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Biliary small intestinal submucosa covered Z-stents: preliminary results in an animal model

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Background. *Purpose of the study was to test the function and biological response of metallic stents covered with small intestinal submucosa (SIS) in the swine biliary system.*

Materials and methods. *A total of 9 SIS-covered single Z-stents were placed in the common bile duct (CBD) in 6 pigs. Stents were delivered into the CBD at laparotomy via the gall bladder and the cystic duct. Animals were sacrificed or died at 2 weeks (n=1), 4 weeks (n=1), 8 weeks (n=2), and 10 weeks (n=2) after stenting and histological studies were performed.*

Results. *Nine stents were deployed in 6 animals. During follow-up, 3 stents in 3 animals (2, 4, and 10 weeks) remained stable, while one stent shifted distally in CBD and 5 of them turned sideways. All stents remained patent. Duct dilatation and bile slugging were noted at 10 weeks. The SIS-membrane was present at 2 weeks, but was not histologically distinct at 4 weeks and later. Histological study showed no significant inflammatory changes in the bile duct in any pig. Mucosal hyperplasia was absent in 2 of 3 stable stents at 2 and 10 weeks, and 1 distally shifted stent at 10 weeks. Mild mucosal hyperplasia was seen at the distal stent end in 1 stable stent at 4 weeks and in 5 dislodged stents at 8 and 10 weeks.*

Conclusions. *Even when the study is limited by dislodgment of high percentage of placed stents, the results in stable stents conducting the bile flow suggest that SIS helps to prevent bile duct inflammation and mucosal hyperplasia typical for uncoated stents. Further studies, particularly with improved wet SIS are warranted.*

Key words: bile ducts, stents; intestinal mucosa

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Introduction

Expandable metallic stents have been established as useful devices for the treatment of large bile duct obstructions whether caused by benign or malignant processes.[1-7] Their long-term patency, however, remains a major problem. Mucosal hyperplasia, bile sludging and stone formation resulting from significant foreign body-type inflammatory reaction often block expandable stents.[1-4] In malignancies, direct tumor ingrowth or overgrowth also obstructs stents.[8-10]

Stents coated or covered with synthetic polymer material have been explored experimentally for potential improvement in a long-term biliary stent patency.[11-15] Generally, stent coated or covered with polyester, polyurethane or polycaprolectone resulted in lesser degree of mucosal hyperplasia and reactive inflammatory or dysplastic changes than with bare stents.[11-13] The results with stents coated or covered with silicone were less uniform showing a decrease of reactive changes in some experiments [11,12,14], while leading to stent occlusion in others.[15] A few clinical studies with prototype stents coated or covered with polyurethane showed promise in prevention of tumor ingrowth into the stent and all investigators called for an improvement of stent covering.[16-18]

We explored a biomaterial- small intestinal submucosa (SIS) as stent cover for potential biliary use. SIS is a relatively acellular, collagen-rich, degradable biomaterial harvested from pig small intestines. It is resistant to infection, does not produce an adverse immunologic response and is remodeled and replaced by host tissue.[19-26] SIS has been successfully used on grafting arteries[19-21], veins[22], and in the defects of the urinary bladder[23,24], diaphragm[25], tendon[26], fascia[27], and abdominal wall.[28]

Material and methods

Animals

Six young domestic swine weighing from 26 Kg to 28 Kg underwent SIS covered stent placement into the common bile duct. The study was approved by the institutional animal care and use committee of Oregon Health Science University in accordance with the guideline established by the Animal Welfare Act.

Covered stents

Single Gianturco-Rösh type Z-stents were used (Figure 1). They were hand-made in our research laboratory of 0.075-inch stainless steel wire and consisted of six legs. They were

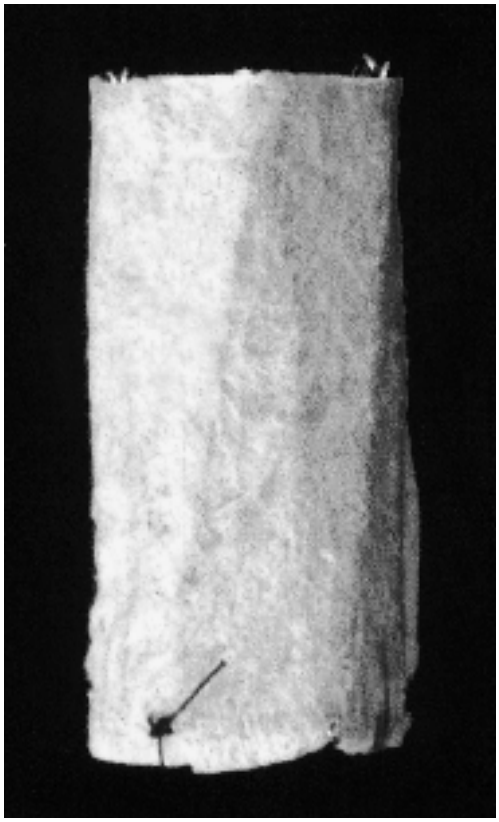


Figure 1. Biliary endograft. A single Z-stent 6-mm in diameter covered with lyophilized dry small intestinal submucosa-sheet.

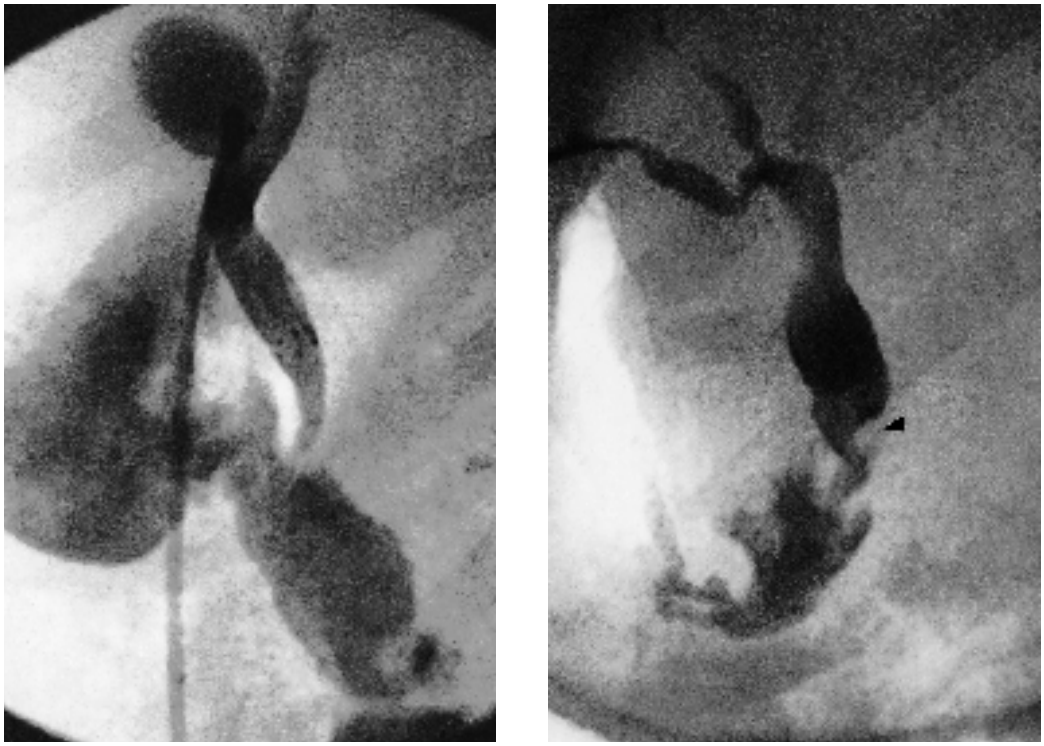


Figure 2a-b. Placement of SIS endograft in the common bile duct. (a) Cholangiography immediately after endograft placement. (b) Cholangiography at 8 weeks before sacrifice shows slightly dilated common bile duct. The stent turned sideways (arrow), and a defect corresponding to mucosal hyperplasia is seen in the bile duct wall where the stent end was located (arrow head).

11 mm long and 6 or 7 mm in diameter, depending on the size of the swine common bile duct. The stent cover was cut out of a 0.1 mm thick, dry SIS-sheet (Cook, Biotech Inc, Lafayette, IN). The sheet was cut to match the stent length and diameter and was rolled into a tube that was wrapped around the outside of the stent and attached to it at both ends with 7-0 polypropylene (Prolene, Ethicon Inc, Somerville, NJ).

Stent placement

Each animal was tranquilized with 1.5mL tiletamine hydrochloride, intubated and maintained with 2% isoflurane and 2L/min O_2. After a small central incision of the abdominal wall just below the sternum, the gallbladder was mobilized with forceps, and the tip of its fundus was pulled up to the central incision. There, it was punctured with an 18G needle, and a 0.035-inch guidewire (Roadrunner, Cook Inc, Bloomington, IN) was advanced through the cystic duct into the common bile duct. A 4-F catheter was then advanced over the guidewire into the common bile duct. After the Roadrunner guidewire was exchanged for a 0.035-inch Super-Stiff guide wire (Medi-Tech/Boston Scientific, Watertown, MA), a 5-F vascular sheath was inserted over the wire deep in the common bile duct. Cholangiography was performed using the sheath for injection. The 5-F sheath was also used for stent placement into the common bile duct between the ampulla and entrance of the cystic duct. The stent diameter was selected to be about 1-1.5 mm

larger than the diameter of the common bile duct. Altogether 9 single stents were implanted in 6 animals. Four animals received six stents of 6 mm in diameter, two receiving one stent and two receiving two stents. The other two animals received three stents with the diameter of 7 mm, one receiving one stent and the other two stents. When two stents were placed into the common bile duct, they were separated only by a few millimeters.

After the placement of stents, a cholangiogram was repeated and the sheath was removed (Figure2a). After the puncture site in the gallbladder was sutured, the gallbladder fundus was pulled up to the incision and secured to the inner abdominal wall. A metallic ring was then sutured together with the secured gallbladder fundus. This ring facilitated the identification of the fixation area for fluoroscopically guided percutaneous puncture for follow-up cholangiography.

Follow-up

Animals were followed up for a maximum period of 10 weeks with a plan to sacrifice two animals at each 4, 8 and 10 weeks after stenting. Cholangiography was obtained at the second weeks of follow-up and at the sacrifice or immediately after death. The two-week cholangiography was performed percutaneously using a 21-G needle. The terminal cholangiogram was performed by the same method as during the original stent placement. A 4-F catheter was inserted into the common bile duct through the gallbladder and cystic duct under general anesthesia. Cholangiography was then performed (Figure 2b). Euthanasia was carried out with a solution of pentobarbital and phenytoin sodium (Euthasol, Delmarva Lab, Inc., Midlothian, VA).

Histology

Segments of the bile duct proximal and distal to the stent, as well as at the center of the stent were placed in neutral-buffered zinc formalin. After a minimum of 24 h of fixation, the specimens were further sectioned into tissue cassettes, processed through alcohol and xylene, and embedded in paraffin. Five-micron paraffin sections were cut and stained with hematoxylin and eosin, or with Masson's trichrome stain.

Results

Stent deployment and clinical course

Stents were successfully placed in the common bile duct in all animals. The diameter of the stented common bile ducts ranged from 5.0 mm to 5.5 mm (mean, 5.3 mm) before stent placement. Stents with a diameter 1-1.5mm larger than the common bile duct remained in place during the initial study. During the follow-up, none of the animals developed jaundice. One animal developed ileus caused by a gauze pad left in the peritoneal cavity during initial laparotomy and died 16 days after stenting. Five animals were doing well, eating and gaining weight, and were sacrificed at the planned intervals of 4 weeks (n=1), 8 weeks (n=2), and 10 weeks (n=2) after stenting.

Cholangiography

The two-week percutaneous cholangiography was successfully performed in 4 out of 6 animals. In the other two animals, it was abandoned because of the failure to enter the gallbladder. One of these two animals, however, died 2 days later and cholangiography was performed immediately after death. Therefore, cholangiograms were obtained at approximately 2 weeks in five animals with 7 stents. They showed good patency and normal size of the stented common bile duct. Three stents remained in their original site of placement. Four stents slipped distally. There was no defect suggesting mucosal hyperplasia in any stent. Two stents in one animal could not be evaluated.

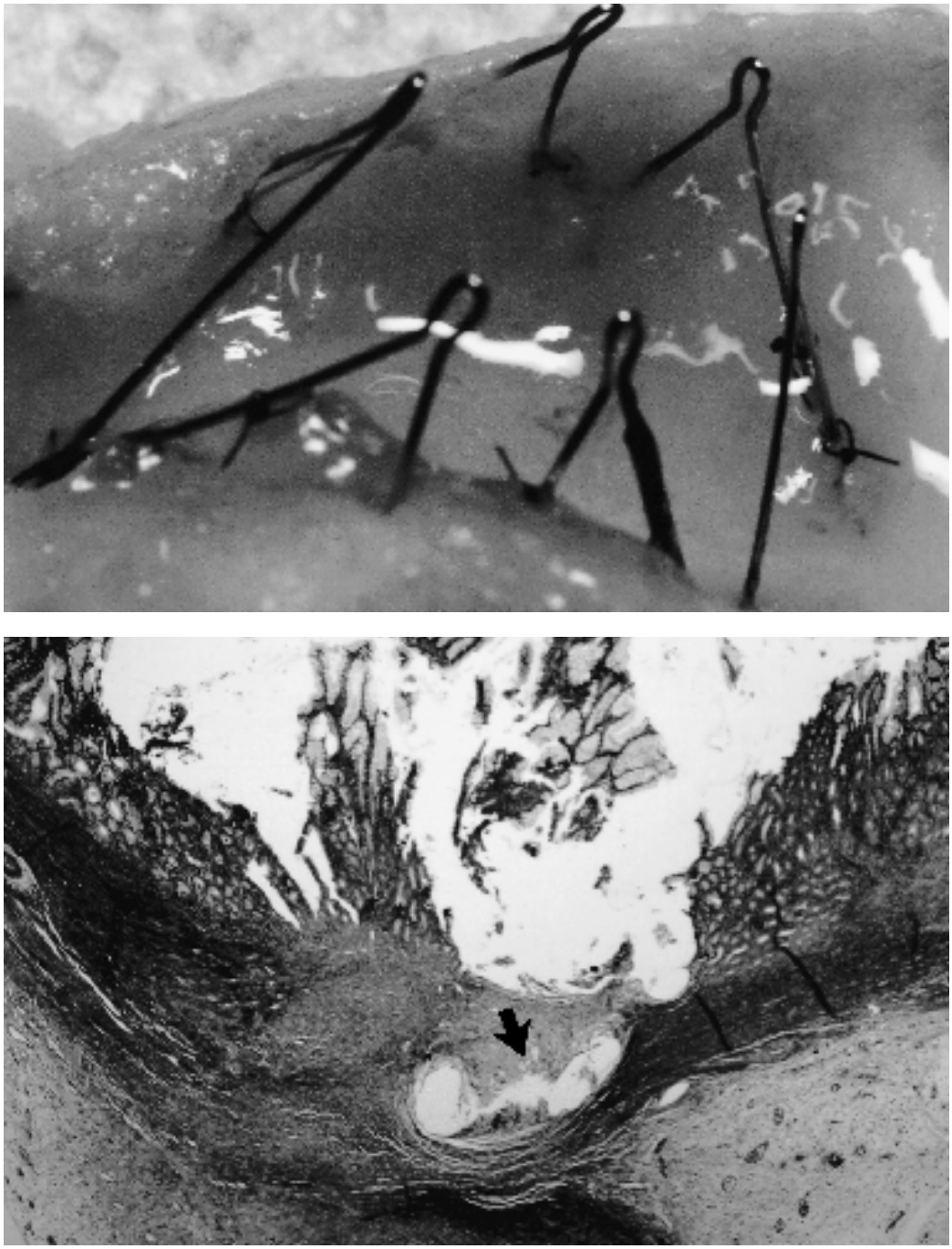


Figure 2c-d. (c) Explanted bile duct after 8 weeks after stenting. The stent ends of the sideways turned stent are embedded in the bile duct wall. (d) Photomicrograph of common bile duct wall in area of the stent. The defect in the wall was caused by two stent struts (arrow). Their mucosal hyperplasia and the adjacent wall show slight compression atrophy. [Masson Trichrome, 25x original magnification]

The four week follow up cholangiogram done in one animal showed well patent normal sized common bile duct with stent remaining in its original place. A smooth small defect suggesting mucosal hyperplasia was found at the distal end of the stent. The eight-week cholangiograms done in 2 animals showed slightly enlarged and well patent common bile ducts. All three stents were found dislodged from their original place, turned sideways and laying across the common bile duct. There were defects, which were considered to be mucosal hyperplasia at the bile duct wall where stents turned sideways (Figure 2b). The ten-week cholan-

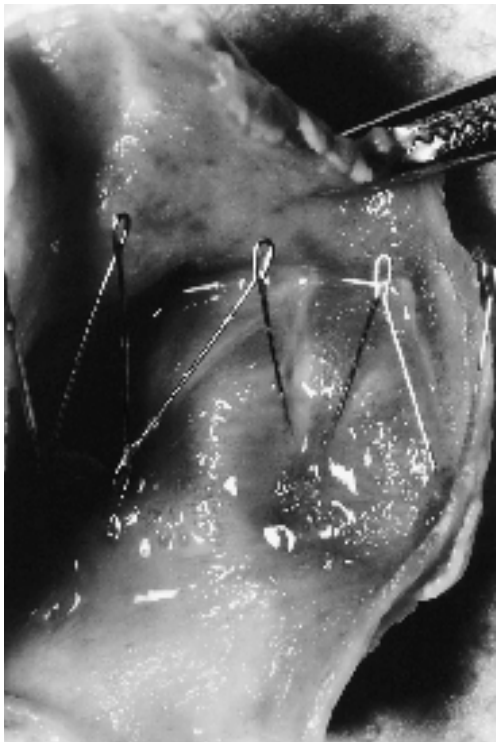
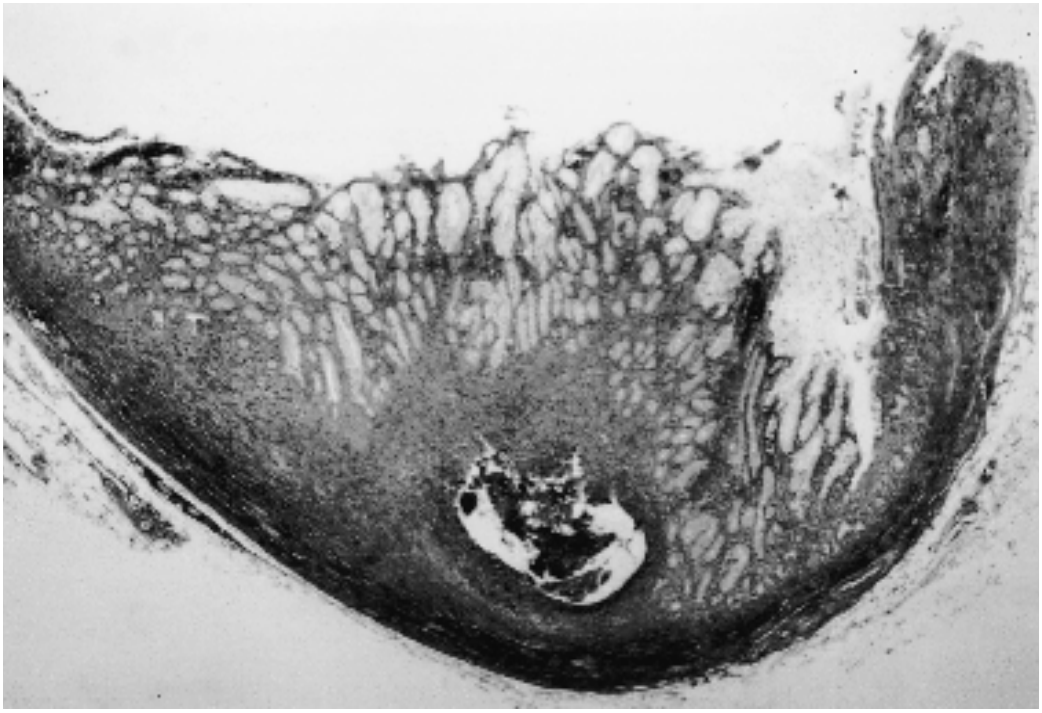


Figure 3a-b. Biliary endograft ex-vivo 4 weeks after placement in the common bile duct. (a) Dissected bile duct specimen shows partially embedded distal end of the Z-stent without SIS-cover in the bile duct wall. (b) The defect at the base of the mucosa corresponds to the location of a stent strut. There is mild inflammation and hyperplasia of the overlying biliary mucosa. [Hematoxylin & eosin, 25x original magnification]



giograms in two animals showed common bile duct dilation and some defects from sludge formation around the stents. Two stents in one animal were turned sideways. In the other animal, one stent stayed in its original position while the other was dislodged distally from the original site of placement. Mucosal hyperplasia was not evaluated cholangiographically because of associated bile sludge.

Histological study

Macroscopically, the SIS sheet covering the stent was identifiable at 2 weeks. Its color turned from white to blackish green, consistent with bile staining. After 4 weeks, the SIS-sheets were not grossly nor microscopically detectable (Figure 3a,b). The two stents, which did not dislodge at 2 and 10 weeks, had intact bile duct mucosa with no apparent hyperplasia (Figure 4a). In the third stent, which had not dislodged for 4 weeks, there was mild mucosal hyperplasia at the distal end (Figure 3a). Beneath this mucosa, there was a localized foreign body-type inflammatory reaction to the stent strut (Figure 3B). Mucosal proliferation was observed adjacent to the stents that had turned sideways (Figure 2c,d); in no case did the hyperplasia significantly narrow the duct lumen. In the stent dislodged distally, the bile duct wall was normal and no mucosal hyperplasia was seen. (Figure 4a,b).

Discussion

For the evaluation of the function and biological response of SIS covered metallic stents in the swine biliary system, only the data from four (45%) stents which remained in the longitudinal position in the common bile duct can be used. Three of them remained in the original position and one slipped distally from the original position. Their average implantation time in the common bile duct was 6.5 weeks with the range from 2 to 10 weeks. Only in these 4 stents, SIS cover was continuously in contact with the common bile duct mucosa and exposed to bile flow. SIS cover prevented focal denudation and reactive proliferation of the mucosa, inflammation in the submucosa, and narrowing of the bile duct lumen often seen after the placement of bare, non-covered stents.[15,29] SIS cover helped to decrease the foreign body-type inflammatory response to Z-stents and, of these four stents, only one showed mild inflammation and mild mucosal hyperplasia of overlying mucosa in a focal area of distal struts. There was no narrowing of common bile duct lumen; on the contrary, a slight, common bile duct dilatation developed at 10 weeks. At four weeks and later, the SIS cover was not microscopically detectable. Whether the membrane simply dissolved or was incorporated into the duct wall as a result of tissue remodeling remains unclear. It is interesting that even after 10 weeks of stenting when SIS membrane was no longer detectable the Z-stent wires did not cause a significant reaction. Whether hyperplasia and obstruction develop at a later time remains to be tested.

Five stents (55%) which dislodged from their original position and turned sideways across the common bile duct caused mucosal proliferation in the wall adjacent to the struts at their ends. This mucosal proliferation, however, was only mild and did not cause significant narrowing of the common bile duct lumen. The type, size, and smooth surface of SIS covered stents, absence of reactive changes in common bile duct wall together with rapid growth of animals must be considered as the main causes of frequent stent dislodgment found in our animals. Single Z-stents used in our animals have a tendency to jump off of the catheter during delivery and remain unstable after their placement, unless their diameter is significantly larger than the lumen of the stented structure. We selected only the stents that were about 15% larger

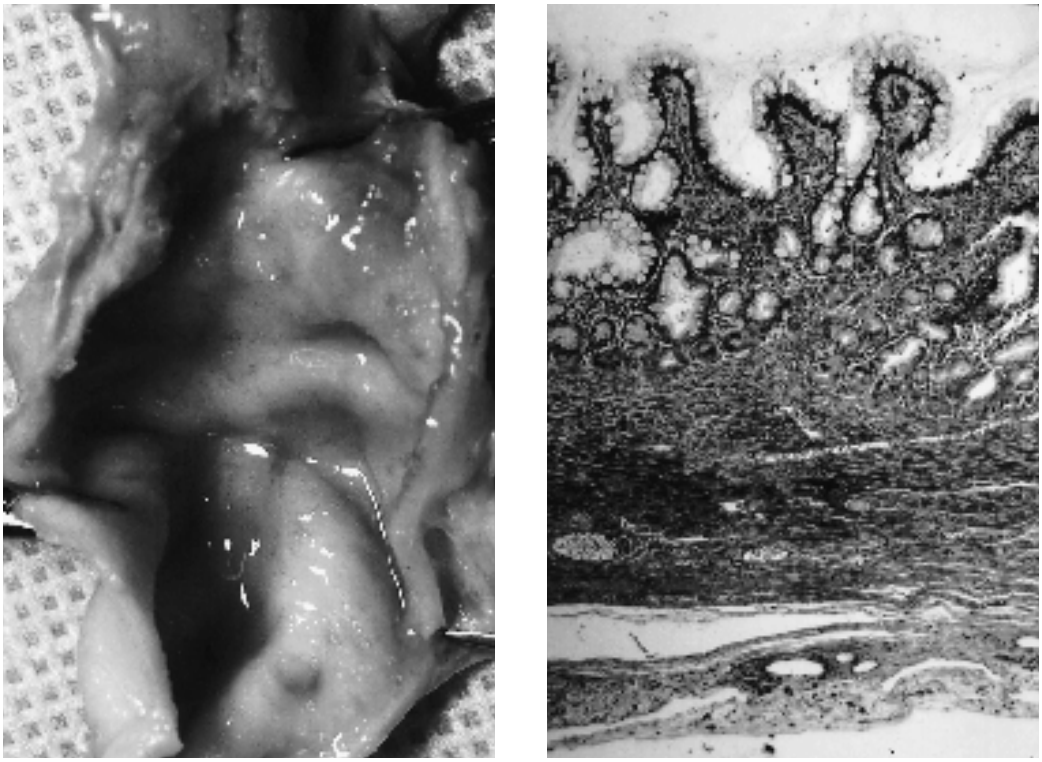


Figure 4a-b. Specimen and low power photomicrograph of common duct wall after 10 weeks of stenting. (a) Dissected bile duct after stent removal demonstrates intact mucosa. (b) The biliary mucosa is intact and shows only mild chronic inflammation. [Masson Trichrome, 200x original magnification]

than the common bile duct diameter. Yet, that was not sufficient in the fast growing animals. For future work, we plan to use larger double body Z-stents which are more stable at the delivery and after the placement. Tendency for migration of covered or coated stents with their minimized surface friction and minimal reactive changes in the duct mucosa, however, will be always a problem, particularly with their use in a nonstenotic duct.

Even when our study is limited in scope, it showed promise of SIS cover for biliary stenting. It showed that SIS is biocompatible and helps to decrease the foreign body-type inflammatory reaction to metallic stents. Further detailed study will be necessary to confirm our initial results and particularly evaluate long term effect of SIS covered stents. For our study we used a dry form of SIS which has several disadvantages, particularly difficulty in attachment and suturing to the stent base. It is also fragile and may break during catheter delivery. For future work we plan to use most recently available and improved wet form of SIS which can be easily and safely attached to the stent, does not leak and can be easily introduced through a catheter.

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