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Study of biodegradable and self-expandable PLLA helical biliary stent *in vivo* and *in vitro*

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Abstract Biodegradable stents have advantages for the treatment of benign and malignant biliary stricture, especially eliminating the need for stent removal. In our present work, helical poly-l-lactic acids (PLLA) stent was fabricated and evaluated *in vivo* and *in vitro*. For *in vivo* study, bile duct injury canine models were made by transection of common bile ducts. Duct to duct anastomosis was done with helical PLLA biodegradable stents. Scanning electron microscopy (SEM) and histopathology were performed after three months. For *In vitro* study, sludge attachment assessment was performed. Polyethylene (PE) and PLLA membranes were immersed in human bile for two months. The samples were taken out and characterized by SEM. Self-expanding property of the helical stent was tested in 37°C water. The results demonstrate that the biodegradable stent had not only good biocompatibility, but also self-clearing effect to clear the attached sludge away. The self-expanding property facilitated stent implantation and also suggested possibility to be implanted endoscopically.

1. Introduction

Bile duct obstruction generally requires placement of stent to enhance unidirectional flow and to facilitate tissue remodeling. Biliary endoprosthesis include T tube, U tube, fixed-diameter plastic stent (FDPS) and Self-expanding metallic stent (SEMS). T tube and U tube are often used after bile duct injury or bile duct stricture. The presences of the tubes impair quality of life of patients significantly. FDPS diameter is limited by the accessory channel of the duodenoscope and easily to be occluded by biofilm accumulation. SEMS is more durable because its diameter goes up to 10 mm after deployment. SEMS cannot be removed because it becomes embedded into the biliary wall after placement. However, epithelial hyperplasia and sludge accumulation may induce stent occlusion. Thus, SEMS is unsuitable for treatment of benign biliary stricture [1].

Recently, biodegradable biliary braided stents have been designed and tested [1–3]. The advantages of biodegradable stent include large stent diameter, decrease of biofilm accumulation and proliferative changes, elimination of the need for stent removal.

In the present work, helical poly-l-lactic acid (PLLA) stents were fabricated and tested in bile duct injury model. The helical wall was designed to fully cover the bile duct and prevent duct hyperplasia into the lumen. Histology and scanning electron microscopy (SEM) were performed after three months. Sludge attachment and self-expanding properties of the biodegradable helical stent were tested *in vitro*.

2. Materials and method

2.1. Stent fabrication

PLLA(99.9%) whose weight average molecular weight, Mw was 1.0×10^5 g/mol was supplied by Shandong Medical Corporation. Chloroform (AR) was bought from Beijing Chemical Company. PLLA was dissolved in chloroform in

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ratio of 2% (g/ml) and stirred for 48 h. The PLLA membrane was prepared by pouring PLLA solution on glass substrate and free drying. Then the membrane was cut into strips. Right before the implantation, the strip was twisted helically on 1.8 mm tube and be fixed for 5 min to form a helical stent. Then the stent was unfixed and inserted into bile duct immediately.

2.2. Animal model

The institutional animal care and use committee of General Hospital of People's Liberation Army approved the study protocol. The procedures were performed under general anesthesia. Common bile ducts of three canines were transected for the model of bile duct injury. Duct to duct anastomosis was done with the helical PLLA stent. Especially, in order to evaluate clinical effect of the stent, another canine bile duct was first transected and sutured without implantation of the stent. After three months, the canine underwent second surgery to be implanted in a PLLA stent. After the surgery, jaundice, serum bilirubin levels, alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (γ-GT) were evaluated every two weeks. After 3 months of the stent implantation, all the canines were sacrificed and histology was performed.

2.3. Sludge attachment test

The human bile was collected from a patient with liver cancer, who had U tube to drain bile after surgery. The human bile was stored hermetically. Sludge appeared in the bile after one or two days after drainage from the patient. The sludge was separated by centrifuge and washed with deionized water to remove the bile residues. The composition of the sludge was analyzed by fourier transform infrared spectroscopy (FTIR) (USA, Spectrum GX).

Strips of PE and PLLA were immersed into human bile for period of 7, 14, 21 and 56 days respectively. The bile was kept at 37°C and was changed with new bile every three days. The membranes were taken out, rinsed in deionized water slightly, and then dried in vacuum desiccator. By SEM (Japan, Hitachi S-800), bile sludge attached on the polymer surface was observed.

2.4. Self-expansion test

The PLLA strips were first helically twisted to be 6F or 8F in perimeter and fixed in 37°C water for 5 min or 10 min. Then stents were unfixed and free expanded in 37°C water. The outside diameters of the samples were measured at 0, 5, 10, 30, 60, 120, 240 and 360 min after unfixation.

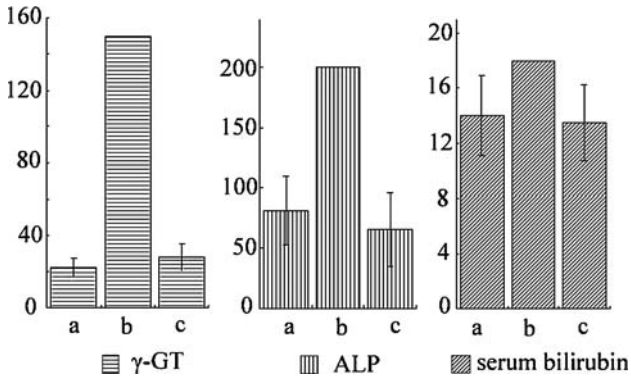

Fig. 1 γ-GT, ALP and serum bilirubin levels of the canines. (a) one week before first surgery (four canines); (b) two weeks after the surgery with no stent implanted (one canine); (c) two weeks after stents implantation (four canines).

To show the maximum expanding rate of the stent, balloon was used to help stent expanding more quickly. During the expansion, the stent and balloon were both immersed in 37°C water. The balloon expanding rate was controlled to avoid cracking of the stent.

3. Result

3.1. Animal model

γ-GT, ALP and serum bilirubin levels of the canines were shown in Fig. 1. For γ-GT, ALP and serum bilirubin levels, no statistic differences were found between blood samples taken one week before first surgery and two weeks after stents implantation ($p > 0.05$). All the figures measured before the first surgery or after stent implantation were at normal level. The canine whose bile duct was transected and sutured without stent had normal serum bilirubin level (normal level: 0-21 Umol/L) two weeks after first surgery. However, ALP and γ-GT were elevated to 200 U/L (normal level: 0–130 U/L) and 150 U/L (normal level: 0-50 U/L).

The canine whose bile duct was transected and sutured without stent had anorexials and jaundice in the first four weeks. Cholangiogram showed bile duct stricture. After second surgery to implant a PLLA stent, the anorexia and jaundice disappeared. All the other three canines had no altered general status with weight loss, anorexia, jaundice or clinical sepsis.

Just before the animals were sacrificed, Cholangiogram was done and no duct stricture or dilation was observed. Fig. 2a was the photograph of the stent in the bile duct after sacrifice. There was no proximal duct dilation. The stent was integrated and has already expanded to 3mm to fully support the duct. No filling defects were observed and the stent was still transparent, implying that no bile sludge accumulation occurred.

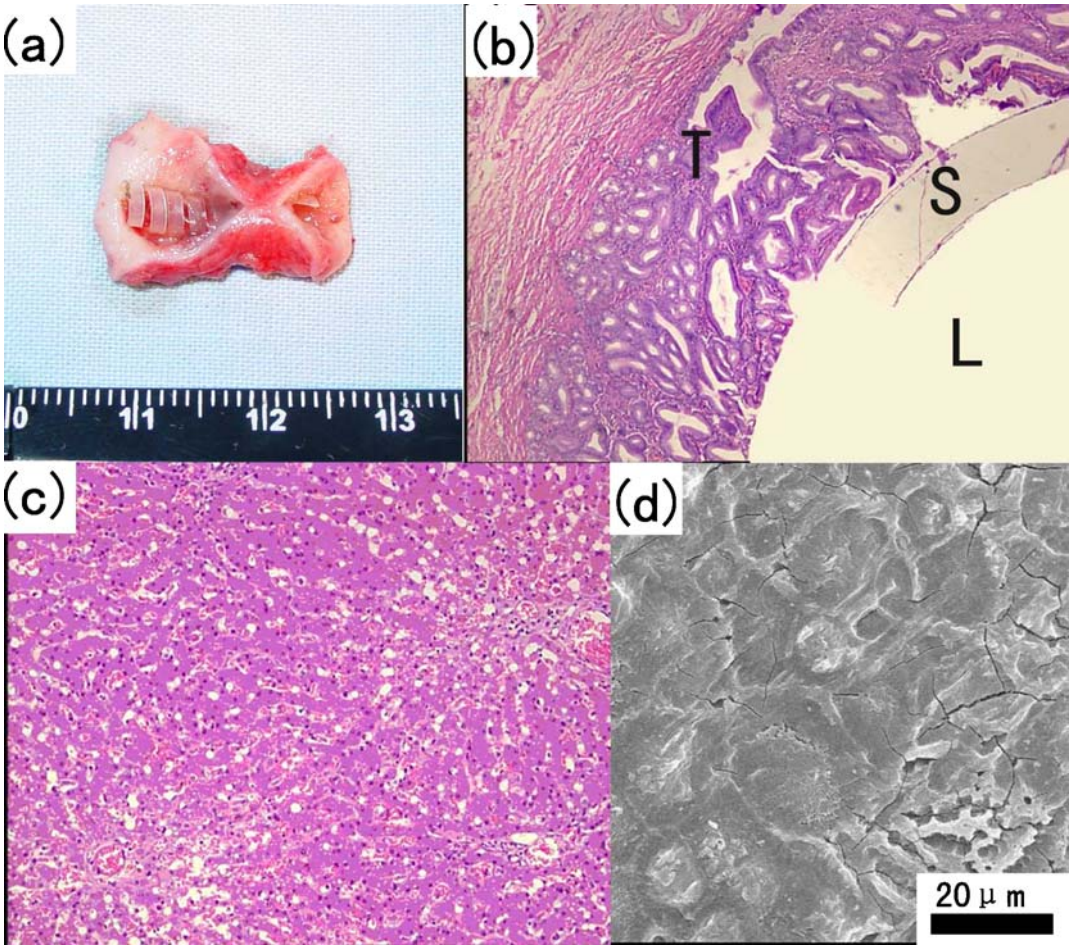

Fig. 2 (a) the photograph of the stent in the bile duct; (b) Histopathology of bile duct; (c) Histopathology of liver tissue; (d) SEM image of the PLLA stent.

Histopathological examination (Fig. 2b) revealed normal bile duct epithelium with no stent impression marks. No inflammation or epithelial hyperplasia was induced by stent. Similar to the report of poly-lactic acids stent by Ginsburg *et al.*, the stent did not become endothelialized, i.e., integrated into the bile duct lamina [1]. Histopathology (Fig. 2c) showed that the liver tissue of the canine was normal.

To investigate the stent surface performance in vivo thoroughly, SEM image of the PLLA stent was shown in Fig. 2d. Microholes and cracks were observed. Obviously, the whole surface had sloughed off. Little sludge was observed.

4. Sludge attachment test

The infrared spectra for the sludge of human bile were shown in Fig. 3. The major bands of the spectrum appeared as calcium palmitate (2914, 2847, 1576, 1539, 1470, 1111 and 721 cm), unconjugated bilirubin (3400 cm) and proteins (3400, 2955, 1655, 1540 and 1418 cm). The result was consistent with the reported results that main constituents of sludge of most patients were calcium palmitate and bilirubin [4].

SEM images of the polymers before contacting with bile were shown in Fig. 4. PE surface were smooth. PLLA had granule pattern surface.

The SEM images of samples after immersion in bile for 7 and 14 days were shown in Fig. 5. With an increased immersion period, the amount of sludge that attached on both polymers increased. After 7 and 14 days, PLLA membrane attracted more sludge than PE did, respectively.

During immersion period of 2 months, the amount of sludge that attached on PE increased continuously. After 2 months, the sludge that attached on PE sample surface almost fully covered the sample surface, as shown in Fig. 6c. However, PLLA membrane surface degraded severely after 3 weeks immersion. Cracks were observed on the surface. Thus, the sludge sloughed off with corrosion of the PLLA surface. After 56 days, the sludge increased slightly. The sludge on the PLLA membrane was obviously less than that on the PE with the same immersion period.

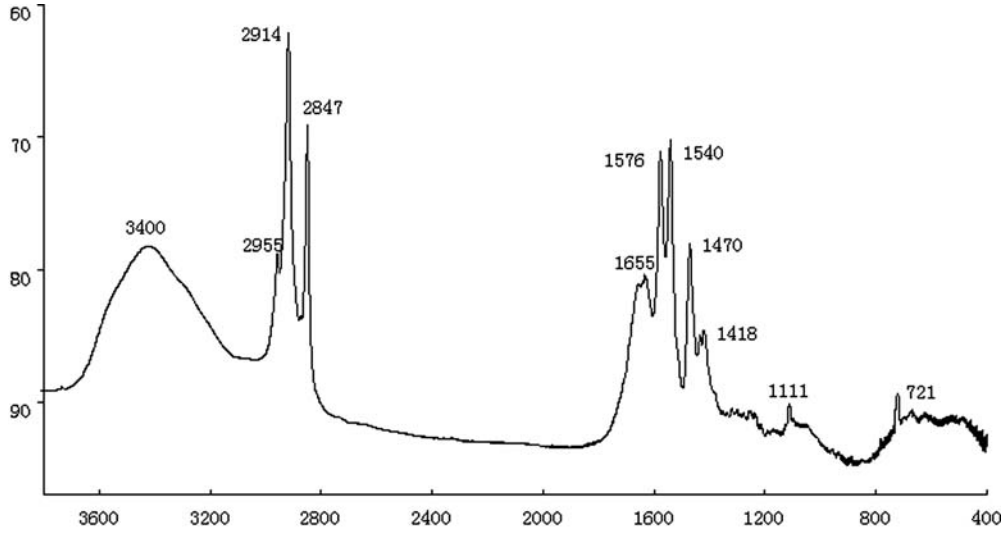

Fig. 3 FTIR analysis of the sludge separated from human bile *in vitro*.

4.1. Self-expansion test

The self-expansion experiment demonstrated that the helical PLLA stent expanded significantly (Fig. 7). The expanding rate depended on fixing diameter and fixing time. Larger fixing diameter and shorter fixing time led to greater expansion. All the samples nearly doubled their diameters in the first 5min. Then the stents expanded slowly in the following 6 h. For example, the stent was twisted with diameter of 2.5 mm (8F) for 5 min, expanded to 3.7 mm just after unfixation. In 5 min, it expanded to 5.8 mm in diameter. After 360 min, its diameter grew slowly up to 8 mm.

In addition, stent self-expansion was accelerated by the help of balloon. The balloon must be dilated slowly to avoid fracture of the stent. With the help of balloon, the stent expanded to 8 mm in 2 min.

5. Discussion

Biodegradable braided stents have been studied by Haber, Freeman and Ginsburg *et al.* [1–3]. These works proved that the polylactide polymer degraded in tissue completely, just as in other tissues. In the present work, helical PLLA stent was designed to replace T tube or U tube used for bile duct injury. The wall of the helical formation blocked out hyperplasia of injury bile duct. Similar to previous report by Ginsburg *et al.* [1], the biodegradable stent did not become endothelialized. In histopathology examination, the stent can be easily taken out of the bile duct. This offered possibility of removing stent when necessary.

The biodegradable PLLA has free COOH group at the end of molecule chains. The COO^- may absorb Ca^{2+} and thus facilitate palmitate, unconjugated bilirubin attachment.

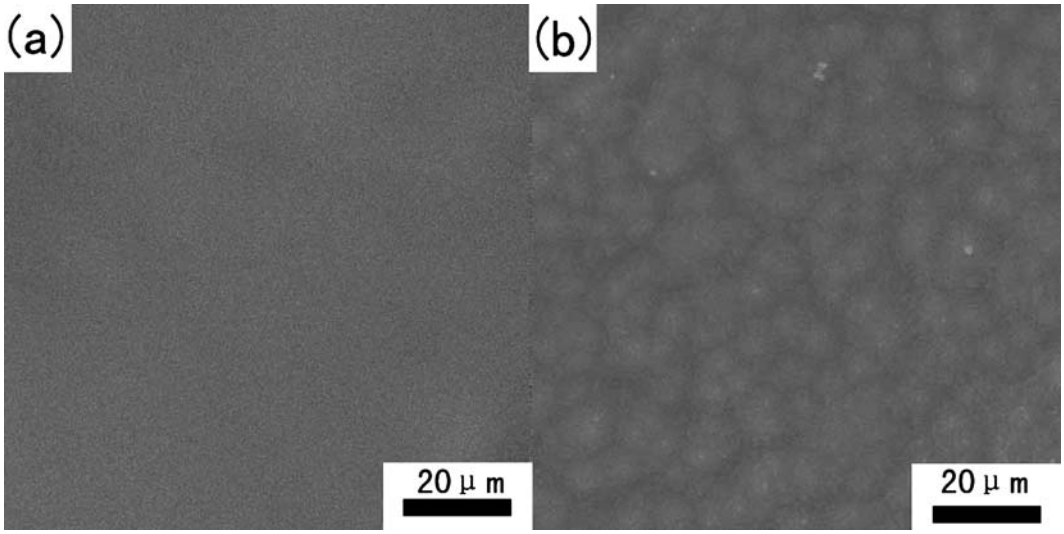

Fig. 4 The SEM images of samples before immersion in bile: (a) PE; (b) PLLA.

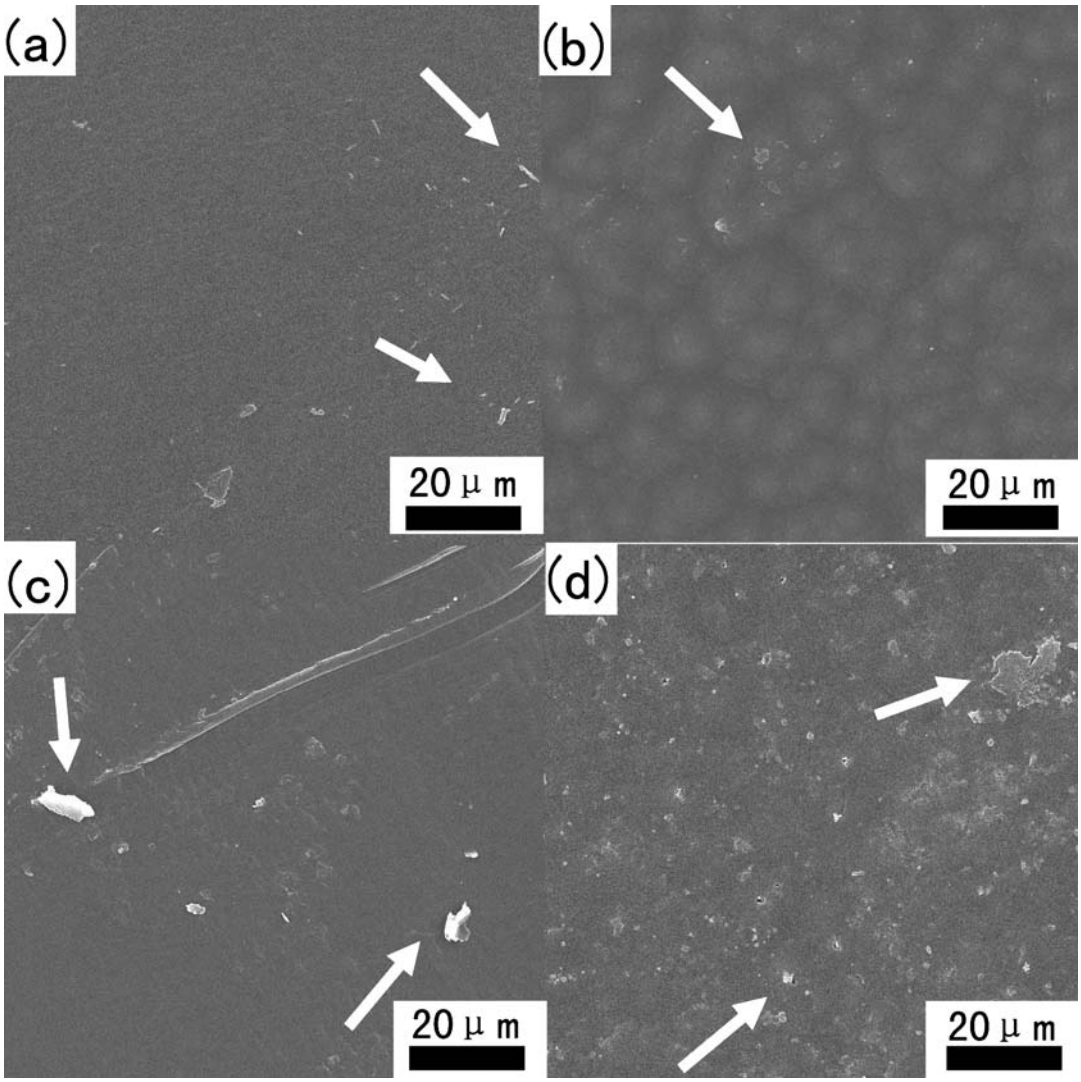

Fig. 5 The SEM images of samples after immersion in bile for 7 and 14 days: (a) PE for 7d; (b) PLLA for 7d; (c) PE for 14d; (d) PLLA for 14d. The white arrows point out some of the sludge that attached on the sample surface.

PE is polymerized by ethylene and has no group to absorb Ca^{2+}. Thus, in the first two weeks, PLLA sample were more attractive to bile sludge than PE.

After immersed in bile for three weeks, sludge continued to attach on PE surface and covered almost the full sample surface after two months. But PLLA surface started to degrade severely at the third week. Crack was observed on the surface. Thus, the sludge detached from the PLLA with surface corrosion. Pétas A postulated that biodegradable polymer refused encrustion formation in urethra, for it surface kept slough off [5]. The present work proved the self-clearing effect of PLLA in bile duct.

The results of animal model study confirmed self-clearing effect of the PLLA stent. In the present work, on all the four stents implanted in animal model, no sludge was observed under optic microscopy in histopathologic evaluation. The PLLA stent performance in bile duct was further studied by SEM. The SEM image showed that the stent surface kept sloughing off in the animal model. Thus no sludge kept attaching on the polymer surface. The detached sludge was not observed in the bile duct after the canines were sacrificed. The detached sludge was quite small and easily evicted with bile.

The viscoelastic behavior of biodegradable polymers makes the memory effect of the material possible. Self-reinforced PLLA stents can be made self-expanding by merit of the viscoelastic memory of the material [6]. In our work, twisting PLLA strips right before implantation, the PLLA stents also gained self-expanding property. The *in vitro* study showed that the stent extended to 5.8 mm in 5 min. In the *in vivo* study, the stents expanded to about 3 mm after being restricted by canine bile duct wall. Thus the stents were fixed in the bile duct.

The property offered convenience to be easily inserted into bile duct in open surgery. The property also suggested

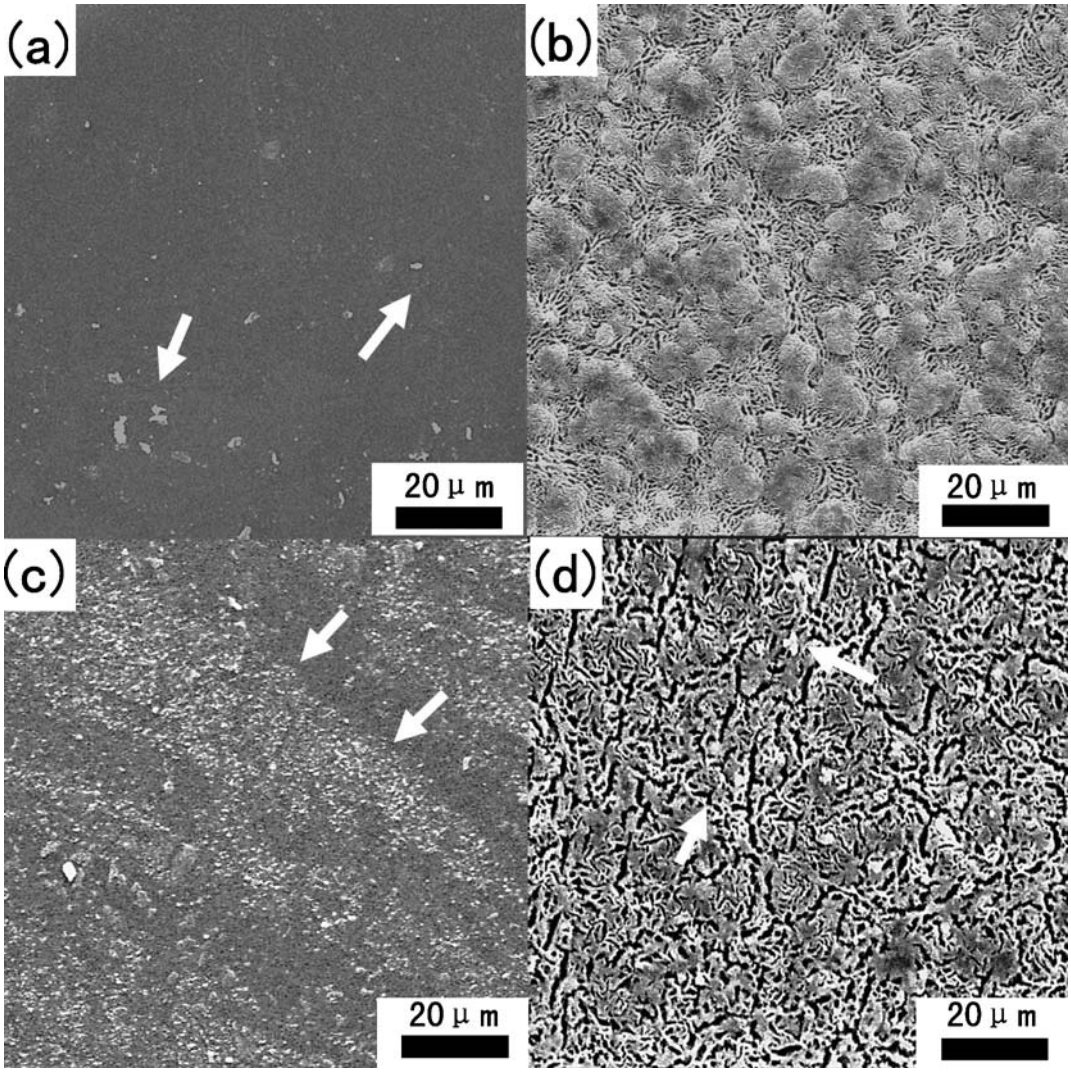

Fig. 6 The SEM images of samples after immersion in bile for 21 and 56 days: (a) PE for 21d; (b) PLLA for 21d; (c) PE for 56d; (d) PLLA for 56d. The white arrows point out some of the sludge that attached on the sample surface.

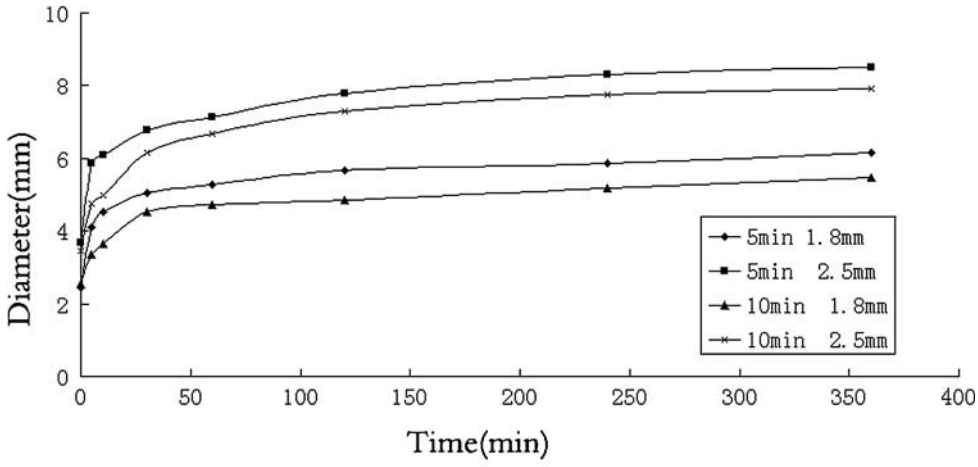

Fig. 7 *In vitro* self-expansion study of PLLA helical stents in 37°C water. The table in the figure indicates fixing time and diameter of the samples.

that the helical stent could be used as duodenoscopical stent. In the *in vitro* study, the stents were fixed to be 6F or 8F in outer diameter. The diameters were small enough for the stents to be easily inserted into bile duct, even duodenoscopically. The times of fixation of stents with small diameters were 5 or 10 min and the times were enough for endoscopic surgery.

In the present work, the PLLA stent performance was good in the first 3 months. However, Haber *et al.* [2] reported that the bioabsorbable stent broke up into fragments and the fragments subsequently occluded the bile duct. Thus, further study with longer experiment period is needed to determine how long the stent would broke and whether the fragments occlude the bile duct.

6. Conclusion

Biodegradable polymer PLLA has good biocompatibility in bile duct. The biodegradation offers the stent self-clearing effect that helps to prevent bile sludge attachment. The self-clearing property would prolong stent function period in bile duct. The self-expandable helical stent was not only a feasible stent for open surgery insertion, but also candidate for endoscopic surgery.

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