Development of Bile Direct Stent Having Antifouling Properties by Atmospheric Pressure Low-Temperature Plasma

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Biomimetics (or biomimicry) is a field of technologies based on imitating various functions and properties of organisms. Waterproof products, which are inspired by lotus leaves with super-water-repellent fine structures, are a well-known example of biomimetics. The present study examined the surface structure of snail shells, which exhibit oil repellency (oleophobic property). Snail shells have nanoporous structures with nanoholes on the scale of 200–400 nm. When water enters these nanoholes, the surface is covered by thin water films. The oil can be repelled by the water film. These structures are known as superhydrophilic nanostructures. An earlier report discussed our efforts to create such nanostructures using a nanoimprinting method and assessed the feasibility of application to the inner walls of biliary stents. This involves a labor-consuming two-stage process involving creating nanostructures on a film surface, then rolling the film into a tube. In addition, the nanoimprinting mold made via electron beam lithography is costly and unsuitable for mass production.

To overcome these issues, we sought to develop elemental technologies for providing antifouling properties to biliary stents, which are made of polyethylenes (PEs), by forming nanostructures directly on the inner surface, using atmospheric pressure low-temperature plasma. We formed nanostructures on the inner walls of PE tubes of varying diameters under varying plasma conditions. We then examined the resulting structures and effects of the antifouling properties thus imparted.

Keywords: Biomimetics, Snail shell structure, Super-nanohydrophilic (structure), Bile duct cancer, Biliary obstruction, Biliary stent, Atmospheric pressure low-temperature plasma

1. Introduction

Applying biomimetics, we sought to develop technologies to improve the antifouling properties of a substrate surface independent of substrate shape. Biomimetics seeks to create artificial structures that imitate diverse functions exhibited by living
organisms [1–3]. As the saying goes, there are no dirty snails, snail shells have long been known to exhibit superior antifouling performance. Snail shells feature nanoporous surface structures on the scale of 200 nm–400 nm (Fig. 1).

Water entering these nanoholes forms a thin film of water on the surface, which repels oil and other fouling substances. Referred to as superhydrophilic nanostructures, these films exhibit antifouling properties, repelling oils containing proteins, etc. (Fig. 2).

Thus, we might expect to produce antifouling properties (super-nanohydrophilic effects) by forming such convex-concave nanoscale structures replicating snail shells on polymer surfaces. An earlier report discussed our efforts to form nanostructures on the surface of an acrylic polymer substrate by nanoimprinting, after which the polymer sheet was rolled into a tube to produce prototypes of biliary stents with antifouling properties [4, 5]. However, this approach involved creating a nano-mold using electron beam lithography and forming nanostructures using a nanoimprinting method. Lithography requires special facilities and equipment and entails high costs. Additionally, current nanoimprinting and lithographic technologies are generally suitable only for flat substrates; they are not designed to create nanostructures directly on the inner walls of a tubular substrate. In the present study, we used the atmospheric pressure low-temperature plasma method [6, 7] to develop a technology for imparting enhanced antifouling performance to the surfaces of tubular materials, regardless of substrate shape.

2. Biliary Stents with Antifouling Properties

Figure 3 shows the relative positions of the liver, gall bladder, and bile duct. Bile is a fluid secreted by the liver that activates lipase, a digestive enzyme that facilitates the dissolution of oils in water and assists in the digestion and absorption of lipids. The main constituents of bile are bilirubin (an end product of red-blood cell breakdown), cholesterol, and bile acid [8]. Bile is temporarily stored in the gall bladder before being excreted to the duodenum. Biliary strictures attributable to bile duct cancer [9] or bile duct obstructions inhibit the flow of bile from the gall bladder to the duodenum, bile may flow back into the liver, resulting in icterus or, if left untreated, even fatal hepatic failure. Treatment to secure a passage for bile flow often involves a surgical procedure called endoscopic biliary stenting (EBS) [10, 11].

Two types of biliary stents are currently available: metallic stents and plastic stents [11]. Most EBS procedures involve plastic stents. Figure 4 is a photograph of a straight plastic stent. Each end has a flap, and each section of the tube beneath the flap has an opening.
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Fig. 1. Snail shell structure.

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Fig. 2. Mechanism of the production of antifouling properties by superhydrophilic nanostructures.

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Fig. 3. Biliary tract structure and example of endoscopic biliary stenting procedure.

Fig. 4. Plastic stent (straight type, Boston Scientific Corporation).

Fig. 5. Comparison of conventional stent and antifouling stent (schematic image).
Figure 5 is a schematic image comparing an antifouling stent to a conventional stent. In efforts to produce a surface that repels fluids containing oils, such as bile, we believed the structures found on snail shells, which exhibit super-nanohydrophilic effects in the presence of water, appeared likely to prove effective in creating an occlusion-resistant biliary stent.

We sought to develop elemental technologies for imparting antifouling properties to the inner walls of PE biliary stents by forming nanostructures directly onto the inner surface of polyethylene (PE) biliary stents with atmospheric pressure low-temperature plasma. We formed nanostructures on the inner walls of PE tubes of varying diameters under varying plasma conditions and examined the resulting structures and effects of the antifouling properties thus imparted.

3. Processing of Inner Walls of PE Tubes by the Atmospheric pressure low-temperature plasma Method

Atmospheric pressure low-temperature plasma can generally be categorized into two types: thermal equilibrium plasma (hot plasma) and nonequilibrium plasma (cold plasma) [12, 13]. A representative example of the former is arc discharge, in which the plasma is at high temperature, with both electron temperature and gas temperature on the order of 10,000 K. The latter is represented by glow discharge. Although the electron temperature of the plasma is 10,000 K or more, the gas temperature is around room temperature. Given the high gas pressure (the high number of molecules in the gas state) at atmospheric pressure, the number of collisions between electrons and gas molecules is also high, tending to result in plasma in a state of thermal equilibrium. While PE biliary stents cannot be exposed to hot thermal plasma, we believe cold plasma that can be sustained near room temperature is suitable.

Two well-known examples of atmospheric pressure low-temperature plasma are streamer discharge and dielectric barrier discharge. Since a uniform spatial distribution of discharge inside the biliary stent is required, we chose to use an RF power supply as the power source and helium as the dielectric barrier discharge gas. This combination suppresses electron density and is sufficient to form atmospheric pressure plasma of uniform distribution at low temperatures [12]. The diagram in Figure 6 illustrates the principle of this apparatus. The basic components are the power source, electrodes, and a glass tube through which helium gas flows. Two electrodes are placed on the opposite sides of the glass tube facing each other at some distance from the glass tube. High-frequency power is applied to the electrodes to generate atmospheric pressure low-temperature plasma inside the glass tube. As shown in Figure

![Diagram of atmospheric pressure low-temperature plasma generation unit and plasma being generated.](image)

Fig. 6. Overview of the atmospheric pressure low-temperature plasma generation unit and plasma being generated.
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When small amounts of oxygen are mixed into the He plasma, the dissociation of oxygen molecules generates atomic oxygen. Since the excitation level of atomic oxygen is the same as the metastable level of helium, previous studies suggest the Penning ionization reaction shown in the reaction formula below will occur. The oxidation reaction attributable to the atomic oxygen generated (O*) holds the promise of various applications for material surface processing technologies [13-20]. Mixing N₂ or NH₃ at concentrations of around 1 % in place of oxygen generates N radical (N*), amino radical (NH₂*), or imino radical (NH*), which can then be used to induce nitridation or amination of material surfaces [14, 15].

\[
\text{He + electron} \rightarrow \text{He}^* + \text{electron} \quad (1)
\]
\[
\text{He}^* + \text{O}_2 \rightarrow \text{He} + \text{O}^* + \text{O}^+ + \text{electron} \quad (2)
\]

4. Experimental

4.1. Examining plasma generation conditions

We observed the state of plasma generation inside the tube for varied He and O₂ flow rates (Figure 8). The results showed a stable glow discharge can be formed at an He flow rate of 1.4 slm and O₂ flow rate of 2–10 sccm. We then adjusted the power to determine the conditions at which stable plasma can be achieved.

Figure 9 shows the dependency of glow discharge inception power on O₂ flow rate. The He flow rate was fixed at 1.4 slm. We found that at O₂ flow rates of 0–6 sccm, a glow discharge can be stably formed at a power of 20–100 W. This power range may be regarded as the process window for glow
discharge inception. We surmised that stably forming a plasma inside the stent within this process window would allow processing of nanoporous structures on the inner wall of the stent.

Figure 9. Dependency of glow discharge inception power on \(O_2\) flow rate (process window) at a fixed \(He\) flow rate of 1.4 slm.

Figure 10 shows photographs of the state of plasma formation within PE tubes of varying inner diameters. We achieved stable plasma formation with inner diameters of 2–4 mm.

4.2. Observations of plasma processed surfaces

To observe nanostructures on the inner walls of the PE tube, we rolled a piece of PE sheet into a tube and inserted it into a guide tube to simulate a PE biliary stent with an inner diameter of 2 mm, then exposed it to plasma processing. We observed the structures on the inner wall of the PE sheet via AFM (Figs. 11 and 12). We made observations for power settings of 45 W and 60 W. To prevent tube overheating due to plasma exposure, we limited the duration of a single plasma irradiation to 5 seconds and allowed cooling intervals of 30 seconds before subsequent irradiation. This irradiation cycle was repeated multiple times.

Fig. 8. State of plasma generation at various \(He\) and \(O_2\) flow rates.

Fig. 9. Dependency of glow discharge inception power on \(O_2\) flow rate (process window) at a fixed \(He\) flow rate of 1.4 slm.

Fig. 10. State of plasma generation for different PE tube sizes.
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Figure 11 shows the results of examination for irradiation at 45 W. Two irradiation cycles reduced surface roughness. Four to six irradiation cycles resulted in surfaces with satisfactory convex-concave nanoscale structures.

Figure 12 presents the results of examination for irradiation at 60 W. As with a power of 45 W, the nanostructures achieved after two irradiation cycles were insufficient. However, we could confirm that four to six irradiation cycles created surfaces with satisfactory convex-concave nanoscale structures.

These results indicated it was possible to form nanostructures on the inner wall of a stent by exposing it to plasma at a power of 45–60 W for four to six cycles for a duration of 5 seconds per cycle.

We examined the oil repellency of the inner wall of a PE tube of 2 mm in diameter processed with four cycles at an applied voltage of 45 W (Figure 13). According to the results, the contact angle was 107.7 degrees for unprocessed surfaces and...
32.4 degrees for plasma processed surfaces, which rendered them hydrophilic. We evaluated oil repellency in water and found that oil bound strongly to the unprocessed surfaces, while oil droplets failed to adhere to the plasma processed surfaces, confirming the oil repellent effects of the processed surfaces. We confirmed antifouling effects can be achieved through plasma processing.

4.3. Liquid passage test for PE stents

To evaluate the antifouling performance of PE tubes with nanostructures, we prepared an artificial bile solution from bovine bile powder and oil. The 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.%, and then heating to 40 °C. We used a pump to feed this artificial bile solution into PE tubes for observations of liquid passage. (Figure 14(a) is a photo of the apparatus used for the liquid passage test.) We allowed the bile solution to pass through the tube for 5 seconds at a flow rate of 6 mL/min, followed for 5 seconds by water. Then, once again, we passed the bile solution for 5 seconds, followed by water for 5 seconds. We observed the inner wall of the tube when water passed for the second time. For comparison, the photos show the tubes as the bile solution passes and as the water passes. We performed tests for tubes of three diameters: 2 mm, 3 mm, and 4 mm. The plasma processing conditions were four cycles of 10 seconds of irradiation and 10 cycles of 10 seconds of irradiation at 20 W and four cycles of 10 seconds of irradiation at 50 W. Under all processing conditions, we allowed a 30-second cooling interval after each 10-second plasma irradiation. Figure 14(b) presents the results of the liquid passage test.

The results confirmed that for unprocessed tubes of 2 or 3 mm in diameter, bile adhering to the inner wall of the tube was rinsed out with water. We observed no turbidity of the bile solution for surfaces processed by plasma for 10 cycles of 10 seconds of irradiation at 20 W and four cycles of 10 seconds of irradiation at 50 W; the surfaces were judged to have antifouling properties. For tubes of 4 mm in diameter, the amount of bile adhering to the inner wall was excessive, and the bile solution was confirmed to be turbid for all conditions.

5. Summary

We sought to develop elemental technologies for imparting antifouling properties to the inner walls of PE biliary stents by forming nanostructures directly onto the inner surface with atmospheric pressure low-temperature plasma. Nanostructures were formed on the inner walls of PE tubes having different diameters under varied plasma conditions, and the resulting structures and effects of the imparted antifouling properties were examined. The results show that plasma processing at an He flow rate of 1.4 slm, an O₂ flow rate of 0–6 sccm, and a power of 20–60 W forms satisfactory nanostructures on the inner walls of PE tubes that allow them to exhibit antifouling properties, especially oil repellency in water. In future studies, we plan to form nanostructures directly onto the
inner surface of PE biliary stents with atmospheric pressure low-temperature plasma and confirm the imparted antifouling effects by performing animal experiments.

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