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Original Article

New polyglycolic acid fabric for the prevention of postoperative pancreatic fistulas

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Summary *Background:* The incidence of postoperative pancreatic fistula (POPF) after distal pancreatectomy is approximately 30%. The most serious complications of pancreatic resection, such as mortality and prolonged hospitalization, are unresolved despite the proposal of various surgical procedures. We developed a new polyglycolic acid (PGA) fabric composed of fine diameter fibers to prevent POPF, and macroscopically and microscopically evaluated the effects of applying it to the pancreatic remnant.
Methods: The ventral pancreatic surface was cauterized to create the experimental model of POPF in 33 female Wistar/ST rats. The injured sites were wrapped with nonwoven PGA fabrics of different fiber diameters and porosities in the treated rats; one group of rats remained untreated. Survival, incidence of generalized peritonitis, and microscopic findings around the pancreas were investigated.
Results: The PGA fabrics acted as a scaffold for tissue repair and resulted in superior survival. Generalized peritonitis was milder in the PGA treated groups. With the new PGA fabric, abundant fibroblast infiltration and a uniformly-developed, self-organized barrier wall prevented both pancreatic leak and spread of inflammation.

Conflicts of interest: No author has any financial or personal relationship with people or organizations that could potentially and inappropriately influence our work and conclusions.

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Conclusion: Application of the newly developed PGA fabric to the pancreatic remnant prevented POPF, and the essential factor for preventing pancreatic leak was the early formation of a self-organized barrier.

1. Introduction

The most serious complications of pancreatic resection are postoperative pancreatic fistula (POPF), leak, and/or abscess.[1–3] The incidence of POPF is approximately 5% following pancreaticoduodenostomy and approximately 30% following distal pancreatectomy.[4,5]

With a pancreatic leak, pancreatic juice leaks out of the pancreatic ductal system via an abnormal connection into the peri-pancreatic space or the peritoneal cavity. The leaked pancreatic juice contains digestive enzymes such as pepsin and lipase that digest the abdominal organs and vessels, potentially resulting in generalized peritonitis, paralytic ileus, bleeding from the digested vessels, intraabdominal abscess, and/or multiorgan failure. Patients experience morbidity from abdominal pain, ileus, fever, possible abscess, sepsis, and hemorrhage, and subsequent prolonged hospitalization.

Localizing the pancreatic leak and suppressing pancreatic juice production are the key factors for preventing POPF. Surgical techniques used to manage the pancreatic remnant to reduce the rate of POPF after distal pancreatectomy include suture closure, the use of various stapling devices, a combination of staple closure and reinforcement with different materials, pancreatic enteric anastomosis, and the use of fibrin sealants; however, a superior technique has not been identified.[6–10] Furthermore, despite the availability of these surgical techniques, the morbidity associated with distal pancreatectomy remains high.[11] Several pharmacological and technical interventions have also been suggested to decrease the rate of POPF but the results have been controversial.[12–15]

Polyglycolic acid (PGA) is not enzymatically degraded, but is instead degraded by hydrolysis after 3–4 weeks. Nonwoven PGA fabric functions as a scaffold for tissue regeneration or repair. We developed a new scaffold composed of a nonwoven PGA fabric made of fine-diameter fibers. The present study aimed to evaluate this newly developed PGA fabric and a commercially available PGA fabric (NEOVEIL; Gunze Co., Kyoto, Japan) to determine their efficacy in localizing the inflammation, preventing POPF, and reducing the morbidity in a rat model of surgically injured pancreas.

2. Materials and methods

The animal experiments were approved by the Doshisha University Animal Experimentation Committee. All surgical and anesthetic procedures were performed in accordance with the animal care guidelines of Doshisha University and European Commission Directive 86/609/EEC for animal experiments.

Thirty-seven female, 8–10 week-old Wistar/ST rats weighing 200–220 g were used. Before the experimental period, the rats were housed in the laboratory for 1 week. During the experimental period, all rats were housed separately and maintained under standard specific pathogen-free conditions: light-dark cycle of 12:12 hours, mean temperature of 23°C, and mean humidity of 50%. Standard laboratory rodent chow and water were available *ad libitum*. On the experimental day, the health condition of the rats was checked, including evidence of diarrhea, mucous discharge from the eyes or anus, or emaciation and the condition of their body hair (hair loss or filthy hair).

The rats were divided into four groups at random: three treated groups (injured pancreatic remnant was wrapped with a sheet of each material) and one nontreated group. Survival and findings of the abdominal cavity were evaluated. Following the surgical procedure, the rats were housed under the same standard conditions for 5 days.

2.1. Preparation of materials

Two types of nonwoven PGA fabric sheets, with differing fiber diameters and porosity, and a copolymer [lactic acid/caprolactone; p(LA/CL)] sheet were prepared.

The first PGA fabric had a mean fiber diameter of 20 μm (PGA-L) (NEOVEIL) and was produced using the needle punch method.[15] The second PGA fabric had a mean fiber diameter of 0.9 μm (PGA-S) and was produced using the melt blowing method.[14] Briefly, the PGA polymer was extruded through dies with small nozzles, attenuated with heat and a high-velocity airstream, and spun into fibers. The fibers were then randomly deposited onto a collector to form a nonwoven fabric. The two types of nonwoven PGA fabric were cut into square sheets measuring 20 mm × 20 mm in size and weighing 50.0 mg. The square sheets were sterilized by soaking them in 99.5% ethanol for 60 seconds followed by two rinse cycles in saline just prior to surgery.

The 0.1-mm thick p(LA/CL) sheet was produced by melt pressing at 15 MPa and 110°C for 5 minutes followed by quenching in ice water. To remove the water, the sheet was dried under a vacuum at room temperature for 1 day.

2.2. Surgical procedure for pancreatic injury

All procedures were performed under sterile conditions by specific surgeons responsible for the assigned procedure. The rats were administered isoflurane (Escain; Mairan Seiyaku, Inc., Osaka, Japan) via inhalation for anesthesia. They were also administered 6.48 mg of sodium pentobarbital (Somnopentyl; Kyoritsu Seiyaku, Inc., Tokyo, Japan) via an intraperitoneal injection.

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The stomach, spleen, omental sac, and pancreas were removed from the abdominal cavity through a 5-cm long median transabdominal incision; then, the anterior wall of the omental sac was opened to expose the pancreas. The ventral surface of the pancreas (80% of the ventral surface) was cauterized with an electric scalpel (Hyfrecator 2000; CONMED, Utica, NY, USA) in coagulation mode (high frequency output, 35 W) without direct contact with the pancreas. In each treatment group, the cauterized surface of the pancreas was fully wrapped by the PGA-L or PGA-S fabric, or the p(LA/CL) sheet was fixed by three-point sutures with 7/0 polyvinylidene fluoride monofilament sutures for microsurgery (Asflex; Kono Seisakusyo Co., Chiba, Japan). In the nontreated group, no further procedures were applied. The laparotomy incision was closed with 4/0 polyamide sutures.

2.3. Evaluation after surgical injury of the pancreas

To determine the survival rate, the rats were observed for 5 days after the surgery. According to the experimental model, all deaths should occur within 5 days after surgery, and the humane endpoint was set as such. The rats were killed humanely when signs of moribund status (impaired mobility) were observed. The survivors were killed humanely on Day 5.

Macroscopic evaluation was conducted for the abdominal findings associated with pancreatic leak. All of the rats (deceased and survived) were autopsied to assess the pathologic findings of the cause of death and the spread of injurious tissue changes caused by pancreatic leak, including generalized peritonitis (inflammation of the entire peritoneum from the diaphragm to the pelvis), intestinal expansion (paralytic ileus), and tumor formation by all organs.

For the microscopic evaluation, all of the abdominal organs were surgically excised *en bloc* as specimens. The sample materials were formalin-embedded and stained with hematoxylin and eosin for histological assessment according to a standard protocol. All specimens were assessed microscopically from two points of view: cell infiltration and granulation formation based on the scaffold of the materials and histological differences between the inside (pancreas side) and outside zones of the materials.

2.4. Statistical analyses

Data was analyzed using IBM SPSS Statistics Base (Armonk, NY, USA). The survival rates, incidence of generalized peritonitis, and incidence of paralytic ileus were compared between the groups using Fisher's exact tests and Bonferroni correction. In the PGA-L group, one rat that died from gastric perforation due to the electrocauterization was excluded from the analyses.

3. Results

3.1. Survival rates

Survival was greater in both the PGA-L and PGA-S groups compared with the nontreated group (Table 1). There were no significant differences between the PGA-L and PGA-S groups. Copolymer p(LA/CL) did not improve the survival.

3.2. Macroscopic evaluation

Generalized peritonitis, intestinal dilation, and strong adhesion of the intraperitoneal viscera occurred significantly less frequently in both PGA treatment groups compared to the nontreated group (Table 1).

3.3. Microscopic evaluation

In the rats that survived in all of the groups, POPF and generalized peritonitis were mild or absent, and inflammatory granulation tissue with abundant cell infiltration formed barrier walls throughout the cauterized surfaces of the pancreas. In contrast, the barrier wall formed by granulation tissue was incomplete or absent in the deceased rats.

In the PGA-L group (Figure 1A), inflammatory granulation tissue formed nonuniformly over the full thickness of the scaffold. Injurious tissue changes and the spread of inflammation by pancreatic leak were less severe in the outer zone of the fabric than in the inner zone. Cell infiltration was observed inside the scaffold.

In the PGA-S group (Figure 1B), regarding barrier formation, a granulation wall formed uniformly throughout the full wall thickness. Fibroblast infiltration inside the scaffold was more abundant in the PGA-S group than in the PGA-L group. In contrast, the barrier wall was irregular and partially rough in the PGA-L group (Figure 1A). Very little inflammatory cell infiltration was present in either side of the fabric in the PGA-L group.

In the p(LA/CL) group (Figure 1C), abundant inflammatory mononuclear cells accumulated, but no self-organized granulation tissue band was formed.

In the nontreated group (Figure 1D), no barrier wall was formed. Injurious tissue changes and severe inflammation spread widely to sites distant to the cauterized pancreas.

4. Discussion

The present study demonstrated that wrapping the pancreas remnant with a nonwoven PGA fabric is effective for preventing pancreatic leak and POPF and for decreasing the generalized peritonitis due to postoperative pancreatic leak. The digestion of tissue by pancreatic enzymes was avoided, and the spread of peritonitis, adherence to adjacent organs, and paralytic ileus were rarely observed in the groups treated with the PGA fabrics. PGA fabrics are safely and widely used in clinical cases. Endogenous barrier formation by inflammatory granulation tissue surrounding the pancreas is an essential contributor to the suppression of POPF and generalized peritonitis because it stops pancreatic juice from leaking and spreading to the peritoneal cavity.

Using fibrin glue when wrapping with PGA fabrics[12,16] and reinforcement of PGA fabrics with surgical stapling reportedly decreases the severity, but not the incidence, of POPF.[4,6,8,17] The precise mechanisms of fabric reinforcement for the prevention of POPF have not been clear. The

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Table 1 Postoperative survival of rats with a surgically injured pancreas.

	Survival (*n*)	Generalized peritonitis	Intestinal expansion	Tumor formation by all of the organs
Nontreated group ($n = 11$)	3	8	8	6
PGA-L treated group ($n = 10$)	9*	1**	1*	1*
PGA-S treated group ($n = 10$)	9*	1*	1*	1*
p(LA/CL) treated group ($n = 6$)	3**	3	1*	2

*$p < 0.05$ compared with the nontreated group. There were no significant differences between the PGA-L and PGA-S groups.
** $p < 0.05$ compared p(LA/CL) with the PGA-L and PGA-S.
PGA-L = polyglycolic acid fabric with a mean fiber diameter of 20 μm; PGA-S = polyglycolic acid fabric with a mean fiber diameter of 0.9 μm; p(LA/CL) = copolymer of lactic acid and caprolactone.

present histological investigation provides useful information: the PGA fabrics provided scaffolds for early tissue repair, and well-built barriers of inflammatory granulation developed at the site of contact with the pancreas remnant. The spread and severity of inflammation inside the PGA fabrics (pancreas side) were mild; therefore, early enclosure of the pancreatic leak might have been achieved, and the barrier formed by the abundant fibroblast infiltration in the scaffold of the PGA fabrics likely prevented POPF.

Early barrier formation with abundant cell infiltration is considered the most important factor for POPF prevention

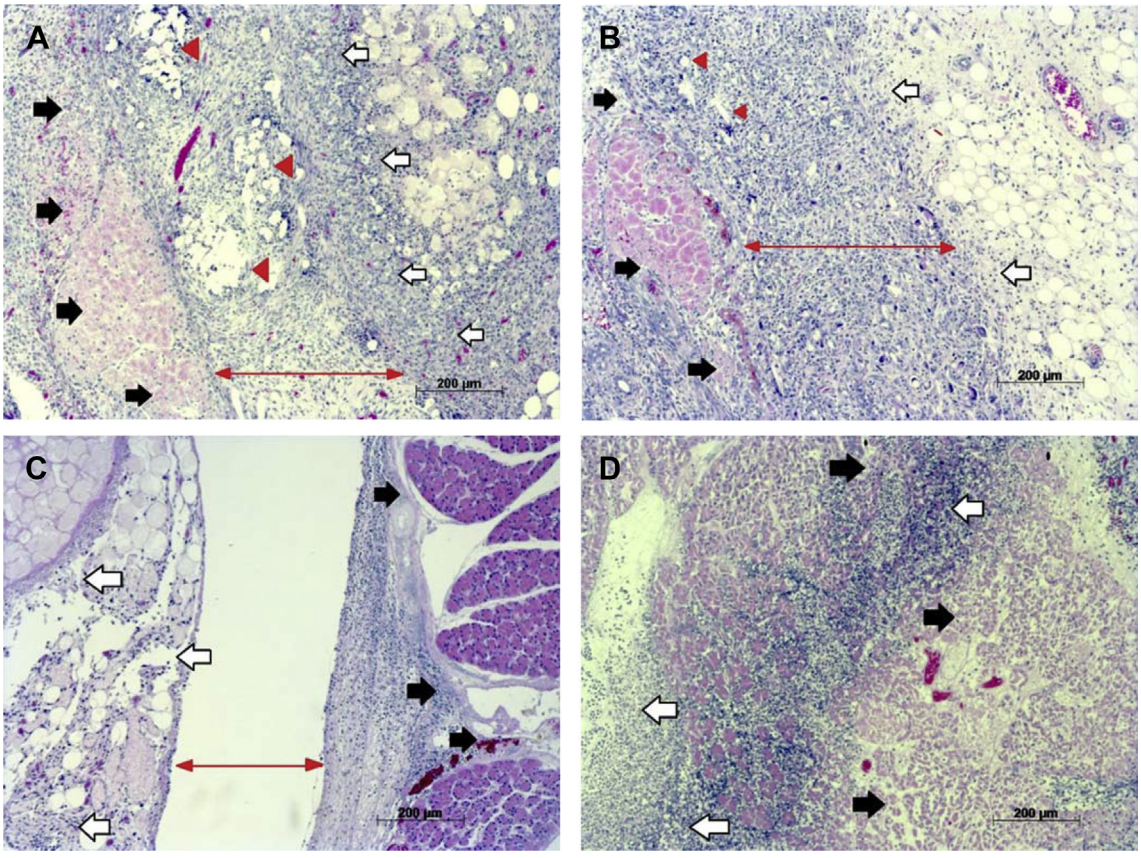

Figure 1 Microscopic evaluation of the pancreas in a rat model of surgically injured pancreas to determine the efficacy of PGA fabrics in localizing the inflammation and preventing postoperative pancreatic fistula. (A) PGA-L group (mean fiber diameter of 20 μm): red ◂—▸, PGA-L fabric placement, showing a barrier wall of nonuniform granulation tissue; black ➡, necrotic pancreatic tissue; red ◂, remaining PGA-L fibers; and white ⇦, inflammatory changes outside the barrier wall; (B) PGA-S group (mean fiber diameter of 0.9 μm): red ◂—▸, PGA-S fabric placement showing a barrier wall of abundant cells and uniform granulation tissue; black ➡, necrotic pancreatic tissue; red ◂, remaining PGA-S fibers; and white ⇦, very little inflammatory cell infiltration outside the barrier wall because the spread of inflammation was enclosed inside the barrier wall; (C) p(LA/CL) group: red ◂—▸, p(LA/CL) placement showing no granulation tissue formation on the inside of the sheet; black ➡, inflammatory cells are abundant inside the barrier wall; and white ⇦, tissue necrosis and inflammation are observed outside of the sheet; (D) nontreatment group: black ➡, necrotic pancreatic tissue; white ⇦, spread of severe necrosis and inflammation to the tissue surrounding the pancreas. PGA = polyglycolic acid; p(LA/CL) = lactic acid/caprolactone copolymer.

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by PGA fabrics. In our previous study, we showed that PGA fabrics degrade very little by hydrolysis and continue to work as a scaffold for tissue repair over a 1-week period.[18] In contrast, p(LA/CL) sheet, which has low water permeability, did not significantly improve the mortality rate (50%, 3 of 6 cases) when compared with the nontreated group. We evaluated p(LA/CL) because it is known to act as a scaffold for tissue regeneration. In the p(LA/CL) group, very little cell infiltration was observed in the material, and no barrier formation developed in the 5 days after the surgery. Therefore, tissue repair in the scaffold is considered indispensable for POPF prevention. The newly developed PGA fabric made of fine fibers induced early cell infiltration and barrier formation, which could act as a scaffold. In the late stage after surgery, degradation of the PGA fabrics at 2—4 weeks postoperative possibly avoids continuous inflammation caused by foreign bodies. The delayed POPF after the degradation of PGA may be possible, but it was thought that the endogenous barrier developed enough to prevent pancreatic leak before the PGA disappeared.

In our previous study (unpublished) using the same experimental model as the present study, different PGA fabrics with fibers of a smaller diameter (0.7 μm) and a narrower space between the fibers showed poor cell infiltration and barrier formation, indicating that fiber diameter and the space between the fibers are important factors for tissue repair in the scaffold. In the present study, the cell infiltration in the PGA-S fabric, which was composed of fine fibers, was more substantial and dense than that in the PGA-L fabric, which was composed of thick fibers. In addition, compared with the PGA-L fabric, the tissue structure of the barrier band in the PGA-S fabric was uniform and thin (300—800 μm), and excessive inflammatory granulation development was not apparent. Therefore, the barrier band of the PGA-S fabric had a structure that restricts the spread of pancreatic juice. With the PGA-L fabric, the cell infiltration and fiber tissue inside the scaffold were coarser, but the band was thick, and the inflammatory responses around the scaffold are stronger. Therefore, based on these histological findings, the PGA-S fabric might be superior to PGA-L fabric for POPF prevention, by stopping pancreatic leak earlier and successfully preventing the spread of pancreatic juice. This is noteworthy because there were no differences in morbidity or mortality between the PGA-S and PGA-L groups despite these differences in the microscopic structure of the barrier.

In the present study we used widely cauterized pancreas to evaluate the preventive effects of the newly developed PGA fabric against POPF and pancreatic juice leakage. Our preliminary experiments showed that distal pancreatectomy in rats does not always produce POPF or major pancreatic juice leakage. Our cauterized pancreas model brought on severe and wide pancreatic juice leakage without fail. According to the experimental results POPF in rats could be brought about by the injury of numerous pancreatic duct branches and the main pancreatic duct. The extent of the pancreatic duct injury is considered to be more severe in the present experimental model than in human surgery. POPF in humans develops in various conditions. The severity and clinical course are substantially different for each POPF case, so that evaluation of the ability of the developed PGA fabric to prevent POPF in humans is necessary. We are developing a revised model with the new fabric and reinforcement using a surgical stapler that can be safely used in pancreatic surgery.

In conclusion, application of the newly developed PGA fabric composed of fine fibers to the pancreatic remnant was effective in preventing POPF, and the essential factor was the early and secure formation of a self-organized barrier.

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