Int Surg 2015;**100:**1408–1413
DOI: 10.9738/INTSURG-D-15-00037.1

An Artificial Bile Duct Made of Bioabsorbable Polymer: A Viable Substitute for Narrowed Portion of the Extrahepatic Bile Duct

Mitsuo Miyazawa[1], Masayasu Aikawa[1], Katsuya Okada[1], Yukihiro Watanabe[1], Kojun Okamoto[1], Shrestha Santosh[1], Shigeki Yamaguchi[1], Isamu Koyama[1], Yoshito Ikada[2]

[1]*Department of Surgery, Gastrointestinal Center, Saitama Medical University, International Medical Center, Saitama, Japan*

[2]*Division of Life Science, Nara Medical University, Nara, Japan*

The aim of this study was to investigate whether an artificial bile duct made of bioabsorbable polymer could serve as a substitute for narrowed portions of the bile duct. The experiments were performed using hybrid pigs (n = 11). After laparotomy under general anesthesia, the extrahepatic bile duct was identified and ligated around the confluence with the cystic duct. A week later, repeat laparotomy was performed on the animals, and the bile duct on the hepatic side of the ligature was resected. The cut end was connected to the duodenum using a bioabsorbable artificial bile duct fabricated from a copolymer of polycaprolactone and polylactic acid fibers. The grafts were recovered for gross, histologic, and blood chemical studies at 4 months after the surgery. All recipient pigs survived until they were humanely killed for collection of the implants. A week of ligation of the extrahepatic bile duct dilated the duct to approximately 1 cm in diameter and increased total bilirubin. Total bilirubin had returned to the pre-implantation level in all animals at 4 months post implantation. Examinations of the grafts revealed complete freedom of stricture and the regeneration of a neo-bile duct of approximately 1 cm in diameter from the graft site in 10 of 11 animals. Gross observation of the graft from the 1 remaining animal revealed stricture at the anastomosis site and poor bile duct epithelization. We have concluded that this bioabsorbable polymer bile duct can serve as a replacement for narrowed portions of the bile duct.

Corresponding author: Mitsuo Miyazawa, MD, PhD, FACS, Department of Surgery, Gastrointestinal Center, Saitama Medical University, International Medical Center, 1397-1, Yamane, Hidaka-shi, Saitama, 350-1298, Japan.
Tel.: 81 42 984 4531; Fax: 81 42 984 4741; E-mail: miyazawa@saitama-med.ac.jp

Key words: Artificial bile duct – Tissue-engineering – Bioabsorbable polymer – Jaundice – Bile duct stenosis

Extrahepatic bile duct stenosis as a result of inflammation or cancer is now treated by removing the affected area and anastomosing the hilar bile duct to the intestine to provide an outlet for bile drainage. In the case of common bile duct lithiasis, the bile duct is opened, the stones are removed, and a T-tube is implanted for approximately 1 month to prevent duct stricture in the aftermath of stone removal. The surgical approach through anastomosis of the hilar bile duct to the intestine bears the risk of retrograde infection and possible restricturing as a consequence.[1,2] Treatment procedures using T-tubes are subject to various inconveniences. The tubes must often be left in place for unduly long periods, for example, and in some cases, they may accidentally slip out of place as a result of accidental impacts and so on.[3,4] In treating extrahepatic bile duct stenosis, the biologic function can be supported to the maximum extent possible by removing only the narrowed portion and replacing it with some type of prosthesis that can act for the bile duct. We previously reported that when porcine normal extrahepatic bile duct was replaced by an artificial bile duct (ABD) made of bioabsorbable polymer, the bile duct similar to the native one was regenerated at the substituted part.[5–7] However, such regeneration occurred in these past models where there was no infection or bile duct stricture. Therefore, this does not apply to actual clinical settings, where bile duct regeneration is required in patients with bile duct stricture and a certain level of jaundice and intraperitoneal infection. In this study, we created a jaundice model by ligating the extrahepatic bile duct and examined whether favorable bile duct regeneration could be induced at the strictured bile duct using an artificial bile duct made of bioabsorbable polymer developed by us.

Materials and Methods

Bioabsorbable polymer

The ABD used as the prosthesis was a tube made of a porous high-molecular copolymer of polycaprolactone and polylactic acid (50:50) reinforced with polyglycolic acid fibers.[8] The mesh-like structure of the polyglycolic acid fiber is highly resistant to splitting when penetrated by suturing needles. Based on the expected dilation of the bile duct on the hilar side of stenosis, we chose an ABD with a diameter of approximately 8 mm and a wall thickness of 1 mm (Fig. 1). The tubular polymer had an air porosity of 95% or more before the operation and was designed to be absorbed by the body within 6 to 8 weeks of implantation.

Animal operation

Hybrid pigs weighing 15 to 30 kg ($n = 11$) were used as the recipients of the bioabsorbable polymer bile duct. After laparotomy (via a midline incision in the upper abdomen under general anesthesia induced with 20 mg/kg of ketamine and maintained with a continuous infusion of 0.2 mg/kg/min of propofol), the common bile duct was identified in the hepatoduodenal ligament and ligated using a 6-0 Prolene suture around the confluence with the cystic duct. One week after the first surgery, a repeat laparotomy was performed on the animals by the same method, and the dilated section of the ligated common bile duct on the hepatic side of the ligature was removed. The hepatic cut end of the native common bile duct was anastomosed to the ABD duct using a 6-0 Prolene suture (Ethicon, Somerville, NJ). The length of the ABD was carefully adjusted in order to prevent any infections. After reconstruction, it measured approximately 3 cm (Fig. 2). The ABD was recovered 4 months after implantation and examined for morphology, length, diameter, and histology. Blood chemistry was conducted before and 1 week after bile duct ligation and 4 months after implantation.

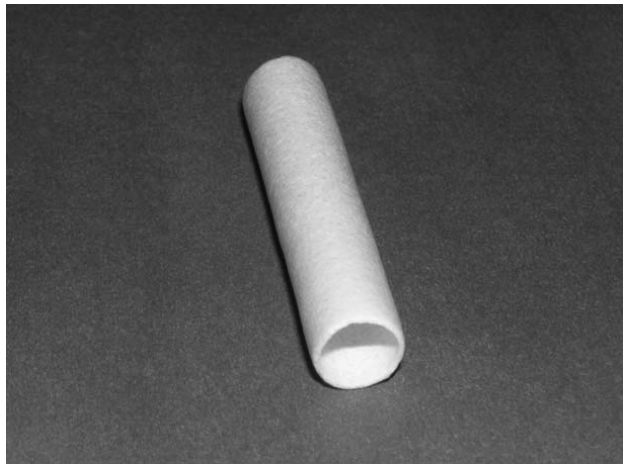

Fig. 1 Artificial bile duct. Tubular porous artificial bile duct (8 mm in diameter).

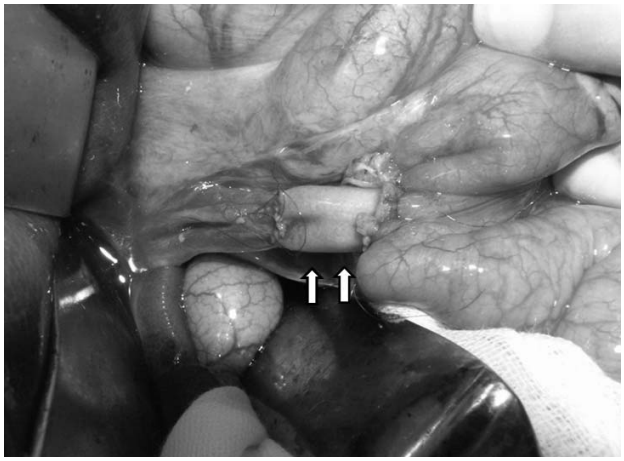

Fig. 2 Intraoperative picture taken after implantation of the ABD (arrows). The dilated bile duct on the hepatic side up to the duodenum was replaced by the ABD.

The abdominal cavity surrounding the graft site was examined for inflammation both macroscopically and histologically. Animal experiments were all performed in compliance with the guideline for the care and use of laboratory animals of Saitama Medical University (No. 1428).

Morphologic quantification

The ABD anastomosed to the native common bile duct was isolated for histology 4 months after implantation. The isolated tissue was formalin-fixed, stained with hematoxylin and eosin (H&E), exposed to antibodies against cytokeratin 19 (CK 19) (Progen, Heidelberg, Germany),[9] and compared with the native bile duct under optical microscopy.

Statistical analysis

The values of the data were expressed as mean ± SD. Statistical analysis were performed using the JMP 8.0 software (SAS Inc, Cary, North Carolina). Comparisons between the 2 values were analyzed by the Fisher exact test or the Mann-Whitney *U* test. Probability values less than 0.05 were considered significant.

Results

All 11 recipient pigs survived until they were humanely killed at 4 months for graft recovery. The mean of biliary enzymes before ABD implantation was total bilirubin (T-Bil), 0.8 ± 0.5; alanine aminotransferase (ALT), 23.0 ± 8.5; and alkaline phosphatase (ALP), 25.8 ± 9.6. Serum levels of T-Bil (1.9 ± 0.7 mg/dL) and biliary enzymes (ALT, 265.3 ± 79.6; ALP, 328.3 ± 69.3) increased 1 week after ligation of the common bile duct in all animals with significant changes before the ligation ($P < 0.05$) (Table 1). Four months after implantation, serum ALT (26.6 ± 13.6), ALP (27.4 ± 14.9), and T-Bil (0.9 ± 0.8) showed no significant changes compared with these levels before the ligation (Table 1).

Gross observation of the abdomen

The common bile duct was dilated to a diameter of approximately 1 cm on the hepatic side of the ligature after 1 week of ligation. Though surrounded by mild inflammation, the site of implantation was easily accessible by detaching the small intestine and greater omentum manually 4 months after implantation. A neo-bile duct dilated to a diameter of 1 cm was formed in the graft site. Though the polymer constituting the prosthesis appeared to have been completely degraded, the neo-bile duct was thicker than the native duct. Ten of 11 recipients were free of macroscopic stricture (Fig. 3), while 1 showed a narrowed anastomosis between the duodenum and ABD.

Immunohistochemistry

H&E staining

Accessory glandular structures were abundant in the lumen of the implant at 4 months after

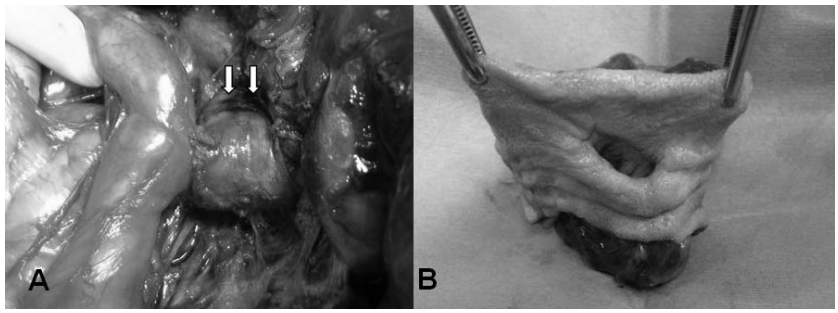

Fig. 3 (A) Neo-bile duct (arrow) generated after implantation of ABD; a dilated new extrahepatic bile duct runs from the porta hepatic to the duodenum in continuity. (B) The excised specimen; as viewed from the duodenum, the opening of the neo-bile duct into the duodenum measured 1 cm in diameter.

Table 1 Changes in biliary enzymes over time after ABD implantation (n = 11)[a]

	Before Tx	1W After Tx	4M After Tx
ALT (IU/L)	23.0 ± 8.5	265.3 ± 79.6	26.6 ± 13.6
	*	*	
	NS		
ALP (IU/L)	25.8 ± 9.6	328.3 ± 69.3	27.4 ± 14.9
	*	*	
	NS		
T-Bil (mg/dl)	0.8 ± 0.5	1.9 ± 0.7	0.9 ± 0.8
	*	*	
	NS		

M, months; NS, not significant; Tx, implantation; W, weeks.
[a]Results are presented as mean ± SD.
*$P < 0.05$.

implantation. No polymer was detected. Profuse inflammatory cells were observed around the accessory glandular structures. The tunica adventitia of the neo-bile duct contained connective tissue. The neo-bile duct was structurally similar to the native duct in the 10 recipients free of macroscopic stenosis, with more inflammatory cells in the former duct. Re-epithelization was comparable among the duodenal, hepatic, and intermediate parts of the neo-bile duct (Fig. 4A). Inflammatory cells far outnumbered epithelial cells in the lumen of the neo-bile duct from the recipient with macroscopic stricture (Fig. 4B). In the comparison between the ABDs implanted as replacements for narrowed bile ducts and for normal bile ducts, the former exhibited relatively increased inflammatory cells around the accessory glandular structures, a decreased rate of bile duct regeneration, and delayed epithelization (Fig. 4).

Staining for CK 19

The same pattern of CK 19 positivity was observed in both the native bile duct and neo-bile duct at 4 months post implantation, with CK 19 cells detected in portions possibly corresponding to epithelial cells and accessory glandular structures (Fig. 5).

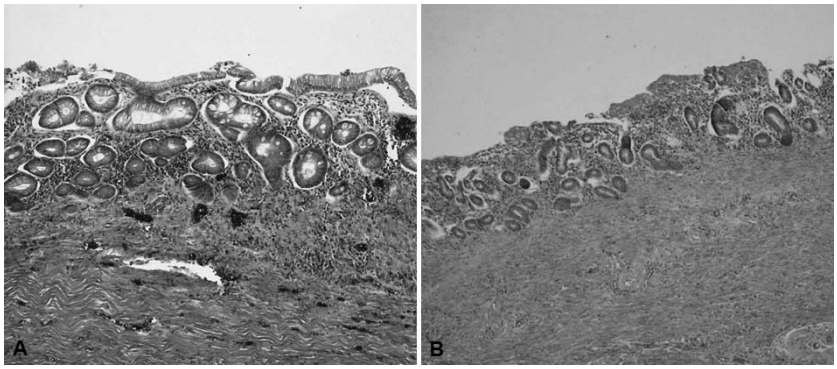

Fig. 4 Neo-bile duct at 4 months after implantation of the artificial bile duct (H&E stain; original magnification ×200). (A) Neo-bile duct without macroscopically apparent stricture; the neo-bile duct showed cuboidal-columnar epithelium on the luminal surface. (B) Neo-bile duct with macroscopically apparent stricture; the neo-bile duct showed no epithelium on the luminal surface.

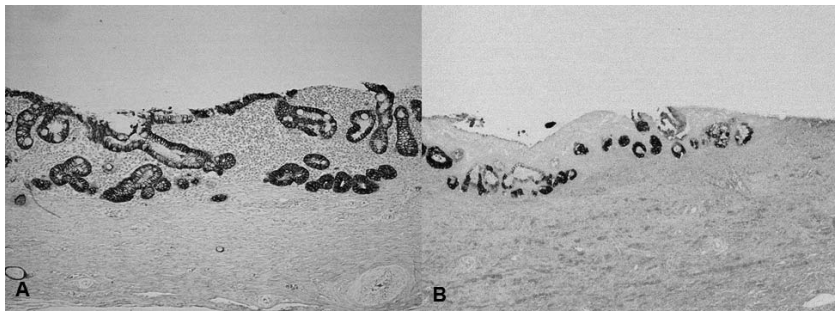

Fig. 5 Neo-bile duct at 4 months after implantation of the artificial bile duct (CK19 stain; original magnification ×200). (A) Neo-bile duct without macroscopically apparent stricture; the portion possibly corresponding to epithelium and accessory glandular structures was stained positive. (B) Neo-bile duct with macroscopically apparent stricture; A few relatively small accessory gland–like structures were stained positive.

Discussion

The implantation of the bioabsorbable polymer graft to replace the narrowed section of the bile duct effectively generated a neo-bile duct similar to the native duct. Hematologic data were within normal range at 4 months after implantation in all recipient pigs, confirming the proper functioning of the neo-bile duct as an extrahepatic bile duct. This innovative approach was therefore concluded to show promise as a replacement for the current surgical treatments of bile duct stenoses in patients with diseases of the biliary system. The increasing use of laparoscopic cholecystectomy for the treatment of cholelithiasis has led to an increased incidence of iatrogenic bile duct injuries or stenoses.[10,11] In most cases, the reconstruction of the bile duct in a laparoscopic cholecystectomy is achieved by anastomosing the hilar bile duct to the small intestine to allow the bile to flow out into the intestine. The ABD fabricated through the tissue engineering techniques pioneered by our group makes it possible to perform bile duct substitution limited to the affected area. The current treatment for congenital biliary stenosis in children consists of formation of an anastomosis between the hilar bile duct and the jejunum.[12] The surgery is associated with recurrent postoperative cholangitis resulting in aggravation of jaundice. In extreme cases, liver transplantation may be required.[13] The use of our tissue-engineered bile duct in this context can reduce the risk of such an outcome. The bioabsorbable polymer composing the ABD is degraded inside the body and replaced with autologous cells in 2 or more months after implantation. It also appears that the neo-bile duct grows as the body grows. If this is so, the graft will prove especially beneficial in children with diseases of the biliary system.

Several conditions must be met to confirm the proper function of an ABD implanted in the body. In the short term after implantation, the subject must be free of jaundice resulting from obstruction of the reconstructed duct and biliary peritonitis resulting from bile leakage from the bile duct. In the long term, the subject must remain free from inflammation-related stricture formation in the neo-bile duct and at the site of anastomosis joining the neo-bile duct to the native duct or duodenum. We recovered the graft at 4 months after implantation in the expectation that the polymer would be degraded and absorbed and that a neo-bile duct macroscopically similar to the native duct would be generated by that time. Our previous studies have confirmed that once an implanted ABD can achieve regeneration at 3 moths, the neo-bile duct subsequently regenerated will resemble the native duct.[5] The neo-bile duct was formed from the ABD that had been implanted in the presence of experimental jaundice more or less complicated by inflammation. Hematology at 4 months showed that biliary enzymes were within normal range in all 11 animals, and histology confirmed the development of epithelization similar to that in the native duct in 10 of 11 animals. We thus confirmed that our ABD is applicable to clinical practice as a substitute for narrowed portions of the bile duct.

We demonstrated that the implantation of a bioabsorbable polymer duct as a replacement of a normal bile duct generated a neo-bile duct similar to the native duct. Having thus succeeded, we repeated our experiment under conditions adapted to actual clinical settings—settings where bile duct stenosis, jaundice, and cholangitis are common—in order to investigate the feasibility of using this ABD in clinical practice. The ABD implanted in the presence of some degree of jaundice and inflamma-

tion induced by bile duct ligation was found to function well enough to generate a neo-bile duct similar to the native duct at the graft site. The results seem to support potential for wide application in the treatment of accidental bile duct injuries, cancer, benign bile duct stenosis, and other disorders. Hematologic data at 4 months post implantation returned to preligation levels in 11 recipient pigs, while histology revealed narrowing at the anastomosis of the ABD to the duodenum in 1 of 11 pigs. The other 10 formed a neo-bile duct slightly larger in diameter than the ABD. Stricture following biliary-enteric anastomosis is not uncommon in clinical practice.[14] The postoperative stricture observed in our experiment was attributable to prolonged inflammation of the anastomosis site and poor epithelization. The other 10 animals showed sufficient regeneration of bile duct epithelium. The general precautions we currently take during intraperitoneal anastomosis for gastrointestinal diseases (*e.g.*, precautions against anastomotic leakage or against tension on the anastomosis) will prevent stricture of the anastomosis of ABD to native duct or intestine.[15,16]

This study demonstrated that implantation of the tissue-engineered ABD as a substitute for the narrowed section of the bile duct generated a neo-bile duct that functioned properly as an extrahepatic bile duct. These results suggest new treatment possibilities with this ABD for extrahepatic bile duct stenosis.

Acknowledgments

This study was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan (No. 25462013). We thank Naoe Akimoto for her excellent technical assistance. Mitsuo Miyazawa received funding for this work from the Japanese Ministry of Health, Labor and Welfare (No. 25462124) and the Maruki Memorial Scholarship for Special Research.

References

1. Ammori BJ, Joseph S, Attia M, Lodge JP. Biliary strictures complicating pancreaticoduodenectomy. *Int J Pancreatol* 2000; **28**(1):15–21
2. Egawa H, Inomata Y, Uemoto S, Asonuma K, Kiuchi T, Fujita S *et al*. Biliary anastomotic complications in 400 living related liver transplantations. *World J Surg* 2001;**25**(10):1300–1307
3. Maghsoudi H, Garadaghi A, Jafary GA. Biliary peritonitis requiring reoperation after removal of T-tubes from the common bile duct. *Am J Surg* 2005;**190**(3):430–433
4. Wills VL, Gibson K, Karihaloot C, Jorgensen JO. Complication of biliary T-tubes after choledochotomy. *ANZ J Surg* 2002;**72**(3): 177–180
5. Miyazawa M, Torii T, Toshimitsu Y, Okada K, Koyama I, Ikada Y. A tissue-engineered artificial bile duct grown to resemble the native bile duct. *Am J Transplant* 2005;**5**(6):1541–1547
6. Aikawa M, Miyazawa M, Okamoto K, Toshimitsu Y, Torii T, Okada K *et al*. A novel treatment for bile duct injury with a tissue-engineered bioabsorbable polymer patch. *Surgery* 2010; **147**(4):575–580
7. Aikawa M, Miyazawa M, Okamoto K, Toshimitsu Y, Okada K, Akimoto N *et al*. An extrahepatic bile duct grafting using a bioabsorbable polymer tube. *J Gastrointest Surg* 2012;**16**(3):529–534
8. Watanabe M, Shin'oka T, Tohyama S, Hibino N, Konuma T, Matsumura G *et al*. Tissue-engineered vascular autograft: inferior vena cava replacement in a dog model. *Tissue Eng* 2001;**7**(4):429–439
9. Green JA, Carthew P, Heuilet E, Simpson JL, Manson MM. Cytokeratin expression during AFB1-induced carcinogenesis. *Carcinogenesis* 1990;**11**(7):1175–1182
10. Nuzzo G, Giuliante F, Giovannini I, Ardito F, D'Acapito F, Vellone M *et al*. Bile duct injury during laparoscopic cholecystectomy: results of an Italian national survey on 56591 cholecystectomies. *Arch Surg* 2005;**140**(10):986–992
11. Sicklick JK, Camp MS, Lillemoe KD, Melton GB, Yeo CJ, Campbell KA *et al*. Surgical management of bile duct injuries sustained during laparoscopic cholecystectomy: perioperative results in 200 patients. *Ann Surg* 2005;**241**(5):786–792
12. Endo M, Masuyama H, Hirabayashi T, Ikawa H, Yokoyama J, Kitajima M. Effects of invaginating anastomosis in Kasai hepatic portoenterostomy on resolution of jaundice, and long-term outcome for patients with biliary atresia. *J Pediatr Surg* 1999;**34**(3):415–419
13. Davenport M, Puricelli V, Farrant P, Hadzic N, Mieli-Vergani G, Portmann B *et al*. The outcome of the older (> or =100 days) infant with biliary atresia. *J Pediatr Surg* 2004;**39**(4):575–581
14. He ZP, Hou WL, Bie P, Dong JH, Wang SG, Han BL *et al*. Etiology and surgical treatment of hilar bile duct stricture. *Hepatobiliary Pancreat Dis Int* 2003;**2**(4):587–593
15. Moosa AR, Mayer AD, Stabile B. Iatrogenic injury to the bile duct: who, how, where? *Arch Surg* 1990;**125**(8):1028–1031
16. Northover JM, Terblanche J. A new look at the arterial supply of the bile duct in man and its surgical implications. *Br J Surg* 1979;**66**(6):379–384