Original Article

# Bile duct reconstruction using scaffold-free tubular constructs created by Bio-3D printer

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## ARTICLE INFO

## ABSTRACT

Introduction: Biliary strictures after bile duct injury or duct-to-duct biliary reconstruction are serious complications that markedly reduce patients' quality of life because their treatment involves periodic stent replacements. This study aimed to create a scaffold-free tubular construct as an interposition graft to treat biliary complications.
Methods: Scaffold-free tubular constructs of allogeneic pig fibroblasts, that is, fibroblast tubes, were created using a Bio-3D Printer and implanted into pigs as interposition grafts for duct-to-duct biliary reconstruction.
Results: Although the fibroblast tube was weaker than the native bile duct, it was sufficiently strong to enable suturing. The pigs' serum hepatobiliary enzyme levels remained stable during the experimental period. Micro-computed tomography showed no biliary strictures, no biliary leakages, and no intra-hepatic bile duct dilations. The tubular structure was retained in all resected specimens, and the fibroblasts persisted at the graft sites. Immunohistochemical analyses revealed angiogenesis in the fibroblast tube and absence of extensions of the biliary epithelium into the fibroblast tube's lumen.
Conclusions: This study's findings demonstrated successful reconstruction of the extrahepatic bile duct with a scaffold-free tubular construct created from pig fibroblasts using a novel Bio-3D Printer. This construct could provide a novel regenerative treatment for patients with hepatobiliary diseases.

## 1. Introduction

Biliary strictures are among the most troublesome complications following hepatobiliary surgery, for example, iatrogenic bile duct injury (IBDI) is a critical complication associated with laparoscopic cholecystectomy [1]. Despite greater experience and improvements in surgeons' laparoscopic skills, the incidence of IBDI is higher for laparoscopic cholecystectomy (3%) than for open cholecystectomy (0.1–0.5%) [2]. Additionally, postoperative biliary stenosis is a serious complication of liver transplantation [3–6]. Biliary complications are more frequent during living donor liver transplantations (LDLTs) than during deceased donor liver

transplantations; the incidence of biliary strictures as a consequence of LDLT ranges from 10% to 50% [7].

The standard treatment for IBDI or biliary complications associated with liver transplantation is endoscopic stent placement [3]. However, as these indwelling stents are in situ for prolonged periods of time, they become clogged and must be replaced periodically to maintain their patency over the remainder of most patients' lives [8]. Furthermore, patients with severe biliary stenosis, which is difficult to manage endoscopically, require a Roux-en-Y hepaticojejunostomy [9]; however, this technique is associated with risks of retrograde infection, scar tissue formation, and stenosis [10]. Repeat surgical repairs for biliary restenosis are more complex [11], and percutaneous transhepatic biliary drainage markedly reduces patients' quality of life [12].

To overcome these biliary complications, many types of artificial bile ducts have been evaluated as potential substitutes for native bile ducts [10,13–17]; however, a range of problems, including immune reactions, the safety of degradation products from the artificial bile ducts, disease or infection transmission, and acute allergic responses, have hindered their clinical application [18].

Nakayama et al. developed the Bio-3D Printer (Cyfuse Biomedical K.K., Tokyo, Japan) that creates scaffold-free tubular constructs using cell spheroids only [19,20]. To date, the Bio-3D Printer has been used to produce blood vessels [21,22], peripheral nerves [23], diaphragms [24], tracheas [25], and esophagus [26]. However, the creation of a bile duct using the Bio-3D Printer has not yet been described.

We hypothesized that transplanting a tubular construct as an interposition graft to replace stenotic bile ducts could treat biliary complications. Using a pig model, this study aimed to create scaffold-free tubular constructs as interposition grafts to treat biliary complications.

## 2. Materials and methods

The hypothesis was tested using a pig model. For allogeneic transplantation, two pigs were used in each experiment. The skin was collected from one pig, while another pig was used for the implantation of the fibroblast tube. Since fibroblast tube implantation was performed in 5 pairs, a total of 10 pigs were used. All experiments were approved by Nagasaki University's Institutional Animal Care and Use Committee (No. 1711131424), and the experiments were performed in accordance with institutional and national guidelines.

### 2.1. Explant fibroblast cultures

Porcine skin was obtained under general anesthesia. The pigs fasted for at least 24 h before surgery. After pre-medication with intra-muscular ketamine (5 mg/kg) and midazolam (5 mg/kg), general anesthesia was maintained with continuous sevoflurane inhalation (2–3%) while the pigs lay in the supine position. The skin around the femoral region was excised and put in collection tubes containing phosphate-buffered saline (PBS) (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) and transported to the laboratory on ice.

To isolate the fibroblasts, the skin was prepared and cultured using previously described methods [27–29] with modifications. In brief, the excised skin was removed from the collection tube in the laboratory, soaked in povidone-iodine for 1 min, allowed to dry, and washed twice with PBS. The skin was then washed three times with PBS containing 100 μg/mL gentamicin (Fuji Pharma Co., Ltd., Toyama, Japan) and 50 μg/mL fungizone (Bristol-Myers Squibb Company, New York, NY, USA). Then, the skin's layers were cut with surgical scissors to isolate the dermis, and the subcutaneous tissue,

including the loose connective tissue, was removed. The dermis was cut into pieces (1–2 mm$^2$) and placed in 10-cm diameter cell culture dishes containing 5 mL Dulbecco's Modified Eagle's Medium (DMEM) (Fujifilm Wako Pure Chemical Corporation) supplemented with 10% fetal bovine serum (FBS) (Invitrogen Corporation, Carlsbad, CA, USA), 100 U/mL penicillin, 100 μg/mL streptomycin (Invitrogen Corporation), and 40 μg/mL gentamicin. The explants were incubated at 37 °C in an atmosphere of 5% $CO_2$ for 1 week without a medium change, and when the primary fibroblasts had migrated radially from the explants, the explants were removed. The medium was replaced by DMEM supplemented with 10% FBS and 100 U/mL penicillin 2–3 days after the inoculation. When the cultures reached 90% confluence, the fibroblasts were suspended by treating them with 0.25% trypsin-ethylenediaminetetraacetic acid (Invitrogen Corporation). The cells were passaged every 5 days, and they were used by the fifth passage in this study.

### 2.2. Tubular construct creation using the Bio-3D printer

The fibroblast suspensions were adjusted to a concentration of $4.5 \times 10^5$ cells/mL, and 100 μL aliquots were plated into each well of ultra-low-attachment, round-bottom 96-well plates (Sumilon PrimeSurface; Sumitomo Bakelite, Tokyo, Japan) and incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$. After 72 h, the fibroblasts had aggregated to form spheroids with a mean (standard deviation) diameter of 750 (50) μm.

To assemble the construct, the spheroids were automatically picked up one-by-one and placed into a 7-mm needle array using the Bio-3D Printer. Approximately 1 week after 3D printing, the spheroids had fused together to form a viable single tubular construct. The needle array was removed, and the fibroblast tubular construct was transferred onto a 5-mm polydimethylsiloxane tube. An additional incubation of >90 days was required for the fibroblasts within the tube to reorganize themselves and strengthen the construct. At this stage, the fibroblast tube was 0.5-mm wide and about 10-mm long (Fig. 1).

### 2.3. Tensile strength test

A uniaxial tensioning apparatus with 5 kN capacity (EZ-L; Shimadzu Corporation, Kyoto, Japan) that pulls ring-shaped tissue constructs to failure was used to test the tensile strength of the fibroblast tubes. To conduct the test, a construct that was 5-mm long was placed around two parallel stainless steel wire hooks that were 3 mm apart. The hooks were then pulled apart at a rate of 40 mm/min until tissue failure. The force required was measured digitally using a data acquisition system (Trapezium; Shimadzu Corporation). The tests were filmed to verify that construct failure had occurred in the middle of the tissue away from the hooks.

### 2.4. Implantation of the fibroblast tube into a pig model

Five pigs that weighed approximately 12 kg were used for the transplantation experiment. After inducing general anesthesia, the area around the surgical site was covered with sterile drapes, and a laparotomy involving an upper mid-line incision was performed. The liver was lifted cranially, and the intestine was moved caudally to identify and expose the common bile duct within the hepatoduodenal ligament. Next, the common bile duct was cut below a junction of a cystic duct and the fibroblast tube was implanted (Fig. 2). The common bile duct and fibroblast tube were anastomosed using PDS-II 6-0 (Ethicon, Inc., Somerville, NJ, USA). Before completing the anastomosis, a polyvinyl chloride tube with an inner diameter of 2 mm (RTBD Tube; Sumitomo Bakelite, Akita,
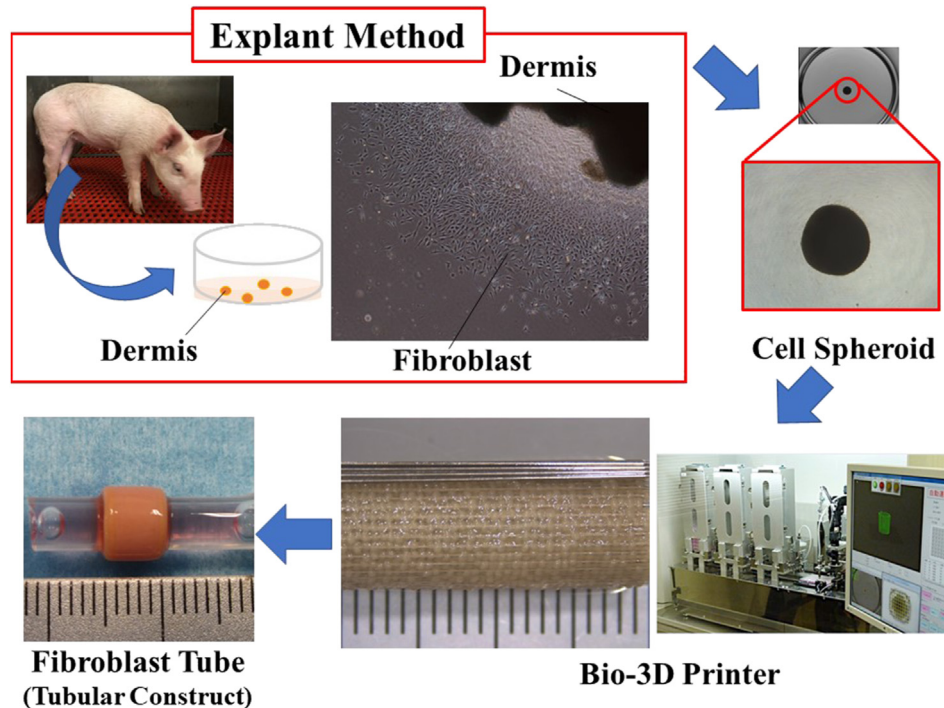
**Fig. 1. Fibroblast tube creation**. The skin was obtained from the femoral region of pigs. The allogeneic fibroblasts multiplied in explant cultures. The fibroblasts aggregated to form spheroids, and the scaffold-free tubular construct was created using a Bio-3D Printer. After printing, over 90 days were required to culture the mature fibroblast tube. 3D: three-dimensional.

Japan) that was approximately 1 cm longer at each end of the anastomosis was inserted inside the fibroblast tube and attached to the native common bile duct with a single stitch as a temporary stent to prevent early deformation. After closing the abdomen, imipenem (0.5 g) (Sandoz, Tokyo, Japan) was administered intravenously. The pigs were allowed access to water and food from postoperative day 1, and each day from postoperative day 1–13, the pigs ingested tebipenem pivoxil (5 mg/kg) (Sandoz, Tokyo, Japan) and tacrolimus (0.04 mg/kg) (Astellas Pharma Inc., Tokyo, Japan) [30]. On postoperative day 14, the pigs were sacrificed by administering potassium chloride intravenously while they inhaled sevoflurane (2–3%), and the liver and duodenum were removed en bloc with the graft.

### 2.5. Biochemical assays

Blood samples were collected from the pigs' cervical veins on the day of the transplantation, on postoperative day 7, and on postoperative day 14 before they were sacrificed. The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), and creatinine (Cr) levels were measured using standard laboratory methods. The results of the liver function test (T-Bil, AST, ALT, ALP and γ-GTP) were compared to those measured in the pigs that underwent duct-to-duct biliary reconstruction without transplantation.

### 2.6. Radiological examinations

Before the radiological examinations began, the biliary stent was removed. Cholangiography was performed using a contrast agent that comprised 60% diatrizoate (20 mL) (Urografin; Bayer Yakuhin Ltd., Osaka, Japan) after inserting a 21-G silicon catheter (Terumo, Tokyo, Japan) into the ampulla of Vater. After the

cholangiography, 3D micro-computed tomography (CT) (Rigaku R_mCT; J. Morita Manufacturing Corporation, Kyoto, Japan) was performed; the micro-CT settings were as follows: X-ray source voltage: 90 V; current: 100 mA; scanning time: 17 s; and resolution: 20 mm/pixel. The micro-CT images of the graft were analyzed using 3D image reconstruction software (i-view; J. Morita Manufacturing Corporation).

### 2.7. Histology

The liver tissue was fixed with 4% paraformaldehyde (Fujifilm Wako Pure Chemical Corporation) for 24 h, dehydrated using high concentrations of ethanol, embedded in paraffin (Paraplast Plus; Leica Biosystems Inc., Richmond, IL, USA), and cut into 5-μm thick sections. The sections were stained with hematoxylin and eosin (H&E) (Muto Pure Chemicals Co. Ltd., Tokyo, Japan) and Heidenhain's azan (AZAN) (Muto Pure Chemicals Co. Ltd.). All staining procedures were performed using standard staining protocols.

### 2.8. Immunohistochemical analysis

The sections were deparaffinized in xylene and rehydrated in graded ethanol solutions. After treatment with a pre-heated target retrieval solution (pH 9.0) (Agilent Technologies, Santa Clara, CA, USA), the slides were cooled in water for 20 min. The sections were rinsed in PBS and incubated for 10 min at room temperature in a peroxidase-blocking solution (Agilent Technologies). The primary antibodies used were a mouse monoclonal anti-pig cytokeratin (CK) 7 antibody (Abcam plc, Cambridge, United Kingdom) diluted 1:100, rabbit polyclonal anti-pig CK19 antibody (LSBio, Seattle, WA, USA) diluted 1:100, and rabbit polyclonal anti-pig cluster of differentiation (CD) 31 antibody (Abcam plc, Cambridge, United Kingdom) diluted 1:50. The sections were incubated with the primary antibodies at 4 °C overnight. After washing, the sections were
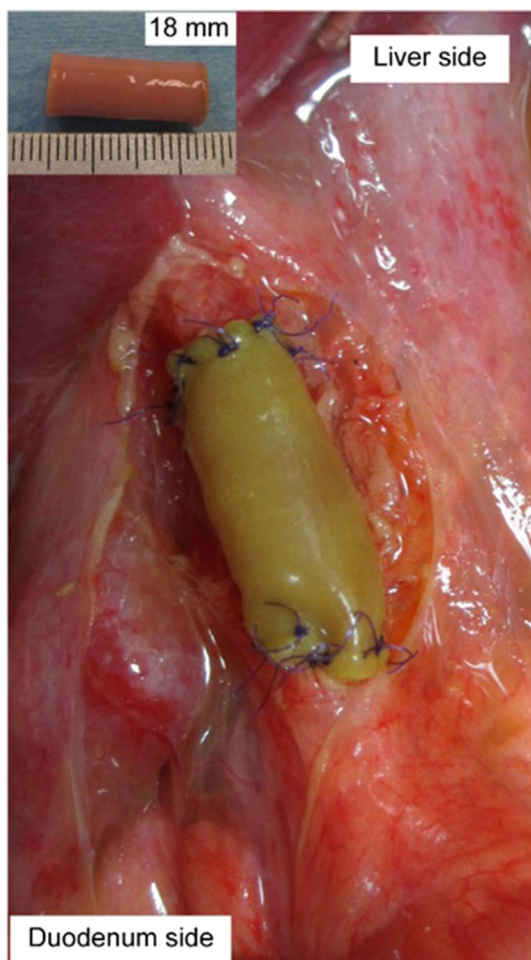
**Fig. 2. Fibroblast tube**. An 18-mm long scaffold-free tubular construct comprising allogeneic pig fibroblasts was created using a Bio-3D printer. The fibroblast tube was implanted in the common bile duct.

treated with a horseradish peroxidase-labeled polymer conjugated to goat anti-mouse and rabbit immunoglobulins (Agilent Technologies) at room temperature for 30 min. Then, the sections were stained with 3,3′-diaminobenzidine tetrahydrochloride containing hydrogen peroxide (Agilent Technologies). The sections were examined using an inverted microscope (DP-74; Olympus Corporation, Tokyo, Japan).

### 2.9. Statistical analysis

All statistical analyses were performed using an Excel statistical software package, version 2.21 for Windows (Bellcurve for Excel; Social Survey Research Information Co., Ltd., Tokyo, Japan). All data are expressed as the means $\pm$ standard deviation. Body weights before and after the operations were compared using Wilcoxon's t test. Means of the continuous numerical variables were compared using one-way analyses of variance. The Mann−Whitney U-test was used to perform the biochemical assay analyses. A value of $p < 0.05$ was considered statistically significant.

### 3. Results

All recipient pigs were healthy and had gained a small amount of weight during the 14-day experimental period (Table 1). The pigs with fibroblast tube implantation showed a similar increase in their

body weight as the pigs without transplantation. Postoperative complications and signs of obstructive jaundice, for example, white stools, were not evident until the animals were sacrificed.

### 3.1. Biochemical assay results

In the pigs that underwent fibroblast tube implantation, the concentrations of the serum T-Bil, AST, ALT, ALP, and γ-GTP levels did not change significantly during the experimental period, and all pigs showed normal Cr levels at all time points (Table 1). Comparisons of the biochemical assay results between the pigs underwent fibroblast tube transplantation and those that underwent duct to duct biliary reconstruction without transplantation did not show any significant difference in total bilirubin, AST, and γ-GTP, but in ALT and ALP (Fig. 3A). As for ALP, since there was a significant difference on POD 0, we compared the results based on the rate of increase (POD7/POD0, POD14/POD7, and POD14/POD0). The postoperative rate of increase in ALP did not show any significant difference (Fig. 3B).

### 3.2. Tensile strength test

We tested the tensile strength of the fibroblast tubes in vitro using a cyclic tension test. The maximal forces applied were 4.575 N to the fibroblast tubes and 13.375 N to the native bile ducts (Fig. 4A). Although the tensile strength of the fibroblast tube was lower than that of the native bile duct, its strength was sufficiently high to enable suturing. From these results, we concluded that the tensile strength of the fibroblast tube was relatively high and adequate for anastomoses.

### 3.3. Imaging findings

Cholangiography on postoperative day 14 showed that the intrahepatic bile duct was not dilated. Consistent bile flows and an absence of bile leakages were confirmed by the excretion of the contrast medium into the duodenum. Micro-CT evaluated the implanted segment in detail. Although pressure from the contrast medium had caused the expansion of the native bile duct and the junction between the native bile duct and the fibroblast tube appeared narrower, the patency of the implant was maintained (Fig. 4B).

### 3.4. Macroscopic examination of the fibroblast tube

Loose fibrous adhesions were randomly distributed around the graft site, and the reconstructed fibroblast tube was easily accessed. The graft site showed slight thickening, and the outer layer of the graft was indistinguishable from that of the native duct. Furthermore, the shape of the fibroblast tube did not noticeably change (Fig. 5A); the tubular structure was maintained in all resected specimens. None of the pigs showed signs of biliary leakage or peritonitis.

### 3.5. Histological examinations

A histological examination of the H&E and AZAN-stained sections showed that the fibroblast tube had remained at the implantation site (Fig. 5B). AZAN-stained sections showed continuous fibrous tissue around fibroblast tube. Granulation tissue was present under the fibroblast tube, and fibrous scar tissue had thickened the bile duct wall. Although the coagulative necrosis tissue existed in the luminal side of the fibroblast tube, the contiguity of the fibroblast tube with the native bile duct was confirmed. Immunohistochemical evaluation of sections that were incubated with CK 7 and CK19 antibodies showed that the biliary epithelium had not

**Table 1**
Body weight and biochemical assay results

|  | Postoperative day 0 | Postoperative day 7 | Postoperative day 14 |
|---|---|---|---|
| Body weight (kg) | 12.2 | – | 14.36 |
| AST (IU/L) | 34 | 42 | 46 |
| ALT (IU/L) | 50 | 55 | 68 |
| T-Bil (mg/dL) | 0.04l | 0.03l | 0.09l |
| ALP (IU/L) | 691 | 653 | 550 |
| γ-GTP (IU/L) | 36 | 52 | 50 |
| Cr (mg/dL) | 0.67 | 0.69 | 0.67 |

AST: aspartate aminotransferase, ALT: alanine aminotransferase, T-Bil: total bilirubin, ALP: alkaline phosphatase, total bilirubin, Aranspeptidase, Cr: creatinine. Value are expressed as the mean LP: alkalinee aminotr
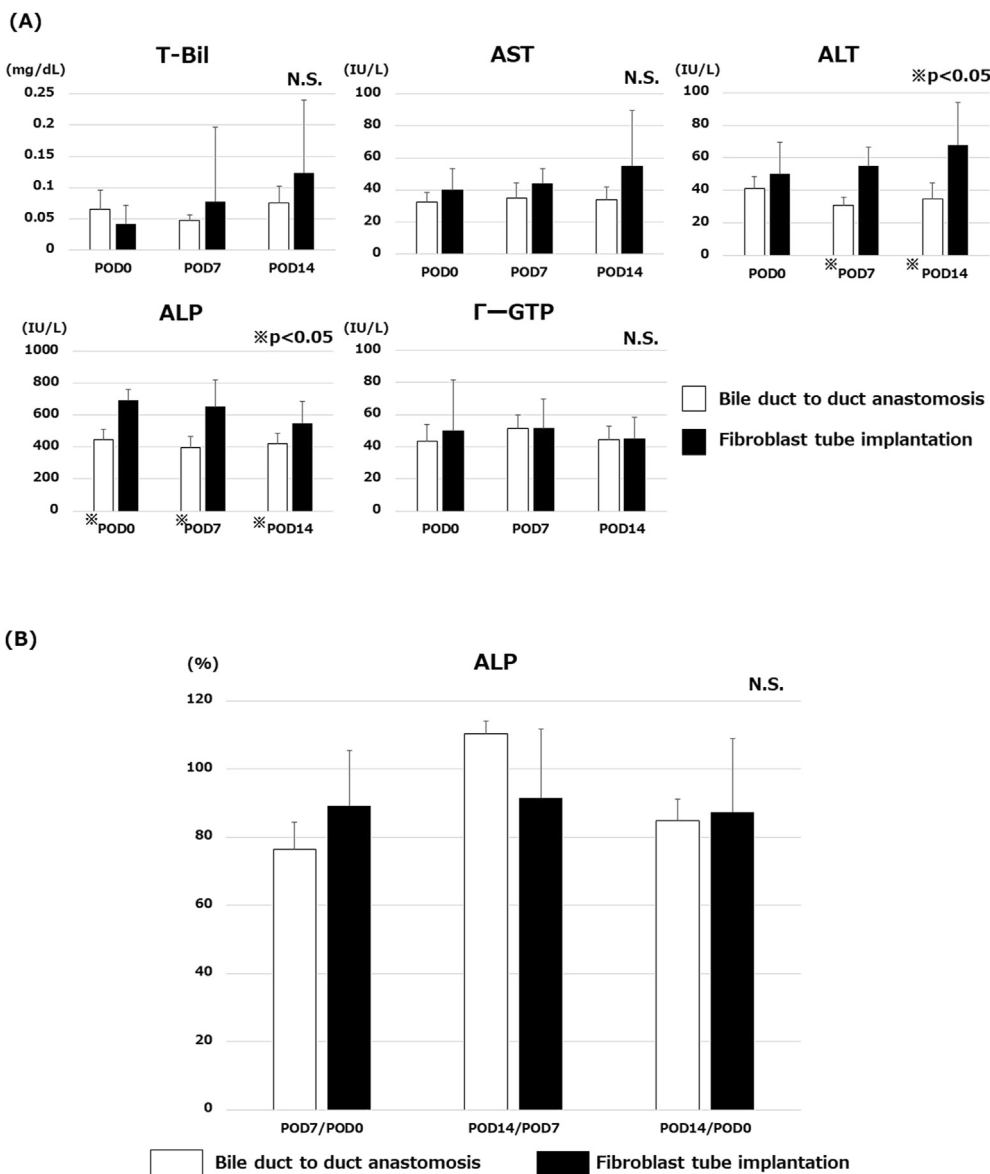


Fig. 3. (A) Biochemical assay results of the pigs underwent bile duct to duct anastomosis and fibroblast tube implantation (B) The rate of change on ALP.

extended into the lumen of the fibroblast tube (Fig. 6A, B). An evaluation of sections incubated with the CD31 antibody showed the presence of blood vessels in the outer layer of the fibroblast tube (Fig. 6C). Furthermore, angiogenesis was evident throughout the outer layer of the fibroblast tube and at the sides of the anastomosis near the native bile duct.

## 4. Discussion

In this study, we developed a scaffold-free tubular construct from pig fibroblasts using a novel Bio-3D Printer, and the tubular construct was implanted into pigs. Furthermore, we examined whether or not fibroblast tubes were transplantable and how they
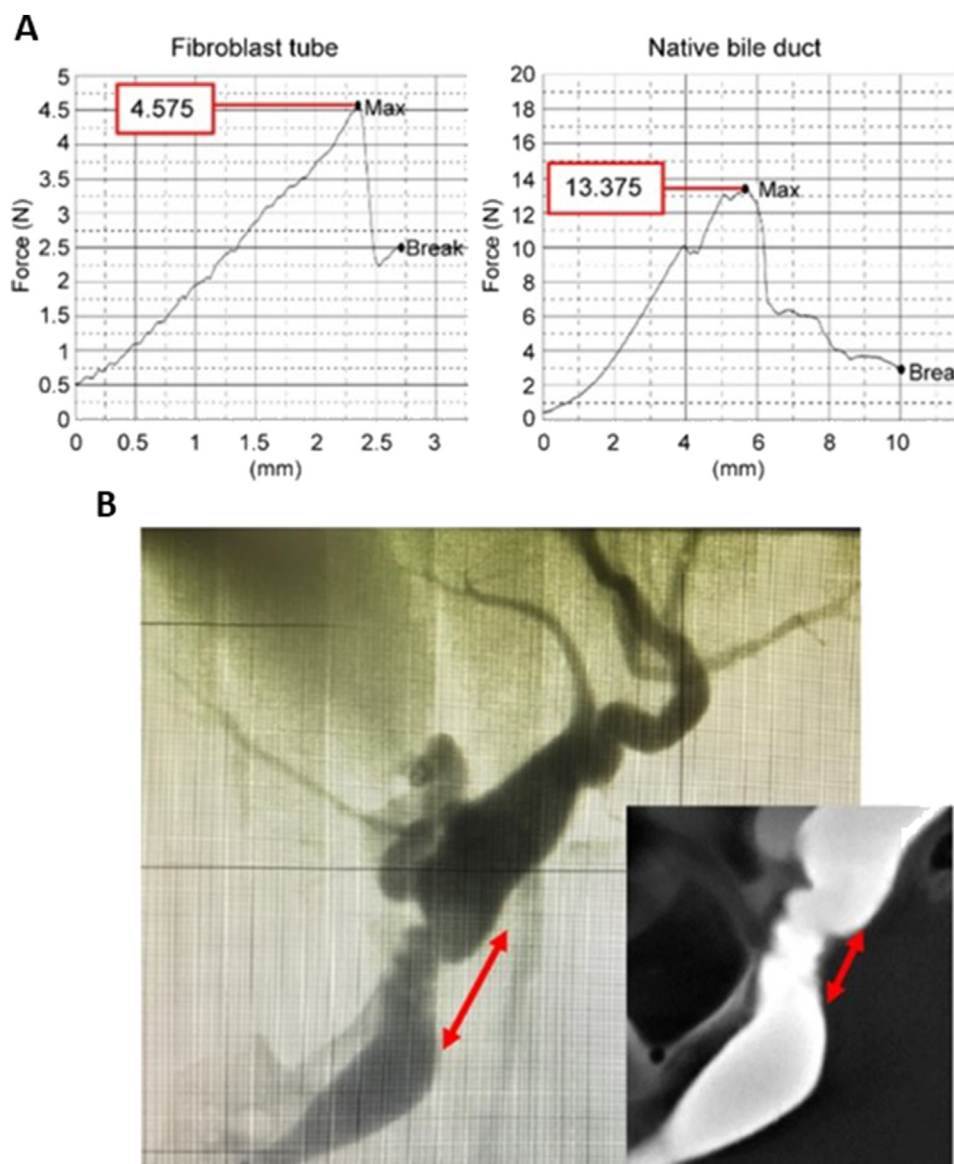
**Fig. 4.** **(A) Tensile strength (B) Cholangiography and micro-computed tomography at 14 days after fibroblast tube grafting**. Arrows (↔) indicate the site of the anastomosis.

affected the surrounding tissue. Although several investigators have attempted to transplant artificial bile ducts [10,13–17], to the best of our knowledge, this is the first report that describes the efficacy of bile duct regeneration using an entirely biological scaffold-free tubular construct. This technology may be useful for treating several bile duct disorders, including IBDI and biliary complications in liver transplantations.

While the Bio-3D Printer has been used to produce many types of tissues and organs [22–26,31–33] most of the cells used originated from humans. This is the first time that a pig fibroblast tubular construct has been produced using the Bio-3D Printer. We used fibroblasts to generate the tubular constructs because they could be easily cultured in vitro [27–29], and previous studies' findings have demonstrated their potential mechanical strength [23,24]. Considering their clinical applications for autologous transplantation, fibroblasts are considered a promising cell source, as they can be collected from a patient's skin easily and less invasively than other sources and efficiently cultured. In this study, the strength of the fibroblast tube was sufficiently high to enable

suturing. Further, the fibroblast tube could tolerate bile. Considering clinical application, since biliary stenosis is a chronic complication and not an acute one, stent placement to manage stenotic lesions can provide us with sufficient time to create a fibroblast tube and thereafter perform autologous transplantation. The morphology of the transplanted tubular structure was similar to that of a native biliary duct. In the present study, the fibroblast tube was used successfully as an extrahepatic bile duct without any leakages or stenoses after transplantation. Consequently, the bile flowed into the duodenum through the fibroblast tube. Risk factors associated with anastomotic stenoses include an unsatisfactory surgical technique, arterial complications, and using external or internal drainage [34]. Moreover, a key cause of anastomotic stenosis is local inflammation caused by ischemia [35]. Although this study did not involve long-term observations, stenosis at the site of the anastomosis did not occur. Immunohistochemical findings demonstrated the presence of blood vessels in the fibroblast tube and at the sides of the anastomosis, and the new vessels provided sufficient blood to the site of the anastomosis, which may explain
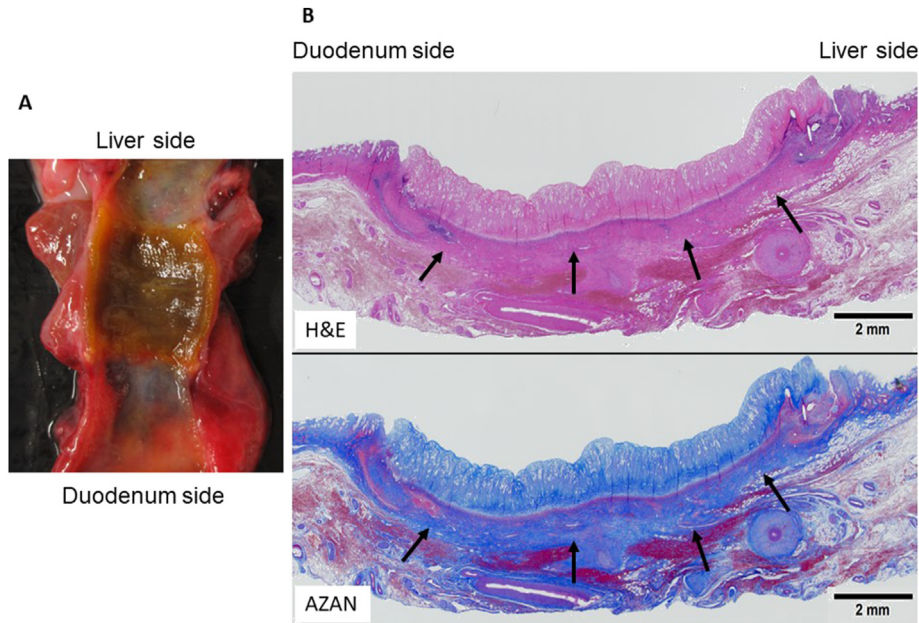
**Fig. 5.** (A) Lumen of the graft site within a resected specimen at 14 days after fibroblast tube grafting (B) The fibroblast tube at 14 days after transplantation. Back arrows point at the fibroblast tube (×2).
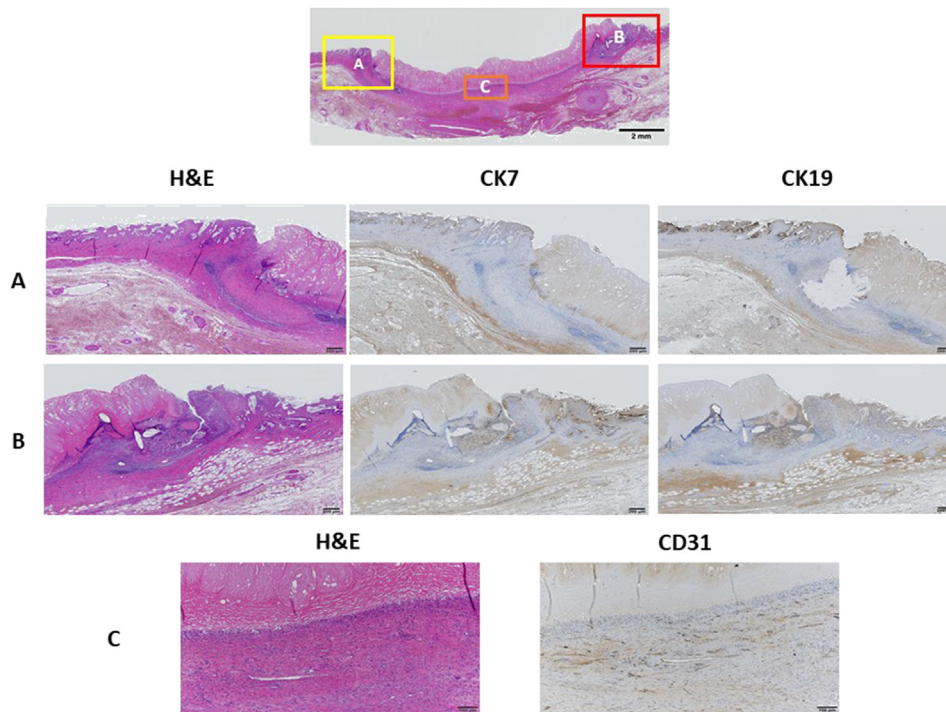


**Fig. 6.** (A) Duodenum side, and (B) liver side of hematoxylin and eosin (H&E), cytokeratin (CK) 7, and CK19 staining of the implant. Immunohistochemical analyses of the sections stained for CK7 and CK19 did not detect the extension of the biliary epithelium into the lumen of the fibroblast tube (×200). (C) Middle of H&E and cluster of differentiation (CD) 31 staining of the implant. Immunohistochemical analyses of the sections stained for CD31 showed angiogenesis in the outer layer of the fibroblast tube and around the native bile duct anastomosis (×200). H&E: hematoxylin and eosin, CD: cluster of differentiation.

why stenosis did not occur. The angiogenic effect of the fibroblast tube may play an important role in future clinical applications.

Although biliary epithelium was not found in the lumen, the tract was contiguous histologically. Smith et al. reported that fibroblasts produce collagen and other connective tissue components and merge with the surrounding tissues [36]. In addition to implementing a series of absorptive and secretory processes that dilute and alkalinize the bile flow during its passage along the biliary tract, the biliary epithelium is involved in several processes essential for liver physiology [37]. Bile ducts establish intimate anatomical and functional associations with the vasculature during liver development and repair; these associations are finely

regulated by signals exchanged between the biliary epithelial cells and vascular cells [38]. Angiogenesis, which is essential to the biliary epithelium, was evident in the fibroblast tube. Although biliary epithelial cells did not extend into the lumen of the fibroblast tube until 14 days in this study, the findings of angiogenesis in the fibroblast tube may affect the development of biliary epithelial cells in the fibroblast tube during a longer observation in the pig model. Further investigations are needed to clarify whether the biliary epithelium will regenerate in this model. Furthermore, it is considered technically possible to coat the lumen of tubular structures with vascular endothelial cells [22]; therefore, a tubular structure coated with biliary epithelium could be another option as a bioartificial bile duct with a similar histological structure as the native bile duct.

The development of a scaffold that incorporates stem cells is the gold standard of tissue engineering [39]. Scaffolds comprise a variety of biocompatible materials, including animal-derived collagen [40] and synthetic polymers [41]. These scaffolds provide templates for tissue formation, and they are seeded with cells and contain growth factors. While the clinical potential of biocompatible scaffolds generated by tissue engineering may be inestimable, safety concerns that cannot be ignored exist, including recipient immune responses [42], the long-term safety of scaffold degradation products [43], and risks of transmission of disease or infection [44,45]. In contrast, the scaffold-free tubular constructs created using the Bio-3D Printer comprised cells only; therefore, biocompatible materials that could induce immune reactions, infections, or acute allergic responses were not present. The present study's findings showed that the fibroblast tube could be implanted without the occurrence of allergies or infections, and as it did not contain any scaffold degradation products, there were no concerns regarding toxicity.

The most notable and potentially beneficial features of this model are that it does not incorporate any artificial components or require donors. Although bile ducts have been reconstructed experimentally using blood vessel or ureter grafts [14], donor tissues are required in a clinical setting; consequently, the clinical application of the technique was hardly realized. Even if decellularization is performed to produce a scaffold and create an artificial bile duct, a scaffold donor is required. In contrast, as the tubular construct comprises cells only, if cells could be cultured from a patient's own tissue, a donor would not be required for autologous transplantation. Patients' cells can be cultured and will proliferate indefinitely without difficulty. Furthermore, no reports have described mature grafts and scaffolds that have grown and developed successfully over long periods of time. If a structure can be produced that incorporates immature cells, it might grow after transplantation. Given the potential impact of a scaffold on the surrounding microenvironment and the impediments encountered when transplanting other live luminal structures, the development of cell-only technology is desirable.

Several limitations of this study should be acknowledged. First, this study involved allogeneic transplantations. Although we are considering autologous transplantations to treat patients with biliary strictures using a similar model to that described in this report, the animal experiment involved allogenic transplantations because 5 months is required to sample the fibroblasts and create and transplant the fibroblast tubes, and keeping pigs for this length of time was not feasible at our institution. Second, we did not collect data describing biliary epithelial regeneration factors associated with the artificial bile duct. Elucidating the mechanism underlying bile duct regeneration will be necessary to create more suitable artificial bile ducts. Third, the postoperative observation period was only 14 days, and the internal stent was not removed during this time. If this technology is to be applied to clinical practice, additional studies that include long-term follow-up

intervals and removal of the stent after a specified period are needed. Comparing the biochemical assay results of the pigs that underwent duct to duct biliary anastomosis and the pigs that underwent fibroblast tube implantation in this study, no significant difference was observed except for ALT. Hence, no significant cholestasis developed in the pigs that underwent fibroblast tube transplantation in comparison to those that underwent conventional biliary anastomosis. The results, such as the elevation of ALT which has been shown to have a longer half-life compared to AST in a short-term model suggested the need for long-term evaluations regarding not only the morphology but also regarding the findings of biochemical assays.

We conducted the present study to verify whether or not the fibroblast tube was transplantable using a short-term model. To determine the superiority of implantable fibroblast tubes over other types of therapeutic intervention, a comparison of outcomes, such as morphological, biochemical and histological evaluations of the biliary system and liver parenchyma, between models with fibroblast tubes and conventional plastic stents should therefore be conducted. In conclusion, we demonstrated the successful reconstruction of an extrahepatic bile duct using a scaffold-free tubular construct generated from pig fibroblasts and a novel Bio-3D Printer. The scaffold-free tubular construct created using the Bio-3D Printer could provide innovative regenerative treatments for hepatobiliary diseases.

## Authors' contributions

T.H., A.N., A.S., Y.S., A.K., K.N., and S.E. conceived the study and its design; T.H., A.N., A.S., T.M., S.Y., M.H., T.H., T.K., M.T., A.K., K.N., and S.E. acquired or analyzed the data; T.H. and A.N. wrote the manuscript's first draft; and T.H., A.N., A.S., Y.S., A.K., K.N., and S.E. wrote, reviewed, and edited the manuscript.

## Submission declaration and verification

All authors agree with the content of the manuscript and its submission.

## Declaration of competing interest

K. Nakayama is a cofounder and shareholder of Cyfuse Biomedical KK. The other authors declare no conflicts of interest.

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