Doctor of Philosophy

A Novel Biodegradable Tubular Stent for Pancreaticojejunal Anastomosis after Pancreaticoduodenectomy: An Experimental Study

The Graduate School
of the University of Ulsan

Department of Medicine
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ABSTRACT

Purpose: Pancreaticoduodenectomy (PD) with pancreaticojejunal (PJ) anastomosis is the standard surgical management for benign and malignant disease of the pancreatic head and periampullary regions. Placement of transanastomotic stent is commonly used to decrease the complications. Stent related complications such as stent migration, stent obstruction or fracture, stricture of the pancreatic duct, and intestinal obstruction have been reported. We developed a novel biodegradable tubular stent (BTS) and investigated its safety and efficacy in a rat model as well as in a porcine model.

Aims and methods: The BTS was developed from teropolymer which is originally approved for manufacturing non absorbable surgical sutures. In a rat model study, 42 Sprague Dawley rats were randomized into 7 groups of 6 rats each after placement of the BTS into the duodenum. The rats were sacrificed 1, 2, 3, 4, 6, 8, 12 weeks after stent placement. After sacrifice, the BTSs were retrieved from the duodenum and dried using the freeze drying technique; the mass loss of the samples was evaluated and scanning electron microscopy was performed to evaluate the surface changes. In a porcine model study, ten pigs were randomized into two groups of five pigs each. The control group had PJ only, while the BTS group had PJ with BTS placement across the anastomosis. Pancreaticography was performed before surgery and after sacrifice to evaluate the changes in the pancreatic duct diameter. Follow up CT was done 1, 4 and 8 weeks after surgery in the BTS group to check any stent migration and any changes in the pancreas and the pancreatic duct. Endoscopic examination of the site of the anastomosis was done just before sacrifice using a portable Rhino-Fiberscope. After sacrifice, Hematoxylin and Eosin and Masson's Trichrome staining were performed to evaluate the histological changes.

Results: In the rat study the technical success rate of BTS placement was 100%. During the follow up, 4 rats died from sepsis and 6 BTSs migrated. The mean mass losses (± standard deviation) of the BTSs were 2 ± 0.5, 7 ± 1.2, 11 ± 2.1, 18 ± 2.9, and 28 ± 3.6 % in group W1, W2, W3, W4, and W6, respectively. The BTSs retrieved from W8 group were very fragile and fragmented into small pieces during extraction from the duodenum or after having vacuum drying, therefore, the mass loss was not accurately determined in this group. The BTSs were completely decomposed at 12 weeks follow-up. The scanning electron microscopy of the BTS showed minor change in the texture in the first two weeks, and minor cracks started to appear from the third week. After 8 weeks, it was difficult to prepare samples for the SEM because they were broken down during extraction from the duodenum or after vacuum drying. In the porcine model, the surgical procedure and BTS placement were technically successful in all pigs (100% technical success rate). Pancreatitis occurred in two pigs in the control group. The pancreatography showed significantly enlarged pancreatic duct luminal diameter in the control compared to the BTS group at different locations ($p = <0.001$). On follow up CT scan, two stents in the BTS group migrated into the small bowel at 4 weeks, however, the pancreatic duct was significantly smaller than in the control group and no pancreatitis occurred with complete healing of the anastomotic site. On endoscopic examination, the anastomotic site in the control group was narrower than that of the BTS group. On histological examination, the mean luminal area of the pancreatic ducts was significantly larger in the control than the stent groups. There was no significant changes in the pancreatic histology in the control and the BTS groups.

Conclusion: Biodegradable tubular stent (BTS) development from commercially available polymers is feasible. The BTS seems to be safe and effective for the management of pancreaticojejunal anastomosis after pacreaticoduodenectomy. We surmise that the optimal

duration of stenting after surgery may be between 4 and 8 weeks. Future studies to optimize the BTS for human application is needed.

Keywords: Pancreaticoduodenectomy, Pancreaticojejunal anastomosis, Biodegradable tubular stent.

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LIST OF ABBREVIATIONS

BTS	biodegradable tubular stent
pBTS	porcine biodegradable tubular stent
rBTS	rat biodegradable tubular stent BTSs
CT	computed tomography
PD	pancreaticoduodenectomy
PDO	poly p-dioxanone
PJ	pancreaticojejunal
SEM	Scanning Electron Microscopy
TMC	trimethylene carbonate

INTRODUCTION

Pancreaticoduodenectomy (PD) is the standard surgical management for benign and malignant diseases of the pancreatic head and periampullary regions (1-7). In recent years, mortality rate of pancreaticoduodenectomy has declined to less than 5% in many institutions around the world (2, 4, 8, 9), however, the reduction in perioperative mortality over the past few decades has not been translated into a notable improvement of post-operative complication (30-50%) (4, 10-13). Anastomotic leakage from the pancreaticojejunal (PJ) anastomosis after PD and subsequent fistula formation (5-41%), or later, anastomotic strictures (5-11%) are the most common causes of morbidity (3, 10, 14-16). Hence, it is imperative to conduct research to identify effective strategies to reduce the complications following the anastomosis. Various modalities have been tried to overcome these complications including the use of fibrin sealants (17, 18), octreotide therapy (19-22), and varied suturing techniques (23). Also, various methods of pancreatoenteric anastomosis have been developed as alternatives to standard pancreaticojejunostomy in an attempt to reduce the leak rate. These modifications include: (a) The invagination anastomosis, also known as a 'dunking' procedure, (b) ligation or obliteration of the pancreatic duct, (c) occlusion of the duct with Ethibloc (Ethicon, Norderstedt, Germany) or neoprene, and (d) pancreaticogastrostomy (9, 10, 24-29). However, current therapeutic strategies remain insufficient and controversial due to inconsistent results from clinical trials and experimental studies (19, 23, 29, 30).

Placement of a trans-anastomotic stent is commonly used for pancreatico-enteric anastomoses (8, 31-35). This technique is used almost exclusively in pancreatic anastomoses because of the high risk of a leak as the pancreatic enzymes can disrupt the anastomotic site (10). Theoretically, the stent may provide some protection of the PJ anastomosis as it facilitates precise

placement of sutures through the pancreatic parenchyma or duct when performing the anastomosis. Additionally, it can direct the pancreatic juice away from the anastomotic site (36-38). Moreover, these stents assure anastomotic patency and decompress the pancreatic duct during the early postoperative period while anastomotic edema resolves (10). Prospective randomized trials have shown that external drainage with a stent decreased the incidence of PJ anastomotic leakage and/or fistula formation after PD, and reduced the median hospital stay (8, 39, 40). Nonetheless, stents made from non-biodegradable materials (e.g. silicone or polytetrafluoroethylene) may be associated with stent-related complications, for example, stent migration, stent obstruction or fracture, stricture of the pancreatic duct, or intestinal obstruction (10, 24, 41-43).

An ideal pancreatic stent for pancreaticoenteral anastomosis would be the one that has a large diameter, does not cause stent-induced ductal or parenchymal injury, and does not need a repeat intervention for removal (44). Additionally, the stent should also be the one that has adequate radial force and diameter recovery for a sufficient period of time, so as to perform its mechanical functions efficiently (45). A properly designed biodegradable stent has the potential to provide these ideal characteristics, and avoid complications as they spontaneously disappear after healing of the anastomotic site (10, 43). So, in this study, we developed a novel biodegradable tubular stent (BTS) for pancreaticojejunal anastomosis from a commercially available polymer material that is FDA approved for manufacturing absorbable surgical sutures.

We hypothesized that the BTS may prevent stent-related complications such as pancreatic duct stricture and leakage at the anastomotic for patients undergoing pancreaticoduodenectomy with pancreaticojejunal anastomosis. Therefore, the purpose of this study was to investigate the biodegradable behavior of the BTS in a small animal model and to assess the preclinical safety and efficacy of the BTS in a porcine model.

MATERIALS AND METHODS

Biodegradable Tubular Stent

The BTSs used in our study were designed and manufactured from a terpolymer consisting of poly *p*-dioxanone (PDO), trimethylene carbonate (TMC), and glycolide. The BTSs used for the rat small animal study (rBTS) were 2 mm in diameter and 15 mm in length. One gold radiopaque marker was attached to its middle part to evaluate the location under fluoroscopy (Fig. 1). The BTSs used for the porcine animal study (pBTS) were 2 mm in diameter, and 30 mm in length. Two gold markers were attached to both ends of the pBTS to evaluate the location under computed tomography (CT) examination and fluoroscopy. Two side holes were made at the both ends of the pBTS to facilitate drainage of the pancreatic secretion and prevent stent obstruction (Fig. 1a).The pBTS was introduced using a 5 Fr dilator (Cook, Bloomington, Indiana) (Fig. 1b). The BTSs were made to our specifications by a local manufacturer (S&G biotech, Yongin, Korea). The BTSs are not commercially available elsewhere.

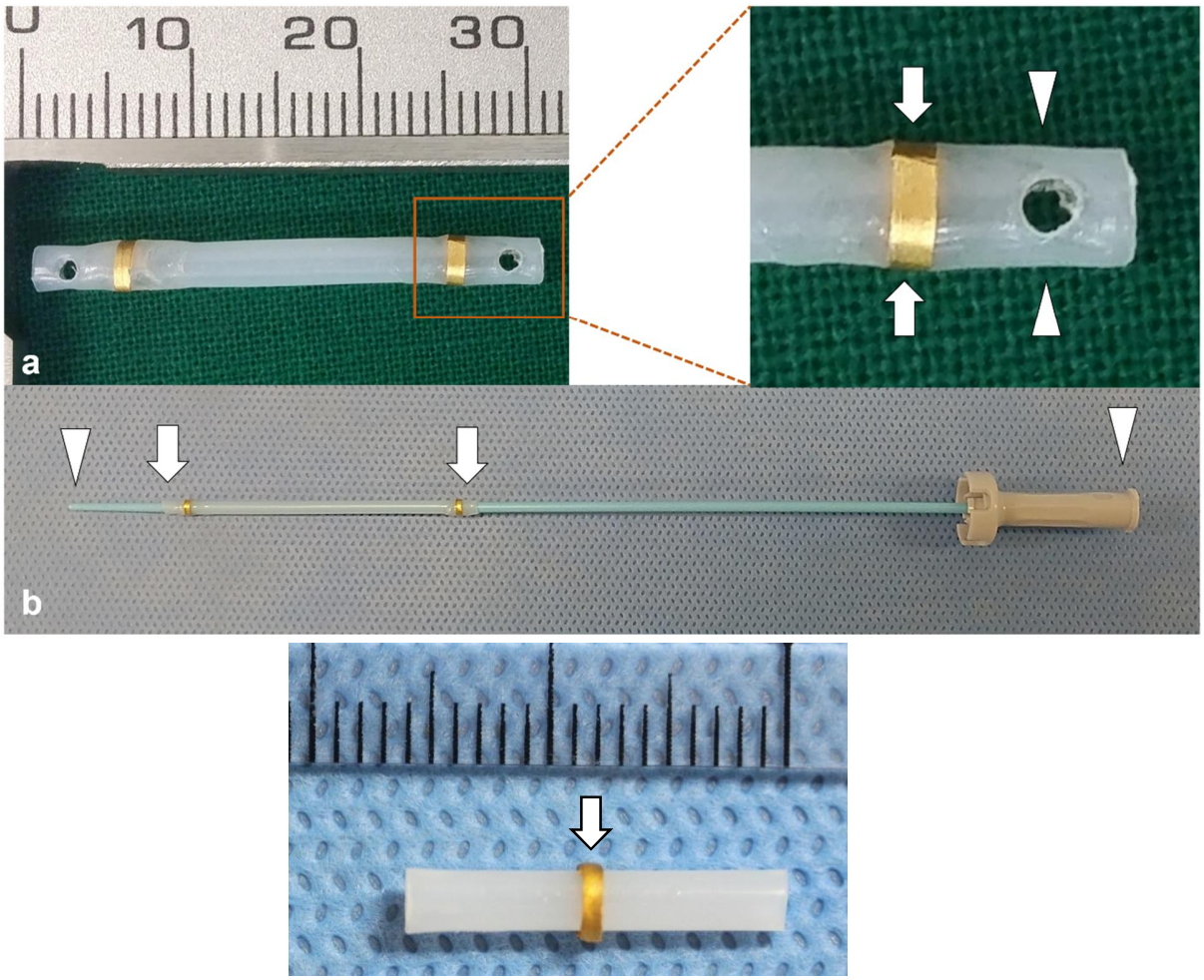

Figure 1. Photographs showing the biodegradable tubular stent and the delivery system. (a) A photograph showing the porcine biodegradable tubular stent (pBTS) with two gold markers (arrows) and side hole (arrowheads) made of a terpolymer consisting of poly p-dioxanone, trimethylene carbonate, and glycolide. (b) A photograph showing the 5 Fr dilator (arrowheads) and loaded biodegradable tubular stent (arrows). (c) Photograph showing the rat biodegradable tubular stent (rBTS) with one gold marker at the central part (arrow).

Cytotoxicity evaluation

An in-vitro assessment of the cytotoxicity of the BTSs on human cells (NCTC Clone 929) was performed. The procedure was conformed to the ISO 10993-5 protocol for examining the toxicity of medical devices. Cell monolayers were grown to near confluence in flasks. Control material [Negative control (high density polyethylene film), and Positive control (0.1 % ZDEC polyurethan film)] and BTS samples were placed in separate cell culture media (6 cm^2/ml at 37 ± 1°C for 24 ± 2 h). The cell proliferation rate was calculated based on the 3-(4, 5-dimethyl-2-thiazol)-2, 5-diphenyl-2H tetrazolium bromide (MTT) assay to evaluate the cytotoxicity of the samples. Table 1 shows the grading system used to assess cell viability (46).

Table 1. Standard of cytotoxicity evaluation

Grades	Relative cells proliferation rate (%)	Cytotoxicity
0	100	Not cytotoxic
1	75-99	Not cytotoxic
2	50-74	slightly cytotoxic
3	25-49	Moderately cytotoxic
4	1-24	Moderately cytotoxic
5	0	Severely cytotoxic

Animal studies

All the animal studies were approved by the committee for animal research at our institution and conformed to the Guide for the Care and Use of Laboratory Animals.

Rat Model

Study design and procedure details

A total of 42 male Sprague-Dawley rats (Orient Bio, Seongnam, Korea) weighing 300–350 g at 10 weeks of age were used for this study. They were randomized after rBTS placement using computer generated random numbers into seven groups of six rats each. Groups W1, W2, W3, W4, W6, W8, and W12 were sacrificed at 1, 2, 3, 4, 6, 8 and 12 weeks after rBTS placement, respectively. The body weights of the rats were measured before the procedure and just before sacrifice.

After overnight fast, animals were anesthetized with intramuscular injection of a mixture of 50 mg/kg zolazepam and 50 mg/kg tiletamine (Zoletil 50; Virbac, Carros, France) and 10 mg/kg xylazine (Rompun; Bayer HealthCare, Leverkusen, Germany). The anterior abdominal wall hair was shaved and antisepsis was provided with 0.05% chlorhexidine hydrochloride. A 2.5 cm midline incision was performed at the upper abdomen and the duodenum was identified. After releasing the intestine from the ligament of Trietz, duodenotomy was performed 8 cm from the pylorus. This site was chosen to avoid obstruction of the biliopancreatic duct which opens at the medial side of the duodenum (22-28 mm from the pylorus) (47-49). The rBTS was placed through the duodenotomy and its distal end was sutured to the duodenal wall using 5-0 non-absorbable

sutures (Prolene, Ethicon, Johnson & Johnson Intl, Brussels, Belgium). The duodenotomy was then repaired and the proximal end of the stent was sutured to the duodenal wall (Fig. 2). The abdominal wall fascia was closed with 4-0 absorbable sutures (Vicryl, Ethicon) using the continuous suturing technique, and the skin was closed with 4-0 non-absorbable sutures (Prolene, Ethicon). Antibiotics were given after the operation (0.06 ml of 50 mg/ml ceftriaxone per 100 g of body weight) and was continued for 48 hours after the operation. All rats were supplied with food and water *ad libitum* and were maintained at 22 ± 2°C with a 12-hour day night cycle. A translucent animal restrainer was used, without anesthesia, to obtain fluoroscopic images on a weekly basis to ensure the rBTS retention (Fig.3). Rats in each group were euthanized by administrating inhalable pure carbon dioxide.

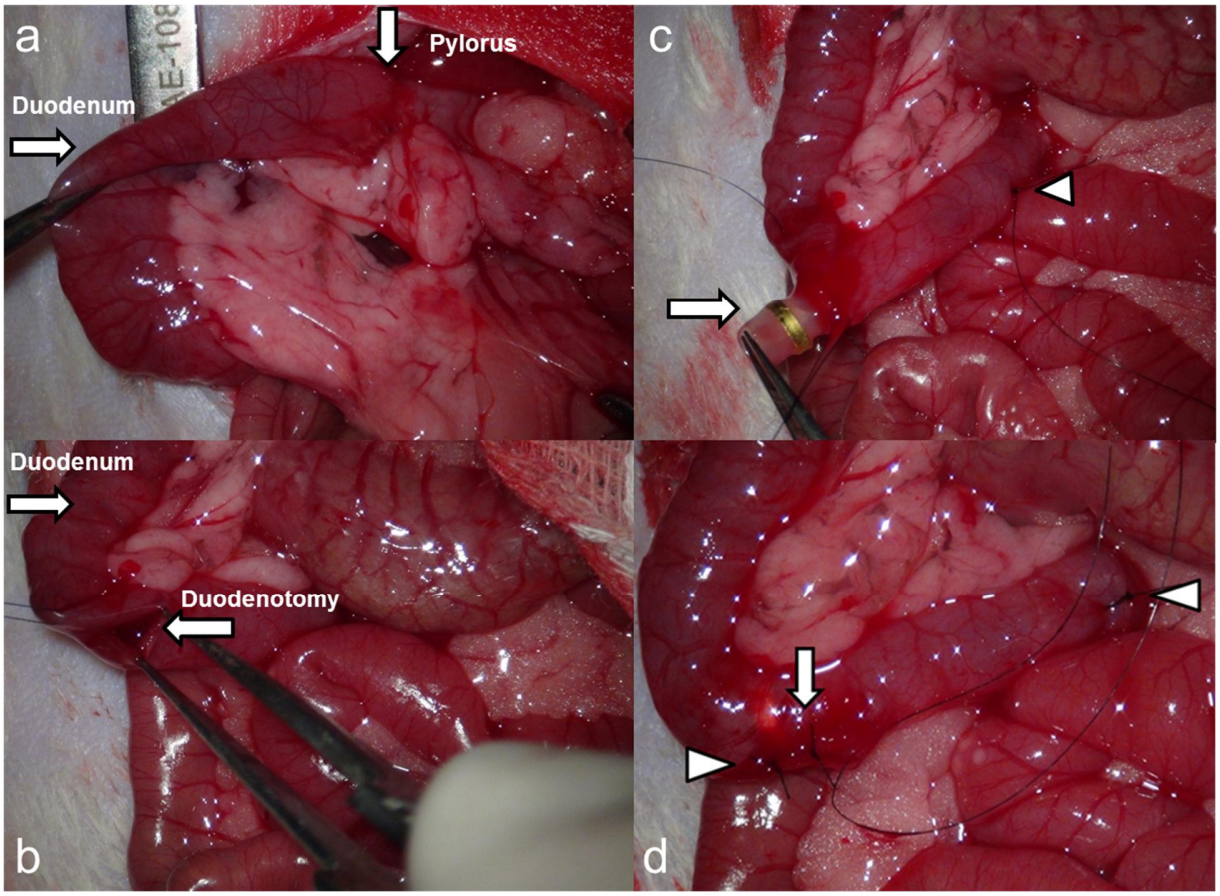

Figure 2. Photographs showing the technical steps of the rBTS placement in a rat duodenum. (a) Identifying the duodenum and pylorus. (b) Duodenotomy performed 8 cm distal to the pylorus. (c) The rBTS is placed through the duodenotomy (arrow), and the distal end is sutured to the duodenal wall (arrowhead). (d) The duodenotomy is closed (arrow), and both ends of the rBTSs are sutured to the duodenal wall (arrowheads).

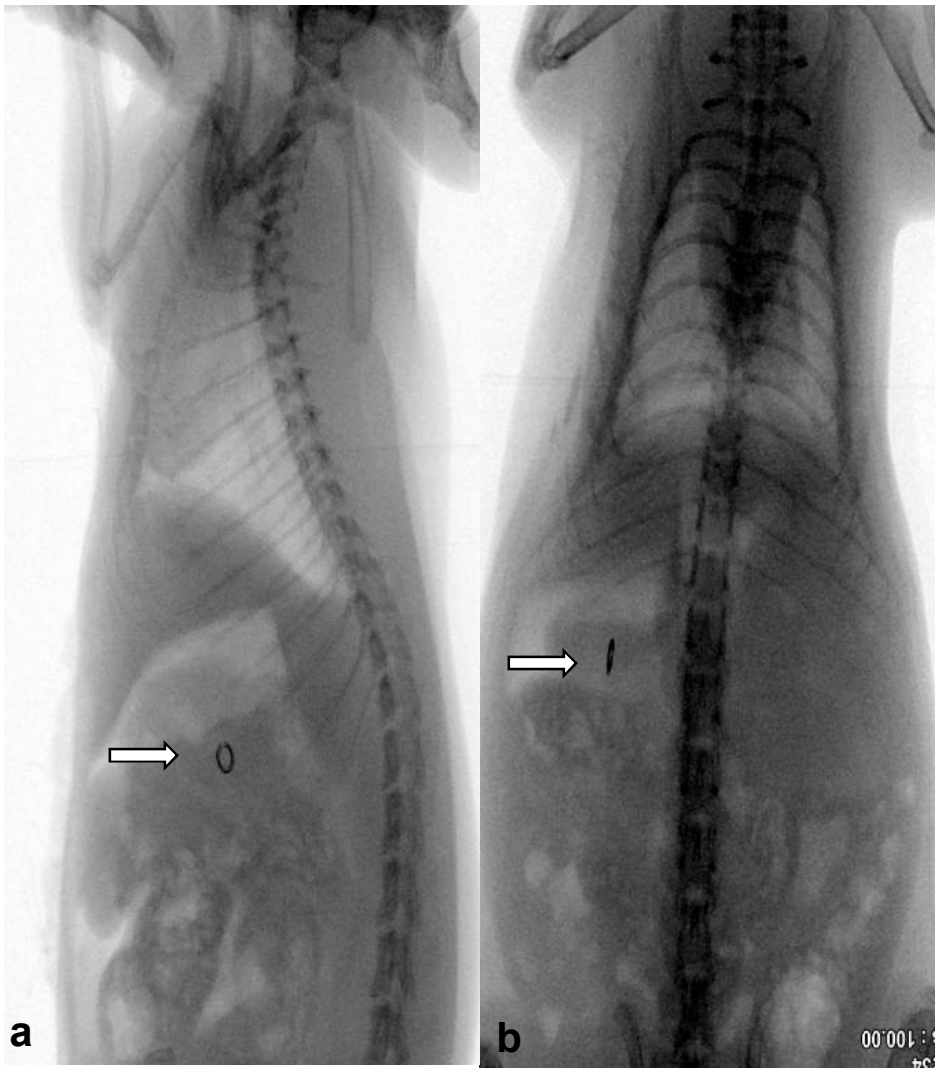

Figure 3. Fluoroscopy images showing the rBTS in the rat duodenum immediately after surgery.

(a) Lateral view. (b) Anteroposterior view.

Evaluation of the Degradation of the rBTS:

1. Weight change

After sacrifice, all the samples were retrieved from the duodenum and dried using the freeze drying technique (Christ freeze dryer type Alpha 1–4 LSC, Martin Christ Gefriertrock-nungsanlagen GmbH, Osterode am Harz, Germany). The process consisted of preliminary freezing for one hour at -80 °C and subsequent drying. The latter process, in turn, consisted of two stages: primary drying and secondary drying. During primary drying or sublimation stage, frozen water was removed from the samples under vacuum and at a temperature below 0°C. The secondary drying stage began after the end of the sublimation stage and occurred at temperatures above 0°C (50). The goal of the secondary drying was to bring the residual moisture of the stent to an optimum level. The dry weight of the sample was then measured using an ultrasensitive electronic balance (Ohaus corp., pine Brook, NJ, USA), and the percentage mass loss was calculated according to the following equation: *Mass loss (%) = [(W-W_d) / W] x 100 (51),* where W was the original weight of the sample and W_d was the weight of dried sample after a certain time interval.

2. Scanning electron microscopy (SEM)

After freeze drying, the rBTSs morphology was examined using a field emission scanning electron microscopy (SEM, Hitachi S4800, Tokyo, Japan). The dried samples were sprayed with platinum using a metal plating instrument. Briefly, they were placed on a stub into a platinum sputtering system. The stub was then removed, and the SEM chamber was then ventilated, allowing the chamber to reach a nominal pressure. The sample stub containing the rBTS was then inserted onto the stage. Pumps were turned on to allow the system to reach vacuum. Magnification was set to a

minimum zoom level of 50X, and the focus was adjusted until the desired focus was acquired. To optimize image clarity, the magnification was then increased close to the maximum level, and the focus was readjusted. A low-voltage anode (10 kV) was adopted to ensure a clear observation.

Porcine model

Study design and procedure details

A total of 10 pigs weighing 33.7 – 37.2 kg (median weight = 35.4 kg) were used for this study. Pigs were randomly divided into two groups using computer generated random numbers as follows: control group (n = 5) had PJ, and BTS group (n = 5) had PJ with pBTS placement. All pigs were euthanized by means of administration of an overdose of xylazine hydrochloride (Rompun; Bayer, Seoul, Korea) 8 weeks after surgery and histopathological examination was performed. All pigs were supplied with food and water *ad libitum* and were maintained at 22 ± 2°C. The body weights of the pigs were measured before surgery and then weekly until sacrifice.

After 24 hours of fasting and under the supervision of a veterinarian, the pigs were premedicated with 50 mg intramuscular ketamine; an endotracheal tube was placed and anesthesia was given by inhalation (0.5–2% isoflurane: Ifran®; Hana Pharm. Co., Seoul, Korea) with oxygen, 1:1 (510 mL/kg per min). All pigs underwent PJ anastomosis of duct-to-mucosa method. Following longitudinal abdominal incision in the midline, the duodenum and pancreas were identified. Through tracing along the duodenum, the pancreatic duct was identified at the level of opening in the ampulla. Following ligation of pancreatic duct at the duodenal side, the opened duct was anastomosed with an adjacent jejunal loop. Before PJ anastomosis, an 18 gauge angiocath (BD Angiocath Plus; BD, Mississauga, Canada) was inserted into the pancreatic duct to perform a pancreatic ductography. Three ml of contrast medium (Omnipaque 300; GE Healthcare, Cork, Ireland) was injected through the duct to identify the pancreatic duct and to exclude the presence of an accessory pancreatic duct. For duct-to-mucosa technique, pancreatic duct-to-jejunal mucosa was stitched with interrupted monofilament synthetic absorbable 5-0 suture material (Monosyn, B.

Braun, Melsungen, Germany). In group B, the pBTS with a 5 Fr dilator was inserted into the cut pancreatic duct, and then the sheath was removed with the pBTS left in place. The remaining distal part of the pBTS was inserted into the jejunal loop. The BTS was sutured to the jejunal mucosa to prevent stent migration during pancreatic duct-to-jejunal mucosa anastomosis (Fig. 4). A row of non-absorbable continuous stitches with polypropylene 4-0 suture was placed in the pancreatic capsule and jejunal serosa in both anterior and posterior walls of the anastomosis. Finally, the abdominal incision was closed using layer-by-layer manner. Fluoroscopic images were routinely obtained immediately after the surgical procedure to confirm the location of the pBTS (Fig.4f).

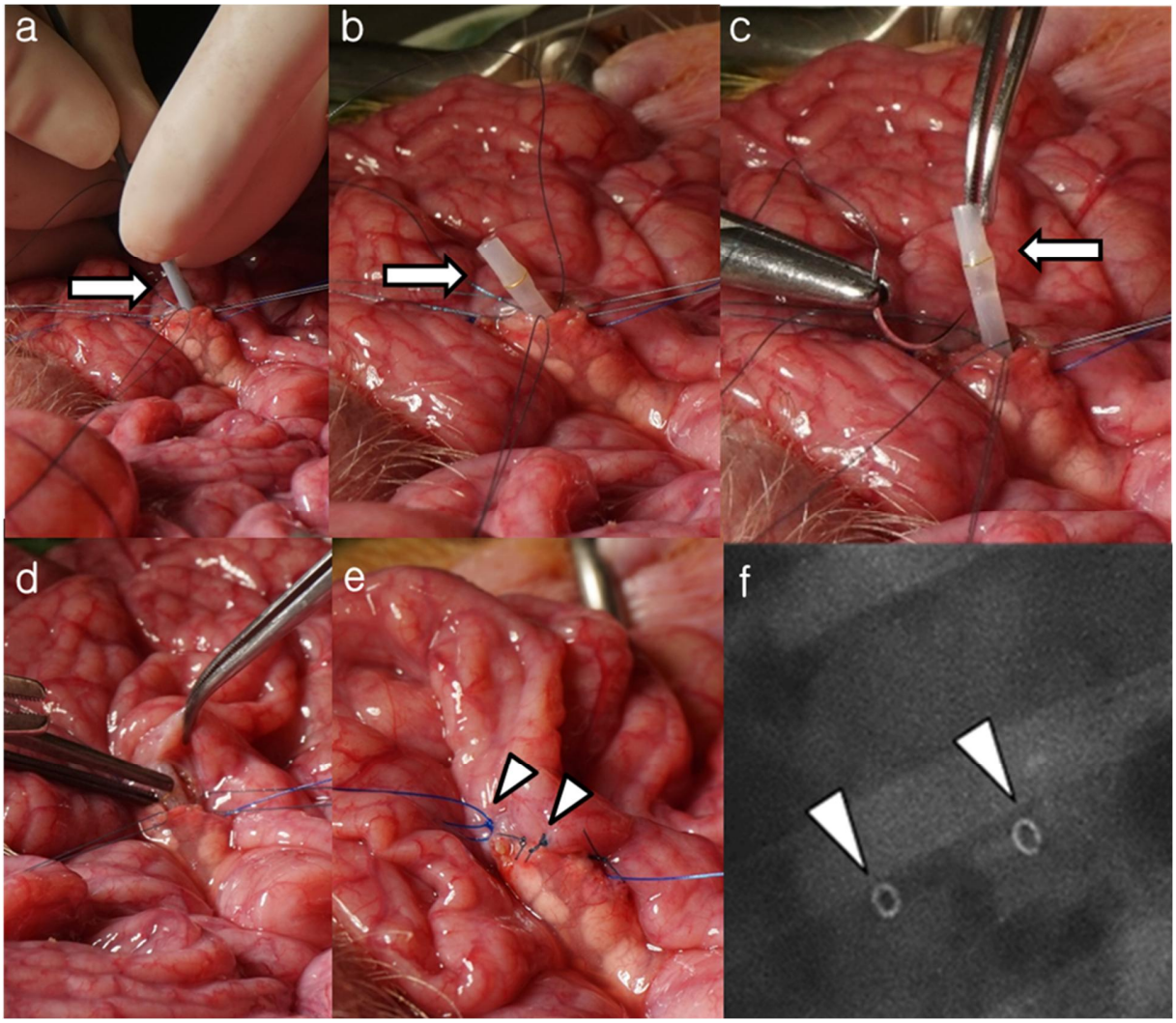

Figure 4. Photographs showing the technical steps of the pBTS placement in a porcine model. (a) A 5 Fr dilator (arrow) was used to insert the pBTS into the cut pancreatic duct. (b) The pBTS (arrow) is seen inside the pancreatic duct. (c) The pBTS (arrow) was sutured to the jejunal mucosa to prevent stent migration. (d) The remaining distal part of the pBTS was inserted into the jejunal loop. (e) Pancreatic duct-to-jejunal mucosa anastomosis (arrowheads) was successfully performed after the pBTS placement. (f) Radiograph obtained immediately after the surgery showing the two gold markers (arrowheads) of the pBTS. pBTS; porcine biodegradable tubular stent

Pancreatic ductography,

The initial pancreatic ductography was performed intraoperatively before the PJ anastomosis. Follow-up pancreatic ductography was performed immediately after sacrifice to verify the degree of the dilatation in the pancreatic duct and the patency of the anastomotic site. The luminal diameter was measured with the use of calibrated catheter (Cook, Bloomington, Indiana, USA). A software (Photoshop, version 6.0; Adobe Systems, Palo Alto, Calif, USA) was used to acquire digital measurements of the inner luminal diameter of the pancreatic duct at the anastomotic site, head, body, and tail regions of the pancreas. Measurements were repeated three times at each level, yielding an average value per level, and these values were subsequently averaged to obtain an overall average diameter of the segment. Analysis of the pancreatic ductographic findings were performed on the basis of the consensus of three observers blinded to the study groups.

Computed tomography (CT)

Abdominal CT (Sensation 16, Siemens, Muenchen, Germany) was performed before surgery, and 1, 4, and 8 weeks after the surgery to verify the location of the pBTS, detect procedure-related complications, and evaluate any changes in the pancreatic duct.

Endoscopic examination

Endoscopic examination was performed to identify the changes at the PJ anastomotic site immediately after sacrifice in all pigs using Rhino-Fiberscope (Karl Storz, Sunrise, FL, USA).

Histological Analysis

Surgical exploration of the pancreas and jejunal loops was followed by gross examination to evaluate the surgical outcomes with/without pBTS placement. The pancreas was sectioned transversely at the anastomotic site, head, body, and tail regions of the pancreas for histological analysis to assess the difference between the two groups (Fig. 5). Tissue samples were fixed in 10% neutral buffered formalin for 24 h, which was then embedded in paraffin and sectioned. The slides were stained with hematoxylin-eosin and Masson's Trichrome. Diameters of the pancreatic duct lumen at the head, body, and tail portion were measured to compare the pancreatic duct size between the two groups. Histological analysis of the pancreas was performed using a BX51 microscope (Olympus, Tokyo, Japan). Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA) was used for measurements. Histological findings were evaluated based on the consensus of three observers blinded to the group assignments.

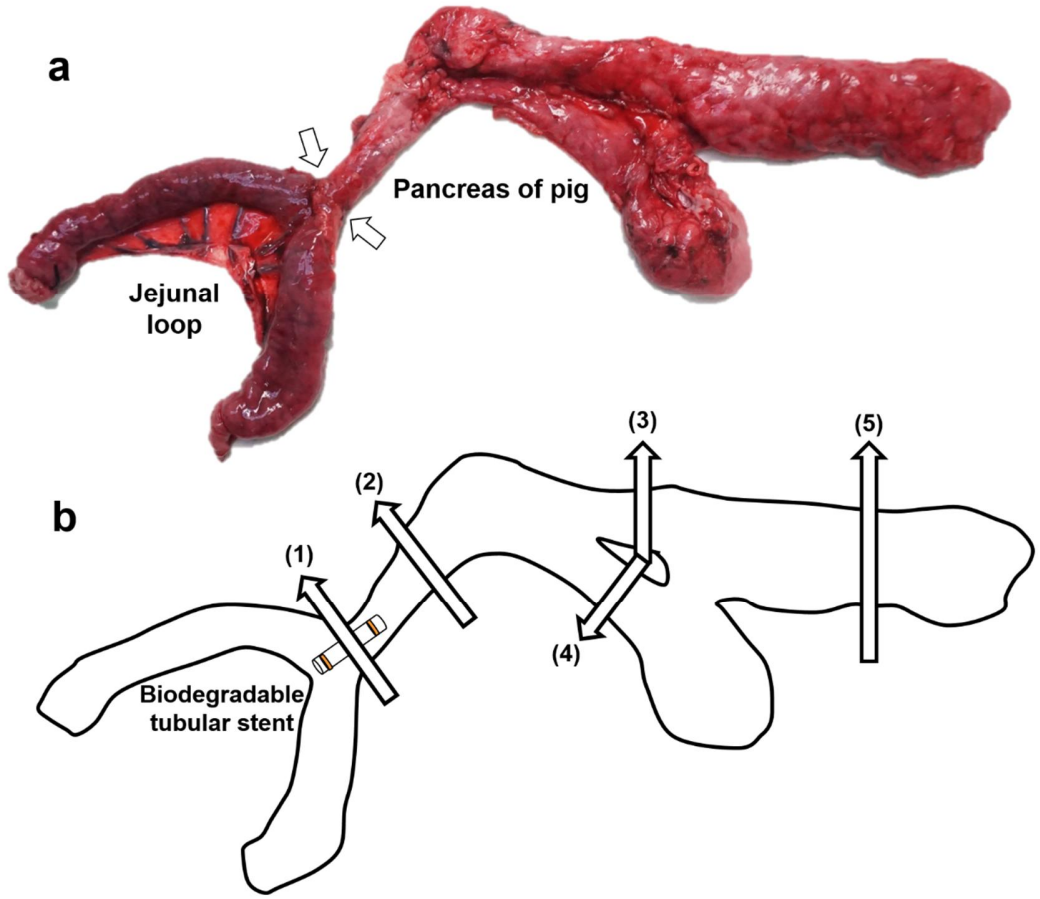

Figure 5. Pathologic findings and locations of tissue sampled for histologic examinations. (a) Photograph obtained immediately after sacrifice showing the pancreas of pig, jejunal loop, and the anastomotic site (arrows). (b) Schematic image showing the locations of tissue samples from the anastomotic site (1), and head (2), upper body (3), low body (4), and tail (4) portions of the pig pancreas.

Statistical Analysis

Differences between groups were analyzed using Mann–Whitney U test, as appropriate. A p value of < 0.05 was considered statistically significant. The statistical analyses were performed using SPSS software (version 24.0; SPSS, Inc., Chicago, IL, USA).

RESULTS

Cytotoxicity test

The human cells viability after incubation with the BTS samples ranged from 84.9 to 97.8% (grade 0 to 1) compared to a range of 5 to 7.5% (grade 4) in the positive control group denoting that the BTS samples were not cytotoxic to human cells.

Rat model

Technical outcome

Placement of the rBTSs was technically successful in all rats (100%). During the follow up, four rats died within the first week after surgery. Autopsy showed a leak at the duodenotomy closure site and peritonitis which mostly lead to death secondary to sepsis. A total of six rBTSs were not detected during the fluoroscopic follow-up (between one and three weeks after placement, median of two weeks). The six rats were immediately scarified and the rBTSs were found to be migrated and spontaneously eliminated through the anus (Fig.6).

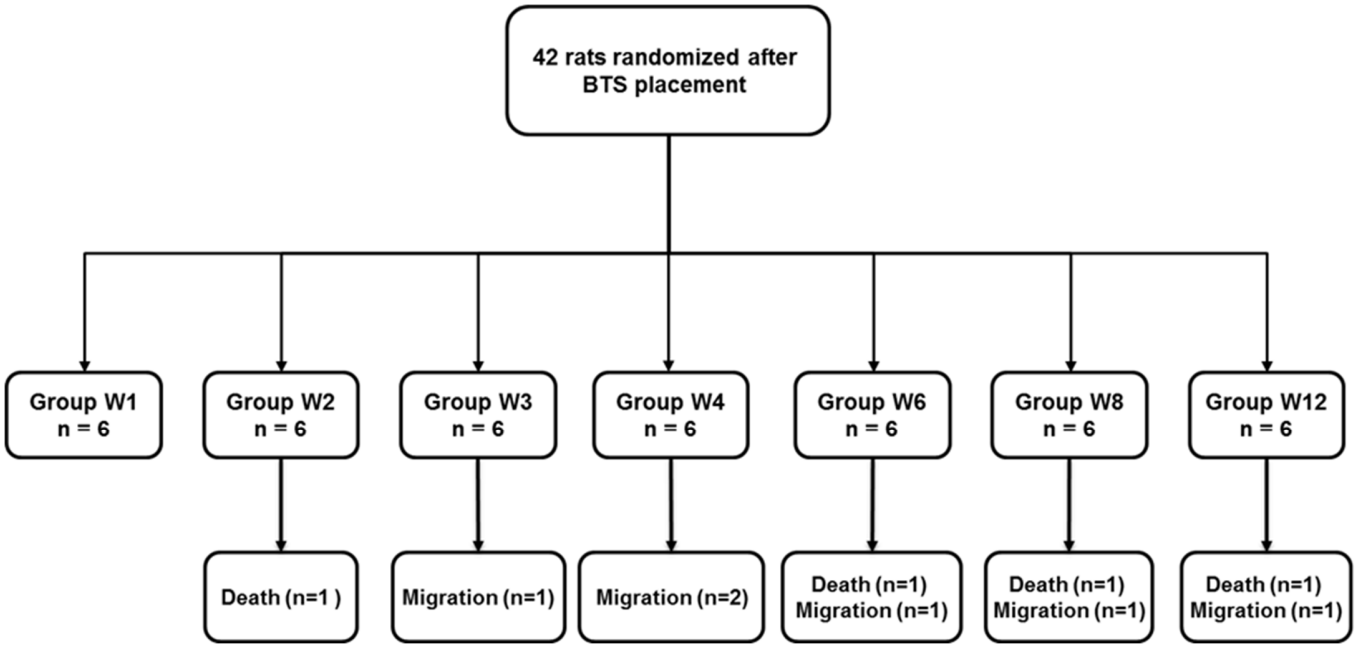

Figure 6. A flowchart showing the randomization and the technical outcome of rBTS placement in 42 Sprague-Dawley rats. rBTS; rat biodegradable tubular stent.

Degradation of the rBTS:

1. Weight change

The mean mass losses (± standard deviation) of the rBTSs were 2 ± 0.5, 7 ± 1.2, 11 ± 2.1, 18 ± 2.9, and 28 ± 3.6 % in groups W1, W2, W3, W4, and W6, respectively (Fig.7). The removed rBTSs from group W8 were very fragile and fragmented into small pieces during extraction from the duodenum or after having vacuum drying, therefore, the mass loss of group W8 was not accurately evaluate. In group W12, all rBTSs had disappeared from the duodenum at time of sacrifice and only the sutures used for fixation were found.

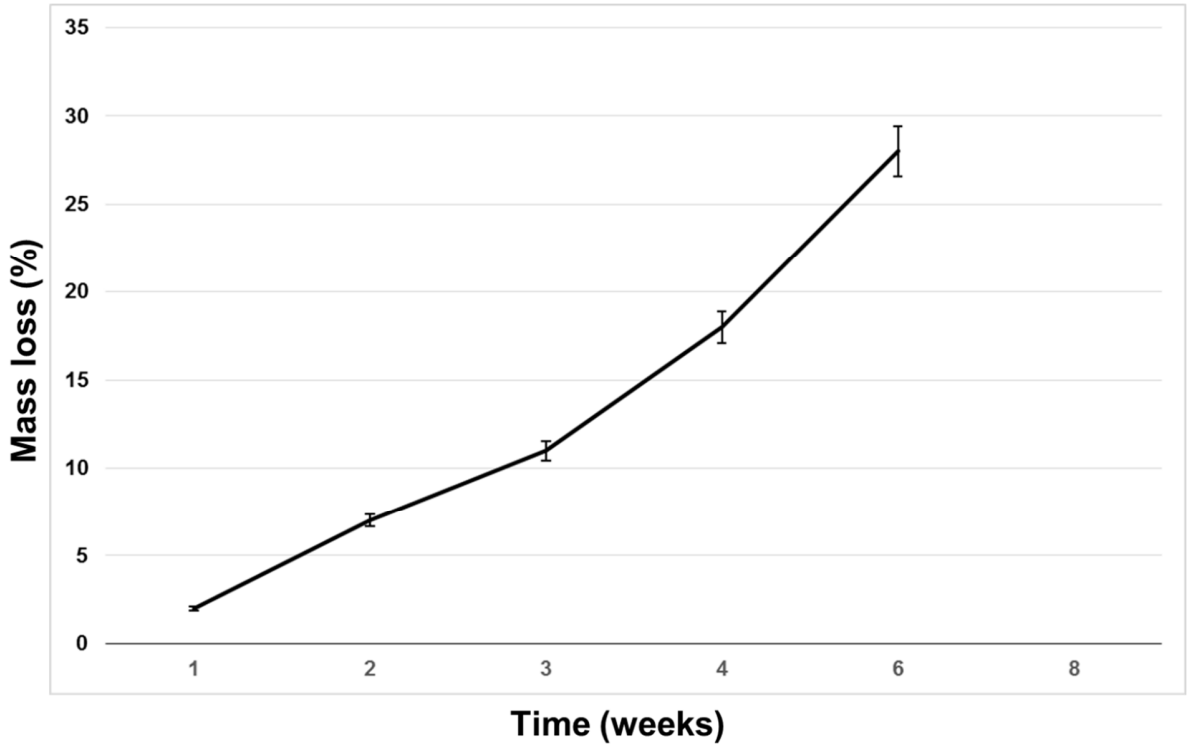

Figure 7. A line graph showing the percentage mass loss of the rBTS. The error-bars represent the standard deviation. The rBTSs that were retrieved from group W8 were very fragile and fragmented into small pieces during extraction from the duodenum or after having vacuum drying, therefore, we could not accurately evaluate the mass loss of this group. rBTS; rat biodegradable tubular stent.

2. Scanning electron microscopy

The surface morphology of the rBTS showed minor change in the texture in the first two weeks, and minor cracks started to appear from the third weeks (Fig.8). After 6 weeks, small cracks were found on the surface of the removed rBTSs. After 8 weeks, it was difficult to prepare samples for the SEM as they were broken down during extraction from the duodenum or after vacuum drying. The rBTS completely decomposed at 12 weeks follow-up.

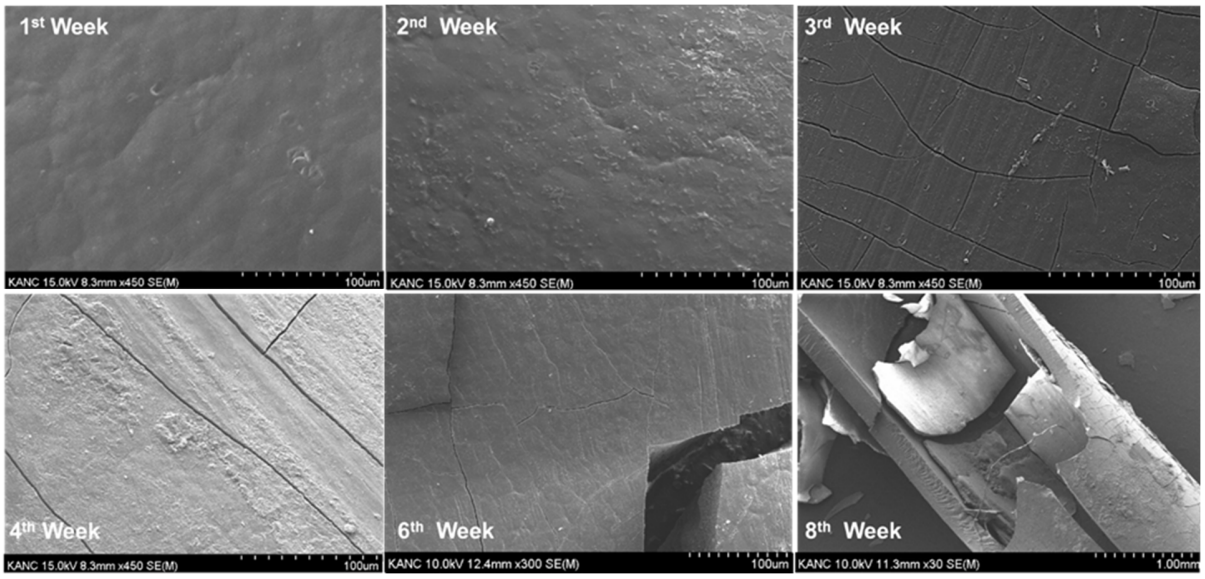

Figure 8. SEM images showing the morphology changes of the rBTSs in-vivo: (a)–(f) representative images from groups W1, W2, W3, W4, W6, and W8, respectively. It was difficult to prepare the samples for SEM at 8 weeksas the rBTSs material was broken down into very small and fragile pieces after extraction from the duodenum or after vacuum drying. SEM; Scanning Electron Microscopy.

Porcine model

Procedure outcome

The technical success rate of the surgery was 100% (10/10). Two pigs had incisional hernia two days after the surgery that were surgically repaired. Pancreatitis occurred in two pigs in the control group and none in the BTS group 2/10 (20%). No significant changes in the animal behavior, food intake, or body weight compared to other animals was noted, however, there were visible signs of pancreatitis in the abdominal cavity (adhesions, fatty necrosis, and pancreatic tissue changes) at the time of sacrifice 8 weeks after surgery (Fig. 9). No leak or abscess formation occurred in any pig during the study.

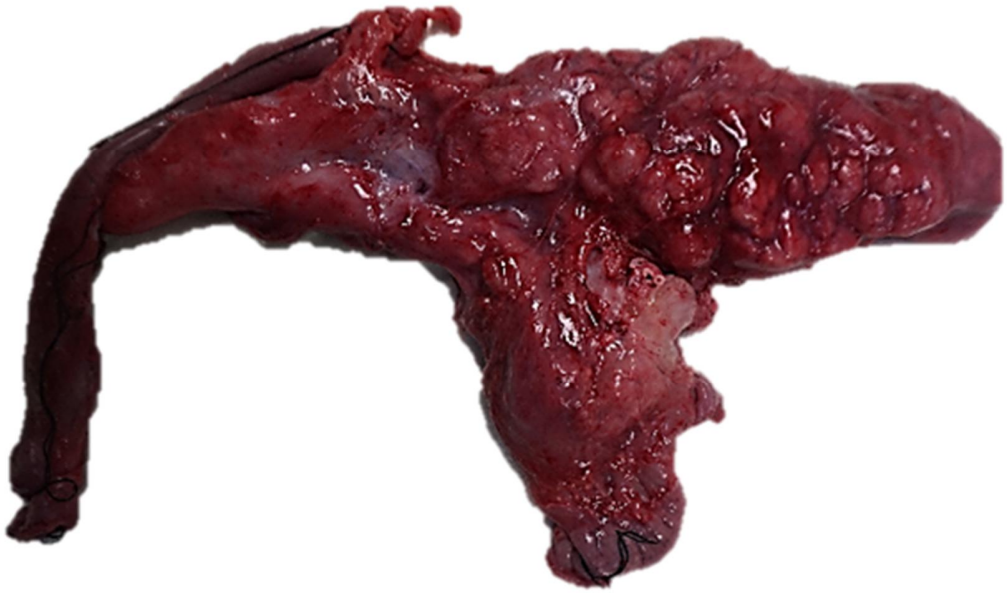

Figure 9. Photography image showing severe pancreatitis secondary to obstruction at the PJ in pig number 4 in the control group. PJ; pancreaticojejunostomy

Pancreatic ductography

The mean luminal diameter of the pancreatic ducts in the control group was significantly larger than the BTS group (3.44 ± 1.08, and 1.6 ± 0.48; $p < .001$). This included the diameter measured at the anastomotic site, pancreatic head, upper, and lower body, and the pancreatic tail (Fig. 10) (Table 2).

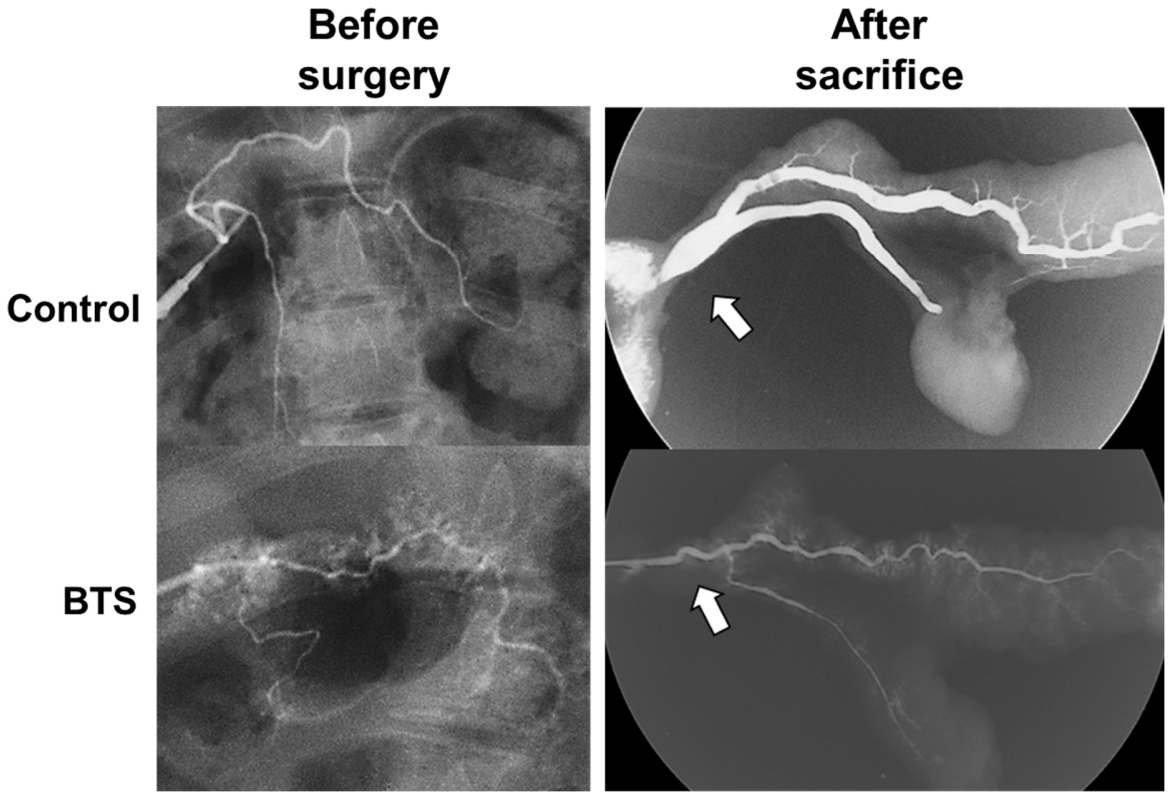

Figure 10. Representative pancreatic ductography images showing the significant difference in the pancreatic duct luminal diameter between a pig from the control and one from the stent group. Arrows are pointing to the site of the anastomosis.

Table 2. Pancreatic ductography measurements of the mean luminal diameter of the pancreatic ducts:

Locations	Control Group* (n = 5)	BTS Group* (n = 5)	P-valve†
Anastomosis	0.67 ± 0.43	2.15 ± 0.28	< 0.001
Head	5.65 ± 0.89	1.79 ± 0.46	< 0.001
Upper body	3.74 ±1.32	1.58 ± 0.43	0.002
Lower body	3.59 ± 1.29	1.22 ± 0.67	< 0.001
Tail	3.56 ± 1.48	1.25 ± 0.55	0.003

** Data are mean ± standard deviation (mm).*

†*Mann-Whitney t test.*

Computed tomography

The pBTSs were seen in place in all of the five pigs in the BTS group at 1 week follow-up. At 4 weeks follow-up, two pBTSs had migrated into the distal small bowel (Fig. 11). The migrated stents passed through the anus and could not be visualized at 8 weeks follow-up CT examination. No differences in the behavior, feeding, and weight changes were found between the two pigs with the pBTSs migration and the other three pigs. The pigs with the migrated pBTSs had significantly smaller pancreatic duct diameter compared to the control group at the time of sacrifice 8 weeks after surgery (Fig. 12).

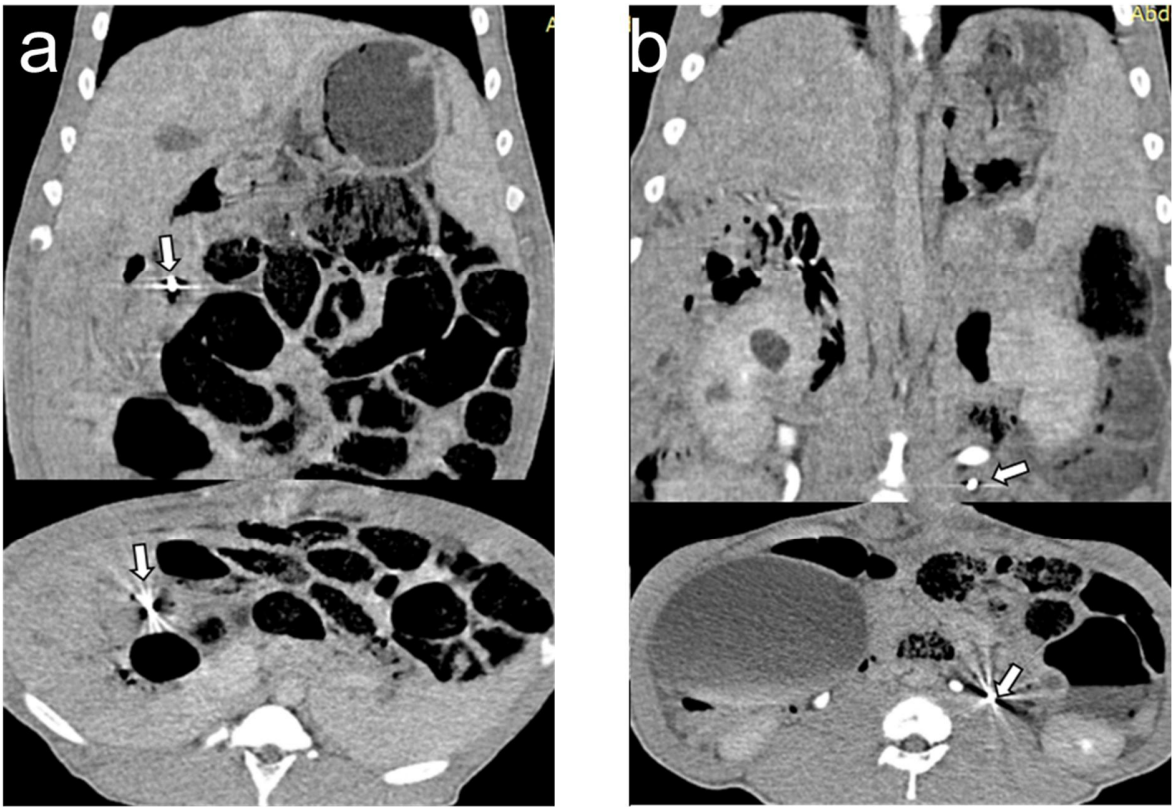

Figure 11. CT images showing pBTS migration into the distal small bowel in pig number 3 in the BTS group (a) coronal and axial CT images at one week follow up showing the gold marker of the pBTS at the PJ anastomosis site. (b)Follow up CT scan at four weeks showing pBTS migration into the small bowel. PJ; pancreaticojejunostomy.

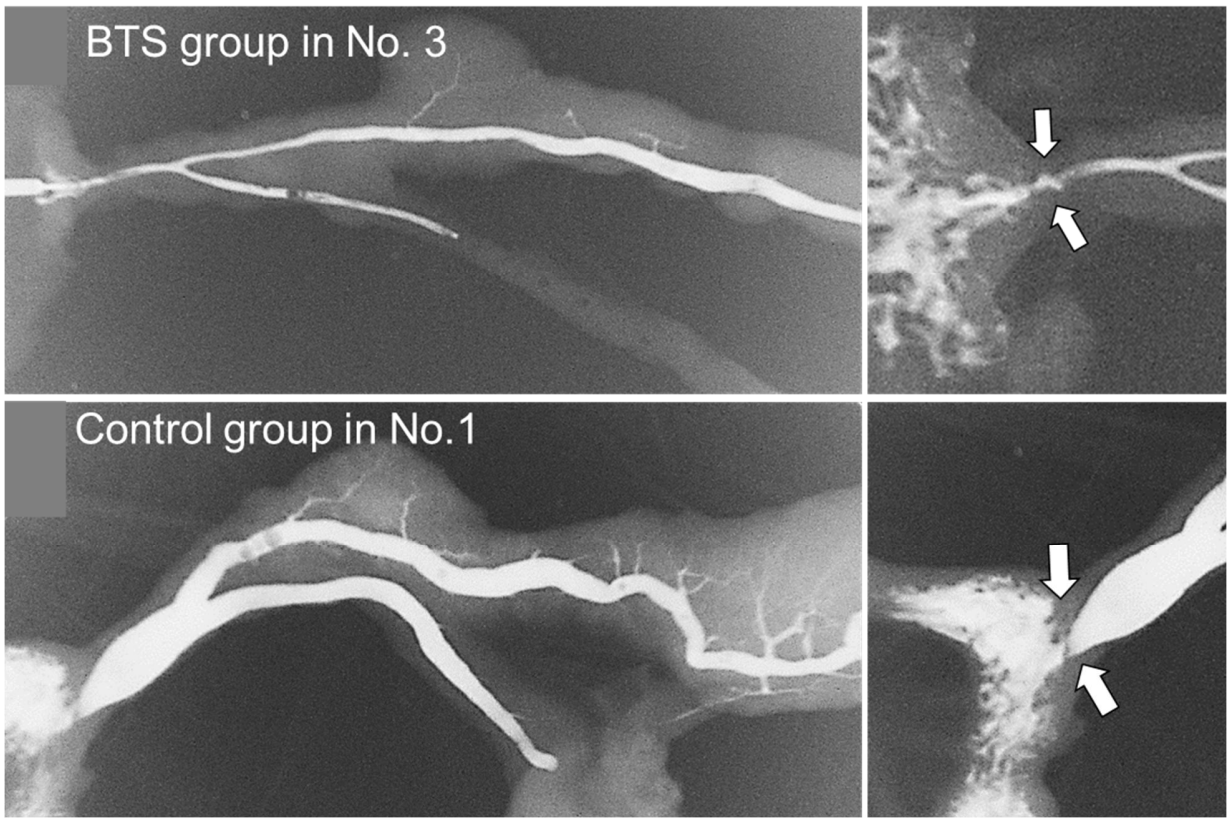

Figure 12. Pancreatography images showing the dilated pancreatic duct in pig number 1 in the control group compared to relatively smaller duct in pig number 3 in the BTS group. Arrows are pointing to the site of the anastomosis.

Histological Analysis

The mean luminal area of the pancreatic ducts was significantly larger in the control compared to the stent groups (Fig.13) (Table 3).In the histologic analysis of the pancreas, only mild changes were detected in the pancreatic parenchyma in the BTS and the control group. The measurement of the diameter at the anastomotic site was not feasible as the lumen was collapsed after staining.

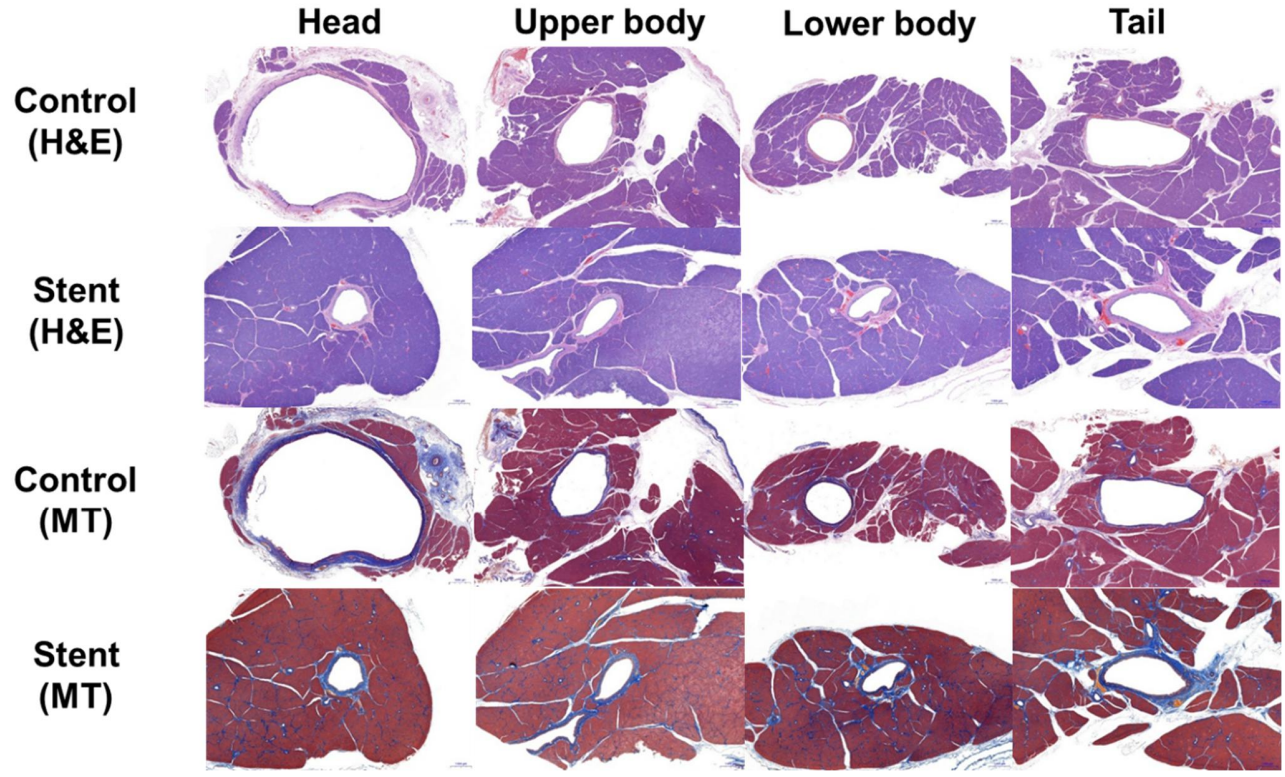

Figure 13. Representative histological images (H&E and MT, x4) showing the significant difference in the pancreatic duct luminal diameter between the control and the stent groups at the head, upper, lower body and tail of pancreas. H&E, Hematoxylin and Eosin stain; MT, Masson's Trichrome stain.

Table 3. Histological results of the pancreatic duct luminal area:

Locations	Control Group* (n = 5)	BTS Group* (n = 5)	*P*-valve†
Head	69.44 ± 52.78	4.58 ± 6.95	< 0.001
Upper body	39.44 ± 21.24	3.96 ± 3.17	< 0.001
Lower body	15.74 ± 10.48	3.60 ± 2.03	< 0.001
Tail	23.90 ± 16.82	3.34 ± 2.05	< 0.001

** Data are mean ± standard deviation (mm2)*

† *Mann-Whitney t test*

Endoscopic examination

The endoscopic examination showed a narrow anastomotic site in the control group compared to the BTS group (Fig.14).

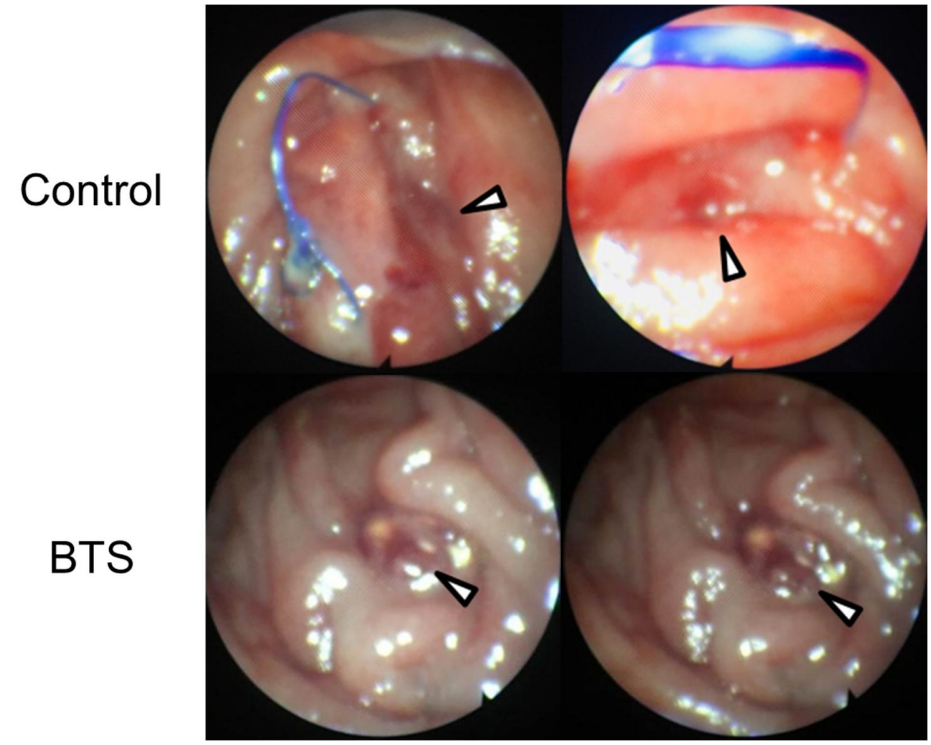

Figure 14. Endoscopic images showing the differences at the anastomotic site between the BTS and the control groups. Arrowheads are pointing to the anastomotic site.

Discussion

In this study, we developed a novel biodegradable tubular stent (BTS) to reduce the incidence of complications after pancreaticoduodenectomy and pancreaticojejunal anastomosis. Two animal models were used in our study. In a rat model, the rBTSs started to degrade 3 weeks after placement and completely degraded between 8 and 12 weeks. In a porcine model, pBTSs placement was successful in all pigs. Two stents migrated into the small bowel between 1 and 4 weeks after the surgery. Nonetheless, all the pigs had patent anastomotic site and a pancreatic duct diameter that was significantly smaller than the control group.

The use of transanastomotic pancreatic stent after PJ anastomosis is recommended by some surgeons in order to reduce the risk of post-operative anastomotic leakage (35). The stent can also maintain the anastomotic patency, and thus prevents edema-induced postoperative pancreatitis and/or partial dehiscence of the pancreaticojejunal anastomosis. Additionally, it may prevent late complications such as stricture formation at the pancreatic duct, and facilitate long-term patency of the anastomosis, thus helping to avoid secondary pancreatic fibrosis, atrophy, and exocrine dysfunction (10). In our porcine model, the pBTS maintained the patency of the PJ anastomosis in all pigs. Moreover, compared to the preoperative diameter, the pancreatic duct was significantly less dilated in the BTS group than the control group. This was also the case in the two pigs which had pBTSs migration into the small bowel denoting that our stent prevented stricture at the anastomotic site even with short stent-retention time. Furthermore, two pigs in the control group had pancreatitis, and no events occurred in the BTS group. This can be explained by the marked narrowing of the PJ anastomotic site in the control group, as detected by endoscopic examination and the pancreatic ductography results, thus causing reflux of the pancreatic

secretions and autodigestion of the pancreatic tissue by the pancreatic enzymes followed by secondary pancreatitis. This did not happen in the BTS group as the patent anastomotic site allowed adequate drainage of the pancreatic secretion. In concordance to our results, Kasuya et al. (52) investigated a novel technique of anastomosis with a short-term degradable stent for pancreaticojejunostomy. The stent was made form Monocryl and PDS-II and was evaluated in 8 patients. It preserved its strength for about 3 weeks, and few post-operative complications were reported. Nordback et al. (53) investigated a radiopaque biodegradable stent in 29 patients. No in-hospital mortality was reported with an overall fistula rate of 3%, and an overall hemorrhage rate of 7%. Six patients (17%) developed clinically significant delayed gastric emptying. Most patients had well opened (57 %), or slightly narrowed (13%) anastomotic site.

The optimal duration of stent placement in PJ anastomosis is still debated (37). Short stent-retention time is likely to ameliorate the risk of leakage, because the epithelium of the jejunal mucosa becomes well healed with the pancreatic ductal epithelium 2 weeks after surgery. However, because the pancreaticojejunal anastomosis may require up to 6 months to become mature, prolonged stent placement may ensure patency. The potential benefit of prolonged stent placement must be balanced against the potential risks. Limited data support the long-term use of pancreatic stents, and some studies suggest that long-term trans anastomotic stent placement is of no benefit and even harmful (10). Biodegradable materials varies in their degradation time, while some can degrade in few weeks, others can take up to several months or even years (54-59). Unpublished data from our in-vitro study showed that the BTS started to degrade at around 20 days when placed in pancreatic or bile juice. In our rat study, the stent started to show signs of degradation after 3 weeks and completely degraded after 8-12 weeks. The results from our porcine study showed complete healing of the PJ anastomosis with no stricture or obstruction in the pancreatic duct in

the BTS group. This occurred even after two pBTSs had migrated before the end of the study. This denotes that the stent remained in place for enough time for the anastomotic site to heal. According to these observations, we can suggest that between 4 to 8 weeks could be an optimum timing for stenting after PJ anastomosis.

The incidence of stent-related complications after PJ is not well documented (42). Mari et al. (42) reviewed the studies that reported such complications. Stent retention was reported in six studies (the number of the patients ranged from 1 to 4). The diagnosis was made after variable duration from surgery (6 weeks up to 7 years). Stent retention usually resulted in steatorrhea, pancreatitis, weight loss, or abdominal pain. In all cases, endoscopic or surgical removal of the stents was required. In the same review, seven studies have reported stent migration (the number of the patients ranged from 1 to 8). The time range until diagnosis was even wider than with stent retention (several months up to 19 years). Stent migration resulted in small bowel obstruction or perforation, bezoar formation, dehiscence of the PJ anastomosis with migration to the peritoneal cavity with peritonitis, liver abscess, and cholangitis. Endoscopic or surgical procedure was needed in most of these cases. In our porcine study, we did not have any cases of stent retention which can be explained by the short degradation time of our stent. Nonetheless, two out of the five pBTSs had migrated into the distal small bowel (40%), albeit all were sutured to the jejunum during the surgery, between 1 and 4 weeks after placement. We believe that the reason of migration is the degradation of the pBTS at the site of the suture attachment. The migrated pBTSs were seen in the small bowel at 4 weeks follow-up, and completely disappeared at 8 weeks. They may had passed through the anus, however, as they biodegrade spontaneously and in relatively short duration (less than 12 weeks), we did not expect further sequelae. Other reported stents related complications include stent obstruction, or later stricture of the pancreatic duct secondary to inflammatory

reaction to the stent. None of these was encountered in our study mostly due to the short stent-retention time, and also the two holes at each end of the pBTS allowed drainage of the pancreatic secretion and decreased the risk of obstruction.

This study has some limitations. First, small number of the animals was used. Second, the follow-up duration was relatively short. Last, preventing the bias during the surgery in the porcine study was not possible. However, this study gives as an insight about the potential benefits of biodegradable stent placement in the management of pancreaticojejunostomy after pacreaticoduodenectomy.

Biodegradable tubular stent (BTS) development from commercially available polymers is feasible. The BTS seems to be safe and effective for the management of pancreaticojejunal anastomosis after pacreaticoduodenectomy. We surmise that the optimal duration of stenting after surgery may be between 4 and 8 weeks. Future studies to optimize the BTS for human application is needed.

REFERENCES

1. Tran KT, Smeenk HG, van Eijck CH, Kazemier G, Hop WC, Greve JW, et al. Pylorus preserving pancreaticoduodenectomy versus standard Whipple procedure: a prospective, randomized, multicenter analysis of 170 patients with pancreatic and periampullary tumors. Ann Surg. 2004;240(5):738-45.
2. Koh FH, Kow AW. Minimally Invasive Whipple's Technique for Laparoscopic-Assisted Pylorus-Preserving Pancreaticoduodenectomy. Journal of the American College of Surgeons. 2017;224(2):e1-e3.
3. Song KB, Kim SC, Hwang DW, Lee JH, Lee DJ, Lee JW, et al. Matched Case-Control Analysis Comparing Laparoscopic and Open Pylorus-preserving Pancreaticoduodenectomy in Patients With Periampullary Tumors. Ann Surg. 2015;262(1):146-55.
4. Zhong X, Wang X, Pan J, Zhu H, Gu L, Shi Z. Mesh-reinforced pancreaticojejunostomy versus conventional pancreaticojejunostomy after pancreaticoduodenectomy: a retrospective study of 126 patients. World journal of surgical oncology. 2018;16(1):68.
5. Warshaw AL, Thayer SP. Pancreaticoduodenectomy. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2004;8(6):733-41.
6. Zhao Z, Yin Z, Hang Z, Ji G, Feng Q, Zhao Q. A systemic review and an updated meta-analysis: minimally invasive vs open pancreaticoduodenectomy. Scientific reports. 2017;7(1):2220.
7. Yang C, Wu H-S, Chen X-L, Wang C-Y, Gou S-M, Xiao J, et al. Pylorus-Preserving Versus Pylorus-Resecting Pancreaticoduodenectomy for Periampullary and Pancreatic Carcinoma: A Meta-Analysis. PLOS ONE. 2014;9(3):e90316.
8. Poon RT, Fan ST, Lo CM, Ng KK, Yuen WK, Yeung C, et al. External drainage of pancreatic duct with a stent to reduce leakage rate of pancreaticojejunostomy after pancreaticoduodenectomy: a prospective randomized trial. Ann Surg. 2007;246(3):425-33; discussion 33-5.
9. Nahm CB, Connor SJ, Samra JS, Mittal A. Postoperative pancreatic fistula: a review of traditional and emerging concepts. Clinical and experimental gastroenterology. 2018;11:105-18.

10. Levy MJ, Chari S, Adler DG, Clain JE, Gostout CJ, Harewood GC, et al. Complications of temporary pancreatic stent insertion for pancreaticojejunal anastomosis during pancreaticoduodenectomy. Gastrointestinal endoscopy. 2004;59(6):719-24.
11. Penumadu P, Barreto SG, Goel M, Shrikhande SV. Pancreatoduodenectomy - preventing complications. Indian journal of surgical oncology. 2015;6(1):6-15.
12. Karim SAM, Abdulla KS, Abdulkarim QH, Rahim FH. The outcomes and complications of pancreaticoduodenectomy (Whipple procedure): Cross sectional study. Int J Surg. 2018;52:383-7.
13. Gouma DJ, van Geenen RC, van Gulik TM, de Haan RJ, de Wit LT, Busch OR, et al. Rates of complications and death after pancreaticoduodenectomy: risk factors and the impact of hospital volume. Annals of surgery. 2000;232(6):786-95.
14. Cioffi JL, McDuffie LA, Roch AM, Zyromski NJ, Ceppa EP, Schmidt CM, et al. Pancreaticojejunostomy Stricture After Pancreatoduodenectomy: Outcomes After Operative Revision. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2016;20(2):293-9.
15. Total Laparoscopic Pancreaticoduodenectomy: Feasibility and Outcome in an Early Experience. Arch Surg. 2010;145(1):19–23.
16. Demirjian AN, Kent TS, Callery MP, Vollmer CM. The inconsistent nature of symptomatic pancreatico-jejunostomy anastomotic strictures. HPB : the official journal of the International Hepato Pancreato Biliary Association. 2010;12(7):482-7.
17. Gong J, He S, Cheng Y, Cheng N, Gong J, Zeng Z. Fibrin sealants for the prevention of postoperative pancreatic fistula following pancreatic surgery. The Cochrane database of systematic reviews. 2018;6:CD009621.
18. Orci LA, Oldani G, Berney T, Andres A, Mentha G, Morel P, et al. Systematic review and meta-analysis of fibrin sealants for patients undergoing pancreatic resection. HPB : the official journal of the International Hepato Pancreato Biliary Association. 2014;16(1):3-11.
19. Does Prophylactic Octreotide Decrease the Rates of Pancreatic Fistula and Other Complications After Pancreaticoduodenectomy?: Results of a Prospective Randomized Placebo-Controlled Trial. Annals of Surgery. 2000;232(3):419-29.

20. Alghamdi AA, Jawas AM, Hart RS. Use of octreotide for the prevention of pancreatic fistula after elective pancreatic surgery: a systematic review and meta-analysis. Canadian journal of surgery Journal canadien de chirurgie. 2007;50(6):459-66.

21. Yeo CJ, Cameron JL, Lillemoe KD, Sauter PK, Coleman J, Sohn TA, et al. Does prophylactic octreotide decrease the rates of pancreatic fistula and other complications after pancreaticoduodenectomy? Results of a prospective randomized placebo-controlled trial. Annals of surgery. 2000;232(3):419-29.

22. Jin K, Zhou H, Zhang J, Wang W, Sun Y, Ruan C, et al. Systematic Review and Meta-Analysis of Somatostatin Analogues in the Prevention of Postoperative Complication after Pancreaticoduodenectomy. Digestive Surgery. 2015;32(3):196-207.

23. Bai M-D, Rong L-Q, Wang L-C, Xu H, Fan R-F, Wang P, et al. Experimental study on operative methods of pancreaticojejunostomy with reference to anastomotic patency and postoperative pancreatic exocrine function. World journal of gastroenterology. 2008;14(3):441.

24. Distal migration of a transanastomotic pancreatic stent resulting in bowel perforation 19 years after pancreatoduodenectomy:report of a case. Surg Today. 2015;45(3):374-7.

25. Kennedy EP, Yeo CJ. Dunking pancreaticojejunostomy versus duct-to-mucosa anastomosis. Journal of hepato-biliary-pancreatic sciences. 2011;18(6):769-74.

26. Kilambi R, Singh AN. Duct-to-mucosa versus dunking techniques of pancreaticojejunostomy after pancreaticoduodenectomy: Do we need more trials? A systematic review and meta-analysis with trial sequential analysis. 2018;117(5):928-39.

27. Tran K, Van Eijck C, Di Carlo V, Hop WCJ, Zerbi A, Balzano G, et al. Occlusion of the pancreatic duct versus pancreaticojejunostomy: a prospective randomized trial. Annals of surgery. 2002;236(4):422-8.

28. Papachristou DN, D'Agostino H, Fortner JG. Ligation of the pancreatic duct in pancreatectomy. The British journal of surgery. 1980;67(4):260-2.

29. Azumi Y, Isaji S, Kato H, Nobuoka Y, Kuriyama N, Kishiwada M, et al. A standardized technique for safe pancreaticojejunostomy: Pair-Watch suturing technique. World journal of gastrointestinal surgery. 2010;2(8):260-4.

30. Aikawa M, Miyazawa M, Okamoto K, Okada K, Akimoto N, Yamaguchi S, et al. Novel pancreatoenteric reconstruction using a bioabsorbable polymer sheet and biocompatible bond. The Journal of surgical research. 2013;183(1):1-7.

31. Kimura W. Pancreaticojejunal anastomosis, using a stent tube, in pancreaticoduodenectomy. Journal of hepato-biliary-pancreatic surgery. 2009;16(3):305-9.
32. Wang Q, He XR, Tian JH, Yang KH. Pancreatic Duct Stents at Pancreaticoduodenectomy: A Meta-Analysis. Digestive Surgery. 2013;30(4-6):415-24.
33. Zhao Y, Zhang J, Lan Z, Jiang Q, Zhang S, Chu Y, et al. Are Internal or External Pancreatic Duct Stents the Preferred Choice for Patients Undergoing Pancreaticoduodenectomy? A Meta-Analysis. BioMed research international. 2017;2017:1367238-.
34. Sachs TE, Pratt WB, Kent TS, Callery MP, Vollmer CM, Jr. The pancreaticojejunal anastomotic stent: friend or foe? Surgery. 2013;153(5):651-62.
35. Biehl T, Traverso LW. Is stenting necessary for a successful pancreatic anastomosis? The American Journal of Surgery. 1992;163(5):530-2.
36. Is Stenting Necessary for a Successful Pancreatic Auastomosis? The American Journal of Surgery. 1992;163(5):530-2.
37. Winter JM, Cameron JL, Campbell KA, Chang DC, Riall TS, Schulick RD, et al. Does pancreatic duct stenting decrease the rate of pancreatic fistula following pancreaticoduodenectomy? Results of a prospective randomized trial. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2006;10(9):1280-90; discussion 90.
38. Dong Z, Xu J, Wang Z, Petrov MS. Stents for the prevention of pancreatic fistula following pancreaticoduodenectomy. The Cochrane database of systematic reviews. 2016(5):CD008914.
39. Motoi F, Egawa S, Rikiyama T, Katayose Y, Unno M. Randomized clinical trial of external stent drainage of the pancreatic duct to reduce postoperative pancreatic fistula after pancreaticojejunostomy. British Journal of Surgery. 2012;99(4):524-31.
40. Pessaux P, Sauvanet A, Mariette C, Paye F, Muscari F, Cunha AS, et al. External pancreatic duct stent decreases pancreatic fistula rate after pancreaticoduodenectomy: prospective multicenter randomized trial. Ann Surg. 2011;253(5):879-85.
41. Pancreaticojejunal stent migration resulting in "bezoar ileus". Am J Surg. 2000 180(2):115-6.
42. Pancreaticojejunal Anastomotic Stent-Related Complications after Pancreaticoduodenectomy. Review of the Literature. JOP J Pancreas. 2015;16(5):413-6.

44. Bhasin DK, Rana SS. Biodegradable pancreatic stents: are they a disappearing wonder? Gastrointestinal endoscopy. 2008;67(7):1113-6.
45. Venkatraman S, Poh TL, Vinalia T, Mak KH, Boey F. Collapse pressures of biodegradable stents. Biomaterials. 2003;24(12):2105-11.
46. Meng H, Zhang L, Chen Q, Wei J, Wang Y, Dong A, et al. Preparation of poly(aspartic acid) superabsorbent hydrogels by solvent-free processes2015.
47. Kara ME. The anatomical study on the rat pancreas and its ducts with emphasis on the surgical approach. Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft. 2005;187(2):105-12.
48. Martins PN, Neuhaus P. Surgical anatomy of the liver, hepatic vasculature and bile ducts in the rat. Liver international : official journal of the International Association for the Study of the Liver. 2007;27(3):384-92.
49. Vdoviakova K, Petrovova E, Maloveska M, Kresakova L, Teleky J, Elias MZ, et al. Surgical Anatomy of the Gastrointestinal Tract and Its Vasculature in the Laboratory Rat. Gastroenterology research and practice. 2016;2016:2632368.
50. Alkeev N, Averin S, von Gratowski S. New Method for Monitoring the Process of Freeze Drying of Biological Materials. AAPS PharmSciTech. 2015;16(6):1474-9.
51. Son SR, Franco RA, Bae SH, Min YK, Lee BT. Electrospun PLGA/gelatin fibrous tubes for the application of biodegradable intestinal stent in rat model. Journal of biomedical materials research Part B, Applied biomaterials. 2013;101(6):1095-105.
52. Kasuya K, Shimazu M, Abe Y, Kikuchi S, Itoi T, Ikada Y, et al. A newly developed degradable stent for pancreaticojejunostomy after pancreatoduodenectomy. Int Surg. 2010;95(3):247-56.
53. Nordback I, Raty S, Laukkarinen J, Jarvinen S, Piironen A, Leppiniemi J, et al. A novel radiopaque biodegradable stent for pancreatobiliary applications--the first human phase I trial in the pancreas. Pancreatology : official journal of the International Association of Pancreatology. 2012;12(3):264-71.
54. Wang Z, Li N, Li R, Li Y, Ruan L. Biodegradable intestinal stents: A review. Progress in Natural Science: Materials International. 2014;24(5):423-32.

55. Sheikh Z, Najeeb S, Khurshid Z, Verma V, Rashid H, Glogauer M. Biodegradable Materials for Bone Repair and Tissue Engineering Applications. Materials (Basel, Switzerland). 2015;8(9):5744-94.

56. Waksman R, Pakala R. Biodegradable and bioabsorbable stents. Curr Pharm Des. 2010;16(36):4041-51.

57. Lorenzo-Zúñiga V, Moreno-de-Vega V, Marín I, Boix J. Biodegradable stents in gastrointestinal endoscopy. World journal of gastroenterology. 2014;20(9):2212-7.

58. Park J-S, Jeong S, Lee DH. Recent Advances in Gastrointestinal Stent Development. Clinical endoscopy. 2015;48(3):209-15.

59. Mao L, Shen L, Chen J, Zhang X, Kwak M, Wu Y, et al. A promising biodegradable magnesium alloy suitable for clinical vascular stent application. Scientific reports. 2017;7:46343.