The Study of Bile Duct Stent Having Antifouling Properties Using Biomimetics Technique

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Biomimetics is a field of technologies based on imitating the functions and properties found in living organisms. The application of the super-water-repellent fine structure of lotus leaves to create waterproof products is a well-known example of biomimetics. The present study examined the surface structure of snail shells, which exhibit oleophobic properties oil repellency and explored the feasibility of recreating this structure on the inner surfaces of conventional biliary stents. Observations of snail shells under an electron microscope show a covering of extremely fine protrusions of around 200 nm in size. When water enters the pores between these fine protrusions, a film of water exhibiting super-nanohydrophilic structure forms on the shell. Because water and oil are immiscible, this film repels oil. We would expect stent occlusion to be less likely with a biliary stent having this structure on its inner surface. Biliary stricture caused by bile duct cancer or bile duct obstruction can lead to icterus and may, in serious cases, induce fatal hepatic failure. A surgical procedure that places indwelling biliary stents inside the biliary tract is sometimes performed to secure a passage for bile flow. However, conventional stents are prone to occlusion due to the accumulation of biliary sludge, resulting in the need for a second surgery to replace the stent. This problem is attributable to the polyethylene used to make the biliary stents; polyethylene is susceptible to the adhesion of cholesterol and fats found in the bile, eventually leading to stent occlusions. This paper reports our efforts to develop biliary stents that feature antifouling properties inspired by biomimetics to address this problem; specifically, the development of oleophobic inner stent surfaces featuring super-nanohydrophilic structures inspired by snail shell surfaces.

Keywords: Biomimetics, Snail shells, Super-nanohydrophilic structure, Bile duct cancer, Biliary stent obstruction, Biliary stent

1. Introduction

The positional relationship of the liver, gallbladder and bile ducts is shown in Fig. 1. Biliary stricture caused by bile duct cancer [1] or bile duct obstruction obstructs the flow of bile from the gallbladder to the duodenum. When this occurs, the bile flows back into the liver, resulting in icterus. Left untreated, this condition can lead to hepatic failure, a potentially life-threatening condition. The condition is surgically treated by implanting an endoscopic biliary stent (EBS) (see Fig. 1) [2,3].

Two types of biliary stents are currently available: metallic stents and plastic stents [4]. Plastic stents are used to treat most EBS cases.

Figure 2 is a photograph of a straight plastic stent (PS). Each end has flaps; each section of the tube beneath the flap has an opening.

As is well-known, because bile is a viscous fluid containing fatty components such as cholesterol, bile sludge tends to collect within the stent, resulting in stent occlusion. To date, investigations seeking
to prevent stent occlusion have failed to produce an adequate solution. In most cases, the problem is addressed by performing a second procedure to remove and replace the stent [2].

Undergoing multiple surgeries places significant stress on a patient. Developing a method to suppress the occlusion of biliary stents would reduce the number of surgeries per patient, translating into reduced burdens not just for the patient, but for clinical practitioners, including doctors. Therefore, the method and technical development to suppress the occlusion of biliary stents are desired.

2. Biomimetics technologies

As the observation There are no dirty snails suggests, snail shells have long been known to exhibit superior antifouling performance. The snails encountered during the baiu rainy season in Japan always have clean, shining shells.

Fig. 1. Structure of the biliary tract and example of endoscopic biliary stenting.

Fig. 2. Plastic stent (straight type, Boston Scientific Corporation).

Fig. 3. Snail with clean shell surface encountered during baiu rainy season.
Snail shells are known to have convex-concave nanoscale structures on their surface measuring approximately 200–400 nm (Fig. 3) that promote the formation of a film of water on the shell surface, creating a super-nanohydrophilic structure that repels oils and stains [5]. This super-nanohydrophilic structure has properties to repel oils containing proteins, etc. Figure 4 shows a schematic representation of this principle.

It follows that by creating a structure that imitates the shell structure of snails, it should be possible to produce a super-nanohydrophilic structure that exhibits this oil-repelling effect. The field of technologies based on imitating various properties and structures observed in living organisms in nature is called biomimetics [6].

The structure of the snail shell was examined in detail. Figure 5 is an SEM image of the snail shell. The snail shell has structures resembling craggy mountain ranges on its surface scale of 20–40 μm. An enlarged view of the surface shows that these structures are formed by grains approximately 200 nm in size. Enlarging the surface still further shows nanoscale holes between the grains measuring approximately 200 nm.

Figure 6 shows the comparison of water repellency and oil repellency in water of snail shells to those of Si substrate.

The principle instrument used in the present study...
is a contact angle meter (model PAC-11 manufactured by Kyowa Interface Science Co., Ltd.) modified to allow the measurement of oil drops that come into contact with surfaces in water. A sample is placed in water, after which an oil droplet is pressed out from the tip of a syringe needle and placed in contact with the sample to observe what happens: whether the oil droplet is repelled or adheres to the surface [7,8].

When an oil droplet was pressed against Si substrate, the droplet was found to adhere to the substrate (oil stain). In contrast, the snail shell exhibited oil repellency in water.

3. Biliary stents with antifouling properties

An occluded biliary stent must be surgically replaced, typically endoscopically rather than by open surgery. If conditions contraindicate endoscopic surgery, the alternatives are percutaneous transhepatic biliary drainage (PTBD) [9], in which the biliary tract is approached percutaneously (through the skin), or open surgery. However, repeated stent replacement procedures can be extremely stressful for the patient. A potential solution is to recreate the oleophobic fine structure of snail shells on the inner walls of biliary stents.

Lotus leaves are a well-known example of super-water-repellency [6]. Inspired by the structures observed on these leaves, Nissan Motor Company is currently performing studies to produce super-water-repellent glass for automobiles. Such glass would remain free of water and ensure continuously clear views. Conventionally, fluorine-based hydrophobic coatings are applied to automobile glass for the same purpose, but these coatings inevitably peel off with time, and the glass loses its water repellency. Water repellent structures that would achieve permanent hydrophobicity have been explored. However, such a technology would not repel oil. Bile, a fluid secreted by the liver, assists in the digestion and absorption of lipids by activating the digestive enzyme (lipase) to facilitate the dissolution of oil in water. The main constituents of bile are bilirubin, an end product of red-blood cell breakdown, cholesterol, and bile acid [10]. Bile is temporarily stored in the gallbladder before being excreted from the duodenum. In cases like the one above in which oleophobic properties would be beneficial, the structure of the snail shell, which exhibits super-nanohydrophilic effects in the presence of water, may help fabricate an occlusion-resistant biliary stent (Fig. 7.).

4. Production of mold with nanohole structures based on snail shell surface structures

To fabricate nanoscale structures inspired by snail shells, we formed 200 nm nanoholes on Si substrate by electron beam lithography. We spin-coated an electron beam resist (the ZEP-520 positive tone resist manufactured by Zeon Corporation) onto Si substrate and baked it to form a resist coating measuring 300 nm thick. We performed electron beam lithography using a mask design with an electron beam lithography unit.

We developed and etched the mold to create 200

![Conventional stent](image1.png) ![Stent developed in this study](image2.png)

**Fig. 7.** Comparison of conventional stent and antifouling stent (schematic image).
nm nanohole patterns on Si substrate. Figure 8 shows the design of the lithographic pattern created for electron beam exposure and an image obtained by SEM of the resulting nanohole patterned mold.

We produced a mold patterned with nanoholes measuring 200 nm in diameter and 200 nm in depth.

5. Producing antifouling sheets for stents

Using the obtained nano-scale structure as a mold and nanoimprint technology, we transferred the structure onto UV-curable acrylic polymers [11,12].

The nanoimprinting method is described in Fig. 9. UV-curable acrylic polymer [13] is dropped onto the substrate (PET film), after which the mold is pressed down onto the polymer. UV light is irradiated from below through the substrate. The nanoimprinting device used here is the LTNIP-5000 manufactured by Litho Tech Japan Corporation [14]. The stamping pressure was 1,000 N, the illuminance of UV light (365–436 nm) 7 mW/cm², and exposure time 5 minutes.

Figure 10 shows a photograph of the transfer film obtained and an SEM image. SEM observations confirmed the formation of nano-pillar patterns at a scale of 200 nm on the surface of the PET film.

Examinations confirmed that both the nanostructure mold and polymer film with the transferred pattern exhibited oil repellency in water (Fig. 11).
6. Fabricating the biliary stent and liquid passage test

The resulting polymer sheet with antifouling properties was rolled to form a tube and placed inside a biliary stent.

Figure 12 illustrates how we produced the biliary stent and shows a photograph of the finished product. The iridescent region is the section with the biomimetic structure. We passed an artificial bile solution consisting of a mixture of bovine bile and oil through the finished biliary stent to observe the manner of the liquid passage. (The artificial bile solution was prepared by dissolving a powder of bovine bile in pure water to achieve a concentration...
of 10 wt%; adding lard to this solution at a concentration of 10 wt%; then heating to 40 °C.) Figure 13 gives an external view of the apparatus for the liquid passage evaluation.

In the liquid passage test, the artificial bile solution was passed through the tube with a pump and the passage of the liquid observed in the section with the biomimetic structure. (See Fig. 14 for test results.) Our examination of the inner surface of the tube after the passage of the bile-oil mixture showed oil adhered to the inner surface of the tube without the biomimetic structure; the section of the
tube with the structure had repelled the oil.

These findings suggest that these efforts to create a highly oleophobic biliary stent were successful.

7. In vivo study

We placed indwelling biliary stents based on the biomimetic structure in the bile ducts of pigs and made SEM observations of the resulting stent conditions.

7.1. Overview of in vivo study

The animals used in the study were female pigs (N = 2 animals, 20–30 kg). All were fasted preoperatively for 12 hours (Category C). All procedures were performed under general anesthesia with mechanical ventilation, induced by intramuscular (IM) administration of ketamine hydrochloride (10 mg/kg) and atropine sulfate (IM, 0.05 mg/kg) administered as a preanesthetic to
suppress tracheal and salivary secretions before endotracheal intubation. General anesthesia was maintained with sevoflurane (2–3%) inspiration and Musculax (0.5 mg/kg). The experiment was performed in pigs and was performed in compliance with the National Institutes of Health Guidelines and the Animal Research Protocol of Saitama Medical University.

7.2. In vivo study procedure

After transportation, the pigs were acclimatized with a period of regular diet. To place the stents, we restricted the pigs to a supine position, then accessed the intraperitoneal cavity with an upper-abdominal median incision, by the method described in the above reference to reduce suffering. After exposing the common bile duct and duodenum, we made an incision to open the anterior duodenum wall, located the duodenal papilla, and inserted the biliary stent tube being evaluated through it to deliver the stent from the common bile duct into the duodenum (Fig. 15). The stent was thereafter fixed to the duodenum wall using 4-0 PDS (0.15 mm absorbable synthetic monofilament). We used the 4-0 PDS to suture and close the duodenum wall after completing the stent implant procedure (Category D). The pigs were started on a liquid diet one day after this procedure and were switched to regular diet three days after this procedure, taking into consideration observations of their general condition. Seven days after the operation, the pigs were euthanized and their intraperitoneal cavities reopened to retrieve the stent. Euthanasia was performed via intravenous administration of ketamine hydrochloride (20 mg/kg).

7.3. Results of animal testing (antifouling evaluations)

We assessed antifouling based on visual and electron microscope observation of the regions of the biliary stent with and without the biomimetic structure.

![Biomimetic structure](image1)

![Bacteria](image2)

![Atheroma (Dirt)](image3)

Fig. 17. Results of SEM observations of biliary stents removed after indwelling for one week in bile duct of pig.

Visual observations with the unaided eye showed less fouling on the surface regions with biomimetic structures. Electron microscope observations confirmed a clear difference in extent of fouling between regions with and without the biomimetic structure (Fig. 16).

We examined SEM images to determine the area fouled in each region (Fig. 17). Three areas were observed in each region. Table 1 presents the results.

Mean fouling rate (%) = 100 × (fouled surface area/total surface area of observed region)

The results indicate the antifouling performance of surfaces that feature the biomimetic structure is 58–85% better than surfaces without the structure.

8. Summary

We produced a polymer film with antifouling properties by replicating a biomimetic structure on
a polymer surface. We rolled the flat polymer sheet into a tube for use in biliary stents. These stents were implanted in the bile ducts of pigs to examine antifouling performance. The results show the polymer sheets are suitable for use in biliary stents and exhibit strong antifouling properties. Future studies will address mold production and stent manufacturing methods suitable for mass production.

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References


Table 1. Summary of antifouling effects.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Structure</th>
<th>Fouled surface area (μm²)</th>
<th>Mean fouling rate (%) *1</th>
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*1 Total surface area of observed region 1,260 (μm²).