-w-cap group.

Discussion

Pharyngoesophagus tissue engineering is becoming quite relevant, and there is certainly a need for gastrostomy in the case of transplantation to continue enteral feeding and minimise morbidity. Gastrostomy tube insertion has also been attempted for the investigation of probiotics to regulate intestinal inflammation and reduce mortality in animals after transplantation. In order to develop and establish a tissue engineering technique for pharyngoesophageal reconstruction, it would be indispensable to have an appropriate gastrostomy model for the study of novel promising strategies [10, 19, 20].

Non-oral feeding is technically easier to perform for large animal models of pharyngoesophageal reconstruction. This is because these animals can maintain feeding by intravenous parenteral nutrition for up to 4 weeks. A appropriate method of non-oral feeding should also be established in small animal models because they have a number of advantages, including cost efficiency, the ability to adequately statistically power studies, and the ability to assess several innate physiologic variables that cannot be mimicked in vitro [21]. In the past 20 years, several intragastric infusion techniques have been studied for providing nutrition in rodent models [11, 12, 22]. For instance, Zhang et al. demonstrated the possibility of gastrostomy tube insertion for effectively providing probiotics to regulate intestinal inflammation in an infant rat model [14]. Li et al. also investigated the effects of glutamine and glutamate on the developing rat small intestine using a gastrostomy-fed rat infant "pup-in-a-cup" model [15]. However, most gastrostomy studies have been carried out on infant rats or mice, and there have been no successful cases in adult rats. In the present study, we attempted to examine a new gastrostomy model for stable non-oral feeding in adult rats.

In this study, we applied two devices, a balloon catheter (Foley catheter) and T-shaped tube, for feeding gastrostomy. The Foley catheter and T-tube were comprised of the same material (silicone) and had the same diameter (3 mm). The Foley catheter has a completely different structure from a T-tube, as its feeding port is very large (Fig. 1). Both of these gastrostomy devices also differ in terms of their structural and functional conditions. We selected the balloon catheter because it is a gastrostomy apparatus used primarily in clinical research [23, 24]. In fact, we originally tried to develop a rat gastrostomy device with a Foley catheter in preliminary research. After inserting a balloon catheter into the stomach, inflation of the balloon helps to prevent it from falling out from the gastric wall. In much out of the literature, most of the animal studies have reported about the use of Foley catheters as urocatheters [25, 26]. In humans, Bendel et al. reported the short-term safety and efficacy of a co-axial angioplasty balloon technique for percutaneous radiologic gastrostomy catheter placement in the human body [27]. There was no occurrence of bleeding or skin infection while using this technique. Thus, we decided to investigate whether a Foley catheter could be applied to a rat model for feeding gastrostomy. However, because the Foley catheter's feeding port was too large, it was impossible to place it in a location where the rats could not reach it with their teeth. Therefore, damage occurred to more than 90% of the implanted devices due to the self-harm behaviour of the rat models. Next, we attempted to used a T-shaped tube and confirmed the possibility of using it in rat feeding gastrostomy. However, there was a problem with food backflow, which was solved by installing a cap at the end of the tube. In both cases, the devices were fixed to the stomach wall through the same surgical process. To prevent abdominal infections, antibiotics were administered for 3 days. It was also necessary to isolate the animals in individual cages to prevent breakage of the catheter by their mates. Flushing was performed daily with 1 ml of saline to prevent clogging of the catheter lumen. The funnel part was kept outside the cage because of the self-harm behaviour of the rats. To overcome these issues, the silicone tubes were covered with stainless steel sleeves to prevent biting. The Foley catheters implanted in most of the animals were damaged by self-harm behaviour within a week. As a result, the animals rapidly lost weight within a few days and showed moribund symptoms such as water intake difficulties at this time. Autopsy revealed that most of rats that received a balloon died of peritonitis. This is because the balloon apparatus was not properly maintained in the stomach due to damage of the silicone tube from self-harm behaviour. On the other hand, we fabricated a T-type tube that was designed to prevent the intragastric tube from being pulled out from the gastric wall. There was a little bleeding at the intubation site while the T type tube was inserted into the stomach. Additional sutures were performed to reinforce the anastomosis in the gastric wall.

Without a special apparatus at the distal port of the gastronomy tube, this system occasionally resulted in regurgitation of gastric contents. This is turn resulted in contamination of the surrounding cage, which led to secondary complications, such as bacterial infection. Therefore, a heparin cap was placed on the distal end of the catheter to solve this problem (Fig. 2). This heparin cap provides a stable supply route for the injection of nutritional supplements. In addition, it protects the silicone tube from damage due to self-harm behaviour. As shown in Fig. 3A, the weight loss rate was significantly reduced in the TT-w-cap group compared with that in the other groups. These results indicate that this system is able to maintain intragastric infusion of nutrients for a long time. Its effectiveness was also demonstrated by the blood parameters (Fig. 4). BUN is associated with dehydration of the animal body [28]. Both the TT-w/o-cap and FC groups showed severe dehydration which, in turn, may have decreased their resistance to bacteria. These groups also had a significantly higher WBC values when compared with the normal group. We can infer this was because of the inflammatory response in the abdominal regions. HGB is another key parameter that is related to anaemia [29]. Anaemia can also be caused by not supplying the right nutrients. By 7 days, the FC group only exhibited significantly lower HGB values than the normal group. No significant difference, however, was observed between the other experimental groups and the normal group. In addition, low ALB levels are caused by stress or nutritional deficiency. Significantly lower levels of ALB were detected in all experimental groups, but the levels of the animals in the TT-w-cap group returned to normal at 4 weeks. These results were consistent with the urinalysis results. In particular, a large amount of blood was detected in all experimental groups immediately post-operation. Furthermore, both the FC and TT-w/o-cap groups showed levels of ALB indicative of albuminuria within 1 week. These results are presumed to be the result of dehydration and the initial conditions of the animals being abnormal. Likewise, elevated urobilinogen levels are indicative of intestine infection. These levels may also increase with haemolytic anaemia. Moreover, the group of rats that received our target technique (TT-w-cap) showed relatively lower values for the urinalysis parameters tested than those in the other experimental groups at 7 days. This indicates that the T-tube with a cap might provide physiological stability as a promising gastrostomy technique in this rat model. Histological evaluation revealed thick collagen and scar tissue at the anastomotic site in the FC group (Fig. 6A). This scarring can be the result of anastomotic leakage after gastrostomy surgery. The TT-w-cap

group was observed to have the best tissue layer in the gastric wall. These results demonstrate that gastrostomy was stably maintained without any leakage for a month. Images of the gross appearance, shown in Fig. 5, also did not demonstrate any inflammation at the intubation site in the TT-w-cap group. In conclusion, a modified gastrostomy technique using a silicone T-tube plugged with a cap was found to be a stable and effective option for non-oral feeding in an adult rat model.

Conflict of Interests

The authors declare that there are no competing interests with respect to this manuscript.

Acknowledgments

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