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Feasibility of biodegradable PLGA common bile duct stents: An in vitro and in vivo study

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Abstract The current study investigates the feasibility of using a biodegradable polymeric stent in common bile duct (CBD) repair and reconstruction. Here, poly(L-lactide-*co*-glycolide) (PLGA, molar ratio LA/GA = 80/20) was processed into a circular tube- and dumbbell-shaped specimens to determine the in vitro degradation behavior in bile. The morphology, weight loss, and molecular weight changes were then investigated in conjunction with evaluations of the mechanical properties of the specimen. Circular tube-shaped PLGA stents with X-ray opacity were subsequently used in common bile duct exploration (CBDE) and primary suturing in canine models. Next, X-ray images of CBD stents in vivo were compared and levels of serum liver enzymes and a histological analysis were conducted after stent transplantation. The results showed that the PLGA stents exhibited the required biomedical properties and spontaneously disappeared from CBDs in 4–5 weeks. The degradation period and function match the requirements in repair and reconstruction of CBDs to support the duct, guide bile drainage, and reduce T-tube-related complications.

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1 Introduction

Prototype degradable polymer materials have been previously employed as urologic and vascular stents, and are currently being developed for application in gastrointestinal devices such as biliary tracts or esophageal stents [1–3]. Because simple suturing of the common bile duct (CBD) can potentially induce hemorrhaging and biliary tract narrowing, the insertion of a CBD stent to support the reconstruction and prevent stenoses is quite important [4–7]. Generally, a T-tube (made of silicone rubber) has been used in clinical treatments to relieve CBD pathological obstructions; however, complications caused by T-tube placement have been reported to be as high as 15%, and include biliary leakage, water-electrolyte disturbance, sepsis, biliary fistula formation, and benign biliary strictures [8–11]. In addition, problems using plastic CBD stents (made of PE, PVC, PTFE or PU), such as accumulation of bacteria-laden biofilms, occlusions, and infections have recently been reported [12–16]. Furthermore, another major limitation of plastic biliary stents is due to their occlusion with sludge, resulting in recurrences of jaundice or cholangitis, which then requires stent replacements for 30–60% of patients [17–19].

Degradable polymer stents are expected to overcome these noted disadvantages. To this end, poly(glycolide) (PGA),poly(lactic acid) (PLA) and other degradable aliphatic polyesters have attracted attention because they are

hydrolysable with excellent mechanical properties, and can also be used in important biomedical applications approved by the FDA; for example, as surgical sutures, in drug delivery, tissue scaffolds, and implants for interior bone fixation [20–25]. Degradation is a crucial property in biomaterial design and selection, as the rate of degradation may affect the duration of supporting effects, drug delivery, cell adhesion, and tissue-guided regeneration.

Absorbable CBD stents have a number of advantages for the treatment of benign and malignant biliary duct strictures, especially in eliminating the need for stent removal. In our previous study, the degradation behaviors of various molar ratios of PLGA were determined in bile in vitro [26]. The results revealed that PLGA stents can provide temporary support to the biliary tract and guide bile drainage, and the stents are then absorbed and removed from the body 4–5 weeks after implantation. In the present work, changes in the mechanical properties of PLGA (molar ratio LA/GA = 80/20) are investigated in bile degradation. Here, a PLGA tubing stent, with an ~10.0 mm outer diameter and a wall thickness of 2.0 mm was prepared and subsequently employed in conjunction with common bile duct exploration (CBDE) and primary suturing in a canine model to explore the effects of absorbable stents in human CBD repair.

2 Materials and methods

2.1 Materials

For these experiments, lactide (LA) and glycolide (GA) were purchased from Purac, Holland, and stannous octoate ($Sn(Oct)_2$, 95%) was obtained from Sigma-Aldrich. Monomers were then purified twice by recrystallization from ethyl acetate. Random PLGA copolymers (with a copolymerization ratio of LA/GA = 80/20) were synthesized by bulk ring-opening copolymerization of LA and GA using $Sn(Oct)_2$ as catalyst. Next, low-molecular-weight residuals were removed using a dissolution-precipitation method with chloroform and methanol as the solvent and precipitant, respectively. Finally, PLGA was dried under reduced pressure for one week at room temperature to remove any remaining solvent.

2.2 Specimen preparation

For in vitro degradation experiments in bile, PLGA was extruded into circular tubing using an XSS-300 extruder (screw diameter 20 mm and length-to-diameter ratio 25) at 155°C. This tubing was then cut into CBD stents; outer diameter of 10.0 mm, inner diameter of 6.0 mm, and length of 30.0 mm. For animal examinations, PLGA stents containing $BaSO_4$ (20 wt.%) as a radio-opacity indicator were fabricated based on the above processing methods and sterilized with ^{60}Co radiation prior to CBD implantation in canines.

2.3 In vitro degradation of samples in bile

The circular PLGA stents were immerged into vials filled with fresh bile (collected and mixed from T-tube-drained clinical patients) and the samples were then incubated at 37°C under an oscillation of 70 r min^{-1}. The bile was changed every day and the samples were withdrawn from the bile every two days; the samples were rinsed three times with distilled water and dried under a vacuum to obtain a constant weight ($n = 3$).

2.4 Physical-chemical characterizations

The specimen degradation was monitored by examining morphological changes and by measuring mass loss and molecular weight of the specimens. In addition, surface and cross-sectional changes of the specimen were determined using scanning electron microscope (SEM, SS550, Shimadzu) after gold-coating, with weight loss ($\Delta W_{weight}\%$) calculated according to the equation:

$$\Delta W_{weight}\% = 100 \times \left(W_o - W_{dry}\right)/W_o$$

where W_o and W_{dry} are the original weight of the specimen and dry weight after degradation, respectively.

The molecular weight was then determined by size exclusion chromatography (SEC); a Waters high pressure liquid chromatograph (HPLC) pump combined with the columns of HT5, HT4 and HT3 in series was used for this purpose. The measurement was carried out at 25°C using chloroform as the mobile phase at a flow rate of 1.0 ml min^{-1} and polystyrene as a standard.

Next, the mechanical properties of PLGA before and after degradation were measured using an Instron-1121 (UK) machine at 25% and 50% relative humidity. For preparation of mechanical property specimens, polymeric materials were laminated into the sheets with a thickness of 4.0 mm and 1.0 mm using a hot press molding at 135°C and 10 MPa pressure. Then, the sheets were cut into 55 × 6.0 × 4.0 mm^3 for the bending test and a dumbbell shape with effective dimensions of 75 × 5.0 × 1.0 mm^3 for the tensile test, and the in vitro degradation of the specimens in bile was evaluated as mentioned above. Finally, the stress-strain curve of the tensile test specimens was recorded at a stretching speed of 5 mm/min ($n = 5$); the anti-bending test was investigated at a speed of 10 mm/min and a span of 40 mm ($n = 5$).

2.5 In vivo stent biodegradation

Six canines were obtained from the Jilin University Research Resource Center. They were used to determine dominant degradation behaviors and biomedical functions of in vivo CBD stents; the University Animal Care and Use Committee approved all animal procedures. As a first step, experimental animals were anesthetized by intraperitoneal injection of ketamine [90 mg (kg body weight)$^{-1}$]. After a standard laparotomy, the CBD was liberated, and a longitudinal incision was made. A PLGA stent (with $BaSO_4$ as a radio-opacity indicator) was then inserted into the lumen of the CBD through the incision. The position of the stent was adjusted such that the incision was located in the middle of the stent, and was then fixed to the CBD using sutures. As the final step, PLGA sutures (polyglactin 910, VICRYL®, ETHICON, US) were used to close the incision, and changes of the in vivo stents were measured based on X-ray images.

To investigate the liver function of canines, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT), and the serum liver enzyme values of each canine model were determined. In brief, a 1 mL blood sample was collected prior to the surgical procedures and postoperatively for 1–8 weeks (weeks 0–4, $n = 4$; weeks 5–8, $n = 2$) using a vein puncture into a heparinized syringe. The samples were then immediately analyzed using a blood biochemistry analyzer.

For histological analysis, mid-portions of CBD and the surrounding tissues near the implantation in canine were harvested at Weeks 1, 4, and 8 postoperation. The tissues were fixed in 2.5% glutaraldehyde and cut into blocks of

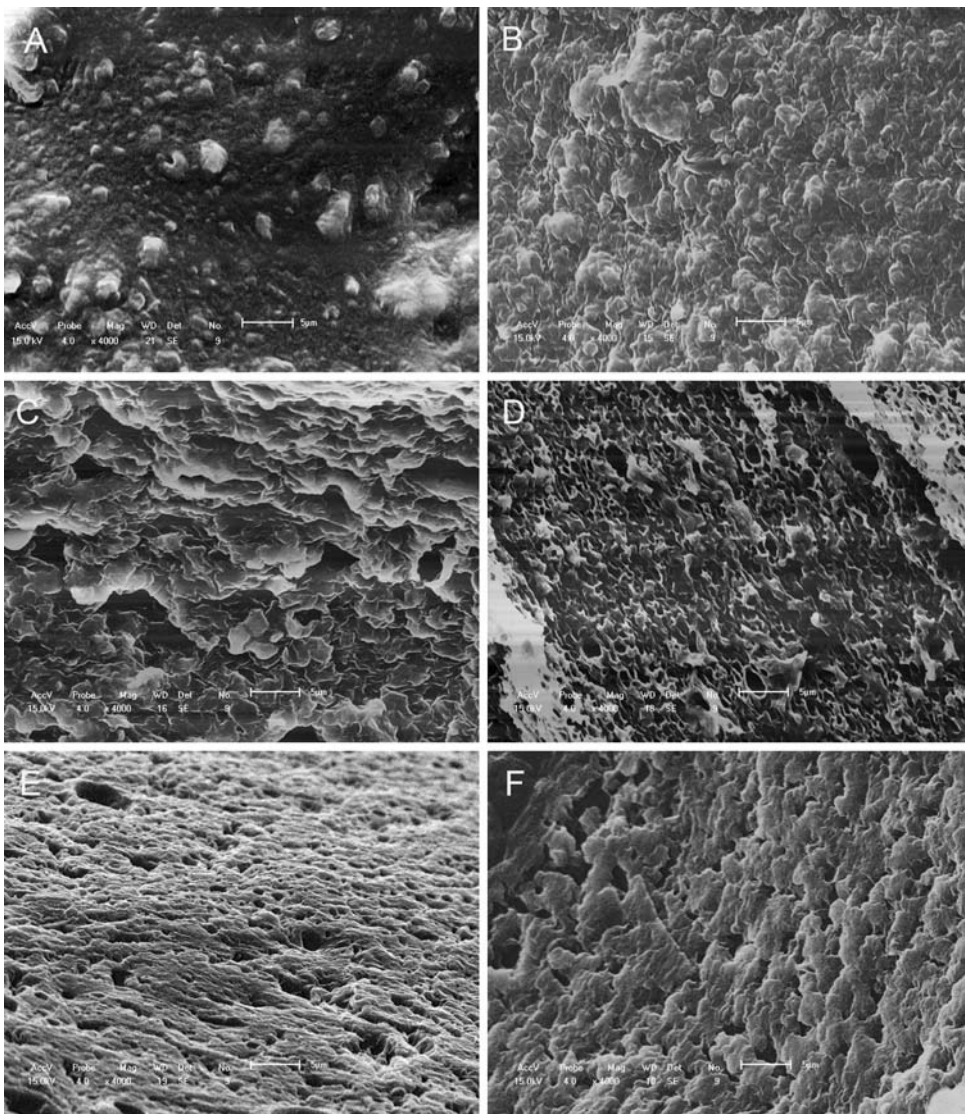

Fig. 1 SEM images of PLGA stents for surfaces (**a**, **c**, and **e**) and cross-sections (**b**, **d**, and **f**) during bile degradation at 37°C in vitro as a function of time (**a**, **b** 0 week; **c**, **d** 1 week; **e**, **f** 3 weeks)

around 5 × 5 mm. before embedding in paraffin wax. Then, the samples were dehydrated by passing them through a series of gradually increasing percentages of alcohol. After drying, the specimens were sectioned at 7 μm-thickness (RM2135, Leica) and the sections were hematoxylin and eosin (H & E, Sigma) and Mallory stained, respectively, then observed using optical microscopy (E400, Nikon).

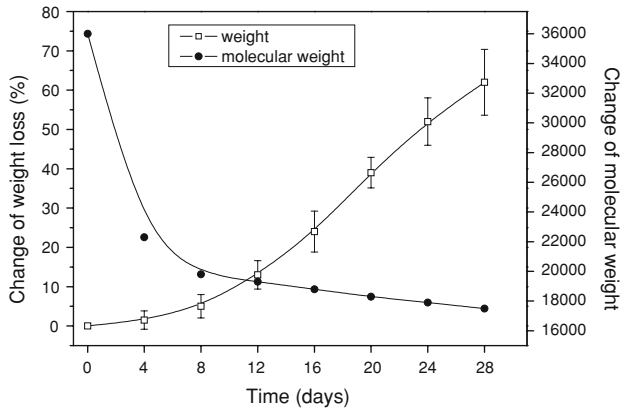

Fig. 2 Change of weight loss (%) and average molecular weight of PLGA stents in bile at 37°C in vitro

3 Results and discussion

3.1 In vitro degradation of PLGA stents

Due to the simple hydrolysis of ester bonds, PLGA has been shown to degrade into lactic acid and glycolic acid, which are then removed from the body by normal metabolic pathways [27–29]. In this study, bile (pH = 7.2–7.6) was used as the degradation medium. On gross inspection of the stent, the color first changed from translucent to yellowish and then became opaque. As further changes, the stent was slightly expanded on Day 10, though its strength and toughness were maintained. After 16 days of treatment, the stent became rigid, deformed, and cracked, and shape and integrity could not be fully maintained; at Day 24, the stent was quite fragmentized. These results indicate that PLGA was suitable for use as a CBD stent in animal experiments.

From SEM images, small holes could be seen on the samples after 3 weeks of incubation with bile (Fig. 1c), with an increase in the number of pores observed on the stent surface and cross-section at Week 4 (Fig. 1d–f). These images also revealed that the lumen surface of the stent degraded faster than the surface, thereby implying the autocatalytic cleavage of the ester bond. As a result, the eroded PLGA degraded into glycolic acid and lactic acid, which confirmed that its water-soluble oligomers could catalyze the degradation inside the polymer.

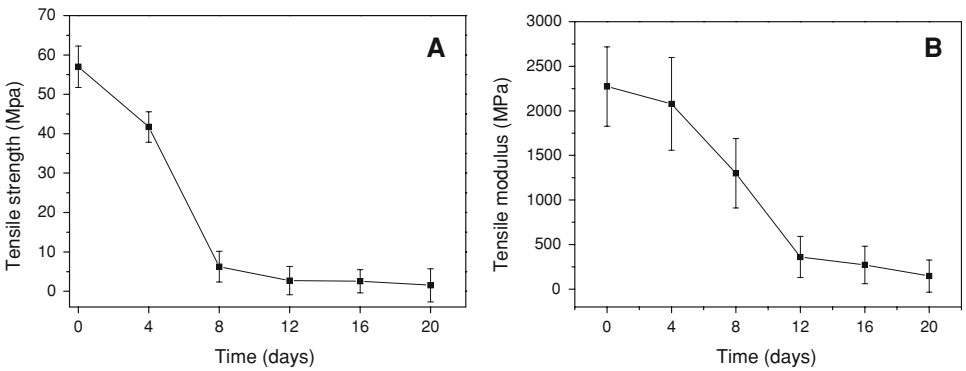

Fig. 3 Change of PLGA in tensile strength (**a**) and tensile modulus (**b**) in bile at 37°C in vitro

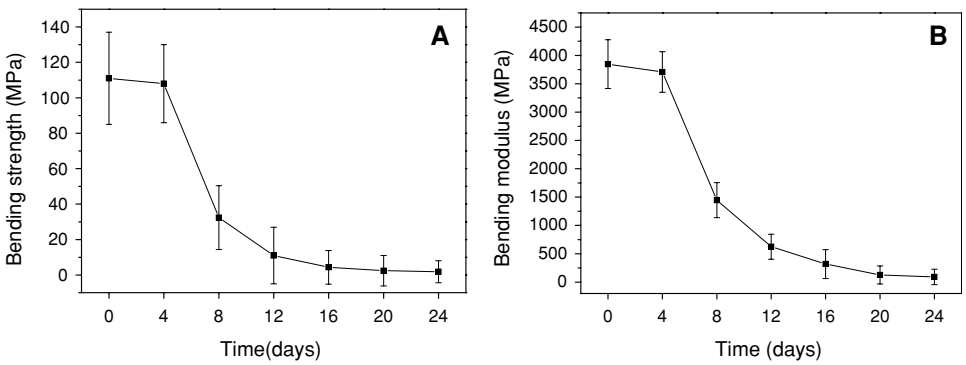

Fig. 4 Change of PLGA in bending strength (**a**) and bending modulus (**b**) in bile at 37°C in vitro

The weight loss and molecular weight curves are shown in Fig. 2 for the PLGA specimens examined, where it can be seen that there was a 5% loss in the original weight within 8 days. As indicated by the slope of the weight loss curve, the mass loss was slow during the first 8 days, and increased for the next three weeks until reaching a final weight loss of 65%.

The original average molecular weight (Mw) of PLGA was around 1×10^5 g mol^{-1} when synthesized, though changed to 4×10^4 g mol^{-1} due to thermo degradation during fabrication. As illustrated in Fig. 2, the decrease of molecular weight of PLGA in bile was quite rapid during the first 6 days; from Day 7 onwards, the rate of molecular weight reduction slowed, especially during the later stage (Day 28). These changes in mass loss and molecular weight imply that bulk hydrolysis and random cleavages of polymer chains occurred during the degradation.

The mechanical properties of PLGA samples are shown in Figs. 3 and 4; Fig. 3 reveals the decrease of dumbbell specimens in terms of tensile strength and Young's modulus with advances in the degradation process. From the figure, it can be seen that both tensile strength and tensile modulus were maintained until Day 4 and then quickly decreased to Day 12. The results indicate that PLGA samples maintained their mechanical strength during the early stage of degradation (0–4 days) and became more brittle and harder at the later period (5–12 days); similar data were obtained for bending strength and bending modulus (Fig. 4a and b). In summary, the mechanical properties of PLGA were found to depend on the degradation period and maintained the requirements of CBD repair and reconstruction. Compared with commercial stents such as PTFE or metal devices, used for long-term implantation or in cancer patients, the PLGA stent can provide temporary support for a 2-week period. Thus, the mechanical properties of this degradable device are suitable for bile duct repair.

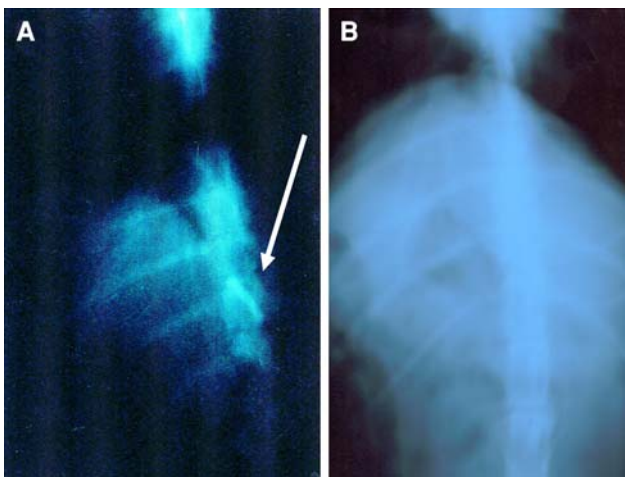

Fig. 5 X-ray images of PLGA stents in a canine at 0 week (**a**) and 5 weeks (**b**) with $BaSO_4$ (20%, w/w) as a radio-opacity indicator

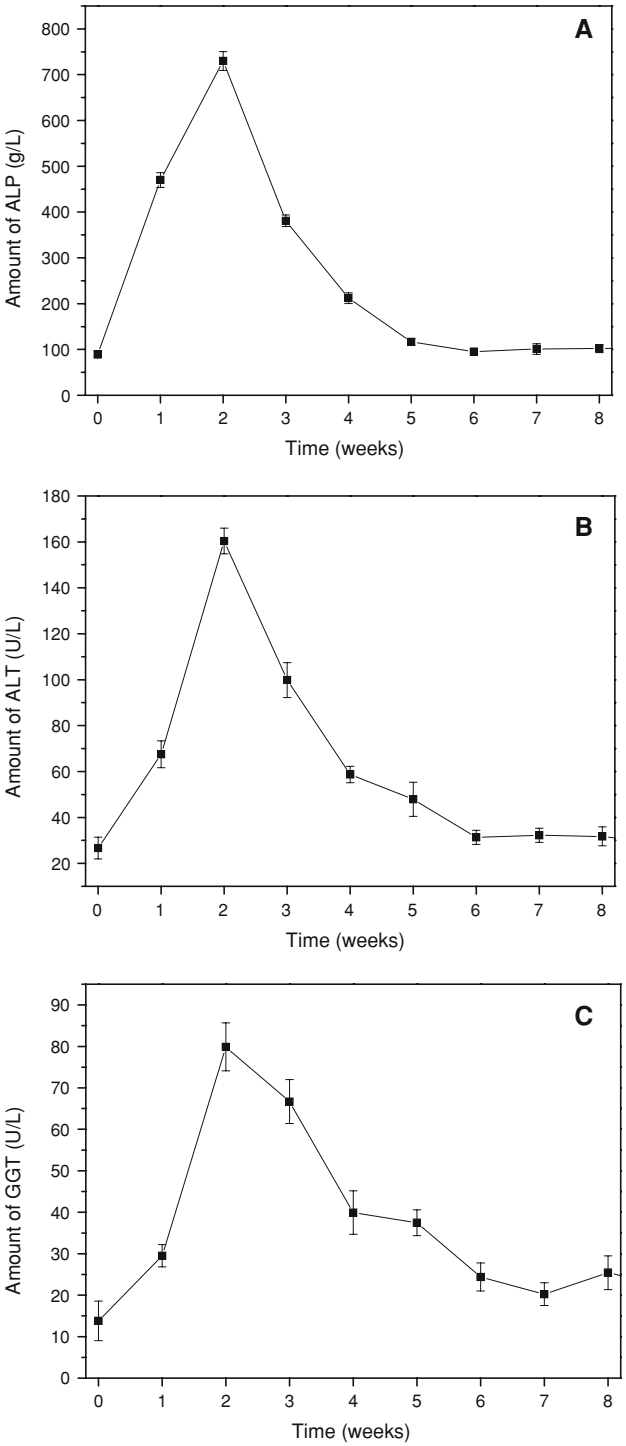

Fig. 6 Change of serum liver enzyme levels in canines before and after operation. **a** alkaline phosphatase (ALP g/L); **b** alanine aminotransferase (ALT U/L); and **c** γ-glutamyltransferase (GGT U/L)

3.2 Animals tests using PLGA stents

As an iatrogenic emergency, biliary leakage is a relatively serious and vital complication of hepatobiliary surgery. In this study, two canines were randomly chosen to determine the best location of the instruments and the incident rate of postoperative bile leakage after one week. From a gross inspection of necropsy, the PLGA stent was found to be fully patent. There was no indication of device movement or evidence of biliary leakage; bile could flow freely through the stent. These results suggest that the PLGA stents could play a role in preventing bile leakage.

PLGA stents with X-ray opacity ($BaSO_4$) were implanted into the CBD of canines. As seen as Fig. 5, the stent could be observed after the surgical procedures (Fig. 5a), and disappeared after postoperative Week 5 (Fig. 5b). This data confirmed that the PLGA stent degrades in vivo, and that the hydrolysis period of the stent matched the period required for the biliary duct prosthesis and reconstruction.

The levels of serum liver enzymes in the canine models, including ALP, ALT, and GGT, were then examined as a function of postoperative time (Fig. 6). Obviously, it can be seen that the enzyme levels increased after the surgical procedure and reached peak levels at postoperative Week 2. This effect was due to bile duct narrowing and cholestasis after simple CBD suturing. After this initial increase, a significant reduction in liver enzymes was observed, with the enzyme levels becoming normal 5–6 weeks post implantation. Similarly, serum enzymes reached a maximum during Week 2 and then decreased in such a way that they returned to normal levels in the fifth week, indicating that the biophysical functions of both the CBD and liver completely recovered in five weeks. The observed elevation of liver enzymes after surgery was only temporary, as they gradually declined and returned to their normal values. Based on this fact, we could conclude that the degree of bile duct narrowing after operation was significantly improved using PLGA stents.

A histopathologic evaluation was subsequently conducted, the results of which are shown in Fig. 7. During the inspection of Week 1, there is a slight exudation, granulation tissue, and epithelial hyperplasia on the anastomosis mucosa, with broken elastic fibers observed in the Mallory stain specimen. In the Week 4 sample, a small number of chronic inflammatory reactions and minimal bile encrustation were present. In this sample, some parts of the bile tract mucosa were repaired and the bile duct wall thickened because of fibrous scar tissue hyperplasia. Compact collagen fibers hyperplasia could also be seen in the Mallory-stained sample (Fig. 7d). At Week 8, the bile tract mucosa was generally repaired and integrated, though some inflammatory invasion was observed.

The results of this study show the efficiency and safety of PLGA stents in the CBD repair and reconstruction. We were initially concerned that PLGA stents, containing 20%

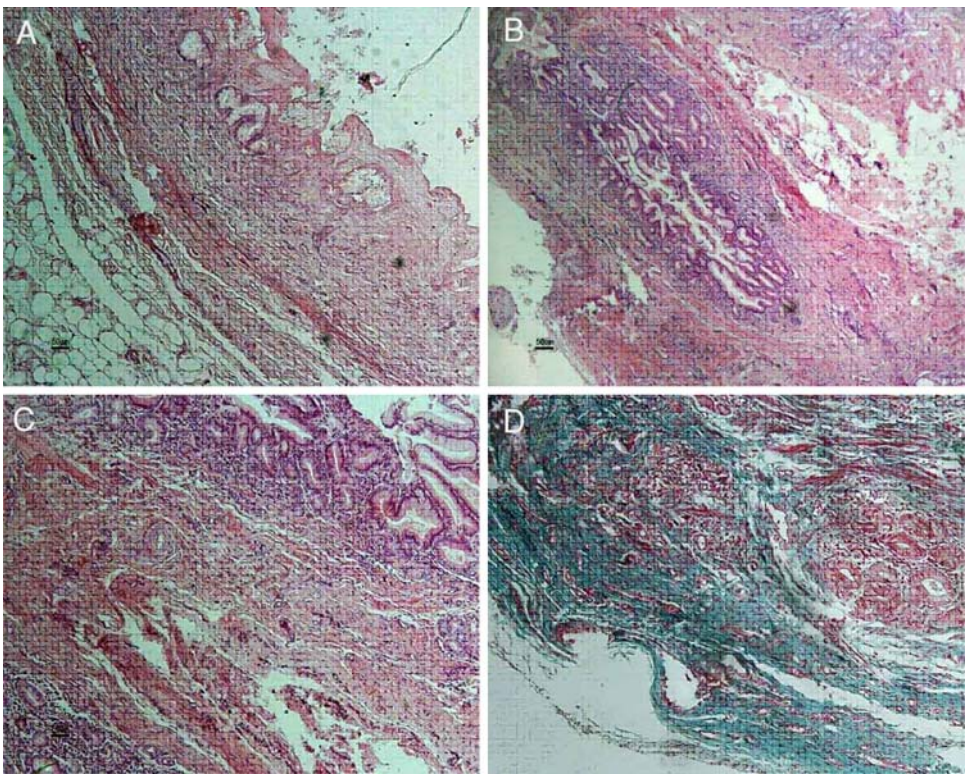

Fig. 7 Histopathological images of H & E stained (**a**, **b**, and **c**) and Mallory stained (**d**) CBD specimens (×100). **a** 1 week; **b** and **d** 4 weeks; **c** 8 weeks

$BaSO_4$, might block bile ducts during in vivo decomposition. However, it was found that radio-opacity indicator enters into the intestine smoothly as PLGA degrades. Thus, bile can drain normally into the intestine through the bile duct after stent insertion; there was no fibrous tissues observed in CBD, though further animal experiments and long term estimates are still needed before clinical trials can begin.

4 Conclusion

In the present study, we chose PLGA as the stent material because its in vivo biodegradation time matches the healing time required for CBD support. A small PLGA tube was prepared and inserted in a canine CBD via a laparotomy to ensure the correct placement of the device in the biliary tract. The results of canine models indicate that the present strategy to employ biodegradable stents in CBDE and PLGA materials with an in vivo life-time of 2–3 weeks is of great interest for temporary therapeutic applications. Prior to the application of this device in humans, however, special attention should be paid to the shape, structural design, and implementation process of the device, as well as to ensure effective measures are undertaken to prevent possible slippage of the stent inside CBD.

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