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ORIGINAL RESEARCH

Initial study of sediment antagonism and characteristics of silver nanoparticle-coated biliary stents in an experimental animal model

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Objective: Plastic biliary stents used to relieve obstructive jaundice are frequently blocked by sediment, resulting in loss of drainage. We prepared stents coated with silver nanoparticles (AgNPs) and compared their ability to resist sedimentation with Teflon stents in a beagle model of obstructive jaundice.

Methods: AgNP-coated Teflon biliary stents were prepared by chemical oxidation–reduction and evaluated in an obstructive jaundice model that was produced by ligation of common bile duct (CBD); animals were randomized to two equal groups for placement of AgNP-coated or Teflon control stents. Liver function and inflammatory index were found to be similar in the two groups, and the obstruction was relieved. Stents were removed 21 days after insertion and observed by scanning and transmission electron microscopy. The AgNP coating was analyzed by energy dispersive X-ray analysis (EDXA), and the composition of sediment was assayed by Fourier-transform infrared (FTIR) spectroscopy.

Results: Electron microscopy revealed a black, closely adherent AgNP stent coating, with thicknesses of 1.5–6 μm. Sediment thickness and density were greater on Teflon than on AgNP-coated stents. EDXA confirmed the stability and integrity of the AgNP coating before and after in vivo animal experimentation. FTIR spectroscopy identified stent sediment components including bilirubin, cholesterol, bile acid, protein, calcium, and other substances.

Conclusion: AgNP-coated biliary stents resisted sediment accumulation in this canine model of obstructive jaundice caused by ligation of the CBD.

Keywords: silver nanoparticles, biliary stent, stent sediment, characterization, animal model

Introduction

Nanomaterials are increasingly widely used in the biomedical field,[1–5] and silver nanoparticles (AgNPs) have good biocompatibility with biological tissue.[6] In the clinic, there are many patients with obstructive jaundice caused by benign or malignant disease. In addition to surgery, endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic retrograde biliary drainage (ERBD) are important treatment methods to treat obstructive jaundice. They can restore the enterohepatic circulation, block inflammatory reactions, and improve the patient prognosis. However, plastic biliary stents often become clogged 2–3 months after implantation, with the sediment primarily composed of pathogenic microorganisms and biliary sludge.[7–13] The reason may be related to the diameter of biliary stents, rough bracket and bile interface, change in biliary fluid dynamics, and bacterial growth.

Teflon is the most widely used material in clinically available plastic biliary stents. We prepared AgNP-coated biliary stents using a chemical oxidation–reduction

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method and compared the antisedimentation behavior of AgNP-coated and Teflon biliary stents in an experimental obstructive jaundice model in beagles. The aim was to evaluate the antagonism of AgNP coating to sedimentation.

Materials and methods

Preparation of AgNP-coated biliary stent using chemical oxidation–reduction method

Silver–ammonia complex ion reduction was used to prepare the AgNP coatings with sodium–naphthalene as the roughing solution. An 8.5 Fr Teflon biliary stent with a length of 12 cm (COOK, Bloomington, IN, USA) was used as the host material (Figure 1A), and was cut into 3 cm lengths for use in the experimental model. We began by removing surface grease by ultrasonic oscillation and then soaking the Teflon biliary stent in a sodium–naphthalene solution for roughening and $SnCl_2$ for sensitization. Finally, AgNPs were deposited on the Teflon stent surface by the oxidation–reduction method to prepare the coating (Figure 1B). Scanning electron microscopy (SEM, JSM-6700F, JEOL Ltd, Tokyo, Japan) and energy dispersive spectrometry (EDS, Oxford INCA X sight, Oxford Company, Oxfordshire, UK) were used to characterize the coating morphology and determine the optimal dose proportion; the optimal concentration of the silver–ammonia solution was found to be $AgNO_3$ 0.3 g + H_2O 15 mL + $NH_3{\cdot}H_2O$ 1 mL + H_2O 9 mL. The optimal sensitizing solution was found to be $SnCl_2$ 0.5 g + HCl 2 mL + H_2O 48 mL + Sn.

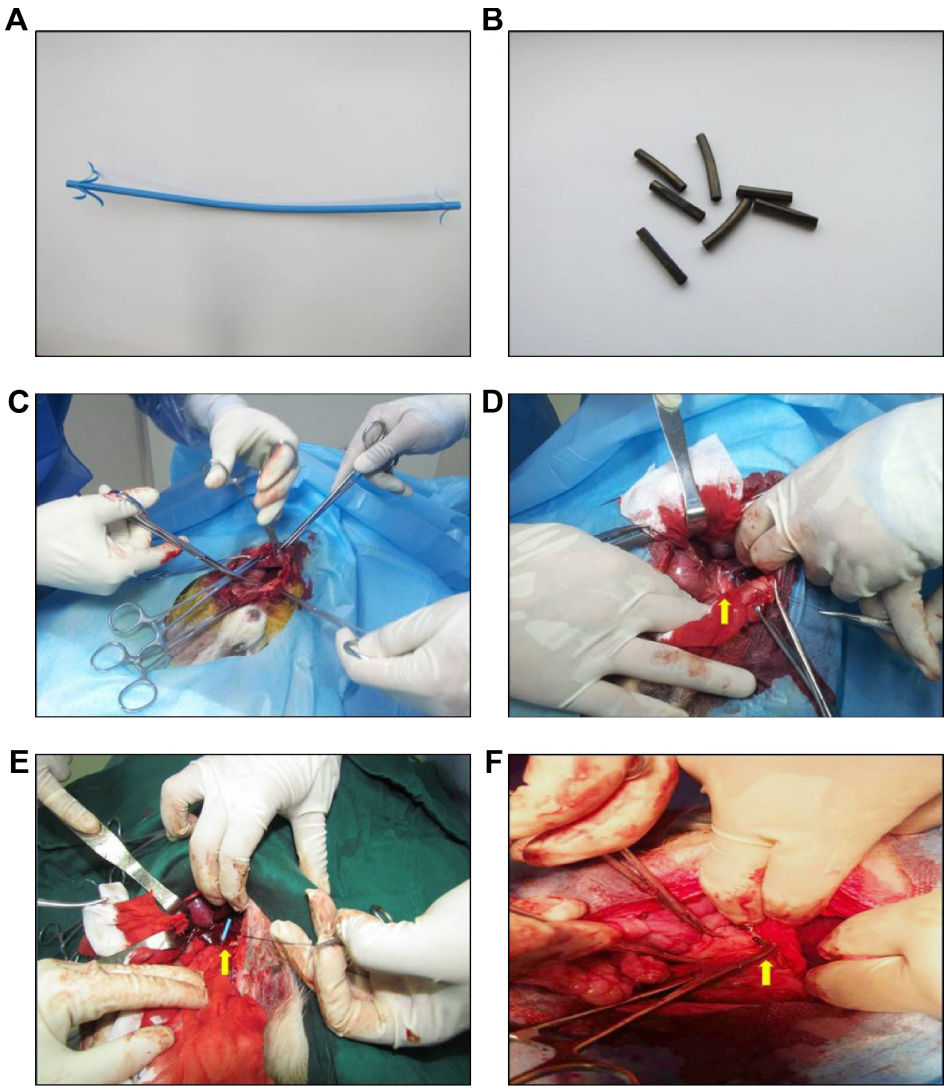

Figure 1 AgNP-coated biliary stents were produced and implanted in dogs in an experimental model of obstructive jaundice.
Notes: Teflon (12 cm, 8.5 Fr) and AgNP-coated (3 cm, 8.5 Fr) biliary stents were produced by a chemical oxidation–reduction method (**A**) and (**B**). Modeling procedure involving opening the abdominal cavity and CBD ligation (**C**). Yellow arrow indicates CBD (**D**). Biliary stent placement: Teflon (blue) and AgNP-coated (black) biliary stents. Yellow arrow indicates CBD (**E**) and (**F**).
Abbreviations: AgNP, silver nanoparticle; CBD, common bile duct.

Canine obstructive jaundice model

Twelve healthy male and female beagle dogs weighing 10–13 kg were selected for use in the experimental obstructive jaundice model. The dogs were fasted for 6 hours before surgery and anesthetized by intravenous administration of 3% pentobarbital sodium via the cephalic vein in a front leg. An 8–10 cm midline abdominal incision (Figure 1C) was made to expose the serosa of the hepatoduodenal ligament above the superior border of the duodenum. The common bile duct (CBD) was then separated and ligated with a 2–0 suture (Figure 1D). This study was approved by the medical experiments ethics committee of Shandong Provincial Qianfoshan Hospital affiliated to Shandong University. The institutional guidelines for this study regarding the animals welfare are as follows: Laboratory Animal Welfare and Animal Experimental Science, Science Press, Version 1 (June 1, 2011), ISBN: 9787030311757.

Controlled study of sediment antagonism by AgNP-coated biliary stents

Animals were randomly assigned to experimental AgNP-coated (n=6) or control Teflon biliary stents (n=6) groups 3 days after modeling. Experimental and control animals did not differ in liver function and inflammatory index. All the statistical tests were conducted using *t*-test, $\alpha=0.05$ was the inspection level, and $P<0.05$ was considered as statistically significant. All computations were performed with SPSS Complex Samples™ 17.0 software (SPSS Inc., Chicago, IL, USA).

During the second surgical procedure, we found cholestasis in the beagle livers, and expansion of the CBD above the ligature. The anterior wall of the CBD was cut open and a 3 cm AgNP-coated or Teflon biliary stent was positioned (Figure 1E and F), ensuring that it passed through the ligature and removed the bile duct obstruction. After 21 days, the abdominal cavity of surviving animals was opened again to remove the stents. The entire CBD including the biliary stent, artificial narrowing in the area of ligature, ampulla, and the duodenum are shown in Figure 2A–C. A bile duct stone observed after extraction of a Teflon biliary stent in a control animal is shown in Figure 2D.

Biliary stents were cut into pieces of suitable size for SEM, cleaned, and critical-point dried. The morphology of the AgNP-coating and biliary stent sediment was observed

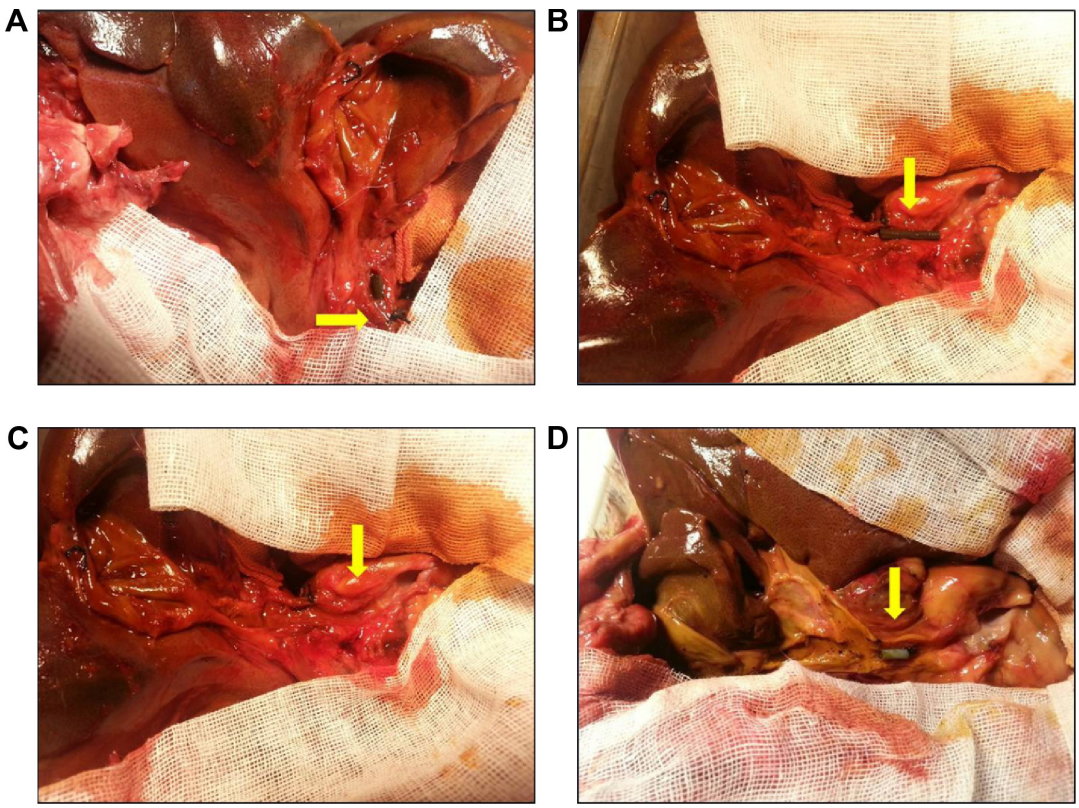

Figure 2 Open abdominal cavity of an experimental animal.
Notes: Yellow arrows point to the AgNP-coated biliary stent and CBD ligation suture (**A**); AgNP-coated biliary stent, with the left end in the CBD, the right end in the duodenum, and the artificial narrowing formed by the ligature in the middle (**B**); the ampulla structure (**C**); the location of CBD stones in an animal with a Teflon biliary stent (**D**).
Abbreviations: AgNP, silver nanoparticle; CBD, common bile duct.

by SEM (3.0–5.0 kV, working distance =8 mm) and EDS before and after animal experiments.

After stents were extracted from the experimental animals, sediment was removed from biliary stents, ground, and cleaned ultrasonically in absolute alcohol and in deionized water. Following centrifugation at 3,500 rpm, sediment was resuspended in absolute alcohol and samples were collected on 200 mesh copper grids and air dried for transmission electron microscopy (TEM; 100 kV; JEM-101, JEOL Ltd).

Small amounts of sediment were prepared for Fourier-transform infrared (FTIR) spectroscopy by mixing with potassium bromide (1:50), grinding in an agate mortar, and pelleting into slices. Sediment composition was compared with that of previously analyzed biliary stones.

Results

Experimental characteristics of animals after modeling

After establishing the experimental obstructive jaundice model, there were no significant differences in alanine aminotransferase, aspartate aminotransferase, total bilirubin (TBIL), or direct bilirubin (DBIL) in animals with AgNP-coated and Teflon biliary stents ($P>0.05$, Table 1). There were also no differences in the concentrations of tumor necrosis factor α and interleukin-6 ($P>0.05$, Table 2). General data of beagle canine model are listed in Table S1.

Weight of biliary stent sediment

The weight of biliary sediment collected from Teflon stents was significantly greater than that of the sediment collected from AgNP-coated stents ($P<0.05$, Table 3).

SEM morphology of AgNP stent coating

Different concentrations of silver–ammonia solution produced AgNP coatings of differing thickness, which increased with the concentration of the silver–ammonia solution. We found the optimum reaction temperature and silver–ammonia solution concentration through repeated trials. The boundary between the AgNP coating and Teflon biliary stent was clearly observed by SEM, permitting easy measurement of the AgNP coating thickness (Figure 3A–C). The thickness of AgNP coating ranged from 1.5 to 6.0 μm.

Table 1 Liver function in experimental animals after modeling (n=6, $\bar{x} \pm s$)

Group	ALT (U/L)	AST (U/L)	TBIL (μmol/L)	DBIL (μmol/L)
Teflon	176.83±19.69*	270.00±25.78*	77.76±2.26*	69.47±6.80*
AgNPs	172.00±22.78	293.33±27.35	78.17±6.70	73.45±6.58
P-value	0.70	0.16	0.92	0.33

Note: *$P>0.05$, there were no statistical differences between both groups after modeling.
Abbreviations: AgNPs, silver nanoparticles; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBIL, direct bilirubin; TBIL, total bilirubin.

Table 2 Concentration of TNF-α and IL-6 of experimental animal after modeling (n=6, $\bar{x} \pm s$)

Group	TNF-α (pg/mL)	IL-6 (pg/mL)
Teflon	64.05±2.49*	322.60±22.92*
AgNPs	66.72±2.84	325.93±19.38
P-value	0.12	0.79

Note: *$P>0.05$, there were no statistical differences between both groups after modeling.
Abbreviations: AgNPs, silver nanoparticles; IL-6, interleukin-6; TNF-α, tumor necrosis factor α.

SEM characterization of biliary stent sediment

SEM revealed that the sediment layer that accumulated on Teflon biliary stents was thicker than that on AgNP-coated stents (Figure 4A and B). The sediment on Teflon stents had a more regular structure than the sediment on AgNP-coated stents. At high magnification (Figure 4C and D), the surface of Teflon stent sediment was relatively flat and appeared more compact than the sediment on AgNP-coated stent, which had a loose, irregular structure with many bumps and angles.

Composition of AgNP coating before and after animal experiments

Energy dispersive X-ray analysis confirmed the stability of Ag in the AgNP coating, with little change after animal experiment (Figure 5).

TEM morphology of biliary stent sediment

TEM revealed that, at the same magnification, sediment from Teflon stent had a denser appearance than sediment from AgNP-coated stents (Figure 6A and B) and had a smooth, flat surface. Sediment from AgNP-coated stents had an irregular surface and appeared to be less compact (Figure 6C and D).

Table 3 Biliary stent sediment weight (n=6, $\bar{x} \pm s$)

Group	Sediment weight (g)
Teflon	0.325±0.026*
AgNPs	0.261±0.045
P-value	0.017

Note: *$P<0.05$, there were statistical differences between both groups after modeling.
Abbreviation: AgNPs, silver nanoparticles.

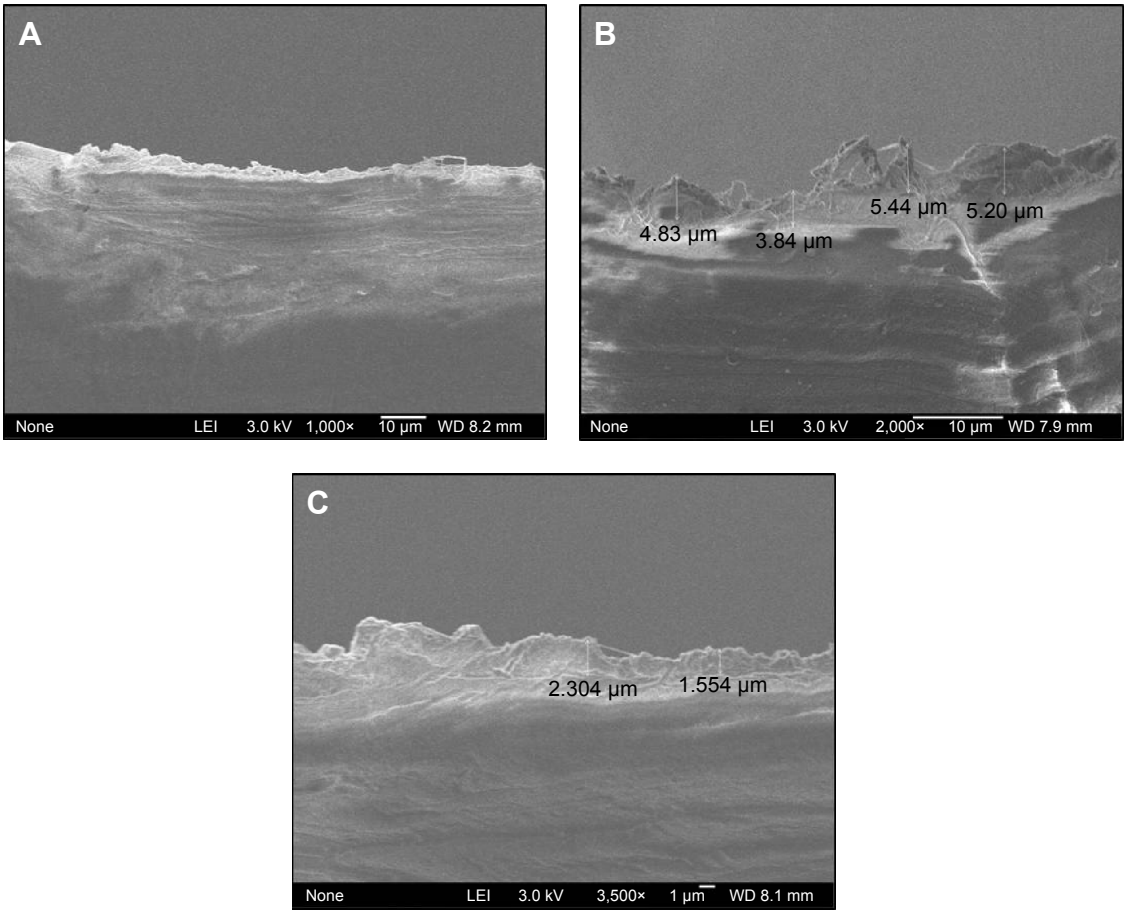

Figure 3 Morphology of AgNP-coated stents observed by SEM.
Notes: (**A**) AgNP-coated and Teflon biliary stents clearly show host material boundaries (1,000×). (**B**) AgNP coating with thickness of 3.84–5.44 µm (2,000×). (**C**) AgNP coating with thickness of 1.554–2.304 µm (3,500×).
Abbreviations: AgNP, silver nanoparticle; SEM, scanning electron microscopy.

FTIR spectroscopy of stent sediment

FTIR absorption spectra (Figure 7) indicated that the biliary stent sediment included bilirubin (absorption peaks at 3,411, 2,915, 1,592, 1,466, 1,250, 699 cm^{-1}), cholesterol (2,868, 1,377, 1,057 cm^{-1}), bile acids (1,645, 613 cm^{-1}), proteins (3,400, 1,235 cm^{-1}), and bilirubin calcium salts (1,627 and 1,573 cm^{-1}). The sediment compositions were confirmed by comparing the absorption peaks and vibration wave number ranges obtained with human mixed-type biliary stones, which were as follows: bilirubin (3,411, 2,915, 1691–699 cm^{-1}), cholesterol (3,431, 2,933, 2,867–1,466, 1,377–1,056 cm^{-1}), bile acids (3,416, 1,645, 1,563–613 cm^{-1}), proteins (3,400, 2,955, 2,915–1,235 cm^{-1}), and bilirubin calcium salts (1,696, 1,663, 1,627, 1,573 cm^{-1}).

Discussion

The main cause of biliary restenosis is the formation of bacterial biofilms[14,15] that combine with other substances in bile form "biliary mud", which accumulates and eventually blocks the stent. There is a need for new types of biliary stents with a combined effect of bacteriostasis and resistance to deposition of bile sediment. This preliminary study describes the morphological characteristics and sediment resistance of a novel AgNP-coated stent.

We prepared AgNP-coated biliary stents by a chemical oxidation–reduction method, and used Teflon as a matrix material. Dilute nitric acid was used as the coarsening agent, naphthalene was used as dispersant, $SnCl_2{\cdot}2H_2O$ was the reductant, silver nitrate was the raw material, and ammonia ($NH_3{\cdot}H_2O$) was the buffer solution. $NH_3{\cdot}H_2O$ is a weakly alkaline buffer, ensuring uniform attachment of Ag^+ to the inside and outside walls of the stent. In both humans and animals, bile is a weakly alkaline liquid; we thus prepared the AgNP-coated biliary stents in a weakly alkaline system to mimic the physiological environment in the hope of achieving stable performance.

Many types of biliary stents are used clinically for endoscopic treatment of benign and malignant obstructive

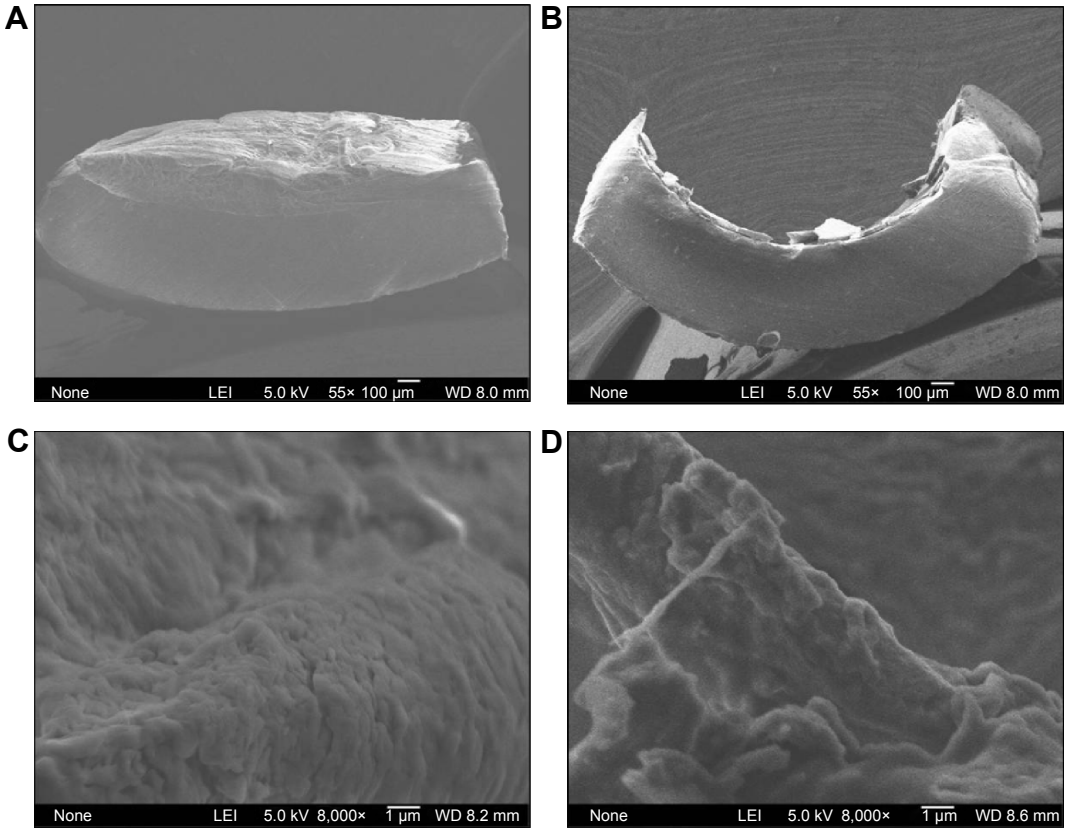

Figure 4 SEM of sediment from both types of stent.
Notes: (**A**) Teflon biliary stent sediment (55×). (**B**) AgNP-coated biliary stent sediment (55×). (**C**) Teflon biliary stent sediment with compact structure (8,000×). (**D**) AgNP-coated biliary stent sediment with loose structure (8,000×).
Abbreviations: AgNP, silver nanoparticle; SEM, scanning electron microscopy.

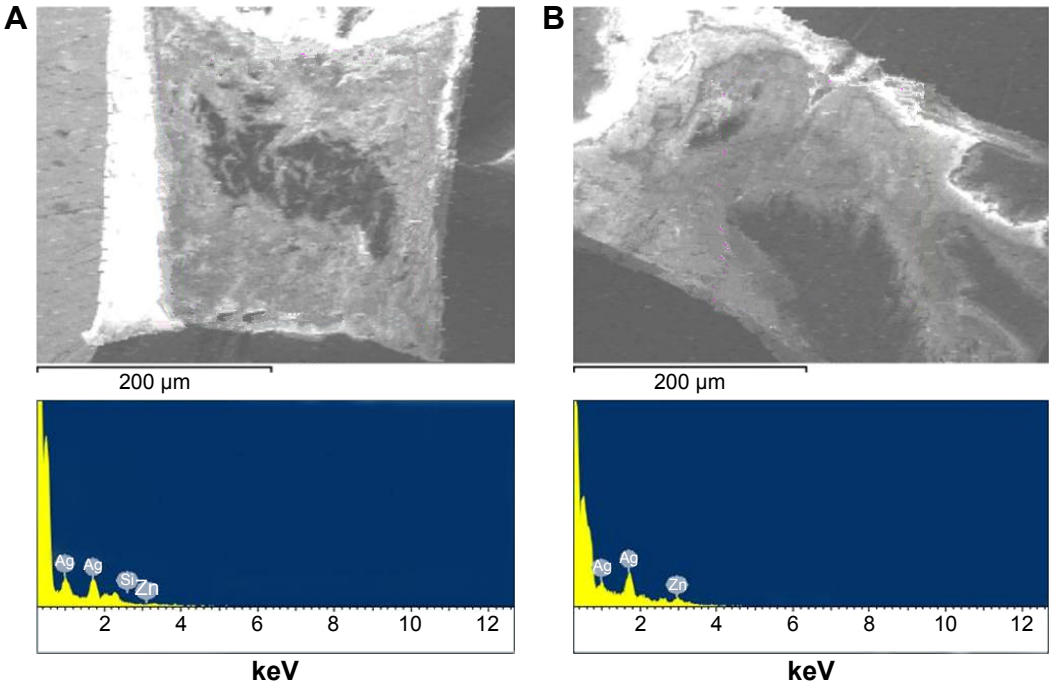

Figure 5 EDXA results before and after the contrast experiments show that the main metal component was Ag mixed with Si and Zn impurities.
Notes: The horizontal axis shows keV; the vertical axis shows the metal content. (**A**) AgNP-coated biliary stent before animal experiments with Ag as the main coating component. (**B**) AgNP-coated biliary stent after animal experiments with Ag as the main coating component.
Abbreviations: AgNP, silver nanoparticle; EDXA, energy dispersive X-ray analysis.

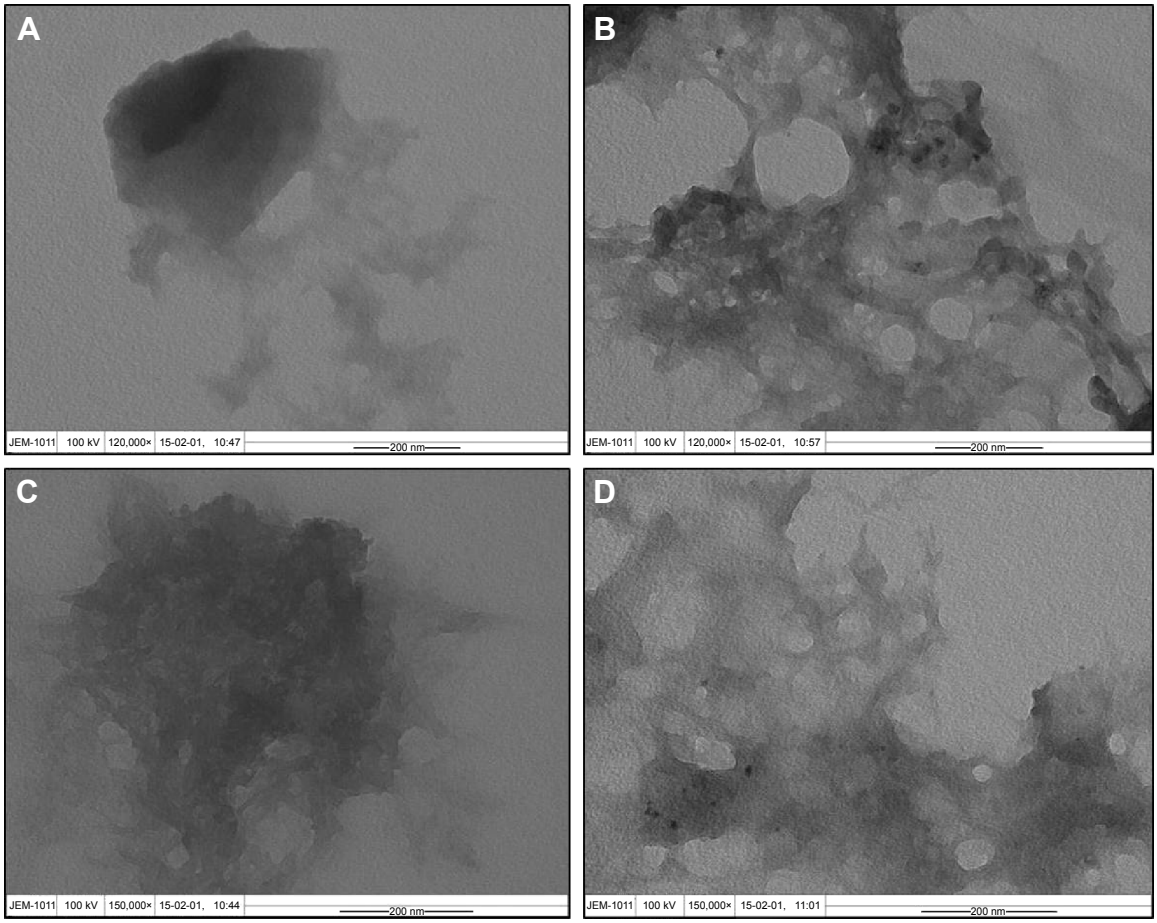

Figure 6 TEM of stent sediments in both types of stent.
Notes: (**A**) Teflon biliary stent sediment (120,000×). (**B**) AgNP-coated biliary stent sediment (120,000×). (**C**) Teflon biliary stent sediment (150,000×). (**D**) AgNP-coated biliary stent sediment (150,000×).
Abbreviations: AgNP, silver nanoparticle; TEM, transmission electron microscopy.

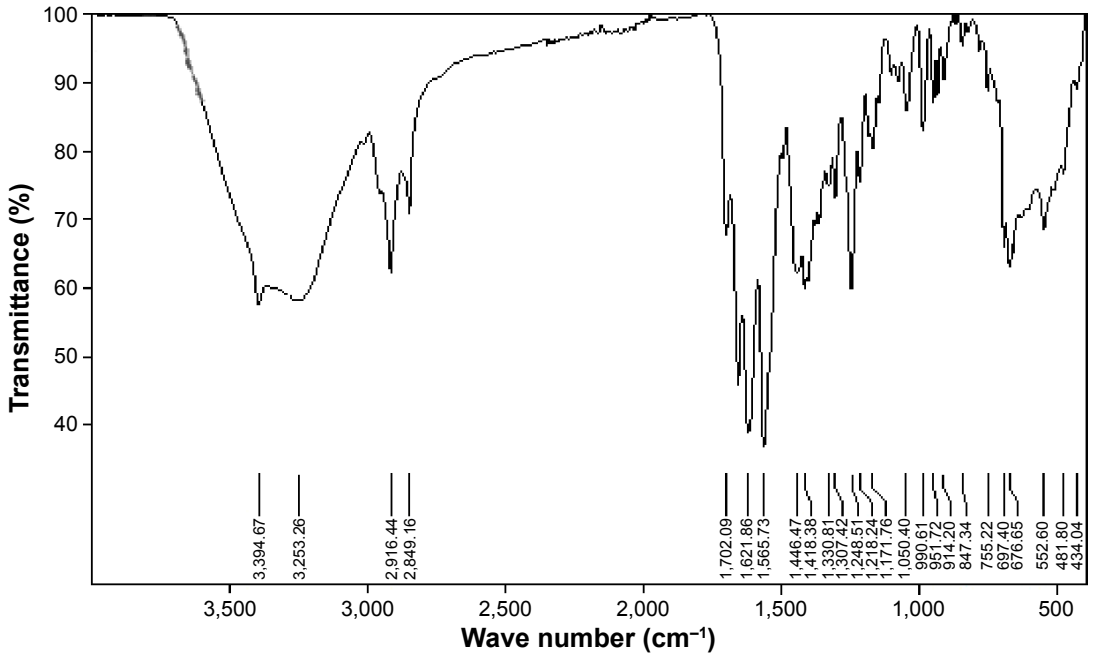

Figure 7 Infrared spectrum analysis of biliary stent sediment.

jaundice.[16–20] The applications include ERCP, endoscopic sphincterotomy (EST),[21] endoscopic nasobiliary drainage,[22,23] and ERBD.[24] Endoscopic procedures have the obvious advantages of safety, minimal invasiveness, convenience, few postprocedural complications, and good tolerance. They are especially advantageous for aged or weak patients who cannot tolerate surgery.

Stent obstruction, retrograde infection, duct wall inflammation, bleeding, perforation, and stent displacement are common complications of ERCP. Benign biliary stricture can be caused by bile duct inflammation, a history of bile duct surgery, sclerosing cholangitis, pyogenic cholangitis, and chronic pancreatitis. Stent placement is also an ideal treatment for bile duct anastomotic stenosis following liver transplantation.[25] With the increasing use of liver and gallbladder surgery, iatrogenic bile duct injury is increasing in clinical importance, and endoscopic treatment might become the first treatment of choice. Malignant biliary obstruction may be caused by compression of malignant tumors or lymphatic metastasis of digestive tract tumors. Biliary stent placement can significantly relieve malignant obstructive jaundice and can also relieve jaundice to improve perisurgical safety.[26]

Coated and noncoated metal, biodegradable, and drug-eluting biliary stents, as well as stents coated with radioactive particles are all in clinical use. The length, diameter, number, material, type, placement method, and disease should all be considered when choosing a suitable biliary stent. The stent type should be selected according to the disease, and in case of benign diseases, noncoated metal biliary stents should be avoided in favor of plastic or removable coated metal stents. The appropriate stent length and diameter depend on the extent of drainage and degree of stenosis, especially in cases of high bile duct obstruction, such as hilar cholangiocarcinoma.

The clinical evidence shows that plastic biliary stents can lead to edema of the CBD. Sung et al[27] found that implantation of stents with one end positioned in the duodenal cavity could result in dysfunction of the sphincter of the ampulla. Reflux of pathogenic bacteria from the duodenal cavity into the bile duct along with intestinal juice increased the chance of retrograde biliary infection and stent blockage. Slivka and Carr-Locke[28] found that 20%–30% of plastic stents became obstructed within 3 months of placement, and 70% became obstructed in 6 months.

Reducing the formation of stent sediment, bacterial adhesion, and biofilm formation are key targets in the antagonism of biliary stent clogging mechanisms. If clogging can be reduced, significant improvements can be had in 1) bile flow rate;[29] 2) inhibition of bacterial adhesion and biofilm formation;[30] 3) change in bile composition;[31] and 4) materials, composition, and properties of biliary stents.[32] The AgNP-coated stents were antagonistic to sedimentation, as shown by the reduced thickness of the sediment adhering to the stent.

We established a satisfactory experimental obstructive jaundice animal model. Experimental variables were constant, and the in vivo environment was stabile, all of which contributed to the reliability of the data. After modeling, the enterohepatic circulation of experimental animals was blocked, which quickly resulted in jaundice. Index assays indicated an increase in TBIL- and DBIL-typical obstructive jaundice. We used Teflon and AgNP-coated biliary stents to relieve biliary obstruction to model the human biliary obstruction environment before and after stent placement. SEM and TEM showed that the AgNP coating antagonized stent sediments. The results obtained with this experimental model can provide data for the further development of AgNP-coated stent in basic research and for future clinical applications.

Both SEM and TEM confirmed that the sediment layer that adhered to AgNP-coated stents was thinner than that on the Teflon stents. The AgNP coating thus increased antagonism to sediments. Stones also developed in the bile duct of animals with Teflon stents but not in those with the AgNP-coated stents. Extensive investigation of the time course of this reaction is needed to determine the reason for secondary stone formation in such a short interval. We have already observed this kind of stone formation in the clinic. Some patients with choledocholithiasis and confirmed absence of residual biliary tract stones by t-tube cholangiography after ERCP + EST or laparoscopic cholecystectomy plus CBD exploration developed recurrent CBD stones within 3 months. Whether the mechanism of stone formation is the same in all patients needs to be confirmed in follow-up studies.

van Berkel et al[33] used confocal laser scanning microscopy and SEM to evaluate the performance of two polythene and hydrophilic polyurethane polymer-coated stents. After 3 months, they found homogeneous tissue on the surface of the stents that consisted of biofilms consisting of living and dead bacteria. The stent lumen was full of free floating bacterial colonies and crystalline material surrounded by foliated mucus structures. This open-mesh fibrous structure included food due to duodenal reflux and was considered to be responsible for clogging.

The composition of stent sediment was analyzed by FTIR spectroscopy, which showed that it contained bilirubin,

bile acid, calcium bile salts, and other substances similar to hybrid calculi. Costa et al[34] analyzed the chemical composition of biliary stent sediments and found that they comprised calcium, bilirubin, and other components, such as protein and unconjugated bilirubin, which was confirmed in our study.

Successful modeling of clogging that follows placement of biliary stents in appropriate animal models is highly significant. Such experimental systems permit systematic research on the influence of different components on sedimentation and search for a more suitable bile duct stent composite materials. Many solutions have been proposed to solve the stent clogging phenomenon, such as increasing the stent diameter, removing flanks, changing the placement method, and changing the composition of the stent surface. Each has a clinical effect, but none have proven ideal.[35,36]

Conclusion

In this experimental animal model of obstructive jaundice, we found that coating stents with AgNPs was antagonistic to sediment. This novel type of composite bile duct stent effectively reversed the obstruction symptoms and may prolong stent life and delay recurrence of obstruction.

In clinical use, clogging of plastic biliary stents is a common occurrence. Prolonging the time for clogging to occur has significant clinical benefit for patients. Our preliminary results with AgNP-coated stents in this experimental model were positive. With ongoing research, we believe that we will succeed in developing biliary stents with antagonistic properties to sediment deposition.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 General data of beagle canine model

Earmark	Sex	Birth date	Father	Mother	Weight (kg)
A071	F	2014-1-20	A831	A0619	11
A072	F	2014-1-20	A831	A0619	10.5
A073	M	2014-1-20	A831	A0619	10
A074	M	2014-1-20	A831	A0619	10
A075	M	2014-1-20	A831	A0619	10.5
A076	M	2014-1-22	A677	A4121	11
A077	M	2014-1-22	A677	A4121	11
A078	F	2014-1-22	A677	A4121	12
A079	F	2014-1-22	A677	A4121	12.5
A080	F	2014-1-22	A677	A4121	11.5
A081	F	2014-1-25	A725	A4053	10.5
A082	M	2014-1-25	A725	A4053	12

Abbreviations: M, male; F, female.

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